Delayed metamorphosis and diapause in the sugar cane borer *Diatraea saccharalis**

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Summary. The problem of arrested or delayed development was examined in the sugar cane borer, *Diatraea saccharalis*. It was found that the insect can either enter diapause or exhibit a period of delayed metamorphosis according to the photoperiod conditions prevailing. We have observed the development characteristics of *D. saccharalis* and conclude that a distinction should be made between a delayed metamorphosis phase and a diapause stage.

Diapause is generally regarded as an arrested period of development allowing insect species to survive adverse environmental conditions[2]. This programming of the organism[3] is induced primarily by abiotic factors, particularly photoperiod and temperature, prevailing before the arrival of adverse conditions. In species inhabiting areas where sub-tropical conditions occur, insects will enter a period of arrested development after 3 or 4 non-diapause generations. Such is the case with species living in the southern United States.

Larval diapause has been described by Chippendale[4] in *Diatraea grandiosella* as a phenomenon where the insect exhibits stationary moults. The entry into what he described as diapause is characterized by the transition from a spotted larva to an immaculate (non-spotted) morph[5].

The sugar cane borer, *Diatraea saccharalis* (Lepidoptera: Pyralidae), is a multivoltine species occurring in all areas of the western hemisphere where sugar cane is cultivated[6]. There are 4–5 generations a year under Louisiana conditions, the last generation entering diapause in the last larval stage, starting in late fall (November and December) and lasting to early spring (March).

Materials and methods. 8 groups of 25 larvae were reared singly from hatching in clear, capped acetate cups containing a soyflour wheat germ diet[7]. They were placed in an incubation chamber fitted with F20Ti2 Indorsun fluorescent tubes (Verd-a-Ray Corporation) emitting 25 W/m^2 at 20 cm. The following photoperiod and temperature regimes were used: (1) 12L:12D and 21 ± 1 °C; (2) 14:10 and 21 ± 1 °C; (3) 14:10 and 27 ± 1 °C; (4) 12:12 and 21 ± 1 °C followed by 14:10, 27 ± 1 °C according to the scheme given in the figure.

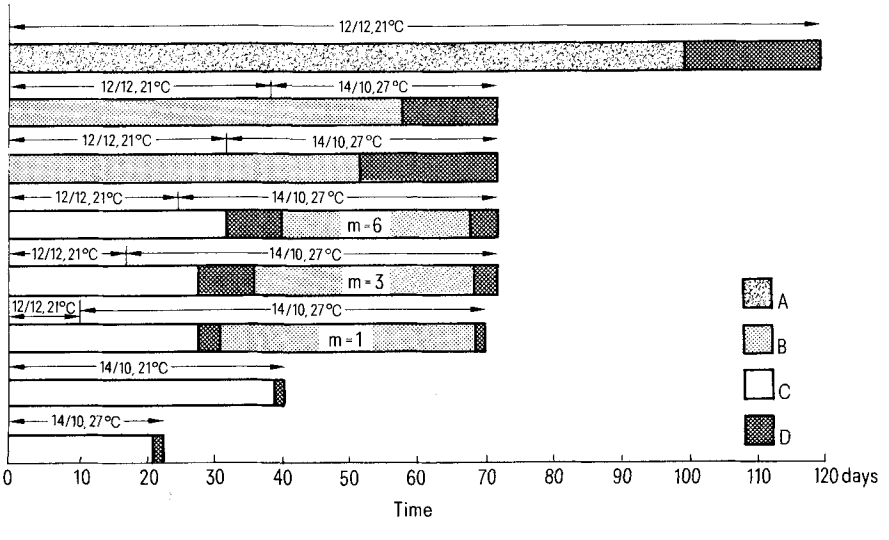
Results. All larvae reared at 14:10, 21 °C and 14:10, 27 °C completed their development without interruption if the diet supplied was adequate (fig.). If the diet dried up before the larvae had completed their fifth instar then they spun a hibernaculum. Such larvae tended to take a bracketed appearance: there were no pinacular spots[8] on the cuticle and the underlying fat body layer was broken up into regular blocks separated by clear areas. When fresh food was supplied to larvae as they began to spin the hibernaculum, they resumed feeding and pupated within a week.

