throughout development, while the damaged spikelet becomes white and dry. The above damage, perhaps occurs because of the toughening of lemma and palea.

Most striking and characteristic third type of damage observed at CRRI farm was the oozing of milk over the grain without any external feeding marks. The systematic examination of the affected grains in different stages of damage revealed the injury caused to the ovary just after fertilization. Some layers of the kernel were damaged by the larvae resulting in the gradual rupturing of the membrane. Soon afterwards the milk flows out through the tip and dries up over the hull (figure 1). There was considerable degree of variation in the extent of damage to individual grains and ranges from a completely chaffy grain with white coating to an almost normal grain. Invariably, in all the damaged spikelets, the tip was slightly open through which the larvae make their exit in search of a tender spikelet. The affected panicles become erect due to the dropping of damaged grains in contrast to a normal panicle. This kind of damage was observed in varieties that flowered in the first fortnight of March. In Zhong Hua 1, a very short duration (80 days to flowering) *japonica* variety nearly 60% of the grains were lost due to this type of damage by the pest. In varities that flowered in April only first and second types of damage were recorded. Another interesting observation was that the varieties with non-synchronous flowering were more susceptible to this pest.

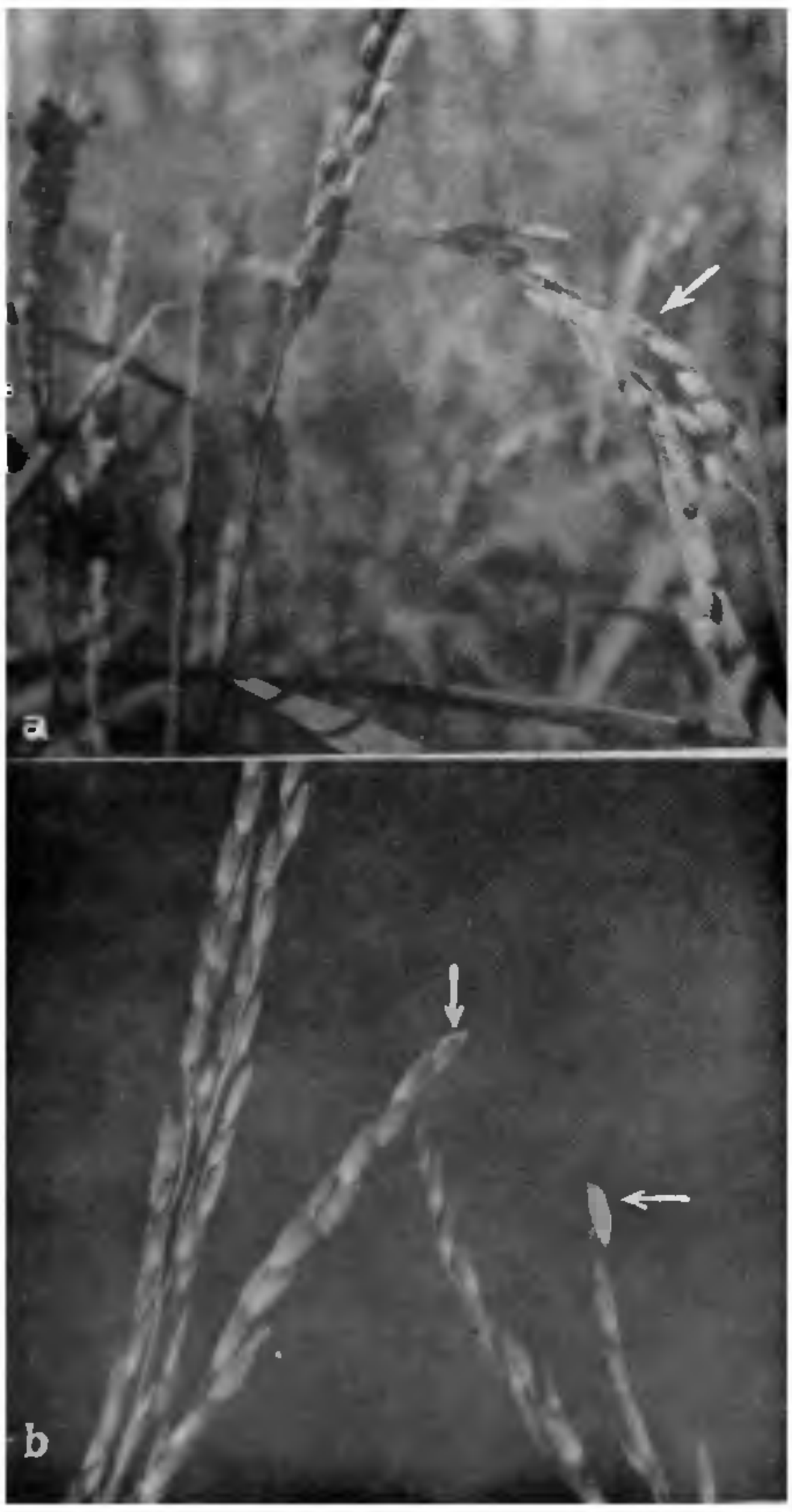

Figure 1. a–b Grains showing damage caused by thrips.

Weather during the months of February and March was generally overcast sky with 8.2 and 7.7 average sunshine hours and 94.5 mm and 90.2 mm total rainfall respectively. This is the first report about the occurrence of *H. ganglbaueri* Schmutz on paddy from Orissa and Eastern India.

