



Diapause Termination in Casuarina Moth (*Lymantria xyli*) Eggs **【Research report】**

黑角舞蛾(*Lymantria xyli* Swinhoe)休眠卵滯育打破之研究 **【研究報告】**

Shaw-Yhi Hwang*, Chao-Chih Jeng, Tse-Chi Shen, Yi-Shan Shae and Chen-Shawn Liu
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Abstract

The casuarina moth, *Lymantria xyli* Swinhoe, is an important pest of hardwood and fruit trees. Overwintering casuarina moths enter an obligatory diapause as a pharate first instar larva. We assessed the relationship between chilling duration and temperature in terminating the diapause stage of casuarina moth eggs. Eggs of the casuarina moth were exposed to a variety of artificial chilling conditions that differed in duration and temperature. Results indicated that diapausing eggs require exposure to cold temperatures (9~15°C) followed by warm temperatures (27°C) for successful emergence in the laboratory. Eggs exposed to longer chilling periods required shorter incubation times to emerge than eggs exposed to shorter chilling periods. Regression analyses revealed a negative relationship between emergence time and chilling duration. The use of a proper chilling temperature and duration can effectively shorten diapause of casuarina moth eggs and make the routine rearing of casuarina moth populations possible.

摘要

黑角舞蛾 (*Lymantria xyli* Swinhoe) 是一嚴重的林木和果樹害蟲，此蟲會以休眠卵的方式越冬。本研究主要是探討冷藏處理對打破黑角舞蛾休眠卵的影響，研究的方法是將黑角舞蛾的卵置於不同的冷藏箱及冷藏不同的時間後，觀察卵的孵化情形。結果發現黑角舞蛾的休眠卵要先經一段冷藏 (9~15°C) 時間，再經一段回溫期 (27°C) 後，才能在實驗室中順利孵化。結果也顯示，冷藏越久的卵，回溫後卵孵化所需要的時間越短。同時，回歸分析顯示孵化時間與冷藏的時間呈現負相關的關係。因此，使用適當的冷藏溫度及冷藏時間，可以有效的縮短黑角舞蛾休眠卵所需休眠的時間。

Key words: *Lymantria xyli*, egg diapause, chilling treatment

關鍵詞: 黑角舞蛾、滯育卵、冷藏處理

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ABSTRACT

The casuarina moth, *Lymantria xyлина* Swinhoe, is an important pest of hardwood and fruit trees. Overwintering casuarina moths enter an obligatory diapause as a pharate first instar larva. We assessed the relationship between chilling duration and temperature in terminating the diapause stage of casuarina moth eggs. Eggs of the casuarina moth were exposed to a variety of artificial chilling conditions that differed in duration and temperature. Results indicated that diapausing eggs require exposure to cold temperatures (9~15°C) followed by warm temperatures (27°C) for successful emergence in the laboratory. Eggs exposed to longer chilling periods required shorter incubation times to emerge than eggs exposed to shorter chilling periods. Regression analyses revealed a negative relationship between emergence time and chilling duration. The use of a proper chilling temperature and duration can effectively shorten diapause of casuarina moth eggs and make the routine rearing of casuarina moth populations possible.

Key words: *Lymantria xyлина*, egg diapause, chilling treatment

Introduction

The casuarina moth, *Lymantria xyлина* Swinhoe (Lepidoptera: Lymantriidae), is a major pest of casuarina (*Casuarina equisetifolia*) and acacia (*Acacia confusa*) forests, natural areas, and fruit tree orchards in Taiwan and the eastern coast of mainland China (Chao *et al.*, 1996). The number of recorded host plants for this moth includes and probably exceeds 69 species of trees and shrubs, belonging to 29 families (Chang and Weng, 1985; Chao *et al.*, 1996). Current infestations of

fruit trees and other hardwoods by *L. xyлина* indicate the real threat posed by this moth.