There were 5 instars at 27 °C and 6 at 21 °C, at a photoperiod of 14:10. The longer time required to complete development at 21 °C is obviously a temperature effect.

At 12:12, 21 °C all larvae entered a period of delayed development followed by diapause. They did not start pupating until at least 100 days after hatching (fig.). All these larvae entered the sixth instar in the immaculate form[5] as opposed to the spotted preceding instar. These larvae kept feeding and went through at least 3 more stationary molts (no head capsule enlargement), gaining weight with each additional instar. They finally spun a hibernaculum in which they stayed for 30–40 days before metamorphosing to a pupa.

At varying conditions as defined in the figure, there were 2 periods of pupation. When the larvae were placed for 25 days or less at 12:12, 21 °C and then moved to 14:10, 27 °C, the majority pupated without interruption (see illustration for actual numbers). The remaining individuals exhibited delayed metamorphosis: they entered the immaculate stage, kept feeding and molting at least once, did not spin a hibernaculum and pupated within 75 days. All larvae placed at 12:12, 21 °C for more than 25 days, but less than 39 days, and then moved to 14:10, 27 °C had only 1 pupation period occurring after a period of delayed development (immaculate larva, molting, no hibernaculum). The bracketed appearance was also present in some individuals.

Discussion. Our results indicate that diapause in *D. saccharalis* is not a phenomenon which follows the usual arrested development pattern reported in other insects exhibiting larval diapause e.g. *Isia isabella*[9]. A photoperiod of 14L:10D is sufficient to prevent any delay in development at both 21 and 27 °C. At 12:12 and 21 °C the insect will enter a period of prolonged development characterized by the immaculate morph, and supernumerary instars, and a period of diapause initiated



Influence of temperature and photoperiod on the development and diapause of *D. saccharalis*. *A* Conditions favoring the onset of diapause; larvae require more than 100 days to reach the pupal stage. *B* Conditions where delayed development prevails; larvae undergo stationary moults after reaching the immaculate morph; m indicates the actual number of larvae involved. *C* Absence of diapause; pupation occurs within 40 days. *D* Time over which pupation occurs.

by hibernaculum spinning. Between the 2 development patterns i.e. no interruption and prolonged development-diapause one can produce, with proper manipulation of temperature and photoperiod, a situation of delayed metamorphosis characterized by an immaculate morph, stationary molting but no spinning. For such an intermediate development situation to occur in the whole population, the insect must spend at least 32 days at 12:12, 21 °C and then experience conditions that do not induce arrested development (14:10, 27 °C). This also indicates that the fifth instar is quite sensitive to photoperiod/temperature conditions. In conclusion then *D. saccharalis* is capable of 1) averting diapause; 2) entering a period of delayed metamorphosis, and 3) entering diapause but the latter cannot be considered to start with the appearance of the immaculate morph according to the generally accepted definition of diapause. It is really initiated at hibernaculum spinning.

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2 Beck, S.D., Insect Photoperiodism, 2nd edn. Academic Press, New York 1980.
3 Ismail, S., and Fuzeau-Braesch, S., J. Insect Physiol. *22* (1976) 133.
4 Chippendale, G.M., A. Rev. Ent. *22* (1977) 121.
5 Chippendale, G.M., and Reddy, A.S., Ann. ent. Soc. Am. *65* (1972) 882.
6 Roe, R.M., Hammond, A.M. Jr, Reagan, J.E., and Hensley, S.D., A bibliography of the sugarcane borer, *Diatraea saccharalis* (Fabricius), 1887–1980. U.S. Department of Agriculture 1982.
7 Brewer, F.D., Development of the sugarcane borer on various artificial diets, U.S. Department of Agriculture (ARS-S-116) 1976.
8 Katiyar, K.P., and Long, W.H., J. econ. Ent. *54* (1961) 285.
9 Goettel, M.S., and Philogène, B.J.R., J. Insect Physiol. *24* (1978) 523.

