Review of Literature

III. REVIEW OF LITERATURE

3.1. POPULATION DYNAMICS

Natural complexes of plant and animal species together with their physico-chemical environment constitute ecosystems. The interactions between the environment and population of each species determine the extent of the fluctuations and these interactions form the basis of population ecology studies. Member of any one species are affected by a large complex of factors, the biotic part being composed of a web of interactions involving many different species. A comparison of the total insect population in any agro – ecosystem under the impact of cultivation should serve to sufficiently emphasize the need for taking an overall picture of the population trends of insect pests confined to crops and weeds around (Ananthakrishan *et al.*, 1986). There are greater variety of species in the grasslands enabling a continuous inflow into the cultivated fields, where some species become better adapted, so that the role of grasses and other weeds as the original source or alternate host of pest species cannot be ignored. (Uvarov,1964). The association between insects with its host plants can often be dynamic, involving the mutual adaptations between them resulting in phytochemical diversity on the one hand, as well as the tolerance of insects to the chemical protection of plants on the other (Pitkin, 1976, Tietz, 1972).

Thus the diversity and variability of insect–plant relationships would comprise (a) the study of feeding behaviour of plant eating insects in response

to the primary and secondary substances present in the host plants, (b) the impact of the quality of the host plant on the growth and fertility of the insect, and (c) the influence of crop and weed hosts on the rate of colonization and population dynamics of phytophagous insects (Ananthakrishan, 1986).

3.1.1. COMMON THRIPS

Thrips population in an agro-ecosystem is primarily determined by the host plant, ideal climatic conditions, protection from natural enemies and the density of immigrants or an equilibrium stage of immigrant and emigrant populations (Varatharajan and James Keisa, 2000). If the above factors are favourable, the density of the pest increases and at times reaches the economic threshold level. Thrips are generally known to be more active during dry season. But, at least a small proportion of them is maintained throughout the year in one or the other host due to their polyphagous nature (Ananthakrishnan, 1984).

Though considerable works has been done on population dynamics of different species of thrips on tea, very little has been done in this area for *Mycterothrips setiventris* in Darjeeling after the early study by Lefroy (1909), Antram (1909) and then Andrews (1925). Andrews (1925) reported that as a general rule, thrips began to attack the bushes towards latter part of May, which extended through June –July followed by a lull and then a second attack towards the end of the season in October. Most of the damage was caused in the early part of the season before plucking. With the commencement of plucking considerable number of young and adult insects and eggs were

removed, controlling the population to some extent.

In general, adults of thrips are said to be phototactic i.e. they prefer places where intensity of illumination is greatest (Bedford, 1943). Hence, it's infestation is severe during hot dry period particularly on unshaded tea. Though Antram (1909) considered that shade encouraged the thrips species in Darjeeling, but Lefroy (1909) found the pest less active under shade.

In the plains of North-East India, the species *Scirtothrips dorsalis* Hood reported to occur almost throughout the year on tea, but rapid build up started from March – April when prolonged droughty conditions prevailed and the peak of incidence was observed during May. After the monsoon there was a decline in population. During cold weather very few thrips might prevail in the pruned sections, but in the unpruned and young tea sections it persisted (Anonymous 1994). In a population dynamic study on young tea clones (TV 1, TV 18, TV 25, TV 26) at Darjeeling plains, infestation of *S. dorsalis* was observed throughout the year on all the clones, with a population rise in the winter months (November to January) and a sharp increase in population in the summer months (May to July) (Sannigrahi and Mukhopadhyay, 1993 and Mukhopadhyay, Sannigrahi and Biswas, 1997). Maitra (1994) also observed that *S. dorsalis* occurred in highest number in May to July and the population declined with onset of heavy showers by end of August.

In tea fields of Japan, adults of *Scirtothrips dorsalis* were found in the leaf zone, branch zone, litter and soil throughout winter of 1979-1980 suggesting that the thrips hibernate in the adult stage during winter. The adult

21

showed minimum activities from mid December to mid March, but some were active in tea grown throughout the winter. The over wintered adults emerged from litter and moved to the leaf zone in late March after a few days of high temperature. After feeding on and laying eggs in new leaves, most of them died before the end of April and some before mid May. The life span of the over wintering adults appeared to be about five months (Okada and Kudo, 1982). Sakakibara and Nishigaki (1988) also considered that adult of *S. dorsalis* over wintered as adult in the tea fields of Japan.

Sasidhar *et al.* (1999) reported that the population of thrips species, *Scirtothrips bispinosus* Bagnall, active in South India, started to build up by November – December and reached the peak in March–April, when temperature was 27 to 32 ^{0}C. Intensity of shade played a predominant role in determining the population density of thrips. Unshaded tea fields always harbour more number of thrips.

Mkwalla (1982) reported from Central Africa that *Scirtothrips auratii*, the South African citrus thrips, occurred in tea all the year round. The bulk number occurred on the host in dry season between September and December. Though the period between January and March had the optimum temperature, but wetness kept the number down. A few days of dryness in this period was likely to be reflected in a rise in number. Population was lowest during April–August, because of low temperature. All developmental stages of the insect were present at any time resulting in overlapping of generation. There was no resting stage and it was likely that the few individuals, who survived the unfavourable temperature during April –July, formed the basal population for

the following period of September – December.

Population studies on *S. kenyensis* in Kericho, Kenya revealed that the pests remained in the field throughout the year, highest and lowest population being observed during dry and wet season respectively. The lowest population during wet season had been attributed to rain wash or other natural control agent. There appeared to be a negative relationship between the monthly fluctuation of thrips populations and rainfall data. However, the minimum and maximum temperature did not have any relationship with thrips population. (Sudoi, 1985). He also reported that number of thrips in unshaded area was significantly higher than the area under *Grevillia* shade.

