4.MATERIALS AND METHODS

4.1. Recording of common insect herbivores and their natural enemies occurring seasonally on the shade tree:

Study areas were selected based on the information obtained from the G.B. Pant Institute of Himalayan Environment and Development and the Spices Board of India, Sikkim branch. The incidence of the insects on the shade tree, *Alnus nepalensis,* were recorded at the experimental plots of large cardamom agroforestry managed by the Spices Board of India (Sikkim branch), at Pangthang (2160m amsl) and Kabi (1630m amsl). Periodic surveys were done round the year at these two altitudes for three years i.e. 2000-2002, to record the insects associated with the tree foliage and branches of the lower accessible bough of the tree belonging to the age group of 2 to 10 years. Since, the sampling was done from the field that had large cardamom plantation, it may be assumed that all the relevant types of insects, i.e., whether true feeders or temporary visitors, have been included in the collection process. The insects were identified to their family to Recognizable Taxonomic Unit (RTU) levels.

Collection techniques like hand picking, beating and aspirating, were applied during survey after the methods prescribed in the handbook "Collection and Preservation Of Animals" (Anonymous, 1990). The eggs and larvae collected in the fields were brought to the laboratory and were reared on the host plant leaves to identify their adults. The adult specimens were brought in the polythene packets and containers and

poisoned (ethyl acetate poisoning) in killing bottles. Some insects suspected to be parasitised in nature were kept in the laboratory for emergence of the parasitoids. Identification of major folivores was confirmed by the Zoological Survey of India, Calcutta. Some of the insects causing maximum damage to the shade tree, *A. nepalensis* and those common to *A. nepalensis* and large cardamom both, were identified to their genus/ species level by the help of the expert authorities from the Spices Board of India (Sikkim Branch). Due to the difficulty in identifying a good number of insect associates of *A. nepalensis* to the species level, their study has been done using morphospecies characters to recognizable taxonomic unit (RTU).

4.2. Incidence and population dynamics of the two major folivores attacking the shade tree (*A. nepalensis*).

Although major work related to the present study, was carried out during the three years 2000-2002, some observations on the seasonal incidence of the major folivores, particularly of *G. chrysolopha*, were made towards the end of 1999.

For assessing the pest population, twenty trees were randomly selected and marked during each year i.e. 1999- 2000 to 2002 at two experimental sites, Kabi and Pangthang.

4.2.1. Lepidopteran folivore *Gazalina chrysolopha*: It was found that larvae of *G. chrysolopha,* climbed tall trees during the night to reach the canopy (foliage) for food (Plate 4). During the daytime they rested at the

Plate 4. Caterpillars of *Gazalina chrysolopha* climbing the tree *A. nepalensis* for food (foliage)

Plate 5. Caterpillars of *G. chrysolopha* in resting position

Plate 6. Experimental rearing jars

plate - 4

plate - 5

plate - 6

base of the tree. It was easier to take a count of the larvae at the tree base as they were sluggish and did not move. A quadrate of 10 cm^2 was designed and put on the resting larvae to take a physical count from each marked tree, adopting the method prescribed by Pedigo (1989). Larval count was recorded based on 10 samples from each tree at fortnight interval (life cycle being too long) during the period of occurrence of *G. chrysolopha* at both the experimental plots.

4.2.2. Coleopteran *Chrysomela chlorina*:

Per leaf population count of larvae of *C. chlorina* was taken from each selected and marked tree after Pedigo (1989) at an interval of one week (life cycle of the pest being comparatively shorter), during the period of their occurrence. From each tree, 10 leaves were randomly selected for population- count.

4.3. Influence of weather parameters on the population of the major folivores:

To study the influence of the abiotic factors such as maximum and minimum temperatures, low and high relative humidity, rainfall and cloud cover (day and night) on the pest population, the information on such parameters, was obtained from Agrometeorological Advisory Bulletin, jointly issued by The Indian Meteorological Department and The Department of Agriculture, Government of Sikkim (Year, 2000-2002). Correlation coefficients (using multiple regression) for the

relations between the herbivore population and the weather parameters were calculated.

4.4. Study of natural enemies of the major folivores:

To study the natural enemies (predators and parasitoids) associated with the major folivores *G. chrysolopha* and *C. chlorina,* a close observation was made on eggs, larvae and adults in the fields as well as in the laboratory. Eggs and larval stages of the folivores were maintained in the laboratory to observe the emergence of parasitoid or, their acceptability by a predator.

