Chapter 2
Review of Literature

Introduction

The coffee white stem borer (CWSB) - *Xylotrechus quadripes* Chevr is very well known to all arabica planters, as a pest of major importance in Karnataka. Stokes (1838) was the first person to report the borer attack on coffee in Mysore (now Karnataka) but he does not include a reference. The insect was formally described by Chevrolat in 1863. Dr Bidie was specifically commissioned to study this pest on behalf of the Madras Government and his report was published by the Government of Madras in 1869. In between, Richter (1867) made careful observations on the life history and habit of this insect and Taylor (1868), a planter, studied the life history and suggested a few control measures in his book entitled “A Short Campaign against the White Stem Borer in the Coffee Districts of Coorg, Manjarabad and Nagar”. Cameron (1899) suggested maintenance of shade for checking the pest. Pringle (1907) studied the effect of moisture and temperature on the development. The major work on the biology and behavior of *X. quadripes* was carried out by Anstead (1915), Kunhi Kannan (1917, 1918, 1925, and 1929), Nicolson (1913), Coleman (1925), Subramanyam (1940), Subramaniam (1934, 1941), Pattabhiraman (1983) and Veeresh (1993). When its life history and control measures were studied by the Division of Entomology of the Mysore Department of Agriculture, and published in bulletin No. 11 in 1934 by Subramanian and revised in 1941, the destruction of borer attacked plants one among other measures like scrubbing of stems; hand picking of beetles, etc. was practically tested in more than one estate. All the planters were advised to adopt these measures. However, the efforts at propaganda and persuasion on the part of the department did not yield the desired results.

Biology of coffee white stem borer

The adults of *X. quadripes* lay eggs in crevices in the bark. The larvae tunnel into the trunk, blocking the tunnel with frass behind them to prevent predators. The tunneling in the trunk and roots rapidly kills young plants of up to 7-8 yr old (Hall et al. 2006). The
behavior of beetle after emergence from the tunnels was studied in detail by Subramaniam (1941) and Veeresh (1993) in India and Visitpanich (1994) in Thailand. Studies on productiveness, durability and field behavior of CWSB were carried out in Northern Thailand by Visitpanich (1994). The study of the biology of the CWSB is very difficult due to its concealed nature, with the breakthrough in making the borer to lay eggs in the laboratory in large numbers it became possible to study and understand the biology of this insect both in the laboratory and in the field on live plants. The adult beetles after their emergence were able to fly from one plant to another for a long distance with a buzzing sound and had the habit of suddenly dropping down to the ground when disturbed and quickly taking shelter under dry leaves and cracks of the soil. The beetle emergence was more during the morning hours from 9 to 12 noon. Observations of the head caps indicated that there are five larval instars; emergence pattern of adult beetles revealed that the beetles emerge in two batches in a year. The total life cycle may vary depending on the condition of the coffee stem. The larval development is influenced to a great extent by the microclimate and the condition of the host stem. Moisture availability and the size of the stem appear to be the two major factors deciding the duration of development from egg to adult. Invariably, the borer takes longer time to complete its life cycle on coffee plants in the field.

The adults are slender, elongate with a pair of long antennae. The fore wings are black with characteristic white bands. Males are generally smaller than females. Adult CWSB varied generally from 10 to 19 mm in length, with large variations from 10 to 17 mm (Gahan 1906), 9 to 19 mm (Subramanyam 1940), and 8 to 17.5 mm (Visitpanich 1994). The female beetles are very active, flying freely and laying eggs on stems, mainly in the afternoon in bright sunshine. Mated female lay eggs in small groups of 1 to 10 in the cracks and crevices of the bark and under the loose scaly bark of the main stem and thick primaries, preferring the plants exposed to sunlight. Each female lays about 100 eggs (Figure 2.1B).