Shortly after emergence and mating in the summer, females of the casuarina moth lay a single egg mass consisting of 100~1000 eggs, which they cover with hairs from their abdomens (Shen *et al.*, 2003). The eggs then enter an obligatory diapause as a pharate first instar larva, prior to the complete consumption of the extra embryonic yolk (Shen *et al.*, 2003). The diapause mechanism and how this stage of development is regulated for the

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casuarina moth, however, are poorly understood. The casuarina moth is closely related to the gypsy moth, *L. dispar*, which is a very serious forest pest in North America. Like the casuarina moth, the gypsy moth also undergoes diapause as a pharate first-instar, and its eggs hatch in April after an 8~9-month dormancy (Williams *et al.*, 1990; Lee and Denlinger, 1996, 1997; Gray *et al.*, 2001). Past research has revealed that gypsy moth oviposition occurs in early to mid summer when temperatures are near yearly highs, and the developing embryos complete the prediapause phase in approximately 16 days at 25°C (Gray *et al.*, 1991). Well-developed gypsy moth embryos then enter a diapause phase that typically lasts 8~9 months (Gray *et al.*, 2001). Research has also indicated that diapause is more rapidly terminated with exposure to low temperatures (Masaki, 1956; Pantyukhov, 1964; Giese and Cittadino, 1977). After sufficient exposure to low temperatures to terminate diapause, embryos will hatch within 11~18 days at 25°C (Gray *et al.*, 1995). In summary, gypsy moth egg phenology and hatching have been well studied and are generally understood, and this information has aided gypsy moth management programs (Gray *et al.*, 2001).

As with any other insect management program, development of appropriate artificial rearing methods for the casuarina moth in the laboratory depends on sufficient knowledge of its life cycle and basic developmental biology. The objective of this study was to investigate the relationship between chilling duration and temperature in terminating the diapause stage of casuarina moth eggs. Eggs of the casuarina moth were exposed to a variety of artificial wintering treatments that differed in duration and temperature. We were interested in determining how these variables interact to influence hatching rates and emergence timing. We believe that this information will be

useful in effectively shortening the diapause stage of the casuarina moth and making the routine rearing of casuarina moth populations possible.

Materials and Methods

Casuarina moth eggs

Casuarina moth egg masses were collected from two heavily infested areas in central Taiwan in early July 2002. These infested areas were located at Mingchien, Nantao County (23°49.1'N, 120°39.3'E) and Erhshui, Changhua County (23°50.0'N, 120°36.4'E). Vegetation was composed primarily of *Acacia confusa* and *Sterculia foetida* in the first area and *Euphoria longana* in the second area. About 100 egg masses were collected from each site (Shen *et al.*, 2003).

In the laboratory, egg masses were first surface-sterilized by soaking in 0.1% sodium hypochlorite with 1% Tween 80 solution for 10 min., then rinsing under running tap water for 5 min. The egg masses were then air-dried. For the assessment of individual eggs, egg masses were first broken up, and the hairs were removed by rubbing the masses against sticky tape. The dehaired eggs from different egg masses were pooled and placed into Petri dishes (500 eggs per dish). To ensure that all eggs had entered diapause, the dehaired eggs were placed in an environmental chamber at 25°C with a 12:12 (L:D) photoperiod for 10 days (Gray *et al.*, 1991, 1995, 2001). After this treatment, 50 diapausing eggs were placed into a 1.5-ml Eppendorf vial and capped with cotton; vials were then assigned to an experimental treatment.

Experimental treatments

Upon diapause initiation, five vials (50 eggs per vial) were randomly assigned to each of 165 rearing regimes. Each regime was composed of an exposure to an experimental chilling treatment. Chilling treatments combined 33 durations

(60, 65, 70, 75, ..., 215, and 220 d) and five constant temperatures (6, 9, 12, 15, and 27°C). All treatments were subjected to a 12:12-h (L:D) regime.

Upon completion of each chilling treatment, five vials of eggs (50 eggs per vial) from each chilling treatment were removed and placed at a constant 27°C in a Percival growth chamber with a 12:12-h L:D photoperiod. Egg hatching was checked for each vial every 2 days for 60 days following treatment. Means and standard errors of the hatching rate and the average emergence time were calculated for each treatment.

Statistical analysis

The egg hatching rates and the average emergence times among different experimental treatments were analyzed by analysis of variance (ANOVA; PROC GLM; SAS Institute, 1998), followed by comparisons of means by the least-significant difference (LSD) test. Linear regression analyses were used to relate average hatching rates and average emergence times with the durations of different chilling treatments (PROC REG; SAS Institute, 1998).

Results

Effects of chilling duration and temperature on hatching rates

There were significant differences in the average hatching rates of casuarina moth eggs among the different chilling treatments (Table 1). Generally, average hatching rates were very low for all of the experimental treatments. No eggs hatched from the 27°C treatment. At the 6°C treatment, almost all eggs failed to hatch through the exposure period. Average hatching rates varied significantly among different exposure periods for the other three temperature treatments (Table 1). Average hatching rates increased with increasing temperature treatments, and the average hatching rates for the 9, 12,

and 15°C treatments were about 2~3%, 4~6%, and 7~9%, respectively. Correlation and linear regression analyses of average hatching rates versus chilling durations for all different temperature treatments revealed weak relationships (Fig. 1). Therefore, egg hatching rates of the casuarina moth were not correlated with the cold exposure periods.

