https://dx.doi.org/10.4314/ijs.v25i1.2

Ife Journal of Science vol. 25, no. 1 (2023)

ACCUMULATION AND UTILIZATION OF FOOD RESERVES IN DIAPAUSE-DESTINED PALLID EMPEROR MOTH, *Cirina forda* (WESTWOOD)

Oriolowo, O. B.[1,*], Ande, A. T.[2] and Azeez, M. O.[3]

[1]Department of Biology Federal College of Education, PMB 39, Kontagora, Niger State, Nigeria.

[2]Department of Zoology, Faculty Life Sciences, University of Ilorin, PMB 1515, Ilorin, Nigeria.

[3]Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ilorin, PMB 1515, Ilorin, Nigeria.

*Corresponding Author's Email: oriolowo.ob@fcekg.edu.ng

(Received: 24th February, 2023; Accepted: 23rd April, 2023)

ABSTRACT

Diapause is a complex life history strategy aimed at tolerating or circumventing stress in insects. It is accompanied by alterations in the food reserve levels to cope with the energy demand of this life stage. In the pallid emperor moth (*Cirina forda*), little is known about the patterns of accumulation and utilization of food reserves before and during diapause, respectively. The accumulation and utilization of food reserves in the haemolymph and the whole body of diapause-destined larvae and diapausing pupae of *Cirina forda* were progressively compared using photometry and HPLC. The quantity of glucose, total carbohydrate, fats, protein, and amino acids measured were taken as indices of food reserve accumulation and utilization by the immature stages of the insect in this study. Data collated were analyzed using Analysis of Variance (ANOVA), and the means were separated using Fisher's Least Significance Difference (LSD). The results showed progressive accumulation of glucose from 46.20 to 105.06 mg/100 g, total carbohydrates from 175.39 to 212.54 mg/100 g, lipids from 440.49 to 641.57 mg/100 g, and protein from 306.04 to 378.83 mg/100 g in the haemolymph of diapause-destined larvae. The reserves decreased progressively (72.1%, 60.3%, 5.7%, and 6.9%) in diapausing pupae. Total amino acids increased by 2.8%, while 61.1% of amino acids in the whole pupae increased significantly ($P<0.05$). The study concluded that *C. forda* accumulates food reserves in preparation for diapause, and reserves decline as they are probably utilized during diapause development.

Keywords: Diapause, Food reserves, Haemolymph, Pupae, Larvae.

INTRODUCTION

Diapause is a state of developmental arrest and metabolic depression exhibited by many animals, especially insects. It is associated with several physiological, biochemical, and molecular changes that generally enhance tolerance to stress and synchronization of reproduction with the resumption of favourable environmental conditions. During diapause, energy production and consumption are adjusted, energy reserves are increased, and biochemical pathways are diverted toward the production of protective molecules. Diapause is anticipatory, and many insects accumulate food reserves in preparation for this stage of their life. Insects generally store metabolic reserves such as lipids, carbohydrates, and amino-acid. However, there are significant alterations in the quantity of the nutrients stored and their utilization in diapause-destined individuals. These reserves are often used in cellular processes such as protein turnover and cell maintenance (Hahn and Denlinger, 2011).

Diapausing insects enter an alternative developmental pathway that has its metabolic demands (Kostal, 2006). This pathway occurs by increased regulation of the production of lipids and several classes of proteins which require a substantial quantity of amino acids and carbohydrates precursors (Denlinger and Lee, 2010). Lipids remain one of the most important energy reserves in most diapausing insects because of their caloric content, low hydration state, and perhaps relatively high yield of metabolic water. They are obtained from food or synthesized from carbohydrates and amino acid precursors via the tricarboxylic acid cycle intermediate (Canavoso *et al.*, 2001). Their storage has been an important factor in mitigating the metabolic challenges of diapause (Hahn and Denlinger, 2007).

Cirina forda, a Lepidoptera of the family Saturniidae, is univoltine and experiences obligatory summer diapause in which the 5th instar larva digs, pupates, and remains inactive in the soil for 9 to 10 months (Oriolowo *et al.*, 2023). This phenomenon is common to other related *Cirina*

species (Bama *et al*., 2018; Payne *et al*., 2019). The pupa remains in this state until favourable conditions of humidity, food, and temperature. There is a paucity of information on the patterns of storage and utilization of food in preparation for diapause and its development. These factors could be crucial to its survival during the less active and non-feeding period of pupa development. This study is aimed at identifying the types of food reserves such as glucose, total carbohydrate, lipids, and protein, and their patterns of accumulation in the haemolymph and whole body of larva and pupa *C. forda*.

