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METAMORPHSIS OF *PRINCEPS DEMOLEUS* (L.) ON *CITRUS TRIFOLIATA* (L.) (FAMILY: RUTACEAE)

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ABSTRACT
The papilionidae butterfly *Princeps demoleus* (L.) (lime butterfly) it occurs throughout the year. The Metamorphosis of *Princeps demoleus* was studied at Rani Chandra Mani Devi bio-diversity Park, china waltair, Visakhapatnam using the leaves of *Citrus trifoliata* as the larval host both in laboratory and in the natural conditions. The behavior and morphological characters of eggs, caterpillars, pupae and adult emergence were observed in the laboratory at 28°-30°c. The life cycle was completed in 31-32 days, with egg hatching 3-4 larvae 17-19, and pupae 10-11 days. The values of consumption index (CI), growth rate (GR), and approximate digestibility (AD) across the instars decreased as the larvae aged. The average values of the CI and GR are 3.86, 0.40 respectively, and that of AD is 81.78. But the values of both efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI) either increased or decreased from instar to instar.

KEYWORDS: Metamorphsis, Instars, Food utilization indices, Growth rate consumption index, Consumption Index, approximate digestibility.

INRODUCTION
Among the insects, the lovely and graceful butterflies provide economic and ecological benefits to the human society. Having multihued colours on their wings, they enhance the earth's beauty incontestably and add immense aesthetic value to the ambient environment. They accomplish pollination, a keystone ecological process in natural sustainability throughout the world. Being dependent on vegetation both as adults and larvae, they involve themselves in complex feeding relationships with green plants. As adults they require a succession of adequate nectar resources. Nectar provides energy for flight, which is vital to find mates and to disperse the species. As larvae, butterflies are typically host specific and often show a "botanical instinct", in that closely related butterfly species choose closely related plants.

If the requirements of the butterfly species in the wild are thoroughly understood, it is possible to conserve them in captivity or wild. A suitable habitat for butterflies should include mating site(s) for the adults, nectar sources for adults and larval food plants for oviposition. As butterflies are holo-metabolous with distinct developmental stages as egg-larva-pupa-adult, their reproductive output is dependent on the combined effect of larvae-derived and adult-derived nutrients or energy. These findings require a study of adult nectar resources, larval food plants and food consumption and utilization by the larvae. The needs for conservation of insects are increasing with each year, and the butterflies are considered to be the important flag ships for insect conservation (New *et al.*, 1995; Smetacek, 1996). The rapid, bounding flight of these butterflies are given them their name (Kehimkar, 2008). But most butterfly ecologists and conservationists express that the existing knowledge of the exact requirements of butterflies in India is woefully inadequate, and the life history of 70% of Indian butterflies is still unknown. Therefore, efforts are being made in the Rani Chandra Mani Devi bio-diversity park to study the biology, ecology and conservation of butterfly fauna available at Visakhapatnam and its surroundings.

The present study relates to the Lime Butterfly - *Princeps demoleus* (L.) (Lepidoptera: Papilionidae) and describes its adult food resources, Oviposition and larval host plant on Citrus trifoliata (L.) egg deposition pattern, life history including the duration of early life history stages.

MATERIAL AND METHODS
Study Region& Site: The present study was carried out at Visakhapatnam during 2011 and 2012. Visakhapatnam (17o 42' N latitude and 83 o 20' E longitude) is located on the east coast of India in the State of Andhra Pradesh. The present study was conducted at Rani Chandra Mani Devi bio-diversity Park which spreads over an area of 5 acres and is in proximity to the coastline. The wilderness

on Rani Chandra Mani Devi bio-diversity Park was searched for the reproductive activity of the butterflies. A large number of tree, shrubs, herbs and medicinal plant species occur on Park. Some of the plants occurring on the park are heavily foraged by adult butterflies for nectar. Many herbaceous taxa serve as the larval hosts for the butterfly species distributed on the park.

Field Study: Regular walks in different patches of the study site were undertaken at 10-day intervals or when required even at shorter intervals or even daily. Butterflies are day active mostly during 0830 – 1500 h. Hence walks were made during these hours of the day to record the flight behaviour, foraging and ovipositing activity of the *Princeps demoleus* chosen for the present study. These activities were observed during the entire period of adults on wing. Nectar resource plants and Oviposition plants used by this species were recorded.