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Oxygen consumption in rat skeletal muscle at various rates of oxygen delivery

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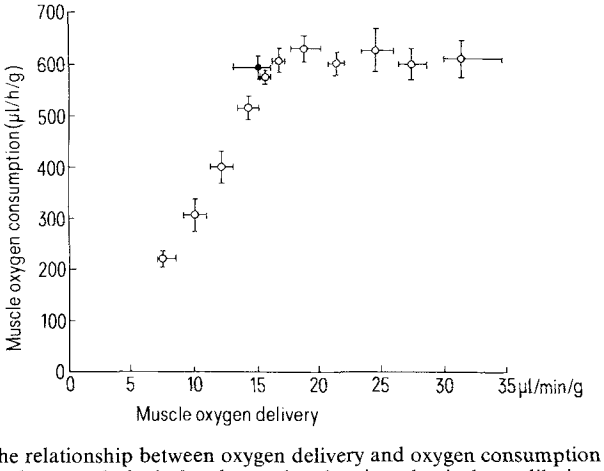
Summary. Resting oxygen consumption of the vascularly isolated gracilis anticus muscle of the rat perfused by natural circulation via the femoral artery with diluted or undiluted blood depends on oxygen delivery (the product of flow rate and arterial oxygen concentration - $\dot{D}O_2$) only when $\dot{D}O_2$ falls below 16 µl/min/g.

The role of blood flow ($\dot{Q}$) as a determinant of the resting muscle metabolic rate ($\dot{V}O_2$) has been studied on many experimental objects, using different techniques. The values of $\dot{Q}$ in rat skeletal muscles at which $\dot{V}O_2$ was measured have varied between 2.0 and 432.5 µl/min/g[1,2]. The dependence of $\dot{V}O_2$ on muscle perfusion rate has been clearly demonstrated[1,3,4], but the mechanism responsible for this effect has not yet been clarified. The aim of the present work was to evaluate the role of $\dot{Q}$ and $\dot{D}O_2$ as determinants of the resting $\dot{V}O_2$ and to find out the range in which this control takes place. In order to induce different $\dot{Q}$ and $\dot{D}O_2$ values of the gracilis muscle preparation an isovolemic hemodilution was applied and $\dot{V}O_2$ was measured under stationary conditions.

Materials and methods. Male Wistar SPF rats, 355–430 g b.wt, anesthetized (Thiopental Spofa, 50 mg/kg, i.p.) and heparinized (Heparin Spofa, 2500 IU/kg, i.v.) were used for the experiments. Before the experiments, rats were kept at 6±1 °C for at least 21 days in order to obtain cold-adapted individuals. These animals are generally used to study the adrenergic control of muscle nonshivering thermogenesis in our laboratory. The left gracilis anticus muscle was completely isolated vascularly, with the exception of the femoral artery through which the muscle was perfused with blood from natural circulation. Venous blood leaving the muscle was returned by a cannula from the left femoral vein into the right jugular vein. The muscle surface was kept moist with a Krebs-Henseleit solution at 36–37 °C. The diluting solution (6% dextran Spofa) was injected into the left jugular vein at the rate of 1 ml/min and the corresponding volume of arterial blood was taken out simultaneously from the right femoral artery. $\dot{Q}$ through the muscle was measured by timed collection of femoral vein effluent in calibrated capillary tubes. $\dot{V}O_2$ was calculated from $\dot{Q}$ and the arteriovenous difference in O_2 concentration according to the Fick principle. Samples of arterial blood were taken from the right femoral artery. Blood O_2 concentration was analyzed using a Clark O_2 electrode (Radiometer Copenhagen) in the 0.2% ferricyanide solution saturated with nitrogen. Muscle $\dot{D}O_2$ was calculated as the product of $\dot{Q}$ and arterial O_2 concentration. The O_2 extraction coefficient was taken as the ratio of arteriovenous difference in O_2 concentration to arterial O_2 concentration.

Results. Mean resting $\dot{Q}$ in the muscle perfused with undiluted arterial blood containing 23.4±1.1 ml O_2/100 ml was 64.0±3.6 µl/min/g. Both parameters were kept un-



The relationship between oxygen delivery and oxygen consumption in the vascularly isolated muscle after isovolemic hemodilution. Closed circle represents the mean resting oxygen consumption of the muscle perfused with undiluted blood. Vertical and horizontal bars indicate SEM and variation of the argument, respectively. n=8–12.