Adult female sensitivity to day-length conditions in terms of winter-egg production in *Schizotetranychus brevisetosus* Ehara (Acari: Tetranychidae)

Katsura ITO[*] and Eri HAMADA

Faculty of Agriculture and Marine Science, Kochi University, 200 Monobeotsu, Nankoku, Kochi 783-8502, Japan

(Received 2 January 2018; Accepted 22 April 2018)

[*]Corresponding author: e-mail: ktr@kochi-u.ac.jp; phone +81-864-5136; fax: +81-864-5136

Short title: Winter eggs in *Schizotetranychus brevisetosus*

Key words: diapause, hibernation, sensitivity, summer egg, winter egg

ABSTRACT

Schizotetranychus brevisetosus Ehara lives on the leaves of *Quercus glauca* L. In Tosayamada, Kochi Prefecture, Japan, females in the field stop producing yellowish-white summer eggs by early November, and after several weeks of reproductive arrest, they produce bright orange winter eggs, from late November or early December until March, when they die. In this study, adult female sensitivity to day-length conditions was evaluated at the beginning of the season in which they produce winter eggs. Females collected from the field in late November and early December were reared under one of two sets of day-length conditions (10L:14D or 15L:9D) for 16 days at either 15 °C or 25 °C. Both groups of field-collected females increased their winter-egg production under long day-length conditions, and December females laid significantly more eggs than November females at 25 °C. In contrast, females from the laboratory strain produced summer eggs at the higher temperature, but were not sensitive to day length. The trend whereby long day-length conditions promote the production of eggs might partly explain the field observation that the number of eggs produced increases during the period over which day length increases, towards the vernal equinox (late March).

INTRODUCTION

Day length is a primary cue for diapause induction and termination in temperate arthropods. The intensity of diapause gradually decreases during the period between diapause onset and termination (Andrewartha, 1952; Hodek, 2002). Exposure to long-day conditions rapidly terminates diapause in many arthropods (Danilevskii, 1965; Veerman, 1977b; Tauber *et al.*, 1986; Danks, 1987).

Sensitivity to day length has long been studied in spider mites (Acari: Tetranychidae). In adult-diapausing species such as *Tetranychus urticae* Koch, diapause is induced by short day-length conditions (Veerman, 1977a), and is terminated rapidly under long day-length conditions (Veerman, 1977b, 1985). In egg-diapausing species such as *Oligonychus castaneae* Ehara and Gotoh, previously called the chestnut strain of *O. unuguis* (Jacobi), the proportion of females producing diapausing eggs increases by the exposure to short day-length during development (Shinkaji, 1975). Although immature stages are usually sensitive to photoperiod, adults are also sensitive both in egg-diapause species (e.g. *Panonychus ulmi* Koch in Lees, 1953; *Panonychus mori*, the diapausing strain of *P. citri* in Fujimoto and Takafuji, 1986) and adult-diapausing species (*T. kanzawai*, *Stigmaeopsis longus* etc. in Saito et al., 2005). In egg-diapausing species, part of adult females producing winter eggs begin to produce summer eggs if exposed to long day-length conditions (Lees, 1953; Fujimoto and Takafuji, 1986).

Despite our present understanding of the importance of day length, the effect of seasonal change in day length has been little investigated. In the field, diapausing females stay on host leaves, if they are available, throughout winter (Osakabe, 1967; So and Takafuji, 1991; Takafuji *et al.*, 1991; Takafuji and Morishita, 2001). Species that feed on evergreen host plants are suitable for investigating sensitivity to day length, as adult females can deposit eggs on the host leaves during winter and spring if climatic conditions allow (Gotoh, 1986a, b; Saito, 2010). However, how the reproductive schedule during hibernation is regulated by day length and other environmental conditions remains unknown.