4.5. Life cycle studies of the two major folivores on *Alnus nepalensis:*

To study the life cycle of *G. chrysolopha* and *C. chlorina*, the adults of both were collected from the field and reared in the laboratory conditions. Pairs of male and female were kept in breeding jars (one pair in each jar of height 80 cm., dia. 65 cm.) to lay eggs. Newly emerged larvae were transferred to rearing jars (height 10 cm., dia. 6.5 cm.) (Plate 6) using moist brush and forceps. The lids of the containers were provided with only a few pores to minimize water loss from the food (leaves of *A. nepalensis*).

Duration of each larval and pupal stage was recorded. Size of the head capsule and the length and maximum breadth of each larva and pupa, were measured using stage occulometer. For other general observations in the field, hand lens was used. Freshly emerged pairs of male and female were kept for mating in breeding jars. Cotton balls moist

with 5% sugar solution, were provided in the breeding jars as nourishment for the adult moths of *G. chrysolopha* and fresh leaves of *A. nepalensis* were provided to the adults of *C. chlorina*. Premating, mating and preoviposition periods, fecundity and male and female longevity, were observed. The number of eggs laid per day by each female was recorded. The study was conducted using 10 replicates.

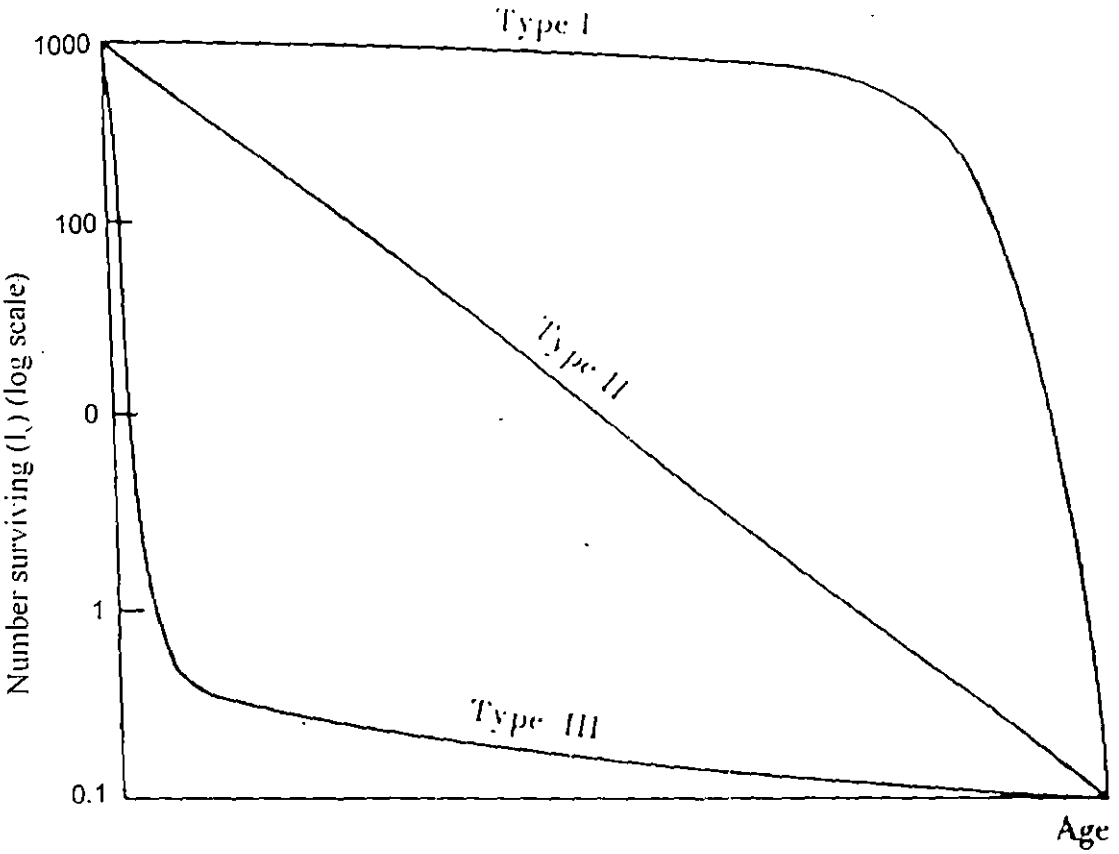
4.6. Survivorship of the major folivores:

Survivorship study was done under laboratory conditions. Rearing cages measuring 10 cm. height, 6.5 cm dia.,were used. Observations were made on the larvae of *G. chrysolopha* and *C. chlorina* at an interval of (age x) 96 hrs. (4 days) and 24 hrs. (1 day) respectively, till pupation. For each observation, 1000 individuals of newly hatched larvae, were reared on leaves of *A. nepalensis* separately in batches of 100 individuals each. The number of larvae surviving at the start of x age interval (l_x), number dying within that age interval (d_x), average number of individuals alive during that age interval (L_x), expectation of life for individuals of X age (e_x) and mortality rate for that age interval (q_x), were determined using formula prescribed by Das (1993). The observations on survivorship were compared using reference curves after Pearl (1928) (Fig 2).