Both sexes have distinct raised markings on the head (Gahan 1906). In the past, CWSB adults were sexed based on their body size, marking on the head and the relative length of the hind femora to the elytra (Le Pelley 1968). As these morphological characteristics are not similar in a population (Venkatesha and Seetharama 1999), adults
can be best sexed perfectly based on the shape and size of the last segment of the abdomen. In the ventral view, the last abdominal segment of female beetles is long and tapered with a semicircular tip, whereas it is short and broad with a slight rectangular tip in males (Figure 2.1A). In addition, the last segment of the abdomen is slightly longer than that of the preceding segment in females, but these two segments are almost equal in males. The mean length of male and female beetles is 11.8 and 13.5 mm, respectively (Venkatesha and Seetharama 1999; Seetharama et al. 2005). Eggs hatch in 3–15 days under different climatic conditions (Subramaniam 1934; Le Pelley 1968; Visitpanich 1994). CWSB larvae pass through five instars in India and six instars in Thailand, and complete their development in about 70 days on the dry stems (Visitpanich 1994; Seetharama et al. 2005). The larval developmental period is about 120 days in standing plants in the field (Seetharama et al. 2005). When the larva is fully grown, it tunnels towards the surface of the stem and cuts a small circular disc below the bark for adult emergence, and then returns to its chamber and pupates. Enclosed adults remain in the pupal chamber for 3–7 days and emerge through an exit hole (Le Pelley 1968; Visitpanich 1994). The developmental period of the pupa is about 30 days on the living plants, while it is only 9 days on dry stems (Visitpanich 1994; Seetharama et al. 2005). Under field conditions, the life cycle of CWSB in India takes 1 year. However, depending upon the age and size of the plants, the life cycle of CWSB can vary from 142 to 390 days in the field. Within 2.5–7 months, the borer completes its life cycle faster on drying/dried coffee stems (Subramaniam 1934; Pattabhiraman 1983; Visitpanich 1994; Seetharama et al. 2005). Further work on an artificial diet is needed for the successful mass rearing of CWSB to enable more studies on the bioecology of the pest and the rearing of its natural enemies. The mean hatching rate of CWSB eggs is 78%, and mortality is high during the first and second larval instars compared with subsequent larval stages (Visitpanich 1994).

The eggs were small, elongate oval with one end more acutely pointed, translucent and with a smooth chorion. The egg measured 1.27± 0.019 mm in length and 0.43± 0.005 mm in width. The larva, on hatching was whitish in color, 1.21± 0.022 mm long and 0.42± 0.005 mm wide, broad at the head end and tapering towards the posterior.
Figure 2.1: A: Posterior end of CWSB beetles; B: Eggs laid by CWSB beetles on a paper in vitro

Figure 2.2: Symptoms and damage by CWSB infestation. A: Infected coffee stem showing ridges; B: CWSB grubs tunnel inside the stem; C: grub developed into pupa in the tunnels; D: coffee stem affected severely by CWSB grubs; E: infected stems showing exit holes
The incubation period of egg was five to six days in the laboratory and seven to eight days in the field. This may be due to temperature differences. (Seetharama et al. 2005)

**Flight periods**

There are two peak emergences or flight period; one during April-May and the other during October-December. Cloudy and wet weather delay the emergence of the beetles (Venkatesha and Dinesh 2012).

**Impact of coffee white stem borer**

Affected plants show externally visible ridges around the stem. They may also exhibit signs like wilting and yellowing (Anonymous 2009) (Figure 2.2). CWSB crisis is major for areas growing 70-80% of arabica. As per survey records it was found that about 50% of plantations in Karnataka have high incidence of white stem borer. About 35 lakh plants were uprooted in the first flight period. Several measures are taken to tackle the problem of CWSB (Anonymous 2014).

After initial tunneling & feeding in the bark, the larvae enter wood and make extensive galleries in the main stem and thick primary branches. Tunneling may also continue to some extent in the main roots. Apart from affecting the quality and quantity of the produce, severe infestation can heavily damage the main stem up to roots and kill the plants. Infested plants die in a year, while older plants withstand the attack for a few seasons. However such plants are less productive, yielding more floats. Young plants of up to 7–8 year old are killed easily, whereas, older plants may survive for a few more seasons. Conventional breeding has not been successful in developing arabica varieties resistant to CWSB. Currently available control measures have not been able to control the pest effectively. As a consequence, CWSB has become major factor contributing to the continuous decline of area under arabica plantations in India. The severity of the pest has increased in recent years possibly due to changing climatic conditions caused by global warming. The pest causes substantial capital loss and heavy financial distress to the arabica coffee farmers as millions of severely infested arabica plants are killed or have to
be uprooted every year to prevent further spread of the pest. As a result arabica is getting replaced by robusta.

