Int. J. Indust. Entomol. Vol. 8, No. 1, 2004, pp. 73~76

International Journal of *Industrial Entomology*

Influence of Chilling Duration on Oxygen Consumption and Hatchability in Eggs of the Silkworm, *Bombyx mori*

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(Received 27 November 2003; Accepted 25 January 2004)

The rate of oxygen consumption of the silkworm eggs was measured to set up the barometer for measuring the effect of egg chilling on diapause termination. In diapause eggs, O_2 uptake showed a maximum of 79.2 μl/mg eggs/hr, at one day after oviposition and then gradually decreased to 2.2 μl/mg eggs/hr at 9 days. The rates of oxygen uptake of eggs raised immediately after HCl-treatment and reached to a maximal level of 484.5 μl/mg eggs/hr in 9-day-old eggs, which corresponds to 220 fold that of diapause eggs, and the hatching ability was also over 50%. In order to break diapause, eggs incubated at 25°C for 30 days after oviposition had to be kept at least for 45 days at 5°C, but chilled eggs for 90 days or longer hatched at 10 days as normal hatching periods. We also investigate periods which the value of O_2 uptake of eggs chilled during 15 to 120 days at 5°C reached at 200 μl/mg eggs/hr. As a result of that, O_2 uptake of eggs chilled during 15 to 45 days were not reached at 200 μl/mg eggs/hr and longer the chilling durations are, the higher the rate of O_2 uptake is and the longer the chilling durations are, the shorter the periods up to hatching is. And also, hatchability increased rapidly as O_2 uptake reaches over 20 / eggs/hr.

Key words: *Bombyx mori*, Chilling, Egg, Hatchability, Oxygen uptake, Silkworm

Introduction

Lowered oxygen consumption is generally accepted to be a characteristic phenomenon of insect diapause (Yaginuma and Yamashita, 1999). Studies on oxygen consumption during diapause of insect have been performed on eggs of the domestic silkworm (Chino, 1958), *Cecropia* silkworm (Schneiderman and Williams, 1953), Colorado potato beetles (May, 1989), and other diapause insects (Waku, 1965; Ellingsen, 1978; Crozier, 1979).

Embryonic diapause in the eggs of silkworm, *Bombyx mori,* is predetermined during oogenesis by a diapause hormone, which is secreted from the suboesophageal ganglion at the stage of female pharate adult stage (Fukuda, 1951; Hasegawa, 1951; Sonobe *et al.*, 1977). Due to exposure to the diapause hormone, oxgen uptake by the eggs decreases abruptly after 24 hrs after oviposition (Chino, 1958) and then embryonic development ceases (Kitazawa *et al.*, 1963). In eggs of *B. mori,* the decrease in oxygen uptake which accompanies initiation of diapause has proposed to be result from the decrease in the permeability of barrier(s) of the chorion and/or serosal cuticle to oxygen gas outside during the early stages of development (Okada, 1971). This oxygen barrier hypothesis was supported by some experiment results. For examples, in diapause eggs of *B. mori,* sorbitol and glycerol accumulate at high concentration in the anaerobic condition (Chino, 1958; Furusawa *et al.*, 1982; Yaginuma and Yamashita, 1999). In contrast, in non-diapause eggs, glycogen is consumed as an energy source and little or no accumulation of sorbitol and glycerol is detectable (Sonobe *et al.*, 1979; Kim, 1987). However, some doubts have recently been cast on this hypothesis (Sonobe and Nakamura, 1991).
It has been generally known that rates of oxygen consumption of pupae of insect represent U shaped curve pattern showing its minimum consumption during metamorphosis period and the rates of oxygen consumption increased markedly and progressively during cold winter and chilling period(Waku, 1965; Denlinger *et al.*, 1972). This tendency was universally found in lepidopterans

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except for *Philosamia cynthia ricuni* which does not undergo true pupal diapause (Waku, 1965) and *B. mori* (Okada, 1971). In the case of diapause insect, exposure to low temperature is essential for the diapause development that lasts for a fixed minimum duration (Kai, 1983). It is difficult to judge diapause development in the chilled silkworm eggs. We, therefore, measured the rate of oxygen consumption of silkworm egg to set up the barometer for measuring the effect of egg chilling on diapause termination.

