【Research Notes】

How does the red flour beetle, *Tribolium castaneum*, obtain chill hardiness under room conditions in Kyoto, Japan?

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Abstract

Adult beetles of *Tribolium. castaneum* were exposed to 5 ℃ after being in unheated seasonal room conditions in a concrete apartment building or constant temperature conditions of 27 ℃ in 2013 and 2014 in Kyoto. No beetles in the room could tolerate the chill treatment for 75 days when the minimum room temperature decreased from 21 to 14 ℃ from Nov. 3 to Dec. 16. However, 42.5% of beetles at 27 ℃ could survive the 75-day chill treatment after being exposed to minimum room temperatures of 12–14 ℃ for 40 days. These results suggest that the beetles need to be exposed to temperatures lower than 14 ℃ for a certain period in order to overwinter in Kyoto.

Key words: red flour beetle, overwintering

INTRODUCTION

The red flour beetle *Tribolium castaneum* Herbst. overwinters as an adult in aggregation. In Kyoto, the overwintering beetles begin to oviposit in late April to May and new beetles begin to emerge in July. The development from eggs to adults takes 40 to 60 days. The new beetles live for 200 days to one year, with females laying eggs before October. More than 50% of beetles emerging in or later than August overwinter, and lay eggs again the next year. However, no other stages tolerate winter cold, even under room conditions (Tsuji, 1997). This suggests that this species develops chill hardiness at the adult stage, entering a dormant or diapause state before winter. However, this process has not been well studied.

In order to determine when the beetles get ready to overwinter, preliminary experiments were carried out under unheated room conditions in Kyoto in 2013 and 2014.

MATERIALS AND METHODS

Insects were obtained in an unheated room in Kyoto and were bred generation after generation there. A 9:1 mixture of flour and dried yeast powder was used as food for the insects.

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Experiment A:

First-generation insects emerging into adulthood in the room in July 2013 had been kept in a cup with new food. The temperatures in the room ranged 26–31℃ in July, 30–32 ℃ in August, and 29–31 ℃ in September. On October 19, sets of 10 beetles (naturally 3 months old in the room) were confined with 1 g of food in 1 of 12 glass tubular bottles (14 mm diameter, 90 mm long, capped with cotton; **Fig. 1**), and these bottles were further kept under the same room conditions. To check the chill hardiness of the beetles under seasonally descending temperatures, 2 bottles each containing 10 beetles were moved to a refrigerator on each of Oct. 19, Nov. 3, Nov. 18, Dec. 3, and Dec. 18, kept at 5 ℃ for 75 or 60 days, and then moved to an incubator at 27 ℃. The percentages of surviving beetles were recorded 15 days after they were moved to the 27℃ incubator.

Two other bottles were moved to the incubator on Jan. 2 (75 days after Oct. 19), without chilling treatment. The room temperatures on and after Oct. 18 are shown in **Fig. 2**.

Experiments B and C:

Insects raised from parents in a 27 ℃ incubator and emerging as adults were used in experiments B and C.

In experiment B, final-instar larvae just before

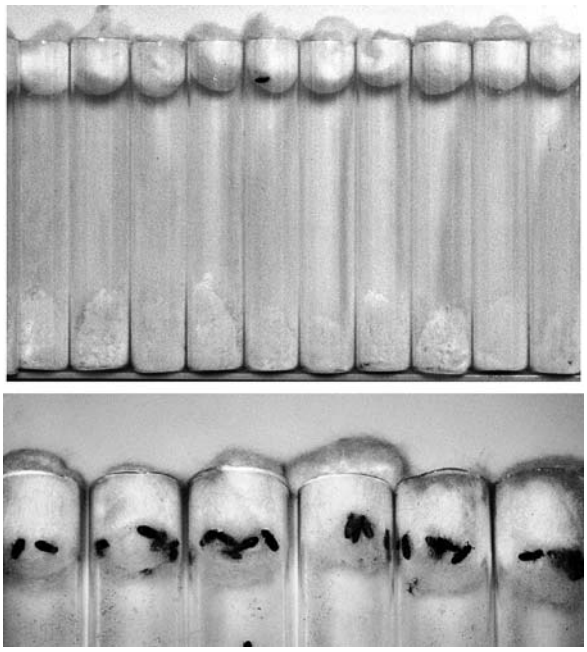

Fig. 1 Tubular bottles used in experiment A
Upper: Just after the beetles had been confined
Lower: Forty-four days after the beetles had been confined. More than 50% of the beetles had stayed outside the food on and after the 10th day since the confinement.