**Management practices followed**

*Maintenance of shade*

It is recommended that two tier system of shade was essential. The shade trees should be regulated in such a way that the coffee plant is not exposed during the flight period of the beetles.

*Tracing, stumping/uprooting and destruction*

Uprooting and destruction of borer infested plants should be completed before the beetles emerge out. Before uprooting, the infested plants should be stumped.

*Scrubbing*

Loose scaly bark of the main stem and thick primaries has to be removed to reduce the cracks and crevices in which the eggs are deposited. Scrubbing should be done before the flight period.

*Use of pheromone traps*

For monitoring and trapping the beetles, pheromone traps can be installed on coffee estates just before the peak flight period to reduce infestation.

*Chemical measures*

Swabbing or painting the stem with chemicals is a popular way of controlling the pest. The main stems and primaries of each plant are swabbed with insecticide such as fenithrin or chloropyrifos. Lime spraying is also recommended to be done before the flight period (Anonymous 2009).

Though different measures are recommended, there is no proper control for CWSB. Susceptibility to certain biotic and abiotic stresses is the major problem to be tackled for improvement of coffee. The concept of stress has been originally derived from physics, where it is exactly defined as relation between inputs and output of a system and can be exactly determined (Lubliner 2008). During their lifespan, plants have to deal with a multitude of stress factors originating from the abiotic as well as the biotic environment. Main abiotic environmental cues influencing the plants performance and fitness include drought and salt stress, ozone and UV-radiation, cold stress and many
others. Biotic stress factors originate from many different groups of organisms like pathogens, nematodes, microorganisms, and also from feeding insects. Given the fact that over 50% of all insects show herbivorous feeding behavior, plants have to adapt to them by developing and modulating different defense strategies (Schoonhoven et al. 1998; Van Poecke 2007). Attack of insects, especially with chewing feeding behavior, cause a massive loss of plant tissue and viability leading to low reproduction rate (Stowe et al. 2000). Attack of herbivorous insects combines different stress stimuli inducing plant defense. Perception of herbivore by the plant consists of recognition of wounding of plant tissue and of elicitors provided by the insect’s oral secretion (Maffei et al. 2004; Mithofer et al. 2005; Mithofer and Boland 2008; Wu and Baldwin 2010).

**Mechanical defense against herbivore attack**

Plants have the ability to differentiate the damage caused by herbivore and mechanical damage, such as rain and wind. This quality is more important to avoid wasting defense resources, since it is expensive, as well, as the production and release of defense responses only benefits herbivore-challenged plants. Plants are also able to recognize compounds in insect oral secretions, which elicit more intense volatile responses than mechanical damage alone (Arimura et al. 2004; Moraes et al. 2001). An open wound caused by mechanical injury is a potential infection site for pathogens; thus, expression of defense genes at the wound site is necessary for the plants to build a barrier against opportunistic microorganisms. In addition to pathogen resistance, wounding pathways may also interact with other signaling processes involving abiotic stress responses (Cheong et al. 2002). The plasma membrane is in direct contact with the environment, and is therefore able to recognize outer changes and initiate cascade events leading to a possible response. Biotic and abiotic stress will lead to an immediate change in the cell membrane potential (V_m), or modulate the ion flux at the plasma membrane level (Ebel and Mithofer 1998; Shabala 2006). As soon as insect herbivores initiate to feed on a plant, several defense signals are induced, leading to different defense responses. Each plant cell has the capability to activate protective mechanisms upon injury sensing. The capacity of cells to activate defense responses upon “danger” sensing and recognition of non-self microbe-associated molecular patterns (MAMPs) and/or
endogenous damage-associated molecular patterns (DAMPs) is characteristic of the plant innate immunity (Akira et al. 2006). Defense responses activated by wounding are similar and overlapping with those activated by MAMPs and DAMPs, indicating that both injury and pathogens are limited by plants in a similar manner.

