3. Review of Literature

3.1. Major lepidopteran defoliators and sucking pests of tea and related crop loss

In the world more than a thousand species of arthropod are known to be associated with different parts of tea plants. Out of them Lepidoptera form the largest order containing 31.53% of the pest species followed by Hemiptera 26.29% (Chen and Chen, 1989). But, only around 300 species of insects and mites are recorded in India as tea pests under Acarina, Lepidoptera, Hemiptera and Coleoptera. A few minor pests have also been reported under Diptera, Hymenoptera and Orthoptera (Muraleedharan *et al.,* 2001). The monographs by Green (1890), Watt and Mann (1903) are the earliest contributions to the study of tea pests. Information on tea pest biology in N.E. India is given by Hainsworth (1952), Das (1965), Banerjee (1983a, b) and that of South India and Sri Lanka by Muraleedharan (1983) and Cranham (1966) respectively.

Cramer (1967) estimated that tea in Asia suffers 8% crop loss due to pests. Glover *et al.* (1961) reported 13% crop loss, where as Banerjee (1993) reported a steady loss of 10% due to overall pest attack as a generally accepted figure, which may at times go up to 40% in devastating attack by defoliators. Sivapalan (1999) also reported that various assessments on crop loss by respective tea pest had been done from time to time in the different tea growing countries. This loss ranges from 5 to 10 to as high as over 50%. Reports from N.E. India suggested that in tea 6 to 12% crop loss annually may be due to red spider mite, *Oligonychus coffeae* alone (Sen and Chakraborty, 1964; Banerjee, 1971). Scarlet mites *Brevipalpus phonicis* and red spider mites are reported to cause 0.56 to 4.79% decrease in yield in Sri Lanka (Danthanarayana and Ranaweera, 1970). Some 8 to 17% increase in tea crop could be obtained following efficient mite control in South India (Rao and

Subramaniam, 1968). The crop loss due to *Helopeltis theivora* in south India varied from 11-100% during high cropping season (Rao and Murthy, 1976). Average crop loss due to *Helopeltis* was 150 kg made tea per ha in Bangladesh (Ahmed, 1996). Damage to cocoa by *H.theivora* resulted in almost 95% crop loss if no biological or chemical control methods were adopted (Way and Khoo, 1989). The economic threshold level (ETL) for *H.theivora* was almost similar to that recommended for *H.schoutedeni* affecting tea in Central Africa during main cropping season (Rattan, 1987) and *H.schoutedeni* in Malawi was capable of causing a phenomenal loss in crop upto 55% (Rattan, 1984).

The common looper *Buzura (Biston) suppressaria* Guen. caterpillar is one of the most destructive pests of tea and in recent years its activities have greatly increased becoming endemic to many gardens where it was unknown in the past (Anonymous, 1994). In the earliest record of tea pests by Cotes (1895), this pest was reported to have been collected first from Nowgong district of Assam. Subsequently Antram (1911) had reported looper caterpillar as one of the common pests of tea. Beeson (1941) in 'The Ecology and control of the forest insects of India and the neighbouring countries' recorded looper caterpillar on alternate hosts such as *Acacia modesta, A.catechu, Aleurites montana, Bauhinia variegata, Cassia auriculata, Carissa diffusa, Dodonaea viscosa, Lagerstroemia indica, Dalbergia assamica, Deris robusta, Albizzia chinensis, A.odorotissima, A.lebbek, Cajanus indicus* and *Priotropis cytisoides.*

Danthanarayana and Kathiravetpillai (1969) related the outbreaks of looper (*Ectropis bhurmitra*) with the presence of shade trees and use of dieldrin which killed many species of parasitic hymenopterans insects in Sri Lanka. Looper caterpillar was not considered to be a pest of major importance in North-East India

until 1990, when it caused considerable damage to tea in the Dooars and Cachar. Since then it has occurred from time to time and has been responsible for considerable losses in many estates of Upper Assam and the Dooars (specially Eastern and Central Dooars) (Anonymous, 1994). Borthakur (1975) had also reported looper caterpillar as one of the major pests of tea. Banerjee (1983b) outlined that the absence of natural enemies was the major contributing factor in looper outbreaks.

