

**EXPLORATION OF NATURAL ENEMIES OF THE OIL PALM LEAFMINER
COELAENOMENODERA LAMEENSIS BERTI AND MARIAU (COLEOPTERA:
CHRYSOMELIDAE) IN OIL PALM PLANTATIONS**

BY

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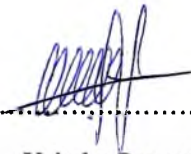
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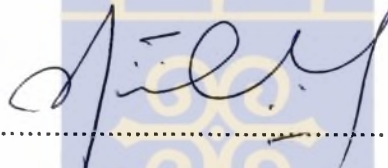
DECLARATION

I hereby do declare that, with the exception of references to other scholars' work which have been duly acknowledged, this thesis consists of my original work and has neither in whole or in part been presented, to any other institution for the award of any degree.



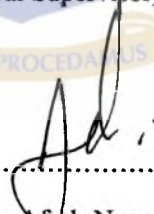
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DEDICATION

To my dad, Philip K. Yakubu, who taught me never to feel limited at any thing and that the most important thing in life is to have a positive mindset at any thing one attempts to do.

Also dedicated to Prince Gabriel Waabu, Kunzinle Roger and Abdullai Naim Alhassan and the rest of my siblings.



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ABSTRACT

Surveys were conducted to identify the natural enemies of the oil palm leafminer, *Coelaenomenodera lameensis* Berti and Mariau (Coleoptera: Chrysomelidae). Different developmental stages were recovered from infested leaflets and reared for the emergence of parasitoids. Four parasitoids all belonging to the order Hymenoptera and family Eulophidae were identified. They were *Neochrysocharis* species, *Chrysocharis* species, *Pleurotroppopsis* species and *Apleurotropis* species. Parasitism on *C. lameensis* developmental stages by the parasitoids was 13.51%. Predatory ant survey was based on the presence of the nests on the oil palm. *Oecophylla* species, *Crematogaster* species and *Tetramorium* species were observed to be the predatory ants of the oil palm leafminer on the plots. Entomopathogenic studies were based on the collection of dead and/or dying *C. lameensis* stages for further culturing and identification. The entomopathogenic fungi identified in this study was the Zygomorpha *Syncephalastrum racemosum* Cohn belonging to the order Mucorales.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Oil palm, *Elaeis guineensis* Jacq is indigenous to the tropical rainforest belt of West and Central Africa between Guinea and northern Angola and has spread throughout the tropics (NewCROP, 1996; Maley and Chepstow-Lusty, 2001; Sheil *et al.*, 2009). It is now grown in over 16 countries. However, the major centre of production is in South East Asia with Malaysia and Indonesia together accounting for about 90% of world palm oil production (Poku, 2002; IPOC, 2006; Yusoff, 2006; USDA, 2008b). Since 2005, Indonesia has been the world's largest and most rapidly growing producer of palm oil (USDA, 2008b).

In 2005, palm oil overtook soybean as the world's main vegetable oil, and in 2007/2008, production reached 41 million tonnes (USDA, 2008b). Other African countries with significant palm oil production are Côte d'Ivoire, Democratic Republic of Congo, Cameroon, and Ghana. Others include Angola, Guinea, Sierra Leone, Benin and Togo (Koh and Wilcove, 2008a; Sheil *et al.*, 2009). Oil palm generally grows and yields well in the tropics and subtropics with evenly distributed rainfall pattern ideally with zero evaporation deficit, good soil, gentle terrain, reliable planting materials and good cultural practices (Gyasi, 1992a; Sheil *et al.*, 2009). In Ghana, the current oil palm growing areas are located in parts of the forest zone which receive an average rainfall of about 1200 to 2200 mm per annum. Large parts of these regions offer suitable climate and soils favourable for oil palm cultivation. Oil palm production in these areas consists of four types, namely; Estates (plantations), smallholders, outgrowers and small-scale farmers' level (Gyasi, 1992a; NewCROP, 1996).

World demand for vegetable oils is rising sharply, from 100 million tonnes in 2005 to an estimated 150 million tonnes in 2020, as the world population continues to grow and the standards of living increase in many developing countries (Sheil *et al.*, 2009).

The role of oil palm as a supply of relatively inexpensive and versatile edible oil is, therefore, expected to become even more prominent. Oil palm and its products have a variety of uses which can be classified as traditional and industrial uses (Essiamah, 1985; Fairhurst and Mutert, 1999). In most West African societies, unrefined red palm oil is an essential ingredient in their diet. Palm fruits are boiled and macerated and used to prepare a nutritious soup, served after removal of the seeds, fibre and part of the oil. In West Africa it is common practice to produce palm wine by tapping the unopened male inflorescences, or the stem just below the apex of felled oil palms. The leaflet midribs are made into brooms and the rachises used for fencing. Young leaflets produce a fine strong fibre for fishing lines, snares and strainers. The palm oil obtained from the mesocarp, and palm-kernel oil from the endosperm have several industrial uses ranging from edible products, such as cooking oils, margarine, vegetable ghee, shortenings, frying and bakery fats, and for preparing potato crisps, pastry, confectionery, ice-cream and creamers. Palm oil is used to manufacture soaps, detergents, candles, resins, lubricating greases, cosmetics, glycerol and fatty acids (Essiamah, 1985; Sheil *et al.*, 2009). Palm oil is employed in the steel industry for tin plating and sheet-steel manufacturing and epoxidized palm oil is a plasticizer and stabilizer in PVC plastics. Palm oil and more particularly its methyl- or ethyl-ester derivatives have potential as biofuel for diesel engines. (Essiamah, 1985; Sheil *et al.*, 2009). Palm oil has also contributed to global biodiesel production (Rupilius and Ahmad, 2007) and its contribution to the industry is swiftly expanding. In Malaysia, palm biodiesel already fuels buses

and cars (Reijnders and Huijbregts, 2008) and most major automotive and oil companies are researching biofuel (Herrera, 2006).

The prospects of the oil palm industry in any economy are bright but for certain production constraints among which is the incidence of insect pests. The oil palm leafminer, *Coelaenomenodera lameensis* Berti and Mariau (Coleoptera: Chrysomelidae) is the most important pest of oil palm, *E. guineensis* Jacq in Ghana (Maulik, 1920; Bernon and Graves, 1979). The beetle is endemic in West Africa and outbreaks have been recorded in many countries in the West African sub region including Cameroon (Shearing, 1964), Cote d'Ivoire (Jover, 1950), Ghana (Maulik, 1920; Bernon and Graves, 1979; Appiah and Yawson, 2003) and Nigeria (Golding, 1946; Agwu, 1977). Both the adult and larvae cause damage to the foliage. However, serious and most damage are caused by the larvae because of the number of mines formed (Berti and Mariau, 1999). The larvae live and develop within the leaflets, feeding by mining the upper epidermis of the palm leaflets (Cotterell, 1925). Adults easily swarm and feed on the underside of the leaflets, scooping out grooves parallel to the veins leading to the partial drying up of fronds (NRI, 1996). In severe infestation, only the spears may be unaffected while the other fronds appear desiccated, leading to a reduction in photosynthetic activity of the leaves (NRI, 1996). Successive cycles of the infestation by *C. lameensis* could lead to inflorescence abortion, bunch failure and considerable economic loss (Wood, 1976). Heavily attacked trees may lose up to 90% of their fronds with 50% consequent loss in yields of fresh fruit bunches (FFB) over two year period (Owusu-Appiah, 2007).

1.2 Justification

Various methods have been used for the control of *C. lameensis*. Chemical application has been used through trunk injection (Mariau *et al.*, 1979; Philippe and Diarrassouba, 1979; Yawson *et*

al., 2009), fluid air spraying using tractor-drawn Tecnomat Twin cannon, aerial spraying using light aircraft or helicopter and through root absorption (Philippe, 1990). Chemicals have also been applied by hot fogging (Philippe, 1990; Yawson *et al.*, 2009). Cultural method of control by leaf pruning has also been practised (Agwu, 1977, 1981). The mining behaviour of the larvae of *C. lameensis* makes chemical control very difficult (Mariau and Philippe, 1983). In addition, chemical control is very expensive (Timti, 1991), pollutes the environment, induces the development of pest resistance and is dangerous to human and animal health.

The management of pest population below the economic threshold level using natural enemies is another viable option of consideration especially in the oil palm industry. The Roundtable on Sustainable Palm Oil (RSPO) stipulates that pests, diseases, weeds and invasive introduced species are effectively managed using appropriate Integrated Pest Management (IPM) techniques. Agrochemicals are used in a way that does not endanger health or the environment. Natural enemies comprising predators, parasitoids and entomopathogens provide less expensive, environmentally-friendly and sustainable pest management option. Biological control using some insect species have been investigated, example, *Crematogaster* sp in Cameroon (Timti, 1991), *Chrysonotomyia* sp (Lecoustre *et al.*, 1980) and *Pediobius* sp (Mariau *et al.*, 1979) in Cote d'Ivoire. Natural enemies, however, are ineffective especially in outbreak situations (Mariau and Morin, 1972; Timti, 1991). In this regards, the exploration of natural enemies in the control of the oil palm leaf miner is expedient. It provides an avenue for oil palm products devoid of insecticides to be produced.

1.3 Objectives

The aim of this study was to explore natural enemies of the oil palm leafminer *Coelaenomenodera lameensis* Berti and Mariau (Coleoptera; Chrysomelidae) in Ghana.

Specific objectives

1. To identify and characterize the parasitoids of the oil palm leaf miner.
2. To identify and characterize predators of the oil palm leaf miner.
3. To determine the entomopathogens of the oil palm leaf miner.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

The oil palm of commerce, *Elaeis guineensis*, is believed to be indigenous to Central and West Africa. There is a general consensus that commercially planted palms in Indonesia, Malaysia and other South–East Asian locations were derived from four West African palm varieties (F.A.O., 2002a).

The history of oil palm as a commercial crop is rather short, dating back to 1807 on the West African Coast where its cultivation commenced. It came to the East via the Island of Mauritius in 1848, to Indonesia (the Botanical Garden at Bogor) where four seedlings of four West Africa palm varieties were planted. At about 1870, the seedlings were transferred to the Singapore botanical gardens and then to Malaysia in 1875 (Moll, 1987). The African oil palm has also been taken to Central and South America where it was first cultivated in Brazil, and later to Colombia and other neighbouring countries.

World production of palm oil increased from 1.3 million tonnes in 1960 (78% from Africa) to 12.1 million tonnes in 1980 (83% from South-East Asia) and it almost doubled again in the subsequent two decades. It continued to increase substantially, from 25.4 million tonnes in 2001 to 34.8 million tonnes (from 12.6 million ha) in 2005 and in 2008 it had increased to 41 million tonnes, largely as a result of further expansion of oil palm cultivation in South-East Asia (Sheil *et al.*, 2009).

In Ghana, Ghana Oil Palm Development Company (GOPDC), Twifo Oil Palm Plantations Limited (TOPP), and Benso Oil Palm Plantations Limited (BOPP), have developed rapidly and contributed significantly towards the expansion of Ghana's oil-palm hectares from 18,000 to

103,000 between 1970 and 1990 (Gyasi 1992a). This growth of 24 per cent per annum has resulted in the re-emergence of the palm as a major commercial crop rivalling cocoa; has served as a basis for the fast-developing palm oil and other agro-industrial processing industries; and rendered the country more than self-sufficient in palm-oil production. In 1989, GOPDC, TOPP, and BOPP contributed about 20 per cent of the national palm hectares and 40 per cent of the palm-oil output, which traditionally had been dominated by small home-based producers (Gyasi, 1992a).

2.2 Oil palm pests

Until the time of the Second World War, it was true to say that the oil palm was largely free from serious diseases and pests. Since that time, however, there have been serious and at times devastating outbreaks of diseases and pests in several parts of the world (Hartley, 1988). A large number of insects have been recorded on oil palms in Ghana, but only a few have become major pests (Obeng-Ofori, 1998; Owusu-Appiah, 2007). The red striped weevil *Rhynchophorus phoenicis* Fabricius (Coleoptera: Curculionidae), the rhinoceros beetle *Oryctes monoceros* Oliver (Coleoptera: Dynastidae), Elephant beetles, *Augosoma centaurus* Fabricius (Coleoptera: Dynastidae) burrow into the cluster of central spear leaves of the oil palm and thereby predispose the palms to secondary attack by the palm weevil. Pheromone-based mass trapping of the *Rhynchophorus phoenicis* Fabricius (Coleoptera: Curculionidae) and the rhinoceros beetle *Oryctes monoceros* Oliver (Coleoptera: Dynastidae) and manual collection of the weevils can be effective methods of pest control. Insect pests that cause occasional damage are the African spear borer *Pimelephila ghesquierei* Tams (Lepidoptera: Pyralidae) and the Oil palm weevil *Temnoschoita quadripustulata* Gyll. (Coleoptera: Curculionidae). These cause considerable damage on young palms in Central and West Africa in particular. The variegated grasshopper *Zonocerus variegates* L. (Orthoptera: Acrididae) is also another insect pest of the oil palm

(Owusu-Appiah, 2007). The oil palm leafminer, *Coelaenomenodera lameensis* Berti and Mariau, (Coleoptera: Chrysomelidae) is noted to be the most important pest of oil palm in West Africa causing widespread defoliation (Coterell,1925; Duodu, 1974; Hartley,1988; NRI,1996; Forest, 2001).

2.3 *Coelaenomenodera lameensis*

Coelaenomenodera lameensis Berti and Mariau (Coleoptera: Chrysomelidae: Hispinae) is an oil palm leaf miner that has been recorded from all the humid tropical countries of West Africa. Outbreaks, characterized by high and extensive defoliation of palms, have been reported in most of those countries (Maulik, 1920; Cachan, 1957; Owusu-Appiah, 2007) and have become increasingly frequent with the development of oil palm cultivation over the last 30 years.

2.3.1 Biology

The life cycle of *C. lameensis* ranges from 96 to 100 days depending on factors such as temperature and the environment (Morin and Mariau, 1971; Hartley, 1988; Owusu-Appiah, 2007). In a year it is possible for four generations to be produced. The adult female lays eggs in a small cavity on the underside of the leaflets and then covers them with a mass of fibrous debris. They are usually laid in groups of 2-4 and sometimes up to 12. A female can lay as many as 350-400 pale yellow eggs each about 0.80mm long and 0.28-0.30mm in diameter (NRI, 1996; Afreh-Nuamah, 1999). Incubation periods range from 20-30 days with an average of about 20-21 days (Owusu-Appiah, 2007).

Larvae

The larvae are uniformly bright yellow in colour and are flattened dorsoventrally with the head embedded in the prothorax. There are four larval stages (Plates 1-4). The first stage larva is the

smallest and measures about 0.8mm. The fourth instar larva measures up to 6.5mm. The larval period ranges from 41-47 days (Cotterell, 1925; Owusu-Appiah, 2007).



