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Effects of environmental humidity on the survival and development of pine caterpillars, *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae)

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Abstract The pine caterpillar, *Dendrolimus tabulaeformis* is one of the most important pests on *Pinus tabulaeformis* and other pine species in North China. In the present study, effects of relative humidity (RH) on the development and survival of pine caterpillars and soil moisture (SM) on their diapausing larvae were investigated. Low RH (20%) deferred the development of eggs and larvae, reduced egg hatching and larval surviving compared to 40%, 60% and 80% RH. Both low (20%) and high (100%) RH reduced egg hatching, but only 20% RH deferred the development of larvae, prolonged developmental duration and reduced the body mass and body length of larvae. The SM influenced the survival of diapausing larvae significantly. The dry treatment significantly reduced the supercooling points (SCPs), whereas increased the mortality and reduced body mass from 56.9 to 36.5 mg and body water content from 78% to 63% after 2 weeks' exposure. Therefore, higher RH is more favorable for the development of early instars and survival of diapausing larvae of the pine caterpillars.

Key words cold hardiness, *Dendrolimus tabulaeformis*, development, mortality, relative humidity

Introduction

Environmental moisture is an important factor that affects different aspects of insects, but the effect may vary between species or developmental stages (Willmer, 1982). Low relative humidity (RH) can prolong the developmental period, but the physiological mechanism of this phenomenon is unknown (Smith, 1993; Guarneri *et al*. 2002). Environmental humidity can also affect winter survival of insects (Danks, 1991) due to its influence on water balance (Hadley, 1994) and physiological cold hardiness (Ring & Danks, 1994). Reductions in body water content can enhance cold hardiness by depressing hemolymph freezing point, and probability of spontaneous freezing (Salt, 1966). Therefore, the ability of insects to survive chilling episodes depends on complex interactions between environmental moisture and temperature.

The pine caterpillar, *Dendrolimus tabulaeformis* Tsai et Liu is one of the most important pests on *Pinus tabulaeformis* Carr. and other pine species in North China. Its larvae feed on needles and overwinter in soil around pine trees (Chen, 1990). Previous studies have shown that the pine caterpillars of the first generation lay eggs on the pine needle during June and August. Most larvae can develop to a new generation, but some larvae enter winter diapause as 3rd or 4th instar larvae due to the exposure of short day lengths in 1st instar larvae (Li & Gia, 1989; Han *et al*., 2005).

In the field, the moisture fluctuates frequently and can affect the pine caterpillars differently. This might be important for us to understand the population fluctuations of this pest under natural conditions. However, only few studies have been reported on the effect of atmospheric RH on growth and development of its lower instar larvae. Influ-

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ence of soil moisture on the overwintering larvae is also neglected (Chen, 1990). In this study, we investigated the effects of RH on eggs hatching, survival and development, the 1st, 2nd and 3rd instar larvae in *D. tabulaeformis*. Moreover, the effects of soil moisture (SM) on the diapausing larvae in soil were also investigated in this species.

Materials and methods

Insects

Eggs of *D. tabulaeformis* were collected from pine trees in the suburbs of Huairou County, Beijing, China (40˚54' N, 116˚37' E). The insects were reared with fresh needles of *P. tabulaeformis* at 25 ± 1°C for one generation. The larvae of the second generation of the lab colony were used.

Effects of relative humidity on eggs and 1st−3rd instar larvae

Different amounts of distilled water and sulfuric acid (H_2SO_4) were used to form 20%, 40%, 60%, 80% and 100% RH by the methods described by Solomon (1951), Winston *et al.* (1960) and Feng *et al.* (1999). One hundred newly deposited eggs, 50 newly exuviating 1st, 2nd and 3rd instar larvae were placed in beakers (500 mL), respectively. Beakers were placed in airproof glass boxes (5 000 mL) with different proportions of H_2SO_4 solutions (RH). The RH was measured by a thermohygrometer (IP68 RH logger, Tinytalk II 99030304, accuracy 3% RH) continuously. We observe this for six time-points over 1 day, and we regulated it based on the variation of RH. The eggs or larvae were exposed to RH treatments (each glass box) at 28°C and under 16:8 L:D photoperiod. The larvae were fed with fresh pine needles from the same pine tree daily, avoiding the influence of food deterioration on larvae. Each treatment (RH levels, glass container) was replicated three times (beakers).

