Discussion
5. DISCUSSION

Crop losses caused by pests are a major problem in both developed and developing countries. Increasing awareness of the environmental consequences of indiscriminate use of chemical pesticides has provided new impetus to the search for alternative ways of managing pests. Particular emphasis has been placed on strategies that cause less pollution to the environment and those that are affordable, especially for the less developed countries. One concept that has received a lot of attention is integrated pest management (IPM), which seeks to manage pests and minimise crop losses by using methods that are economically viable and less harmful to the environment. At least three distinct classes of new biotechnologies can have impacts on integrated pest management. These include microbial biotechnologies, plant molecular biology and genetics, and insect molecular biology and genetics. For instance, recent improvements in molecular biology have enabled scientists to overcome species barriers and to genetically alter plants, animals and microorganisms in ways that were not possible before. Already, several genetically altered plants which express genes that confer protection against pests have been produced. The techniques of biotechnology have also played important roles in elucidating pest populations and in studying the population dynamics of biological control agents and other types of organisms that live in association with crop plants.

Pest management approach, methods and discipline have experienced over time developments and advancements to minimize environmental impact. This thesis includes comprehensive treatments of biology, molecular systematics, isolation and identification of pheromones, biological control using Bt in case of Myllocerus a serious coleopteran pest genus that is in the family Curculionidae. The main findings and problems of the study will be summarized and discussed in this chapter.

The coleoptera are the largest order of insects, with about 40% of the known species in the Hexapoda. These vary in length from less than a millimetre up to about 75mm. Some tropical species reach a length of about 125mm. The beetles vary considerably in habits and are found almost everywhere. Many species are of great economic importance.
The family Curculionidae is the largest family of organisms known today. More than 47,930 species in 4300 genera are currently recognized (Kuschel, 1995). The vast majority of weevil species are strictly phytophagous as adults and larvae although a few are saprophytic. Even those taxa that are hypogean in habits appear to feed primarily on roots (Osella, 1979). They are recognized by their distinctive long snout and geniculate antennae with small clubs. The genus *Myllocerus* are most important.

The members of the genus *Myllocerus* of the family Curculionidae are all plant feeders both as adults and grubs, and are potentially injurious to crop. Several species such as *M. undecimpustulatus*, *M. subfasciatus*, *M. viridanus*, *M. dentifer*, *M. discolor* are known to be pests of crops in South India. Adults feed on the leaves of different plants including fruit crops and other crops also. The grubs are subterranean and are extensively root feeders. The information pertaining to the subterranean habits is less known because of their hidden way of life. There are a few species like *M. subfasciatus* and *M. undecimpustulatus* which continues to damage crops as adult feeding almost entire year. The genus *Myllocerus* is confined to the old world and the species are especially abundant in the tropical and subtropical regions. Since the members of this genus are largely dependent on cultivated fields for their breeding, the local distribution in any particular locality is confined to areas around agro systems.

**Name**

*Myllocerus undecimpustulatus* Marshall

**Taxonomic position**

Insecta: Coleoptera: Curculionidae

**Common names**

Cotton grey weevil
Sri Lanka weevil
Mango ash weevil
Grey weevil

**Name**

*Myllocerus subfasciatus* Guérin-Méneville

**Common name**

Ash weevils
Since our knowledge of the fauna of this economically important genus *Myloccerus* so far as Karnataka is concerned was limited. Therefore, it was felt that there was scope and need to study this further. In order to fill in the lacunae in the areas concerning the weevils which are considered as of great importance in agriculture in our country, the present investigations were undertaken at the Indian Institute of Horticulture Research (IIHR), Bangalore.

The biology of *M. undecimpustulatus* has been studied in detail. The eggs take about a week or more to hatch and so can become vulnerable to natural parasitism/predation. A preliminary trial done at NBAII has shown that *Trichogramma japonicum* is able to parasitize the eggs of *Myloccerus* (Venkatesan et al., personal communication). This may be a vulnerable weak link that can be looked into from a management perspective.

This study showed that winter duration of larvae is 70 days which is almost twice as that of summer. This implies the longer feeding by the grubs in winter may require repeated control. However, longer duration in winter also implies a lower population due to probably one lesser generation subsequently. Longer periods in soil, may perhaps render the grubs more vulnerable in slow acting entomopathogens like *Beauveria, Metarhizium* or even *Bt*. Thus the potential of this need to be explored.

Successful management of *M. undecimpustulatus* Marshall and *M. subfasciatus* Guérin-Méneville requires a detailed knowledge of its biology and ecology. The present study was conducted in the field to elucidate developmental durations of immature stages, adult emergence, and to identify the breeding and hiding areas and source of infestation of the adult weevils in Karnataka, India. *In vitro* studies of *M. undecimpustulatus* were conducted during the period November 2010 to November 2011, to determine the biology and ecology of the weevil. Average oviposition rate by a single female was 111.05 eggs per day. The oviposition lasted for a period of 7.5 days. In general eggs hatched between 7-9 days with a mean of 7.5 days, after which larval and pupal stages lasted a mean of 12 and 23 days respectively. The highest rate of oviposition was recorded during the first four days after mating and none after ninth day. Egg to adult
development stages of *M. undecimpustulatus* under *in vitro* conditions took an average of 74 days.

Based on this, we can suggest at which stage of insect development and at what time of the year control should be targeted. The larvae could be considered as good targets for control through mechanical destruction, the practical application is difficult due to their hidden nature. The study clearly suggested that management in summer and winter require different approach.

The damage on the different host crops by the economically important pest *M. undecimpustulatus* was observed at several locations of Southern India. The host plants like *Mangifera indica*, *Delonix regia*, *Muntingia calabura*, *Passiflora edulis*, *Magnolia champaca*, *Quisqualis indica*, *Chrysanthemum morifolium*, rose, *Tagetes patula* etc., are reported in the present investigation. These pests were also identified and their damaging stage, part of tree damaged and pattern of damage were also observed. Leaf preference by adults indicated that more feeding was noticed at the new leaf stage when the leaves are still tender and purplish. This polyphagy also ensured that the population is able to survive throughout the year and thus more economically important crops like brinjal and mango can become vulnerable.