Hill (1983) in 'Agricultural insect pests of the tropics and their control' had mentioned looper as an active defoliator of Indian and South-East Asian tea. Das and Gope (1987) had also suggested control measures against defoliators of tea and shade trees. Growing resurgence of pest and their resistance in N.E. India had prompted Das *et al.* (1988) to suggest non-conventional approach in tea pest management. Chen and Kunshan (1988) in China, were successful in controlling of tea looper by the field release of the laboratory-reared parasitic wasps, *Trichogramma dendrolini.* Singh *et al.* (1990) had reported olfactory behavioural response in both sexes of the moth, *B.suppressaria* possibly mediated by pheromone. Muraleedharan (1991) has a given account of the biology of looper caterpillar and their occurrence in shade trees in the book 'Pest Management in Tea'. Further, Muraleedharan (1993) had recorded rare occurrence of looper caterpillar in the tea growing areas of south India.

A sketchy account of natural occurrence and control measures of looper caterpillar is given in the book 'Pests of tea in North-East India and their control' (Anonymous, 1994). The book also includes names of other members of the family Geometridae that are known to occur on tea; these are *Buzura (Biston) bengaliaria*, *Boarmia sclenaria*, *B.acaciaria*, *Medasina strixaria*, etc, but none of them has attained the

status of a pest (Anonymous, 1994). Chakravartee (1995) had mentioned that looper infestations might become devastating within a short period, so timely control of looper is very important.

Eterusia magnifica Butl. (Red slug caterpillar) is one of the major pests of tea in the Terai, the Dooars, and North-East India. Early in 1906, Mann and Antram had reported approximate time of occurrence of different broods of caterpillars and adults of red slug. Hutson in 1932 had reported the occurrence of different species, *Et.singala* from Sri Lanka and Rau (1952) reported occurrence of *Et.virescens* Butl. on tea from South India. During nineteen fifties and sixties this pest was recorded to be a sporadic pest (Anonymous, 1994). Hill (1983) in 'Agricultural insect pests of the tropics and their control' has reported *Eterusia magnifica* as defoliators from India and South-East Asia. Muraleedharan (1991) has given brief biology of this pest and suggested its control measures. Again in 1993, Muraleedharan had mentioned about rare occurrence of this folivore in the tea growing areas of South India. An account of biology of red slug is available in the treatise 'Pests of tea in North-East India and their control' (Anonymous, 1994). Chakravartee (1995) had mentioned that a destructive pest like red slug should not be allowed to grow to an epidemic stage and hence control measures should be undertaken once the pest is seen in tea.

Of the 41 recognised species of *Helopeltis*, 26 are restricted to Africa and 15 are distributed in Austrasian region (Stonedahl, 1991; Stonedahl *et al.,* 1995). *Helopeltis* spp. are serious pests of various cultivated plants in old world tropics. The damaging effects of these insects on tea plants in India was documented over a century ago by Peal (1873) and Wood-Mason (1884). Subsequently, the species responsible for causing damage to tea was identified as *H.theivora* Waterhouse,

1886. In south India, its outbreak was reported around 1920 on tea (Shaw, 1928; Rao, 1970).

Tea shoots were damaged by *H.antonii* in Sri Lanka (Mann, 1907; Ballard, 1921), *H.bradyi* in Indonesia and Malaysia (Leefmans, 1916; Lever, 1949), *H.bergrothi* in Malawi–Africa (Leach and Smee, 1933), *H.cinchonae* in Malaysia (Lever, 1949), *H.clavifer* in New Guinea (Smith, 1978), *H.fasciaticollis* in China (Xie, 1993), *H.sumatranus* in Sumatra (Miller, 1941), *H.schoutedeni* in Malawi (Rattan, 1988) and *H.theivora* in India and Indonesia (Mann, 1907; Leefmans, 1916; Ballard, 1921). *H.schoutedeni* in Malawi was capable of causing a phenomenal loss in crop, up to 55% and short-term loss can be considerably higher (Rattan, 1984). Due to its infestation, an average of 25% of the total crop was lost. The loss due to the attack of *Helopeltis theivora* in south India varied from 11-100% during the high cropping season (Rao and Murthy, 1976). In North-Eastern India, a miximum loss of 7.5 lakhs kg. of made tea / year has been reported (Das, 1984). Prior to use of modern chemical pesticides, crop losses on tea plantations due to *H.theivora* in India sometimes reached 100% (Muraleedharan, 1987).