Plate 1: First instar larvae



Plate 2: Second instar larvae



Plate 3: Third instar larvae

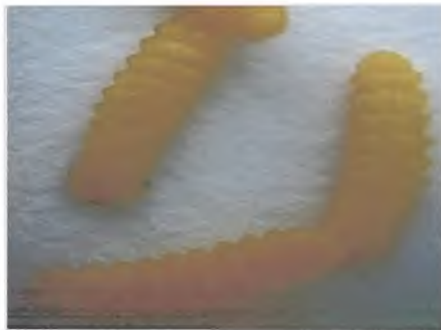


Plate 4: Fourth instar larvae

Pupae

The pupae are orange red in colour and are mobile (Plate 5). They are found in the centre of the galleries mined by the larvae and are not enclosed in any covering. Pupal development takes about 11-14 days (Cotterell, 1925; Owusu-Appiah, 2007).



Plate 5: Pupae of *C. lameensis*

Adult

The young internal adult remains in the mines for about 3 days before emergence into matured adults. Adults are usually orange-yellowish in colour (Plate 6) with the females slightly bigger than males. The females measure about 5.6mm while the males are usually about 5.2mm long exclusive of the antennal length. The body is covered with numerous puncture-like spots. The pronotum is rounded interiorly and projecting forwards, with a pair of lateral depressions (Maulik, 1920).



Plate 6: Adult *C. lameensis*

Adults easily swarm and feed on the underside of the leaflets making grooves of about 1 -2cm long, parallel to the central rib. The adult can be ready for egg laying 17days after emergence and continues to live for three to four months during and after laying eggs (Morin and Mariau, 1971; Hartley, 1988; Afreh-Nuamah, 1999; Owusu-Appiah, 2007).

2.3.2. Destructive behaviour of *Coelaenomenodera lameensis*

Morin and Mariau (1971) and Afreh-Nuamah (1999) reported that, the growing larvae mine under the upper epidermis of a leaflet, producing a mine of about 20cm in length and 1-2cm wide. The mean area mined by a larva is about 10cm². The mined area soon turns necrotic. The adults easily swarm and feed on the underside of the leaves, scooping out grooves parallel to the veins and continue to live for three to four months leading to partial drying up of fronds. Destruction of leaves starts from lower fronds. Infestation tends to radiate from an initial focus of just a few trees. The larvae by their mining behaviour for an average period of 44 days are the main cause of damage whereas, the length of the adult life cycle accounts for the incidence of the pest reappearing in some cases every three or four months (Hartley, 1988). In heavy infestations only the central spear remains uninfested while the leaflets appear desiccated leading to a reduction in photosynthetic activity of the leaves (NRI, 1996). Secondary infestation leads to toppling of the trunk and reduces the plant's resistant ability to diseases (Owusu-Appiah, 2007). Morin and Mariau (1971) noted that the outbreak of the leaf miner varies in extent, but they can spread over several hundreds of hectares. An outbreak can defoliate a plantation in 6 months reducing output for 3 years. Heavily attacked trees may lose up to about 90% of their leaves with a consequent loss in yields of about 50% over a two year period (Owusu-Appiah, 2007).

2.3.3 Economic importance of *C. lameensis* in Ghana

All the large plantations/estates in Ghana; Benso Oil Palm Plantations Limited (BOPP), Ghana Oil Palm Development Company (GOPDC), National Oil Palm Limited (NOPL), Twifo Oil Palm Plantations Limited (TOPP) and the State Oil Palm Plantations Limited (SOPP) have at various times been severely attacked by *C. lameensis* (Appiah *et al.*, 2007).

The Jukwa Complex of the SOPP has had an unbroken outbreak since 1985. This has resulted in the premature felling of palm trees, which have not reached the end of their economic period. NOPL by 1993 had only 1300 out of a total plantation of 4300 hectares that were fruit bearing to feed the rehabilitated mill. The bulk of the area had to be felled as an interim measure to contain the menace of the pest that had severely defoliated the palms (Anonymous, 1993).

The plantations of GOPDC at Kwae were severely attacked by *C. lameensis* in 1986-87 and about 2000 hectares out of the total 4200 hectares of the plantation suffered severe defoliation. A report on the state of the plantations estimated production losses at about 13000 tonnes of fresh fruit bunches or 2600 tonnes of palm oil. This amounted to a revenue loss of about GH¢ 20,800 (20,800 US dollars). In 1989, production of fresh fruit bunches (ffb) had still not yet returned to its normal level. An average of 25% production loss each year over three years was estimated in addition to the high cost of chemical control by aerial spraying (Anonymous, 1993).

The Twifo Oil Palm Plantations Limited (TOPP) importation of Thiocyclam hydrogen oxalate (Evisect S) insecticides for *C. lameensis* control for the year 2008 was GH¢27,395 not considering the loss in yield which is inevitable (Ahiabie, personal communication) and corresponding additional cost of labour and time that will be involved in applying the insecticide.

2.4 Management of *C. lameensis*

Several methods are employed in the management of the Oil palm leaf miner, *C. lameensis* population from reaching an outbreak within most plantations in Ghana and across West Africa. All these methods are in line with the current Integrated Pest Management strategy which seeks to ensure that chemical use is minimised as much as possible. These management strategies are phytosanitary, cultural, chemical as well as biological control methods. Studies conducted by Appiah *et al.* (2007); Morin and Mariau (1971) revealed that there were no significant differences in the duration of developmental stages of the oil palm leaf miner on oil palm progenies. Host-plant resistance has thus not as yet been proven to be an efficient management strategy in oil palm.

2.4.1 Chemical control

Chemical insecticides have provided the principal means of insect pest control since 1945 with the advent of DDT and Gamma-HCH (Dent, 1991). The use of lindane (Gamma-HCH), Ethyl parathion DDVP among others has been the backbone of *C. lameensis* control since its first outbreak (Ruer, 1964; Shearing, 1964; Bescombes, 1968; Hartley, 1988). Morin and Mariau (1971) reported that these chemicals give immediate amelioration of *C. lameensis* attacks. However, the well publicized drawbacks associated with their use are insecticide resistance, destruction of beneficial insects, environmental contamination and increased cost of production (Dent, 1991). The insecticide application also harms natural enemies of *C. lameensis* (Morin and Mariau, 1971; Timti, 1991). Since natural enemies are often responsible for regulating the population of *C. lameensis*, their destruction has exacerbated the pest problem which has led to outbreaks.

Other chemical control methods available are treatment by trunk injection and treatment by root absorption which target the larvae and adults. Hot fogging is another chemical control option which has recently gained popularity due to its ease and convenience in use, however, hot fogging only target the external adult population (Appiah and Yawson, 2003). Systemic insecticides like Azadirin (55% monocrotophos) including other organophosphorus and carbamate insecticides have been used for most of the control. Evisect S (thiocyclam) a member of the nereistoxin analogues is generally a stomach poison with some contact action and often shows some systemic action. It acts as an acetylcholine receptor agonists at low concentrations and as channel blockers at higher concentrations. Increasing demand for organic oil palm products in world trade has made the use of chemical control a management option less desirable in Ghana (Buabeng, 2002).

2.4.2 Biological control

Biological control is the use of living organisms as pest control agents. The most frequently used biological control agents are insects, which are released to control both insect pests of crops and weeds. The desirable qualities of these agents include their great diversity, high degree of specificity and ease of handling (Waage and Greathead, 1992).

Waage and Greathead, (1992) acknowledge that biological control may provide long term or even permanent results. Since it causes no pollution, and poses no risk to human health, it should always be a preferred control measure when economically competitive with other methods. In developing countries, it will have special application where the cost of chemical control is prohibitively expensive and where the inappropriate or excessive use of chemicals has resulted in high levels of pest resistance.

2.5 Predators

The important characteristics of predators are that individuals consume a number of prey during their lifetime and that they are active organisms which seek their food. There is a range from species consuming a wide range of prey species (polyphagous) to extreme specialists (monophagous). Polyphagous arthropod predators, like vertebrate predators, are usually of little use in biological control as they do not concentrate their attention on the target pests, tending to feed on the most abundant and easily captured prey. They may also include useful species in their diet. Monophagous predators or species with a narrow range of prey (oligophagous) are more likely to have desirable characteristics. Ladybird beetles, many species of predatory bugs, lacewings and the larvae of hover flies are common insect predators (Waage and Greathead, 1992).

2.6 Parasitoids

These insects parasitically develop in a single host which is eventually killed. Thus, individual parasitoids only consume one prey (or host) during their lifetime. This unique lifestyle is remarkably exhibited by a diverse group of small wasps and flies, comprising about 300,000 species, or about one tenth of all species of multicellular organisms. Although the larval stage is parasitic, the adults are free-living and highly mobile so that they are able to search actively for hosts in which to lay eggs, and may themselves feed as predators. Many are entirely monophagous.

Cornell and Hawkins (1995) reported that enemy attack is the most frequent cause of death for immature herbivores and is more frequent than all other factors combined for late immature

stages (late stage larvae and pupae). Hawkins *et al.* (1997) indicates that enemy-induced mortality increases with herbivore age. Parasitoids kill more herbivores than either predators or pathogens. The dominance of parasitoids as mortality agents is most pronounced in the mid/late larval and pupal stages of their hosts.

2.6.1 Parasitoids of leafminers

Leafminers are in the phytophagous guild (group of organisms that consume the same resource in the similar manner), with the highest number of parasitoid species per host species, and have the highest average rate of parasitoidism (Hawkins, 1994). Hochberg and Hawkins (1992) reported that certain characteristics of leafminer habits make them more vulnerable to parasitoid attacks. These characteristics include the scarce mobility of the larvae, the clear visibility of the mines produced, and the scarce physical protection provided by the leaf epidermis. On the other hand, leafminers are herbivorous insects characterized by a distinct homogeneity of both ecological and taxonomic aspects (Connor and Taverner 1997), which facilitates the development of a diverse community of parasitoids that share hosts, and explains why this insect group as a whole has an elevated load of parasitic species (Godfray, 1994).

Most of the leafminer parasitoid species correspond taxonomically to the superfamilies Chalcidoidea (Families Eulophidae and Pteromalidae), Ichneumonoidea (Family Braconidae), and Cynipoidea (Family Figitidae) (Salvo, 2007).

Biological control has been envisaged in the control of *C. lameensis* in West Africa. Some parasitoids of *C. lameensis* have been identified and studied by Waterston (1925); Kerrich (1970); Morin and Mariau (1971). These include *Achrysocharia leptocerus* (egg parasitoid); *Sympiesis aburianus*, *Pediobius steigenus* and *Closterocerus africanus* (larval parasitoids); *Oligosita longiclavata* (egg parasitoid) and *Perislitus persimillis* (adult parasitoid). Other

parasitoid species identified in Ghana in the control of *C. lamensis* include *Phymasticus* spp., *Neochrysocharis* spp., *Pleurotroppopsis* spp., *Apleurotropis* spp. and *Cirrospilus* spp., with more yet to be identified (Yawson *et al.*, 2009). Some of the identified parasitoids species have been found to parasitize other species of leafminers and also have been used in bio-control programmes. *Neochrysocharis* spp. had been used before in a trial to control the oil palm leafminer in Cote d'Ivoire (Lecoustre *et al.*, 1980). *Phymasticus* spp. including *P. coffeae* LaSalle (Hymenoptera; Eulophidae) was among a number of parasitoids of the coffee borer *Hypothenemus hampei* Ferrari (Coleoptera; Scolytidae) collected from Africa (Benoit, 1957; Ticheler, 1961; Borbon-Martinez, 1989) for use on the coffee borer in Latin America. *Neochrysocharis* spp. including *N. Formosa* Westwood and *N. okazaki* Kamijo have been found to parasitize the vegetable and ornamental leaf miner *Liriomyza chinensis* Kato, *L. huidobrensis* Blanchard, *L. sativae* Blanchard and *L. trifolii* Burges (Greathead and Greathead, 1992; Johnson, 1993; Lynch and Johnson, 1987; Stegmaier, 1966). *Pleurotroppopsis* spp are also known to parasitize vegetable (tomatoes and watermelon) leafminers including *L. sativae* Blanchard, *L. trifolii* Burges and *L. huidobrensis* Blanchard (Poe *et al.*, 1978; Getzin, 1960). *Apleurotropis kumatai* Kamijo has been found to parasitize the vegetable leaf miner *L. sativae* Blanchard, *L. trifolii* Burges and *L. huidobrensis* Blanchard in Japan (Konishi, 1998). *Cirrospilus* spp. including *C. quadristriatus* has been found to be among parasitoids that parasitize the citrus leaf miner *Phynocistis citrella* Strainton in Mexico (Ruiz and Mateos, 1996; Perales *et al.*, 1996). LaSalle (1996) reported fifteen species of *Cirrospilus* associated with the citrus leaf miner and stated that this is a cosmopolitan and polyphagous genus although it prefers to parasitize insects in the family Gracillaridae.



2.6.2 Factors affecting parasitoidism

The efficiency of parasitoids as agents of leaf miner mortality varies depending on the species, and it may even vary between different larval stages of the same species, as well as in relation to environmental conditions (Grabenweger, 2003). For example, parasitoids are likely to be a more important cause of mortality in temperate zones than in the tropics (Hawkins *et al.*, 1997; Queiroz, 2002). Similarly, parasitoidism rates would be higher at the end of the growing season, at least in cultivated systems (Parrella, 1987; Murphy and LaSalle, 1999; Urbaneja *et al.*, 2000). The presence of other organisms may also affect leaf miner parasitoidism rates; in this regard, there is evidence of effects caused by other herbivorous insects, such as externally chewing herbivores (Faeth, 1985), and even by the presence of endophytic fungi (Preszler *et al.*, 1996). Tritrophic plant-herbivore-parasitoid interactions are important in these systems (Price *et al.*, 1980). It has been observed that parasitoidism may also vary depending on plant species (Olivera and Bordat, 1996; Rauf and Shepard, 1999), or even between cultivars or genotypes of the same plant species in which the leaf miner develops (Fritz *et al.*, 1997; Braman *et al.*, 2005). For example, *L. huidobrensis* parasitoidism in potato is very low compared to that observed in other crops (Shepard *et al.*, 1998). According to Johnson and Hara (1987), effective biological control of certain leaf miners may depend on the plant species on which the leaf miner feeds. One aspect of the host plant which may affect their parasitoidism level is leaf abscission. In some cases, leaf abscission causes mortality to both the leaf miner and parasitoid (Potter, 1985); while in other systems, the abscission reduces larval parasitoidism due to the fact that parasitoids do not search for hosts in fallen leaves (Kahn and Cornell, 1989).