In the above reviews, some behaviours and characters are found to be common in all the thrips species infesting tea in different parts of the world. These are :

   i) They prefer unshaded condition;

   ii) Their population is the highest during dry period, lowest during rainy period and winter with low temperature and

   iii) They prevail throughout the year with drastic reduction in population during adverse period.

3.1.2. GREEN FLY

This insect is highly polyphagous infesting several cultivated crops. *E. flavescens* attacks tea in Bangladesh, China, Japan and Vietnam, but it is rarely seen on tea in south India (Muraleedharan, 1991). It is widely distributed

in all the tea growing district of North East India including Darjeeling. It was reported from plains of North–East India where the insect occurred throughout the year. During cold weather very few active stages were found on pruned and deep skiffed tea bushes while a large number of them occurred in unpruned, newly planted young tea, and nursery having some new growth. The pest was found more active during March-July. Along with increase in temperature from March the insect multiplied rapidly to become severe in May – June. The infestation continued up to July. From August the population suddenly declined to a negligible number followed by a slight increase in November. The attack however commenced later in Darjeeling, where June – July was regarded as the green fly season (Anonymous 1994). Andrews (1923) also reported that the seasonal cycle of greenfly synchronized well the flushing period of the bushes.

E. formosana, the important pest of tea in Taiwan, was reported to cause heaviest damage from May to July which varied with tea variety (Chen *et al.*, 1978).

The green leafhopper, *E. pirisuga* was found to attack tea in china. Zhang *et al.* (1994) reported that the population density of tea jassid (*E. pirisuga*) in tea field of China was negatively correlated with the thickness of the palisade tissue, the spongy tissue and the hypoderma and the number of collenchyma layer in the undersurface of the main vein of tea shoots. The population density was found significantly and negatively correlated to the caffeine content and the soluble protein content of the shoots. A very significant and positive correlation existed between the damaging index by the

pest and the amount of pubescence on tea shoots. Cheng and Cheng (1994) using the data from 9 years' observation on population of *E. pirisuga* on tea in China and related meteorological data found that the sum of 10-day average temperatures in January and February as the most appropriate factor for use in a regression equation to predict the date of the start of the first population peak of the pest. Two peaks of population of *E. perisuga* were reported to occur annually in china on tea, the first peak causing serious damage to tea production in April (Lu, *et al.* 1994).

The peak period of adult occurrence of *E. onukii* in tea field of Japan was in early July, based on captures with yellow sticky traps (Mochizuki, *et al.* 1994).

Working on incidence of *E. kerri* on pigeonpea cultivars, Sekhar *et al* (1993) reported that among the different weather factors, daily mean temperature and relative humidity (morning) had significant positive influence on the population of *E. kerri*. A good rainfall followed by a dry spell with daily mean temperature in the range of 17 – 28 ^{0}C was found favourable for *E. kerri* on pigeon pea cultivars. The population increased as the crop growth progressed with a peak from first fortnight of September to first fortnight of November.

3.1.3. APHID

The tea aphid, *Toxoptera aurantii* Boyer de Fons (Homoptera, Aphididae) is one of the most polyphagous pests attacking a number of

cultivated crops. It is one of the most common pests in tea and widely distributed throughout the tea growing district of North-East India. In plains, pruned tea was reported to suffer from mid January onwards because during that period migration of winged aphid started and they settled on almost all the new growth of buds and shoots (Anonymous 1994). At Tocklai, Assam, India, it was observed that the alate females started appearing in winter months during January–February, with a highest population in February, followed by a gradual decline until August when an increase was noticed. The highest and lowest populations were recorded in the outer and inner zone of tea bushes (Das and Kakoty, 1992). Sannigrahi and Mukhopadhyaya (1993) also reported from Darjeeling plains that the months of February and March appeared to be favourable for incidence of aphid on four tea clones studied namely, TV1, TV18, TV 25 and TV 26 followed by a higher incidence during the month of July excepting that on TV 1.

Han (2002) reported that in Chinese tea garden *T. aurantii* generally reproduced 20-30 generations, whilst the young tea shoots sprouted four or five times per year and therefore always remained colonized by aphid population.

3.2. ALTERNATE HOST

Alternate hosts play a very important role in maintaining the life cycle of any pest by supplying food during the period of paucity in the main host. Van Emden (1965) had cited about 442 examples of weeds or wild plants as hosts of crop pests or disease organisms. Alternative host plants are particularly important where the cropping season is short and followed by a cold or dry season of fallow or total replacement by a different cropping system (Van Emden, 1981). The nature of weed–crop interactions with respect to the seasonal fluctuation of phytophagous Thysanoptera has been investigated in detail. Ananthakrishan and Thirumalai (1977) have mentioned the role of the weed *Chloris barbata*, abundant in *Pennisetum typhoides* fields as an alternate host of *Chirothrips mexicanus*. The inflorescence of *Echinochloa crusgalli* also harbours numerous adults and larvae of *Haplothrips ganglbaueri*, the weed acting as an important alternate host for the thrips in paddy fields. *Caliothrips indicus* an important thrips pest of groundnut, *Arachis hypogaea* was also found to colonize in large numbers on the weed host *Achyranthes aspera* (Daniel *et al.*, 1984), almost throughout the year although its infestation on *A. hypogaea* was seasonal.

However, there was no record available on any alternate host of the thrips species *M. setiventris* under this study. The greenfly (*E. flavescens*) was also reported to attack castor (Regupathy, 1989, Anonymous, 1980) other than tea. The aphid *T. aurantii* was observed to attack a number of hosts like citrus (Anonymous,1980, Regupathy, 1989), jackfruit, coffee (Regupathy, 1989) etc, but it is most common on citrus and is commonly known as citrus aphid.