The number of hatched eggs were recorded every 12 h until no hatch occurred in two consecutive days. The development and survival of larvae were determined by recording numbers of exuviations and dead larvae every 12 h. The body length was measured by a Vernier caliper (sensitivity 0.01 cm) and the body mass was determined by weighing individuals on an electronic balance (sensitivity 0.1 mg, Sartorius, R200D.A.G., Göttingen, Germany) just after the larvae exuviated to the new instars.

Effects of SM on diapausing larvae

To evaluate the effects of SM on diapausing larvae, the newly hatched larvae were reared at 25 ± 1°C combined with 9:15 L:D which triggers larval diapause (Han *et al.*, 2005). The diapausing larvae were exposed to 10 °C for 30 days, simulating the overwintering field conditions before testing.

The soil used in the experiment was taken from the forest, where the caterpillars were collected, and sifted by a sifter with 60 meshes per cm^2. The sifted soil was oven-dried at 100 °C for 24-h. Dry, moist, and wet substrates were prepared by reconstituting aliquots of the oven-dried soil with de-ionized water to 0%, 15%, 25% dry mass, respectively to represent the moisture levels of 0% (dry), 60% (moist), 100% (wet) (Costanzo *et al.*, 1997), which also represent local soil water content (Wu *et al.*, 1997). Three hundred larvae and ample treated soils were placed in each box (20 × 17 × 15 cm). The boxes were then sealed with foam and kept at 5 °C. Three boxes were used for each RH treatment. Fifty larvae (all of the remainder at the last time) were removed randomly from each box at 2, 4, 8, 12 (total 200) weeks after exposure, and were kept at 25 °C for 24 h and the numbers of live and dead individuals were recorded. Larvae with no movement and loose body segments were assumed to be dead (Goto *et al.*, 2001).

To determine water content of individuals, the fresh weights of 15 living larvae from each box were measured, and then the larvae were dried at 65 °C for 24 h and weighed. The water content was expressed as the percentage of loss of body weight after drying. Each treatment was replicated three times (boxes).

To determine supercooling points (SCPs), defined as the lowest temperature caused by the heat of crystallization are the representations of the limit of low temperature tolerance (Bale, 1987), diapausing larvae were fixed with a thermocouple connected to a temperature recorder (uR100, Model 4152, Yologama Elect. Co., Seoul, Korea). The larvae were then placed into a styrofoam tube (1 cm diameter, 6 cm length) to protect the larvae from breaking, and put into a freezing chamber cooled at a rate of 1°C/min. The SCP was assessed as the temperature that initiated spontaneous freezing as indicated by the liberation of latent heat (Chen & Kang, 2002). Thirty larvae were used to determine SCP in each treatment.

Statistical analysis

Statistical analysis was performed with SAS (SAS Institute, 1996). Data were analyzed using one- or two-way analysis of variance, and means were separated by Tukey test. Data were tested for normality.

Results

Effects of RH on hatching and developmental duration of eggs

Relative humidity affected the egg hatching and the lowest RH (20%) caused a significant decrease ($P < 0.05$) in number hatching compared with the other RH treatments (Fig. 1). The 100% RH also decreased egg hatching compared with the 40%, 60% and 80% RH. The developmental duration of eggs in the 80% RH was significantly shortened compared to other treatments ($P < 0.05$), whereas no significance was found among other treatments.

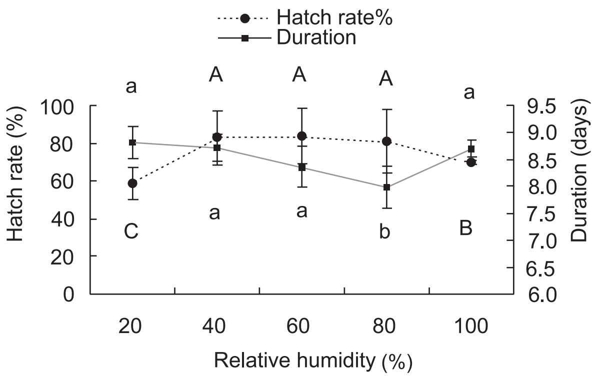

Fig. 1 Hatching (%) and developmental duration of eggs (days) of *Dendrolimus tabulaeformis* under different relative humidity (RH) regimens. Bars (SE) with different letters (capital letter for egg hatching and lower-case letter for developmental duration, respectively) are significantly different by Tukey test ($P < 0.05$).