Other factors that affect leaf miner parasitoidism include droughts (Staley *et al.*, 2006), leaf age (Facknath, 2005), and leaf miner position in the plant (Barrett, 1994; van der Linden 1994; Brown *et al.*, 1997).

2.6.3 Effects of insecticides on parasitoids

In regard to chemical control, most leaf miners are resistant to organophosphates, carbamates, and pyrethroids, and at the same time, their natural enemies are severely damaged by these chemicals, which leave few options for their chemical control. Insecticides that do not penetrate the leaf surface are practically ineffective (Weintraub and Horowitz, 1998). For this reason, insecticides with good translaminar action (i.e. cyromazine and abamectin) are the most widely used against leaf miners (Civelek and Weintraub, 2003). Growth inhibitors are useful in controlling leaf miners, and at the same time, are potentially compatible with biological control agents due to their low toxicity and high specificity.

There are numerous studies on the susceptibility of leaf miner parasitoids to different types of insecticides. Some species of leaf miner parasitoids have developed resistance, mainly to organophosphates. For example, *Diglyphus begini* (Ashmead) (Hymenoptera: Eulophidae) is tolerant to oxamyl, methomyl, permethrin, and fenvalerate (Rathman *et al.*, 1992). Insecticide application time and method of application may affect the susceptibility of the parasitoids (Kaspi and Parrella, 2005), which varies between species (Mafi and Ohbayashi, 2004) and even between genders (Rathman *et al.*, 1992).

In studies with different leaf miner species, crops treated with low doses or without insecticides had higher percentages of parasitoidism (Galantini Vignez and Redolfi de Huiza, 1992; van Driesche *et al.*, 1998; Adachi, 2002; Chen *et al.*, 2003a).

Likewise, in organic crops, greater parasitoid species richness together with improved densities of leaf miner parasitoids has been observed (Balázs, 1998). However, in other cases, there was no difference on leaf miner parasitoids between plots treated and not treated with synthetic insecticides (Mafi and Ohbayashi, 2004).

2.6.4 Parasitoids and cultural control

It is possible to increase the action of enemies of leaf miner through habitat management (Price and Harbaugh, 1981). Different studies cite the importance of weed patches near crops as possible reservoirs of parasitoids (Murphy and La Salle, 1999). For this reason, it has been suggested that the management of weeds and other plants in or at the edge of an agroecosystem can improve the availability of pollen and nectar for natural enemies of leaf miners (van Mele and van Lenteren, 2002). In some cases, the presence of flowering plants in nearby habitats produces an increase in leaf miner parasitoidism (Chen *et al.*, 2003b). However, in other cases, the increase in plant diversity had no effect on leaf miners or their parasitoids (Johnson and Mau, 1986; Letourneau, 1995).

Although some weeds may act as reservoirs of pests of leaf miner (Smith and Hardman, 1986; Schuster *et al.*, 1991), others give refuge to leaf miner specialists, which do not harm crops.

In the latter case, weeds provide alternative hosts for the parasitoids, thereby increasing the biological control in crops. These plant species could be used for the implementation of open field rearing of parasitoids (Parkman *et al.*, 1989), which consist of favouring the presence of plants and harmless leafminers, in the same environment as the crop, to increase populations of natural enemies. Rearing parasitoids in the open field has been successful for controlling some leaf miners (van der Linden, 1992). Thus, the knowledge of the relationship between leaf miners and their parasitoids is critical (Parkman *et al.*, 1989; Chen *et al.*, 2003b; Rizzo, 2003).

In some cases, the study of trophic webs in different environments has made possible the theoretical proposal of open-field rearing systems, taking into account the possible interactions between the species at three trophic levels (Valladares and Salvo, 1999). Policultures and planting additional plant species with the main crop may also have a positive effect on parasitoids. For example, it has been observed that at the beginning of the sweet potato crop cycle, growing kidney bean plants in adjacent strips attracts *L. huidobrensis* parasitoids, and advances and increases their presence in the sweet potato crop (Da Paixão Pereira *et al.*, 2002). Likewise, potato cultivated along with wheat has less leaf miner damage because wheat attracts parasitoids (Ebwongu *et al.*, 2001).

Compost application to the crop may have an indirect effect on leafminers, increasing their predator's diversity and efficiency (Brown and Tworkoski, 2004). However, nitrogen fertilizers must be used with care because they can stimulate pest development, augmenting not only the plant's vigor, but also the survival of leaf miner larvae and pupae (van Lenteren and Overholt, 1994). There might be fertilization thresholds above which the numbers of leafminer pupa are augmented and the parasitoidism diminishes, as shown in citrus leafminers (Ateyyat and Mustafa, 2001). On the other hand, elevated levels of nitrogen fertilization in kidney bean crops decreased the development time and increased the fertility of the parasitoid *Chrysocharis oscidinis* (Hymenoptera: Eulophidae) on the leafminer *Liriomyza trifolii* (Kaneshiro and Johnson, 1996). Other studies have evaluated the effect of fertilization on parasitoidism rates, without analyzing the mechanisms involved (Yarnes and Boecklen, 2006).

One practice recommended for reducing leaf miner populations is the destruction of plant residues from the previous harvests that were attacked by pests, which can be burned or buried (Larraín, 2004), but this practice also reduces parasitoid populations (Vincent *et al.*, 2004).

Pruning has proved to be a useful cultural practice in the management of *C. lameensis*, but pruning is only useful upon the timely detection of *C. lameensis* infestation when they are still within the galleries. During an outbreak, pruning does not provide an efficient crop protection.

Traps are used in integrated pest management programs for leafminer flies (Agromyzidae), where the number and surface area of traps for effective control is known in some detail (Braun and Shepard, 1997). However, there is a possibility that yellow sticky traps decrease parasitoid populations (Gonçalves, 2006). The combination of yellow traps with attractive substances for the leafminers, such as extracts of leaves of host plant decreases the capture of other insects and possibly also reduces the number of parasitoids trapped (Harand *et al.*, 2004).

2.7 Entomopathogens

Natural pathogens of arthropods often play an important role in the regulation of insect and mite populations in agroecosystems (Ignoffo, 1985; Steinkraus 2007); however, their main impact on pests may occur after economic thresholds are surpassed. Inundatively or inoculatively, applied microbial control agents have been developed as alternative control methods for a wide variety of arthropod pests (Alves, 1998a; Lacey *et al.*, 2001; Kaya and Lacey, 2007), which include pests of tropical tree fruits.

2.7.1 Viruses

Many viruses have been identified from hundreds of arthropod species. For most of them, those with particles occluded in protein bodies (OBs) [Baculoviridae, Entomopoxviridae, Reoviridae (Cypoviruses)] have been successfully used in microbial control programs. The Baculoviridae (nucleopolyhedroviruses and granuloviruses) are the most studied and used microbial control agents (Hunter-Fujita *et al.* 1998; Moscardi, 1999; Cory and Evans, 2007). They are normally

transmitted *per os* and gain access to host tissues via the midgut where the OBs that surround the virus rods are dissolved. The currently unclassified virus of *Oryctes rhinoceros* L. is the most successfully used non-occluded virus. Viruses comprise some of the key host-specific entomopathogens but their main drawbacks are the requirements for *in vivo* production and their sensitivity to ultra-violet degradation.

2.7.2 Bacteria

Although several species of bacteria have been used as microbial control agents of a variety of insects, only *Bacillus thuringiensis* Berliner (Bt) has been used for practical pest control. It is the most widely used inundatively applied microbial control agent (Lacey *et al.*, 2001). Several isolates of Bt are commercially produced with activity against Lepidoptera, Coleoptera, and Diptera. The safety of Bt for applicators and vertebrate and invertebrate non-target organisms is well documented (Lacey and Siegel, 2000). Its insecticidal activity is associated with delta-endotoxins located in parasporal inclusion bodies (or parasporal crystals), produced at sporulation and, which must be ingested by the target organism in order to be active. A limiting factor of Bt is its fairly narrow host range.

2.7.3 Fungi

The majority of fungi that naturally regulate insect and mite populations is in the order Hypocreales (Ascomycetes, including the vast majority of conidial entomopathogens in more than two dozen genera formerly classified among either the Hyphomycetes or Fungi Imperfecti) and Entomophthorales. The latter are difficult or impossible to mass-produce and, hence, they have not been commercially produced or applied inundatively on a large scale. On the other hand, several asexually reproducing species in the Hypocreales are amenable to mass production and commercialization. The most studied for control of insects and mites belong to the genera

Beauveria, *Metarhizium*, *Paecilomyces*, *Aschersonia*, *Hirsutella*, and *Lecanicillium* (formerly *Verticillium*) (Alves, 1998b; Inglis *et al.*, 2001; Goettel *et al.*, 2005). Since the normal route of invasion is through the cuticle, fungi are especially suitable microbial control agents for sucking insects (Hemiptera).

Phylogenetic studies now confirm that Microsporidia are highly derived organisms correctly placed among the lower fungi rather than extremely ancient and simple organisms classified with the Protozoa (Hirt *et al.*, 1999).

Although many microsporidians are common pathogens of arthropods, few have been included in microbial control programs because certain fundamental characteristics (complex life cycles, obligate parasitism, and chronic rather than acute effects) inhibit their use (Solter and Becnel, 2007).

2.8 Entomopathogens in Integrated Pest Management

Integrated Pest Management plays a significant role in crop protection since it is an important aspect of sustainable agriculture that attempts to minimize the negative environmental impacts and other deleterious effects due to the use of chemicals (Huffaker, 1985; Dent, 2000). Individual components of IPM are often evaluated as stand-alone tactics without consideration of their interactions with other components of the agroecosystem.

An integrated approach that is based on pest densities and their relation to economic injury thresholds will ultimately be required for each cropping system and location before agriculture will be truly sustainable. When selective insecticides are used, the negative impact on beneficial insects is reduced. Biopesticides provide an alternative means of control that further minimizes impacts on beneficial and other non-target organisms (NTOs). This is due to the specific nature of many microbial control agents. Safety testing data for entomopathogens indicate that they are



generally safe for most NTOs, especially vertebrates (Laird *et al.*, 1990; Akhurst and Smith, 2002; Hokkanen and Hajek, 2003); however, it will be necessary to determine their effects on the beneficial organisms under specific conditions in each agroecosystem.

Whether entomopathogens are utilized in classical biological control or conservation will depend on the characteristics of the pest and the fruit crop in which it causes damage or yield loss. Fruit crops are stable agroecosystems where any of the above strategies for pathogen use could be considered. In addition to the use of commercially available biopesticides, it may be useful to consider employing native entomopathogens. Surveys should be undertaken in different agro-ecological zones to identify prevailing environmental conditions and the presence of native pathogens and natural enemies that may be better suited for the targeted location than an exotic species or strain (Dolinski and Moino, 2006).

On the other hand, an exotic pest may require importation of natural enemies from its native range. In classical biological control, natural enemies, including entomopathogens, are sought in the region of origin of the invasive pest, imported and established in an area where they do not naturally occur.

2.9 Entomopathogens and biopesticides

When microbial control agents are formulated as biopesticides, they are predominantly used for inundative applications and often treated much like chemicals, with the expectations that they will perform at the same standards. In general, this has not always been possible. On the other hand, there are biological control agents capable of doing what chemicals are not able to do. They have a capacity to find their pest host, kill it and reproduce in it. In fact, many entomopathogens have the capacity to reproduce in the host and hence produce secondary inoculum which is able to attack and kill other individuals in the pest population. This numerical

increase in response, of which chemicals are incapable, needs to be better exploited in tropical conditions. Several other advantages of entomopathogens over chemicals are presented by Alves (1998c); Lacey *et al.*, (2001); Kaya and Lacey, (2007).

The cost of producing natural enemies must be judged in terms of the value of the crop protected by using the agent and in comparison to the cost and side effects to the ecosystem of competing control options such as chemicals (van Driesche and Bellows, 1996).

2.9.1 Interaction of Entomopathogens and Other Biological Control Agents

Parasitoids and predators can interact synergistically (e.g. enhanced transmission and dispersal of insect pathogens) or antagonistically (e.g. parasitism/infection, predation and competition) with entomopathogens. In most studies examining the interaction between entomopathogens and other natural enemies, the pathogen almost always dictates the population dynamics of other guild members (Brooks, 1993; Begon *et al.*, 1999). Most studies indicate the positive nature of these interactions with respect to the control of insect populations (Brooks 1993; Begon *et al.*, 1999; Roy and Pell, 2000). Various studies have shown the capacity of parasitoids to identify and avoid oviposition in hosts infected by the different entomopathogenic groups (Brooks, 1993). This rejection is usually due to visual changes that occur in hosts and/or chemical cues associated with biochemical changes in hosts during the disease development. As for predators, this does not appear to be true (Wraight, 2003; Koppenhöfer and Grewal, 2005).

2.9.2 Interaction of Entomopathogens and Chemical Pesticides

Unlike parasitoids, entomopathogens are generally compatible with chemical pesticides (Croft, 1990). Certain combinations of entomopathogens with chemical pesticides can be synergistic, such as reported by Quintela and McCoy (1998) and Koppenhöfer *et al.* (2000) for sublethal concentrations of imidacloprid and fungi or EPNs, respectively.

The economic feasibility of such combinations will depend on how much of each component can be reduced compared with their recommended application rates when they are applied individually. The compatibility of chemical pesticides with arthropod natural enemies will be another consideration when integrating pesticides and entomopathogens into a pest management program.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental site

The plantations of the Twifo Oil Palm Plantations Limited, (TOPP), a subsidiary of Unilever Ghana, in the Twifo-Hemang – Lower Denkyira District of the Central region of Ghana, were surveyed from September, 2008 to March 2009. The plantations which cover 4250 hectares of land in the district is located between latitudes 5°50'N and 5°51' N and Longitudes 1°50'W and 1°10'W. It is bounded on the North by the Upper Denkyira East Municipality, on the South by the Abura Asebu Kwamankese Dipistrict, Cape Coast and Komenda-Edina-Eguafo-Abirem, on the West by the Mpochor Wassa East District and the East by the Assin North Municipal. The district lies within the semi-equatorial zone marked by double maximal rainfall in June and October, with the mean annual rainfall being 175cm. The district is between 76m and 91m above sea level. The Pra River and its tributaries including Obuo, Bimpong and Ongua drain the area. The drainage pattern is dendritic and has given rise to the dissected nature of the topography. The District's vegetation consists basically of semi-deciduous forest that has been largely disturbed by the activities of man through farming, logging and mining among others.