MATERIALS AND METHODS

Insect collection and rearing

Cirina forda larvae (1st instar) were collected by hand from host plants on the field at Kutigi (Latitude 9° 10´N; 9°, 13´ and Longitude 5°35´E; 5°, 39´), Lavun Local Government Area, Niger State, Nigeria in late July 2014, 2015, and 2016. They were supplied with fresh *Vitellaria paradoxa* (Sapotaceae) twigs and transported to the Zoology Department laboratory, University of Ilorin in a perforated paper carton. Immediately after reaching the laboratory, the larvae were transferred to four culturing wire netted cages of 100 cm x 60 cm x 45 cm. Each cage was filled with loamy soil to a height of about 45 cm to provide the larvae with pupation substrate. Fifty larvae were maintained under ambient temperature and relative humidity (25-32 °C; 75-85% RH) and daily supplied with fresh twigs of *V. paradoxa* until pupation in each cage.

At the onset of signs of wandering among the last instar larvae, they were transferred into a paper carton pupation cage of size 60 cm x 40 cm x 70 cm filled with loamy soil to a height of about 45 cm. The paper pupation cage makes it easy to remove pupae from the soil without any damage when the need arises. Pupae were left in this cage for four weeks (August 15 to September 10) before being removed for further investigation.

Haemolymph Collection

A 1 mL volume of haemolymph was collected into a pre-chilled 25 mL polypropylene tube containing 9 mL of 1% (w/w) ascorbic acid solution by cutting the last abdominal proleg of 3rd, 4th, and 5th instar larvae. In the case of pupae, haemolymph was collected once every month by puncturing the last abdominal segment with a hypodermal needle. Little pressure was applied at the thorax to enhance haemolymph exudation. Ascorbic acid is an antioxidant that prevents endogenous phenoloxidase activity and leads to coagulation and blackening of the haemolymph or protein melanization. Sometimes, it was necessary to pool together the haemolymph sample from some larvae to obtain the required volume. After collection, the haemolymph sample was stored in a refrigerator until further analysis.

Total lipid concentration

A 5 mL volume of haemolymph solution was dissolved in concentrated sulphuric acid, digested at 100 °C for 10 min, and treated with phosphoric acid/vallin reagent. The resulting solution was assayed for total lipid spectrophotometrically (Spectrumlab 752s; product standard Q/SEEK3) at a wavelength of 540 nm using cholesterol as standard (Zoellner and Kirsch 1962, Holwerda *et al*., 1977). Three replicates were obtained for each sample.

Total soluble sugar in the haemolymph

The haemolymph solution (5 mL) was added to 2.5 mL of freshly prepared anthrone reagent in a test tube. The mixture was boiled in a water bath at 97 °C for 4 min and cooled in ice-cold water. The resulting solution was filtered through a Whatman No1 filter paper. Total carbohydrate was then measured using a spectrophotometer (Spectrumlab 752s; product standard Q/SEEK3) at wavelength 640 nm using glucose solution as standard (Arslan *et al*., 1986). Three replicates were obtained for each sample.

Total haemolymph protein concentration

The haemolymph solution (5 mL) was added to the biuret reagent. Total protein was measured from the resulting mixture in a spectrophotometer (Spectrumlab 752s; product standard Q/SEEK3) at an absorbance of 540 nm (Rameshkumar *et al.,* 2009). Bovine Serum Albumin (BSA) was used to prepare a standard calibration curve. Each treatment comprised triplicate samples.

Haemolymph glucose concentration

The glucose concentration in a drop of haemolymph from larvae and pupae was

quantified using the One Touch UltraEasy glucose monitoring system (AW 06651502A; Lifescan Johnson and Johnson Company, United Kingdom). Haemolymph glucose concentrations were obtained by transforming the values in mmol/L from One Touch device to mg/mL.

Determination of Amino Acids

The amino acid profile in dry samples of larvae and pupae was determined as described by Benitez (1989). Briefly, 5 g was dried to a constant weight, defatted (AOAC, 2006), hydrolyzed, evaporated in a rotary evaporator, and loaded into the Amino Acid Analyzer (Applied Biosystems PTH). The mean values for the larvae and pupae were recorded for each amino acid, and three replicates were measured for each sample.

