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Implications of Temperature and Humidity on the Moulting Patterns and Moulting Survival in the Silkworm, *Bombyx mori* L.

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The implications of temperature (25, 30 and 35 ± 1°C) and relative humidity (60, 70 and 80 ± 2%) on the moulting pattern, moulting duration and moulting survival were studied in the silkworm, *Bombyx mori* L. Larvae of two pure silkworm breeds, Pure Mysore (PM) and NB_4D_2 and their hybrid, PM × NB_4D_2 were reared under experimental conditions under natural day photoperiodic (LD 12:12) condition. Two developmental marker events in the fourth moulting, settling for moult (SM) and completion of moult (CM) occurred at or around the middle of the photophase. The computed mean vector ($\bar{\phi}$), based on the circular statistics also confirmed the above. Temperature and humidity did not alter the moulting rhythmicity much. However, extreme temperature and humidity conditions reduced moulting survival in PM and PM × NB_4D_2. Further, moulting survival reduced below the economic level in NB_4D_2. The temperature and humidity together seem to exert synergic impact on the moulting survival of the silkworm *Bombyx mori*, at least in NB_4D_2.

Key words: Silkworm, *Bombyx mori*, Temperature, Humidity, Moulting, Survival

Introduction

Growth of the silkworm is characterized as discontinuous and is manifested by a series of moults. At moulting stages, the caterpillars of silkworm will be inactive, showing complete lack of interest in available food. Apart from moulting hormones, moulting is influenced by many environmental factors like photoperiod (Sivarami Reddy *et al.*, 1990) and temperature (Morohoshi, 1975; Murai, 2000), affecting the growth and development of the silkworm. In *Bombyx* silkworm, moulting was reported to be instar-dependent (Sivarami Reddy *et al.*, 1990). Krishnaswami (1986) indicated that apart from temperature, humidity may also influence the moulting patterns in the silkworm larvae. Nonetheless, studies on the combined effects of temperature and humidity on the moulting patterns and moulting survival in the silkworm are scanty. Further, Reynolds (1980) strongly viewed that very little published information seems to be available on mortality of larvae during larval to larval ecdysial process, especially in the commercial silkworm, *B. x mori* which directly accounts for the productivity of cocoon crop (Sivarami Reddy *et al.*, 1994). In the present study, decisive involvement of temperature and relative humidity on the moulting patterns and moulting survival in the *Bombyx* silkworm larvae are reported.

Materials and Methods

The silkworm, *Bombyx mori* L. was used as the test material in the present study. Three silkworm breeds/hybrids, *viz.*, PM (Pure Mysore), a multivoltine pure breed, NB_4D_2, a bivoltine pure breed and their hybrid, PM × NB_4D_2 were selected. The DFLs (disease free layings) of the silkworm were procured from the Government Grainages, Hindupur and Madakasira (Andhra Pradesh, India). Five DFLs of each silkworm breed/hybrid were introduced into experimental conditions (Sivarami Reddy and Sasira Babu, 1990; Lakshminarayana Reddy *et al.*, 2002). The silkworm rearing was conducted as per Krishnaswami (1986) under LD 12:12 (photophase from 06.00 h to 18.00 hrs of the day; around 50 lux and scotophase from 18.00 hrs to 06.00 hrs). Three temperature conditions, *viz.*, 25, 30 and 35°C (± 1°C) were imposed in the laboratory throughout exper-

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imentation, using an environmental chamber (Kolarstat). Further, three levels of relative humidity (RH) conditions, *viz.*, 60, 70 and 80% (± 2%) were induced.

All the three silkworm breeds/hybrids are of tetramoulters. Two developmental marker events in the moulting process of fourth moult were identified and selected for the study. The first marker event was the phase of settling for moult (SM), when the larvae stop feeding with its thoracic segment bulged and fixing legs to the substratum, adopting an S shaped posture for moulting, from which time the larvae are not supposed to be disturbed (Krishnaswami, 1986; Sivarami Reddy *et al*, 1990; Sivarami Reddy, 1993). The second marker event was the completion of moult (CM), the time at which the larvae cast their old skin (cuticle) and enter into the next larval instar (Sivarami Reddy *et al.*, 1990). For counting convenience, 100 larvae per each replication (5 replications) per each silkworm breed/hybrid (3) were maintained. Precise number of larvae that complete the SM and CM was noted at one-hour intervals. The moulting duration, from SM to CM (between peak hours) for all the experimental conditions for fourth moult was determined based on the observations made for SM and CM. Data on SM and CM were represented in distribution diagrams (hourly histograms resolved for 24 h; $\omega = 360^{\circ}$). From these values, the circular variables like mean vector ($\bar{\phi}$), length of the mean vector (r) and angular standard deviation (s) corresponding to SM and CM were computed through circular statistics (Chassé and Théron, 1988) for comparison with observed values. Date on these values ($\bar{\phi}$, r and s) were then analyzed statically using the test of Mardia-Watson-Wheeler (Mardia, 1972) to test the significance of difference, if any. However, data on $\bar{\phi}$, r and s are not presented in the present paper, as the test of Mardia-Watson-Wheeler showed no significant differences between treatments. Finally, the data on moulting duration and moulting survival (moulting percentage) were recorded and analyzed statically (ANOVA, 2-way classification with 5 observations per cell).