The tea red spider, *Oiigonychus coffeae* is a serious pest of tea throughout the world. Out of the 12 species recorded, the red spider mite is the major one (Banerjee, 1988, 1993). The genetics of mite reproduction (Banerjee, 1979) also underlines the interplay of biological and physical factors that have made this species ubiquitous on tea the world over (Banerjee and Cranham, 1985). Although it primarily attacks tea, the mite causes considerable damage to jute and is also known to attack cotton, rubber, citrus, mango, oil palm and many other tropical plants and weeds (Das, 1959). The mite attacks *Grevillea robusta* and *Albizzia falcata* in tea plantations of Sri Lanka (Cranham, 1966); has also been reported

from *Deris robusta* and *Tephrosia candida* in India (Andrews, 1928). In severe damage the leaves get dried, leading eventually to defoliation (Das, 1959; Banerjee, 1965, 1980). Red spider mite causes considerable loss in tea production (Lima *et al.,* 1977; Das, 1983; Mkwaila, 1983; Kilavuka, 1990). Red spider mite has gained economic importance because of crop failure for a period of two or more months due to complete defoliation. It is reported to cause crop loss between 6-11% in the Dooars and 5-7% in Assam (Sen and Chakraborty, 1964). Crop loss also varies between 5-20% in North-East and south India (Aswathy and Venkatakrishnan, 1977; Muraleedharan and Radhakrishnan, 1989).

3.2. Pesticide use patterns in tea

Sustainable and cost effective crop protection requires the optimum use of chemicals and non-chemical pest control techniques (Chakravartee, 1995). It has been estimated that annual loss in agriculture due to pests, diseases and weeds in India is around Rs. 6,000-9,000 crores. The consumption of pesticide is estimated to be 90,000 tonnes and around 0.336 kg / hectare as against 10.75 kg or more in developed countries (Atwal, 1986). The annual loss of tea crop in N.E. India due to pest, diseases and weeds is estimated to be about 85 million kg valued at Rs. 425 crores (Barbora and Biswas, 1996). Of the total pesticide applications, only 3.5 rounds were acaricides and the rest were other insecticides. It was also indicated that nearly 65% of the pesticide is used during the first half of the year (January-June) and balance 35% between July and December. 80% of the acaricides was used between January and June and in case of insecticide, the seasonal use was observed to be almost uniform which apparently means that the activities of insect pests remain consistent in the region throughout the year (Sannigrahi and Talukder, 2003).

It has been estimated that tea in India harbours about 125 species of pests and in order to tackle these, extreme care must be taken before a pesticide is introduced to tea for pest control (Das, 1962) specially to avoid residue build up. The problems of pest resurgence, pest resistance and secondary pest outbreak were reported in the past (Das, 1959). He also inferred that poor control of *Helopeltis* with chlorinated hydrocarbon (DDT) might be due to the outbreak of resistant strains of *Helopeltis*. De Bach (1974) in his classical book "Biological control by natural enemies" mentioned the cause of upsets and outbreaks of tea tortrix and leaf-eating caterpillars during late fifties in Sri Lanka due to insecticide use. The continuous use of copper fungicides against blister blight (*Exobasidium vexans*) disease had been proved to be responsible for mites build up in Sri Lanka, India and Indonesia (Cranham, 1966; Venkata Ram, 1966; Oomen, 1982). Compounds such as methoxychlor and carbaryl were found to increase red spider mite infestation (Cranham, 1966).

3.3. Hydrolytic and oxidoreductase enzymes of lepidopteran, sucking and mite pests

Digestive enzymes are nearly universal in Animalia, but a means of delivering them efficiently to an external food source requires special modifications. The source of the digestive enzymes may be specialized structures, such as maxillary or salivary glands found in many insects and mites, or the midgut, as in some beetles and lepidopteran larvae (Evans, 1992; Snodgrass, 1935; Christeller *et al.,* 1992). The fundamental objective of digestion is to render macromolecules into simple compounds that can be absorbed and circulated (Gilmour, 1961; House, 1974). The digestive enzymes are all hydrolases (Baldwin, 1967), including proteinases, lipases, carbohydrases and nucleases (Gilmour, 1961; House, 1974). Proteinases probably are the most important liquefaction enzymes for insect predators (Cohen, 1993; Miles, 1972). The digestion mainly seems to occur in the midgut where a variety of