RESULTS

The concentration of food reserves: glucose, total carbohydrate, lipids, and proteins in the haemolymph of *C. forda* from larva to pupa is shown in Table 1. The glucose concentration in the haemolymph of *C. forda* increased from 46.20 to 105.06 mg/100 g, total carbohydrate from 175.39 to 212.54 mg/100 g, lipids from 440.49 to 641.57mg/100 g, and protein from 306.04 to 378.83 mg/100 g. There was a gradual accumulation of reserves as the insect developed from larva III to a one-month-old pupa (PI). The highest concentration of glucose of 150 mg/100 g occurred at PI, while the highest accumulation of total carbohydrates of 234.44 mg/100 g, lipids of 828.76 mg/100 g, and proteins of 597.32 mg/100 g occurred at PII. The lowest value for glucose of 11.45 mg/100 g occurred at PIV after its initial gradual accumulation from LIII-PI. There were significant differences in the haemolymph concentration of each food reserve across the stages of larva and pupa development. They include glucose ($F_{2,\ 30}$ = 83.13, ANOVA, LSD = 14.31, $P<0.05$), total carbohydrates ($F_{1,\ 20}$ = 82.73, ANOVA, LSD = 43.04, $P<0.05$), total lipids ($F_{2,\ 30}$ = 3757.2, ANOVA, LSD = 5.98, $P<0.05$) and total proteins ($F_{2,\ 30}$ = 245.3, ANOVA, LSD = 19.9, $P<0.05$). Between PII-PV, all reserves declined at different rates. Glucose declined by 72.1% at PII, total carbohydrate by 60.3% at PIII, lipids by 5.7% at PIII, and protein by 6.9% at PIII. However, haemolymph concentrations of lipids and proteins were more stable than carbohydrates.

Table 1: Haemolymph food reserves of diuapause-destined larvae and pupae of *C. forda* (mg/100 g).

Stage	Glucose	Carbohydrates	Lipids	Protein
LIII	46.20 ± 2.62^{a}	175.39 ± 0.34^{a}	440.49 ± 1.95^{a}	306.04 ± 10.12^{a}
LIV	64.81 ± 6.63^{b}	203.70 ± 1.67^{a}	510.12 ± 6.95^{b}	376.93 ± 14.01^{b}
LV	105.06 ± 16.89^{c}	212.54 ± 4.81^{a}	641.57 ± 8.76^{c}	378.83 ± 3.68^{b}
PI	150.50 ± 46.65^{d}	233.85 ± 1.76^{b}	648.54 ± 1.60^{d}	437.90 ± 8.78^{c}
PII	41.93 ± 6.85^{ae}	234.44 ± 0.75^{bc}	828.76 ± 0.86^{e}	597.32 ± 7.92^{d}
PIII	40.41 ± 6.52^{ae}	93.12 ± 0.74^{d}	781.40 ± 0.03^{f}	556.30 ± 8.99^{e}
PIV	11.45 ± 1.88^{f}	88.20 ± 0.25^{d}	764.46 ± 2.34^{g}	544.85 ± 4.72^{ef}
PV	33.70 ± 13.59^{gh}	87.37 ± 0.26^{d}	711.77 ± 0.71^{h}	540.45 ± 0.81^{fg}
PVI	32.48 ± 2.28^{hi}	85.00 ± 2.75^{d}	665.15 ± 3.88^{i}	529.92 ± 6.62^{gh}
PVII	29.73 ± 10.20^{ik}	59.46 ± 0.72^{d}	633.41 ± 5.36^{j}	461.77 ± 3.68^{hi}
PVIII	25.77 ± 9.61^{kj}	55.03 ± 0.56^{d}	619.80 ± 1.83^{k}	357.16 ± 2.42^{bj}

Values are the mean of three replicates
Mean carrying different superscripts are significantly different (ANOVA, LSD, $P<0.05$)
LIII-LV represents larva instar III-V
PI-PVIII represents 1st-8th month old pupa