3.2 Field survey

Field surveys were conducted in the plantations to collect differently developmental stages of the oil palm leafminer for parasitoid rearing as well as for entomopathogenic studies. During the survey, observations were also made for predators of the developmental stages. Plots that were surveyed in a particular month were not surveyed again for that month. Some of those plots were, however, surveyed in subsequent months. On the average two plots were surveyed each session. Trees used for the survey were planted between 1981 and 1985 and were generally very

tall. Surveys were done by cutting fronds from the lower ranks of oil palm trees. For evenness of distribution, the first frond was cut from oil palm trees within rows one to ten. The second frond was cut by counting ten palm trees from the palm on which the first frond was cut. Subsequently the third frond was cut ten palm trees from the palm on which frond two was cut. Within one row, six fronds were cut. Subsequently, 10 rows were counted from the previous row along which another batch of six fronds was cut as in the manner described above. Most of the plots that were used for the survey were 40 hectares. This implies that a maximum of 78 fronds were cut evenly within each plot.

The oil palm leafminer, *C. lameensis*, infested leaflets on the cut fronds were detached. They were sent to the laboratory where the mines were opened up and the number of larvae and pupae found in each mine were recorded. They were kept in petri-dishes.

Surveys were conducted from 6:30am to 10:00am daily and five times in a week.

3.2.1 Entomopathogens

During the survey, any *C. lameensis* developmental stage observed with any form of abnormal behaviour (loss of coordination or loss of orientation) were collected in sterile plastic containers and kept until they died. The dead insects were then incubated in a moist environment. Dead oil palm leafminers that were found attached to the oil palm leaflets were also collected and sent to the laboratory for entomopathogenic studies.

3.2.2 Predator survey

The presence of predators of *C. lameensis* was also investigated as already indicated in Section 3.2. When the palm fronds were cut, the presence of ant nests was recorded. Based on the figures

obtained, the predator index for each ant species was determined for each plot as follows: the number of nests for each ant species divided by the number of fronds cut on each plot. This gave a figure which was always less than one. A higher index indicates higher predatory ant abundance.

3.3 Laboratory work

3.3.1 Collection of Parasitoids

Host (*C. Lameensis* stages) samples brought in from the field were kept free from diseases and not exposed to excessively high or low temperatures. Laboratory conditions of 29⁰ C and 82% relative humidity were maintained. The larvae collected were separated into their various stages using head width and body length measurements done with a pair of callipers (Yawson *et al.*, 2009) as shown in Table 1.

Table 1: Mean body length and head width of *C. lameensis* larval stages (Yawson *et al.*, 2009).

	Larval stages			
	1st	2nd	3rd	4th
Head width (mm)	$H \leq 1.2$	$1 < H \leq 1.4$	$1 < H \leq 1.4$	$H > 1.1$
Body length (mm)	$1.3 < B \leq 3.9$	$4 \leq B < 4.9$	$5 \leq B < 5.9$	$B \leq 5.9$

H = Head width : B = Body length

In the laboratory, the larvae of the same stages were transferred into test tubes where they were observed until parasitoids emerged. Each test tube contained a maximum of five insects. The test tubes were blocked with cotton wool to prevent insects and other predatory arthropods from entering or escaping. The emerged adult parasitoids were collected, counted and preserved in

70% alcohol. All test tubes were kept at room temperature of about 29⁰ C and 82% relative humidity.



Plate 8: *C. Lameensis* stages in test tubes for parasitoid emergence

Emerged parasitoids were identified using Bland and Jaques, (1978); Boucek (1988); Gibson (1997); Sharkey and Wharton (1997); Reina and LaSalle (2003); Fisher and LaSalle (2005) and later sent to the museum of the Zoology Department of the University of Ghana, Legon for confirmation.

3.3.2 Preparation of Potato Dextrose Agar (PDA) for fungi culturing

Potato was obtained, peeled and washed. 200g of the potato was weighed and boiled with 500ml of distilled water. The boiled potato with the distilled water was strained through a straining cloth. 10g of dextrose and 15g agar were added to the strained potato. This was made up to one litre with distilled water and brought to the boil to dissolve the agar completely. This was placed

in appropriate containers and autoclaved at 121°C at 1.1kilogram pressure for 15 minutes. The medium was allowed to cool to between 45-50 °C.

3.3.3 Fungal culturing

Under aseptic conditions, about 20ml of the media (PDA) was poured into petri dishes and allowed to settle. The insect specimens with the fungi were picked with a pair of forceps and plated on the potato dextrose agar and placed in an incubator at temperatures between 20 and 25 °C for four days. The petri dishes were observed after the fourth day for fungal growth and the fungi were then identified using morphological keys as in Hobot and Gull (1980); McCoy *et al.*, (1988); Arora *et al.*, (1991); Chitarra, (2003).

CHAPTER FOUR

4.0 RESULTS

4.1 *Coelaenomenodera lameensis* stages sampled

A total of 45,412 *C. lameensis* stages were sampled during the entire study period. The month of March, 2009 recorded the highest number of samples with 42.38% of total samples collected and October, 2008 the month with the least number of collections representing 2.85% of samples collected during the survey as can be seen in Figure 1. Average monthly collection was 14.29% of the total collection made during the survey.

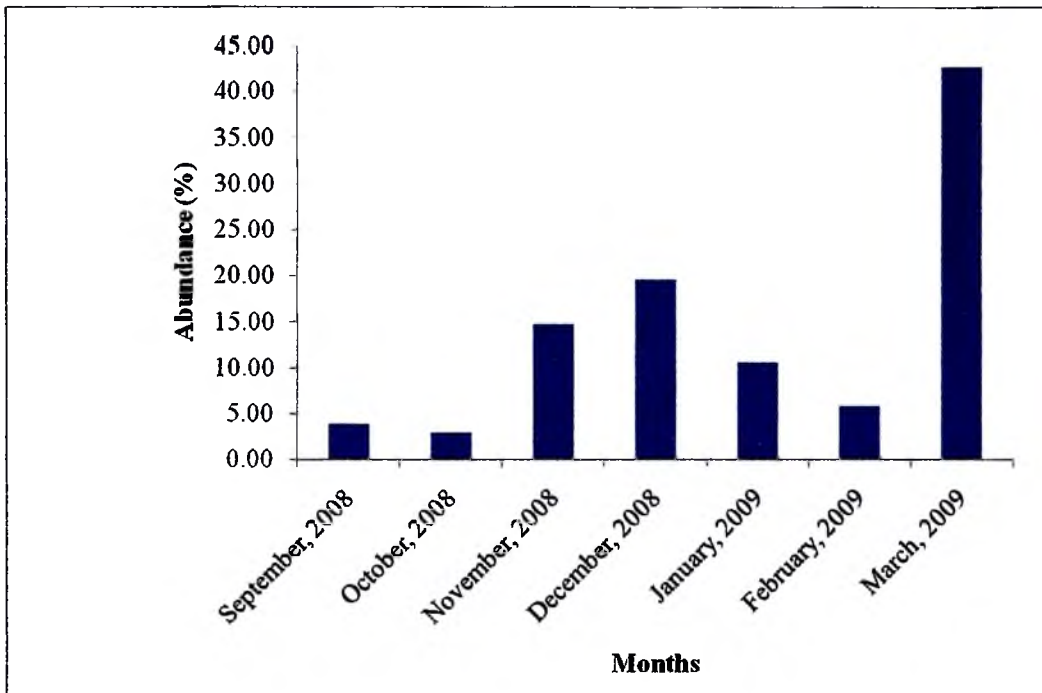


Figure 1: Distribution of *C. lameensis* hosts sampled during the study period.

Figure 2 shows that about 48% of samples were third and fourth larval stages. First stage larvae accounted for 18.64% while second stage larvae were 16.46% of the total *C. lameensis* host stages collected. Third larval, fourth larval and pupal stages collected were 23.69, 23.79 and 17.42 % respectively.

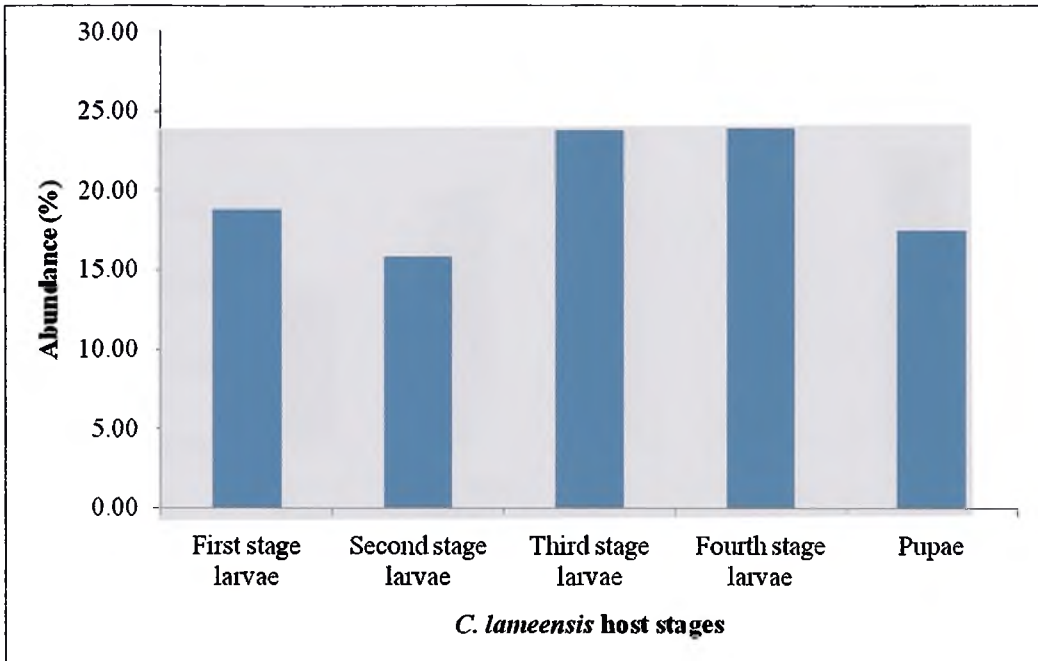


Figure 2: Distribution of *C. lameensis* host stages sampled during the study period

Out of the forty five thousand four hundred and twelve (45,412) developmental stages sampled, six thousand one hundred and thirty four (6,134) of them were parasitized by various species of parasitoids, representing a parasitism rate of 13.51%.

From Figure 3, it is observed that parasitoid attack is more at the third and fourth larval stages (55%). Early larval stage (first and second stage larvae) accounted for about 16% while pupal stage was approximately 19%.

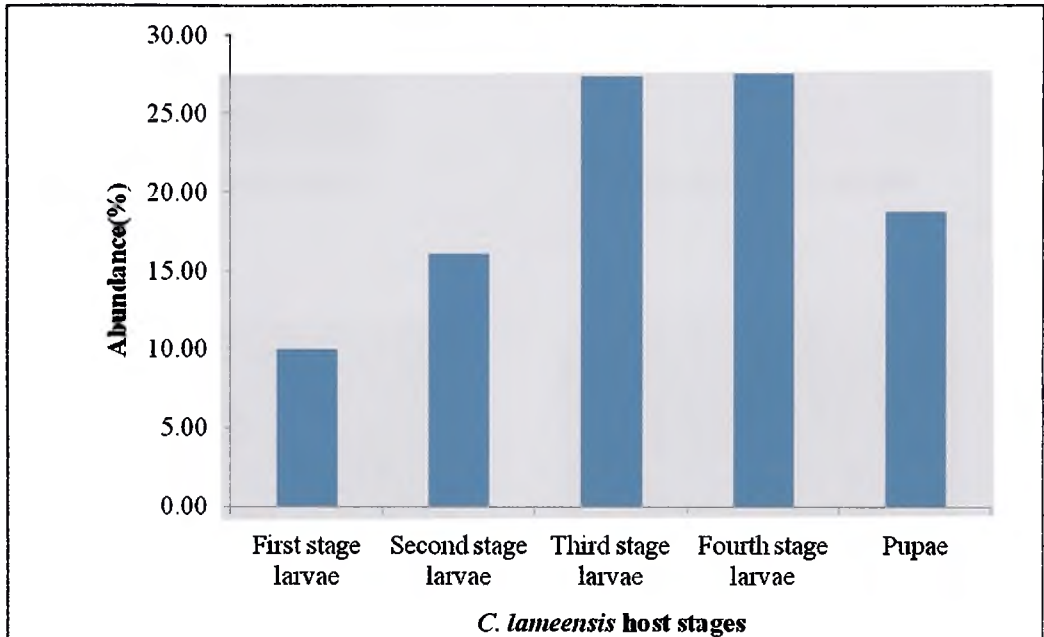


Figure 3: Distribution of parasitized *C. lameensis* stages

4.2 Parasitoids recovered from *Coelaenomenodera lameensis* host stages

The complex of parasitoids recorded on *C. lameensis* in the Twifo Oil Palm Plantations Limited was represented by *Neochrysocharis* species, *Chrysocharis* species, *Pleurotroppopsis* species and *Apleurotropis* species which are all members of the hymenopteran family Eulophidae.



Plate 9: *Neochrysocharis* species



Plate 10: *Apleurotropis* species



Plate 11: *Pleurotropsis* species



Plate 12: *Chrysocharis* species

Table 2: Species of parasitoids associated with the oil palm leafminer, *C. lameensis*, at Twifo Oil Palm Plantations Limited

	Host stages utilized	Ecto/Endoparasitoid
<i>Neochrysocharis</i> species	First stage larvae	
	Second stage larvae	
	Third stage larvae	Endoparasitoid
	Fourth stage larvae	
	Pupae	
<i>Chrysocharis</i> species	First stage larvae	
	Second stage larvae	
	Third stage larvae	Endoparasitoid
	Fourth stage larvae	
	Pupae	
<i>Pleurotropsis</i> species	Third stage larvae	
	Fourth stage larvae	Endoparasitoid
	Pupae	
<i>Apleurotropis</i> species	Third stage larvae	
	Fourth stage larvae	Endoparasitoid
	Pupae	

Neochrysocharis sp (Eulophidae: Entedoninae) was observed to emerge from all stages of the host. Figure 4 below shows the percentage emergence of *Neochrysocharis* sp from the host stages. The second, third and fourth instar host larva stage served as hosts for over 80% of *Neochrysocharis* species that emerged while the first larval stage and pupal stage served as host for about 20% of *Neochrysocharis* species. *Neochrysocharis* species is an endoparasitoid with 4-7 parasitoid larvae emerging per *C. lameensis* stage.