Identification of the specimens by Dr. T. N. Ananthakrishnan is gratefully acknowledged. We thank Dr. H. K. Pande, Director, CRRI for facilities and Dr. Y. S. Rao, Head, Entomology Division for encouragement.

7 August 1982

1. Ananthakrishnan, T. N., *Thrips: Biology and Control*, MacMillan, India, 1973, p. 109.
2. Chaudhary, J. P. and Ramzan, M., *J. Res. Punjab Agr. Univ.*, 1971, **8**, 214
3. Abraham, C. C., Thomas, B., Karunakaran, K. and Gopalakrishnan, R., *Curr. Sci.*, 1972, **41**, 721.

VARIATION OF PROTEIN AND NUCLEIC ACID DURING LARVAL DEVELOPMENT OF *CHIRONOMUS BARBATITARSIS* IN NORMAL AND APOSYMBIOTIC CONDITIONS

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CORRELATION of different biochemical parameters with various ontogenetic events in insects has been

receiving increasing attention[1-3]. Protein and nucleic acid deserve special attention among other nutrients, since they constitute the raw materials of enzymes and hormones which largely regulate the ontogenetic events[4,5]. Role of symbiotic bacteria to provide essential nutrients to the host is well established[6,7]. Since the last few decades *Chironomus* has been widely used in genetical research but biochemical studies of the post-embryonic development are relatively meagre. The present investigation was undertaken to report the variation of protein and nucleic acid during larval development in normal and aposymbiotic conditions.

Experiments were conducted with a laboratory cultured stock of *C. barbatitarsis* maintained at 20±1°C[8]. Duration of four larval instars were 4, 4, 5 and 12 days respectively. Larval instars were identified as early, mid and late periods on the basis of chronological age. Larvae were rendered aposymbiotic with the application of terramycin at the doses of 5%, 10% and 15% (v/v) with the culture media. Aposymbiosis was also caused by treating the freshly laid eggs with 1% aqueous solution of Na-hypochlorite.