3.3. NATURAL ENEMIES OF TEA PESTS

Muraleedharan *et al.* (2001) recently published a detailed list of parasitolds and predators of tea pests in India. They reported that an array of parasitic and predatory arthropods was active in the tea ecosystem. Predatory mites belonging to Acaridae, Anystidae, Ascaidae, Bdellidae, Cunaxidae, Phytoseiidae and Stigmaeidae fed on phytophagous mites. Aelothripids and thripids were the main predators of thrips and mite pests. Coccinellidae, Carabidae and Dermestidae had several representatives feeding on mites, aphids and scales. Many anthocorids, pentatomids, reduviids and lygaeids acted as general predators in tea fields. Members of Chrysopidae, Hemerobildae and Coniopterygidae were the natural enemies of aphids, spider mite, and microlepidoptera. Under Diptera syrphids larvae were the main aphid predators. Parasitic Diptera included species of Tachnidae, Muscidae and Sarcophagidae. There were large numbers of hymenopteran parasitoids exerting control over caterpillar pests. Aphelinids, aphidiids, bethylids, braconids, ceraphronids, chaicidids, elasmids, eulophids, eurytomids, ichneumonids, pteromalids and scelionids played a vital role in the suppression of several lepidopterous pests. Das and Mukhopadhyay (2002) and Anonymous (2003 b) listed the natural control agents that were active in Darjeeling Terai, Dooars and lower elevation of Himalayan foot hills. In these areas, aphids, jassids and thrips were found to be predated by both adult and grub of coccinellids, *Coccinella septempunctata, Coccinella transversalis, Scymnus* sp.; *Aspidimerus circumflexa* (NR), *Oenopia sexareata* (NR) *and Ola* sp. (Anonymous 2003 b). The above workers also reported that the minor status of many of the tea pests in India was obviously due to the influence of

biocontrol agents.

3.3.1. COMMON THRIPS

No earlier record was found on natural enemies of Darjeeling thrips - *Mycterothrips setiventris*. Muraleedharan *et al.* (2001) reported from South India that nymphs and adults of tea thrips *Scirtothrips bispinosus* was attacked by predators – *Aeolothrips intermedius*, *Mymarothrips garuda*, *Anthocoris sp.* and *Orius sp.* Predation of *Scirtothrips dorsalis* by the geocorid predator *Geocoris ochropterous* Fieber has been reported by Sannigrahi and Mukhopadhyay (1992). The predators of *Scirtothrips* spp. on tea in Kenia included Araneae, coccinellids, syrphids and formicids (Sudoi, 1987).

3.3.2. GREENFLY

A study on mortality of *Empoasca vitis (=flavescens)* caused by natural enemies in Hunan, China revealed that spiders were the most effective predators (Zhang *et al.* 1992). Chen (1992) also observed in laboratory that *E. pirisuga* was one of the main preys of spiders.

E. onukii was reported to be a major pest of tea in Japan, which was attacked by the egg parasite *Anagrus* sp. (Takagi,1978).

3.3.3. APHID

Extensive works were done on natural enemies of tea aphid, particularly

on its predators and parasitoids. In Chinese tea field, population of the ladybug, *Leis axyridis* (Coleoptera : Coccinellidae), closely followed the population of tea aphid, especially from May to July and September to November. Under laboratory conditions, an adult of the lady bug could consume 90-110 tea aphids and an adult of the lacewing, *Chrysopa septempunctata* (Neuroptera, Chrysopidae) could consume 80-100 tea aphids. The aphid parasite, *Aphidius* sp. (Hym., Braconidae, Aphidiinae) also attacked both nymphal and adult aphids. The average percentage of parasitism was around 10 % in May to June and 15 % in September to October. The pattern of parasitisation usually appeared to be highly aggregated with parasitism on groups of adjacent tea branches reaching over 90 % (Han, 2002).

In tea field of Tocklai, Assam, as high as eleven species of coccinellids attacking tea aphid were recorded. They were *Cryptogonus bimaculata* Kapur, *Scymnus* sp., *Coleophora biplagiata, Verania vincta, Menochilus sexmaculatus, Coccinella repanda, Leis dimidiata* var *quindecimmaculata, Leis dimidiata, Paeudaspidimerus circumflexus, Coccinella septempunctata* var *divaricata* and *Jauravia quadrinotata.* The feeding capacity of an adult of different species in its life span was found within the range of 1300 – 7640 aphids. The syrphid predators found to feed on tea aphid were *Syrphus balteatus, Syrphus serarious, Paragus indicus, Paragus verburiensis, Xanthogramma scutellare.* and *Asarcina aegrota.* The feeding capacity of the individual larva during the larval period was 305 – 590 aphids. The larvae of ant-lion, *Micromus timidus* Hagen (Hemirobidae, Neuroptera) was also recorded to feed on tea aphids with a maximum feeding capacity of an individual larva up to 116 aphids. The internal parasites *Aphelinus* sp. and

Trioxys sp. were also found to parasitized tea aphid at Tocklai. The degree of parasitism varied in between 4-13% and 22% respectively by both the species (Das and Kakoty, 1992).

Excellent natural control of *Toxoptera aurantii* by coccinellid, syrphid and hemerobid predators and aphidiid parasitoids was reported by Radhakrishnan (1989) from South India. Muraleedharan (1991) and Muraleedharan *et al.* (988 and 2001) also from South India, reported that the tea aphid was to a very large extent naturally regulated by bio-control agents, namely the larvae of syrphids – *Paragus tibialis*, *Episyrphus balteatus*, *Betasyrphis seratus*, *Allobaccha nubilipennis*, *Ischiodon scutellaris*, and *Dideopsis aegrota*; the coccinellids – *Cryptogonus orbiculus*, *Jauravia pubescens*, *Lemnia bissellata*, *Menochilus sexmaculatus*, *Pseudaspidimerus circumflexus*, *Scymnus pyrocheilus*; the neuropteran, *Micromus timidus* and the braconids *Aphelinus* sp., *Aphidius colemani*, *Lipolexis scutellaris*, *Trioxys* sp. and *Trioxys indicus* .

