

# A Preliminary Study on Host Plant Related Changes of General Esterases In *Buzura suppressaria* (Lepidoptera: Geometridae), A Major Defoliator of Tea In The Darjeeling Foothills And Adjoining Plains In India

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## ABSTRACT

The looper, *Buzura suppressaria* Guen. (Lepidoptera: Geometridae) is the major defoliating pest of tea in the Darjeeling foothills and plains in north-east India. Esterase isozymes were found to differ in looper populations maintained on TV-1 and TV-25 tea clones. The major clone-based difference was located in the 'very slow moving band' group (VSM). While the three VSM bands were absent in the phoregrams of the midgut and salivary glands of TV-1 looper populations, they were prominent in the midgut of TV-25-reared specimens. In looper populations of pesticide-treated plantations, the esterase bands of the VSM group were more elaborate, along with deep staining of the slow moving (SM) and the fast moving (FM) bands. Such an enhancement of band intensity possibly reflects a greater tolerance or resistance of the loopers to plant allelochemicals and pesticides. The isozyme profiles of esterases of *B. suppressaria* can therefore be used as a tool for detecting the resistance associated with the tea clones (variety) as well as that which develops in the species on exposure to pesticide spray under field conditions (plantation).

**Key words:** Esterase isozymes, looper caterpillars, tea clones

## INTRODUCTION

The looper caterpillar, *Buzura suppressaria* Guen. (Lepidoptera: Geometridae), attacks mature leaves and defoliates tea (*Camellia sinensis*) bushes causing heavy crop loss. This major defoliator has a wide distribution in Indian subcontinent. While the pest is more prevalent in Darjeeling Terai-Dooars (foothills & plains) and north-east India, its occasional outbreaks are reported from tea plantations of South India (Tamil Nadu and Kerala) but, not at present from Sri Lanka. The species is recorded even from Indonesian tea (Muraleedharan, 1991; Muraleedharan and Selvasundaram, 2002; Danthanarayana, 1967).

To control the depredations of various tea clones and the multiple generations of *B. suppressaria*, regular insecticide spraying is required. The difference in susceptibility of insect pests to insecticides when maintained on specific plants has been related to different levels of metabolizing enzymes, presumably induced by the plants (Yu, 1982; Ambrose and Regupathy, 1992; Tan and Guo, 1996). Many enzymes involved in detoxification pathways act on a broad array of substrates, including both naturally occurring plant allelochemicals and synthetic pesticides (Gordon, 1961).

An enhanced metabolism by esterases is a major mechanism for countering pesticide stress that has been detected in lepidopterans (Beeman and Schmidt, 1982). The objective of the present study was to determine the differences in the general esterase profiles of looper caterpillars when reared on two different Tocklai (Tea Research Association) varieties of tea, namely TV-1 (an early release) and TV-25 (a relatively late release). A further aim was to compare the banding pattern and staining density of esterase bands of looper populations exposed and unexposed to pesticide sprays.

## MATERIALS AND METHODS

### Insect collection and maintenance

200-250 larvae of *B. suppressaria* were collected from tea plantations, mostly comprising TV-1 and TV-25 varieties, in the Darjeeling foothills, Terai and Dooars in West Bengal State, India. These larvae were reared separately on the Tocklai clonal varieties, TV-1 and TV-25, for two generations at  $27 \pm 2^\circ \text{C}$  and  $72 \pm 2\% \text{RH}$  with a photoperiod of L:D, 13:11, in transparent containers (30 x 30 cm) with a supply of fresh tea twigs, obtained from the tea plantation of the North Bengal University campus, culturally maintained without application of pesticides.

### Enzyme extraction and gel electrophoresis

Enzyme extraction was done from laboratory-reared fifth instar larvae of *B. suppressaria*, and from larvae of the same stage collected from natural populations occurring in conventionally managed plantations that were subjected to routine synthetic pesticide spraying.