The amino acid concentration of non-diapausing 3rd, 4th, 5th instar larva and 1st, 2nd, 3rd 4th, 5th 6th, 7th, and 8th diapausing pupa of *C. forda* is presented in Table 2. Except for methionine and threonine, all the amino acids were either significantly increased or decreased between larva and pupa. Increased amino acid in pupa were leucine ($t_{1,\ 3}$ = 10.9, $P<0.05$), lysine ($t_{1,3}$ = 10.0, $P<0.05$), phenylalanine ($t_{1,3}$ = 4.9, $P<0.05$), tryptophan ($t_{1,3}$ = 2.6, $P<0.05$), and valine ($t_{1,\ 3}$ = 13.2, $P<0.05$). Others are arginine ($t_{1,\ 3}$ = 34.7, $P<0.05$), tyrosine ($t_{1,\ 3}$ = 13.9, $P<0.05$), histidine ($t_{1,\ 3}$ = 15.9, $P<0.05$), alanine ($t_{1,\ 3}$ = 9.2, $P<0.05$), glycine ($t_{1,\ 3}$ = 22.3, $P<0.05$), and serine ($t_{1,\ 3}$ = 22.5, $P<0.05$). The amino acids that

significantly decreased in pupa were isoleucine ($t_{1, 3}$ = 21.7, P<0.05), proline ($t_{1, 3}$ = 7.4, P<0.05), cysteine ($t_{1, 3}$ = 6.3, P<0.05), glutamic acid (t_{1}, 3 = 87.2, P<0.05) and aspartic acid (t_{1}, 3 = 12.3, P<0.05). About 61% of the amino acid increased, 27.7% decreased, while 11.1% did not significantly change during pupa diapause. Moreover, about 78% of essential amino Acids (EAAs) increased while 44% of non-essential amino acids (NEAAs) decreased during pupa diapause.

Table 2: Amino acids profile of diapause-destined larvae and pupae *C. forda* (g/100 g of protein).

	Larva	Pupa
Amino acid	Mean ± SD	Mean ± SD
Leucine*	7.30 ± 0.03[a]	7.67 ± 0.08[b]
Lysine*	7.03 ± 0.04[a]	7.48 ± 0.10[b]
Isoleucine**	3.99 ± 0.02[a]	3.53 ± 0.05[b]
Phenylalanine*	3.19 ± 0.03[a]	3.37 ± 0.09[b]
Tryptophan*	2.20 ± 0.03[a]	2.31 ± 0.07[b]
Valine*	4.91 ± 0.03[a]	5.35 ± 0.05[b]
Methionine	1.28 ± 0.02[a]	1.23 ± 0.05[a]
Proline**	4.57 ± 0.03[a]	4.36 ± 0.04[b]
Arginine*	9.63 ± 0.03[a]	10.41 ± 0.07[b]
Tyrosine*	4.30 ± 0.04[a]	4.47 ± 0.05[b]
Histidine*	3.38 ± 0.05[a]	3.70 ± 0.05[b]
Cysteine**	0.54 ± 0.03[a]	0.42 ± 0.02[b]
Alanine*	5.84 ± 0.04[a]	6.22 ± 0.05[b]
Glutamic acid**	9.99 ± 0.03[a]	9.23 ± 0.03[b]
Glycine*	5.27 ± 0.02[a]	5.94 ± 0.04[b]
Threonine	3.88 ± 0.05[a]	3.99 ± 0.04[a]
Serine*	4.19 ± 0.05[a]	4.59 ± 0.03[b]
Aspartic acid**	8.31 ± 0.02[a]	8.15 ± 0.04[b]

***Increased in the pupa **Decreased in the pupa
Values are the mean of four replicates
Mean carrying different superscripts are significantly different (T-test, P<0.05)

DISCUSSION

The haemolymph energy reserve (carbohydrates, protein, and lipids) increased gradually from the third larva instar (LIII) to the fifth (LV). The highest accumulation of glucose occurred at the first-month-old pupa (PI), while total carbohydrates, protein, and lipids had their highest accumulation at the two-month-old pupa (PII). This accumulation is probably in preparation for the non-feeding diapausing pupa stage where they will be required for metabolism, maintenance, and post-diapause development. This result is similar to the report of Zhang *et al.* (2013), where glucose and trehalose were at high levels in diapause-destined sixth-instar larvae of *Helicoverpa armigera*. At PI, the glucose level in the haemolymph increased by about 70%, while at PII, total carbohydrates increased by 25%, lipids by 47%, and proteins by 49%. The increase in glucose concentration in preparation for diapause in *C. forda* could be a result of upward regulation of the gene phosphoenolpyruvate that synthesizes glucose by enhancing glycogenolysis and gluconeogenesis as reported in other insects (Overgaard *et al.*, 2007), especially in response to heat stress (Sorensa *et al.*, 2005).