Here, we focused on *Schizotetranychus brevisetosus* Ehara, which lives on the leaves of the evergreen Japanese blue oak *Quercus glauca* L. (Ehara, 1989). In Tosayamada, Kochi Prefecture, Japan (33.633°N, 133.708°E), the number of summer eggs (yellowish-white in colour) on host leaves diminishes by the beginning of November, and adult females stay on the leaves, without oviposition, for several weeks, although the temperature frequently rises above 20 °C in mid-November. They begin to produce bright orange winter eggs in late November or early December, when the daily maximum temperature decreases to ca. 15 °C (Japan Meteorological Agency, 2017). They survive until the next year while continuously producing winter eggs. Since these eggs do not hatch until spring comes, the density of winter eggs increases from the winter solstice toward March, when the females begin to die (K. Ito, in preparation).

The aim of this study is to clarify sensitivity to day length in females immediately before the deposition of winter eggs. We primarily compared the pattern of egg production in adult females collected from the field under different day-length conditions (10L:14D and 15L:9D) at either 15 °C or 25 °C. These extreme day lengths were selected to emphasize the differences between the response at short and long day lengths (cf. approximately 10L:14D at the winter solstice and 14.5L:9.5D at the summer solstice in Kochi City; Japan Meteorological Agency, 2017). We monitored egg production at 16 days and did not assess lifetime fecundity, because day-length conditions during the adult stage may affect oviposition patterns (Fujimoto and Takafuji, 1986; Saito et al., 2005), and may obscure the original reproductive capacity. The experiments were replicated twice, in late November and early December, to investigate whether the response to day-length conditions changes as development proceeds. These responses were compared with those of summer-egg-producing females from a laboratory strain, to examine the physiological characteristics of the wild females that produce winter eggs.

MATERIALS AND METHODS

Females from the field

The field sample of *S. brevisetosus* was collected from *Q. glauca* trees at the Kochi Prefectural Forestry Technology Research Centre in Kami, Kochi Prefecture (33.633°N, 133.708°E; WGS84) on two occasions (23 November and 7 December 2016). In each month, 10 females were individually isolated on 1 × 1 cm *Q. glauca* leaf squares resting on water-soaked cotton pads in a plastic dish with internal dimensions 91.3 × 38.2 mm (Insect Breeding Dish; SPL Life Sciences, Gyeonggi-do, Korea) within 24 h of collection. This dish was kept in the chambers of a Bio Multi Incubator LH-30-8CT (NK System, Osaka, Japan) under four different combinations of day-length (10L:14D and 15L:9D) and temperature (15 °C and 25 °C) conditions (n = 10 for each treatment). The production of eggs was recorded after 16 days of female isolation. Females that died during this period were excluded from the analyses.

Females from the laboratory strain

More than 100 foundresses were collected from the same place on 1 November 2016 as a laboratory strain. The laboratory strain was maintained on the undersurface of a detached leaflet of *Q. glauca* and kept at 30 °C under a day-length regime of 15L:9D, with 40–60% relative humidity, in a plant growth chamber (MIR-154, SANYO). These females reproduced and their offspring developed well under these conditions (cf. mean ± SD egg-to-adult development time of 22.6 ± 3.1 days (n = 22) under 25 °C and 16L:8D conditions, recorded by Tamura and Ito, 2017). On each of 23 November and 7 December 2016, 10 fertilised adult females were randomly sampled from the next generation of the laboratory strain and assessed for the production of eggs using the method described above.

Statistical analysis

The number of eggs produced by the field- or laboratory-derived females was analysed using multivariate analysis of variance (ANOVA). Day length, temperature and collection date were incorporated as independent variables, and the main effect of each factor and the effect of the interaction between these factors on the number of eggs was evaluated. These analyses were conducted using the *aov* command in R v. 3.3.2 (R Core Team, 2016). Furthermore, the difference between the number of eggs at 25 °C was assessed using a three-way ANOVA in R, with sampling date, source (field or laboratory) and day length as independent variables. The data from the 15 °C trials were not analysed because it included too many zero values, which caused a heavy skew and a high degree of non-normality in the residual distribution.