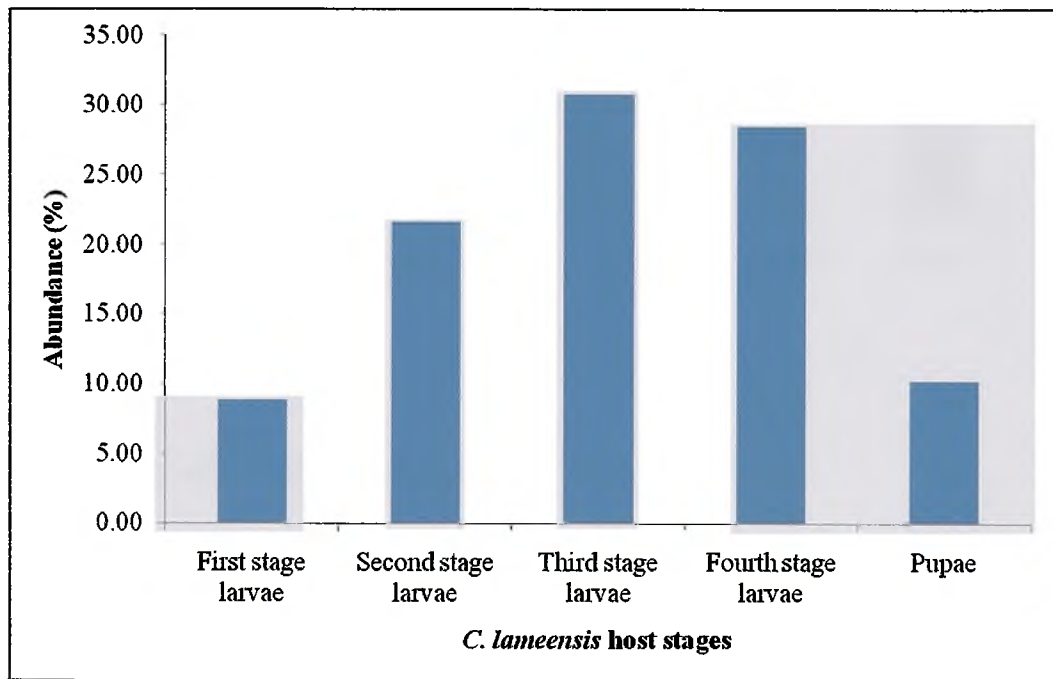


Figure 4: *Neochrysocharis* species emergence from *C. lameensis* host stages

Chrysocharis sp is an endoparasitoid which gregariously develops in a host. This parasitoid emerged from all four larval instar stages as well as the pupal stage (Figure 5). Percentage emergence of first stage larvae was 17.64%.

Second larval, third larval fourth larval stages were 21.73, 27.74 and 22.64 % respectively.

Percentage emergence from pupal hosts was 10.25%.

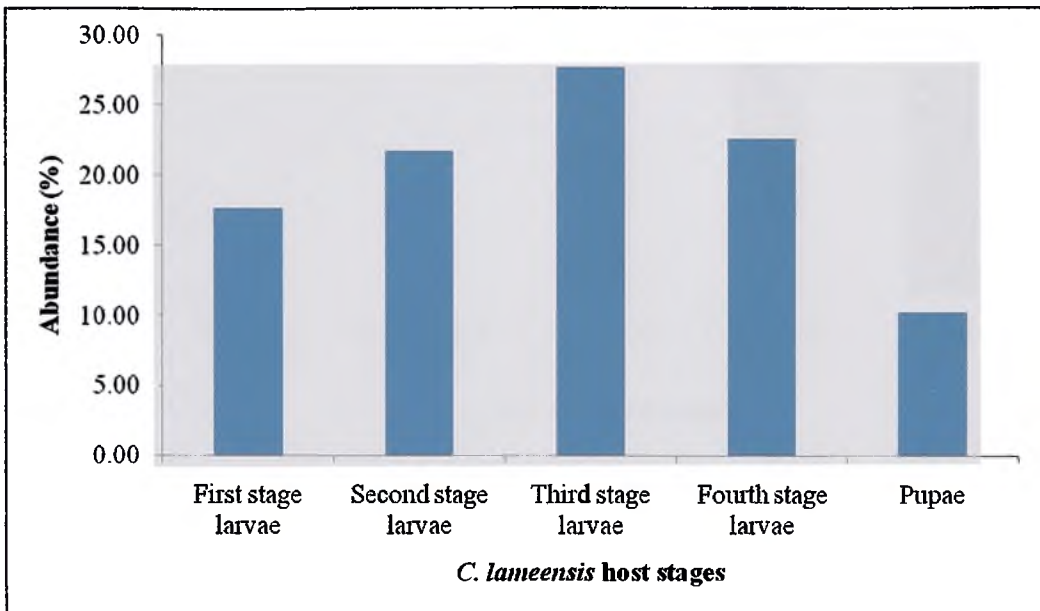


Figure 5: *Chrysocharis* species emergence from *C. lameensis* host stages

Pleurotroppopsis species is an endoparasitoid that emerged solitarily from the host stages. There were no emergences recorded for first and second stage larvae (Figure 6). The third larval stage recorded 41.41% of the total *Pleurotroppopsis* species emergence from the host stages. The third larval stage larvae recorded 33.94% of the *Pleurotroppopsis* species collected from the survey while the pupal host recorded 24.65% of *Pleurotroppopsis* species emergence.

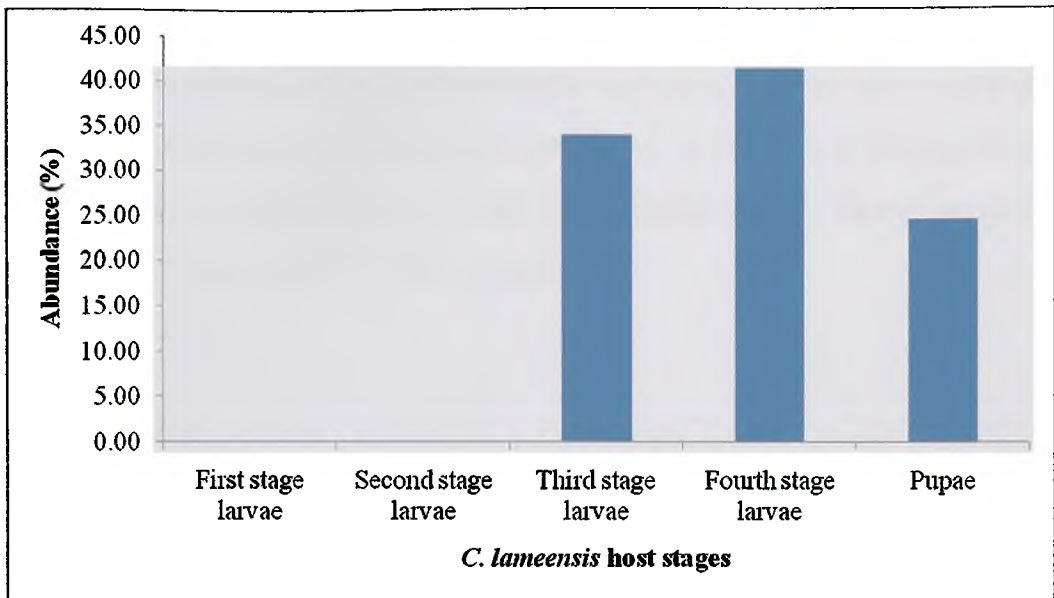


Figure 6: *Pleurotropopsis* species emergence from *C. lameensis* host stages

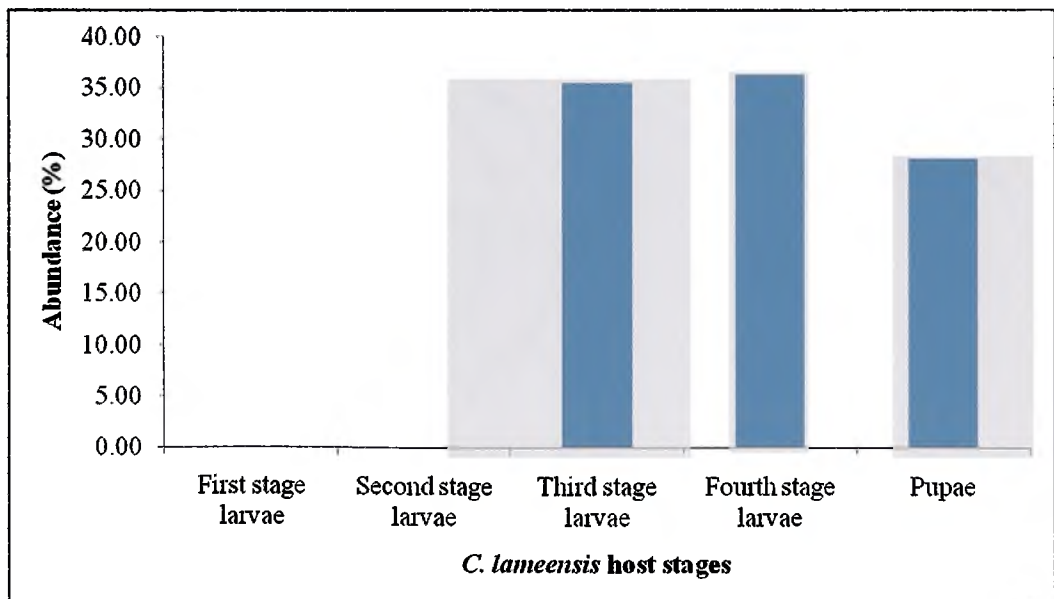


Figure 7: *Apleurotropis* species emergence from *C. lameensis* host stages

Another solitary endoparasitoid, *Apleurotropis* species, emerged from the third and fourth stage larvae and the pupal stage (figure 7). There were no emergences from first and second stage larval stages. Third stage larval emergence accounted for 35.50% while fourth stage larval emergence and pupal emergence were 36.33 and 28.17 % respectively. Thus the third and fourth larval stage emergence was 71.83% of the total emergence.

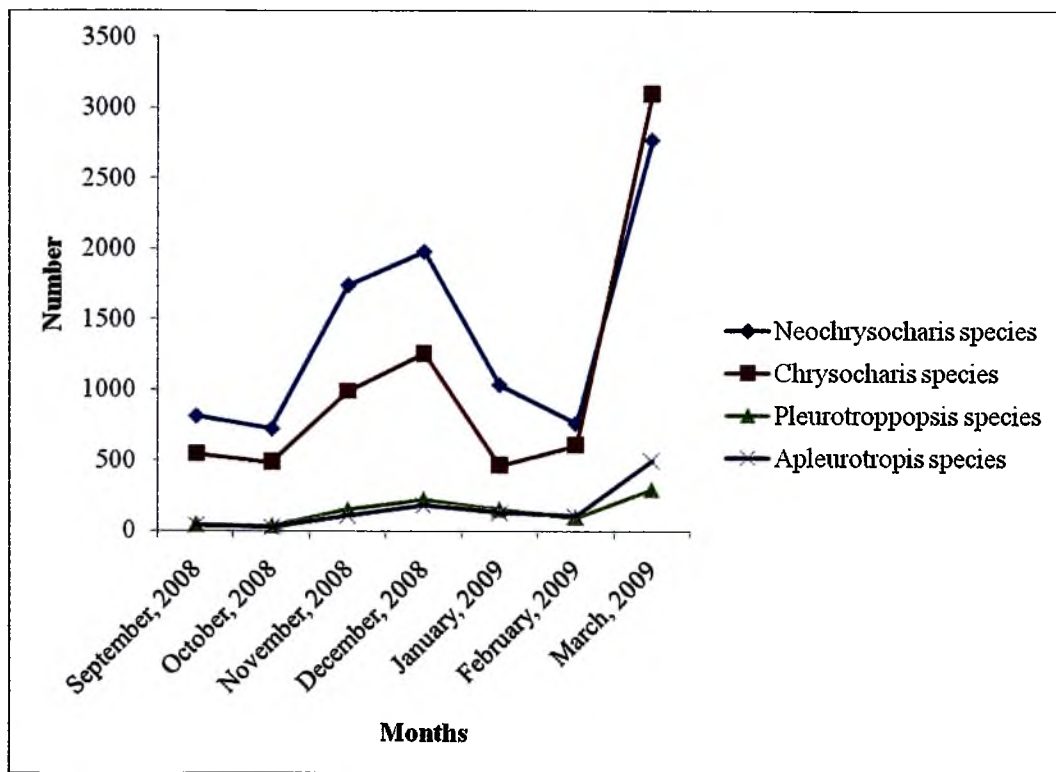


Figure 8: Parasitoid abundance from *C. limeensis* host stages during the 2008- 2009 study period

Figure 8 above represents the dynamics of parasitoid species collected during the entire study period. *Neochrysocharis* species was most abundant with 50.77% of the total parasitoids collected. *Pleurotroppopsis* species was the least abundant with 5.12% of parasitoids collected. *Chrysocharis* species (38.53%) was more abundant than *Apleurotropis* species (5.58%). *Neochrysocharis* species and *Chrysocharis* species together represented 89.30% of the total parasitoids collected.

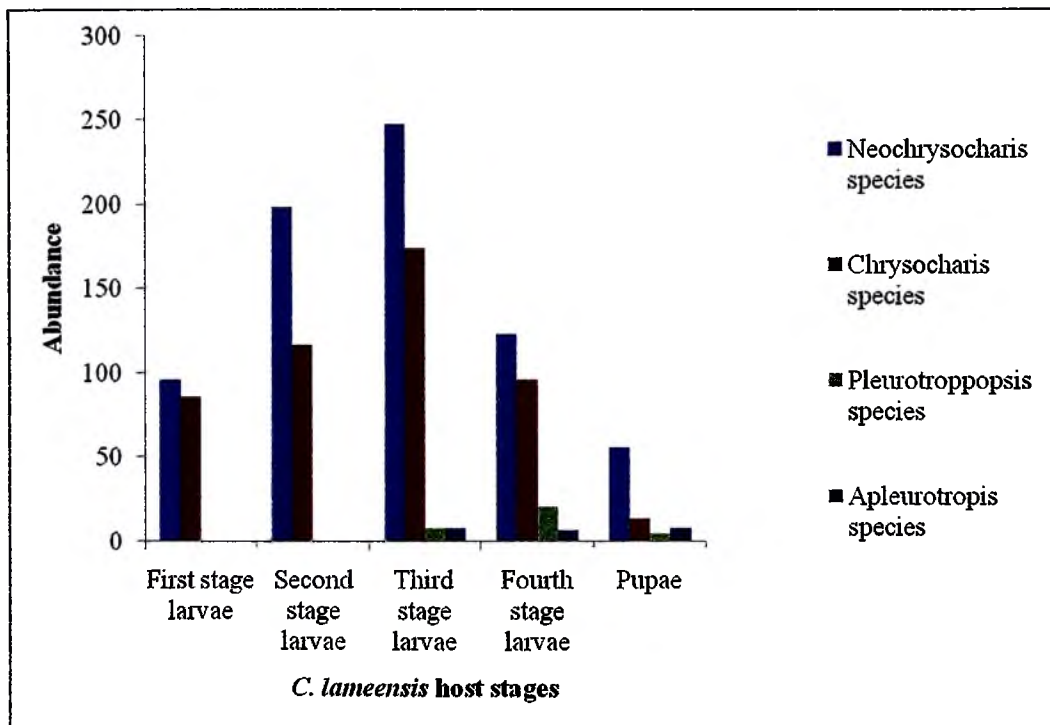


Figure 9: Parasitoids collected from *C. lameensis* host stages in September, 2008