enzymes are available in abundance (Hori *et al.*, 1981). Proteinases and peptidases from the intestinal tract of fifth-instar larvae of *Heliothis zea* have been identified by Lenz *et al.* (1991). Herbivores possess various physiological and morphological traits that enable them to exploit their host plants. All herbivores deal with chemicals that are potentially damaging to their cellular processes, these come from various sources including secondary chemicals of plants that can be toxic or antinutritive to them (Duffey and Stout, 1996). The morphological traits of the gut have been described for most insect groups, physiological traits of the digestive tract are less well known (Appel, 1993). Some herbivores maintain extreme physiochemical conditions in the digestive tract, presumably to enhance digestion and inhibit the activity of some allelochemicals. In the larval Lepidoptera, the midgut is the primary site of digestion and absorption and midgut epithelial cells are modified to generate pHs depending on the species (Berenbaum, 1980; Dow, 1984, 1986; Appel, 1993). In addition physiochemical conditions of the midgut in caterpillars are likely to have a major impact on nutrient digestion and allelochemical activity (Appel and Maines, 1995). Lepidopteran larvae have been the subjects of study largely due to their impact on economically important plants. The digestive enzymes of these larvae are of interest both as a target for insect control and because of their unusual ability to function in the alkaline lepidopteran midgut (Christeller *et al.*, 1992). Digestive proteases catalyse the release of peptides and amino acids from dietary protein and they are found most abundantly in the midgut region of the insect digestive tract (Jongsma and Bolter, 1997). Most of the midgut proteolytic enzymes in lepidopteran larvae have been shown to be extracellular proteases with high pH optima which are well suited to the alkaline conditions of the midgut (Applebaum, 1985). Herbivores also produce salivary enzymes constitutively, prior to ingestion, that minimize the effectiveness of plant defenses, such enzymes are applied to leaf wounds as the herbivores chew

and these may reduce the activation of induced defenses in plants (Karban and Agrawal, 2002). Herbivore saliva is also known to contain oxidative enzymes like peroxidase and polyphenol-oxidase (Felton and Eichenseer, 1999). Relatively little attention has been focused on the antioxidant defenses in the gut lumens of insects. However in the gut lumen, the ingested phenolic compounds may become extensively oxidized (Barbehenn and Martin, 1994; Barbehenn *et al.,* 1996). A variety of antioxidant enzymes protect caterpillar tissues and extracellular fluids from oxidative damage. Among the most widely studied enzyme is catalase. The catalase activity was detected in the midgut tissues and regurgitate of larval *Helicoverpa zea, Spodoptera exigua*, *Manduca sexta*, and *Heliothis virescens* (Felton and Duffey, 1991).

Like caterpillars, other herbivores may also secrete saliva that interferes with plant defenses. The chemical composition of the saliva of heteropteran insects is crucial for effective feeding. These insects rely heavily on saliva for extra-oral digestion (Cohen, 1998) and detoxification of defensive chemicals (Miles and Oertli, 1993). Sucking bugs deposit salivary secretion in or on plants when feeding, which significantly influences the physiology of the affected plant tissues. Some of the secretions result in phytotoxemia (Gopalan, 1976). The ability of insects to use plant materials as food is indicated by the presence of specific digestive enzymes in their saliva (Zeng and Cohen, 2000). In many heteropterans specific digestive enzymes for phytophagy include amylase and pectinase (Cohen, 1996). Proteolytic activity has also been detected in salivary glands of mirid bugs such as *Lygus rugulipennis* (Laurema *et al.,* 1985) and *Creontiades dilutus* (Colebatch *et al.,* 2001). Mirids use their digestive enzymes through the salivary canal to liquefy food into nutrient-rich slurry (Miles, 1972; Hori, 2000; Wheeler, 2001). The food slurry is ingested through

the food canal and is passed into alimentary canal where it is further digested and absorbed (Cohen, 2000).