A large number of syrphid larvae were also recorded as active predators of aphids in lower Himalayas (Anonymous 2003 b). Mukhopadhyaya and Sanigrahi (1993) suggested *Geocoris ochropterus* as an efficient predator and control agent of tea aphids as they kill more prey than they consume. *T. aurantii* was also used as a main prey of spiders in the laboratory in China (Chen, 1992).

3.4. FEEDING IMPACT

Infestation by pests is known to cause several changes in the biochemical and physiological process of the plant affecting the yield and quality of the harvest to a great extent (Cowgill and Prance, 1989; Raffa and Berryman, 1982; and Gangnon, 1967). The changes in host plants due to pests attack may be due to mechanical damage, or certain toxic substances, and / or microorganisms injected into the host body during feeding by the pests (Carter, 1962). These pest-mediated changes are ultimately reflected in yield and quality of agricultural crops (Prestidge, 1982 and Berberet *et al.* 1981). The biochemical changes occur in host tissue as a result of antiherbivore defenses is becoming an important area of research (Lawton and Strong, 1981). It is now clear that many of the plants secondary metabolites protect the plants against attacks of herbivores and pathogens by interfering with their growth or germination (Rice, 1974). From the studies in the recent past a lot has been learned about the expression of plant defenses following insect attack (Green and Ryan, 1972; Karban and Baldwin, 1997; Mathew and Douglas, 1997). Many of these herbivore-induced plant defenses were known to be triggered by the perception of tissue damage and subsequent jasmonic acid (JA) signaling. While plants clearly perceived and responded to insect-associated elicitors (Turlings *et al.*, 1990), it was observed that mechanical damage alone initiated a cascade of plant responses, many of which were regulated by jasmonic acid (JA). Though Jasmonic acid regulated developmental processes ranging from germination to flower development, but its role in plant responses to biotic stress has attracted the most attention (Creelman and Mullet, 1997). Jasmonic acid-induced secondary metabolites

32

included alkaloids, steroids and terpenoids as well as protease inhibitors and polyphenol oxidase that were found to interfere with insect nutrition (Farmer and Ryan, 1990; Baldwin et al., 1994; Hopke et al., 1994; Thaler et al., 1996). Many JA-induced responses were demonstrated to protect plants against future attack and could be triggered by mechanically damaging plant tissues in the absence of actual herbivory (Baldwin et al., 1994; Thaler et al., 1996; Thaler 1999).

Other than JA, pests feeding was also reported to stimulate the production of ethylene. (Kendall and Bjostad, 1990; Kahl et al 2000). Feeding by Thrips tabaci induced greater ethylene production in intact onions than mechanical damage alone (Kendall and Bjostad, 1990). Ethylene regulated a wide array of plant responses including root hair formation, fruit ripening, and senescence (Johnson and Ecker 1998). Ethylene was also found to coordinate dynamic plant responses to both biotic and abiotic stresses including pathogens, insects, flooding, and wind. Ethylene could both inhibit and synergize specific plant defense responses to pathogens and insects (Penninckx et al., 1998; Kahl et al., 2000). In tomato, ethylene acted in part by influencing the production of JA and salicylic acid (SA) during wounding and pathogen infection, respectively (O'Donnell et al., 1996; 2001). Increased SA production during pathogen infection (Yang et al., 1997) resulted in broad-spectrum resistance to subsequent pathogen attack, a response known as systemic acquired resistance (SAR). It was observed that JA alone did not entirely explain the differential plant responses following mechanical damage and insect herbivory. Induced nicotine accumulation was found to be primarily regulated by wound-induced JA levels (Baldwin et al., 1994; 1997). The burst

33

of herbivore-induced ethylene inhibited wound-induced nicotine accumulation by repressing the levels of a key biosynthetic enzyme, putrescine N-methyltransferase, located in the roots (Winz and Baldwin, 2001). Musser *et al.* reported (2002) that glucose oxidase, one of the principal components of the corn earworm, *Helicoverpa zea* saliva was responsible for suppressing production of nicotine in tobacco, which otherwise produced as an induced defense mechanism by wounding. The factor, Glucose oxidase converted the simple sugar glucose into gluconic acid and hydrogen peroxide which reduced nicotine production by 29 and 44 percent respectively. Factors responsible for the induction of ethylene during insect herbivory have not yet been identified.

In many species of plants, systemic accumulation of leaf phenolic compounds to protect themselves from the invaders was observed (Feenstra *et al.* 1963; Feldman and Hanks, 1967; Hori, 1973). The attacked cells released factors for destruction and necrosis of neighbouring cells (hypersensitive reaction leading to metaplasia) to prevent further invasion (Hori, 1973). This might encourage release of enzyme polyphenol oxydase and peroxidase from the injured cells. In *Helopeltis theivora* infested tea leaves, the levels of catalytic enzymes, peroxidase and polyphenol oxidase were found about two times higher than that in uninfested leaves (Mazumdar, 1995). These enzymes were known to catalyze the oxidation of phenol producing quinones, which was toxic to the pests and acted as a plant defense mechanism. However, quinones were reported to be reversed to phenols by quinone reductase or transformed into insoluble brown non-toxic polymers, melanine like substances and coagulative protein complexes due to further oxidation in presence of polyphenol oxidase from the plant or parasites. Some

herbivores were known to have polyphenol oxidase system in their saliva and can transform quinone, into non-toxic substances (Miles, 1968 b, 1969; Mukhopadhyaya et al. 1997). Similar observations were made by Ishaaya (1971) and Hori (1973) in lemon buds infested by Aonidie aurantii and sugar beet leaf by Lygus disponsi respectively.

Wickremasinghe (1974) reported a positive role of sucking pests in flavour development by reducing chlorophyll content of the fresh tea leaves.