A review of literature on the biology of this important pest reveals the dismal lack of information, excepting for the description of copulation given by Stebbing (1914). The biology of the weevil and effect of temperature on its field population study has been studied and described. This constitutes a valuable contribution to the study of immature stages of the species of *Myllocerus*. The adult damage was found to be around 70% during May and June. The present study showed that from November 2010 to December 2010 there was fluctuation in *M. undecimpustulatus* population. From January 2011 to April 2011 no *M. undecimpustulatus* was noticed. From April 2011 to September 2011 gradual increase in population was noticed (Figure. 2). Anstead (1910) stated that *M. undecimpustulatus* is a common insect found occurring injury to economic crops. Marshall (1916) reported the larvae of *M. undecimpustulatus* as a serious pest of sugarcane. Fletcher (1917a, 1971b) stated that they were found commonly at the roots of
maize and wild Amaranthus. Fletcher (1920) stated that *M. undecimpustulatus* is a common species, often occurring sufficient numbers to constitute at least a minor pest. Misra (1920) mentioned that it is found on every crop but it is not serious to any as a rule. Ayyar (1922) considered it as one of the chief species causing injury to economic crops. Heinrichs (1972) stated that the species is a common pest of ragi crop.

Stebbing quoted by Marshall (1916) collected the adults from thorny climber namely, *Acacia intsia* for the first time in July 1902; a few days later he collected them on *Dalbergia paniculata* and teak. The same author reported that the larvae caused damage to dec dar seedlings in the forest nursery. Lefroy (1906) observed the adults hiding away in large numbers during the winter months under bark or in any sheltered crevices. Thus it is clear that the summer population is more pestilent than the winter. He also stated that the adults became active during March. In the present study adult congregation to ward off winter was not observed. Now a century after Lefroy observed that the temperature is probably warmer however, larval duration to 70 days delayed ensures emergence of adults, which probably helps them to tide over winter.

Anstead (1910) mentioned their occurrence during January and February on trees when the leaves were young. A number of workers have reported that the adults feeding on sorghum, maize, sugarcane, ragi, *Hibiscus cannabinus*, *Zizipus*, *Acacia*, *Dalbergia* and *Tectona* (Ayyar, 1922) on green manuring crop, viz., *Glyricidia maculate* (Hutson, 1937) on *Dalbergia sissoo*, *Tamarix*, *Ulmus campestris* and *Carica papaya* (Mathur and Singh 1959, 1960). Wadhí and Batra (1964) stated that the damage caused to the foliage of loquat and mango was of minor importance. Basher and Jayaraj (1964) reported that the species was a minor pest of cashew and the polyphagous adults sometimes defoliate the tender plants. Sohi (1964) listed the species as causing damage to cotton leaves. The species has been reported to feed on *Moringaoleifera* (Subramaniam, 1965) on wheat in Karnataka (Vishakantaiah et al., 1974) Marshall (1916) reported *M. undecimpustulatus* as a general feeder attacking foliage of many different cultivated plants. Fletcher (1917) observed them on groundnut, *Hibiscus cannabinus* in June and young leaves of wheat. Misra (1917) has indexed this as an Indian fruit pest, on bael, ber, guava, loquat and mango. Ghosh (1921) in his list mentioned the adult as feeding on young leaves of
sugarcane, maize and wheat etc., and the larvae among the roots of the above named crops. In the present work the young mango leaves were more preferred.

The biology was studied under laboratory conditions. The eggs were laid singly as well as in cluster slightly embedded in the soil (Figure. 5). The posterior end of the egg which faces the soil is always slightly narrower than the other end. Under laboratory conditions eggs were laid in the space between the cotton wad and the walls of the glass tube. Eggs were obtained in good numbers on confining a large number of adults in small rearing tube. The incubation period of totally 3500 eggs observed was found to vary from 7-9 days during the months of April to June, when the temperature ranged from a mean minimum of 20.04° C to a mean maximum of 32.15° C and relative humidity from 48.24% to 72.38%. In laboratory conditions the egg laying was observed in all the seasons. The egg-laying and emergence were noticed both during day and night. The entire process of egg hatching took 5 to 6.30 hours after the initiation of the process. The percent egg hatch was >95%. It is note that eggs in the field are covered in soil, thus may go undetected by parasitoids and predators like ants. If exposed, they can become vulnerable to Trichogramma japonicum as was observed in the laboratory. However with >95% hatch grubs as potential pests of roots, can cause havoc of eggs are laid in agroecosystems. This is especially true in brinjal fields. Recent studies at NBAII have shown the T. japonicum released for the control of Leucinodes orbonalis in Dharmapuri, Tamil Nadu also reduced damage of M. undecimpustulatus (Verghese, 2014, Pers. Comm.)

Larvae hatching from the eggs feed on the roots for approximately seven days to 22-25 days (depending on temperature) and then pupate (Figure.5). The pupal stage lasts about 23-33 days, after which the new adult emerges. Newly emerged adults feed on young leaves of host plant. Females begin laying eggs three days to five days after they emerge. Generation time from egg to egg averages about 38 days to 56 days, although it can be shorter or longer depending on environmental conditions.

Adult feeding damage is most easily observed, manifested as marginal leaf notching. Adult damage may be visible from a considerable distance. Larvae are
presumed to be root feeders, although further biological data will be necessary to confirm this. However, the larval host plants for *M. undecimpustulatus* are unknown (Mannion *et al.* 2006). As part of a study to learn more about the biology of this insect, population fluctuation and feeding potential studies were carried out. This study provides the first information on the population fluctuations of this pest in South India.