After these initial surges in haemolymph reserves, their concentrations declined as the insect passed through the diapausing pupa stage. Glucose, total carbohydrate, and lipids had their lowest haemolymph concentration at the three-month-old pupal stage (PIII), but protein had its lowest accumulation at PV. A similar decline in glycolytic-related metabolites like glucose and carbohydrates in *Helicoverpa armigera* was associated with a

dramatic reduction in metabolic activities (Xu *et al.,* 2012). Moreover, there were differences in the utilization of these reserves in the diapausing pupa. A similar pattern was reported by Adedokun and Denlinger (1985) for diapausing pupae of the flesh fly *Sarcophaga crassipalpis* in which total fat levels decreased rapidly at the first stage of diapause, while its concentration remained relatively constant at the second half of diapause development. The rate at which these reserves declined was trendy across the developmental stages. The initial percentage decline in glucose was about 72.1%, total carbohydrates decreased by 60.3%, lipids by 5.7%, and 6.9% for proteins. These imply that the insect utilized a mixture of these substrates. A similar result was reported by Yocum *et al.* (2005) for solitary bees, *Megachile rotundata*. Therefore, during diapause development in *C. forda*, there were shifts in nutrient utilization probably due to the differences in the metabolic demands of the various developmental stages of the insects. Apart from the increase in glucose as a metabolic substrate during diapause, it is involved in the production of certain cryoprotectants (Denlinger and Lee, 2010). This may account for the decrease in haemolymph glucose concentration as the insect passes through diapause during its development.

At the period when the *C. forda* pupae were suspected to be diapausing (PIII-PV), the glucose content of the haemolymph declined. This was contrary to the result obtained by Teets *et al.* (2013), where glucose concentration increased during diapause in antarctic midge, *Belgica antarctica*. The difference may be due to differences in environmental conditions. In *C. forda*, heat, drought, and desiccation are prominent diapause-inducing environmental conditions, while in *Belgica antarctica,* diapause was induced by winter conditions.

The amino acid profile of diapausing *C. forda* pupae showed that the concentration of amino acids such as alanine, leucine, lysine, tryptophan, valine, arginine, phenylalanine, tyrosine, histidine, glycine, serine significantly increased. However, the concentration of proline, isoleucine, aspartic acid, glutamic acid, and cysteine declined. About 61% of the amino acid increased, while about 28% decreased. This is similar to the report of Colinet *et al.* (2012) in which amino acids serine, lysine, tryptophan, alanine, glycine, and aspartic acid increased, while proline, isoleucine, glutamic acid, and phenylalanine decreased in diapausing aphid parasitoid *Praon volucre*. Purac *et al.* (2015) also reported the elevation of alanine in diapausing larvae of European corn borer *Ostrinia nubilalis*. The increase of alanine in diapausing *C. forda* could be due to the stress of anaerobic conditions. This observation was attributed to an increase of alanine in diapausing pupae of *Mamestra brassicae* (Goto *et al.*, 2001).

The decrease in proline in this study is similar to the findings of Goto *et al.* (1997), who reported a decline in proline in the diapausing larva of grass borer *Enosima leucolaeniella*. Proline is a potential fuel that powers anaerobic metabolism after conversion to alanine during diapause (Khodayari *et al.,* 2013). Higher alanine enrichment in the diapausing pupa of flesh fly was linked with metabolic depression (Denlinger *et al.,* 1980) and heavy reliance on glycolytic and gluconeogenic pathways for energy generation (Kukal *et al.*, 1991, Machaud and Denlinger, 2007). Alanine also acts as a cryoprotectant in diapausing insects (Denlinger and Lee, 1998). Increased glycolysis, gluconeogenesis, and anaerobic metabolism have also facilitated metabolic depression and stress resistance in diapausing insects (Ragland *et al.*, 2010).

Histidine, a precursor to histamine, was significantly elevated in diapausing pupae of *C. forda*. Histamine is a neurotransmitter released from the photoreceptor synapse of insects and other arthropods (Zhang *et al.,* 2013). A significant increase in histidine could indicate changes in the levels of the photoreceptor transmitter histamine, which participates in the molecular events of diapause development such as daylight signal received by the brain (Zhang *et al.*, 2013).