The plant’s mechanical defenses are the first layer of defense that an herbivorous insect encounters while feeding on them. In A. thaliana, the major component contributing to its mechanical defenses are trichomes. These structures on the plant surface, which are formed by epidermal cells, show a high grade of branching. It was shown that trichomes negatively influence the herbivore feeding behavior via its effect on insect mobility (Reymond et al. 2004). Additionally it was shown that in a population of Arabidopsis lyrata plants lacking trichomes are more susceptible to herbivore than plants with higher trichome density (Loe et al. 2007). The plant surface also harbors additional layers of mechanical defense in form of epicuticular waxes which influence insect’s feeding behavior and egg deposition (Blenn et al. 2012). These mechanical barriers are thus a first line of defense; the major part of the plant’s defense against herbivores is, however, made up by different chemical defenses.

**Direct and indirect defense**

The plant defense triggered after herbivore attack is a multiple network of different pathways, which are constitutively expressed or induced upon stimuli sensitivity (Figure 2.3). Both groups of defense pathways are composed of direct and indirect defenses (Howe and Jander 2008). Direct defense compounds like secondary metabolites, which include glucosinolates, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), alkaloids, phenolics, and other compounds, act as toxins in plant-insect interactions. Plant secondary metabolites are the by-products of primary metabolites and are not essential for plant growth. However, it is widely accepted that plant secondary metabolites are involved in the resistance to pathogens and herbivores (Theis and Lerdau 2003; Mao et al. 2007; Chen 2008). Many plant secondary metabolites are toxic to insects. Even if insects have evolved mechanisms to detoxify the compounds, there is a high cost to overcoming the toxicity. The cost may affect the insect survival. For plant defensive proteins, a major class of defensive proteins includes protease inhibitors (PIs)
that were first found to rapidly accumulate in potato and tomato after insect feeding. (Kant et al. 2009). Indirect defenses like emission of volatile organic compounds (VOCs) after herbivore attack function as attractant for parasitic wasps which in turn predate on the attacker (Van Poecke 2007). Plants release volatiles constantly. Each plant species under insect infestation emits different blends of volatiles. Plants emit a blend of volatiles for growth, development, and responses to the environment stress (abiotic and biotic stresses).

![Diagram of plant defense mechanisms](image)

**Figure 2.3**: Diagrammatic representation of plant defense mechanisms

**Mechanisms of plant defense against herbivores**

While plants develop new defense compounds or mechanisms to enhance the resistance against herbivores, their attackers find new ways to bypass or detoxify these (Jander 2014). Generalist herbivores feed on many different plant species and have to encounter different defenses; specialist insects are limited to a number of food plants and show a higher level of adaptation to the defense mechanism of these specific plants (Ali...
and Agrawal 2012). For example *Manduca sexta* larvae feeding on tobacco plants show a high grade of adaptation to otherwise toxic levels of nicotine (Steppuhn et al. 2004; Pluskota et al. 2007).

Upon insect attack, many chemical reactions change in the plant cell, such as ion fluxes, formation of reactive oxygen species (ROS), protein modification, and phytohormone (jasmonic acid, ethylene, and salicylic acid) biosynthesis and signaling (Mithofer and Boland 2008; Thivierge et al. 2010; Hogenhout and Bos 2011). Although chewing insects cause serious mechanical damage to plant tissues, this does not account for the entire effect of herbivores on plants. Plants respond to insects differently depending on the herbivore feeding style. Piercing-sucking insects (ex: *Sternorrhyncha*) have specialized stylets/tube-like structures to suck liquid contents from plants. Chewing insects (ex: *Coleoptera* and *Lepidoptera*) cause extensive plant tissue damage by chewing, snipping, and tearing. It has been shown that chewing insects cause extensive wound damage to plant tissues, but this mechanical damage does not account for the entire herbivore effect on plants (Howe and Jander 2008). Large-scale analysis of gene expression analysis has surveyed gene transcript differences between mechanical wounding and insect feeding (Reymond et al. 2000; Major and Constabel 2006). Farmer and his colleagues showed that many wound induced genes in *Arabidopsis* may not be induced or are expressed less when plants are under herbivore attack (Reymond et al. 2000). In addition, few genes had a similar expression pattern between water-treated and insect regurgitant-treated tobacco plants (Halitschke et al. 2003).

