Int. J. Indust. Entomol. Vol. 10, No. 1, 2005, pp. 45~49

International Journal of *Industrial Entomology*

Rate of Oxygen Uptake in *Antheraea mylitta* in Various Stages of Development and during Diapause

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(Received 19 January 2005; Accepted 22 February 2005)

Respiration in *Antheraea mylitta* was studied using constant pressure respirometer. Oxygen uptake in different stages of life showed that moth has highest rate of oxygen uptake as compared to larva and pupa. At each stage significant sexual differentiation was observed. Depression in the rate of oxygen uptake in diapausing pupa was found to be higher in male (65.75%) than female (60.65%) as compared to non-diapausing counterparts. During diapause lowest oxygen uptake was recorded in the month of February, and were in the order of February < January < December < March < November < April < May < June in male, and, February < January < December < March < April < November < May < June in female. Significant sexual differentiation in oxygen uptake was recorded throughout the period of diapause (November to June) where female pupae registered lower rate of oxygen uptake than that of male. Oxygen uptake in female pupae declined upto 28.89% in non-diapausing and 18.29% in diapausing broods over male. Highest respiratory quotient value was recorded in the moth of November (0.68 in male and 0.70 in female) and, the lowest in the month of March in male (0.54) and May in female (0.55). Percentage loss in pupa weight always remained at a significantly higher level in male (except in February and March). The lowest oxygen uptake rate and weight loss in the month of February shows that the pupae were at peak of their dormancy during this month.

Key words: *Antheraea mylitta*, Oxygen uptake, Respiratory quotient, Diapause

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Introduction

Oxygen is required in a biological system to release most of the energy in aerobic organisms. Insects, being poikilothermal, their metabolism as expressed by oxygen consumption vary directly with fluctuation in the environmental temperature. The respiratory rate has a linear relationship with body weight and it may vary with temperature, season, age and oxygen concentration (Keister and Buck, 1964). Respiratory rate is greater in adult, than in larva while the pupal consumption is lower than either (Chapman, 1980). The oxygen consumption in diapausing insects remains high during phases of morphogenesis but low during the period of diapause development (Wigglesworth, 1982). Depression in metabolism is always a striking characteristic of diapause in many insects like *Lucilia, Celerio, Platysamia cecropia, Agapema galbina, Antheraea pernyi*, and *Leptinotarsa* (Keisster and Buck, 1964; Stegwee, 1964; Wigglesworth, 1982).

Antheraea mylitta has three distinct stages in life, the larval stage is the only feeding stage, otherwise known as energy gathering stage, and the other two stages, the pupa and the adult are the non-feeding stages and can be referred as energy expending stages. This insect shows facultative diapause and the diapause period extends to about 6 – 8 months. This period is well suited to regions with a long developmental season since it enables the insect to make the best use of the time available. A comparative study on the metabolic rates and their energy conversion in three voltine varieties of *A. mylitta* were reported for the diapausing pupae 15 days prior to emergence assuming the scheduled emergence date as 15th June (Satapathy and Mitra, 2001; Satapathy, 2003). But, particularly for *A. mylitta* such assumption on scheduled emergence does not hold good. No relation between pupa weight and pupa period with adult emergence are observed in *A. mylitta* not even in the same age group

(Rath *et al.*, 2005).

No study was undertaken earlier to find out the rate of oxygen uptake in various stages of development in *A. mylitta* and to establish to what extent the metabolism suffers a set back during the whole period of diapause. The present study was undertaken to find out the rate of oxygen uptake in various stages of development of this insect and to find out the level of metabolism during the long period of diapause. It was also intended to study about the metabolites taking part in releasing energy and weight loss during the long diapause period.

Materials and Methods

The animal is enclosed in a chamber and the changes caused by its respiration in the composition of the air inside are determined. The respirometer used for the experiment was based on the principle that, pressure was kept constant and the volume was allowed to change on the absorption of evolved carbon dioxide by potassium hydroxide (KOH) pellets so that volume of air will change proportionately to the consumed oxygen (Hurket and Mathur, 1974).

