3. MATERIALS AND METHODS

Experimental insects:

Following races/lines of *Bombyx mori* were considered in the experiment.

i) Multivoltine - Race Nistari, indigenous to West Bengal.

ii) Bivoltine - Pure line of KPGB and an evolved hybrid of KPGB X P_5 popular in West Bengal.

Disease free layings were procured from the Central Sericultural Research and Training Institute, Berhampur, West Bengal.

Selection of instar :

For experiment on any single day food deprivation, during fifth instar, fifth instar larvae of different race/lines were considered. For treatment with imidazole compounds fourth instar larvae of KPGB X P_5 was considered.

Seasons :

Experiments on any single day food deprivation during fifth instar on race Nistari; KPGB and KPGB X P_5 were undertaken during both dry part of summer (March to first week of June) and the wet part of summer (second week of June to first week of October). Experiment on the induction of trimoulters in bivoltine silkworm larvae, using imidazoles were undertaken during the wet part of summer only (August-September) for utilization of bivoltine male components for the production of polyvoltine X bivoltine hybrid for commercial use.

Rearing Environments :

		Max	Min
Dry part of summer	Temperature(0°C)	32	25
	Humidity (%)	70	50
Wet part of summer	Temperature (°C)	33	24
	Humidity (%)	99	80

Mulberry plants as food :

All the experiments were carried out on the S_1 variety of mulberry.

Design of the experiments :

(a) Rearing of fifth instar larvae of *Bombyx mori* for any single day food deprivation with and without treatment of methoprene

All rearings of silkworm were done following Krishnaswami (1986).Disease free layings were reared in mass upto fourth moult. After resumption from moult the newly ecdysed fifth instar larvae were carefully separated into batches of randomly selected 100 worms. Each of the batches were replicated suitably. Nistari being the well adopted race, it was replicated thrice. Pure bivoltine, KPGB, was replicated five times and bivoltine hubrid, KPGB X P_5, four times for their susceptibility to adverse climate. The larvae of the batches subjected to starvation were regarded as treatments. Because of variation in the life span of fifth stage the days of starvation (number of treatments) were different in multivoltine and bivoltine larvae and also in two different seasons of rearing. Each of the treatments

with replication were different in multivoltine and bivoltine larvae and also in two different seasons of rearing. Each of the treatments with replication were duplicated so that one of the batches can be treated with the JHa, methoprene. A control batch, each with and without methoprene treatment was maintained to compare with the results of the treatments.

Each treatment with and without methoprene were subjected to daywise starvation. Except the day of food deprivation the larvae were allowed to take food *ad libitum* on other days of fifth instar life (Table-1). The larvae subjected to starvation without treatment of methoprene were marked with T and larvae subjected to starvation with treatment of methoprene were designated as TM. Control larvae of both the batches, with and without methoprene treatment, were allowed to feed *ad libitum* without starvation. On maturity larvae of all the treatment were transferred to bamboo made spinning tray for the purpose of spinning. On fifth day of spinning the cocoons were harvested, pupa sexed (by cutting the cocoon case with blade) and subjected to different assessments.

(b)Dose of JHa

The JHa, methoprene was obtained from Otsuka Chemical Co. , Tokyo, Japan for experimental use. 0.3125 µg of methoprene in 2 µl of acetone solution was applied topically to each larva of 48-hrs-old fifth stage. This dose of 0.3125 µg per larva during fifth instar was found to be most effective for promoting silk production in West Bengal condition (Rao *et al.*, 1988). The age of the larvae for the treatment with JHa was selected through experimental trials. It was found that 48-hr-old fifth instar larvae have given the best performance during the seasons of rearing even afcter starvation on different days.