Materials and Methods

Experimental animals

Bakok-Jam (bivoltine races, Jam 123 × Jam 124) eggs of the silkworm, *Bombyx mori,* were used. The larvae were reared on fresh mulberry leaves in spring, 1999. Eggs laid within a single 30 min period were pooled for diapause eggs and non-diapause eggs. The pooled eggs were soon weighted and then divided into each one g-package. The diapause eggs were controlled by continuous exposure to 25°C to maintain diapause. HCl-treated eggs were soaked into HCl solution (specific gravity 1.075 at 15°C) for 5.30 min at 46.2°C after incubation at 25°C for 20 hrs from oviposition in order to break diapause quickly. In order to measure oxygen consumption during chilling, non-diapause eggs were kept at 5°C for 15, 30, 45, 60, 75, 90 and 120 days respectively, after incubation at 25°C for 30 days after oviposition.

Measurement of oxygen uptake

The basic oxygen uptake technique was followed that used by Kim (1987). Oxygen uptake of eggs (usually one g, about 2,000 eggs) was measured in a volumetric system with O_2 uptester (Daiyo Scientific Industrial Co., Tokyo, Japan). The experimental materials of 2,000 diapause and non-diapause eggs were placed in the 20 ml main chamber of each of the vessels. CO_2 gas was absorbed into a strip of filter paper saturated with 0.5 ml of 20% potassium hydroxide. The eggs were separated from the absorbing agent with porous polyethylene membrane. The oxygen uptake tester was set in a room maintained at 20 – 22°C and the chambers containing the eggs were submerged in the water bath maintained at 22°C. Readings were taken at intervals of 30 min for a period of 2 – 3 hrs. All the eggs were kept at 25°C until they were transferred for measurement. In the case of chilled eggs, O_2 uptake was measured at one hr after transfer to 25°C after chilling. Oxygen uptake was expressed as μl/mg eggs/hr. Each value for the oxygen uptake represents the mean of 3 replicates, one g/replication for diapause eggs and non-diapause eggs.

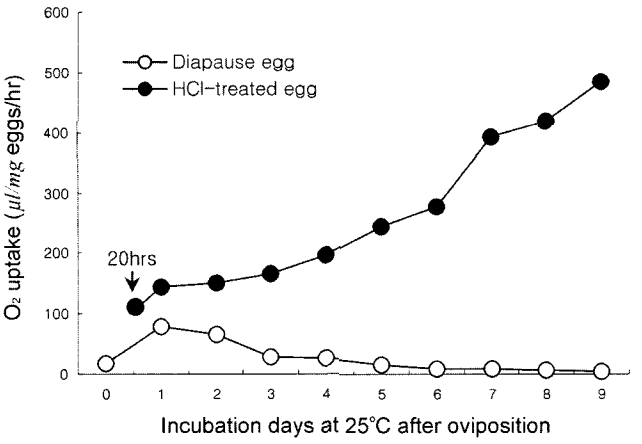

Fig. 1. Comparison of O_2 uptake between diapause eggs and HCl-treated eggs of *B. mori* during embryogenesis. HCl-treatment was performed at 20 hrs after oviposition. O_2 uptake was measured at 25°C. Each value for the oxygen uptake is the mean of 3 replicates: the standard deviations are within 10% of the each mean.

Results and Discussion

Comparison of oxygen uptake between diapause eggs and HCl-treated eggs of *B. mori* during the early stages of incubation

In order to know to changes of oxygen uptake in the diapause eggs and HCl-treated eggs in the course of embryonic development, we measured oxygen uptake using O_2 uptaker. The rates of oxygen uptake varied with the time of incubation and voltinism of the eggs (Fig. 1). Oxygen uptake in the diapause eggs was about 17.1 μl/mg eggs/hr within 1 hr after oviposition. This uptake increased to the level of about 79.2 μl/mg eggs/hr in 1-day-old-eggs and then decreased to 2.2 μl/mg eggs/hr in 9-days-old eggs and stabilized at this level until at least 1 month after oviposition (data not shown).