Effects of chilling duration and temperature on emergence times

There was a significant difference in the average emergence times of casuarina moth eggs among the different temperature treatments (Table 2). At the 6°C treatment, almost all eggs failed to hatch through the exposure period. For the other three chilling treatments (9, 12, and 15°C), average emergence times varied significantly among different exposure periods (Table 2). For these three temperature treatments, the average emergence time decreased with increasing exposure duration. Correlation and linear regression analyses of average emergence time versus exposure duration revealed negative relationships (Fig. 2). The strongest and most negative relationship was found at the 15°C treatment between the average emergence time and the exposure duration ($r^2 = 0.93$, $p < 0.001$) (Fig. 2). Overall, our results suggest that by observing chilling duration, the emergence time can be closely predicted for different temperature treatments ($r^2 > 0.77$, $p < 0.001$).

Discussion

Our results indicated that diapausing casuarina moth eggs require exposure to cold temperatures followed by warm temperatures for successful emergence in the laboratory. This study also clearly demonstrated that eggs exposed to longer chilling periods require a shorter warm temperature incubation for emergence than eggs exposed to shorter chilling

Table 1. Mean hatching rates (%) of casuarina moth eggs under different chilling treatments (mean \pm S.E., n = 5 vials with 50 eggs/vial)

| Chilling duration (d) | Mean hatching rates | | | | <i>F</i> | <i>p</i> |
|--------------------------|---------------------|-------------------|-------------------|-------------------|----------|----------|
| | 6 °C | 9 °C | 12 °C | 15 °C | | |
| 60 | 0.4 \pm 0.4 | 2.0 \pm 0.6 | 7.2 \pm 1.9 | 5.2 \pm 1.0 | 7.48 | 0.0024 |
| 65 | 0.4 \pm 0.4 | 2.4 \pm 0.8 | 3.2 \pm 1.2 | 3.2 \pm 1.4 | 1.75 | 0.1978 |
| 70 | 1.6 \pm 0.8 | 2.8 \pm 1.5 | 2.8 \pm 1.1 | 6.0 \pm 2.1 | 1.73 | 0.2015 |
| 75 | 0.4 \pm 0.4 | 3.6 \pm 1.7 | 4.4 \pm 2.0 | 9.2 \pm 3.1 | 3.09 | 0.0569 |
| 80 | 0.4 \pm 0.4 | 6.4 \pm 0.8 | 2.8 \pm 1.0 | 16.0 \pm 2.5 | 24.25 | < 0.0001 |
| 85 | 0.0 \pm 0.0 | 3.6 \pm 1.2 | 3.2 \pm 0.8 | 16.4 \pm 1.6 | 46.08 | < 0.0001 |
| 90 | 0.0 \pm 0.0 | 1.2 \pm 0.8 | 3.2 \pm 1.0 | 1.2 \pm 0.8 | 3.03 | 0.0597 |
| 95 | 0.0 \pm 0.0 | 0.8 \pm 0.5 | 6.4 \pm 1.2 | 0.8 \pm 0.5 | 19.01 | < 0.0001 |
| 100 | 0.4 \pm 0.4 | 1.6 \pm 0.4 | 5.3 \pm 1.0 | 0.8 \pm 0.5 | 12.00 | 0.0002 |