(c) Rearing of imidazole treated bivoltine

Rearing of bivoltine hybrid, KPGB X P_5 , was dose during the wet part of summer (September) only. Disease free layings of silkworm were reared in mass culture upto the third moult. Freshly moulted fourth instar larvae were separated in batches and subjected to following experiments.

i) Determination of optimum doses of different imidazoles

Larvae in batches of 20 each were replicated five times. Different dosages of KK-42, KK-110 and KK-22 were applied topically to the 0-hr-old fourth instar larvae with the help of a microliter-syringe. The compounds were diluated in acetone and 2 µl of acetone solution containing different concentrations of imidazoles for inducing precocious pupation were applied. A control batch treated with 2 ml of acetone/larva was also maintained for comparing the results. The experiment was conducted thrice to confirm the results and to determine the optimum dosage during the wet part of summer.

ii) Rearing of bivoltine through use of imidazoles

Larvae in batches of 100 each in four replications were topically treated in 2 µl acetone solution/larvae with different doses of different imidazole compounds to induce trimoulter. Rearing performances, consumption of food and its utilization and reproductive performances under different doses of the three imidazole compounds were assessed from the precocious cocoons.

iii) Rearing of multi X bi hybrid eggs

Rearing performances of different combinations of Nistari X KK-

42-treated induced bivoltine males, Nistari X KK-110 treated induced males, Nistari X KK-22 induced bivoltine males and Nistari X normal tetramoulter bivoltine males (control) were assessed through rearing of 20-disease free layings in five replications for each of the combinations.

Assessment of food consumption and utilization

a) Any single day starvation during the life of fifth instar larvae treated with or without methoprene.

For assessment of food consumption larvae were reared treatmentwise in batches of 25 larvae/batch. Each time measured food was provided to the larvae and uneaten leaves and litters were collected carefully during bed cleaning. The uneaten leaves and litters were dried at 60°C for 72 hrs. These dry products were then measured in high precision physical balance for assessment. Same procedure was followed for investigation in both the seasons.

b) Treatment with imidazole compounds

For assessment of food consumption by the imidazole treated bivoltine larvae of KPGB X P_5 , 50 larvae were maintained in four replications for each of the treatments. Otherwise the same procedure as in case of the starved larvae was observed for the assessment of food consumption and utilisation.

Maintenance of reserve pool

A reserve pool was maintained for each of the treatments in different seasons. Unhealthy and sick worms from main experimental lots/replications were replaced from this pool during the experiments. However, utmost

care was taken so that minimum replacement was required and accurate result could be obtained.

Estimation of economic parameters

Economic parameters such as, single mature larval weight single cocoon-shell weight, single pupal weight were taken on dry basis (drying the products at 60°C for 72 hrs.). Silkworm larvae being continuous feeder there is every possibility of retention of leaf residue in gut resulting in error (Barbehenn and Keddie, 1992). To avoid such error larvae after attaining maturity were kept for sometimes away from leaf to allow gut purging (defecation of last litter along with urine) and then was put in deep freeze for sometime and then put in oven for drying at 60°C. After drying mature larvae, cocoon shells and pupae were weighed in an electrical balance (Dhona 100-DS having accuracy built in weights $\pm$ 0.01 mg and the weighing range of 100 mg). For single larval weight, single cocoon shell weight and single pupal weight 25 samples were dried and average weights were taken from both males and females.

Estimation of reproductive parameters

Reproductive parameters considered were live female pupal weight, ovariole length, number of eggs in ovariole, fecundity, weight of eggs and hatching percentage. The observations were based on 20 individuals of each of the treatments, and then the average was calculated. After oviposition the bivoltine eggs were treated with hydrochloric acid (Sp. gr 1.0642 at 46°C) for 4 minutes to assess the hatching percentage (Biram Saheb *et al.*, 1990). The grainage procedure followed for both the experiments i.e, starvation on any single day of the fifth instar larvae and the larvae treated with imidazoles for the induction of trimoulter in tetramoulter bivoltine was that of Narasimahanna (1988).

Estimation of total nitrogen

Estimation of total nitrogen of leaves, litters, mature larvae, cocoon shells and female pupae was done by Kjheldhal method during both the seasons of investigation.