RESULTS AND DISCUSSION

All field females kept at 15 °C produced bright orange eggs as on winter host leaves. The females at 25 °C produced eggs of various colours ranged from pale to bright orange, but those from the laboratory strain produced only yellowish-white eggs at both temperatures.

Egg production in each treatment is summarised in Fig. 1. The females collected from the field and those of the laboratory strain tended to produce more eggs at 25 °C than at 15°C (Table 1A and B, Temperature, both $P < 0.001$). Moreover, the number of eggs laid by the females collected in the December field was significantly greater than that laid by the November females (Fig. 1A, Table 1A, Date, $P < 0.001$), but no difference was found in the females of the laboratory strain (Fig. 1B, Table 1B, Date, $P = 0.099$). Therefore, this difference cannot be attributed to seasonal changes in leaf conditions, but the development of adult females may have proceeded during the period between November and December.

Fig. 1

Table 1

Importantly, day length strongly affected egg production in the field-collected females, irrespective of the month of collection, though the females collected in November did not oviposit in either day length at 15 °C. At 25 °C, the females collected in November oviposited 0.14 eggs, on average, under 10L:14D conditions, but 5.75 eggs under 15L:9D conditions. Similarly, the females collected in December oviposited 2.80 eggs under 10L:14D conditions, but 9.25 eggs under 15L:9D conditions. The ANOVA results established that long day length significantly increased the number of eggs deposited (Tables 1A, Day length, $P < 0.001$). The effect of day-length was not found in the laboratory strain (Tables 1B, Day length, $P = 0.127$).

Table 2

Table 2 shows the results of the ANOVA for the number of eggs deposited at 25 °C. The main effect of day length was highly significant, and the interaction between the source (field versus laboratory) and day length was also significant. These results indicate that long day-length conditions increased egg production, but that the response to long day length in the females from the field was stronger than that of the females from the laboratory strain.

These results demonstrate that adult females of *S. brevisetosus* can perceive photoperiodic changes as reported in several egg-diapausing species, in which adult females producing winter eggs became to produce summer eggs if transferred to long day-length conditions (Lees, 1953; Fujimoto and Takafuji, 1986). However, the change in the egg number has scarcely investigated, e.g. winter females of *P. mori* are less fecund than summer females (Fujimoto and Takafuji, 1993). Although the winter eggs of *S. brevisetosus* are not proved to be in diapause, this species might be different from egg-diapausing species in that long day length accelerates the production of winter eggs. However, colour is now only a cue to distinguish egg types, so that the characteristics of diapause (if they do) and morphology in each type should be further studied. Moreover, we should investigate the life-history traits of winter females, and compare them with those of summer females to comprehend the overwintering strategy of *S. brevisetosus*.

This high sensitivity to long day-length conditions suggests that females from the field are ready to produce winter eggs in response to increasing day-length conditions in the upcoming spring. Winter eggs increase in the period between late December and March without hatching, during which day-length conditions increase from 10L:14D (winter solstice) to 12L:12D (vernal equinox, excluding civil twilights; K. Ito, unpublished data). The present experiments adopted an extremely long day

(15L:9D) to clarify the qualitative differences between responses under long-day and short-day conditions. The precise day-length conditions for inducing winter-egg production should be identified to clarify the reproductive schedule of *S. brevisetosus* during winter and spring.