Diapausing insects are vulnerable to fungal and bacterial infections. Hence, their innate immune system continues to function as it prevents the invasion of microorganisms (Nakamura *et al.*, 2011). Serine protease is responsible for immune functions such as haemolymph coagulation, antimicrobial peptide synthesis, and melanin

synthesis in many invertebrates (Gorman and Paskwitz, 2001). Serine protease is composed of amino acids such as serine, histidine, and aspartic acid, with serine serving as the nucleophilic amino group of the enzyme (Hedstrom, 2002). The increase in serine and histidine in diapausing *C. forda* pupae is connected with the immune response to microbial infection common in *Cirina forda* (Mohammed and Ande, 2014). Serine protease was reported by Zhang *et al.* (2013) to accumulate in sixth instar larvae of diapause-destined *Helicoverpa armigera* in preparation for its pupal diapause. Similar enrichment of this enzyme was reported in diapausing adults of the Colorado potato beetle, *Leptinotarsa decemlineata* (Yocum *et al.,* 2009), and mosquito, *Culex pipiens* (Robich *et al.,* 2007). Thus, an increase in serine and histidine could imply an indirect elevation of serine protease and activation of immune response to facilitate survival during adverse conditions associated with long periods of diapause (Zhang *et al.*, 2013).

CONCLUSION

Food reserves like carbohydrates, lipids, and proteins accumulate in diapause-destined *C. forda*. These are utilized as energy reserves, precursor substrates for immunity, and crypto-protectants during diapause. They declined at different rates during diapause development either because some are used more at a specific period or inter-converted to other substrates when needed. The decrease in proline could be attributed to its conversion to alanine, a substrate needed for anaerobic metabolism during diapause. Also, the increase in serine and histidine is for immune responses to pathogenic microbial infection associated with the long inactive pupa diapausing stage in the insect. Elevated concentrations of glucose, alanine, and leucine are suggested to be involved in glycolysis, while glutamic acid is important in the long-term storage of memories involved in the storage of diapause-induced photoperiodic information. This study concludes that the dynamics of food reserves in *C. forda* are meant to enhance its overall survival during the harsh period of pupa diapause.

DECLARATIONS

Ethics approval and consent to participate

The study was approved by the Postgraduate Ethical Committee (Board), Department of Zoology, University of Ilorin, Ilorin, Nigeria.

ACKNOWLEDGEMENTS

We acknowledge the technologists in the Department of Zoology postgraduate research laboratory, University of Ilorin for their assistance during the period when this research was carried out.

REFERENCES

Adedokun, T.A. and Denlinger, D.L. 1985. Metabolic reserves associated with pupal diapause in flesh fly *Sarcophaga crassipalpis*. *Journal of Insect Physiol*ogy, 31:229-233. doi:10.1016/0022-1910(85)90124-6

AOAC. 2006. Official Methods of Analysis (18th Edition), Association of Analytical Chemists, Washington, DC, USA.

Arslan, A., Standifer, L.N. and Don, H. 1986. Carbohydrates in honey bee haemolymph. *Comparative Biochemistry and Physiology B,* 84:363-367. doi:10.1016/0305-0491(86)90090-8

Bama, H.B., Dabire, R.A., Ouattara, D., Niassy, S., Ba, M.N. and Dakouo, D. 2018. Diapause disruption in *Cirina butyrospermi* Vuillet (Lepidoptera, Attacidae), the shea caterpillar, in Burkina Faso. *Journal of Insects as Food and Feed,* 4:239-245. doi:10.3920/JIFF2017.0068

Benitez, L.V. 1989. Amino Acid and fatty acid profiles in aquaculture nutrition studies, p. 23- 35.in S.S. De Silva (ed.) Fish Nutrition Research in Asia. *Proceedings of the Third Asian Fish Nutrition Network Meeting.* Asian fish. Society Special Publication; 4, 166p. Asian Fisheries Society, Manila Philippines.

Canavoso, L.E., Jouni, Z.E., Karnas, K.J., Pennington, J.E. and Wells, M.A. 2001. Fat metabolism in insects. *Annual Review of Nutrition,* 21:23-46. doi:10.1146/annurev.nutr.21.1.23

Colinet, H., Renault, D., Charoy-Guevel, B., and Com, E. 2012. Metabolic and Proteomic Profiling of diapause in the Aphid Parasitoid, *Praon volucre*. *PLoS One*, 7:e32606. doi:10.1371/journal.pone.0032606

Denlinger, D.L. and Lee, R.E. 2010. *Low-Temperature Biology of Insects*. Cambridge, UK: Cambridge University Press.