Salivary secretions of phytophagous Hemiptera contain various organic and inorganic compounds (Miles, 1972). An important group of them are proteins, mainly various enzymes, playing a fundamental role in food digestion of sucking–piercing insects (Miles, 1968; Miles and Sloviak, 1970; Peng and Miles, 1988a; Baumann and Baumann, 1995). Among these, polyphenol-oxidase uses molecular oxygen to catalyze two different types of reaction that are hydroxylation of monophenols to o-diphenols and oxidation of polyphenols to quinones and further dark brown or black pigments, melanins (Robinson *et al.,* 1991). Peroxidases use hydrogen peroxide to oxidize phenols and other aromatic derivatives (Deimann *et al.,* 1991). Both these oxidoreductases have been identified in the salivary secretions of aphid species (Miles and Peng, 1989; Madhusudhan and Miles, 1993) and they were found to be involved in overcoming the plant defenses by neutralizing phenolics and their derivatives (Miles, 1969; Urbanska and Leszczynski, 1992).

Digestion is the process by which food molecules are broken down into smaller molecules that can be absorbed by the gut tissue. As a general adaptation of the digestive apparatus of stored product mites to starch digestion and utilization (Bowman and Childs, 1982; Bowman, 1984), amylases catalyze the initial hydrolysis of starch into oligosaccharides, an important step towards transforming sugar polymers into simpler units that can be assimilated by the organism. The high level of activity of the digestive α-amylases in the whole body extract from *Acarus siro* was detected by Hubert *et al.* (2005). In *Dermatophagoides pteronyssinus* amylase, protease and lipase activity was associated with digestive processes (Stewart *et al.,* 1992). The endogenous enzymes of protective systems (EEPS) including catalase, peroxidase were found in a number of organisms (Fridovich, 1977). It has been

shown that the elevated EEPS activities were closely related to the resistance of organisms to unfavourable environments (Packer, 1984). The enhanced anti-oxidation enzymes (catalase and peroxidase) activities could reduce the effects of the toxic products on mites, resulting in defensive power strengthened, and survivorship and reproduction power of mites increased, thus density of mites on the host plant increased (Zhang *et al.*, 2004). Grubor-Lajsic *et al.* (1997) reported that exposure to cold conditions significantly increased the activities of the antioxidant enzymes of two larval Lepidoptera, *Ostrinia nubilalis* and *Sesamia cretica.* Aucoin *et al.* (1991) reported that catalase might be inducible defenses against phototoxins by insects.

3.4. An overview of enzyme based biochemical resistance

Nearly 40 years of studies, all over the world, suggest that insecticide resistance could be correlated with quantitative and/or qualitative changes in insecticide metabolizing enzymes or a change in target site or appearance of a protein in cuticle which retards the penetration of an insecticide of easy sequestration of insecticides by binding on a protein. The increased detoxification capabilities occur more frequently than the alteration of the insecticide target sites. The resistance associated enzymes could easily be assayed in laboratory. Efforts have been made to estimate the resistance associated enzyme activity to surrogate substrate usually naphtholic esters, whose products can be measured either in solution or cellulose filter paper or on nitrocellulose membranes. Further more, these surrogate substrates can be used to probe the isozymes and their mobility variance following electrophoresis and electrofocussing. The esterase banding patterns was proved very successful in the diagnosis of insecticide resistance in aphids and jassids. This technique is straightforward to use when resistance is due to the production of a detoxifying enzymes (Mehrotra and Phokela, 1996). There are three major types of detoxification