The emission of volatile compounds due to pest feeding has been well documented in some recent studies and large number of volatile compounds has been identified with their biosynthetic pathways as potential mediators of plant-insect interactions. It was reported that wounding by pests generated green leaf volatiles, such as mixtures of C6 alcohols, aldehydes and esters, through a lipoxygenase pathway mediated oxidation of membrane-derived fatty acids (Blee, 1998), The shikimic acid / tryptophan pathway resulted in the nitrogen containing volatiles, such as indole (Frey et al., 2000). Some volatiles were sequestered as glycosides and immediately released during insect damage through the action of glycosidases (Boland et al, 1992). In contrast, many herbivore-induced volatiles showed a significant delay between the time of feeding and detection of emission. Unlike damage-dependent constitutive volatiles, many insect-induced volatiles display delayed induction, diurnal cycling of emission, continued emission after cessation of herbivory, and de novo biosynthesis (Loughrin et al., 1994; Paré and Tumlinson, 1997). However, the role of herbivore saliva in volatile emission and general expression of direct defenses is unknown at present.

3.4.1. GREEN FLY

The functional mouth of green fly is formed by paired mandible and maxillae, which lies at the tip of the stylet bundles. The maxillary grooves serve as two opposing tubes during feeding. Saliva is pumped downward through one of them and host fluid is sucked upward through the other. To facilitate piercing by stylet into plant tissue while feeding, the salivary enzyme is believed to dissolve or soften pectate layers of mid lamellae (McAllan and Adams, 1961). During feeding the stylet is applied to the substrate first and then the sheath material is discharged as a viscous fluid which begins to jel as soon as it leaves the styletes. The material sticks to the surface of the plant tissue surrounding the tip of the stylet bundle which fills the space between it and the labium up to the level of labial clamp (Auclair, 1963). Then a characteristic structure is formed due to rapid jelling of the secreted material. This is variously called a plug, a collar and a flange (Miles, 1959; Sylvester, 1962; Nault and Gyrisco, 1966). Utilities of stylet sheath studies in assessing host plant susceptibility to a number of leafhopper species was demonstrated by several workers (Miles, 1972; Backus, 1985; Backus et al., 1988).

Earlier workers reported the relation in between leafhopper population and crop loss in many cultivated crops (Parnell et al., 1949; Allan et al., 1940). It was reported that a low density of leafhopper (Empoasca fabae) could reduce the yield of potato to a great extent and its increase did not result in a proportionate damage (Peterson and Granovsky, 1950). However, in case of Empoasca solana, injury of plant tissues varied directly with insect population and the plants recovered after the insect was controlled (Martin and

Pemberton, 1942).

The plant sucking arthropods were reported to inject substances into plant tissues which increased their nutritional quality. For example, the hemipterans were observed to contain polyphenol oxidase enzymes in their saliva which oxidized the defensive polyphenolic compounds of plant (Miles, 1968a, 1968b, 1978). Mukhopadhyay *et al.* (1997) also reported that the levels of enzymes peroxidase and polyphenol oxidase in *Helopeltis theivora* infested tea leaf significantly increased by 1.87 and 1.86 times respectively of the uninfested leaf within first 12 hours of feeding. The changing proportion of the enzymes in the infested leaf tissue indicated gearing up of biochemical mechanism related to the possible resistance in the host plant.

There is a common and age old belief amongst the tea planters of Darjeeling that infestation of tea shoot in field improves flavour in made tea of Darjeeling tea. Long back Grice (1967) tried to establish the correlation in between greenfly infestation and flavour improvement in Darjeeling tea through organoleptic taste of made tea produced from greenfly infested and uninfested tea shoots, but without any biochemical analysis. However, he did not find any significant improvement of flavour in tea made from green fly infested shoots over that made from uninfested ones. But, Borah (1996) reported that polyphenolic contents of tea cultivars increased with jassid infestation. Particularly, the catechins and their gallates namely (-) Epigallocatechin gallates, (-) Epigallocatechin and (-) Epicatechin gallates were found to increase significantly with jassid-infestation in all the cultivars under study. But, the rise in their content was found to slow down gradually with high level of

infestation, though their amounts in no case had been recorded less than their normal uninfested counterparts. He observed a reduction in the protein content of the jassid-infested tea leaves in all the cultivars under study (1.5% to 2.1%). This reduction in the protein content might help to develop tea quality and to increase flavanol content in such jassid-infested leaves. Because, as a result of protein flavanol interactions, insoluble compounds could be produced thus reducing the flavanol levels and affecting the made tea quality adversely. (Bokuchava and Skobeleva, 1969). An enhance in enzyme polyphenol oxydase (PPO) activities was also noticed along with the rise in polyphenolic content. Several other workers (Gagnon, 1967; Finger, 1994; Thipyapong et al., 1995; Matthew and Douglas, 1997) also reported such rise in the PPO activities as a part of plants' defense mechanism. The PPO activity plays a vital role in tea fermentation (oxidation) to convert polyphenols to characteristic pigments namely theaflavin (TF) and thearubigin (TR) of black tea. It was also reported to provide protection to tea plant against pests (Wickremasinghe, 1974).

Tamaki et al. (1990) observed higher ratio of linalool derivatives to linalool in Pomfon oolong tea injured by the cicadellid *Empoasca onukii* than uninjured oolong and black tea, and concluded that the chemical characteristic of aroma were probably formed as a result of damage caused by *E. onukii*.

3.4.2. THRIPS

The characteristic mouth parts of thrips are asymmetrical where only left

mandible is well developed. It is protected within a mouth cone and projecting downwards from the ventral surface of the head. While feeding, the left mandible is used for rasping the leaf surface and the content of the epidermal cells are sucked using the feeding tube formed by coadapted maxillary stylets.