Denlinger, D.L, Campbell, J.J. and Bradfield, J.Y 1980. Stimulatory effect of organic solvents on initiating development in diapausing pupae of the flesh fly *Sarcophaga crassipalpis* and the tobacco hornworm *Manduca sexta*. *Physiology Entomol*ogy, 5:7-15. doi:10.1111/j.1365-3032.1980.tb00207.x

Denlinger, D.L. and Lee, R.E 1998. Physiology of cold sensitivity: Temperature Sensitivity in Insects and Application in Integrated Pest Management, eds Hallman G.J, Denlinger D.L (Westview, Boulder, CO), pp 55-95.

Gorman, M.J. and Paskewitz, S.M. 2001. Serine proteases as mediators of mosquito immune responses. *Insect Biochemistry and Molecular Biology,* 31:257-262. doi:10.1016/s0965-1748(00)00145-4

Goto, M., Fuji, M., Suzuki, K. and Sakai, M. 1997. Factors affecting carbohydrate and free amino acid content in overwintering larvae of *Enosima leucotaeniella*. *Journal of Insect Physiology,* 44:87-94. doi:10.1016/S0022-1910(97)00098-x.

Goto, M., Li, Y-P., Kayaba, S., Outani, S. and Suzuki, K. 2001. Cold hardiness in summer and winter diapause and post-diapause pupae of the cabbage armyworm, *Mamestra brassicae L.* under temperature acclimation. *Journal of Insect Physiology,* 47:709-714. doi:10.1016/s0022-1910(00)00164-5

Hahn, D.A. and Denlinger, D.L. 2007. Meeting the energetic demands of insect diapause: nutrient storage and utilization. *Journal of Insect Physiology,* 53:760-773. doi:10.1016/j.jinsphys.2007.03.018.

Hahn, D.A. and Denlinger, D.L. 2011. Energetic of insect diapause. *Annual Review of Entomology,* 56:103-121. doi:10.1146/annurev-ento-112408-085436

Hedstrom, L. 2002. "Serine protease and specificity" *Chemical Review,* 102:4501-4524. doi:10.1021/cr000033x

Holwerda, D.A., van Doorn, J. and Beenakkers, A.M.T. 1977. Characterization of the adipokinetic and hyperglycaemic substances from the locust corpus cardiacum. *Insect Biochemistry*, 7:151-157. doi:10.1016/0020-1790(77)90008-7

Khodayari, S., Moharramipour S., Larvor, V., Hidalgo, K. and Renault, D. 2013. Deciphering the Metabolic Changes Associated with Diapause Syndrome and Cold Acclimation in the Two-Spotted Spider Mite *Tetranychus urticae*. *PLoS One,* 8:e54025. doi:10.1371/journal.pone.0054025.

Koštál, V. 2006. Eco-physiological phases of insect diapauses. *Journal of Insect Physiology,* 52:113-127. doi:10.1016/j.jinsphys.2005.09.008

Kukal, O., Denlinger, D.L. and Lee Jr., R.E. 1991. Developmental and metabolic changes induced by anoxia in diapausing and non-diapausing flesh fly pupae. *Journal of Comparative Physiology [B],* 160:683-689. doi:10.1007/BF00571268

Michaud, M.R. and Denlinger, D.L. 2007. Shifts in the carbohydrate, polyol and amino acid pools during rapid cold-hardening and diapause-associated cold-hardening in flesh flies (*Sarcophaga crassipalpis*): a metabolomic comparison. *Journal of Comparative Physiology [B],* 177:753-763. doi:10.1007/s00360-007-0172-5

Muhammad, M.I. and Ande, A.T. 2014. Biotic decimating factors affecting the distribution of *Cirina forda* Westwood *(Lepidoptera: Saturniidae*) population in Niger State, Nigeria. *West African Journal of Applied Ecology,* 22:57-68.