For the experiment *Antheraea mylitta* (Lepidoptera: Saturniidae; eco-race: Daba B. V.) larva 1^{st} to 5^{th} instar (L 1^{st} to L 5^{th}), pupa day 0 to day 14 (P 0 to P 14) and moth day 0 to day 7 (M 0 to M 7) were used. Oxygen uptake by larva of different instars, pupa (naked, with out the cocoon case) and moth (wing amputed) of non-diapausing generation were measured during July - August in the prevailing climatic conditions (temperature $30 \pm 3°C$ and $80 \pm 5\%$ relative humidity). There were 10 replications each with 5 samples.

Oxygen uptake in diapausing pupae (naked, with out the cocoon case) was studied during the whole period of diapause (from November to June). Experiment was conducted during mid of every month during the period of study. There were 10 replications each with 10 samples. The minimum and maximum temperatures were recorded every day to find out the temperature available during the period of diapause. Respiratory quotient (R. Q. = CO_2 liberated/O_2 uptake) was studied without using KOH pallets in the respirometer.

A comparison in oxygen uptake by diapausing and non-diapausing pupae were made by subjecting the pupae of same age (7 days old) to experimentation to find out the amount of depression in oxygen uptake following diapause.

Unit body weight was taken for expressing the respiration rate (Keister and Buck, 1964; Edwards, 1985) and was expressed as $\mu l O_2$ uptake/g body wt./h. The results were analysed using student's t-test and ANOVA.

Results and Discussion

Rate of oxygen uptake in various stages of development

Oxygen uptake by *Antheraea mylitta* in various stages of development during non-diapausing generation revealed that the rate of oxygen uptake by the moth was highest as compared to larva and pupa. Pupa recorded the lowest oxygen uptake. During the course of experimentation sex could not be identified in the larvae up to 4th instar and hence sex-wise oxygen uptake could not be studied up to 4th instar. The average rate of oxygen uptake during larval stage were 4875.5 (1^{st}), 3850.75 (2^{nd}), 3237.4 (3^{rd}), 1927.8 (4^{th}) and 1132.69 (5^{th}), during pupa it was 726.55 and moth 3804.12 $\mu l O_2$/g body wt. /h in male; 4875.5 (1^{st}), 3850.75 (2^{nd}), 3237.4 (3^{rd}), 1927.8 (4^{th}) and 747.27 (5^{th}), during pupa it was 535.88 and moth 3679.73 $\mu l O_2$ /g body wt. /h in female. In the larva, the oxygen uptake was significantly declined ($P < 0.001$) with the increase in their developmental stages. Male larvae of 5^{th} instar have significantly higher ($P < 0.001$) oxygen uptake than the female counterpart. In pupa, the oxygen uptake rate increased significantly with age in both the sexes. In male moth, the oxygen uptake significantly declined with age, while in female, it increased significantly up to day 4, and then declined. Significant sexual differentiation ($P < 0.001$) was observed at each stage of life, where males registered a higher rate of oxygen uptake than females, but it was not true for female moths of age day 3 onwards, where the oxygen uptake was significantly higher than their male counterparts. Significant difference between age groups during pupal and moth stage were also observed (Fig. 1).

The regular increase in oxygen uptake as development proceeds was most prominent in most of the insects studied. A very close relation between developmental stages and respiration was observed for *Melanoplus differentialis*. The oxygen consumption rate is greater in adult than in larva while the pupal consumption is lower than either was reported earlier (Edwards, 1985). It corroborates our findings. Male bee moth (*Galleria mellonella*) and *Musca domestica* have higher rate of oxygen consumption than female. But in the gravid female of *Musca domestica* the oxygen consumption rises quite steeply until just before the eggs are laid (Edwards, 1985). Unlike this, our findings points out that although females had lower rate of oxygen consumption than that of males throughout the developmental stages, the female moths of age group day 3 onwards had higher respiration rates than males which shows higher energy requirement for the act of oviposition.