Sections of the mouth cone including the stylet showed that the mandible is a closed tubular structure and that the maxillary stylets are grooved, i.e. C-shaped in section (Mickoleit, 1963). Grasse (1951) stated that although the maxillary stylets did not show the mutual adaptations found in the Hemiptera they formed a tube when fitted together. Grinfel'd (1959) suggested that thrips might suck food through the tube formed by the stylets in addition to applying the mouth cone directly onto the food. Mound (1971) reported the studies with stereo-electron microscope that, though single mandible was a stout structure with a solid apex, the maxillary stylets were not simple needle like structure in any of the major groups of thrips. These stylets were actually complex and mutually adapted, although in a very different way from those of Hemiptera. Lewis (1991) suggested that the mouth cone was pressed against leaf surface while feeding and as a result mandibular stylet pierced the substrate. In the mean time, maxillary stylet formed a groove through which plant sap was sucked in by the action of cibarial pump mechanism.

Working on thrips species, *Scirtothrips dorsalis* and aphid- *T. aurantii*, Mukhopadhyay *et al.*(1997) suggested that the biochemical changes occurred in tea leaf by thrips attack had certain similarities with those of the aphid infested leaf, where the protein in the vascular bundle (mid rib) area and adjacent leaf blade region got depleted. This was possibly due to constant

siphoning of the leaf sap by aphid (protein could be located in the same area of the control leaf) and its break down into amino acids due to extra oral digestion process. Depletion of starches was also evident in the aphid infested leaves. But, an increase in the lipid level in the vascular bundle and especially in the dorsal layer of tea leaf tissue was apparent. Leaf protein and starch resources were found low in thrips infested leaf also. An increase in tannin level possibly indicated the gearing up of the plant defense system (Maitra 1994).

Lefroy (1909) and Antrma (1909) reported that china hybrid *jats* were more sufferer by thrips attack than "good *jats*". But it was not clear what exactly they tried to mean by good *jats*, most probably Assam type *jats*.

Mkwaila et al. (1979) reported as high as 16 % and 36 % damage by thrips in late pruned tea at two different sites respectively in Central Africa within a certain period of the season.

Some tea planters of Darjeeling are of the opinion that like greenfly, infestation of thrips also improves the cup quality of tea by increasing the flavour. However, some are of the opinion that "thrippy" tea is devoid of flavour and it is flat. Though during the quality period i.e. March to June many planters do not like to take control measures against thrips and greenfly, it is doubtful whether the crop loss is compensated by improvement in quality or not. But, all are in agreement that they cause heavy loss of crop every year in Darjeeling (Anon, 1994; Tamaki *et al*,1990).

Though some information are available on the effect of greenfly infestation on made tea quality (Borah, 1996), but nothing has been done so far to study the impact of thrips feeding on made tea quality. Hence, present study was undertaken to investigate if there is any relation between greenfly and thrips infestation and made tea quality in Darjeeling tea.

3.5. CHEMICAL CHARACTERISTICS OF TEA

A typical harvestable tea shoot is composed of all the essential chemical and biochemical constituents like enzyme, intermediates, structural elements, flavour and colour precursors etc. and these determine the final quality of manufactured tea (Table-1). However, their level varies depending on geographic location, agronomic practices, method of plucking etc. (Wood *et al.*, 1964, Bhatia and Ullah, 1968).

The chemical constituents of tea can broadly be grouped in phenolic and non-phenolic compound as described below.

3.5.1. PHENOLIC COMPOUNDS

In tea shoots, the phenolics or polyphenolic compounds are the major constituent making up 25 -30 % of the dry weight. They are responsible to form the precursors of the non-volatile components of black tea which are essential for the character of the beverage. There are four major categories namely the flavanols or catechins, the flavonols, the flavandiols and phenolic acids and their derivatives (Millin and Rustidge, 1967).

41

Table 1. : Chemical composition of green tea shoots (Assam variety)

Substance soluble in hot water	Approx. Dry weight %
Flavanols (-) epi-gallocatechin gallate (EGCG)	9-13
(-) epi-catechin gallate (ECG)	3-6
(-) epi-catechin (EC)	1-3
Other flavanols	1-2
Flavonols and flavonol glycosides	3-4
Flavandiols	2-3
Phenolic acids and esters (depsides)	5
Total polyphenols	30
Caffeine	3-4
Amino-acids	4
Simple carbohydrate	4
Organic acid	0.5
Substances partially soluble in hot water	
Polysaccharides : starch, pectic substances,	1-2
Pentosans, etc.	12
Proteins	15
Ash	5
Substances insoluble in water	
Cellulose	7
Lignin	6
Lipids	3
Pigments (chlorophyll, etc.)	0.5
Volatile substances	0.01-0.02

Adapted from Millin and Rustidge (1967) and Borah (1996)

3.5.1.1. The flavanols or tannins

They are most important components of the tea leaf contributing to the essential characteristic of black tea like colour, aroma and flavour. In the plant, they are produced from simple reducing sugar such as glucose either by acetic acid pathway or the shikimic acid pathway (Bokuchava and Skobeleva, 1969).

3.5.1.2. The flavonols

Though flavonol compounds are quantitatively important, but seem to play a very minor role in taste due to slight astringency or woody nature. They occur as a complex mixture in the fresh tea shoot and their characteristics are changed relatively during fermentation (Millin *et al.*, 1969).

3.5.1.3. Anthocyanin

Anthocyanin are colouring pigments and conspicuous in young shoots of first and autumn flush of growth (Baruah, 1990). They play a major role in the development of characteristic colour in processed tea.

3.5.2. NON-POLYPHENOLIC COMPOUNDS

3.5.2.1. Caffeine

Tea shoot contain caffeine up to 3-4 % which is known to increase

considerably during withering (wood et al., 1964). The terminal bud and first leaf of a tea shoot contain highest caffeine which may range from 4 to 5 %. This may reduce to 3 % in the second leaf and to 1.5 % in the stalk. Tea seed is devoid of caffeine. The tea shoots of assamica are richer in caffeine then those of sinensis variety (Ikeda et al., 1993).

