

**FEEDING BIOLOGY AND DIGESTIVE ENZYMES OF
BUZURA SUPPRESSARIA GUEN. AND *ETERUSIA
MAGNIFICA* BUTL., TWO MAJOR DEFOLIATING
PESTS OF *CAMELLIA SINENSIS*
FROM DARJEELING PLAINS, INDIA**

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ABSTRACT: The common looper caterpillar, *Buzura suppressaria* and the red slug caterpillar, *Eterusia magnifica* are serious defoliators of tea bushes (*Camellia sinensis*) of the Terai and Dooars areas of Darjeeling and N.E. India. While the former species prefers young leaves, the latter feeds on more mature leaves. This study aims to find the difference of the nutritional indices for the two folivores, such as relative consumption rate (RCR), relative growth rate (RGR), gross growth efficiency (ECI), net growth efficiency (ECD) and approximate digestibility (AD) and relate the same with their maintenance cost and production index (body mass). *B. suppressaria* has an edge over *Et. magnifica* as far as RCR and AD values are concerned. However, *Et. magnifica* could make up for the poor food quality (as they feed on mature tea leaves) by increasing their feeding period and better food conversion efficiencies. Higher value of AD in *B. suppressaria* may be due to higher quantity of the digestive enzymes in the midgut of this caterpillar. Significant differences in the activities of amylase, protease and lipase could be detected at salivary and midgut levels in the two folivores. The adaptive strategies in exploiting the different qualities of leaves, from two hampers of tea bushes is important for optimal food utilization by the two folivores with niche segregation.

KEY WORDS: *B. suppressaria*, *Et. magnifica*, *Camellia sinensis*, nutritional indices, digestive enzymes, Darjeeling

The common looper caterpillar, *Buzura suppressaria* Guen. and the red slug caterpillar, *Eterusia magnifica* Butl. are serious defoliating pests of tea, *Camellia sinensis* (L) O. Kuntze from Terai and the Dooars areas of Darjeeling and N.E. India (Anonymous, 1994). Of these folivores the former exercises preference for young and the latter for mature tea leaves. In case of severe infestation however, they may eat the entire leaf, as well as the woody parts of the bush. In order to have a better understanding of feeding biology of both the pests the present study was undertaken on their food consumption, utilization and digestive enzymes. The nutritional requirements of an insect change throughout development and such changes are typically reflected in changes of its food consumption and feeding behaviour (Barton

Browne, 1995). Numerous studies in the field of nutritional physiology have reviewed the effects of nutritive compounds (Mattson, 1980; Felton, 1996) on insect responses. Some of the nutritional responses are adaptive, such as preingestive increase in consumption of nutritionally poor food (Taylor, 1989; Woods, 1999) or postingestive increase in activity of digestive enzymes (Hinks & Erlandson, 1994; Lazarevic, 2000).

As the ability of *B. suppressaria* and *Et. magnifica* to utilize leaves of *C. sinensis* is largely dependent on three basic digestive enzymes viz. amylase, protease and lipase, these have been quantified in the salivary secretions and midgut of the larvae of both the pests. Further, an attempt has been made to relate and compare the enzyme quantity with the nutritional indices of these pests. Such information on digestive enzymes *vis a vis* food utilization can help contemplation of control of these pests through use of enzyme inhibitors and allelochemicals under host-plant resistance programmes.

MATERIAL AND METHODS

A commonly planted high yielding tea clone of Assam x Cambod origin was provided as food for the rearing of the pest larvae in a transparent container (27.5x 27cm) in aseptic conditions. Freshly emerged adults in laboratory were sexed, paired and allowed to mate in glass chimneys (19.5 cm x 8.5 cm), containing a twig with tea plant immersed in water of a conical flask to elicit oviposition. Larvae hatched from these eggs were reared at $28 \pm 2^\circ\text{C}$, $75 \pm 5\%$ relative humidity and 12 hours L: D.