| 105 | 0.0 \pm 0.0 | 4.8 \pm 1.6 | 9.2 \pm 1.7 | 1.6 \pm 0.4 | 11.28 | 0.0003 |
| 110 | 0.0 \pm 0.0 | 4.8 \pm 1.2 | 13.6 \pm 2.0 | 0.8 \pm 0.8 | 24.89 | < 0.0001 |
| 115 | 0.0 \pm 0.0 | 4.8 \pm 1.0 | 9.2 \pm 2.7 | 4.4 \pm 1.9 | 4.77 | 0.0146 |
| 120 | 0.0 \pm 0.0 | 0.8 \pm 0.5 | 5.7 \pm 1.9 | 7.2 \pm 2.3 | 5.31 | 0.0099 |
| 125 | 0.0 \pm 0.0 | 6.4 \pm 1.7 | 4.8 \pm 2.6 | 5.6 \pm 1.2 | 3.02 | 0.0606 |
| 130 | 0.0 \pm 0.0 | 8.0 \pm 2.5 | 4.8 \pm 0.8 | 7.2 \pm 2.6 | 3.90 | 0.0287 |
| 135 | 0.0 \pm 0.0 | 3.6 \pm 0.8 | 6.8 \pm 2.2 | 8.0 \pm 1.7 | 6.43 | 0.0046 |
| 140 | 0.0 \pm 0.0 | 2.4 \pm 0.8 | 8.4 \pm 2.5 | 9.2 \pm 2.9 | 5.43 | 0.0090 |
| 145 | 0.0 \pm 0.0 | 2.0 \pm 1.2 | 6.0 \pm 2.1 | 12.4 \pm 1.7 | 13.98 | < 0.0001 |
| 150 | 0.0 \pm 0.0 | 2.4 \pm 0.4 | 2.4 \pm 0.8 | 10.8 \pm 2.7 | 11.00 | 0.0004 |
| 155 | 0.0 \pm 0.0 | 3.2 \pm 1.4 | 4.0 \pm 1.4 | 10.0 \pm 2.8 | 5.89 | 0.0066 |
| 160 | 0.0 \pm 0.0 | 2.0 \pm 1.1 | 4.0 \pm 1.3 | 9.6 \pm 2.7 | 6.73 | 0.0038 |
| 165 | 0.0 \pm 0.0 | 1.2 \pm 0.8 | 6.4 \pm 1.9 | 9.6 \pm 1.8 | 14.03 | < 0.0001 |
| 170 | 0.0 \pm 0.0 | 3.6 \pm 1.2 | 9.2 \pm 3.1 | 7.6 \pm 1.9 | 4.57 | 0.0170 |
| 175 | 0.4 \pm 0.4 | 2.4 \pm 1.0 | 1.6 \pm 0.8 | 11.6 \pm 1.5 | 27.44 | < 0.0001 |
| 180 | 0.0 \pm 0.0 | 2.4 \pm 1.0 | 0.4 \pm 0.4 | 9.2 \pm 1.5 | 21.65 | < 0.0001 |
| 185 | 0.0 \pm 0.0 | 1.2 \pm 0.5 | 2.4 \pm 1.2 | 7.6 \pm 1.5 | 11.91 | 0.0002 |
| 190 | 0.0 \pm 0.0 | 0.8 \pm 0.5 | 4.4 \pm 2.0 | 9.6 \pm 1.0 | 14.28 | < 0.0001 |
| 195 | 0.0 \pm 0.0 | 2.0 \pm 0.9 | 4.8 \pm 1.6 | 6.4 \pm 1.9 | 4.53 | 0.0176 |
| 200 | 0.0 \pm 0.0 | 2.0 \pm 0.6 | 9.2 \pm 1.9 | 5.2 \pm 1.2 | 12.23 | 0.0002 |
| 205 | 0.0 \pm 0.0 | 0.4 \pm 0.4 | 7.6 \pm 0.8 | 6.8 \pm 1.4 | 25.73 | < 0.0001 |
| 210 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 4.8 \pm 1.4 | 7.2 \pm 2.4 | 6.75 | 0.0037 |
| 215 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.4 \pm 0.4 | 5.6 \pm 1.8 | 8.53 | 0.0013 |
| 220 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | -- | -- |
| | <i>F</i> = 2.06 | <i>F</i> = 3.58 | <i>F</i> = 3.39 | <i>F</i> = 5.04 | | |
| | <i>p</i> = 0.024 | <i>p</i> < 0.0001 | <i>p</i> < 0.0001 | <i>p</i> < 0.0001 | | |

* No egg hatched from the 27°C treatment.

periods.

Generally, in Taiwan the winter temperature range in natural environments of the lowlands is around 8 to 18°C. Low temperatures, from 9 to 15°, have proved

favorable to the eggs of the casuarina moth. However, when we decreased the temperature to 6°, the mortality rate was very high in our experiments, indicating a low resistance to cold temperatures for