Though caffeine dose not play an active role in the chemical changes take place during manufacture, but it has an important function in forming cream or precipitation formed when a tea infusion cools, which is a mixture of caffeine, theaflavin (TF) and thearubigin (TR) (Harler, 1970). Caffeine with other alkaloids like theobromine and theophyllin provide strength in a tea cup.

3.5.2.2. Carbohydrates

There are various types of carbohydrates in tea shoots like glucose, fructose, sucrose, rhamnose etc and complex polysaccharides like cellulose and hemicellulose (Bokuchava and Skobeleva, 1969). It plays the most important role in biosynthesis of polyphenols. Sugars and amino acids through non-enzymic browing reactions contribute to tea colour.

3.5.2.3. Protein and Amino Acids

Protein level is important for determining the tea quality. As a result of protein-flavanol interactions, insoluble compounds are formed reducing flavanol levels and thus affecting the quality of made tea adversely (Bokuchava and Skobeleva, 1969).

Out of the total free amino acid content in tea, theanine alone constitutes 50 %, but its role in tea quality is not clear. The other amino acids present in tea shoots are leucine, isoleucine, valine and phenylalanine.

3.5.2.4. Chlorophylls

Other than photosynthesis, chlorophyll is primarily responsible for the appearance namely blackness and brownness of made tea. It is reported that low level of chlorophyll in tea leaf as a major factor for aroma development in tea (Wickremasinghe, 1974). Low chlorophyll in plucked shoots is a desirable character to make flavoury tea in Darjeeling.

3.5.3. ENZYMES

The two main enzymes involved in tea manufacture are : (1) Pectase and (2) Polyphenol oxidase. Pectase acts on the carbohydrate in the tea leaf cell wall making them soluble. It probably functions to form a kind of varnish on the outside of the leaf which may aid in keeping quality of tea (Agarwal, 1989).

The polyphenol oxidase (PPO) is the most important enzyme which is of copper protein type and most readily acts on the catechin group of polyphenols in tea leaf to change them to orthoquinones. These orthoquinones by a process of dimerization condense to bis-flavanols and these in turn condense rapidly to theaflavins, which are yellow bodies. An additional oxidation not controlled by enzyme action transform these theafavins into thearubigins, which are red brown bodies with tanning properties.

Higher TF value in CTC teas as compared to orthodox teas is associated with higher activity of the oxidoreductase enzyme on the catechin substrate which inhibits the action of the hydrolytic enzyme, reported to be responsible for producing monoterpene alcohols in anaerobic conditions (Takeo, 1981 and Ullah and Roy, 1982).

3.6. TEA FLAVOUR AND TEA AROMA PRECURSORS

Taste and aroma both associated with the term tea flavour. Tea taste is the contribution of non-volatile compounds like catechins and their derivatives, caffeine and amino acids. It is a balanced mixture of astringency, bitterness, umami (brothy taste) and slight sweetness. Catechins and their derivatives contribute astringency and bitterness. Caffeine has simple bitterness. Amino acids contribute the brothy taste with slight sweetness or bitterness depending on amino acids. Aroma of tea is a very complicated mixture of volatile compounds, such as terpenoids, alcohols, carbonyl compounds, etc. The aroma compounds are mostly developed during manufacturing process and composition of these compounds determines the aroma of individual made tea (Yamanishi, 1999).

3.6.1. TEA TASTE

3.6.1.1. Catechins and their derivatives

During manufacturing black tea, some of the catechins are changed to

theaflavis (TF) and thearubigin (TR) by enzymatic oxidation and coupling reactions. Roberts and Smith (1961) giving the terms "theaflavin" and "thearubigin" stated that TRs were equally important for flavour and quality of black tea as the TFs. TFs imparted the mouth sensation of "briskness"', "freshness" and "aliveness" while TRs were responsible for "body " and richness" of tea brew. Ding *et al.* (1992) after HPLC analysis and comparing sensory analysis of five main catechins, TF and TF-gallates in tea brew of various black teas from Darjeeling, Sri Lanka, Kenya and China concluded that the total amount of catechins had a significant effect on astringency, while TF and TF-gallates had no statistical correlation with astringency. In orthodox tea, theaflavins concentration was found in between 0.59 and 1.03 % and thearubigins in between 9.34 and11.48 % (Yamanishi, 1999).

3.6.1.2. Caffeine

Pure caffeine is bitter with a detection threshold of around 3 ppm in water. But, it forms a complex with TFs and TRs in the tea brew losing the bitterness of caffeine as well as TFs and TRs. The complex product contributes to briskness or "liveliness" and thickness of black tea brew (Wood and Roberts, 1964; Millin *et. al.*, 1969). Tea tasters' evaluation for quality of tea is associated significantly with briskness of its liquor (Biswas *et al.*, 1971) and therefore caffeine is considered as one of the quality factors.

3.6.1.3. Amino acids

Theanine (N^5-ethylglutamine) concentration alone is half of the total

amount of amino acid in tea leaves with an average content of 3 % by weight. The other major amino acids are glutamic acid, aspartic acid, arginine and serine (Maeda and Nakagawa, 1977; Kawagishi and Sugiyama, 1992). They give the characteristic brothy taste "Umami". It is significantly important to the taste of green tea and much research has been devoted to the study of amino acids in green tea since Sakato (1949) discovered theamine from the Japanese green tea "gyokuro".

3.6.2. TEA AROMA

The aroma components are essential oil consisting of numerous varieties of volatile compounds popularly known as "Volatile Flavour Constituent" (VFC). They are isolated from tea leaves or made tea by steam distillation and solvent extraction with a concentration of 0.01 to 0.03 % (Yamanishi, 1999). Research on aroma of tea has advanced considerably since 1960s when Gas chromatography (GC) and Mass spectrometric method (MS) are available for separation and structure determination of minor components. Subsequently, over 600 volatile compounds responsible for aroma in different types of tea have been isolated and identified (Yamanishi, 1995).