Due to minute size of the first instar larvae, biochemical estimations were carried out from second instar onwards. Protein was estimated following the method of Lowry *et al*[9]. For the estimation of nucleic acid, extraction was done with 0.2 M perchloric acid and from the aliquot obtained by following the method of Cherry[10], the DNA was estimated using diphenylamine as the colour reagent[11] and the RNA was determined using orcinol as the colour reagent[12] The extent of aposymbiosis was determined by counting the number of bacteria/ larva following the formula of Noda[13]

Protein and nucleic acid content showed highest protein level (7.4 to 14.4 mg/100 mg wt body wt.) followed by RNA (6.3 to 10.0 mg/100 mg wet body wt.) and DNA (5.2 to 9.2 mg/100 mg wet body wt.) during the respective larval periods. Protein and RNA content exhibited gradual increase (with intermittent decline during each moult) throughout the larval periods while DNA showed maximum content around mid instars (table 1). Aposymbiotic insects showed 88.3 to 93.7% reduction in the number of bacteria in the whole body. Of the different doses of terramycin, 10% dose was found to be most effective exhibiting 17.4 to 35.1%, 8.2 to 18.8% and 4.8 to 13.0% depletion of protein, DNA and RNA levels respectively. Egg treatment with 1% Na-hypochlorite depicted 26.8% to 35.6%, 13.0 to 19.0% and 11.3 to 13.3% reduced levels of protein, DNA and RNA respectively. All these variations were recorded during mid periods of respective larval instars (table 2). Duration of respective instars in treated larvae was much prolonged and the rate of pupation was drastically reduced.

TABLE 1

Protein, DNA and RNA contents (mg/100 mg wet body wt.) during early, mid and late larval instars of Chironomus barbatitarsis. Data are mean ±SE of 7 replications

Contents	Larval instars	Moulting cycle periods: Early	Mid	Late
Protein	II	7.1±0.2	7.6±0.5	8.7±0.7
	III	9.7±0.1	10.9±0.5	13.1±0.3
	IV	10.8±0.6	12.4±0.8	14.4±0.4
DNA	II	5.2±0.4	6.6±0.7	6.1±0.4
	III	6.0±0.7	8.8±0.5	6.4±0.6
	IV	6.2±0.9	9.2±0.8	7.7±0.4
RNA	II	6.3±0.4	7.0±0.6	7.8±0.7
	III	6.3±0.7	7.3±0.8	8.1±0.6
	IV	7.2±0.8	9.2±0.6	10.0±0.8

Gradual increasing protein and RNA contents and the maximum level of DNA around mid instars of the respective moulting cycles corroborate the previous findings[14,15]. Such pattern of variations revealed their developmental profiles in relation to diffeent morphogenetic events. The increasing trend of protein and RNA appeared higher in post mid-instar periods in respect of pre-mid instar periods (table 1) which is supposed to be related with the developmental dynamics of various tissues throughout the moulting cycles. It has previously been noted that in *C. barbatitarsis* the rate of dry weight increase was maximum upto mid-instars[8]. During early periods of respective instars increased rate of food consumption caused an influx of protein from dietary source but it was suggested to be largely used to meet the demand for growth and development which is evident from the moderate increase of the contents in early periods. In post-mid-instars it is suggested that active conversion of sugar and lipid into protein consequently raised the levels[1,2]. Moreover it is also reported that in post-mid-instar periods high ecdysone level accelerates protein and nucleic acid synthesis rates[16]. Towards the end of an instar, extra protein synthesis occurs to meet protein requirement for moulting[17] which is reflected in the high protein and RNA levels in late instars. The declining level of DNA in post-mid-instars is presumed to be due to an antagonistic relationship of DNA with protein and RNA levels since it is reported that once protein and RNA levels tend to attain higher levels they block DNA synthesis[18]

TABLE 2

The number of bacteria (BN, Data×10^7 larva), protein, DNA and RNA contents (mg/100 mg wet body wt) during third and fourth larval instars (mid) of C. barbatitarsis in control and aposymbiotic conditions. Data are mean ±SE of 6 replications