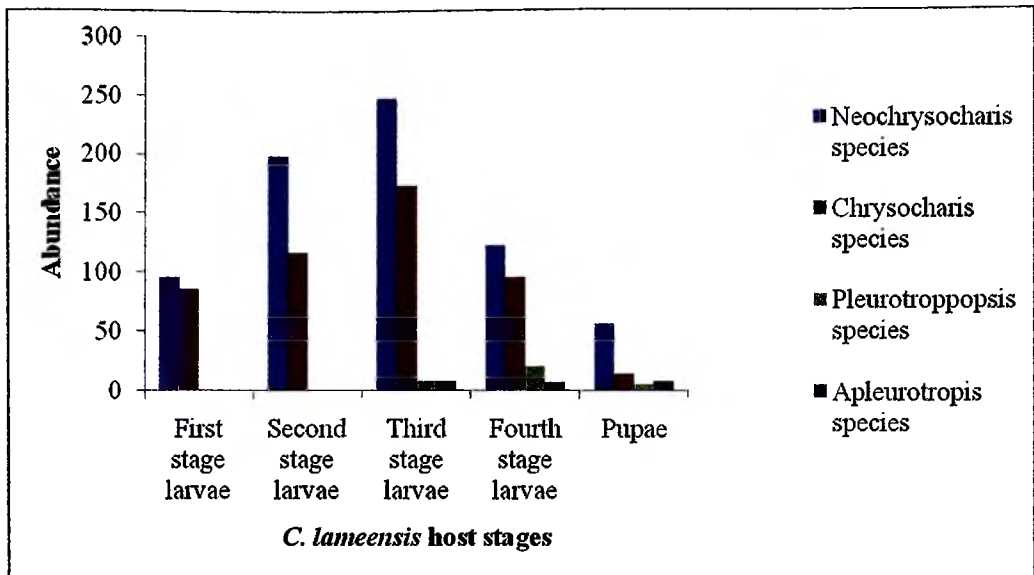


Figure 10: Parasitoids collected from *C. lameensis* host stages in October, 2008

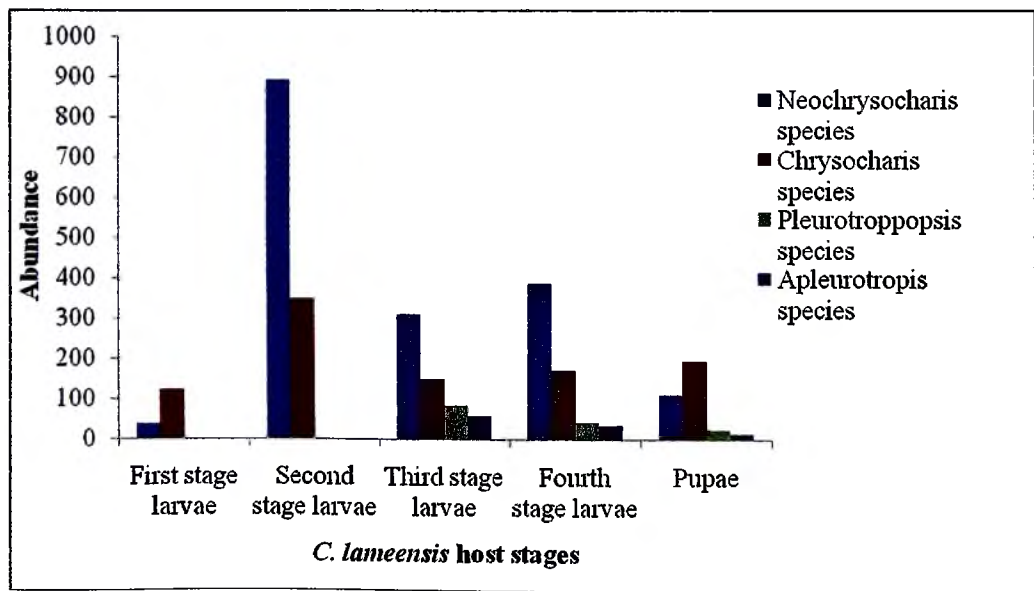


Figure 11: Parasitoids collected from *C. lameensis* host stages in November, 2008

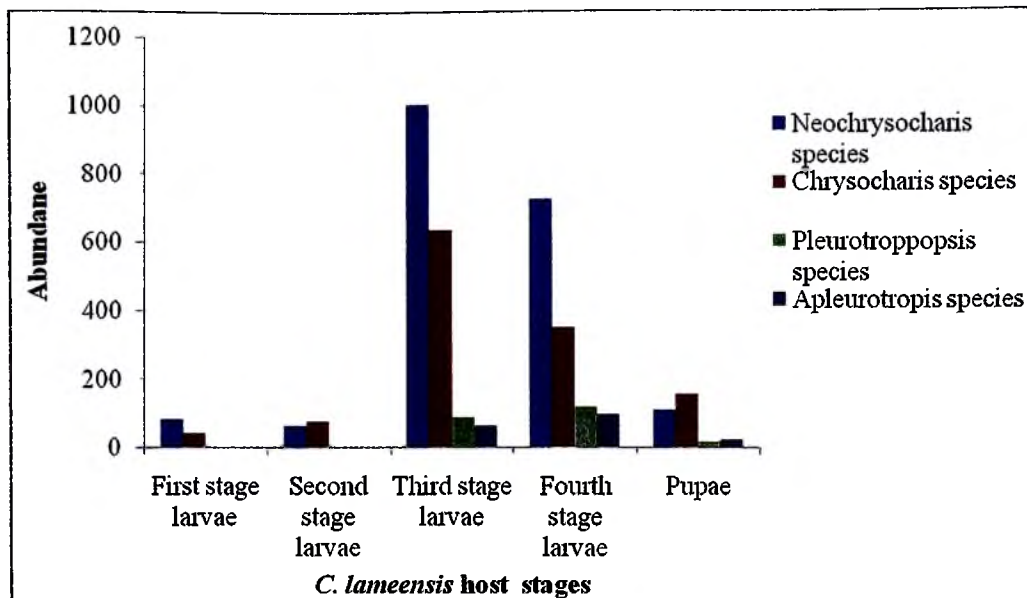


Figure 12: Parasitoids collected from *C. lameensis* host stages in December, 2008

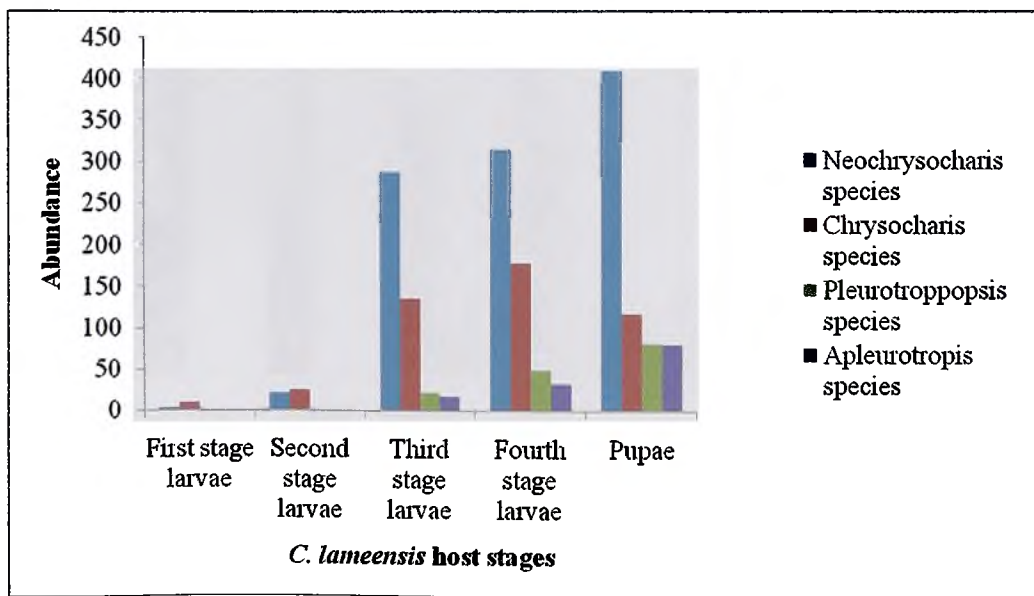


Figure 13: Parasitoids collected from *C. lameensis* host stage in January, 2009

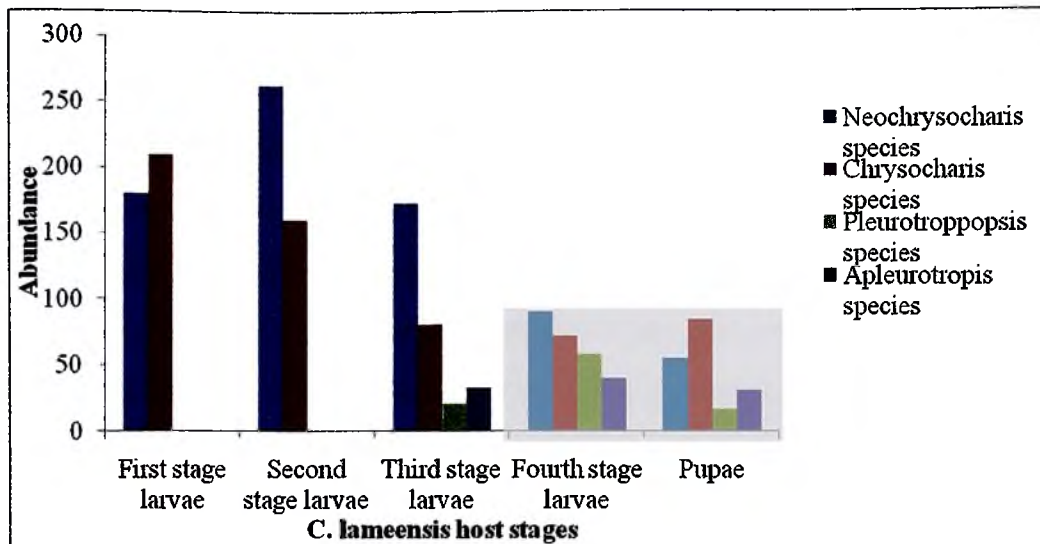


Figure 14: Parasitoids collected from *C. lameensis* host stages in February, 2009

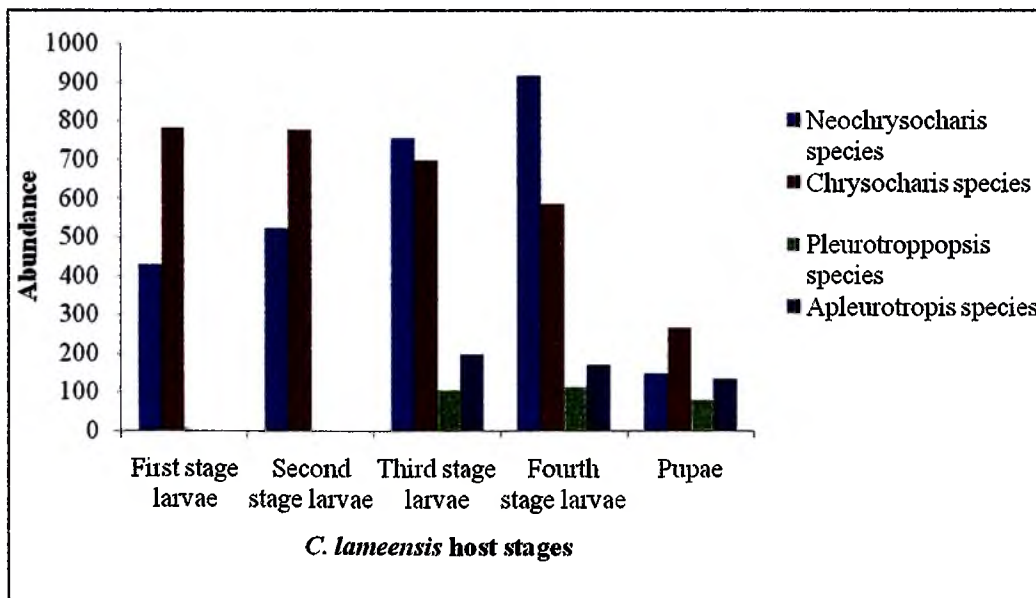


Figure 15: Parasitoids collected from *C. lameensis* host stages in March, 2009

Figures 9 to 15 show the parasitoid species that emerged from *C. lameensis* stages sampled from September 2008 to March 2009. *Neochrysocharis* species and *Chrysocharis* species were the dominant parasitoid species that emerged throughout the period of study and were found in all the stages sampled. *Pleurotroppopsis* species and *Apleurotropis* species did not emerge from first and second stage larvae that were collected for the entire period. They however, emerged in third and fourth stage larvae as well as the pupal host samples. *Pleurotroppopsis* species and the unidentified species comprised about 10% of all parasitoids collected during the entire study period.

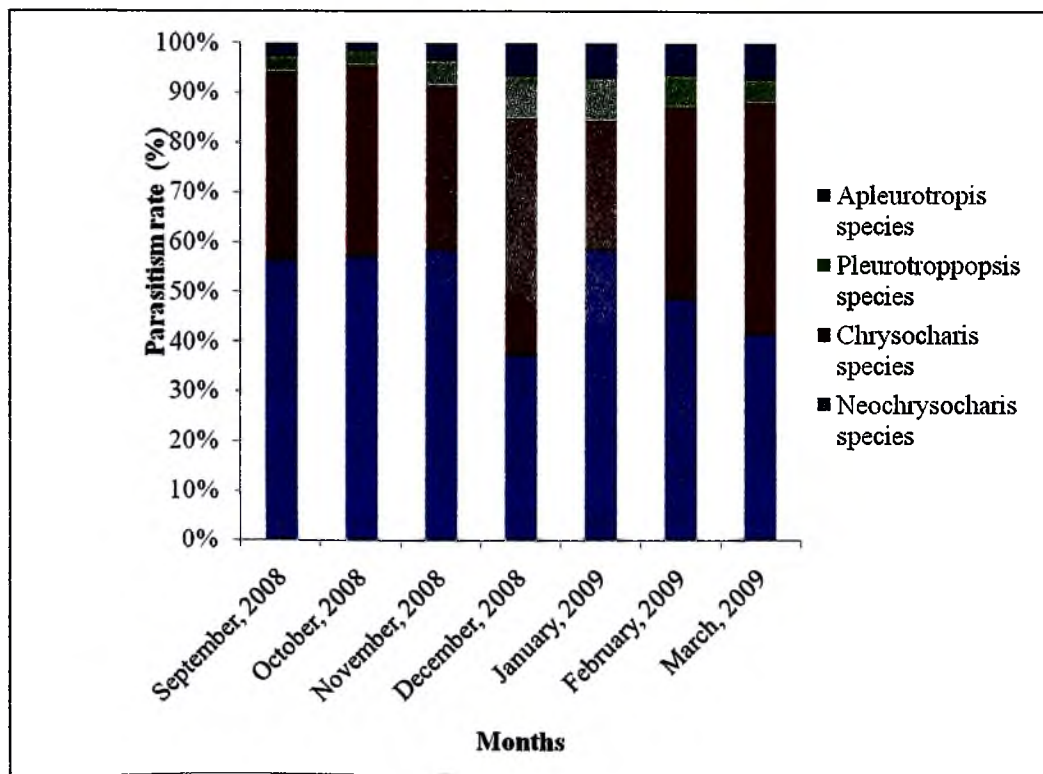


Figure 16: Monthly parasitoid parasitism rate

Figure 16 shows the parasitism rate of parasitoids that emerged monthly for the study period. The highest parasitism rate for *Neochrysocharis* species was in October, 2008 (55.58%) and the lowest was in December, 2008 (11.07%). *Chrysocharis* species recorded its highest parasitism rate in October, 2008 (37.64%) and the lowest in January, 2009 (9.79%). The parasitism rate of *Pleurotropopsis* species was highest in February, 2009 (3.67%) and lowest in March, 2009 (1.53%). *Apleurotropis* species recorded its highest parasitism rate in February, 2009 (3.97%) and the lowest rate in November, 2008 (1.52%).