Laboratory Study

Life History Study: After noting the period of breeding season and the Oviposition plants close and prolonged observations were made of the breeding females laying eggs on its host plants. Freshly laid eggs were spotted, and the plant material (leaves/twigs) on which they were laid, was plucked without causing any damage. The date and time of day of collection were noted. Then the material was transferred to Petri dishes of 10 cm diameter and 1.5 cm depth. The inside of each of these Petri dishes was lined with moist blotting paper to provide moist conditions. They were brought to the Department and incubated in the laboratory having a temperature of 28 ± 2°C and relative humidity of 80 ± 10%. The Petri plates were kept in a clean, roomy cage (60 x 50 x 30 cm) covered with wire gauge. The light condition was the normal indirect sunlight, but its duration was not uniform throughout the year. It varied from a low of about 11 h during November – February (winter months) to a high of about 13 h during May – June.

The eggs thus incubated in the laboratory were examined at 6-hour intervals daily for recording their incubation period and hatchability. The eggs were treated as hatched when the larvae came out from them.

In order to assess the total larval period and the number of instars that may be produced, the newly hatched larvae were transferred individually into the Petri dishes with the help of a camel hairbrush. Fresh young leaves were offered as food to the growing larvae. Moulting was noted and thus the number of instars produced was recorded. Observations as color, shape and size of each instar was maintained. As the larvae grew, they needed more space. Increased space was provided by transferring the growing larvae to bigger Petri dishes (15 cm diameter; 2.5 cm depth).

The full grown larvae pupate and particulars of pupae including color, shape, size, weight and duration were also recorded. Millimeter graph paper was used for taking measurements. Taking the number of eggs studied, their developmental success (%) was calculated. In describing the details of adult characters, the butterflies that have emerged from the pupae in the laboratory, and those caught in the wild were used.

Food Consumption and Utilisation: The larvae represent the main feeding stage. Quantitative data of food consumption and utilization were recorded for each instar of the butterfly species under study using the gravimetric method of.[11] The larvae and the host leaves were weighed separately and then placed in Petri dishes. The larvae were allowed to feed on the leaves for 24 h and then the weights of the larvae and the remaining leaf material, and faecal matter in the Petri dish were determined. Fresh food was supplied and the related weights were also taken every 24 h. From these fresh weight measurements, growth and food utilization indices were calculated. These indices included Consumption Index (CI), Growth Rate (GR), Approximate Digestibility (also called Assimilation Efficiency) (AD), Efficiency of Conversion of Ingested Food (also called Gross Conversion Efficiency) (ECI), and Efficiency of Conversion of Digested Food (also called Net Conversion Efficiency) (ECD). The formulae of[11] used in the calculation of these indices are:

$$\text{CI (Consumption index)} = \frac{\text{Weight of food consumed}}{\text{Weight of instar x Number of feeding days}}$$

$$\text{GR (Growth rate)} = \frac{\text{Weight gained by the instar}}{\text{Mean weight of instar x Number of feeding days}}$$

$$\text{AD (Approximate digestibility)} = \frac{\text{Weight of food ingested} - \text{Weight of faeces}}{\text{Weight of food ingested}} \times 100$$

$$\text{ECD (Net conversion efficiency)} = \frac{\text{Weight gained by the instar}}{\text{Weight of food consumed} - \text{Weight of faeces}} \times 100$$

$$\text{ECI (Gross conversion efficiency)} = \frac{\text{Weight gained by the instar}}{\text{Weight of food ingested}} \times 100$$

The weights are expressed in units of milligrams (mg). The values are based on five different observations for each parameter; standard deviations were also calculated.

Statistical Analysis: The relation between the food consumed and the weight gained per instar by the larvae of each of the butterfly species under study was statistically analysed on the basis of Legenders principle by fitting a straight line. Larval weights are represented on Y- axis and the food consumed on X-axis. Correlation coefficient was calculated in each case along with‗t‘value.