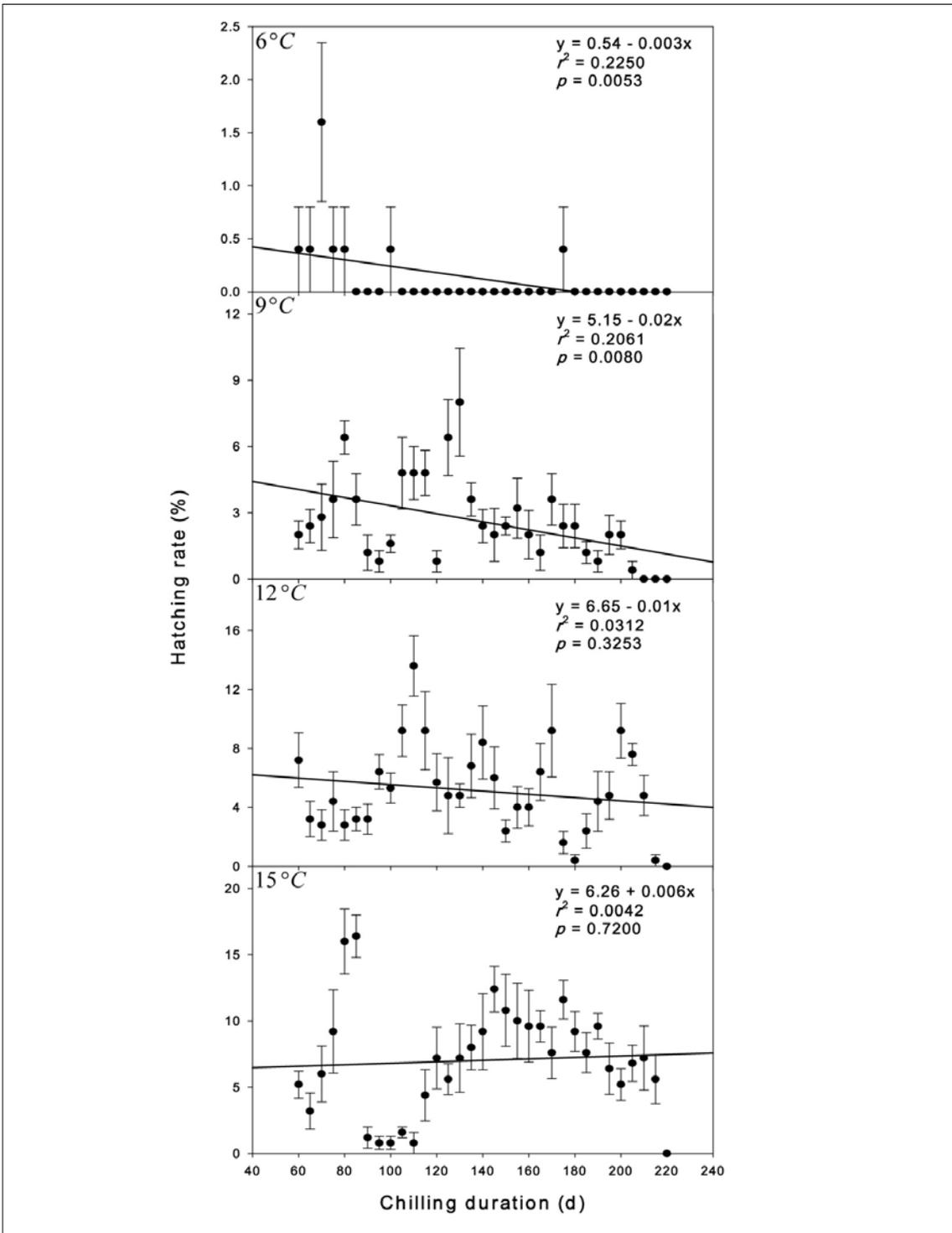


Fig. 1. Relationship between hatching rate and chilling duration under various treatments (duration: 60, 65, 70, 75, ..., 215, and 220 days; temperature: 6, 9, 12, and 15°C), and incubated at 27°C to emergence. There were five replicates per treatment and 50 eggs per replicate.

Table 2. Mean emergence time (d) of casuarina moth eggs after different chilling treatments (mean \pm S.E., n = 5 vials with 50 eggs/vial)