Soon after hatching the grubs looked creamy, cylindrical and wrinkled (Figure 5). The head is hypognathous, exerted, pale creamy yellowish with brownish pointed mandibles. All the segments are beset with numerous asperities and covered with golden tinged, white fine hairs. There were no major structural differences among different larval instars. Direct observation showed that grubs moulted four times before pupation. There were four larval instars, before each moult the larva stopped feeding and constructed an earthen cell a little away from the roots, a day prior to moult. The moulted larva came out breaking the mud cage with the help of mandibles. The first, second, third and fourth instars occupied approximately 8, 15, 21 and 26 days respectively. The total larval period approximately lasted for 35-45 days (under laboratory conditions variations are quite common). In winter the larval period extended upto 70 days where maximum variation was observed in the second and fourth instars. Availability of roots, optimum moisture in the soil and humidity are very important factors that favour the development of grubs.

Insects are poikilothermic animals that are largely affected by various environmental factors. Among all the climatic factors, temperature has probably the greatest effect on insect development (Taylor 1981; Pedigo 1989). Previous studies have shown that temperature influences various biological characteristics of insects such as sex-ratio (Zheng *et al.*, 2008), adult life-span, survival, fecundity, and fertility (Yang *et al.*, 1994; Dreyer and Baumlager 1996; Infante 2000). As a result temperature profoundly affects colonization, distribution, abundance, behavior, life history, and fitness of insects (Cossins and Bowler 1987; Denlinger and Yocum 1998; James *et al.*, 2002; Hoffmann *et al.*, 2003). Therefore, information on the thermal requirements of invasive insect pest development has important implications for control programs as temperature determines the population growth and size of invasive pests and their variation under different conditions (Kang *et al.*, 2009). Insect abundance and distribution
are regulated by several biotic and abiotic factors and their interactions. Survival and thriving at extreme physical conditions require peculiar adaptations and plastic responses. Among abiotic factors, temperature and humidity stand out as the most important ones constraining abundance and distribution of insects. Furthermore, it is well documented that abiotic factors, especially temperature, regulate the ecology of insect communities.

It was evident that *M. undecimpustulatus* weevil was active during morning hours and gradually decreased with rise in temperature. Such effects are obvious from the influence of daily mean temperature and humidity on the presence of these weevils during their daily feeding in the field.

The present investigation showed a pronounced effect of the environmental factors as seen from the daily mean values of the temperature and RH on the population of *M. undecimpustulatus*. The level of activity was observed as related to the differences in weevils numbers during certain period of the year, hence the maximum number were recorded in August then July and September respectively with an average temperature 26.99, 28.01, 27.67°C and RH of 71.50, 71.68, 70.16% respectively. The minimum population density of the weevils was recorded during February, March, April, January and November where the average temperature reached 28.97, 33.77, 32.92, 28.31, 28.03 and RH 44.71, 46.05, 56.70, 54.29, 69.37%. During the rest of the year the average population density falls between the average range. The *M. undecimpustulatus* weevils were affected by variation in the environmental conditions as regards to their activity and different plant growth stages throughout the year. Moreover they thrive well in temperature ranging between 26°C to 29°C, and RH between 66 and 71%.

Generally, *M. undecimpustulatus* weevils were abundant during June to September then the population starts declining from October to April where it reached the minimum population numbers (Fig. 24). It is pertinent to mention that a month prior to these, grubs had shorter larval duration, more generations, which become evident as abundant adults numbers from June to September.
Members of various orders of insects are often found infested by mites of different groups and the association between them may be of temporary nature for passive transport or may be commensalistic, parasitic or may be of predatory nature (Hunter and Rossario, 1988). The parasitic/predatory behaviour of mites, may be exploited in biological control of insect pests and hence, insect associated mites have received importance and studies have been initiated to explore the mites associated with insects, especially of agricultural importance in order to find out the nature of association between them.

Acarid mites, *Caloglyphus* sp belong to a free living mite species. The mites in soil, feed on decaying plant materials, e.g. fruits, bulb, root or rhizome vegetables and ornamental plants, potatoes, and mushrooms. They often occur in compost, green- and mushroom-houses and field cultivation, in damp stored products, e.g. grain, various plant seeds and food-stuffs (Hughes, 1976; Turk and Turk, 1957; Zakhvatkin, 1941). *Caloglyphus* sp was also observed as invader of broiler-houses, colonizing poultry manure and as necrophagous species infesting bodies of dead birds, e.g. chickens, geese. *Caloglyphus berlesei* also utilizes other kinds of animal food; sometimes this species will feed on dead insects, e.g. grubs of scarabeid beetles (Lipa and Chmielewski, 1966; Chmielewski and Lipa, 1967). Its association with other arthropods, mostly beetles and other insects is mainly phoretique in nature. In the present study it was found during biological rearing that the *Caloglyphus* sp was seen on *M. undecimpustulatus*. There role was not clear and will be an interesting line of future study.

DNA barcoding promises fast, accurate species identifications by focusing analysis on a short standardized segment of the genome (Hebert *et al.*, 2003). Several studies have now established that sequence diversity in a 650-bp fragment of the mitochondrial gene cytochrome c oxidase I (cox1; also referred to as COI) provides strong species-level resolution for varied animal groups including birds (Hebert *et al.*, 2004), fishes (Ward *et al.*, 2005) and Lepidoptera (Hajibabaei *et al.*, 2005).

To be practical as a DNA barcode, a gene region must satisfy three criteria: (*i*) contain significant species-level genetic variability and divergence, (*ii*) possess conserved
flanking sites for developing universal PCR primers for wide taxonomic application, and (iii) have a short sequence length so as to facilitate current capabilities of DNA extraction and amplification. A short DNA sequence of 600 bp in the mitochondrial gene for cytochrome c oxidase subunit 1 (CO1) has been accepted as a practical, standardized species-level barcode for animals (see www.barcoding.si.edu). This lack of consensus is in part due to the limitations inherent in a plastid marker relative to plant CO1, and also because a quantitative context for selecting a gene region as a barcode for plants has not been offered. Several factors must be considered and weighted in selecting a plant DNA barcode: (i) universal PCR amplification, (ii) range of taxonomic diversity, (iii) power of species differentiation, and (iv) bioinformatics analysis and application.