RESULTS

ADULT STAGE (Plate.B**)**

Field Characters: Wingspan ranges between 85 - 100 mm. Both sexes black with bright green spots and streaks on wings. Hind wing tails short and stumpy. Underside pale brown with black, suffused with lilac or violet-grey. Green spots and markings less distinct. Two red spots near base each edged with a black crescent and a small red tornal spot on Underside hind wing. Male has scent wool in dorsal fold on Upper side Hind wing. Basal stripes and discal, cells and marginal spots, all bright green. Tail longer in female. Male with scent wool in dorsal fold.

Habit: Common in well wooded regions with good rainfall. Restless flier, flits from flower to flower. In behavious it resembles sarpedon but does not ascend the hills to such a height and is very seldom seen drinking from damp patches. Females are more likely to be caught when looking for their foodstuff.

Food Resources: In the study area its nectar host plants included *Antigonon leptopus* Hk. & A., *Lantana camara* Linn. *Santalum album* Linn. and *Cestrum diurnum* Linn. It held its wings horizontal while foraging at flowers for nectar.

Oviposition Host Plants: The plant used for ovipositing by this butterfly in the study area was *Citrus trifoliate* (L.). The larval host plants of the butterfly belong to the family Rutaceae. The recorded Oviposition host plants of this butterfly including, Aegle marmelos, Atlantia racemosa, Citrus sinensis, C. aurantifolia, C. grandis, C. limon, C. reticulate, Glycosmis pentaphylla, Murryya koenigii, M. paniculata, Ruta graveolens, triphasis trifolia. The present study was carried out on the leaves of *Citrus trifoliate* (L.).

***Citrus trifoliata* (L.) (Rutaceae) (plate.A)**

The plant recognizable by the large 3–5 cm (1.2–2.0 in) thorns on the shoots, and its deciduous leaves with three (or rarely, five) leaflets, typically with the middle leaflet 3–5 cm (1.2–2.0 in) long, and the two side leaflets 2–3 cm (0.79–1.18 in) long. The flowers are white, with pink stamens, 3–5 cm (1.2–2.0 in) in diameter, larger than those of true citrus but otherwise closely resembling them, except that the scent is much less pronounced than with true citrus. As with true citrus, the leaves give off a spicy smell when crushed.

The fruits are green, ripening to yellow, and 3–4 cm (1.2–1.6 in) in diameter, resembling a small orange, but with a finely downy surface.

Biological Observations recorded (Table.1).

EGG STAGE (Plate.C&D): Eggs of the lime butterfly are laid singly on young stems, the petiole are the underside of leaves of the *Citrus trifoliata.* The egg is pale creamy yellow with a finely roughened surface. It laid eggs singly, but on different leaves, mostly during 0830 – 1200h. The egg measured 1.00-1.20 (1.06±0.08) mm in length and 1.80–1.90(1.86±0.05)mm in width. They hatched in 3-4 (3.20±0.44) days of incubation. The larva immediately after emerging consumed as its egg shell. It passed through five instars over a period of 31-33 (31.60 ± 0.89) days.

Larval Stage on *Citrus trifoliata* (Plate.E, F, G, H &I**)**

Instar I: The young caterpillar eats, its way out of the mature egg, and then proceeds to finish up the rest of the egg cell. This stage lasted for 2 days. On the first day the larva was 3.00-3.50 (3.40 ± 0.22 mm in length. And 1.00 - 1.20 (1.10 ± 0.07) mm wide. The body was yellowish brown dorsally and darker brown laterally. The whitish saddle patch on the 3rd -4th abdominal segments, become more evident. The head was dark brown in colour, 0.90-1.00 (0.98±0.04) mm wide.

Instar II: This stage lasted for 2 days. The larva measured 5.20 – 5.50 (5.32 ± 0.16) mm in length and 1.30- 1.80 (1.52 ± 0.21) mm in width. The 2nd instar caterpillar is similar appearance to the late 1st instar caterpillar, except for the more prominent whitish saddle. As the caterpillar grows, small whitish patches appear laterally on the first two thoracic segments. Head size 2mm.

Instar III: This stage lasted for 3-5 days. The larvae attained a length of 10.30 – 14.20 (11.74 ± 1.23) mm and a width of 2.20 – 2.70 (2.40 ± 0.23) mm. The 3rd instar caterpillar has whitish lateral patches on the anterior and posterior body segments. The white saddle is more extensive and prominent than in the last two previous instars. Head measured 2.90-3.20 (3.08 ± 0.10) mm in size.