Effects of RH on the growth of 1st–3rd instar larvae

The mortality of 1st instars increased with a decrease in RH, except at 100% RH. Mortality at 20% RH (69%) was the highest and 80% RH (6%) was the lowest. Mortalities did not differ significantly among the 60%, 80% and 100% RH for 1st instars and among different RH treatments for 2nd and 3rd instars (Table 1).

The developmental durations were affected by RH differently. The greatest influence occurred on 1st instar larvae. RH of 20% and 40% prolonged the developmental duration of the 1st instar larvae significantly compared with those at 60%, 80% and 100% RH. No difference of the developmental durations of the 1st instar larvae was observed at a RH over 60%. Only the 20% RH delayed the development of the 2nd instar larvae significantly (P =

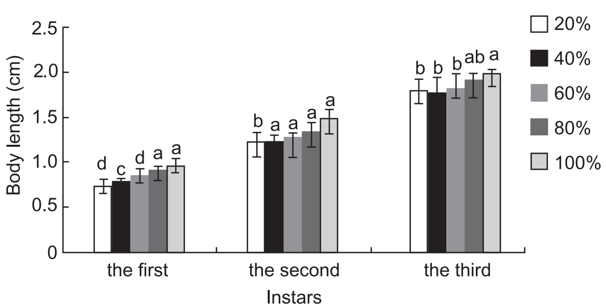

Fig. 2 Body lengths of 1st–3rd instar larvae of *Dendrolimus tabulaeformis* under different relative humidity (RH) regimens. Bars (SE) with different letters are significantly different by Tukey test ($P < 0.05$).

Table 1 Mortality (%) of 1st–3rd instar larvae of *Dendrolimus tabulaeformis* under different relative humidity (RH) regimens.

Instars	Relative humidity regimens				
	20%	40%	60%	80%	100%
1st	69.0 ± 2.48 a	43.0 ± 5.18 b	14.0 ± 1.84 c	5.7 ± 6.57 c	11.8 ± 8.05 c
2nd	14.7 ± 3.32 a	13.6 ± 3.72 a	12.7 ± 8.25 a	9.2 ± 3.69 a	11.6 ± 2.85 a
3rd	24.4 ± 5.67 a	16.1 ± 0.70 a	15.8 ± 2.95 a	18.1 ± 7.93 a	23.8 ± 2.98 a

Values (mean ± SE) followed by different letters in a row are significantly different by Tukey test ($P < 0.05$).

Table 2 Developmental duration (days) of 1st–3rd instar larvae of *Dendrolimus tabulaeformis* under different relative humidity (RH) regimens.

Instars	RH regimens				
	20%	40%	60%	80%	100%
1st	7.8 ± 0.64 a	6.6 ± 0.64 b	5.7 ± 0.97 c	5.5 ± 0.27 c	5.5 ± 0.31 c
2nd	6.6 ± 0.22 a	5.9 ± 0.16 b	5.8 ± 0.28 b	5.5 ± 0.64 b	5.8 ± 0.38 b
3rd	7.3 ± 0.25 a	7.4 ± 0.26 a	7.5 ± 0.10 a	6.9 ± 0.32 a	6.7 ± 0.32 a

Values (mean ± SE) followed by different letters in a row are significantly different by Tukey test ($P < 0.05$).

Table 3 Body mass (mg) of 1st–3rd instar larvae of *Dendrolimus tabulaeformis* under different relative humidity (RH) regimens.

Instars	RH regimens				
	20%	40%	60%	80%	100%
1st	4.5 ± 0.03 d	6.0 ± 0.20 c	7.5 ± 0.30 b	9.5 ± 0.60 a	9.0 ± 0.28 a
2nd	20.5 ± 0.50 c	21.5 ± 5.20 c	42.2 ± 1.30 b	46.7 ± 2.80 b	66.4 ± 2.20 a
3rd	62.2 ± 2.60 b	77.4 ± 5.70 a	76.8 ± 6.30 a	81.5 ± 5.20 a	82.8 ± 2.60 a

Values (mean ± SE) followed by different letters in a row are significantly different by Tukey test ($P < 0.05$).