Transcription is a major regulatory step in the activation of these responses, and JAs trigger an important transcriptional reprogramming of the cells to switch the basal developmental programs into the necessary stress response program (Fernandez et al. 2011) The plant hormones jasmonates (JAs) are fatty acid–derived oxylipins required for the regulation of multiple physiological aspects of plant growth, development, and defense. Jasmonic acid and related compounds, collectively named jasmonates (JAs), are ubiquitously occurring lipid-derived compounds, and function as a master switch in plant responses to several abiotic and biotic stresses such as wounding (mechanical stress), drought and salt stress, ozone and pathogen infection, and insect attack (Wasternack 2007). Methyl jasmonate (MeJA) was first isolated and identified in 1962 as a new
odoriferous component from essential oil of *Jasminum grandiflorum* (Demole et al. 1962). JAs have become accepted as a new class of plant hormones, mainly involved in defense (Browse, 2009). Genes coding for plant defense proteins, so-called jasmonate-induced proteins (JIPs), such as proteinase inhibitors or enzymes of phytoalexin synthesis are up regulated by JA, while those coding for housekeeping proteins such as RUBISCO are down regulated (Wasternack 2007). In addition, JAs regulate many aspects of plant development and growth such as seed germination, fruit ripening, production of viable pollen, root growth, tendril coiling, photo morphogenesis, leaf abscission and senescence (Wasternack and Hause 2013; Robson et al. 2010).

**Phenolics**

Based on the structure phenolics are grouped into benzoates, hydroxycinnamates, furanocoumarins, coumarins, stilbenes, flavonoids, hydrolysable tannins, condensed tannins and lignin (Rehman et al. 2012). Along with protecting the plants from UV radiation, phenolics provide a first line of defense against herbivory wherein they act as feeding deterrents, growth reducers, or toxins (Close and McArthur 2002). The biochemical mode of action of phenolics in herbivores depends on the gut pH and the presence of enzymes that can activate them (Appel 1993). At the alkaline pH level found in herbivore guts, phenolic oxidation leads to the formation of reactive oxygen species that readily oxidize vital biomolecules including lipids, proteins, carbohydrates and nucleic acids (Appel 1993; Felton and Summers 1995; Witzell and Martin 2008). Oxidation of phenolics can also occur by plant polyphenol oxidases forming quinones which react with proteins and result in their precipitation (Felton et al. 1992). A few phenolics like tannins can bind and precipitate gut proteins affecting digestion (Butler and Rogler 1992). The insecticidal activity of coumarins was observed against fall armyworm, *S. frugiperda*, wherein larval growth was significantly reduced and a high percentage of mortality was also observed (Vera et al. 2006). Similar effects were observed against lepidopteran forest pests such as *Plecoptera reflexa, Clostera cupreata* and *Crypsiptya coclesalis* (Sharma et al. 2006).

**Defensive proteins**

Defensive plant proteins contribute to constitutive defenses against herbivores, although the majority of studies have focused on the induction of defensive proteins by
insect feeding (Ryan 1990; Van Loon et al. 2006; Shivaji et al. 2010). Defensive proteins are classified based on the mode of action by which they affect insect pests and are classified in terms of anti-nutrition effects and/or toxicity (Chen 2008). Anti-nutritive proteins include protease inhibitors, oxidative enzymes, and amino acid de-aminases (Ryan 1990; Pechan et al. 2002; Chen 2008). Toxic plant proteins include cysteine proteases, chitinases, lectins and leucine aminopeptidases (Dowd 1994; Zhu-Salzman et al. 2008). Anti-nutritive proteins negatively affect the nutritive value of ingested host material (Chen 2008). Protease inhibitors produced by plants form complexes with proteases in the insect midgut and inhibit the ability of insects to effectively break down nutritive plant proteins (e.g. rubisco) into their individual components, which serve as the primary source of nitrogen, which is essential for growth and development of the insect (Ryan 1990). Oxidative enzymes such as ascorbate oxidases, lipoxygenases, peroxidases, and polyphenol oxidases interact with plant phenolics to form reactive quinones that are capable of polymerizing or forming covalent adducts with certain chemical groups on proteins (Felton et al. 1992; Dowd 1994). In addition to their ability to interact with various phenolic compounds, oxidative enzymes may function via several mechanisms that negatively impact herbivore performance. Toxic plant proteins affect insect pests by disruption of the midgut lining [peritrophic membrane (PM)] which interferes with digestion and absorption of nutrients, ultimately leading to death of the pest (Zhu-Salzman et al. 2008). Cysteine proteases permeabilize the PM of insect pests, most likely by directly degrading PM proteins (Mohan et al. 2006). Leucine aminopeptidases are thought to function in a similar manner by directly damaging the insect midgut (Felton 2005). Negative effect of lectins on the performance of lepidopterans, coleopterans, dipterans and hemipterans has been recently reviewed (Vandenborre et al. 2011).