enzymes: 1) broad-spectrum oxidases such as mixed function oxidases or monoxygenases that include cytochrome P-450 enzyme system, 2) hydrolases that breaks up esters, ethers and epoxides and 3) conjugation systems such as glutathione *S*-transferase, which are mediated to cover up the reactive part of the toxic chemical and further facilitate its removal. Every type of detoxification enzyme has been documented to play a role in the development of some form of resistance against various classes of insecticides. Organophosphorous and pyrethroid insecticides are mainly degraded by hydrolases and the involvement of an increased hydrolytic enzyme activity may be suspected when insects develop resistance against these chemicals (Matsumura, 2003). Incresed carboxylesterase and acetylcholinesterase are often associated with insecticide resistance (Abdel-Aal *et al.*, 1993). Esterases, in general have been noted to play a number of significant role in the hydrolysis of various chemicals that contain ester linkages and degrading various pesticides with carboxyl or amide-groups. These pesticides include organophosphates and synthetic pyrethroids (Krueger and O' Brien, 1959; Needam and Sawicki, 1971; Miyamoto and Suzuki, 1973; Motoyama and Dauterman, 1974; Hama, 1976; Yu and Terriere, 1977; Hughes and Devonshire, 1982; Oppenoorth, 1982; de Malkenson *et al.*, 1984). Differences in the amount of esterase activity between two strains of the same insect species is considered an indicator of relative sensitivity to certain insecticides, subsequently, various biochemical assays have been used for insect populations as possible indicators of insecticide resistance (Brown and Brogdon, 1987). One of the enzyme assays uses alpha-napthyl acetate (α-NA) as a model substrate for general esterase activity in wide variety of insects (Devonshire, 1977; Georghiou and Pasteur, 1978, 1980; Ferrari *et al.*, 1993). Either high α-NA activity or extra esterase bands stained with α-NA have been associated with resistance (Lalah *et al.*, 1995). A polyacrylamide gel electrophoresis assay has

been used to identify and characterize greenbug resistance (Sivakumaran and Mayo, 1992; Shufran *et al.,* 1993; Shufran and Wilde, 1996). Electrophoretic studies on organophosphate resistance of the cotton aphid, *Aphis gossypii* have indicated clearly the involvement of particular distinct isozymatic forms of carboxylesterase in resistant strains (Owusu *et al.,* 1996). The greenbug, *Schizaphis graminum* produced a single esterase band that was either absent or undetectable in susceptible green bug (Ono *et al.,* 1994).

3.5. Specific role of esterases, glutathione *S*-transferases and acetylcholln--esterases in insecticide resistance

Esterases are the most significant enzymes in insect causing insecticide detoxication. Organophosphate (OP), carbamate and pyrethroids contain carboxylester and bonds that are subject to attack by esterase enzymes. These esterases can often be separated into isozymes with different substrate specificities. Insect esterases are very diverse and can include monomers, dimmers and multimers, which mean that their relative molecular mass can cover a wide range. Polymorphism is a notable characteristic of insect esterases. Multiple forms of esterases are present in the soluble, cytosolic fraction of insect (Brattsten, 1992; Dauterman, 1985). Of the multiple forms of esterase isozymes that exist in insects, few participate in insecticide metabolism (Maa and Terrier, 1983). Each isozymes probably has a certain range of substrates. Unlike the monooxygenase reaction, esterases use low energy co-factors (Dauterman, 1985). Different types of esterases (A1, B1, A2, B2) have been recognized in organophosphate (OP) insecticide resistant populations of *Culex pipiens* complex throughout the world (Poirie *et al.,* 1992) and overproduction of nonspecific esterases is a common mechanism of

resistance. For B1, resistance to OP insecticides has been shown to be due to sequestration of insecticide and overproduction of esterase B. This production at enhanced scale is due to gene amplification (Callaghan *et al.*, 1994). The current work of Jayawardena *et al.* (1994) on *Cx.quinquefasciatus* revealed that a strain with elevated A2 and B2 is resistant to a broad range of organophosphate insecticides. Enzymatic assays suggested that sequestration rather than metabolism is the primary mode of operation of these esterases on malathion. The basis of malathion resistance in the adults of *Anopheles arabiansis* from Sudan was a carboxylesterase (Hemingway, 1983) Malathion resistance due to an increase in degradation at the carboxylester linkage is a common detoxication pathway that has been implicated in *An.culicifacies* (Herath and Davidson, 1981); *An.stephensi* (Hemingway, 1982); *Blattella germanica* (Heuval and Cochran, 1965); *Cx.tarsalis* (Matsumura and Brown, 1963); *Tetranychus urticae* (Matsumura and Voss, 1964); *Tribolium castaneum* (Dyte and Rowlands, 1968). Esterase cleavage has been implicated in OP and pyrethroid resistance in *Musca domestica* (Funaki *et al.*, 1994). Resistance to pyrethroids in *Blattella germanica* was partly due to elevated esterases (Atkinson *et al.*, 1991). Esterase dependent cross-resistance between OPs and pyrethroids have been detected in several species. In OP resistant *M.domestlca* and *Culex* mosquitoes, the esterases responsible for cross-resistance are thought to be involved in pyrethroid hydrolysis (Soderlund and Bloomquist, 1990). The best documented example of esterase based metabolic resistance to OPs, carbamate and pyrethroid insecticides is that found in Aphid, *Myzus perslcae* (Devonshire and Moores, 1982) in which the overproduction of esterases FE4 and E4 responsible for insecticide hydrolysis and sequestration have been shown to be caused by amplification of a structural gene. The massive overproduction of any esterase protein by resistant *M.persicae* may