Each larva was dissected and its salivary gland and midgut were removed. Dissections were carried out in ice-cold sodium phosphate buffer, 0.1 M, pH 7.0 using sterilized scissors and needles. Salivary glands and midguts 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 volume of the buffer was adjusted to produce similar protein concentrations in the homogenates of each individual. The homogenate was centrifuged at 10,000 g for 15 min at  $4^\circ \text{C}$ . The supernatant of this preparation was stored at  $-20^\circ \text{C}$  for future use.

Using the technique of Davis (1964), fifteen microlitres of homogenate was dispensed into each well of the electrophoresis gel. Electrophoresis was carried out at constant

current (10 mA) for about 1.5 hr on 8% native polyacrylamide vertical slab gel, using tris-glycine (pH 8.3) as running buffer.

The gel was then stained for 30 min at 36° C for esterase with Fast Blue BB salt in 0.1 M phosphate buffer, pH 7.0, containing 0.03 M alpha naphthyl acetate solution dissolved in acetone.

The relative migration of esterase bands in the zymograms was determined by the Kodak digital science 1D Image Analysis Software, version 2.0.3. Relative mobility ( $R_m$ ) was calculated as:

distance migrated by the specific bands (cm) / distance migrated by the marker dye (cm).

## RESULTS AND DISCUSSION

The general esterase phoregram appeared in three groups: fast moving bands (FM or EST-1), slow moving bands (SM or EST-2), and very slow moving bands (VSM or EST-3), on the basis of relative mobility. These groups were distinctly separate from one another. The first three bands of the VSM group were absent in the salivary homogenates of all three categories of looper populations. In the midgut homogenates, the VSM group was present as five bands in TV-25-reared and field-collected loopers. However, the first three bands of VSM were not present in the midgut homogenate of TV-1 reared populations.

Among four SM bands of esterases, bands 1, 2 and 3 were prominent, and were consistently present both in salivary and midgut homogenates. These appeared deeply stained and with uniform mobility in all the looper populations.

A similar pattern, parallel to TV-25, was observed in field-collected looper populations, in which the bands of VSM, SM and FM were deeply stained, indicating a larger quantity of esterases (Table 1 and Fig.1 a, b, c).

Isozyme analysis has been applied to identify species, biotypes and host-specific populations in many insects such as aphids, egg parasitoids (*Trichogramma* spp.) and others (Loxdale and Hollander, 1989). In the present study, zymograms of esterases failed to show major differences in banding patterns, except for three additional VSM bands which were present in the gut homogenate of the looper populations reared on TV-25 and the field collected loopers. Mullin and Croft (1983) found large differences in general esterase activity of *Tetranychus urticae* on snapbean varieties, ranging from 0.4 fold on a mint to 2.4 fold on umbellifers. Moreover, herbivorous insects metabolize and detoxify insecticides using the same set of enzymes that are involved in the metabolism of ingested plant allelochemicals (Brattsten, 1979; Ahmad *et al.*, 1986).

The bands parallel to the three VSM bands showed intense staining in loopers of field populations exposed to pesticide treatments in conventional plantations. Such band intensity may be related to looper populations with greater pesticide tolerance (*vis-à-vis* resistance).

In similar findings, a higher midgut esterase activity was reported in *Plutella xylostella* exposed to pesticides (Mohan and Gujar, 2003), and also a higher activity of slow moving esterases as apparent in PAGE for the same species (Maa and Liao, 2000). Resistant aphids display a high level of non-specific esterase activity represented by intense esterase bands (Ono *et al.*, 1994) as is evident in malathion resistant *P. xylostella* (Maa and Chuang, 1983; Doichuanngam and Thornhill, 1989). Thus EST-3 or VSM bands appeared to be crucial in utilizing a relatively recent tea clone (TV-25) and development of greater pesticide tolerance / resistance in *B. suppressaria* population. As such, these bands could be used as markers in screening the populations of the pest for resistance / tolerance status as well as screening the clonal varieties that sufficiently differ biochemically (allelochemically).