**Plant defense signaling**

The studies of Baldwin and his group on the interaction between insect herbivores and tobacco (*Nicotiana attenuata*) have provided new insights into the molecular basis of plant defense. They estimate that approximately 500 mRNAs constitute the insect responsive transcriptome in tobacco (Hermsmeir et al. 2001). However, many of these genes are of unknown function, and many changes in gene expression do not represent
induction of defense-related proteins. Photosynthetic genes, for example, are down regulated in tobacco plants in response to insect attack. Further microarray analysis has demonstrated putative up regulation of defense-associated transcripts and down regulation of growth-associated transcripts. This analysis provided evidence for the simultaneous activation of salicylic acid, ethylene, cytokinin and jasmonic acid-regulated pathways during herbivore attack. The signal transduction mediated by the systemin receptor results in activation of phospholipase A2, via a MAP kinase, and as a result leads to the release of linolenic acid from membrane lipids. Added effects such as calcium discharge from vacuoles, calmodulin synthesis and opening of ion channels in the plasma membrane are also stimulated by perception of the signal, and self-evidently take part in the wounding response, but are not part of the direct pathway (Gatehouse 2002).

Deciphering the signals that regulate herbivore-responsive gene expression will afford many opportunities to manipulate the response (Figure 2.4). Signaling molecules such as salicylic acid, jasmonic acid and ethylene do not activate defenses independently by linear cascades, but rather establish complex interactions that determine specific responses. Knowledge of these interactions can be exploited in the rational design of transgenic plants with increased disease/insect resistance (Rojo et al. 2003). However, recent research has shown that induced defenses also involve the plant’s ability to produce toxic or repellent secondary metabolites as direct defenses, and volatile molecules that play an important role in indirect defense. Insect herbivores activate induced defenses both locally and systemically via signaling pathways involving systemin, jasmonate, oligogalacturonic acid and hydrogen peroxide (Kessler and Baldwin 2002). Attack by piercing–sucking herbivores, like aphids, thrips and spider mites, inflicts little physical damage and harms a limited number of plant cells. By contrast, feeding by chewing insects like CWSB causes severe wounding and damage large portions of the plant tissues. Wounding provides nutrients to microbial pathogens and facilitates their entry into the tissue leading to subsequent infection. Plants have evolved sophisticated mechanisms to promptly respond to wounding, rapidly heal the tissue and prevent microbe infections (Savatin et al. 2014; Duran-Flores and Heil 2016).
Gaps identified in the literature survey and overall intention of present study

So far only conventional breeding methods have been adopted by various national and international coffee research centers for genetic improvement of this commercial crop. However, conventional breeding has limitations for coffee improvement because of the genetic barriers of chromosome number (diploids vs. tetraploids), auto incompatible alleles (diploid species) and long breeding cycles. As a result, the transfer of genetic traits from wild outbred species of the genus to cultivated species is quite difficult. A further complication in Coffea is its lengthy period of fruit development and the 2-4 year bean generation time, which make such traditional approaches costly and time consuming. Release of a new coffee variety has been estimated to require a minimum of 24 years of continuous breeding (Vander Vossen 2001).