result in the detoxication of insecticidal esters first by sequestration and then by hydrolysis (Devonshire and Field, 1991, 1995).

Gel electrophoresis techniques have been used extensively in the investigation of the taxonomy, systematics and population genetics of a wide range of animals and plants. The techniques involve the electrophoretic separation and specific histochemical staining of enzymes and allow examination of the variation of immediate protein products of genes (Ayala, 1983; Easteal and Boussy, 1987). The genes at different loci, giving rise to families of enzymes called isozymes and these may be of different molecular weight and electric charge and hence show mobility variation when separated on an electrophoretic gel (Loxdale, 1993). Electrophoresis has also aided the monitoring and investigation of insecticide resistance in populations of some aphid species like *Myzus persicae* (Devonshire, 1975a; Sawicki *et al.,* 1978; Baker, 1979) and in *Aphis gossypii* (Owusu *et al.,* 1996). In *Schizaphis graminum,* using α-napthyl acetate as substrate for nondenaturing polyacrylamide gel electrophoresis (PAGE) showed several strongly stained esterase bands with faster electrophoretic mobility from the organophosphate resistant greenbugs and these esterases were absent or expressed at low levels in the organophosphate susceptible greenbugs (Ono *et al.,* 1994; Zhu and Gao, 1998; Zhu and He, 2000).

Glutathione *S*-transferase (GST) is a family of multifunctional isozymes found in all eukaryotes. One of the main functions of GST is to catalyse xenobiotics, including pesticides in the marcapturic acid pathway leading to the elimination of toxic compounds (Hayes and Pulford, 1995). In insects, this family of enzyme has been implicated as one of the major factors in neutralizing the toxic effects of insecticides (Clark *et al.,* 1986; Grant *et al.,* 1991; Salinas and Wong, 1999). The majority of studies on insect GSTs have focused on their role in detoxifying foreign compounds, in

particular insecticides and plant allelochemicals and more recently, their role in mediating oxidative stress responses (Clark *et al.*, 1986; Wang *et al.*, 1991; Fournier *et al.*, 1992a; Ranson *et al.*, 2001; Vontas *et al.*, 2001; Sawicki *et al.*, 2003). GSTs are important in phase I metabolism of organophosphorous and organochlorine compounds and play a significant role in resistance to these insecticides in insects (Yu, 1996; Clark and Shamaan, 1984; Ranson *et al.*, 1997). Many studies have shown that insecticide resistant insects have elevated levels of GST activity in crude homogenates, and have suggested the possible role of GSTs in insecticide resistance (Armstrong and Suckling, 1990; Reidy *et al.*, 1990; Smirle, 1990; Kao *et al.*, 1989; Clark *et al.*, 1986; Ottea and Plapp, 1984). In insects, GST isozymes are present in three to four forms in house flies and at least two in Australian sheep blowflies (Clark and Dauterman, 1982; Kotze and Rose, 1987). Several insecticide resistant strains of housefly have been reported to have elevated GST activity in crude homogenates against organophosphates (Clark *et al.*, 1986; Wei *et al.*, 2001).

The GSTs are involved in O-dealkylation or dearylation of organophosphates (OPs) (Hayes and Wolf, 1988). High frequencies of profenofos resistance were correlated with GST activity toward 1-chloro-2, 4-dinitrobenzene in larvae of *H.virescens* that were collected in Louisiana cotton fields during the 1995 cotton growing season (Harold and Ottea, 1997).