Instar IV: This stage lasted for 3 days. The larva reached a length of 15.00 – 25.60 (19.90 ± 4.31) mm and a width of 2.60– 4.50 (3.38 ± 0.93) mm. The 4th instar caterpillar resembles the late 3rd instar caterpillar. Initially but with amore slimy appearance and ground colour which is in darker brown. Head was 3.90-4.20 (4.02 ± 0.10) mm in size.

Instar V: This stage lasted for 6-8 days. The full grown larva measured 38.00 – 44.00(40.44± 2.36) mm in length and 5.10 – 7.00 (7.76 ± 1.13) mm in width. The 5th instar was with a drastic change in appearance. There are two lateral eye spots near the leading edge of the 3rd thoracic segments with a milky brown transverse band linking them. Another transverse band can be found at the posterior edge of the same body segment. A long oblique bar, dark brown in colour, stretches from the base of the 4th abdominal segment to the dorsum of the 5th abdominal Segment. A much shorter bar can be found in the 6th abdominal segment. Round dorso – lateral marks, variable in size can be found on the 6th to the 8th abdominal segments. After the moult to 5th instar, the body ground colour is initially mottled yellowish green,

but this change was gradually to a uniform green are yellow green. Head was measured in 5.10-5.30(5.20 ± 0.08) mm.

PUPAL STAGE (plate. I**)**

Prepupa: It lasted for a day. The larva stopped feeding and its body became short and thickened. It was attached to the substratum by its posterior end.

Pupa: This stage lasted 10-11 days. It was 27.00-30.00 (28.80 ± 1.09) mm in length and 8.00 – 9.00 (8.50 ± 0.35) mm in width at its broadest end. Pupation takes place a day later. The pupa suspends itself with a silk girdle from the substrate. There are two color forms. In the green form, the pupa is mainly green segments. In the brown from, the pupa is mainly grayish brown with dark patches. Each pupa has a pair of cephalic horns, a dorsal thoracic hump and is angled in side view. After 9 days of development, the pupa turns black as the development within the pupal case comes to an end. The yellowish spots and band on the forewing becomes visible through the pupal case. Its weight was about 1237.66 mg.

Duration of Life Cycle: It ranged between 31 – 33 days (egg 3 – 4; larva 17 - 19; and pupa 10 - 11).

Development Success Of Eggs, Larvae And Pupae: The data obtained in the laboratory study on the development success of three life stages are set out in Table.3. The eggs of *Princeps demoleus* could be spotted on *Citrus trifoliata* and collected in each month during the period from September – June. The month to month hatching success rate varied from a low of 25% in June to high of 90.9% in February. The success rate of larvae ranged between 60% (February) – 100% (October) and that of pupae between 27.2% (May) – 70% (February).

Food Consumption and Growth: The data on the weight of food consumed and weight gained by the larvae on host plant was incorporated in Tables.2. The amount of food consumed increased from instar to instar, the proportion of total food consumption was of successive instars was: *Citrus trifoliata* 1.52, 3.05, 6.25, 18.89 and 69.32%. Thus, there was a greater consumption of food by the final instar on the host plant. Out of the total weight obtained, the weight proportions of successive instars were: *C. trifoliata* 0.39, 6.15, 12.98, 18.40 and 67.14Mg. Thus in the final instar alone there was more than 37.92 – 85.55 % of growth on the host plant. The weight gain by different instars was plotted against the food consumption (Fig.1). The figures indicated a direct relationship between these two parameters. The values of growth rate (GR) on *C.trifoliata* increased from *II* instar and gradually decreased from all remain instars. And consumption index (CI) decreased from first instar to fifth instar. The values of GR varied between 0.81 – 0.08 mg/day/mg and those of CI between 11.84 - 0.16 mg/day/mg.

Indices of Food Utilization: The estimated AD values for the five instars were high and ranged between 51.36 – 97.18 %, the highest and lowest values being associated with the first and the final instars respectively (Table.2). By contrast, the values of ECI and ECD increased progressively from first instar through successive instars, the former ranging between 1.81-55.66 % and the latter between 3.55 – 30.55%.