		Third Instar				Fourth Instar			
		BN	Prot.	DNA	RNA	BN	Prot.	DNA	RNA
Control		65 ±1.8	10.9 ±0.5	8.8 ±0.7	7.3 ±0.8	71 ±1.6	12.4 ±0.8	9.2 ±0.3	9.2 ±0.5
	5%	7.5[b] ±0.1	8.2[a] ±0.4	7.6 ±0.8	6.9 ±0.5	8.3[b] ±0.4	10.3[a] ±0.9	8.5 ±0.4	8.3 ±0.6
Terramycin treated	10%	6.1[b] ±0.2	7.1[b] ±0.6	7.2[a] ±0.7	6.6[a] ±0.4	7.4[b] ±0.3	9.2[b] ±0.7	8.0[a] ±0.6	8.0[a] ±0.4
	15%	4.1[b] ±0.2	7.1[b] ±0.4	7.2[a] ±0.6	6.7 ±0.6	5.4[b] ±0.3	9.7[b] ±0.8	8.1[a] ±0.4	8.0 ±0.5
Egg treated		5.1[b] ±0.3	7.0[b] ±0.5	7.1[a] ±0.5	6.4[a] ±0.4	7.4[b] ±0.6	9.1[b] ±0.7	8.1[a] ±0.4	7.9[a] ±0.8

[a] and [b] indicate $P<0.05$ and $P<0.01$ respectively.

Decreased level of protein in aposymbiotic larvae often supports the previous report that the symbiotic bacteria supply specific polypeptide to the host[6]. Symbionts' role in the synthesis of nucleic acid is assumed to be an indirect one, they are known to provide folic acid to the host which is the precursor of both purines and pyrimidines[19,20]. Thus decrease of both DNA and RNA might be attributed to the decreased supply of folic acid to the host. Delayed growth rates and failure of pupation of the treated larvae might be due to hormonal imbalance, because the treated larvae could not completely utilize cholesterol[8,21] which serves as the raw material for ecdysone synthesis; it might also be indirectly attributed to the lack of B-vitamins and some important amino acids in aposymbiotic insects[6,7,22].

Financial support by the authorities of CSIR (New Delhi) is gratefully acknowledged.

30 August 1982; Revised 20 November 1982

1. Gilbert, L. I. and Schneiderman, H. A., *Am. Zool.*, 1961, **1**, 11.
2. Agrell, I., *The Physiology of Insecta*, (ed.) M. Rockstein, Academic Press, New York, 1964, p. 91.
3. Lawrence, P. A., *Adv. Insect Physiol.*, 1970, **7**, 197.
4. Chen, P. S., *Adv. Insect Physiol.*, 1966, **3**, 53.
5. Calvez, B. and Fourche, J., *Develop. Growth and Differ.*, 1980, **22**, 93.
6. Brooks, M. A. and Kringen, W. B., *Insect & Mite Nutrition*, (North Holland, Amsterdam), 1972.
7. Brown, J. J. and Chippendale, G. M., *Comp. Biochem. Physiol.*, 1975, **A50**, 83.
8. Islam, A. and Roy, S., *Proc. Indian Nat. Sci. Acad.*, 1982, (In press).
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
10. Cherry, J. H., *Plant Physiol.*, 1962, **37**, 670.
11. Burton, K., *Biochem. J.*, 1956, **62**, 315.
12. Ceriotti, G., *J. Biol. Chem.*, 1955, **214**, 59.
13. Noda, H., *Appl. Ent. Zool.*, 1974, **9**, 275.
14. McDowell, R. E., Feir, D. and Ecker, R. E., *Insect Biochem.*, 1974, **4**, 295.
15. Srivastava, P. H., Auclair, J. L. and Srivastava, U., *Insect Biochem.*, 1980, **10**, 209.
16. Krishnakumaran, A., Oberlander, H. and Schneiderman, H. A., *Nature*, 1965, **205**, 1131.
17. Firling, C. E., *J. Insect Physiol.*, 1977, **23**, 17.
18. Sekeris, G. E., *Mechanism of Hormone Action*, Academic Press, New York, 1965, p. 149.
19. Nogge, G., *Experientia*, 1976, **32**, 995.
20. Pell, P. E. and Southern, D. I., *Microbes Letters*, 1976, **2**, 203.
21. Islam, A. and Roy, S., *Sci. Cult.*, 1982, **48**, 255.
22. Islam, A. and Roy, S., *Sci. Cult.*, 1982, **488**, 148.