Results

The developmental marker events in moulting, SM and CM for the fourth moult of the silkworm, *B. mori* (PM, NB_4D_2 and PM x NB_4D_2) are depicted in Fig. 1, 2, 3. The molting process was observed in the mid-light phase of the LD cycle, with the peak of SM and CM occurring at or around 12 hrs of the day for PM (Fig. 1) and PM x NB_4D_2 (Fig. 3) and after 12 hrs for NB_4D_2 (Fig. 2). Under low temperature (25°C) crests of both SM and CM under three humidity conditions were very distinctive, with a sharp peak at 12 hrs. The duration between peaks of SM and CM was ~24 hrs. Under medium temperature (30°C), however, the chronograms were broadened to cover entire moulting marker events (SM and CM) in 6 to 8 hrs from 10 hrs to 18 hrs. Similar results were seen under high temperature (35°C). The amplitudes of moulting marker events (SM and CM) at the peak were gradually reduced as the rearing temperature increased and humidity reduced.

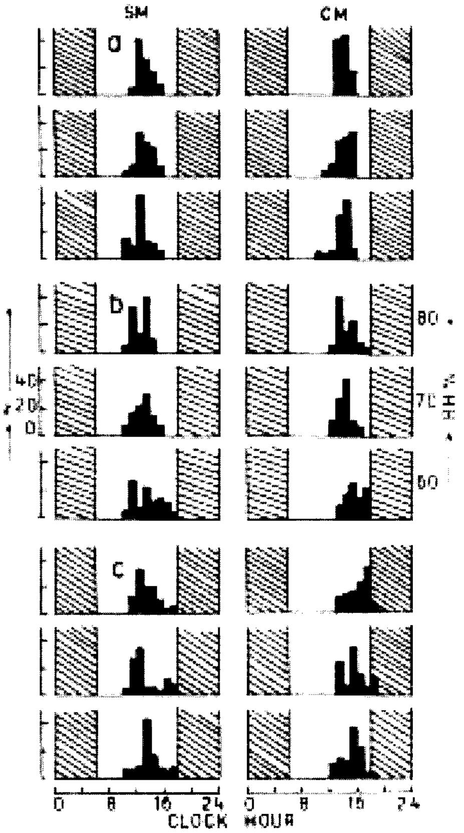

Fig. 1. Molting patterns of fourth molt in *B. mori* (PM) under low (25°C, a), medium (30°C, b) and high temperature (35°C) under LD 12:12 photoperiodic conditions. Crosshatched area represents the imposed scotophase (SM, settling for molt and CM, completion of molt being the molting marker events).

Fig. 2 depicts the results on moulting patterns of fourth moult in NB_4D_2 under low (25°C), medium (30°C) and high temperature (35°C) with three humidities (RH of 60, 70 and 80%). The moulting peak for both SM and CM expressed after 12 hrs of the day, unlike that of PM and PM x NB_4D_2. Under low temperature (25°C), expression of both SM and CM under three humidity conditions, was very distinctive, with a sharp peak at 13 hrs. The duration between peaks of SM and CM, in this case also was ~ 24 hrs. Under medium temperature (30°C), however, the chronograms were broadened to cover the entire SM and CM in 6 hrs from 13 hrs to 17 hrs. Similar results were seen under high temperature (35°C). Further, the ampli-

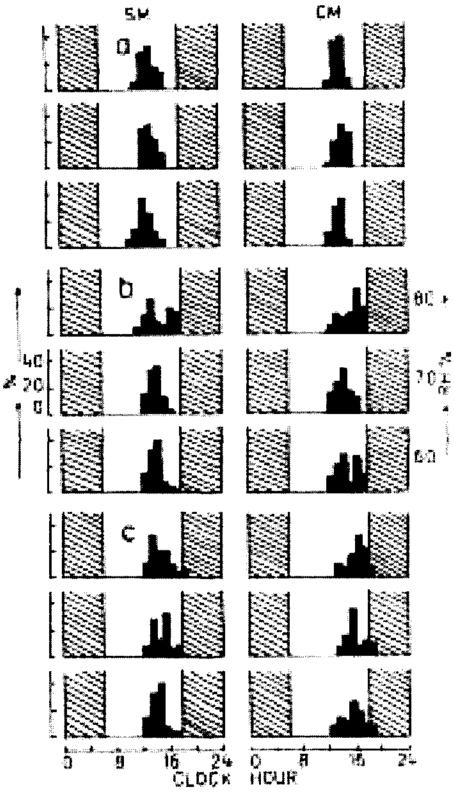

Fig. 2. Molting patterns of fourth molt in *B. mori* (NB_4D_2) under low (25°C, a), medium (30°C, b) and high temperature (35°C) under LD 12:12 photoperiodic conditions. Crosshatched area represents the imposed scotophase (SM, settling for molt and CM, completion of molt being the molting marker events).