0.048). No difference occurred in development of 3rd instar larvae in response to differing RH ($P = 0.26$) (Table 2).

Relative humidity had significant effects on body mass and body length of the larvae. The 1st instar larvae exposed to 20% RH had significantly shorter body length ($P < 0.01$) and small body mass ($P < 0.01$) than those exposed to higher RH. Body mass and body length of the larvae increased with an increase in RH except 100% RH. Similarly, the body mass and body length of the 2nd and 3rd instar larvae increased with the increase in RH (Fig. 2, Table 3).

Influence of SM on the diapausing larvae

The body mass of the diapausing larvae decreased from 56.9 to 36.5 mg ($P < 0.01$) and water content from 77.9% to 63.3% ($P < 0.01$) after a 2-week exposure to dry soil (0% SM), respectively, and then became constant ($P > 0.05$) after this period (Fig. 3A,B). The dry treatment significantly reduced the SCPs ($P < 0.01$) (Fig. 3D). However, the mean mortalities of larvae exposed to dry soil (0% SM) increased significantly ($P < 0.01$) compared with moist (60% SM) and wet (100% SM) conditions. More than 90% of the larvae (94.7%) died after 12 weeks of exposure to dry soil. However, moist and wet conditions had little effect on the survival of diapausing larvae in this period (Fig. 3C).

Discussion

Hatching and development of eggs affected by environmental humidity have been reported in many insect species (Roca & Lazzari, 1994; He *et al.*, 2002). For example, low atmospheric RH decreased the egg hatching of masson pine caterpillars, *D. punctatus* (Walker) (He *et al.*, 2002) and low RH was deleterious to the eggs of some triatomine

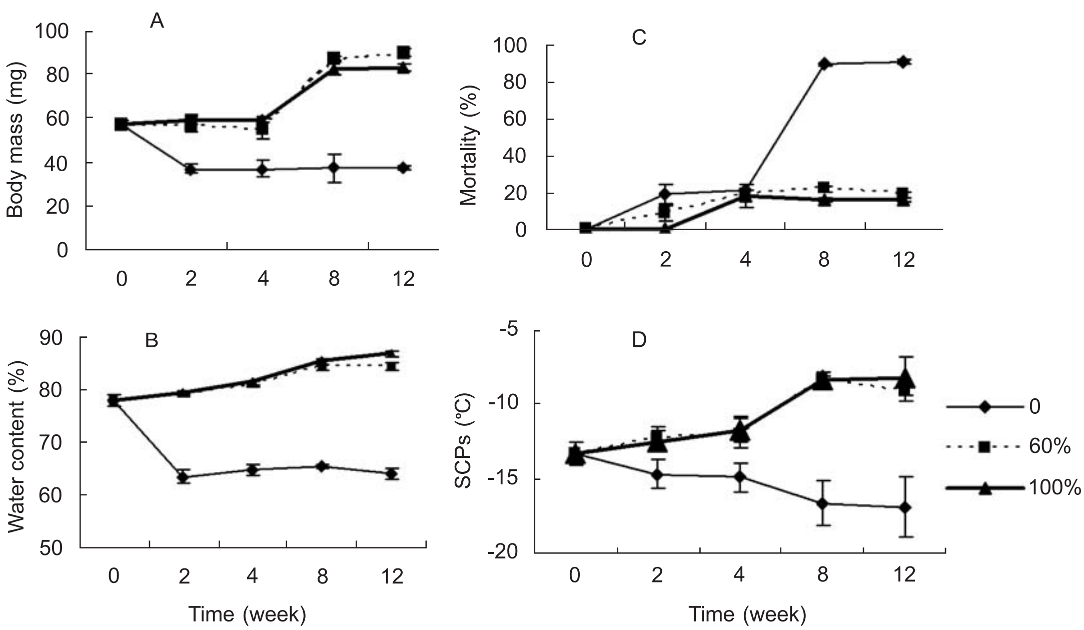

Fig. 3 Changes in body mass (A), water content (B), mortality (C) and supercooling points (SCPs, D) of the diapausing larvae of *Dendrolimus tabulaeformis* in different soil moisture (SM) regimens.

species (Roca & Lazzari, 1994). Present results showed that both low and high RH were deleterious to the egg hatching and low RH (20%) resulted in significantly low egg hatching in the pine caterpillars, *D. tabulaeformis*. Moreover, the development duration of eggs decreased with a decrease in RH and the treatment of 80% RH caused significantly shorter development duration in this species. We presumed that the deleterious effect of RH on eggs might be attributed to the desiccation that leads to abnormal development of the embryo (He *et al*., 2002) or prevents larval release from the eggshell by a loss of lubrication or sufficient cuticular softness (Guarneri *et al*., 2002).