Adult sensitivity and its response to winter egg production may be associated with the availability of *Q. glauca* leaves in winter. The most egg-diapausing species in previous studies lived on deciduous host plants, and adult females produce diapausing eggs on twigs and die before winter comes (Fujimoto and Takafuji, 1993). For example, the winter females of *P. mori* move to twigs for oviposition, and return to leaves for feeding after every oviposition (Fujimoto and Takafuji, 1990). Evergreen host plants enable adult females producing winter eggs to survive late in the year, or even hibernate themselves as found in *P. akitanus* Ehara (Gotoh, 1986a, b) and *Yezonychus sapporensis* Ehara (H. Yanagida, unpublished data). *Schizotetranychus brevisetosus* is similar to *P. akitanus* in that only two stages (adult females and eggs) exist in the coldest month (Gotoh, 1986a, b). However, the females of *P. akitanus* complete the production of winter eggs within the year (before the winter solstice) and survive until the coming breeding season (Gotoh, 1986a, b), while the females of *S. brevisetosus* dies without breeding with the generation of winter eggs (K. Ito, unpublished data). Thus, the overwintering strategies may be variable even among species on evergreen host plants. Gotoh (1986b) argued that species that overwinter in two life stages in *P. akitanus* may represent a transient evolutionary state from egg to female adult diapause, or vice versa. The reproductive pattern in *S. brevisetosus*, which is probably categorized as egg-diapausing species, would also represent favourable study subjects for clarifying how sensitivity to day length or other environmental conditions is associated with the differentiation of overwintering patterns in Tetranychidae.

ACKNOWLEDGEMENTS

We thank Dr. Y. Chae for valuable comments on this study and for experimental assistance.

REFERENCES

Andrewartha, H. G. (1952) Diapause in relation to the ecology of insects. *Biological Reviews,* 27: 50–107.

Danilevskii, A. S. (1965) Photoperiodism and seasonal development of insects. Edinburgh, &c., Oliver & Boyd Ltd.

Danks, H. V. (1987) Insect dormancy: an ecological perspective. Biological Survey of Canada, Ottawa.

Ehara, S. (1989) Four new species of spider mites (Acarina: Tetranychidae) from Japan. *Proceedings of the Japanese Society of Systematic Zoology*, 40: 28–38.

Fujimoto, H. and A. Takafuji (1986) Photoperiodic sensitivity of various stages of the diapausing strain of the citrus red mite, *Panonychus citri* (McGregor) (Acarina: Tetranychidae). *Applied Entomology and Zoology*, 21: 582–588.

Fujimoto, H. and A. Takafuji (1990) Ovipositional behaviour of diapause-Induced females of the citrus res mite, *Panonychus citri* (McGregor) (Acarina: Tetranychidae). *Applied Entomology and Zoology*, 25: 509–514.

Fujimoto, H. and A. Takafuji (1993) Oviposition of diapause-induced females of *Panonychus mori* Yokoyama (Acari: Tetranychidae). *Applied Entomology and Zoology*, 28: 106–108.

Gotoh, T. (1986a) Life-cycle variation in *Panonychus akitanus* Ehara (Acarina:Tetranychidae). *Experimental and Applied Acarology,* 2: 125–136.

Gotoh, T. (1986b) Local variation in overwintering stages of *Panonychus akitanus* Ehara (Acarina: Tetranychidae). *Experimental and Applied Acarology,* 2: 137–151.

Hodek, I. (2002) Controversial aspects of diapause development. *European Journal of Entomology*, 99: 163–174.

Japan Meteorological Agency (2017) Climatic statistics. Available at http://www.data.jma.go.jp/obd/stats/etrn/index.php (8 September 2017)

Lees, A. D. (1953) Environmental factors controlling the evocation and termination of diapause in the fruit tree red spider mite *Metatetranychus ulmi* Koch (Acarina: Tetranychidae). *Annals of Applied Biology*, 40: 449–486.

Osakabe, M. (1967) Biological studies on the tea red spider mite, *Tetranychus kanzawai* Kishida, in tea plantation. *Bulletin of the Tea Research Station*, 4: 35–156 (in Japanese).

R Core Team (2016) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria.

Saito, Y. (2010) Plant Mites and Sociality – Diversity and Evolution. Springer, Tokyo.

Saito, Y., K. Ito and T. Sakagami (2005) Imaginal induction of diapause in several adult-diapausing spider mites. *Physiological Entomology*, 30: 96–101.

Shinkaji, N. (1975) Seasonal occurrence of the winter eggs and environmental factors controlling the evocation of diapause in the common conifer spider mite, *Oligonychus ununguis* (Jacobi), on chestnut (Acarina: Tetranychidae). *Japanese Journal of Applied Entomology and Zoology*, 19: 105–111.