4.7.Age distribution of *G. chrysolopha* and *C. chlorina*:

Using the information from the morphometric studies of the larva, it was possible to analyze the age distribution of the natural population of

Fig. 2



Hypothetical survivorship curves
(After Pearl 1928)

both the folivores in different months of their occurrence. To study this, the number of larvae present in each 10 cm^2 quadrate every fortnight for *G. chrysolopha* and on 10 leaves from each tree after every week for *C. chlorina,* were collected. The samples were then subjected to analysis to find out the average number of larvae present in each instar, using morphometric scale in the laboratory. Repeated sampling (10 replicates) was done to find out the changes in age distribution on selected trees throughout the period of their occurrence after Pedigo, 1996.

4.8. Study of nutritional ecology of the major folivores:

4.8.1. Feeding preference:

Feeding preference of the larvae of *G. chrysolopha* and *C. chlorina* was determined separately by providing them a choice among four types of leaves of varying maturity after Harrel *et al.* (1982). Leaf types ranged from tender, young, mature to senescent leaves. Feeding preference was observed using 10 final instar larvae. Each larva was allowed to feed on weighed quantity of leaf for 24 h. At the end of each feeding period, the left over leaf- weight was noted. This experiment was replicated 10 times and the pooled data was analyzed. Water content of each leaf type used in feeding choice, was determined.

4.8.2. Growth and feeding indices:

Since the early instar larvae of *G. chrysolopha* nibbled on debris/ very tender leaves (in laboratory) found at the base of the host tree, the experimental study on leaf- consumption and utilization was, conducted using the 3rd, 4th & 5th instar

larvae. For *C. chlorina*, the study was conducted on the 2nd, 3 rd & 4th instar larvae as the first instar larvae were too small to weigh and only nibbled the leaves. The insects were reared on host leaves of *A. nepalensis* in plastic containers (height 10 cm., dia. 6.5 cm) with perforated lids to facilitate ventilation. Discs of filter paper were used as towels at the bottom of the containers to facilitate cleaning and to absorb extra moisture. The experiment was repeated in three successive years with 20 replicates. Weighed quantities of host plant leaves were provided to the insects at every 24 hrs. interval throughout their larval development period. The weight of the residual leaf (left after feeding), initial and final weights of the insects and weight of the faecal pellets, were also recorded after every 24 hrs. Since the work was done on the fresh weight basis, a control leaf quantity of the same weight, was kept under the same conditions and weighed after every 24 hrs to calculate the water loss due to evaporation / transpiration. Low room temperature (8^0C to 18^0C) and high humidity (80% - 90%) within the rearing container, in case of lepidopteran *G. chrysolopha*, helped in keeping the leaves fresh with minimum moisture loss during the feeding experiment. In case of the coleopteran, *C. chlorina,* however, the petioles of the leaves were kept dipped in water. The feeding experiment in this case, was conducted at room temperature (18^0C to 25^0C) and humidity 75% - 85%. Weight of the larva after ecdysis and prior to consumption of food, was recorded as initial weight. Larval weight during spinning stage was

taken as the final weight after Waldbauer (1968). Difference in the initial and final weights of larvae in each instar, gave the value of weight gain during the instar.

Consumption and nutritional indices used in this study, were calculated on fresh weight basis after the methods suggested by Ananthakrishnan (1990), Singh and Sehgal (1993) and Shantibala *et al,* (2002). The formulae applied for calculating different consumption, utilization and growth indices were:

Consumption Index (CI) $= \dfrac{\text{Weight of food consumed}}{\text{Mean weight of larva x duration of feeding period (days)}}$

Approximate Digestibility (AD) $= \dfrac{\text{Weight of food Ingested} - \text{Weight of faeces}}{\text{Weight of food ingested}} \times 100$

Efficiency of conversion of ingested food (ECI) $= \dfrac{\text{Weight gained by the larva}}{\text{Weight of food ingested}} \times 100$

Efficiency of conversion of digested food (ECD) $= \dfrac{\text{Weight gained by the larva}}{\text{Weight of food digested}} \times 100$

Growth Rate (GR) $= \dfrac{\text{Weight gained by larva}}{\text{Mean larval weight x duration of feeding period (days)}}$

4.9. Evaluation of the negative and positive ecological role of these major folivores in the cardamom agroforestry.

4.9.1 Positive role:

To evaluate the possible contribution of the folivores in form of faecal urine as manure to the large cardamom agroforestry, soil analysis was done before appearance of the pest, and, thrice at the time of its maximum occurrence. Basic fertility components (N, P, K and organic carbon) of the soil (*in situ*) were analyzed. The same components were also determined of leaves provided as food to, as well as faecal pallets of, the folivores.