Table 1: Biological observations of early life stages of *Princeps demoleus on Citrus trifoliata.*

Stage	Length(mm)			Width (mm)			Duration (days)	
	Min.	Max.	AV.±S.D.	Min.	Max	AV. ±S.D.	Range	AV.±S.D.
Egg	1.00	1.20	1.06±0.08	1.80	1.90	1.86±0.05	3-4	3.20±0.44
I	3.00	3.50	3.40±0.22	1.00	1.20	1.10±0.07	2	2.00±0.00
II	5.20	5.50	5.32±0.16	1.30	1.80	1.52±0.21	2	2.00±0.00
III	10.30	14.20	11.74±1.53	2.20	2.70	2.40±0.23	3-5	4.20±0.83
IV	15.00	25.60	19.90±4.31	2.60	4.50	3.38±0.93	3	3.00±0.00
V	38.00	44.00	40.04±2.36	5.10	7.00	5.76±1.13	6-8	6.60±0.89
Total larval Period							17-19	18.00±0.70
Pupa	27.00	30.00	28.80±1.09	8.00	9.00	8.50±0.35	9-11	10.40±0.89

Table 2: Food consumption, growth and food utilization efficiencies of *Princeps demoleus larva fed with Citrus trifoliata.*

Instar	Wt. of food ingested (mg)	Wt. of faeces (mg)	Wt. gained by larva (mg)	GR (mg/day)	CI (mg/day)	AD (%)	ECD (%)	ECI (%)
I	35.34±11.84	0.92±0.08	2.38±0.60	0.81	11.84	97.18	7.14	6.94
II	68.14±35.35	2.06±1.11	7.38±0.33	0.69	6.25	96.60	12.84	12.36
III	139.22±44.54	46.90±64.91	77.76±33.41	0.32	0.64	86.25	70.19	64.43
IV	420.74±364.10	51.10±10.71	110.24±25.87	0.11	0.42	77.53	84.46	51.84
V	1543.86±576.99	1097.34±481.45	402.20±214.41	0.08	0.16	51.36	219.36	59.65