To overcome the limitations of conventional breeding techniques for genetic improvement of coffee plants, interest has turned to biotechnology approaches as biotechnology has become a very powerful tool in modern biology. It can supplement the
efforts of coffee breeders with additional tools for genetic improvement. Hence, transfer of desirable genes, in particular for disease resistance, from resistant coffee species into Arabica cultivars without affecting quality traits has been the main objective of Arabica breeding (Vander Vossen 2001). Transcript pattern changes in response to herbivores have been generated in many plant species including *Arabidopsis thaliana* (Reymond et al. 2000), *Citrus sinensis* (Mozuruk et al. 2006) and *Coffea* (Mondego et al. 2005 and Cardoso et al. 2014). These studies have provided insights into the molecular basis of insect-plant interactions, but little information regarding the perennial crops are available. Different levels of resistance to the CWSB have been observed among *Coffea* species (Ram et al. 2008).

Review of literature revealed that in recent years lot of information is generated on plant resistance against insect pests. But such information is lacking in coffee plants. When the insect attacks, plants respond to herbivores with the production of toxins and defensive proteins that target physiological processes in the insect. Herbivore-challenged plants also emit volatiles that attract insect predators and strengthen resistance to future threats. This highly dynamic form of immunity is initiated by the recognition of insect oral secretions and signals from injured plant cells. These initial cues are transmitted within the plant by signal transduction pathways that include calcium ion fluxes, phosphorylation cascades, and, in particular, the jasmonate pathway, which plays a central and conserved role in promoting resistance to a broad spectrum of insects. An understanding of plant immunity to herbivores will provide new insights into basic mechanisms of chemical communication and may also facilitate new approaches to crop protection and improvement.

Robusta coffee is resistant to CWSB compared to arabica. Except for rare instances, CWSB is unable to complete its life cycle on robusta plants. Robusta plants occasionally exhibit symptomatic ridges of CWSB incidence on the stem, but there is no further damage to the plants (Santosh et al. 2011). Mostly the pest is arrested and killed at early stages of infestation in the bark tissue or outer most layers of wood. As a result CWSB cannot cause significant damage to robusta plants. Robusta seems to have evolved genetic defense mechanisms against CWSB. It seems to mount strong induced defense response in the bark tissue which is the initial site of infestation and comprises of mostly
living cells, before it enters the wood which mostly comprises of dead cells. Profiling of transcriptional changes in the bark tissue of robusta due to CWSB infestation may be expected to provide an insight into the molecular mechanisms of genetic defense against the pest which in turn can lead to development of arabica cultivars resistant to the pest. While \textit{C. arabica} is highly susceptible to CWSB, \textit{C. canephora} and other species like \textit{C. liberica} are resistant to CWSB. It is essential to understand the genetic mechanisms of defense mounted by the resistant species against CWSB to come out with strategies to breed arabica varieties resistant to CWSB.

It is hypothesized that genetic defense mechanisms are operating in the resistant species. However, there is little information on nature of the genetic defense mechanisms that may be present and activated in the resistant coffee plants to defend against \textit{X. quadripes}. Hence this study was proposed to fill the gap. The purpose of the study was to conduct investigations towards deciphering genetic defenses of coffee plants (\textit{Coffea} sps.) that may be operating against the insect pest coffee white stem borer (\textit{X. quadripes}) using molecular genetics and other approaches. Specific objectives and work plan were planned based on the earlier work (Santosh 2012). Present work was expected to create a profile of the expressed genes in responses to CWSB attack and provide clues on the nature of \textit{Coffea} defense response. The information generated is subsequently expected to be useful for genetic improvement of \textit{C. arabica} with CWSB resistance. A variety of strategies that were planned, are summarized below.

**Objectives**

1. Identifying putative defense related genes, profiling their expression levels and selecting DNA sequences for polymorphic markers by analysis of the Suppression Subtractive Hybridization (SSH) cDNA library prepared in response to \textit{Xylotrechus quadripes} infestation.
2. Obtaining full length sequences of selected genes by RACE and upstream sequences by Genome Walking.
3. Identifying coffee genes related to perception of insect herbivory from public data bases.
4. Exploring constitutive defense systems.