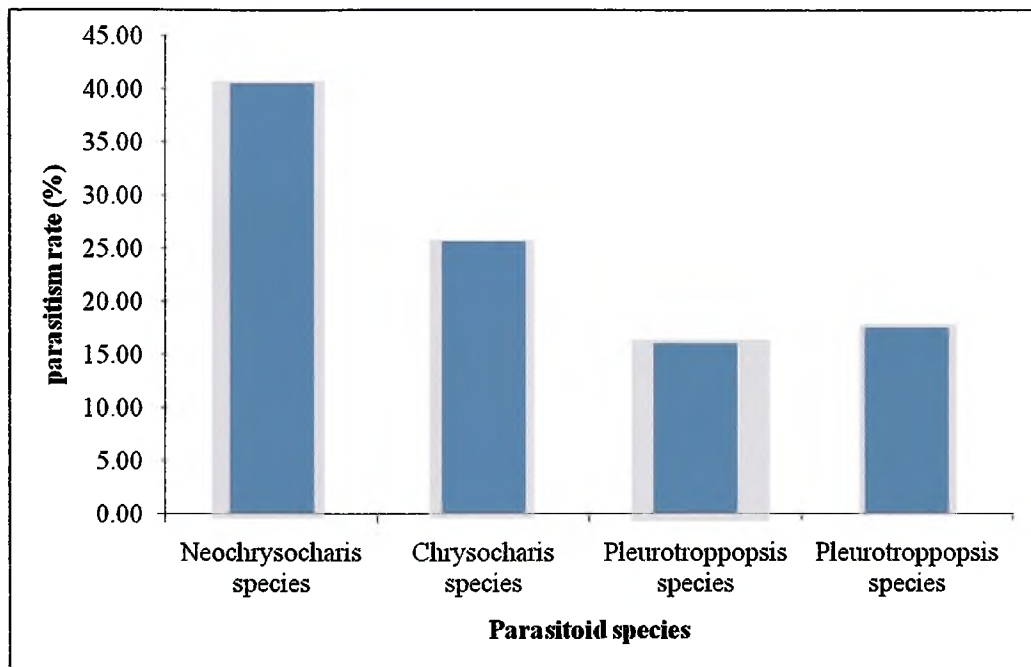


Figure 17: Parasitoid parasitism rate

Out of the 6,134 *C. lameensis* stages which were parasitized by the various parasitoid species, 40.58% (2,489) were parasitized by *Neochrysocharis* species. *Chrysocharis* species parasitized 25.69% (1,576) of the *C. lameensis* host stages while *Pleurotropopsis* species parasitized



16.14% (990) of the host stages parasitized. *Apleurotropis* species parasitized 17.59% (1,079) of parasitized host stages.

4.3 Predatory ant survey

During the study, three predatory ants were found to be natural enemies of the oil palm leafminer, *C. lameensis*. The predatory ants were *Oecophylla* species (Plate 14), *Crematogaster* species (Plate 13) and *Tetramorium* species (Plate 15) all belonging to the order Hymenoptera. The predatory ant species were found in nests on palm fronds (Plates 13, 14, 15). *C. lameensis* stages present on palm frond were reduced when the predatory ants' nests were present on the palm fronds.



Plate 13: *Crematogaster* species



Plate 14: *Oecophylla* species



Plate 15: *Tetramorium* species



Plate 16: Nest of *Oecophylla* species



Plate 17: Nest of *Tetramorium* species



Plate 18: Nest of *Crematogaster* species

Figures 18 to 24 show the monthly predatory index of the *Oecophylla* species, *Crematogaster* species and *Tetramorium* species on the plots.

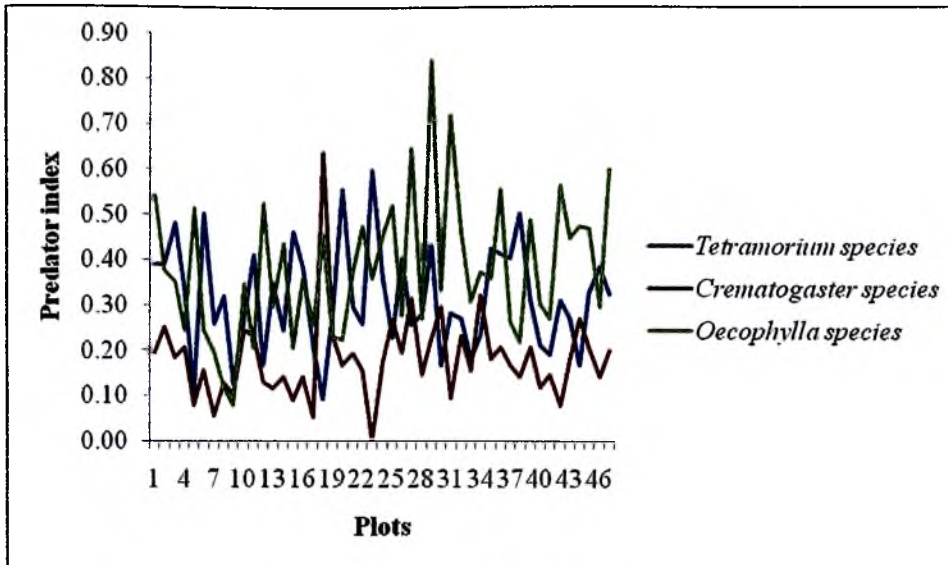


Figure 18: Predator abundance in September, 2008

In September 2008, *Oecophylla* species and *Tetramorium* species were present on all the 46 plots visited (Figure 18). *Crematogaster* species was only missing on one plot. In terms of abundance, *Oecophylla* species was found to be the most abundant while *Crematogaster* species was least abundant (Figure 18).

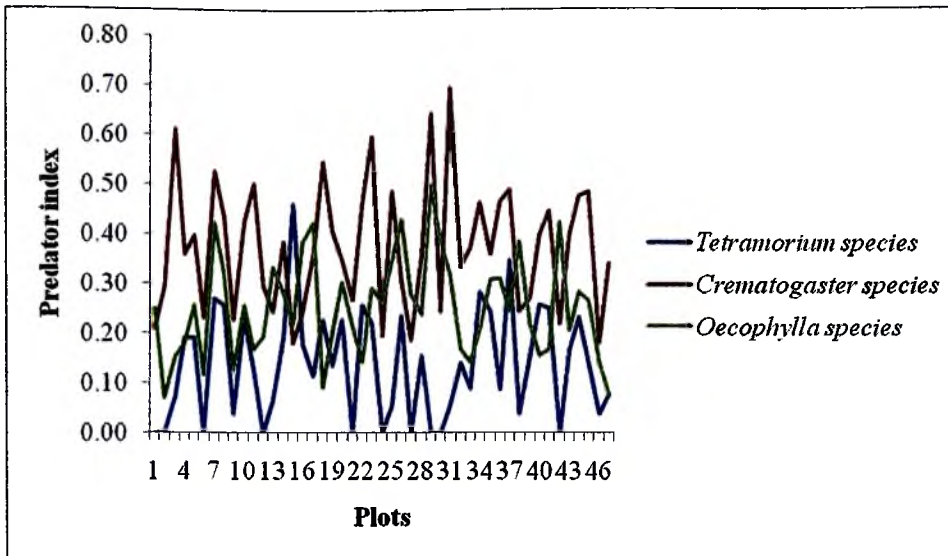


Figure 19: Predator abundance in October, 2008

As depicted in Figure 19 *Crematogaster species* was the most abundant predatory ant encountered in October, 2008. *Tetramorium species* was least abundant and was visibly missing on 10 out of the 46 plots visited for the month. *Oecophylla species* and *Crematogaster species* were present on all the plots.

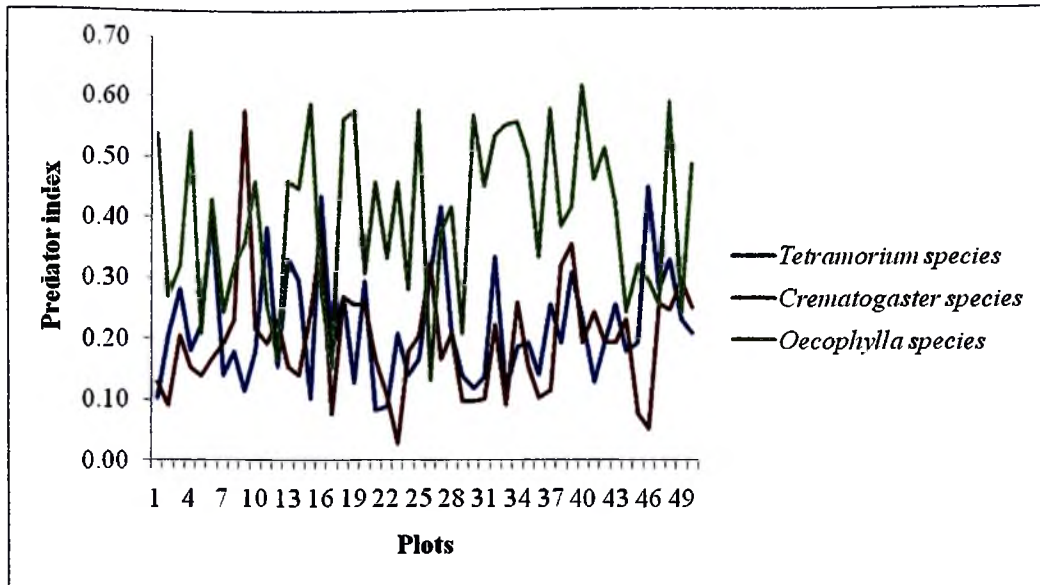


Figure 20: Predator abundance in November, 2008

Figure 20 represents *Oecophylla* species, *Crematogaster* species and *Tetramorium* species abundance on 49 plots visited in November, 2008. All three predatory ants were visibly present on all plots. *Oecophylla* species was most abundant while *Crematogaster* species was least abundant for the month of November, 2008.

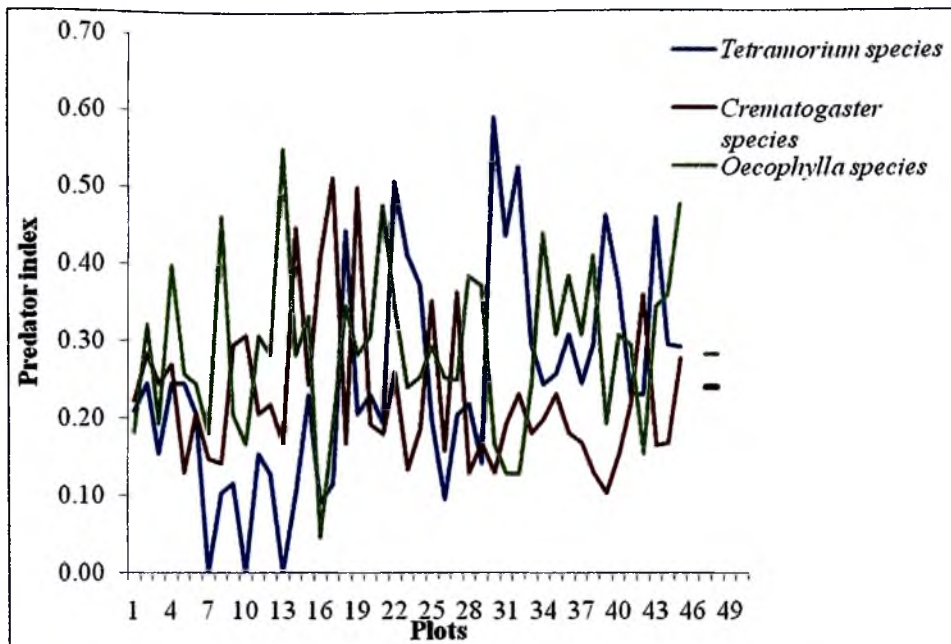


Figure 21: Predator abundance in December, 2008

Figure 21 represents predator abundance on 49 plots visited in December, 2008. On three plots *Tetramorium* species was not present. *Oecophylla* species and *Crematogaster* species were however present on all plots for the month. *Oecophylla* species was the most abundant predatory ant found on the plots with a monthly mean index of 0.43 (Figure 25). For December, 2008, *Tetramorium* species was more abundant than *Crematogaster* species (Figure 25).