Oxygen uptake of HCl-treated eggs was 64.9 μl/mg eggs/hr within 30 min after HCl-treatment at 20 hrs from oviposition. This uptake rose for 1 day, to a value of 144.7 μl/mg eggs/hr and this is about two fold higher than that of 1-day-old-diapause eggs (Fig. 1). The rates of oxygen uptake increased immediately after acid-treatment and then rose to reach a maximal level of 484.5 μl/mg eggs/hr in 9-days-old eggs, which was 220 fold higher than that of diapause eggs, and the hatching ability of this time was also over 50%. This changing pattern at the initiation phase of diapause eggs and HCl-treated eggs confirmed the results of Chino (1958), Sonobe and Okada (1984), Kim (1987), and Yaginuma and Yamashita (1999). Changing pattern of oxygen consumption observed in this experiment appears to represent a physiological phases of embryonic diapause in *B. mori*

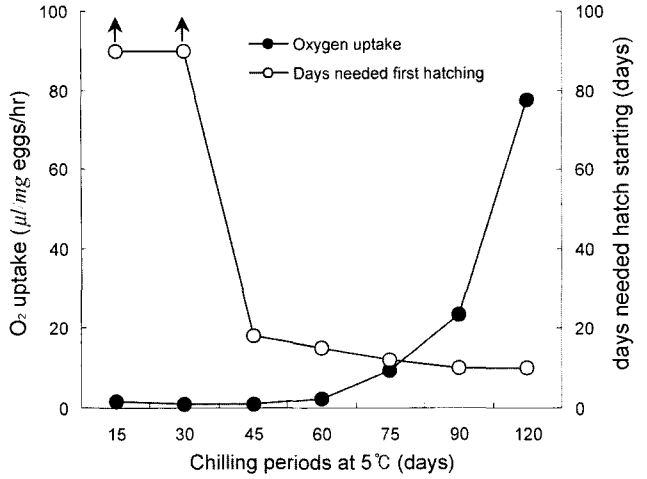

Fig. 2. Changes in O_2 uptake and days needed hatch starting during chilling of silkworm eggs. Eggs were chilled at 5°C after incubation at 25°C for 30 days after oviposition. O_2 uptake was measured at one hr transfer of eggs from 5°C to 25°C. The arrows over open circles indicate that there were no hatching up to 90 days or longer after incubation at 25°C.

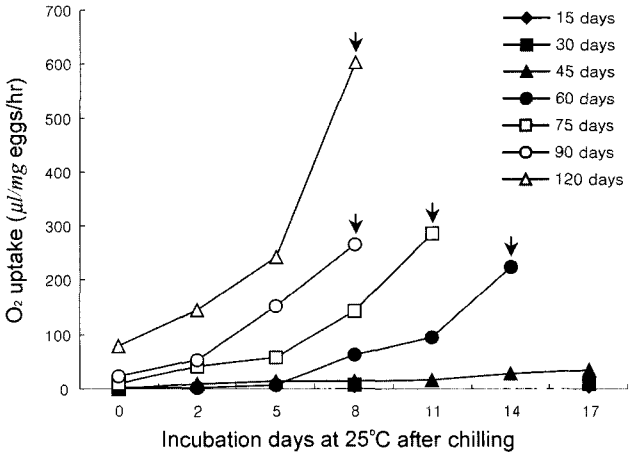

Fig. 3. Changes in O_2 uptake pattern of *B. mori* eggs by chilling duration. Eggs were chilled at 5°C after incubation at 25°C for 30 days after oviposition. O_2 uptake were measured at one hr after transfer to 25°C. Arrows represent first hatching days.