| Chilling duration (d) | Mean emergence time (d) | | | | F | p |
|-----------------------|-------------------------|----------------|----------------|----------------|--------|----------|
| | 6 °C | 9 °C | 12 °C | 15 °C | | |
| 60 | 8.5 \pm 8.5 | 55.0 \pm 7.9 | 36.6 \pm 2.2 | 55.6 \pm 3.3 | 14.41 | < 0.0001 |
| 65 | 24.0 \pm 0.0 | 31.5 \pm 3.0 | 43.6 \pm 2.5 | 48.5 \pm 3.7 | 6.17 | 0.0042 |
| 70 | 37.3 \pm 21.9 | 43.4 \pm 0.7 | 32.6 \pm 4.0 | 44.7 \pm 0.8 | 1.96 | 0.1431 |
| 75 | 13.0 \pm 3.0 | 41.4 \pm 2.4 | 37.8 \pm 3.1 | 45.0 \pm 1.2 | 12.68 | < 0.0001 |
| 80 | 12.0 \pm 1.0 | 35.8 \pm 2.0 | 35.7 \pm 2.8 | 45.1 \pm 1.5 | 15.14 | < 0.0001 |
| 85 | 0.0 \pm 0.0 | 38.3 \pm 3.3 | 27.6 \pm 4.3 | 46.0 \pm 1.6 | 13.07 | < 0.0001 |
| 90 | 0.0 \pm 0.0 | 27.3 \pm 1.5 | 31.4 \pm 1.5 | 39.7 \pm 6.7 | 11.23 | 0.0011 |
| 95 | 0.0 \pm 0.0 | 26.0 \pm 0.0 | 28.2 \pm 2.6 | 33.5 \pm 5.5 | 2.77 | 0.0735 |
| 100 | 23.0 \pm 0.0 | 22.3 \pm 4.0 | 22.3 \pm 1.6 | 24.5 \pm 3.5 | 0.08 | 0.9717 |
| 105 | 0.0 \pm 0.0 | 20.0 \pm 2.0 | 27.0 \pm 2.4 | 27.8 \pm 4.6 | 3.32 | 0.0306 |
| 110 | 0.0 \pm 0.0 | 18.9 \pm 1.7 | 25.0 \pm 1.7 | 26.5 \pm 6.5 | 3.63 | 0.0198 |
| 115 | 0.0 \pm 0.0 | 19.3 \pm 1.0 | 24.6 \pm 2.8 | 29.3 \pm 2.4 | 3.58 | 0.0214 |
| 120 | 0.0 \pm 0.0 | 15.0 \pm 0.0 | 22.9 \pm 1.9 | 21.0 \pm 1.7 | 3.70 | 0.0218 |
| 125 | 0.0 \pm 0.0 | 15.8 \pm 1.1 | 16.9 \pm 1.7 | 20.7 \pm 1.1 | 7.40 | 0.0005 |
| 130 | 0.0 \pm 0.0 | 14.0 \pm 0.4 | 14.8 \pm 1.4 | 20.7 \pm 1.9 | 8.09 | 0.0002 |
| 135 | 0.0 \pm 0.0 | 16.4 \pm 1.2 | 14.5 \pm 0.6 | 18.3 \pm 1.6 | 4.77 | 0.0058 |
| 140 | 0.0 \pm 0.0 | 15.8 \pm 1.3 | 14.4 \pm 0.7 | 16.9 \pm 0.9 | 7.38 | 0.0004 |
| 145 | 0.0 \pm 0.0 | 15.6 \pm 1.0 | 14.0 \pm 0.7 | 16.4 \pm 0.7 | 7.82 | 0.0002 |
| 150 | 0.0 \pm 0.0 | 15.3 \pm 0.8 | 8.8 \pm 0.9 | 12.3 \pm 0.7 | 8.02 | 0.0003 |
| 155 | 0.0 \pm 0.0 | 12.6 \pm 1.1 | 9.8 \pm 0.5 | 9.8 \pm 0.6 | 7.39 | 0.0005 |
| 160 | 0.0 \pm 0.0 | 15.0 \pm 1.5 | 10.3 \pm 0.7 | 10.0 \pm 0.6 | 9.67 | < 0.0001 |
| 165 | 0.0 \pm 0.0 | 13.0 \pm 0.0 | 9.5 \pm 0.7 | 6.7 \pm 0.6 | 9.05 | 0.0001 |
| 170 | 0.0 \pm 0.0 | 13.1 \pm 0.5 | 8.3 \pm 0.4 | 5.4 \pm 0.5 | 42.17 | < 0.0001 |
| 175 | 13.0 \pm 0.0 | 12.5 \pm 0.4 | 8.5 \pm 1.3 | 6.0 \pm 0.4 | 16.84 | < 0.0001 |
| 180 | 0.0 \pm 0.0 | 10.8 \pm 1.2 | 7.0 \pm 0.0 | 2.7 \pm 0.5 | 18.22 | < 0.0001 |
| 185 | 0.0 \pm 0.0 | 14.0 \pm 1.5 | 7.7 \pm 0.4 | 2.0 \pm 0.5 | 33.39 | < 0.0001 |
| 190 | 0.0 \pm 0.0 | 16.5 \pm 0.5 | 10.5 \pm 0.3 | 1.5 \pm 0.3 | 195.30 | < 0.0001 |
| 195 | 0.0 \pm 0.0 | 13.4 \pm 0.4 | 10.0 \pm 0.0 | 2.8 \pm 0.6 | 82.41 | < 0.0001 |
| 200 | 0.0 \pm 0.0 | 9.8 \pm 1.5 | 5.4 \pm 0.2 | 1.2 \pm 0.1 | 64.45 | < 0.0001 |
| 205 | 0.0 \pm 0.0 | 11.0 \pm 0.0 | 7.2 \pm 0.7 | 1.0 \pm 0.0 | 25.06 | < 0.0001 |
| 210 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 6.8 \pm 0.3 | 1.0 \pm 0.0 | 169.29 | < 0.0001 |
| 215 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 6.0 \pm 0.0 | 1.0 \pm 0.0 | Infty | < 0.0001 |
| 220 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | 0.0 \pm 0.0 | -- | -- |
| | F = 0.24 | F = 27.46 | F = 28.03 | F = 164.57 | | |
| | p < 0.9948 | p < 0.0001 | p < 0.0001 | p < 0.0001 | | |