Evolution results from the accumulation of inherited changes in populations. Because DNA is the molecule of heredity, evolutionary changes must be reflected in changes in DNA. Systematics have long known that comparing DNA within a group of species would be a powerful method for inferring evolutionary relationships, but for most of the history of systematics, direct access to genetic information was nothing more than a dream. Today, however, DNA sequencing—determining the sequence of nucleotides in segment of DNA – is comparatively cheap, easy, and widely available. The polymerase chain reaction (PCR) allows systematics to easily accumulate large samples of DNA from organisms, and automated machinery makes sequence determination a comparatively simple task.

Direct benefits of DNA barcoding undoubtedly include

1. Make the outputs of systematics available to the largest possible community of endusers by providing standardized and high-tech identification tools, e.g. for biomedicine (parasites and vectors), agriculture (pests), environmental assays and customs (trade in endangered species);

2. Relieve the enormous burden of identifications from taxonomists, so they can focus on more pertinent duties such as delimiting taxa, resolving their relationships and discovering and describing new species;
3. Pair up various life stages of the same species (e.g. seedlings, larvae);

4. Provide a bio-literacy tool for the general public.

Perhaps another advantage of DNA barcoding is that it will also facilitate basic biodiversity inventories. Indeed, from the premises of molecular phylogenetics to assembling the tree of life, DNA sequences in environmental sampling and reconstruction of phylogenetic trees to place sequences into an evolutionary context have been used in several inventories of cryptic biodiversity (e.g. soil bacteria or marine/freshwater microorganisms).

It is now widely appreciated that a variety of technical issues associated with the use of mtDNA in PCR-based techniques may arise within such studies. The presence of nucleotide ambiguities in mtDNA sequences can be the result of either laboratory contamination or natural biological processes such has heteroplasm or mitochondrial pseudogenes inserted into the nuclear genome (NUMT’s). In any case, co-amplification of these spurious mtDNA fragments can produce misleading and sometimes undetected incorrect results. Perhaps because of the tacit acceptance of the “standard” paradigm regarding the inheritance and biological properties of the mtDNA, researchers are likely to dismiss the idea of heteroplasm or NUMTs when ambiguous sites are encountered during examination of mtDNA sequences. However, as demonstrated in molecular systematics results, when they are identified as the result of these biological processes they can provide valuable insights into the evolutionary history of an organism.

Mergawy and Al Ajlan (2011) reported on the Molecular Phylogeny of Coleoptera: Curculionidae, Staphylinidae and Carabidae Based on the Mitochondrial Cytochrome oxidase I Gene. Phylogenetic relationships among 146 species of Coleoptera (Families: Curculionidae, Staphylinidae and Carabidae) were estimated based upon mitochondrial Cytochrome Oxidase I gene sequences. The monophyletic of the polyphaga and Adephaga was not supported in our study using CO1gene sequences, as family Carabidae (Adephaga) was grouped with family Staphylidae (Polyphaga) with Staphylinidae paraphyletic. The subfamily Scolytinae is the most common ancestor for
the subfamilies: Ceutorhynchinae, Curculioninae and Dryophthorinae and hence the oldest. The subfamily Cryptorhynchinae is the oldest among the five tested Curculionidae families. At the family level, the genetic distances and phylogenetic analysis obtained in this study showed that the family Carabidae was more related to family Staphylinidae than to family Curculionidae with the topology Staphylinida-Carabidae-Curculionidae. The topology was the same when Micromus igorotus from order Neuroptera was used as an outgroup taxon as it was Staphylinida, Carabidae, Curculionidae/Neuroptera. An alternative topology was obtained when Acytolepis puspa from order Lepidoptera was used as an outgroup that was Carabidae, Staphylinida, Curculionidae-Neuroptera/ Lepidoptera, where the species of order Neuroptera placed within family Curculionida. According to the estimated genetic distances and to the standard mitochondrial DNA clock estimated at 2.3% MYA, family Curculionidae separated from family Staphylinidae and Carabidae approximately 112 and 115 MYA respectively.

The molecular taxonomic literature of *Myllocerus* sp is scattered and sparse, and the lack of molecular diagnostic methods means that identification of eggs and larvae is not easy because the immature life stages are morphologically homogeneous. An integrative taxonomic approach was used incorporating both traditional morphological taxonomy and DNA barcode data in an iterative process to both identify beetles and develop robust diagnostics for them. DNA barcodes provide unambiguous discrimination of all species examined in this study, albeit a limited sample, and have the advantage that they can be used to identify all life stages of the species.

Barr *et al.*, 2013 reported on the utility of the cytochrome oxidase I (COI) DNA sequence used for DNA barcoding and a Sequence Characterized Amplified Region for diagnosing boll weevil, *Anthonomus grandis* Boheman. Maximum likelihood analysis of COI DNA sequences from 154 weevils collected from the United States and Mexico supports previous evidence for limited gene flow between weevil populations on wild cotton and commercial cotton in northern Mexico and southern United States. The wild cotton populations represent a variant of the species called the thurberia weevil, which is not regarded as a significant pest. The 31 boll weevil COI haplotypes observed in the study form two distinct haplogroups (A and B) that are supported by five fixed nucleotide
differences and a phylogenetic analysis. Although wild and commercial cotton populations are closely associated with specific haplogroups, there is not a fixed difference between the thurberia weevil variant and other populations. The Sequence Characterized Amplified Region marker generated a larger number of inconclusive results than the COI gene but also supported evidence of shared genotypes between wild and commercial cotton weevil populations.