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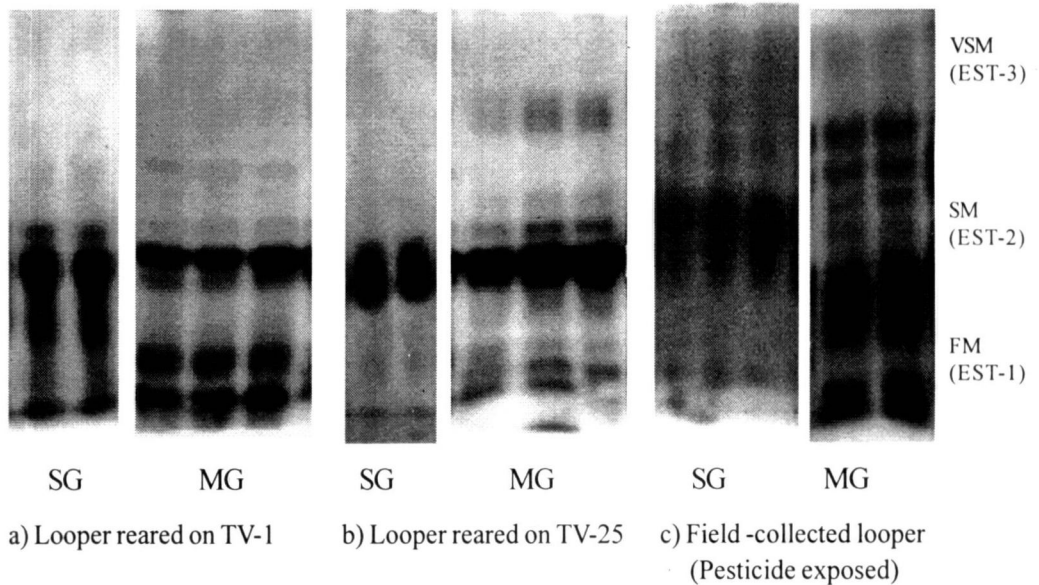
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**Table 1. Electrophoretic variation in relative mobility ( $R_m$ ) (n =15) of esterase isozyme bands of salivary and midgut homogenate of *Buzura suppressaria* larvae on TV-1, TV-25 and field-collected populations from the Darjeeling foothills and adjoining plains**

Esterases	Tea clone				Field-collected population	
	TV-1		TV -25		SG	MG
	SG	MG	SG	MG	SG	MG
VSM (EST- 3)	----	----	----	0.161	----	0.161
	----	----	----	0.184	----	0.184
	----	----	----	0.201	----	0.201
	0.332	0.332	0.332	0.332	0.332	0.332
	0.381	0.381	0.381	0.381	0.381	0.381
SM (EST-2)	0.430	0.430	0.430	0.430	0.430	0.430
	0.458	0.458	0.458	0.458	0.458	0.458
	0.481	0.481	0.481	0.481	0.481	0.481
	0.538	0.538	0.538	0.538	0.538	0.538
FM (EST-1)	----	0.614	----	0.614	0.614	0.614
	----	0.632	----	0.632	0.632	0.632
	0.672	0.672	0.672	0.672	0.672	0.672
	0.684	0.684	0.684	0.684	0.684	0.684

SG: Salivary gland homogenate  
 MG: Midgut homogenate

VSM (EST-3): Very Slow moving bands  
 SM (EST-2): Slow moving bands  
 FM (EST-1): Fast-moving bands



SG: Salivary gland homogenate  
 MG: Midgut homogenate

VSM (EST-3): Very Slow moving bands  
 SM (EST-2): Slow moving bands  
 FM (EST-1): Fast-moving bands

**Fig. 1:** Zymograms of esterases of loopers, *B. suppressaria* fed and maintained on tea clones (a) TV-1, and (b) TV-25, and from (c) conventional plantations exposed to pesticide sprays (Each lane represent the phoregram of a single looper)