Nakamura, A., Miyado, K., Takezawa, Y., Ohnami, N., Sato, M., Ono, C., Harada, Y., Yoshida, K., Kawano, N., Kanai, S., Miyado, M. and Umezewa, A. 2011. The innate immune system still works at diapause, a physiological state of dormancy in insects. *Biochemical and Biophysical Research Communications,* 410:351-357. doi:10.1016/j.bbrc.2011.06.015

Oriolowo, O.B., Ande, A.T., and Azeez, M.O. 2023. Respiratory activity of the pallid emperor Moth, *Cirina forda* (Lepidoptera: Saturniidae) during larva and pupa developments. *Journal of Entomology and Zoology Studies*, 11:37-42. doi:10.22271/j.ento.2023.v11.i1a.9143

Overgaard, J., Malmendal, A., Sørensen, J.G., Bundy, J.G., Loeschcke, V., Nielsen, N.C. and Holmstrup, M. 2007. Metabolomic profiling of rapid cold hardening and cold shock in *Drosophila melanogaster*. *Journal of Insect Physiology,* 53:1218-1232. doi:10.1016/j.jinsphys.2007.06.012

Payne, C.L.R., Badolo, A., Cox, S., Sagnon, B., Dobermann, D., Milbank, C., Scarborough, P., Sanon, A., Bationo, F. and Balmford, A. 2019. The contribution of 'chitoumou', the edible caterpillar *Cirina butyrospermi*, to the food security of smallholder farmers in southwestern Burkina Faso. *Food Security,* 12:221-234. doi:10.1007/s12571-019-00994-z

Purać, J., Koic, D., Popovic, Z.D., Vukasinovic, E., Tiziani, S., Gunther, U.L. and Grubor-Lajsic, G. 2015. Metabolomic analysis of diapausing and diapausing larvae of the European corn borer *Ostrinia nubilatus*. *Acta Chimica Slovenica,* 62:761-767. doi:10.17344/acsi.2015.1370.

Rameshkumar, G., Ravichandran, S., Kaliyavarathan, G. and Ajithkumar T.T. 2009. Comparison of protein content in the haemolymph of brachyuran crabs. *Middle-East Journal of Scientific Research*, 4:32-35.

Rangland, G.J., Denlinger, D.L. and Hahn, D.A. 2010. Mechanisms of suspended amination are revealed by transcript profiling of diapause in the flesh fly. *Proceedings of the National Academy of Sciences,* 107:14909-1491. doi:10.1073/pnas.1007075107

Robich, R.M., Rinehart, J.P., Kitchen, L.J. and Denlinger, D.L. 2007. Diapause-specific gene expression in the northern house mosquito, *Culex pipens L.* identified by suppressive substrate hybridization. *Journal of Insect Physiology*, 53:235-245. doi:10.1016/j.jinsphys.2006.08.008

Sørensen, J.G., Nielsen, M.M., Kruhoffer, M., Justesen, J. and Loeschcke, V. 2005. Full genome gene expression analysis of the heat stress response, in *Drosophila melanogaster*. *Cell Stress and Chaperones,* 10(4):312-328. doi:10.1379/csc-128r1.1

Teets, N.M., Kawarasaki, Y., Richard, E., Lee Jr., and Denlinger, D.L. 2013. Expression of genes involved in energy mobilization and osmoprotectant synthesis during thermal and dehydration stress in the Antarctic midge, *Belgica Antarctica*. *Journal of Comparative Physiology B,* 183:189-201. doi:10.1007/s00360-012-0707-2

Xu, W.H., Lu, Y.X. and Denlinger, D.L. 2012. Cross-talk between the fat body and brain regulates insect developmental arrest. *Proceedings of the National Academy of Sciences of the United States of America*, 109:14687-14692. doi:10.1073/pnas.121287910.

Yocum, G.D., Kemp, W.P., Bosch, J. and Knoblett, J.N. 2005. Temporal variation in overwintering gene expression and respiration in the solitary bee *Megachile rotundata*. *Journal of Insect Physiology,* 51:621-629. doi:10.1016/j.jinsphys.2004.11.008

Yocum, G.D., Rinehart, J.P., Chirumamilla-Chapara, A. and Larson, M.L. 2009. Characterization of gene expression patterns during the initiation and maintenance phases of diapause in the Colorado potato beetle, *Leptinotarsa decemlineata*. *Journal of Insect Physiology,* 55:32-39. doi:10.1016/j.jinsphys.2008.10.003

Zhang Q., Yu-Xuan Lu and Wei-Hua Xu. 2013. Proteomic and metabolomic profiles of larval hemolymph associated with diapause in the cotton bollworm, *Helicoverpa armigera*. *BMC Genomics,* 14:751. doi:10.1186/1471-2164-14-751

Zoellner, N. and Kirsch, K. 1962. Colourimetric method for determination of total lipids. *Journal of Experimental Medicine,* 135:545-550. doi:10.1007/BF02045455