Relative humidity could be a factor influencing development of early instars of *D. tabulaeformis*. Only the lower RH caused prolonging of developmental time and reducing of body mass, body length and survival of larvae consecutive from 1st to 3rd instars. This was agreement with He *et al*. (2002), who found that the larvae of masson pine caterpillars, *D. punctatus* developed more slowly than the larvae exposed to higher RH. Many studies on the effect of RH on insects also suggested that development time decreased with increasing moisture content, for example *Leucania separata* (Walker) (Jin *et al*., 1964), *Oryzaephilus surinamensis* (Linnaeus) (Arbogast, 1976), *Anisopteromalus calandrae* (Howard) (Smith, 1993) and *Triatoma brasiliensis* (Neiva) (Guarneri *et al*., 2002). The physiological reason for development time increase at low RH is unknown. We presumed that it may be related to decreasing rate of feeding or enzymatic reactions at suboptimal osmotic conditions. Another factor could be increasing energy cost of maintaining water balance by producing or conserving free water (Smith, 1993). Water is the universal solvent of living organisms. Biochemical and physiological processes of living organisms require an optimal interaction with water, and organisms possess efficient mechanisms to regulate the amounts and activities of extracellular and intracellular water. The RH of the environment can influence the water balance of a body directly and indirectly, which in turn may affect the metabolic activity of larvae (Danks, 2002). Therefore, dehydration or low RH results in slow development and small survival of the pine caterpillars.

The capacity to survive in different humidity conditions varies between insect species (Tichy, 2003). The present results indicated that the effect of RH decreases with the maturation of larvae of the pine caterpillars. This might be related to the resistance of larvae to low or high RH which increases with their development, that is, the development of cuticles prevents the water from vaporizing from the interior of the body as well as infiltration of water from outside the body (Danks, 2002).

The SM influences the metabolic activity of the diapausing larvae of the pine caterpillars. Dry soil led to a loss of body mass (36%) and water content (15%) and higher mortality compared with the moist and wet conditions. The effect of the dry conditions on larvae occurred as early as 2 weeks after exposure and resulted in a relative steady level after 8 weeks, but the effects of moist and wet conditions occurred 8 weeks after exposure. The results suggested that dissipating water was easier than absorbing water during winter in diapausing larvae of the pine caterpillars. Our results also indicated that dry soil significantly reduced the SCPs of the diapausing larvae. This could be partly explained by concentration of hemolymph cold hardiness substances as body water content declined (Lee & Denlinger, 1991; Worland, 1996). Although the diapausing larvae of *D. tabulaeformis* exposed to dry surroundings have lower SCPs compared with the larvae exposed to moist and wet surroundings, they can survive better under moist and wet surroundings than dry surroundings, suggesting that death might be more commonly caused by dehydration rather than by cold intolerance. This was not in agreement with Ushatinskaya (1978), who reported that winter mortality was sometimes associated with excessive moisture even in soils that do not freeze.

In North China, the rainfall that causes humidity varies between seasons and years. The pine caterpillars lay eggs on needles in rainy seasons (June and August) and overwinter as diapausing larvae in soil from November to April of the following year. Variations of humidity can affect the egg hatching, development and survival of early instar larvae of the pine caterpillars. Increase in rainfall during June and August can cause higher egg hatching, shorter developmental time of eggs and larvae and lower mortality of individuals. Despite the decrease in SCPs, diapausing larvae may not be able to survive dehydration in dry soil in winter. We presumed that environmental humidity was one of the reasons of the population fluctuation in this species in the field. Therefore, humidity level along with development stage may provide important information for predicting and managing the populations of the pine caterpillars, *D. tabulaeformis*.

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