So, P. M. and A. Takafuji (1991) Coexistence of *Tetranychus urticae* (Acarina: Tetranychidae) with different capacities for diapause: comparative life-history traits. *Oecologia*, 87: 146–151.

Takafuji, A. and M. Morishita (2001) Overwintering ecology of two species of spider mites (Acari: Tetranychidae) on different host plants. *Applied Entomology and*

Zoology, 36: 169–175.

Takafuji, A., P. M. So and N. Tsuno (1991) Inter- and intra-population variations in diapause attribute of the two-spotted spider mite, *Tetranychus urticae* Koch, in Japan. *Researches on Population Ecology*, 33: 331–344.

Tamura, K. and K. Ito (2017) Extremely low fecundity and highly female-biased sex ratio in nest-living spider mite *Schizotetranychus brevisetosus* (Acari: Tetranychidae). *Systematic and Applied Acarology*, 22: 170–183.

Tauber, M. J., C. A. Tauber and S. Masaki (1986) Seasonal adaptations of insects. Oxford Univ. Press, New York.

Veerman, A. (1977a) Aspects of the induction of diapause in a laboratory strain of the mite *Tetranychus urticae*. *Journal of Insect Physiology*, 23: 703–711.

Veerman, A. (1977b) Photoperiodic termination of diapause in spider mites. *Nature*, 266: 526–527.

Veerman, A. (1985) Diapause. In: Spider mites. Their biology, natural enemies and control, 1A (eds., Helle, W. and M. W. Sabelis), pp. 279–316, Elsevier, Amsterdam.

Table 1. Summary of the ANOVA of the egg production of female *S. brevisetosus* individuals collected from the field (A) and cultured in the laboratory (B).

(A) Field

	df	SS	MS	F	*P*
Date	1	64.97	64.97	36.86	< 0.001
Temperature (Temp)	1	269.44	269.44	152.85	< 0.001
Day length (Dl)	1	189.80	189.80	107.67	< 0.001
Date × Temp	1	11.25	11.25	6.38	0.014
Date × Dl	1	10.99	10.99	6.24	0.015
Temp × Dl	1	114.22	114.22	64.79	< 0.001
Date × Temp × Dl	1	1.00	1.00	0.57	0.454
Residuals	63	111.06	1.76	-	-

(B) Laboratory

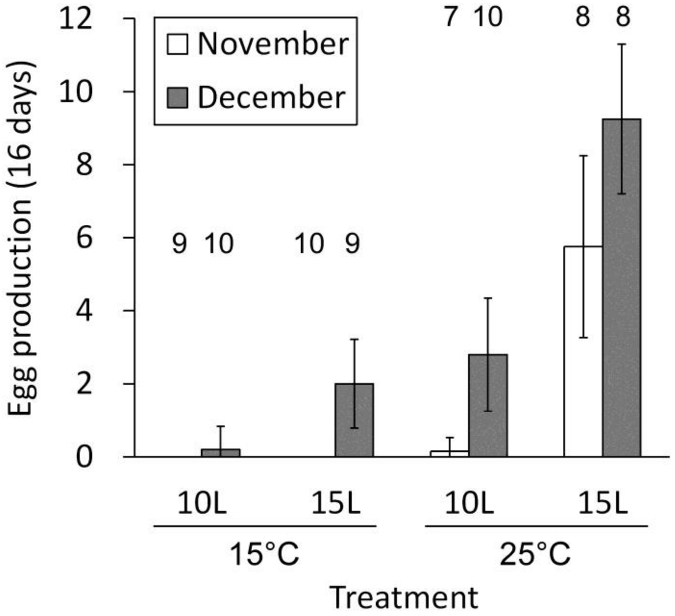
	df	SS	MS	F	*P*
Date	1	6.50	6.50	2.64	0.111
Temperature (Temp)	1	328.20	328.20	134.05	< 0.001
Day length (Dl)	1	5.90	5.90	2.42	0.127
Date × Temp	1	4.00	4.00	1.62	0.209
Date × Dl	1	0.40	0.40	0.16	0.695
Temp × Dl	1	8.10	8.10	3.32	0.075
Date × Temp × Dl	1	0.60	0.60	0.26	0.613
Residuals	47	115.10	2.40	-	-