4.9.1.1 Analysis of leaves and faecal pellets:

The leaves and the faecal pallets obtained from the insects were first air dried at 60 °C for 48 hrs. Then separately, they were ground and sieved (.4 mm) for basic nutrient analysis. Phosphorus (P) was estimated by molybdophosphoric blue color method (Jackson, 1967). Estimation of Nitrogen (N) was done by modified Kjeldahl method (Anderson and Ingram, 1993). Potassium (K) content was determined using flame emission method (Allen, 1974). Organic carbon (C) was estimated by wet oxidation by chromic acid (by titration) method (Jackson, 1967).

4.9.1.2. Analysis of soil:

Soil samples from beneath the shade tree, were collected. The first sample (control sample) was collected before the pest appeared. Again at the time of maximum occurrence of the pest, soil samples (when soil was

naturally mixed with faecal droppings) from the adjoining spots under the tree were collected at a month's interval for three months. For each sampling, soil from 30 cm deep after Sharma *et al.* (2002) was taken. 5 replicates from 5 plots (1m x 1m) were sampled every time from Feb. to April for *G. chrysolopha* and July to Sept. for *C. chlorina*. After collection, the soil samples were taken to the laboratory and passed through 2mm sieve. After separating roots, debris and stones, the soil samples were dried at 60 °C for 48 hrs. Organic carbon was measured after partial oxidation with an acidified dichromate solution, adopting modified Walkley-Blach method (Anderson and Ingram, 1993). Estimation of N was done by modified Kjeldahl method (Anderson and Ingram, 1993). Estimation of P was done by modified ascorbic acid method (Anderson and Ingram, 1993). Potassium was analyzed using flame photometer and the estimation was done through a calibration curve using K standard (Allen, 1974).

4.9.2. Negative role:

During regular field visits it was found that of the two major folivores (*G. chrysolopha* and *C. chlorina)* the coleopteran, *Chrysomela chlorina* inflicted injuries to large cardamom foliage as alternate host. Therefore, to estimate the injury potential of *C. chlorina* to cardamom leaves, nutrition ecology of the folivores was studied, adopting the methods described earlier.

For a direct assessment of the extent of injury caused by both the major folivores, the leaf area consumed or injured (Pedigo, 1996) by the final instar larvae of both the insects, was calculated (*ex-situ*) in laboratory conditions by planimetric method. Fresh leaves of *A. nepalensis* were provided to each set of larvae of *G. chrysolopha* and *C. chlorina* after every 24hrs. Grubs of *C. chlorina* being small, 10 batches having 10 grubs in each batch, were used at a time for the leaf- area injury study. Photocopies of the leaves were made before and after feeding. The experiment was carried through a period of one-week with 10 replications and the food consumption (leaf area) was calculated based on the method suggested by Hendrix and Marquis (1983). The formula applied for calculating the consumption of leaf in terms of area was: Leaf area consumed = A /B x 100

Where, A = Total area of the leaf eaten

B = Total area of the leaf provided to the larvae

The thickness of the leaves and other circular structures had not been considered for simplicity of the calculation.

The estimation of injury in the field condition (*in-situ*) by direct or indirect method in the mountain terrain was a difficult preposition for lone worker (Ph. D candidate), the same could not be taken under scope of study.

4.10. Computer applications:

Studies on Life cycle, fecundity, age distribution, population dynamics, nutritional indices, feeding preference and morphometrics were

subjected to statistical treatments as per requirements which included mean, standard deviation, student's t-test, LSD, ANOVA and multiple regression. SYSTAT and SAS packages in computer were used for computation and Excel was used for graphics.

5. Study area:

Experimental sites selected for the study were located at Pangthang 2160m amsl (East District) and Kabi at 1630m amsl (North District) in Sikkim state in the Eastern Himalayas. These sites are about 70 Km apart, which allowed comparison of field observations at the two sites located at different altitudes. These are major areas of large cardamom cultivation with *A. nepalensis* as shade tree (Fig.1). The study area was covered by monsoon with a temperate climate having winter (November- February), spring (March- May) and rainy (June-October) seasons. The mean monthly maximum and minimum temperature ranged from 23.3-14.3 ^{0}C in spring and rainy seasons and to 15.8-1.4^{0}C during winter. Total rainfall varied with the season. During monsoon it was maximum, with a total of 3000-4500 mm per year at the study site. During rainy season, relative humidity varied between 80-95%, which decreases to about 55% in spring.