Nutritional ecology:

In order to find out the daily food consumption and weight changes in final larval instar freshly ecdysed Vth instar stages, 10 replicates each of *B. suppressaria* and *Et. magnifica* were monitored under controlled conditions (as mentioned earlier) in BOD incubator. Daily-pretweighed fresh food (tea leaves with twig) was offered to each individual kept in (26cmx8.5cm) plastic containers. After 24 hours of feeding, leftover food and excrement were removed, oven dried and weighed. Dry weight of the actual food consumed was calculated by subtracting the dry weight of the leftover food from the dry weight of an equivalent amount of the food offered. Dry weight change of larva was calculated by drying a larva of similar weight in the oven at 50°C for 72 hours. Control was run concurrently by keeping tea leaves with their twig immersed in water of a conical flask having its mouth plugged with a cotton ball. Gravimetric (dry mass) technique was used to determine food consumption, and post ingestive food utilization efficiencies after Waldbauer (1968), Slansky & Scriber (1985), Petruszewicz & MacFadyen (1970), Muthukrishnan & Pandian (1987) and Farrar et al. (1989).

Activity of digestive enzymes:

Enzyme extraction was made from laboratory-reared Vth instar larvae of *B. suppressaria* and *Et. magnifica*. The dissections were carried out in an ice-cold sodium phosphate buffer (0.1 M, pH 7.0). Salivary gland and midgut were homogenized individually in fresh sodium phosphate buffer containing 0.01 M each of EDTA (Ethylene diamine tetra acetic acid) and 0.5% Triton X-100. The homogenate was centrifuged at 10,000g for 15 min at 4° C. The supernatant of this preparation were used for measuring enzyme activities and stored at – 20° C for future use.

Amylase assay:

Amylase activity in the salivary gland and midgut was determined after the method of Madhusudhan et al. (1994) followed by the method of Sadasivam & Manickam (1996) using dinitrosalicylic acid reagent; and quantification of enzyme product was deduced from a standard curve prepared using various concentration of maltose alone at 520 nm using UV-Vis spectrophotometer. The enzyme activity was expressed as $\mu\text{M} / \text{min} / \text{mg}$ of protein.

PROTEASE ASSAY:

Proteolytic activity was assayed after the methods of Kunitz (1947) modified by Jayaraman (1981). 1% (w /v) casein was used as the substrate. 1 ml of casein prepared in 0.1 N NaOH was incubated with equal volume of enzyme. After incubation for one hour, the reaction was terminated by the addition of 10% TCA and the acid-soluble peptides were quantified using the biuret reagent at 520 nm using UV-Vis spectrophotometer. The enzyme activity was expressed as $\mu\text{g} / \text{mg}$ of protein.

LIPASE ASSAY:

Lipase activity was measured following the method of Sadasivam & Manickam (1996). The enzyme activity was calculated as milliequivalent activity of free fatty acid / min/ g sample.

RESULTS AND DISCUSSION

B. suppressaria and *Et. magnifica* showed considerable changes in the quantity of food ingested and development of body mass but with similar trends. Despite a greater quantity of leaf consumed (in total) by *Et. magnifica*, the relative consumption rate (RCR) value of *B. suppressaria* was recorded to be higher. Such a difference may be due to quality of leaf consumed. Leaves of different plants / varieties differ

in their suitability as insect food because of variations in nutrient content, water content, type and concentration of secondary plant compounds and degree of sclerophyll (toughness / fibre) (Gullan & Cranston, 1994). *B. suppressaria* consumed younger leaves of upper tier and *Et. magnifica* preferably fed more on mature leaves of middle tier of a tea bush. A better consumption rate of *B. suppressaria* is possibly due to consumption of leaves of higher nutritional quality, in which the percentage of nitrogen and moisture is more, than the mature leaves consumed by *Et. magnifica*. In a similar finding Scriber & Fenny (1979) showed that Swallowtails had a higher consumption rate on nitrogen and moisture-rich forbs than when feeding on tree foliage having relatively less values of nitrogen and moisture. In the two species, efficiencies of ingested (ECI) and digested food (ECD), showed that *B. suppressaria* had lower ECI and ECD values as compared to *Et. magnifica* (Table 1). This could be explained by a higher metabolic cost of processing the young leaves, which contain more allelochemicals. The young leaf of tea plants contains high levels of plant allelochemicals like polyphenolic compounds, caffeine (Roberts, 1962; Banerjee, 1993). These secondary plant compounds are associated with induction mechanisms at the level of digestion and detoxification. A reduction in ECD associated with allelochemical ingestion is a common phenomenon (Koul et al., 1990; Appel & Martin, 1992). Secondary plant compounds often inhibit growth and development of insects (Todd et al., 1971; Lindroth et al., 1988; Ayres et al., 1997). Secondary plant substances also frequently act at the behavioural level of insects as deterrents and feeding inhibitors (Kraft & Denno, 1982; Kelly & Curry, 1991; Van Dam et al., 1995). The above hypothesis is tested by a comparison of the life histories of two folivores in question on young and mature tea leaves and their adaptations to the different leaf quality and quantity.