Bernhard et al., 2009 studied the phylogenetic analysis of Hydrophiloidae (Coleoptera: Polyphaga) based on molecular data and morphological characters of adults and immature stages. An extensive combined data set comprising 160 morphological characters of adults and immature stages of Hydrophiloidae and sequences of six different genes were analysed using parsimony and a Bayesian approach. Analyses were carried out with equal weight for individual morphological and molecular characters, and alternatively with approximately equivalent weight for the entire partitions, i.e., 147 informative morphological characters $\times 9.5 \approx 1383$ informative molecular characters. With the former approach some conventional groups such as the histeroid lineage (Histeridae and Sphaeritidae), Helophorinae and Sphaeridiinae were recovered. However, the branching pattern as a whole is strongly in contrast to the results of previous studies. The results obtained with the modified weighting scheme (9.5:1) conform more to morphology based analyses. The monophyly of Hydrophiloidae, Histeridae + Sphaeritidae, Epimetopinae + Georissinae, Helophorinae, Sphaeridiinae and of the hydrophiline-sphaeridiine lineage is supported in the parsimony analysis. Spercheinae is placed as sister group of all the remaining hydrophiloid groups and a clade is formed by the subfamilies Epimetopinae, Georissinae, Hydrochidae and Helophorinae. In the Bayesian analysis the monophyly of Hydrophilidae is supported. Georissinae form a clade with Hydrochidae, and Epimetopinae are placed as sister group of a clade comprising Spercheinae + the hydrophiline-sphaeridiine lineage. Berosus is placed as the sister group of the remaining groups of Hydrophilinae-Sphaeridiinae in both analyses, and Sphaeridiinae are always nested within a paraphyletic Hydrophilinae. The divergent results of the different analyses show that important questions in the phylogeny of Hydrophiloidae such as for instance the placement of Spercheinae are still open.
Pheromones are chemical signals from one organism that stimulate a response in another individual of the same species. Insects of different orders respond to some pheromones with aggregation behavior. Male-produced sex attractant have been referred to as aggregation pheromones, because they typically result in the arrival of both sexes at a calling site leading to an increase in density of conspecifics in the vicinity of the pheromone source. Aggregation pheromones have been reported for members of the Coleoptera, Dictyoptera, Hemiptera, Homoptera and Orthoptera and have been identified for hundreds of species. During the last two decades the attention has turned to economically important species especially members of the Coleoptera, Curculionidae.

The concept of IPM is based on the recognition that no single approach to pest control offers a universal solution, and that the best crop protection can be provided by a fusion of various tactics and practices based on sound ecological principles. Pheromones are a commonly used component of many insect IPM programs.

The existence of pheromones has been known for centuries, apparently originating in observations of mass bee stinging in response to a chemical released by the sting of a single bee. The first isolation and identification of an insect pheromone (silkworm moth) occurred in 1959 by German scientists. Since then, hundreds, perhaps thousands of insect pheromones have been identified by increasingly sophisticated equipment. Today we have a much clearer view of the limitations and possibilities associated with insect pheromones in IPM programs. The two primary uses of insect pheromones are for detection and monitoring of populations and for mating disruption. These uses take advantage of sex pheromones on which a vast majority of insect pests rely to mediate reproduction.

The principle use of insect sex pheromones is to attract insects to traps for detection and determination of temporal distribution. In most instances, it is the males who are responders to female-produced sex pheromones. Trap baits, therefore, are designed to closely reproduce the ratio of chemical components and emission rate of calling females. Ideally, trap bait should uniformly dissipate its pheromone content over time and not permanently retain or degrade the pheromone in the process. Trap baits of
many designs have been tested over the years, but the hollow polyvinyl plastic fiber (emit from open ends), closed hollow fiber and bag (emit through walls) and laminated plastic flake (emit through walls and exposed edges) are commonly used today. Trap design is also critical to effective use of traps for monitoring insect populations. Traps vary in design and size dependent on the behavior of the target insects. Consistent trapping protocols are essential for population evaluations, spray thresholds, and year to year comparisons. The information from trap catches can be very useful for decision making on insecticide applications or other control measures. For example, trap catches may indicate a loss of effect of pheromone on mating disruption and the need to reapply a pheromone treatment. Careful monitoring and experience in interpreting collected data are important for success. Traps may also be placed with the objective of destroying males for population control.

Male annihilation is trapping carried to a seemingly logical conclusion. Place enough traps, catch enough males, and leave the females of the species without mates. This approach has been used against pink bollworms in an isolated area of Arizona with low numbers of overwintering moths. A rate of 5 traps per acre was used and the traps were composed of Styrofoam cups containing oil to provide larger capacity for dead moths. These traps were placed on row centers to avoid the cultivator and never serviced again. The grower community paid for this program for a few years, but results were difficult to prove because a control area was not available. Calculations by Dr. Edward Knipling (USDA retired) indicated that almost all (95% +) male pink bollworms would have to be destroyed before they could mate in order to exert significant population control. Any untrapped males simply mate more frequently. Mating disruption does not depend on traps for control, although traps are frequently used to monitor the extent of mating disruption in the population. Failure to trap males is taken as an indication that males are unable to find females which may or may not be true. Thus, trap data must always be related to actual levels of crop infestation.

With the commercial availability of insect sex pheromones for several agricultural pests in the 1970's, scientists and entrepreneurs turned their attention to mating disruption as a "biorational" approach to insect control. In theory, mating disruption may be
accomplished in two principle ways: false trail following or confusion. False trail following results from placing many more point sources of pheromone (hollow fibers, flakes or other point sources) per acre than the anticipated numbers of females in the crop. The odds of males finding females at the end of the pheromone trail must be greatly reduced. Emission of pheromone is relatively low from each source such that a downwind trail is created and not lost in a background of released pheromone. Males following these trails are thought to spend their mating energies in pursuit of artificial pheromone sources. Pink bollworm males were early observed trying to mate with hollow fiber pheromone sources in treated fields. Thereafter, commercial pink bollworm pheromone products were applied in stickem containing small amounts of a contact insecticide. The resulting attract-and-kill formulations (another form of male annihilation) were viewed as a subversion of the pheromone by purists, but in practice the damage was limited to the target species. However, the effectiveness of the added insecticide is largely unknown under field conditions. Growers endorsed the idea that a dead male is better than a confused one. A further combination of pheromones and insecticides is occasionally encountered. Dual applications of pheromone and full strength insecticides (either separately or in tank mixes) are applied with the idea of increasing insect flight activity and thus increasing the chance of insecticide exposure. Full strength applications of pheromone are generally used for this method. The greater the amount of pheromone applied and the greater the release rate, the more likely males are to be confused in the fog of ambient pheromone.