Calculation of results

Estimation of utilisation of food - Different authors have followed different methods for estimation of food utilized by silkworms. But most of the authors (Schroeder, 1976; Periswamy *et al.*, 1985 ; Paul *et al.*, 1992; Anantharaman *et al.*, 1993; Remadevi *et al.*, 1993) have referred the method of Waldbauer (1968). However, Biren *et al.*, (1987) and Tzenov (1993) have referred the IBP formula of Petrusewiez and Mac Fadyen (1970). Here the nutrition (mass) budget have been calculated using formula of Waldbauer (1968), IBP formula after Petrusewiez and Mac Fadyen (1970), Muthukrishnan and Pandian (1987) and Farrar *et al.*, (1989).

The estimations and calculations of nutritional indices and budgets are done on a dry weight basis, Units used are, weight in mg or g, time in days and efficiency in percentage.

Total consumption as represented by Petrusewiez and Mac Fadyen (1970) is $F = P+R+F_1 +U$, where P is production, R is energy lost as heat due to metabolism, F_1 is excreta and U is the nitrogenous excretory products. Due to very negligible quantity U has not been considered in estimation following Waldbauer (1968).

Hence, consumption has been calculated as Egestion(FU) + Production(P) + Respiration(R).

C = FU + P + R

Assimilation (A) = C - FU ; Production (C) = Gain in weight

Respiration (R) = A - P ; Maintenance Cost (MC) = R/P ;

Assimilation efficiency (AE) = A/C X 100

(AD by Waldbauer)

Absolute Consumption rate (ACR) = C/T ;

T=Total feeding duration (days) ;

Absolute Growth Rate (AGR) = P/T

Efficiency conversion of digested food (ECD) = P/(C-FU) X 100

P = Gain in larval weight, Cocoon shell weight , Pupal weight.

Reference ratio (R) = C/(C-FU).

Estimation of economic characters

a) Average cocoon shell weight is expressd in mg or gram

b) Average pupal weight is expressed in mg or gram

c) Average filament length expressed in meters

d) Average filament size expressed in denier.

Estimation of Reproductive performance

a) Average weight of eggs expressed in mg or gram

b) Average fecundity expressed in number

c) Average ovariole length expressed in centimeter

d) Average hatching of eggs expressed in percentage.

Estimation of total Nitrogen (by Kjheldhal)

Nitrogen estimated has been expressed in percent or mg.

Utilization of imidazole treated bivoltine males of KPGB X P_5 for preparation of Nistari X bivoltine hybrid eggs.

Induced trimoulter male components produced by treatment of imidazole compounds of KK-42, KK-110 and KK-22 were allowed to cross with Nistari females for the production of three way cross of Nistari X (KPGB X P_5). Rearing results were compared with those of control larvae for the following parameters : Fecundity, weight of 100 eggs, weight of 100 ants (newly hatched larvae), Hatching percentage, ERR percentage, single cocoon weight, single cocoon shell weight filament length.

Table 1 - Feeding deprivation scheme during fifth instar larval span.

Batches of fifth instar		Feeding deprivation Scheme during fifth instar larval span	Total larval period in control
No Methoprene	With Methoprene		
Control	Control	No food deprivation	
1T	1 TM	Starvation on day 1 only, and feeding on rest days.	KPGB (L.P. 8 days, wet part of summer)
2T	2 TM	Starvation on day 2 only, feeding on rest days.	KPGB X P_2 (L.P. 8 days, both the season)
3T	3 TM	Starvation on day 3 only, feeding on rest days.	KPGB (L.P. -7 days dry summer)
4T	4 TM	Starvation on day 4 only, feeding on rest days.	
5T	5 TM	Starvation on day 5 only, feeding on rest days.	
6T	6 TM	Starvation on day 6 only, feeding on rest days.	NISTARI (L.P. 6 days, both the seasons).
7T	7 TM	Starvation on day 7 only, feeding on rest days.	
8T	8 TM	Starvation on day 8 only, and feeding on rest days.	

L.P. - Larval period

Both seasons - Dry part of summer and wet part of summer.