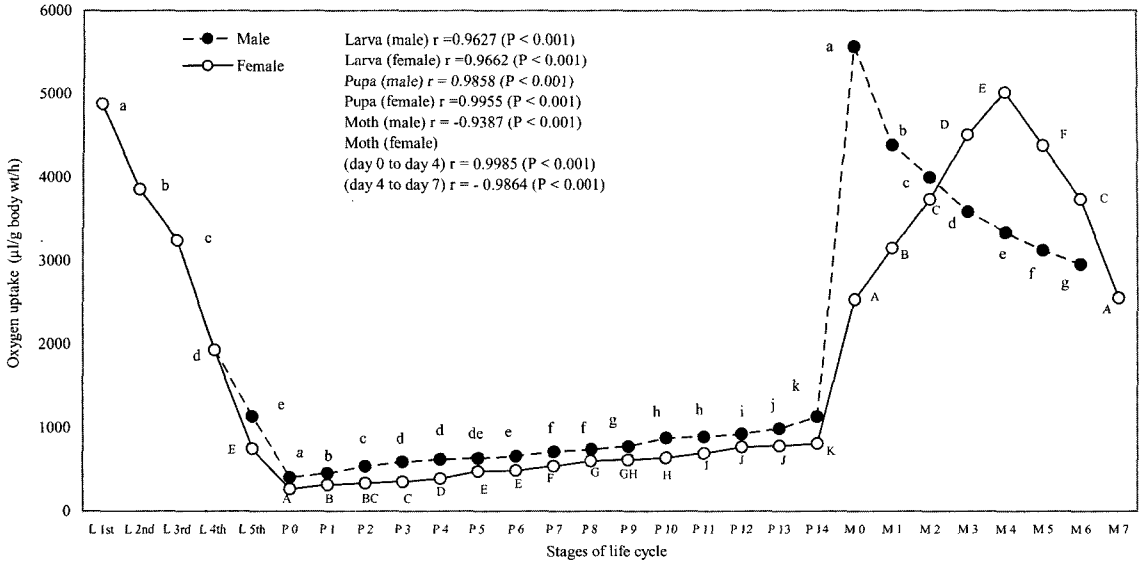

Fig. 1. Oxygen uptake in *Antheraea mylitta* in various stages of development. Sex difference during larval stage could not be made up to 4th instar. L 1st to L 5th, 1st instar larva to 5th instar; P 0 to P 14, pupa day 0 to day 14; M 0 to M 7, moth age day 0 to day 7. Sex difference in different stages (from 5th instar onwards) were significant ($P < 0.001$). Significant difference between age groups in larvae, pupae and moths were indicated by different letters according to the one-way ANOVA (upper case for female and lower case for male, except in larval stages represented by open circle and lower case up to 4th instar represents both the sexes).

Change in temperature and oxygen uptake during diapause

The oxygen uptake during diapause was bi-phasic, a decline phase followed by a phase of increase. In the first phase oxygen uptake gradually but significantly declined as the diapause proceeds. It started falling from the moth of November and reached its lowest level during February when the environmental temperature was lowest. In the second phase, there was a gradual but significant increase in oxygen uptake following increase in the environmental temperature till June. Thus, the temperature-oxygen curve of *A. mylitta* diapausing pupae showed a direct relation-