Male confusion is thought to be the result of ambient pheromone concentrations sufficient to hide the trails of calling females (large doses from diffuse sources such as microcapsules or larger doses of pheromone in point source dispensers such as tie-on polyethylene ropes). Added to the effect, or indeed the effect, is the adaptation of antennal receptor sites and/or habituation of the insect's central nervous system. Specific receptor sites on the antennae respond to only the pheromone molecules (individual component molecules appear to have individual receptor sites on antennae). When a receptor site is continually activated by high ambient concentrations of pheromones, the resulting electrical signal diminishes (measured by an electroantennogram). The receptor site becomes unresponsive and the insect becomes navigationally blind. When the insect's
central nervous system is inundated with signals from the receptor sites it becomes 
habituated: no longer able to provide the directed behavior. All of the above are, to some 
degree, based on known neurophysiology, but exactly what proportion of each occurs in a 
given situation can only be guessed. The net result of confusion is that the male is unable 
to orient to any pheromone source and follow the upwind trail to a mate. For a current 
summary of theory and application of pheromones for control of Lepidopterous pests.

Present commercial formulations of pheromones for both trap baits and mating 
disruption mimic the natural chemical blends of females as clearly as possible. Most 
insect sex pheromones are multicomponent with precise ratios of components which may 
be expensive to manufacture. Thus, insect sex pheromones and products containing 
pheromones are commercially available primarily for insects of economic importance. 
Fortunately, there is hardly an insect species of agricultural importance, among the 
Lepidoptera at least, for which there are not some pheromone products available.

It is impossible to cover here all the insect pests for which IPM programs using 
pheromones are recorded. We have selected agricultural pests for discussion that we are 
familiar with or for which information is readily available.

The boll weevil, *Anthonomus grandis* Boheman, has been a primary pest of cotton 
since its introduction to the United States in 1892. Numerous methods have been used to 
control weevils, but the idea of eradication has always had advocates. It was not until the 
early sixties that it was shown that the male boll weevil produces a sex pheromone in its 
frass that is attractive to females. This pheromone was also shown to act as an 
aggregating pheromone for both sexes (primarily in early and late season). The four 
components of the pheromone were identified and synthesized by the late 1960's and 
called grandlure. Formulations that were attractive as trap baits made survey and 
monitoring possible over wide cotton growing areas of the cotton belt. In 1978, an 
eradication program for the boll weevil began which continues today. Basically, the 
program includes: 1. In-season control using insecticides. 2. Reproduction-diapause 
control in early and late season to reduce the numbers of weevils entering cotton fields 
each spring. 3. Survey and monitoring with pheromone-baited traps. 4. Area-wide
insecticide treatment of cotton at first pin-head flower bud ("hostable square" stage) in the spring. As of this date, December 1995, boll weevils have been eradicated from much of the eastern cotton belt and programs are underway in central states including Mississippi, Louisiana, and Texas. Because small cotton fields are the norm in much of the cotton belt, the use of up to 1 trap per acre (along field borders) is required to detect extremely low residual populations. Detection of a single weevil results in more intense trapping. Capture of a second weevil is considered evidence of a possible reproducing population and spraying is initiated. Pheromone-baited traps are placed on millions of acres of cotton to monitor new and old program areas. During the 1995 season, the program consumed 2 million traps and 25 million trap baits—the largest program of its kind in the world. Malathion (ULV) is the current principle insecticide used in program-directed spraying. The boll weevil eradication program is jointly funded by federal, state, and grower money and has saved billions of dollars in weevil control over its lifetime. Just as importantly, the use of insecticides to control boll weevils, ca. 40% of the total U. S. consumption, is being reduced. Complete eradication of boll weevils from the United States is projected for approximately the year 2010.

*Pectinophora gossypiella* (Saunders), by mating disruption began with the sex attractant "hexalure" in the early 1970's. The discovery of the pink bollworm sex pheromone in 1973 led to the first successful commercial formulation in 1978 (*Baker et al.* 1991). The pheromone, a two component mixture of Z, Z- and Z, E-7,11-hexadecadienyl acetate (called gossypure in commercial products), has appeared in a variety of aerially applied formulations including hollow fibers, flakes, microcapsules, and in hand-applied twist-tie ropes and twist-on spirals. Original applications utilized 0.75 to 1.5 g Al/acre in several thousand point sources and were applied several times during early to mid-season while recent hand-applied formulations utilized ca. 30 g Al/acre and were applied once. These are known as false-trail following and confusion methods, respectively. All formulations are to be applied at first flower bud ("pin-square" or about 8 true leaf stage cotton) which is the earliest fruiting form in which the pink bollworm can reproduce. Applications at first flower bud are made against the lowest seasonal (over-wintered) populations, an aid to efficacy.
Pheromones have played a major role in controlling infestations of the tomato pinworm, *Keiferia lycopersicella* (Walsingham), a primary pest of tomatoes. Larvae attack leaves, but economic damage is greatest when they enter the fruit. Development of a control system using sex pheromone for control of the tomato pinworm was initiated in 1979, soon after the identification of the pheromone as a 96:4 mix of E and Z-4-tridecenyl acetate. A hollow fiber formulation was first successfully developed in Mexico's Culiacan Valley. Commercial use of the pheromone increased during the 1980's as the pinworm became increasingly resistant to insecticides. Problems with insecticide use were several: control became more expensive as repeated applications of chemical insecticides failed to control the pest, insecticide residues resulted in condemnation of tomatoes intended for shipment into the United States and outbreaks of secondary pests were often triggered by repeated applications.