More recent research on the precursors of aroma compounds in tea has resulted in isolation of various glycosides with their absolute chemical structure (Kobayashi's et al., 1993 and Sakata et al.,1995). It was observed that though VFC is a complex mixture, a few components with low threshold values and high concentration could make easy impact on the sensory organs such as

nose and tongue to be of significance to the tea brew during assessment. These few rosy top notes from VFC viz. linalool, their oxides, geraniol, phenyl ethanol and fatty hexenals might well represent an important part of specific tea flavour (Mahanta and Hazarika, 1985).

The main sources of flavour compounds were reported as lipids (Hatanaka and Harada, 1973; Mukherjee, 1966 and Selvendran et al., 1976), amino acids (Wickremasinghe et al., 1979) and terpene glycosides and/or carotenes (Mukherjee, 1966, Selvendran et al. 1978, Wickremasinghe, 1974 and Takeo, T. 1983). These sources changed during manufacture and each change resulted in production of specific new compounds (Owuor, 1986). The major flavour compounds with their odour are presented in the table 2.

The lipids of tea leaf tissue undergo significant changes producing volatile flavour compounds during post harvest chemical changes with a chain of reactions from the time the shoots are plucked from the plant through the process of tea manufacture (Agarwal, 1989). The characteristic volatile flavour constituents are mostly formed by the hydrolytic action of enzyme on cell constituent during leaf maceration (Takeo, 1981; Renold et al., 1974). Thus the processing technique largely determines the quality of black tea. In withered leaves the quantity of volatile flavour compound is low though their formation starts at this stage with lipid degradation. It gains momentum during rolling and fermentation (Hazarika et al., 1984). It was reported that amino acid added to a solution of flavanols undergoing oxidation, produced VFC (Bokuchava and Popov, 1954). Wickremasinghe and Swain (1964) showed that there was an increase in certain volatile aldehydes concomitant with a decrease in the level

Table 2: Volatile flavour compounds with their odour in black tea.

Compound	Odour	Compound	Odour
1. Trans-2-hexenal	Strong greenish	11. Methylsalcylate	Oil of
2. Cis-3-hexenal	Greenish		wintergreen
3. Acetaldehyde	Malt	12. Methyl jasmate	jasmine
4. Methylpropanal	Apple	13. Benzyl alcohol	Rose honey
(isobutyraldehyde)		14. 2-phenylethanol	do
5. 2-methylbutanal	Apple & malt	15. Geraniol	Rose
6. 3-methylbutanal	Chocolate	16. Geranic acid	Rose honey
(Isovalavaldehyde)		17. Linalool	Sweet flowery
7. Methional	Potato or soy sauce	18. Linalool oxides	Do
8. Butanol	Rancid butter	19. Linalool oxide	Woody
9. Pentanol	Greenish	20. Linalool oxide	Lilly of the
10. 1-octen-3-ol	Stimulating (fruity)		valley

Adapted from Owuor (1986)

of related amino acids. These findings suggest that amino acid is involved in the formation of VFC.

Darjeeling orthodox tea has a typical muscatel aroma accompanied by a characteristic woody and complexed heavy note (Yamanishi, 1999). It was reported that in different type of black teas, the major difference in the aroma pattern remained in concentration of linalool, linalool oxides and geraniol (Yamanishi *et al.*, 1968; Horita and Owuor, 1987; Owuor, *et al.*, 1986, Mahanta and Hazarika, 1985; Hara *et al*, 1995). The specific characteristic compounds of Darjeeling tea aroma were identified as 2, 8-dimethyl-3, 7-octadiene-2, 6-diol and reported as the photosensitized oxygenation product of linalool (Kawakami, *et al.* 1995). This was also reported as the precursor of 3, 7-dimethyl-1,5,7-octatriene-3-ol (Matsuura and Butsugan,1968; Hara, 1989).

The ability of tea clones to synthesize the characteristic and essential VFC was observed to be dependent primarily on the geographical location and climatic conditions like cold, dry, windy nights (Wickremasinghe, 1974). The VFC content of Darjeeling teas were found three times higher than the plains tea in general and the amounts of essential and characteristic VFC viz. rosy linalool and its oxides and geraniol were around five times higher than plains teas. Though the nature of reaction was not very clear, but it was understood that terpenoides were synthesized better under Darjeeling weather conditions like, cold dry windy nights and humid day with relatively low temperature. There is a well defined period in Darjeeling during May–June, known as second flush, during which tea has outstanding flavour. The chinary clones were also found to be rich in linalool, geraniol, benzyl alcohol and phenyl

ethanol. Different stages of manufacture like, withering, rolling, fermentation and drying have their own importance and independent contribution toward production of VFC. In orthodox manufacture, at the end of rolling a large amount of VFC was detected, which decreased as fermentation progresses. After optimum fermentation the residual substance might produce small increase in VFC (Mahanta and Hazarika, 1985). It was observed that the orthodox tea contain twice the amount of VFC present in CTC teas (Takeo and Mahanta, 1983)

The flavour of CTC tea was found inferior to that of orthodox black tea due to the presence of high level of carbonyl compounds such as (E)-2-hexenal (grassy aroma) and the low concentration of floral and fruity aroma components, such as linalool, linalool oxides, (z)-3-hexenyl esters and other desirable compounds (Takeo and Mahanta, 1983). Higher theaflavin (TF) content in CTC teas as compared to orthodox teas was found to be associated with higher activity of the oxido-reductase enzymes on the catechin substrate which inhibited the action of the hydrolytic enzyme reported to be responsible for producing linalool and its oxides and methyl salicylate in triturated tea leaf tissues under anaerobic conditions (Takeo,1981).