* No egg hatched from the 27°C treatment.

casuarina moth eggs. Our results are consistent with findings from other winter-diapausing insects that require a period of low-temperature exposure (within certain temperature ranges; Bosch and

Kemp, 2003). Past studies have also indicated that the diapause of gypsy moth eggs is more quickly terminated after exposure to lower temperatures (Masaki, 1956; Pantyukhov, 1964; Gray *et*

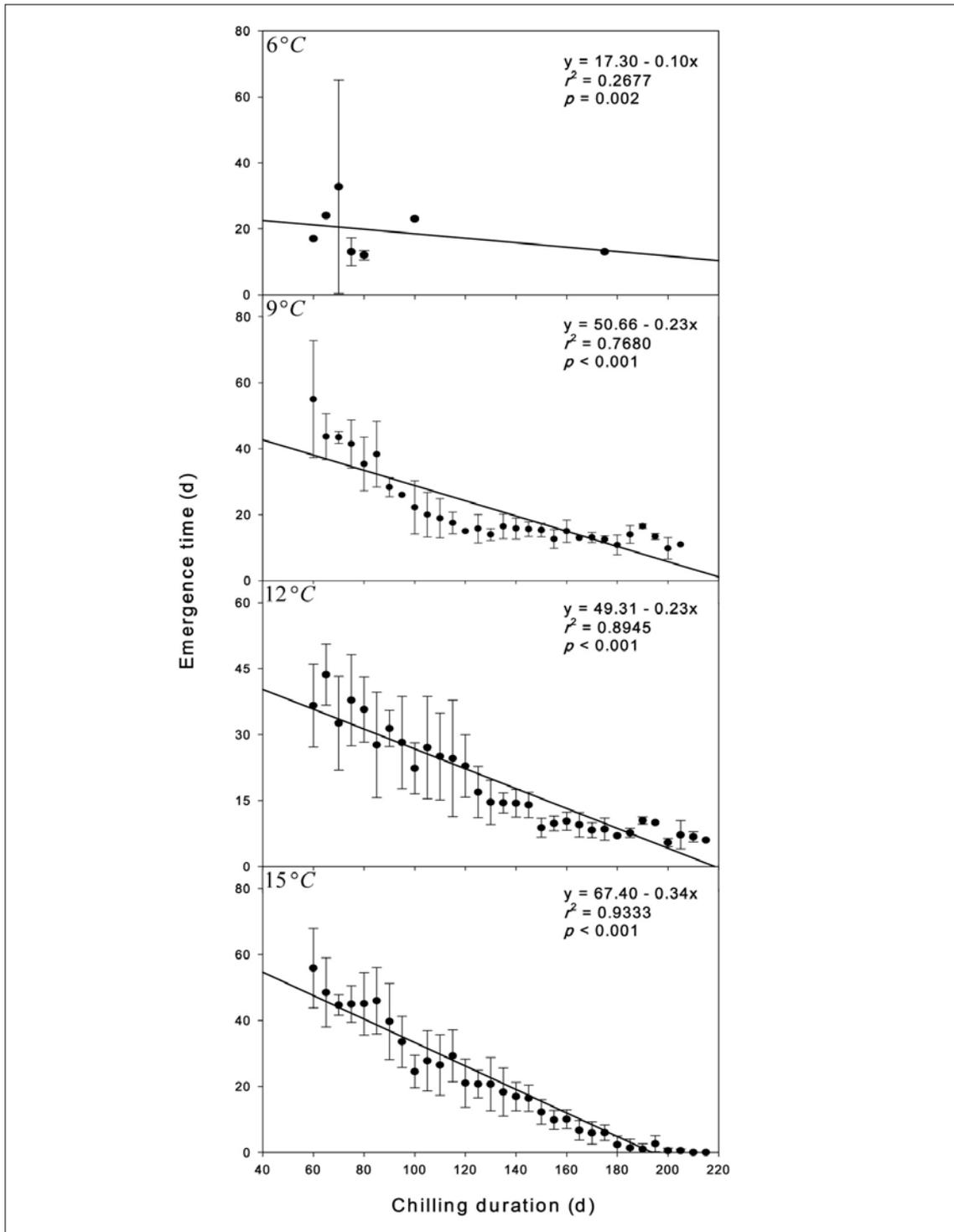


Fig. 2. Relationship between emergence time and chilling duration under various treatments (duration: 60, 65, 70, 75, ..., 215, and 220 days; temperature: 6, 9, 12, and 15°C), with incubation at 27°C to emergence. There were five replicates per treatment and 50 eggs per replicate.

al., 1995).

The time required for egg hatching following diapause initiation is determined by the sum of many developmental responses governing the developmental course throughout the entire period. Many studies have been conducted to study the thermal and physiological regulation of diapause in gypsy moth eggs (Gray *et al.*, 1991, 1995, 2001; Lee and Denlinger, 1996, 1997; Lee *et al.*, 1998). Those studies have shown that after sufficient low-temperature exposure (110 days at 5°C) to terminate diapause, the gypsy moth eggs would hatch within 11~18 days at 25°C (Gray *et al.*, 1991, 1995). No study, however, has revealed the thermal responsiveness during diapause for the casuarina moth. Based upon information from gypsy moth studies that the transition period from diapause to postdiapause is about 14 days (Gray *et al.*, 1991, 1995), we proposed that the diapause and postdiapause transitions for the casuarina moth would be approximately 120, 125, and 140 days after initiation at 9°, 12°, and 15°C, respectively. More studies are needed for a better understanding of the thermal and physiological regulation of the casuarina moth egg diapause.