tudes of moulting marker events (SM and CM) at the peak were gradually reduced in direct relation to temperature and indirectly to humidity. No significant differences were seen between the observed moulting patterns in all the breeds/hybrid and the computed moulting patterns ($\bar{\phi}$, r and s) calculated through circular statistics (therefore, data not presented in the paper).

The molting durations of fourth moult (duration between SM and CM) in PM, NB_4D_2 and PM × NB_4D_2 were ~ 24 hrs (Fig. 4). The moulting durations of PM and PM × NB_4D_2 were very low compared to that of NB_4D_2. Least moulting durations were observed for PM × NB_4D_2. Statistical analysis (ANOVA - 2 way classification with 5 observations per cell) revealed that both temperature and humidity has no significant effects (5%) on moulting durations in all three breeds and hybrids studied. Notably, the interactive effects of temperature and humidity on moulting durations were also not significant.

The molting survival during fourth moult is depicted in Fig. 5. Highest moulting (percentage) was observed in PM × NB_4D_2 followed by PM, while the least in NB_4D_2. The data subjected to ANOVA (2-way classification with 5 observations per cell) revealed that temperature alone has significant (5%) effect on moulting percentage. Humidity, on the other hand, showed no significant effects (5%) on the moulting percentage. Interestingly, the interactions between temperature and humidity have more effects (5%) on the moulting percentage, particularly in NB_4D_2.

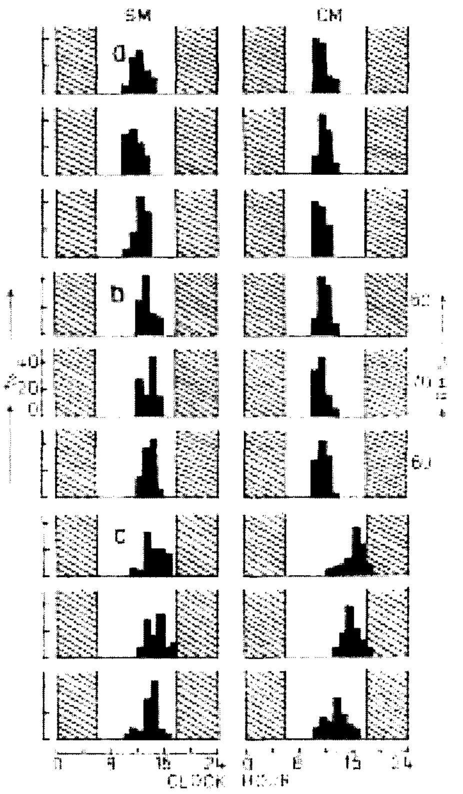

Fig. 3. Molting patterns of fourth molt in *B. mori* (PM × NB_4D_2) under low (25°C, a), medium (30°C, b) and high temperature (35°C) under LD 12:12 photoperiodic conditions. Crosshatched area represents the imposed scotophase (SM, settling for molt and CM, completion of molt being the molting marker events).

Discussion

Trumans (1972) extensive studies revealed that the larval ecdysis in *Antheraea pernyi* and *Manduca sexta* occurs at a particular part of the day depending on species, the instar and photoperiod. He reported that the larval moulting distribution time tends to broaden in *A. pernyi* from initial instars to later ones. The first and second molts occurred during light phase of both LD 12:12 and 17:7, but that of the third moult occurred in the following night and that of the fourth moult was delayed, occurring in the light phase again. Similar results were reported in *B. mori*

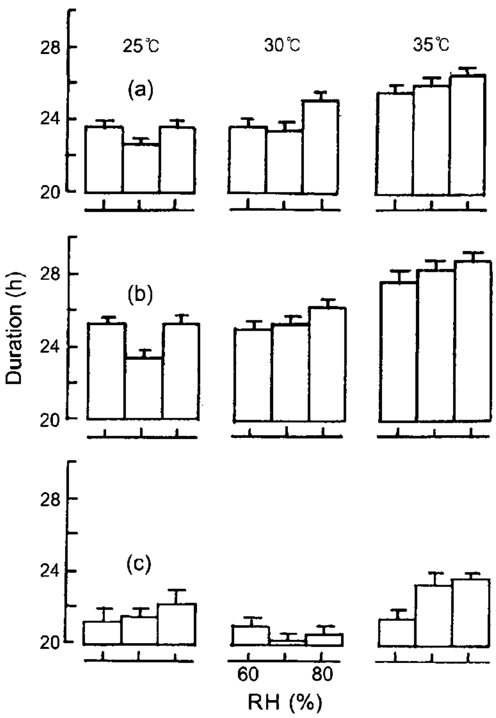

Fig. 4. Molting durations (duration between peaks of SM and CM) the fourth molt of the silkworm, *Bombyx mori* L. Note the moulting duration to be ~ 24 hrs in all cases. The effects of temperature and humidity and the interactions between temperature and humidity are not significant (ANOVA).