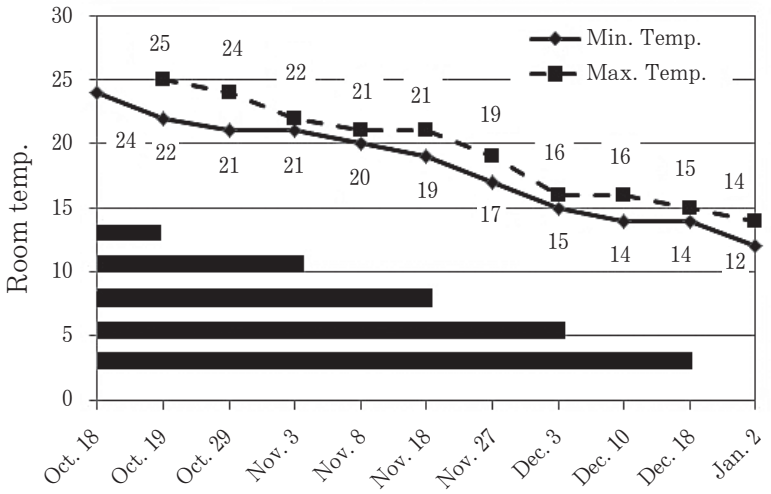

Fig. 2 Maximum and minimum room temperatures on and after Oct. 18, 2013 and duration of each stay in the room in experiment A
▬ : Days when the beetles were kept under the descending room temperature conditions including the summer of the year (2013) before chilling treatment. The beetles had naturally been exposed to temperatures in the room ranging 26–31℃ in July, 30–32 ℃ in August, and 29–31 ℃ in September.

pupation were gathered in a cup on Nov, 15, 2013. All of them were 2–3-month-old beetles on Feb. 11, 2014, when 20 beetles were confined with 4 g of food into each of 12 plastic cases (Fuji Film case, 30 mm diameter, 50 mm height, with a cap, **Fig. 3**), On the same day, they were moved to unheated room conditions for 0, 10, 20, 30, or 40 days before exposure to chill treatment at 5 ℃. After the chill treatment for 75 days or less, the plastic cases with the beetles were moved back to the 27 ℃ incubator. The room temperatures on those days are shown in **Fig. 4**.

Fig. 3 Plastic containers used in experiments B and C
Usually more than 50% of the beetles stayed outside (on the surface of) the food on and after the 10th day from the confinement of beetles.

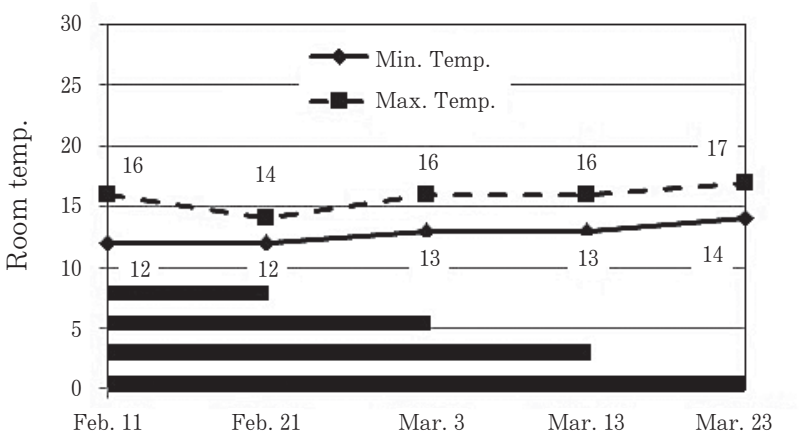

Fig. 4 Maximum and minimum room temperatures on and after Feb. 11, 2014 and duration of each stay in the room in experiment B
▬: Days when the beetles raised at 27 ℃ were kept under the room temperature conditions before chilling treatment at 5 ℃.