Moreover, GSTs are involved in the dehydrochlorination of DDT in *M.domestica* (Clark and Shamaan, 1984) and are the primary metabolic mechanism of resistance to this insecticide. Finally, a recent study suggests that GSTs act as antioxidant-defense agents and confer pyrethroid resistance in *Nilaparvata lugens* and possibly in other insects (Vontas *et al.*, 2001). Enhanced activities of GSTs that confer insecticide resistance result from both quantitative and qualitative alterations in gene expression. First, there is evidence for over-expression of one or more GST isoforms

in resistant insects. For example, the high activity found in an insecticide-resistant strain of *M.domestica* is correlated with high level of GST1 transcript (Fournier *et al.*, 1992a). Similar phenomena were also found in insecticide-resistant *Aedes aegypti* (Grant *et al.*, 1991), *Anopheles gambiae* (Prapanthadara *et al.*, 1993; Ranson *et al.*, 2001), and *Plutella xyostella* (Ku *et al.*, 1994). Moreover, qualitative differences of GSTs were also present between susceptible and resistant insects. For example, most subcellular fractions from susceptible *M.domestica* had higher conjugation activities toward 1-chloro-2, 4-dinitrobenzene than the fractions from the Cornell-R strain, but all fractions from the susceptible strain had lower conjugation activities toward 1, 2-dichloro-4-nitrobenzene than fractions from the Cornell-R strain (Chien *et al.*, 1995). In addition, quantitative and qualitative alterations can be co-expressed and confer resistance in one resistant strain. For example, GSTs from a DDT-resistant strain of *An.gambiae* had an altered GST profile and one of the GSTs was increased 8-fold in a resistant strain compared with the susceptible strain (Prapanthadara *et al.*, 1993).

The major target site for both OPs and carbamates (O'Brien, 1960) is acetylcholinesterase, which acts by binding to the neurotransmitter (acetylcholine) in some synapses of the nervous system. Reduced sensitivity of acetylcholinesterase (AChE) to these insecticides is well-studied and has been expressed in a number of insects (Oppenoorth, 1985), such as *M.domestica* (Walsh *et al.*, 2001), the aphid, *Nasonovia ribisnigri* (Rufingier *et al.*, 1999), the Colorado potato beetle, *Leptinotarsa decemlineata* (Zhu *et al.*, 1996), and the fruit fly, *Drosophila melanogaster* (Mutero *et al.*, 1992). Reduced sensitivity of AChEs to OPs and carbamates is also expressed in *H.virescens* as a major resistance mechanism. For example, compared with a susceptible strain, AChE activity from a methyl parathion resistant strain of *H.virescens* was 22-fold less sensitive to inhibition by methyl paraoxon in both larvae

and adults. This resistant AChE was also less sensitive to structurally analogous OPs, and to the N-methyl carbamates (Brown and Bryson, 1992). Similar results were demonstrated in thiodicarb-selected larvae (Zhao *et al.,* 1996) and OP and carbamate-resistant adults of *H.virescens* (Kanga and Plapp, 1995). Reduced sensitivity of AChE to OPs and carbamates is conferred by point mutations. Although genetic and biochemical bases of reduced sensitivity of AChE have been examined in a number of insects, they have been well-studied in *D.melanogaster, M.domestica* and *L.decemlineata.* In the nucleotide sequence of AChE in *D.melanogaster,* Tyr^{109} was first reported as a potential site of a point mutation conferring resistance to insecticides (Mutero *et al.,* 1992). Subsequently, amino acid substitutions at four different sites (Phe^{115} to Ser, Ileu^{199} to Val, Gly^{303} to Ala, and Phe^{388} to Tyr) were found in OP-resistant *D.melanogaster* (Fournier *et al.,* 1993) and at five different sites (Val^{180} to Leu, Gly^{262} to Ala or Val, Phe^{327} to Tyr and Gly^{365} to Ala) in *M.domestica* (Walsh *et al.,* 2001). The expression of varied combinations of mutations in a single AChE gene resulted in different patterns of resistance among OPs, and levels of resistance increased in an additive way. To date, more mutations in AChEs of *D.melanogaster* have been reported as being possibly involved in OP and carbamate resistance, but their toxicological significance has not been demonstrated (Villate *et al.*, 2000). Interestingly, reduced sensitivity of AChEs to OPs and carbamates also occurs by replacement of a different amino acid (Ser^{238} to Gly) in an azinphosmethyl-resistant strain of the Colorado potato beetle, *L.decemlineata* (Zhu *et al.,* 1996).