The maintenance cost of *B. suppressaria* was higher in comparison with *Et. magnifica*. The increase in food consumption rate that enhanced the cost of maintenance of *B. suppressaria* than *Et. magnifica* may be due to its food quality. In *B. suppressaria* a large part of the ingested food is presumably utilized in maintaining of basal metabolism, resulting in low conversion for growth. In *Pseudaletia unipuncta*, similar phenomenon was observed by Mukerji & Guppy (1970). The suboptimal availability of nutrient often nitrogen or water reduces growth rate, increases maintenance costs and causes a lower metabolic efficiency (Schoonhoven et al., 1998). The production index of *Et. magnifica* was found to be higher than *B. suppressaria* and this might be due to the better suitability of the mature tea leaf as food in supporting the advanced life stages of the former species.

Study on approximate digestibility (AD) showed a higher value in *B. suppressaria* as compared with that of *Et. magnifica*. A higher AD and assimilation are known to be influenced by quality, specially of nitrogen, water and toxin contents of the plant food (Muthukrishnan &

Pandian, 1987). The increased AD in response to tea leaf quality could also be as a result of changes at the levels of digestive enzymes. Higher activity of digestive enzymes in relation to food composition have been reported by Hinks & Erlandson (1994) and Ishaaya & Swirski (1976). Deficiencies in the quality of a food resource can be balanced by various mechanisms of nutritional compensation as is evident in *Et. magnifica* that overcome poor food quality by increase in their feeding period and better food conversion efficiency (Fig. 1 and Table 1). Starch is the main reserve polysaccharide in tea (Banerjee, 1993). The amylase activity found both in salivary and midgut of *B. suppressaria* indicates greater digestion of polysaccharides in midgut than its break down at the time of ingestion in the oral cavity *vis a vis* in *Et. magnifica* amylase activity of equal quantity indicates almost similar polysaccharide digestion at salivary and midgut levels. This is possibly an adaptation for better digestion of starch through an increase of the feeding period and higher conversion efficiencies (Table 2). In unprocessed tea, protein makes up to 20% of the dry weight (Mulky, 1993). The protease activity in oral as well as midgut of *B. suppressaria* and *Et. magnifica* ensure an active protein digestion at both the levels. Nevertheless a higher protease activity in salivary secretion of *Et. magnifica* possibly ascertains a better digestion of the available protein of mature leaves, starting in the oral cavity followed by midgut (Table 2). The activity of lipase is much reduced than the other two digestive enzymes. In *B. suppressaria* the lipase activity is significantly higher than that of *Et. magnifica* both at salivary and midgut levels possibly because the former feeds on young tea leaves in which lipid make up 4% to 9% of the dry matter (Roberts, 1974; Mahanta et al., 1985). The lipase activity has also been reported in the midgut of *Manduca sexta* (Rubiolo et al., 2000) and *Spilosoma obliqua* (Anwar & Saleemuddin, 1997). The digestive enzymes are mainly reported from the midgut of different insects (Hori et al., 1981; Lenz et al., 1991). The present study on feeding biology and digestive enzyme activities reveals different exploitation strategies by the two folivores of two qualities of tea leaves (young and mature). Further, it establishes that *Et. magnifica* has a better adaptive flexibility than that of *B. suppressaria* because of its greater efficiency in converting both ingested and digested food. The study throws-up future research opportunities in non-conventional management of these two pests based on digestive enzyme inhibitors and other HPR strategies, which would be a necessity in developing IPM – programme of tea.