By the end of the decade, growers of both stake and processed tomatoes in Mexico had changed completely to IPM programs using mating disruption for control of the pinworm. This pheromone is especially intriguing because it can be used successfully against heavy infestations of the pinworm. Most pheromone programs require that initial applications start when the pest is at a low numerical density. Traps and lures are also used widely to detect moth emergence cycles so that timely applications of pheromone or insecticides can be applied.

The European corn borer, *Oestrinia nubilalis* (Hübner), is an important pest of corn, cotton, sorghum, and vegetable crops in the eastern United States. The European corn borer has two generations per year in most of the United States which limits population growth and places a premium on control of the first generation. The pheromone of the European corn borer was identified as a mixture of Z- and E-isomers of 11-tetradecenyl acetate in the early 1970's. Interestingly, three distinct populations of European corn borer exist (sympatrically in some areas), separated by ratios of the two isomers of their pheromone. The Z and E strains utilize predominantly these isomers in their pheromones and hybrids utilize intermediate ratios. Pheromone traps are used to monitor populations with large cone traps catching approximately seven times as many males as wing traps which rely on a limited amount of sticky surface. Therefore, trap
type, ratio of Z- and E- isomers and lure strength are critical for consistent results in monitoring the European corn borer. Unfortunately, adjacent areas may require different trap baits for optimum catches. Current practice for IPM management includes population monitoring with pheromone traps and judicious use of biorational materials such as the various *Bt* products and insecticides.

In the present report the male aggregation pheromone response to female *M. subfasciatus* has been reported. Representative GC-EAG recording of female *M. subfasciatus* responses to prothorasic gland extraction volatiles (Fig. 45). The FID peaks marked are those which elicited responses in 20 replications of 200 adults per each time: Z-11- Pentadecenal and 16- Heptadecyn-1-ol. Chemical structure of pheromone components of *M. subfasciatus* revealed and identified as (Z)-10-Pentadecenal, (Z)-9-Tetradecen-1-ol, (Z)-9-Octadecen-1-ol, (Z)-9-Hexadecenoic acid, (E)-11-Tetradecenal (Fig. 46). The chemical structure of pheromone components of *M. subfasciatus* is depicted in Fig. 47.

Previous research on other coleopterous insects showed that topical application of juvenile hormone (JH) or a juvenile hormone analog substantially increased pheromone production in bark beetles (Scolytidae) (Borden *et al.*, 1969; Hughes and Renwick, 1977a,b; Renwick and Dickens, 1979). While application of a juvenile hormone analog (methoprene) increased pheromone production in the boll weevil *Anthonomus grandis* Boh. (Curculionidae) over that of control insects, antennectomy increased pheromone production significantly more within 48 h (Dickens *et al.*, 1989). Since the juvenile hormone analog decreased the sensitivity of antennal receptors for pheromones (Palaniswamy *et al.*, 1979) and for plant odors in the boll weevil (Dickens, 1989), it was proposed that decreased antennal input may be responsible for observed increases in pheromone production.

The male-produced pheromone for Colorado potato beetles is the first to be identified for a chrysomelid beetle; previous pheromones identified for chrysomelids have been female produced sex attractants (Mayer and McLaughlin, 1991). Although the structure of CPB I has been reported (Devi and Bhattacharyya, 1977) as a bacterial
metabolite of geraniol, it is unique for an insect pheromone. Recently, field-trapping experiments indicated that male crucifer flea beetles, Phyllostreta cruciferae (Goeze), may produce an aggregation pheromone, but the nature of the attractant was not elucidated (Peng et al., 1999).

Based on the results of the current investigation, it is evident that the identified compounds can be incorporated into the integrated pest management programme for monitoring M. subfasciatus. The pheromone component identified in the present investigation has scope to be synthesized and commercialized. It can be used as a useful monitoring and surveillance tool to for Mylocerus. The present study is the first such study for M. subfasciatus.

The scientific community working in the field of insect pest management is experiencing an increasing academic and industrial interest in the discovery and development of new bioinsecticides as environmentally friendly pest control tools to be integrated, in combination or rotation, with chemicals in pest management programmes. In this scientific context, market data report a 15% annual growth of the biopesticide segment. This trend is in line with the requirements of new regulations on Integrated Pest Management. After few decades of research on microbial pest management dominated by B. thuringiensis (Bt), novel bacterial species are being discovered and developed into new products.

The global pesticide market is presently growing at a rate of 3.6% per year and is valued around $ 47 billion (BCC Research, 2010). This trend is in relation to the need of protecting the environment, farmlands and the agriculture crops (Oerke and Dehene, 2004) feeding a human population expanding at a rate around 1.15% per year (United Nations, 2011). In this context, environmentally sustainable improvements in technology, agricultural techniques, and pest management are vital to allow farmers to expand crop production and animal farming on the limited land available.

The bioassays of B. thuringiensis have been steadily modified and new bioassays have been designed since the 1960s. The need to modify official bioassays and
develop new ones derives from the discovery of new strains, the development of conventional and genetically manipulated *B. thuringiensis* formulations, the limited knowledge of environmental impact of the microbe and the necessity to study and monitor insect resistance to the microbe. For example, the mortality records (LC\text{50}) used to determine the potency and activity of the microbe, the time-mortality (LT\text{50}) and effective concentration (EC\text{50}) became useful and accurate parameters in bioassay protocols. Standardized dietary bioassays and diverse leaf/plant bioassay procedures have been made more useful. In tritrophic interactions of *B. thuringiensis*, the microbe bioassays are combined with compatibility assays of plant allelochemicals or interactions with the insect host and its larval parasitoid(s). The bioassays for monitoring insect resistance to the microbe have been made more efficient and practical. In the foreseeable future, it is expected that the need for new or improved bioassays as useful tools for developing rational control strategies with *B. thuringiensis* will continue. In the present investigation the toxicity analysis of *Bt* results produces mortality both in grubs as well as adults Fig 49 and 50.