too (Sivarami Reddy *et* al., 1990). In the present experiment, the moulting (SM and CM) occurred during light phase of the day. Saunders (1982) viewed that the period of oscillation controlling pupal eclosion rhythm (pupal to adult ecdysis) did not change over a wide range of constant temperatures ($Q_{10} = 1.02$), which indicates that constant temperature has nothing to change the rhythmicity. The observed patterns of rhythmicity in larval-larval ecdysis, SM and CM under LD 12:12 conditions, and persistence of rhythmic pattern under all the imposed temperatures and humidity conditions, are worth noting. Sivarami Reddy (1993) and Sivarami Reddy *et al.* (1990) reported that the ecdysial rhythm under LD 12:12 condition persisted under all other photoperiodic conditions including continuous conditions (DD/LL). From the present study, it is clear that the rhythmicity of ecdysis persisted under constant temperature and humidity conditions too. Notably, the time of expression for SM and CM and the time between SM and CM (molting duration) did not vary. Therefore, it is generalized that temperature and humidity has nothing to change the rhythmicity in the moulting patterns in the *Bombyx* silkworm.

The productivity of the silkworm in terms of cocoon crop depends on several factors that operate within and outside the body of the silkworm. The success of the silkworm larvae through consecutive moultings is also one of the factors. The moulting survival was more influenced by temperature and humidity and further with the interactions between temperature and humidity (Fig. 5). Thus, the moulting survival was seen directly related to temperature and indirectly to humidity conditions imposed. Such effects, for pupation patterns, have been reported (Lakshminarayana Reddy *et al.*, 2002). Ecdysis is a mechanically difficult task and one can expect that its performance would be accompanied by an appreciable failure rate (Reynolds, 1980) and thus mortality in the larvae under moulting. The influencing factors of larval mortality during moulting period includes temperature, humidity (Reynolds, 1980), photoperiod (Sivarami Reddy *et al.*, 1994) etc. Bursell (1970) viewed that tropical species are more resistant to than temperate species. Thus, high moulting survival in PM, in the present study, is justified. Interestingly, moulting survival in the hybrid, PM x NB_4D_2 has been the highest over its pure parent breeds (PM and NB_4D_2) in the present study, perhaps justified as hybrid vigour (Suresh Kumar and Yamamoto, 1995). Therefore, it can be generalized that, while the silkworm moulting patterns and moulting durations did not change much, moulting survival, in the silkworm, *B. mori* are more influenced by temperature and humidity. Further, the interactions between temperature and humidity showed

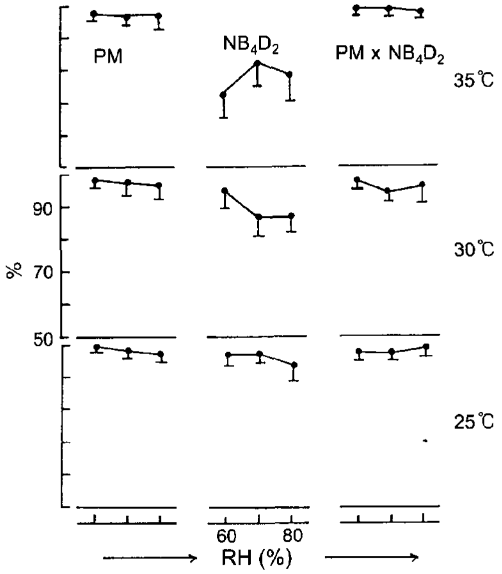

Fig. 5. Molting percentage (molting survival) in the fourth molt of the silkworm, *Bombyx mori* L. of PM (a), NB_4D_2 (b) and PM x NB_4D_2 (c). Note the high molting survival in PM and PM x NB_4D_2, and less survival in NB_4D_2. The effects of temperature are more significant than those of humidity (ANOVA). The interactions between temperature and humidity have significant effects on moulting survival in NB_4D_2 only.

profound effects on the moulting survival of bivoltine silkworm, NB_4D_2. Significant effects of temperature and humidity and their interaction effects on moulting survival strongly support the synergic impact of temperature and humidity on the moulting survival in the silkworm, though not on the rhythmic pattern of the moulting marker events.

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