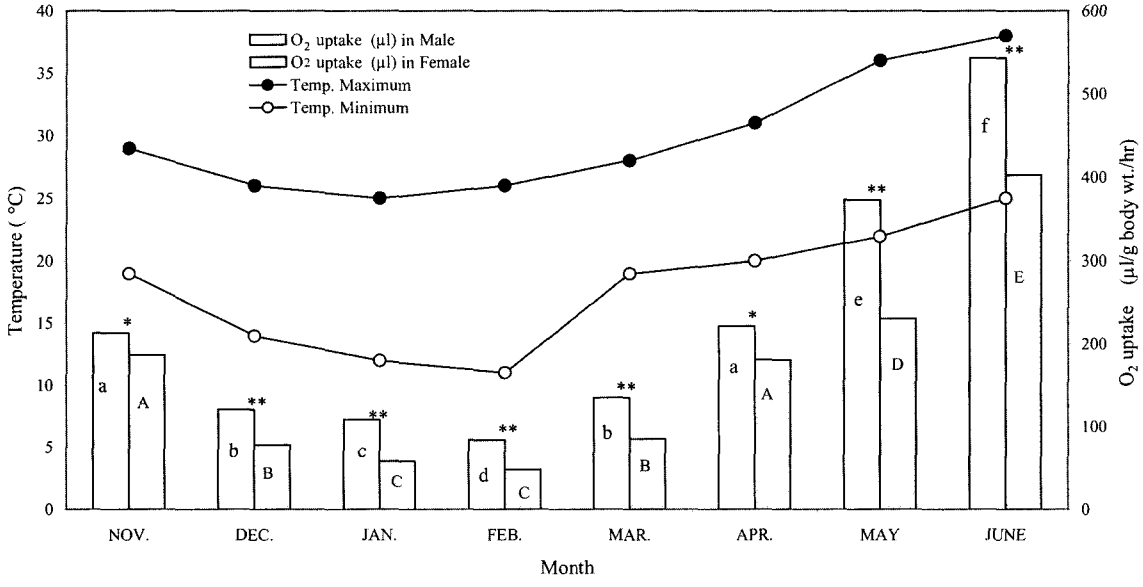

Fig. 2. Change in temperature and oxygen uptake during diapause in pupa of *Antheraea mylitta*. Sex difference in oxygen uptake were significant at $P < 0.02$ (*) and at $P < 0.001$ (**). Significant difference in oxygen uptake between months were indicated by different letters according to one-way ANOVA (uppercase for female and lower case for male).

ship between environmental temperature and oxygen uptake. The trend was same for both the sexes (Fig. 2). Similar reports are available for other insects also (Chapman, 1980; Wigglesworth, 1982; Edwards, 1985).

During diapause the rate of oxygen uptake was lowest during February and were in the order of February < January < December < March < November < April < May < June in male, and, in female it was February < January < December < March < April < November < May < June. Significant sexual differentiation was observed throughout the period of diapause where female pupae registered significantly lower rate of oxygen uptake than that of male (Fig. 2).

In diapausing pupa the oxygen uptake falls up to 65.75% ($P < 0.001$) in male and 60.67% ($P < 0.001$) in female as compared to non-diapausing ones. Sexual differentiation was also significant ($P < 0.001$). Oxygen uptake in female pupa declined up to 28.89% in non-diapausing and 18.29% in diapausing broods over male (Table 1). Depression in metabolism is always a striking characteristic of diapause. Our finding corroborates with the findings in *Celerio* (Wigglesworth, 1982), where the respiration falls up to 1/3rd to 1/4th and also in *Platysamia cecropia, Antheraea pernyi*, and *Agapema galbina* (Keister and Buck, 1964; Rees, 1977). The total respiration of adult *Leptinotarsa* during diapause falls by 75% as extreme degeneration of flight muscle had occurred which is restored at the end of diapause (Stegwee, 1964). All these findings reported earlier agree with our results. The diminished rate of respiration in the diapausing pupae seems to be due to a reduced energy demand during that period of development which was also reported by Rees (1977).

Respiratory quotient (R. Q.)