The biological control paradigm changed some decades later, when the potential of entomopathogenic bacteria was discovered, especially associated to species belonging to the genus *Bacillus* (Glare and O'Callagan, 2000). Initially, the species *Paenibacillus* (former *Bacillus*) *popilliae* Dutky was introduced for the management of the Japanese Beetle *Popillia japonica* Newman (Steinhäus, 1975), but more concrete results were achieved with the discovery of new *B. thuringiensis* (*Bt*) strains showing high toxicity against specific insects at competitive level compared to conventional insecticides in terms of efficacy and costs of production. The strain HD-1, belonging to subsp. *kurstaki*, soon became the main commercial focus for the management of lepidopteran pests in agriculture and forestry. Beside it, today other strains are commercially available, such as SA-11, SA-12, PB 54, ABTS-351 and EG2348, all isolated from insects or soil, and expressing a range of different toxins mostly belonging to the Cry1 and Cry2 families. Subsequently, the discovery of a *Bt* strain belonging to the subsp. *israelensis* (*Bti*) was followed by its commercialization for the management of mosquitoes and simulids.
(Goldberg and Margalit, 1977). Then, a particularly active strain of the subsp. *tenebrionis* was discovered and employed against Coleoptera (Krieg et al., 1983).

Undoubtedly, *B. thuringiensis* is the species on which most of the scientific community and industry efforts have been focused. Main feature of this bacterium is the production of parasporal bodies (crystals) containing specific insecticidal endotoxins (Cry proteins) acting by ingestions through a pore-forming mechanism of action detrimental for the insect gut epithelium (Pigott and Ellar, 2007).

With reference to a strain-specific mode of action, Cry toxins act only if binding to gut receptors varying among insect species. For these reasons, an increasing number of *cry* genes have been identified and sequenced, and more genes continue to be discovered as new bacterial isolates are collected worldwide (De Maagd et al., 2003). Other protein toxins from *Bt* (i.e. Cyt, VIP) have been identified and their genes characterized.

As a result of this continuous search, an official international database including toxins and genes from *Bt* and other etomopathogenic bacteria is continuously being updated (Crickmore et al., 2006). Different studies to evaluate the effects of *Bt* toxins on both insect pests (Pérez-Guerrero et al., 2012) and on non-target species (Marchetti et al., 2012) are continuously being conducted.

Despite the success of many available products, the use of microbial based biological control is still relegated to niche contexts, in relation to the previously described highly specific mode of action and the narrow efficacy spectrum. Biopesticides use in combination or rotation with synthetic pesticides is likely to be enhanced in the near future, but more research is needed to come up with innovative solutions that can really meet farmers and regulator needs in terms of effectiveness and environmental sustainability. At the present state of the art, biopesticides have not yet achieved their potential due to the lack of truly transformational associated technologies that may enhance their effectiveness (Glare et al., 2012).
Conclusion

The main findings of my Ph.D. thesis studies are summarized and discussed under this heading. A comprehensive systematic treatment of the genes Myllocerus has been made in this investigation, including a general introduction to the weevils, general biology and the hosts, phylogenetic analysis using molecular systematics, distribution pattern appraisal, insect pheromone identification and biological control using Bt toxins. This work has provided a much firmer basis of knowledge of the genus than before. Such knowledge is essential for the correct identification of species, better management of pests and understanding of evolution of this increasingly important group of weevils. I hope that my work presented in this thesis is to be used as a foundation on which further research on Myllocerus can be built. The biology and pest status of Myllocerus undecimpustulatus and Myllocerus subfasciatus in Karnataka state, India are critically investigated. New data are presented and the relationships between the weevil and its host plants considered. Despite their pest status and the importance of the damage they causes, no recent or comprehensive review in which their biology is compared has been published. Lacunae in data on their biology are pointed out, and lines of research that could profitably be pursued are indicated. The lack of knowledge of the processes involved in adult dispersal and longevity are highlighted as major areas of concern. The biology and behaviour of the adult and larval stages are investigated and new data presented. The feeding preferences of the adult weevils are considered and the possibility of using deterrents as a pest management strategy discussed. In general as evident from the results the activity of the weevil is at its peak in the month of August; therefor it is recommended that the crop must be protected during the month of July, September and June to control the activity of the weevils. The development of risk assessment and forecasting tools aimed at more effective deployment of pest-management options are discussed. Risk criteria have their origins in important ecological relationships which require new understanding, but the prospects for determining high-risk sites are promising. This study documented preferential distribution of Caloglyphus spp. mites on M. undecimpustulatus weevils. These parasitic mites were numerically abundant on M. undecimpustulatus and the effects of this association may be more parasitic. The possible detrimental role of excessive Caloglyphus spp. mites on the weevils needs further
investigation. In host preference study the hypothesis is bound by two important assumptions. Firstly, that grubs use more than one host plant during development and, secondly, that switching host species during development reduces juvenile fitness. The advantages of this hypothesis are only valid in insect species where both these traits are borne out. In this section we look at evidence which suggests that these particular traits may be widespread throughout the Insecta. This hypothesis leads to an advantage for learning in the oviposition behaviour of adult polyphagous insects which as yet, remained unexplored. Foraging adult females are likely to encounter more abundant host plant species more frequently. Where a more abundant host roots are more suitable for larval development and increasing the fitness of their offspring. To our knowledge, this study is the first record of phoretic mites in India. The options for biological control are evaluated, in particular the use of *B. thuringiensis* and larval and adult mortality. It is concluded that much more research into the biology and ecology of ash weevils is required before a successful Integrated Pest Management (IPM) programme can be initiated. This study has a lot of application value as it has identified some weak links in the life stages of insects, especially eggs and grubs. The study has explained the in post summer population in terms of more number of generations and lower population in winter due to longer larval period. Control strategy for summer and winter populations especially grubs will vary. The pheromone and Bt have as significant components of IPM.