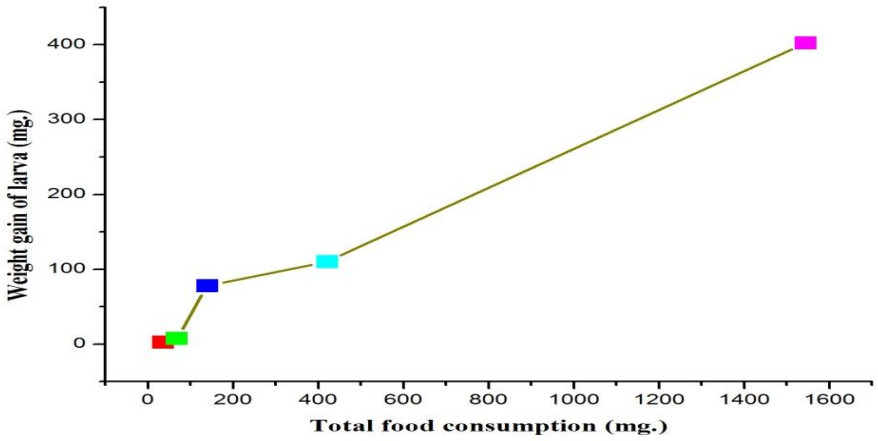
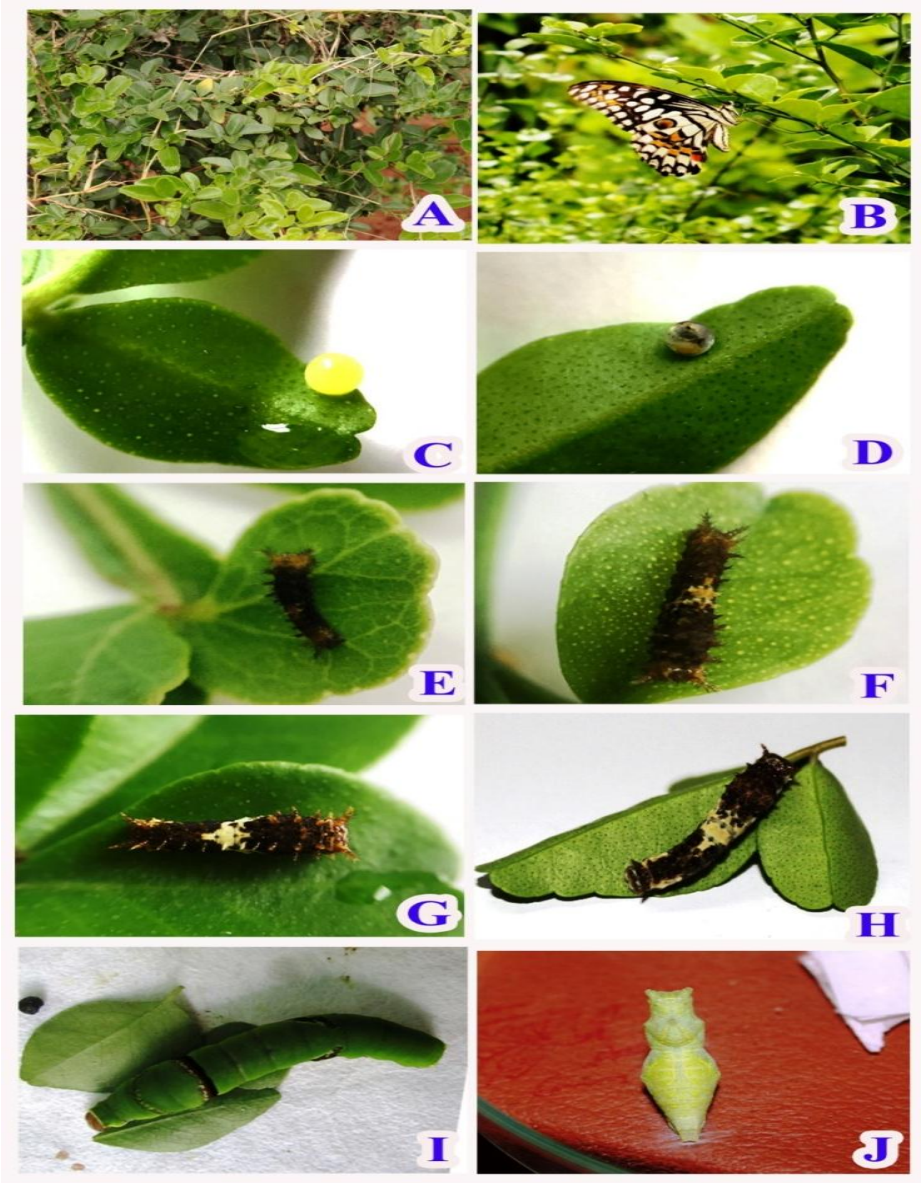


Figure.1: Relation between food consumption Growth in *P. demoleus* on *Citrus trifoliate*



PLATES

Plate: A. ***Citrus trifoliate*** **(L.) host plant; B. *Princeps demoleus* (L.) adult butterfly; C. Freshly layed Egg; D. Hatched Egg. E. First Instar (larva); F. Second Instar (larva); G. Third Instar (larva); H. Fourth Instar (larva); I. Fifth Instar (larva); J. Pupa.**

DISCUSSION

Data were obtained with reference to *Princeps demoleus* on larval host plant, egg-laying patterns, hatching period, the number of instars the larva passed through, their duration and pupal period and development success of eggs to adult in the laboratory. In addition, data were collected on larval performance on the basis of growth rate GR, food consumption index CI, approximate digestibility of food AD, efficiency of conversion of digested food ECD and efficiency of conversion of ingested food ECI. These different aspects of biology and food utilization are discussed below in the light of the relevant information available from temperate and tropical regions of the world.

It is generally understood that the gravid females directly deposit their eggs on the plants on which their larvae later feed. The larval survival, growth rate, development time, pupal weight and the nutritional indices AD, ECI, and ECD have been estimated for *P.demoleus* on its natural Oviposition host plants and the effect of potential host plants on the offspring. The potential host plants chosen for this butterfly species was *Citrus trifoliata.*

Based on the spectrum of plant species utilized by the larvae for feeding, The present report of the host plants of the butterfly species under study in the context of the above concept of food plant utilization indicated that, *Princeps demoleus* could be treated as oligophagous and it has been shown to have a wider range of potential host plants suitable for larval growth than the range of plants now used for Oviposition.

While[5] recognized three categories of butterflies on the basis of their egg-laying habit, most authors considered only two categories: (1) the cluster or batch layers and (2) those laying eggs singly. The species of the present study *P. demoleus* exhibited single-egg laying habit respectively.