Respiratory quotient value was found to be highest during the month of November in both the sexes (male 0.68; female 0.70). The R. Q. falls gradually in both the sexes up to March in male (0.54) and May in female (0.55), and thereafter increased till June. The R. Q. value did not differ among sexes statistically during diapause except December, the R. Q. of male significantly ($P < 0.05$) lower than that of female (Table 2). The chief source of energy during pupal development is fat in many insects where the R. Q. value agrees with the expected value for fat consumption (R. Q. = 0.70). It corroborates with the findings observed in other insects like *Lucilia, Calliphora, Attacus, Popillia, Leptinotarsa* where the R. Q. value declined below 0.70. The R. Q. of *Galleria, Antheraea* and *Mimas tiliae* at rest is 0.69 throughout life (Wigglesworth, 1982) and that of grasshopper egg it remains at 0.60 (Edwards, 1985) agree with our findings. In most animals the male has a higher metabolism than the female. The oxygen consumption of adult male silkworm is three times that of female and the R. Q. is lower (Wigglesworth, 1982). The lower R. Q. in male observed in our study agrees with it. The R. Q. changes vary from 0.4 to 0.7 in pupa during development in Japanese beetle which might

Table 1. Oxygen uptake by diapausing and non-diapausing pupae of *Antheraea mylitta* (value are mean ± SD)

Sex	μlO_2 uptake/g body wt./h			Percentage decline in diapausing pupa
	Non-diapausing	P	Diapausing	
Male	651.16 ± 46.62	< 0.001	223.00 ± 19.89	65.75
P	< 0.001		< 0.001	
Female	463.03 ± 20.27	< 0.001	186.22 ± 13.56	60.65
Percentage decline in female	28.89			18.29

Table 2. Respiratory quotient (R. Q.) and weight loss in *A. mylitta* pupa during diapause

Parameters	Sex	Months								CD at 5%
		November	December	January	February	March	April	May	June	
R. Q.	Male	0.68	0.63	0.61	0.59	0.54	0.57	0.58	0.59	0.05
	P	NS	< 0.05	NS	NS	NS	NS	NS	NS	
	Female	0.70	0.68	0.62	0.59	0.57	0.56	0.55	0.61	0.04
Weight loss (%)	Male	1.33 ± 0.15	1.09 ± 0.12	0.96 ± 0.11	0.72 ± 0.08	0.60 ± 0.07	1.93 ± 0.21	2.65 ± 0.29	4.34 ± 0.48	0.18
	P	< 0.05	< 0.01	< 0.01	NS	< 0.001	< 0.001	< 0.01	< 0.001	
	Female	1.14 ± 0.03	0.85 ± 0.02	0.78 ± 0.02	0.71 ± 0.02	0.93 ± 0.02	1.35 ± 0.03	1.99 ± 0.05	2.92 ± 0.07	0.02
% change in loss of body weight in female over male		-14.178	-21.382	-18.776	-1.521	+53.40	-29.912	-24.887	-32.788	

be due to histogenesis and histolysis in pupa supports our observation (Edwards, 1985).

Loss in live weight

The loss in live weight of diapausing pupa (expressed in percentage weight loss) was initially 1.33% in male and 1.14% in female. This loss gradually fall upto March in male and February in female, but thereafter, the loss percentage increased till June. Total loss percentage during diapause was 13.62 in male and 10.67 in female. Male pupa always loses significantly more weight than female except in February where the loss percentage was same for both the sexes (when the pupae were at peak of their dormancy), but the female pupa loss significantly higher ($P < 0.001$) body weight than male during the moth of March (Table 2).

It was observed that there was a clear cut relationship between weight loss and rate of oxygen uptake in diapausing pupae. The rate of oxygen uptake follows the same trend as that of weight loss and is temperature dependent. The lower R. Q. showed that fat moieties serve as the fuel for various life activities during diapause. Lowest level of oxygen uptake and weight loss in the month of February indicates that the pupae were at peak of their dormancy.

It can be concluded that the rate of respiration was highest in moth followed by larva and pupa. Even in diapausing pupae development continuous but at lowest rate as represented by lowest rate of oxygen uptake during that phase. The higher oxygen uptake in the female moth from day 3 onwards show higher energy requirement for oviposition of eggs.

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