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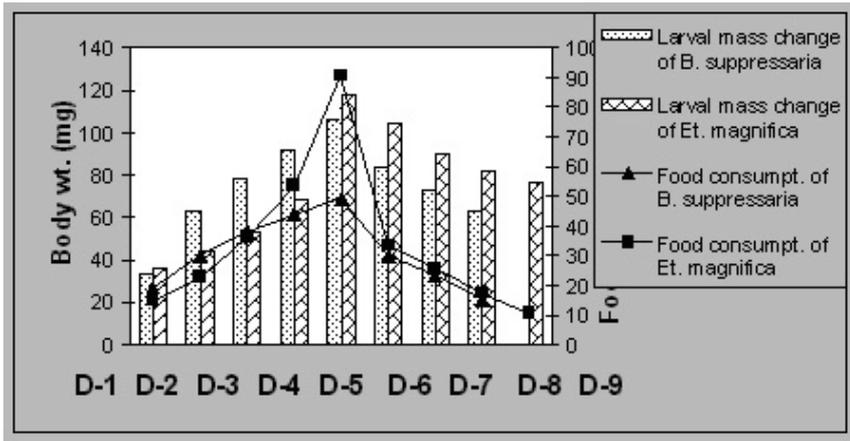


Fig. 1. Relation of dry mass (mg) changes (left ordinate) and daily food consumed (mg) (right ordinate) during development of Vth instar larvae of *Buzura suppressaria* and *Eterusia magnifica* (D = day)

Table 1. Nutritional Indices of *Buzura suppressaria* and *Eterusia magnifica* (Vth instars) on tea leaf (Mean \pm SE).

| V th instars | RCR | RGR | ECI | ECD | AD | Ment. Cost | Prodn. Index |
|-------------------------|--------------------------|--------------------------|---------------------------|------------------------|------------------------|--------------------------|--------------------------|
| B. suppressaria | 0.630a \pm 0.008 | 0.078a \pm 0.002 | 12.355a \pm 0.246 | 24.761a \pm 0.453 | 49.904a \pm 0.441 | 3.056a \pm 0.070 | 0.247a \pm 0.005 |
| E. magnifica | 0.574b \pm 0.004 | 0.080a \pm 0.001 | 13.879b \pm 0.212 | 31.241b \pm 0.509 | 44.453b \pm 0.243 | 2.212b \pm 0.051 | 0.312b \pm 0.005 |

Means followed by the same letter are not significantly different using t-test at $p > 0.05$

Table 2. Digestive enzymes of salivary gland (SG) and midgut (MG) homogenate of *Buzura suppressaria* and *Eterusia magnifica* (Mean \pm SE) (n = 10)

| | Amylase ($\mu\text{M. mg protein}^{-1} \cdot \text{min}^{-1}$) | | Protease (Amount of protein, casein, utilized) | | Lipase (Activity meq. / min /g of sample) | |
|------------------------|--|---------------------------|--|------------------------|---|---------------------------------|
| | Salivary gland | Midgut | Salivary gland | Midgut | Salivary gland | Midgut |
| B. suppressaria | 0.318 \pm 0.71 aA | 0.405 \pm 0.62 bB | 38.22 \pm 0.19 aA | 44.81 \pm 0.38 bB | 0.0076 \pm 0.0002 aA | 0.0328 \pm 0.0013 bB |
| E. magnifica | 0.331 \pm 0.37 bA | 0.348 \pm 0.63 aB | 44.45 \pm 0.46 bA | 43.49 \pm 0.22 aB | 0.0043 \pm 0.0001 bA | 0.0127 \pm 0.0009 aB |

Difference in lower case letters in columns indicate significance difference of mean using t-test at $p > 0.001$; Difference in upper case letters for each enzyme in rows indicate significance difference of mean using t-test at $p > 0.001$