Table 2. Summary of the ANOVA of the egg production of female *S. brevisetosus* at 25 °C. Source indicates whether individuals were collected from the field or cultured in the laboratory.

	df	SS	MS	F	P
Date	1	12.95	12.95	2.84	0.099
Source	1	9.71	9.71	2.13	0.151
Day length (Dl)	1	283.55	283.55	62.24	< 0.001
Date × Source	1	24.10	24.10	5.29	0.026
Date × Dl	1	3.07	3.07	0.68	0.416
Source × Dl	1	49.88	49.88	10.95	0.002
Date × Source × Dl	1	1.84	1.84	0.40	0.529
Residuals	45	205.02	4.56	-	-

Figure legends

Fig. 1. Egg production of female *S. brevisetosus* 16 days after the isolation of females collected from the field (A) and cultured in the laboratory (B). Mean and SD are indicated. The number of females is indicated on each bar.


(A) Field

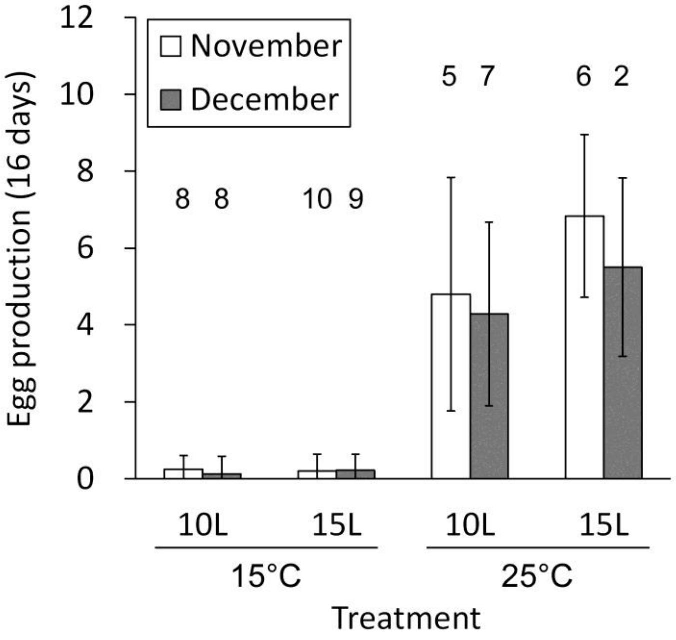

(B) Laboratory

Fig. 1.

摘要

カシノキマタハダニ雌成虫の冬卵産下における日長感受性

伊藤 桂・濱田 英里（高知大学農林海洋科学部）

カシノキマタハダニ（*Schizotetranychus brevisetossus* Ehara）はアラカシの葉に寄生する。高知県土佐山田町では、メス成虫は11月上旬までに夏卵の生産を終え、数週間にわたり繁殖を停止した後、11月下旬あるいは12月上旬から翌3月頃にかけてオレンジ色の冬卵を産み続ける。本研究では冬卵生産直前（11 月下旬）もしくは開始直後（12 月上旬）のメス成虫の産卵における日長感受性を調べるため、それぞれの時期に野外から採集したメス成虫を、15°Cと25°Cのいずれかの温度条件のもとで、異なる日長条件（10L:14D, 15L:9D）において16日間産卵させ、日長条件および温度条件・採集日が冬卵の生産に及ぼす影響を分析した。いずれの時期に採集された雌でも日長の効果は有意であり、特に25°Cの高温条件では15L:9Dの長日条件における卵の生産数は著しく増加した。以上の結果は、冬卵を生産する時期のメスは日長条件に対する感受性が高まっていることを示唆している。これらの結果は、野外のメスが日長が増加する期間に越冬卵を産み続けることと矛盾しない。