(Braune, 1976). Our data were shown that the initiation of diapause occurs around 1 – 2 days after oviposition, and a compete physiological status of diapause is established after incubation at 25°C for 9 days after oviposition (Fig. 1). In the case of silkworm, fertilization occurs about 2 hrs after oviposition at 25°C and after about 13 hrs nuclei reach the periplisum of the egg, and the blastoderm forms thereafter (Takesue *et al.*, 1980; Miya, 1984).

Changes in oxygen uptake and days needed hatch starting during chilling of silkworm eggs

In order to know changes in oxygen uptake and first hatching days of eggs during chilling, we transferred chilled eggs at 5°C to 25°C, and then measured O_2 uptake at one hr after incubation at 25°C and days needed for first hatching (Fig. 2). Chilled eggs for 15 days and 30 days at 5°C were not hatched at 90 days or longer after incubation at 25°C, and O_2 uptake of these eggs at one hr after incubation at 25 was about 1.7 μl/mg eggs/hr and 1.1 μl/mg eggs/hr, respectively. Forty-five days and 60 days-chilling eggs were hatched at 18 days and 15 days, and O_2 uptake of these eggs was 1.1 μl/mg eggs/hr and 2.2 μl/mg eggs/hr, respectively. In chilled eggs for 90 days and 120 days, the duration needed for the first hatching were 10 days, the value of which about 2 fold shorter than that of chilled eggs for 45 days, and O_2 uptake value of these eggs was 23.5 μl/mg eggs/hr and 77.6 μl/mg eggs/hr, respectively. These results show that the duration to break diapause requires for the eggs to be kept at least for 45 days at 5°C after incubation at 25°C for 30 days after oviposition. However, eggs chilled for 90 days or longer hatched at 10 days as normal hatching periods.

Changes in O_2 uptake pattern by chilling duration in the *B. mori* eggs

It was investigated periods which the value of O_2 uptake reached at 200 μl/mg eggs/hr during chilling (Fig. 3). The readings of O_2 uptake were started within 1 hr after transfer to 25°C. Oxygen uptake by chilled eggs for 15 days and 30 days at 5 remained almost constant (about 5.5 μl/mg eggs/hr and 9.9 μl/mg eggs/hr at 17 days), and those eggs were not reached to 200 μl/mg eggs/hr although it has passed over 90 days and 80 days, respectively. The value of oxygen consumption of 45 days-chilling eggs reached about 33.8 μl/mg eggs/hr at 17 days, and was not reached at 200 μl/mg eggs/hr so far after 30 days or longer either. However, after chilling for 60 days and 75 days, oxygen uptake of those increased rapidly at 8 days after 25°C incubation and then the oxygen uptake value reached 223.8 μl/mg eggs/hr and 286 μl/mg eggs/hr, respectively, at 14 days and 11 days after incubation. In the case of chilled eggs until 90 days or longer, oxygen uptake increased markedly from 2 days after incubation, and the oxygen uptake value of 90 days and 120 days-chilling egg was 265.8 μl/mg eggs/hr and 241.8 μl/mg eggs/hr, respectively, at 8 days and 5 days after incubation. The periods reached at 200 μl/mg eggs/hr of these was about 2 – 3 fold shorter than that of chilled eggs for 60 days. We also investigated O_2 uptake at one hr transfer of eggs from 5°C to 25°C (Fig. 3). Oxygen uptake of 15 days, 30 days and 45 days-chilled was 1 – 2 μl/mg eggs/hr and that of 75 days, 90 days and 120 days was 9.5 μl/mg eggs/hr, 23.5 μl/mg eggs/hr and 77.6 h μl/mg eggs/hr,

respectively. And, hatchability increased rapidly as O_2 uptake reached at over 20 μl/mg eggs/hr.

The results so far obtained show that the longer the chilling durations are, the higher the rate of O_2 uptake is and the longer the chilling durations are, the shorter the periods up to hatching is. Kai (1995) reported that the hatching mode depended upon the chilling duration. The ability to break diapause is correlated with the increased of O_2 uptake after transfer to 25°C and an ability to consume oxygen is recovered in the course of termination of diapause in an egg exposed to 5°C (Yaginuma and Yamashita, 1986, 1999).

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