Using chilling as a diapause terminator in the casuarina moth, the incidence of diapause cessation was higher and the ability of the eggs to hatch was lower. This divergence occurred in eggs from almost all temperature treatments, and occasionally no larvae hatched from the treated eggs. Some of the eggs probably perished before or after cooling, thereby raising the overall mortality rate. The reason for the low hatchability is not known. However, we suspect that reduced hatching may have been due to the dehairing process. Each egg mass was vigorously rubbed by hand against sticky tape during dehairing, and this method may have had a harmful effect on the eggs. It is significant to note that eggs in

chill-treated whole egg masses (9°C, >100 days) have very high hatchability (Hwang, unpubl. data).

Chilling is the primary means for diapause termination in many winter-diapausing insect species. The actual mechanisms of chilling treatment on termination of the diapause stage are not clear. However, recent studies have suggested that chilling may activate certain gene expressions in diapausing eggs and lead to initiation of diapause termination (Moribe *et al.*, 2001). In the past, the necessary chilling temperature and duration to terminate the diapause of casuarina moth eggs were not known. Our data permit certain generalizations to be made concerning the termination of diapause in pharate first-instar larvae of *L. xyliana* and other members of the Lepidoptera.

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黑角舞蛾(*Lymantria xylina* Swinhoe)休眠卵滯育打破之研究

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摘 要

黑角舞蛾 (*Lymantria xylina* Swinhoe) 是一嚴重的林木和果樹害蟲，此蟲會以休眠卵的方式越冬。本研究主要是探討冷藏處理對打破黑角舞蛾休眠卵的影響，研究的方法是將黑角舞蛾的卵置於不同的冷藏箱及冷藏不同的時間後，觀察卵的孵化情形。結果發現黑角舞蛾的休眠卵要先經一段冷藏 (9~15°C) 時間，再經一段回溫期 (27°C) 後，才能在實驗室中順利孵化。結果也顯示，冷藏越久的卵，回溫後卵孵化所需要的時間越短。同時，回歸分析顯示孵化時間與冷藏的時間呈現負相關的關係。因此，使用適當的冷藏溫度及冷藏時間，可以有效的縮短黑角舞蛾休眠卵所需休眠的時間。

關鍵詞：黑角舞蛾、滯育卵、冷藏處理