The basic life cycle of a butterfly from egg to adult varies from 3 weeks to 2 years.[8] Most butterfly species in the tropics have short life cycles (Owen 1971). As is the case *Princeps demoleus*: average of 31 - 33 days.

Food consumption and utilisation across the instars

The larvae of *Princeps demoleus* were found to eat firstly the shell, thus getting the valuable nutrients available immediately to them. After finishing the cell the larvae continued to feed on young leaves of the oviposition host plants of *Ctrus trifoliata.* Therefore, the larvae were reared in the laboratory feeding them with fresh young leaves of their natural host plants every day.

The young leaves support better larval growth than older ones.[3];[12] Young leaves are known to be rich in nitrogen content[7] and thus satisfy the nutritional requirement of larvae. Not only the nutrients of the leaf but also its water content is important in relation to larval growth and development (Scriber 1979;.[7] The leaf water contents of the host plants of *P. demoleus* ranged on *C. trifoliata* was 79.2 % and tallied with the values given by.[10]

The data obtained with respective to *P. demoleus* on the quantity of food consumed and growth achieved in terms of larval body weight, and the values of consumption index (CI) and growth rate (GR) showed a definite trend of increasing absolute and declining relative rates of CI and GR under study. There is a straight line relationship between food consumption and growth (Fig.1). Of all instars, the penultimate and final instars together consumed a greater amount of food: *C. trifoliata* 88.21 of total food consumed over the entire larval period. The strategy of increased food consumption with the progression of larval age appears to be characteristic of all Lepidoptera and the same has been reported in other Lepidoptera in general (David and Gardiner 1962.[4]; [2];[9]

Consumption index (CI) of instar I was the highest and the values decreased as the instars progressed (Table.2). This decline in CI as the larvae aged may be related to the increase in body size of the larvae or to the increase in conversion efficiency of ingested food to body mass (ECI). So the high consumption index of early instars is due to the low conversion efficiency. The values of conversion efficiency (ECI) showed an increasing trend as the values of CI decreased across the instars (Tables.2**).** The values of CI obtained in the present study for early and late instars agree well with the values reported for some other butterfly species from the study area.[2]; [9] Like food consumption, a larger proportion of total growth in terms of larval body weight took place during the last two instars. Thus as the instar larvae progressed there was a trend of increasing absolute weights, but the relative rates (values of GR) generally declined (Table.2). Probably GR is size dependent, and therefore its values declined as the instar larvae progressed gaining weight and size. A similar declining trend in GR has been reported in other butterfly species.[2],[9] The GRs of penultimate and final instars of the butterfly species of the present study is in line with the decreasing trend in growth rate from penultimate to final instars.

Like those of CI and GR, the values of assimilation efficiency or approximate digestibility (AD) also declined from early to late instars (Table.2). The inverse relationship of AD profile with the amount of food intake by the successive instars is in conformity with[11] who observed a similar relationship and stated that AD would be at its highest in instar I. In the present study the same expectation has been realized with AD value being at its highest in the first instar. The AD values of the present study ranged between 51.36 – 97.18 % (Table.2). These values appear to be on the higher side of the range 19 – 81% given for 60 species of lepidopteran larvae by.[4] They are comparable to those those (31.0 - 91.5%) estimated by[6], (72.0 - 98.0%) of[1] and those (39.40 – 97.25%) of.[9] An inverse relationship is expected

between assimilation efficiency AD and efficiency of conversion of digested food or net conversion efficiency ECD. The ECD values (6.6 – 46.9%) are low compared to AD values (16.2- 22.1%), but are mostly within the range of 2 - 87% and 2 - 93% estimated for Lepidoptera feeding on forb and tree foliage respectively.[10] The values of ECD across the instars showed no definite trend in the increase or decrease (Table.2)[10], remarked that it is rather difficult to determine the causes of such reduction in ECD. The ECI values in the present study varied between 3.55 – 30.55 % (Table.2). This showed a continuous increase from first instar to final instar. In line with the opinion of[10], it may be said that because both age and size of larvae are increasing during development, and because feeding habits may also be changing, it is frequently difficult to interpret the causes of the changes in performance values of the larvae.

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