In experiment C which was intended to reinforce the B's data with no pretreatment at unheated room conditions, active beetles 2 to 3 months old at 27 ℃ were directly exposed to 5 ℃ for 0, 10, 20, or 30 days. The chill treatment in experiment C started on April 5, 2014 (**Table 2**), and the beetles were directly returned to 27 ℃ after the chill treatment.

In both the B and C experiments, the percentages of surviving beetles were recorded on the 10th and 20th day after the beetles were moved back to the 27℃ incubator.

RESULTS AND DISCUSSION

The results of experiment A are shown in **Table 1**. These results indicate that the exposure of the beetles to room temperatures that gradually decreased from the summer to Dec. 18 (14 ℃) in this room was insufficient to induce chill hardiness against 5 ℃ for 75 days. However, the results of experiments B and C (**Table 2**) indicate that exposure to slightly lower temperatures of 12–13 ℃ for a longer period was effective.

Table 1 Results of experiment A

Dates when chill treatment started	Days at the room temperature after Oct. 19	Days chilled at 5 ℃.	% Survival after being chilled at 5 ℃.	
Oct. 19, 2013	0	75	0	(n = 10)
		75	0	(n = 10)
Nov. 3	15	75	0	(n = 10)
		75	0	(n = 10)
Nov. 18	30	75	0	(n = 10)
		75	0	(n = 10)
Dec. 3	45	75	0	(n = 10)
		75	0	(n = 10)
Dec. 18	60	60	0	(n = 10)
		60	0	(n = 10)
Jan. 2, 2014	75	0	60	(n = 10)
		0	50	(n = 10)

All the chilled beetles were found to be dead 15 days after they were moved to the 27 ℃ incubator. Survivals of non-chilled beetles kept at room temperatures for 75 days were 90 and 70% 15 days after they were moved to the 27℃ incubator, and 60 and 50 % after 45 days.

Table 2 Results of experiment B and C

Days exposed to the room temperature before chilling	Days chilled at 5 ℃. after exposed to the room temperature and % survivals of each 20 beetles tested				
	10	20	30	60	75 days
0 days	65 (%)				
	25 (%) **				
		0			
		0 **			
			0 **		
10			50		
				25	
20					10
					10
30					25
					25
40					40
					45

** Results of experiment C

Room temperature exposure in experiment B started on Feb. 11, 2014 (**Fig.2**), and chilling treatment in experiment C started on Apr. 5, 2014.

Survival % figures on the 10th day were the same as on the 20th day after the beetles were moved back to the 27℃ incubator.

REFERENCES

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【資料】

コクヌストモドキは京都市の無加温室内でどのように冬期低温耐性を獲得するか

辻　英明

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摘　要

2013 年および 2014 年，京都市内のコンクリート製集合住宅内において，無加温室内の季節的な室温条件，あるいは 27℃の恒温条件下に置かれたコクヌストモドキ成虫を 5℃条件に露出する実験を行った．夏季の 30〜32℃から季節的に自然低下する室内に保たれ，11 月 3 日（最低室温 21℃）から 12 月 18 日（最低室温 14℃）までのいずれかの時点に 5℃の冷蔵条件下に移された成虫は，75 日間の冷蔵処理に耐えられずに死亡した．しかし，27℃恒温飼育の成虫は，最低室温の 12〜14℃に 40 日間保たれた後，75 日間の冷蔵処理に 42.5%が生存できた．これらの結果は，コクヌストモドキ成虫が京都で越冬するためには，14℃以下の温度に一定期間さらされる必要があることを示唆する．

Key words: red flour beetle（コクヌストモドキ），overwintering（越冬），chilling（冷蔵）

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