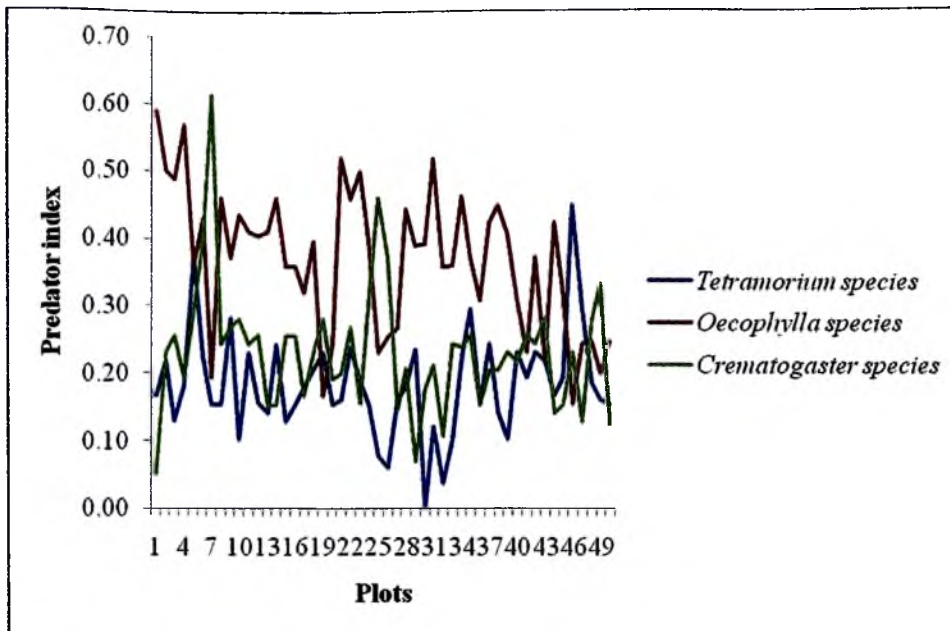


Figure 22: Predator abundance in January, 2009

In January, 2009, all three predatory ants were found on all the plots visited except for one plot where *Tetramorium* species was not present. Generally, *Oecophylla* species was most abundant with a mean monthly index of 0.39. *Tetramorium* species followed with a mean monthly index of 0.26 while *Crematogaster* species recorded a mean monthly index of 0.15 (Figures 22 and 25).

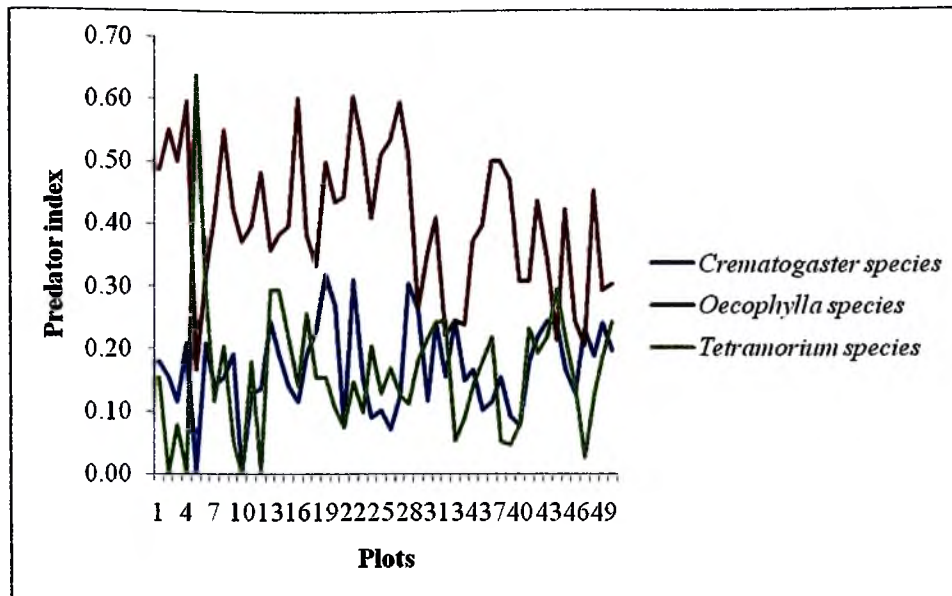


Figure 23: Predator abundance in February, 2009

Oecophylla species was the most abundant predatory ant species in February, 2009 with a monthly index of 0.40 (Figure 23 and Figure 25). *Tetramorium* species recorded a monthly index of 0.33 which was higher than the index for *Crematogaster* species index of 0.19 (Figures 23 and 24).

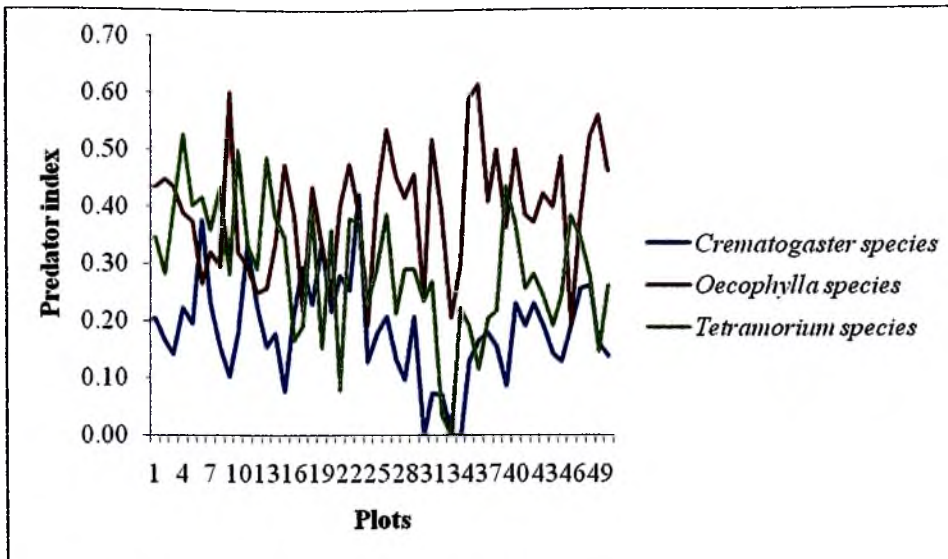


Figure 24: Predator abundance in March, 2009

In March, 2009, the most abundant predator was *Oecophylla* species with a monthly index of 0.43. *Tetramorium* species was also more abundant than *Crematogaster* species with monthly indices of 0.30 and 0.19 respectively (Figures 24 and 25).

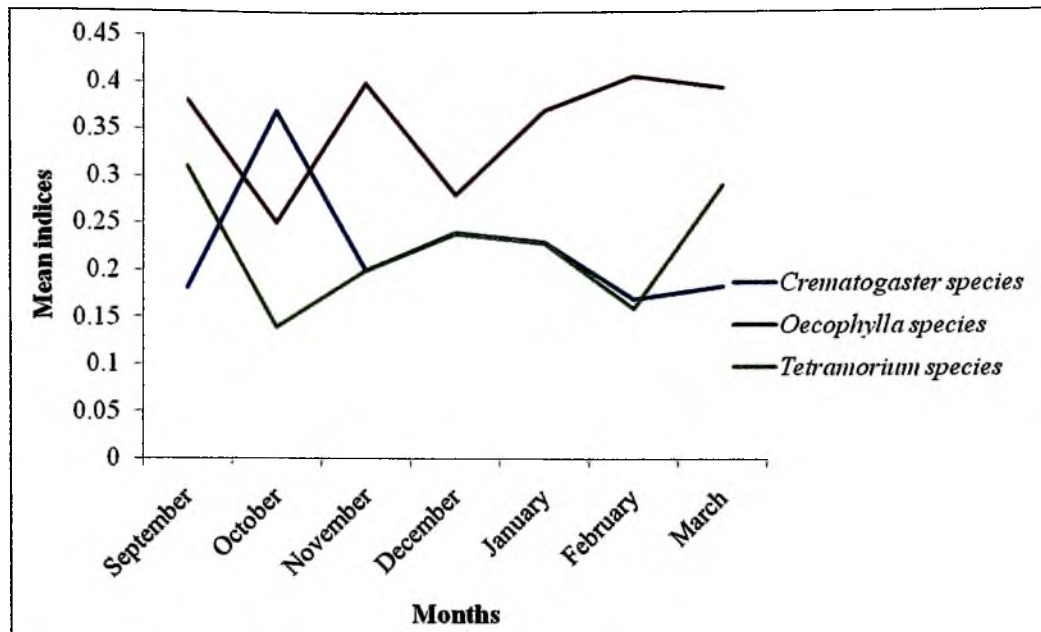


Figure 25: Mean monthly predator abundance

4.4 Entomopathogens

Sixty three (63) fungal infested *C. lameensis* developmental stages were obtained from the survey (Plate 19). One fungal species was recovered from all the 63 infested *C. lameensis*. The fungal species was *Syncephalastrum racemosum* Cohn (plate 20) belonging to the phylum Zygomorpha and order Mucorales (Plate 20).

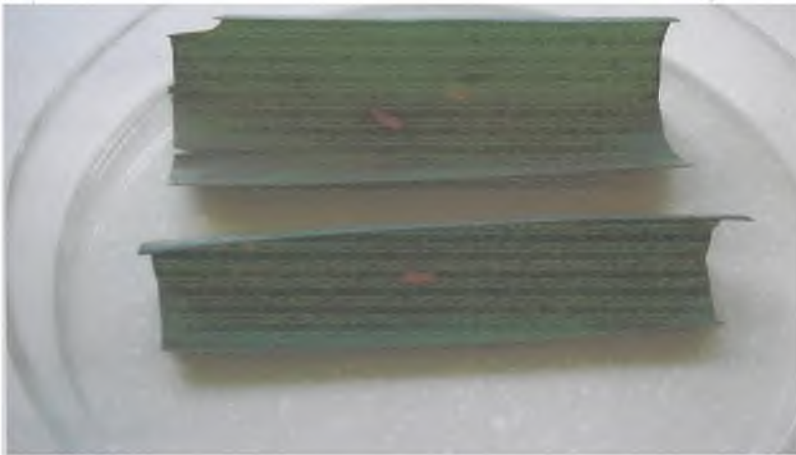


Plate 19: Fungal infested *C. lameensis* adult on Oil palm leaflet

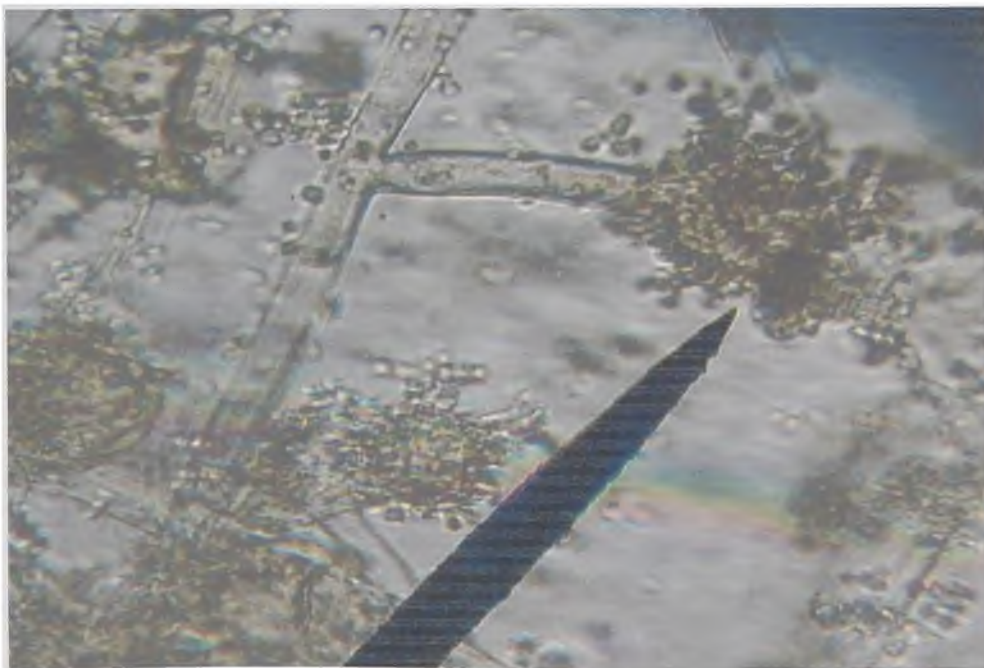


Plate 20: *Syncephalastrum racemosum* Cohn

CHAPTER FIVE

5.0 DISCUSSION

5.1 Parasitoids of *Coelaenomenodera lameensis*

This study has identified four parasitoid genera including *Neochrysocharis* species, *Chrysocharis* species, *Pleurotroppopsis* species and *Apleurotropis* species all belonging to the order Hymenoptera and family Eulophidae from the plantations of the Unilever Twifo Oil Palm Plantations Limited. Minkenbergh and van Lenteren (1986), Waterhouse and Norris (1987), Konishi (1998), Murphy and LaSalle (1999) and Reina and La Salle (2003) had earlier observed that Eulophid wasps were the most common parasitoids recorded on leafminers and the most successful agents used in biocontrol programmes against leafminers. A similar survey carried out by Yawson *et al.*, (2009), however, identified more parasitoid species from the *C. lameensis* host stages. Parasitoids identified by Yawson *et al.*, (2009) were *Phymastichus* spp, *Apleurotropis* spp, *Neochrysocharis* spp, *Pleurotroppopsis* spp, *Closterocerus mirabilis* and *Cirrospilus* spp. *Chrysocharis* species was, however not found in the collection of Yawson *et al.*, (2009). A reasonable speculation to account for the fewer number of parasitoid species collected during this study period could be the extent of insecticide (Evisect S) application in the control of *C. lameensis* outbreak at TOPP. Though the chemical was judiciously used, it still affected the insect species diversity including the parasitoid species present in the plantations.

Neochrysocharis species and *Chrysocharis* species were the most abundant of the parasitoid species collected. This was probably due to the fact that they were the only gregarious parasitoids collected during the study period. Generally, each *C. lameensis* stage that hosted either *Neochrysocharis* species or *Chrysocharis* species recorded about five individuals of these parasitoid species emerging. This significantly contributed to the abundance of *Neochrysocharis*

species and *Chrysocharis* species. These parasitoid species also took shorter times to emerge from the host. In fact, it took between 1-6 days for the pre-pupal stages of *Neochrysocharis* species and *Chrysocharis* species to emerge from the host under captivity. It is however not enough to conclude that *Neochrysocharis* species and *Chrysocharis* species spent about 6 days before they emerged from their host. This is because *C. lameensis* were already parasitized before they were brought from the fields and this study could not exactly determine what time the adult female parasitoids laid their eggs in their host. The adult *Neochrysocharis* species and *Chrysocharis* species lived from 4-5 days when they were kept in the test tubes in the laboratory where room temperature and relative humidity were maintained at 29°C and 82% respectively. It is believed that it is during this 4-5 day period that oviposition occurs in the open field.

Pleurotroppopsis species, a solitary parasitoid, was least abundant. It emerged only from the third and fourth larval stages and the pupal stages with no emergence from the early larval stages. Emergence time for *Pleurotroppopsis* species from *C. lameensis* hosts recorded in the Laboratory ranged from 31-40 days. Again this does not give a guide as to the exact time oviposition took place in the host since the host stages were already parasitized before they were brought in from the field. *Pleurotroppopsis* species lived for about 6 days.

Apleurotropis species, also solitary, emerged from third and fourth larval and pupal stages. Emergence time for *Apleurotropis* species ranged from 14-21 days. Adult life span for this parasitoid was about 6 days.

Pleurotroppopsis species and *Apleurotropis* species were not abundant probably because they are solitary and it takes a relatively longer time for them to emerge.

5.2 *Coelaenomenodera lameensis* stages attacked by Parasitoids

The results indicate that *C. lameensis* stages attacked by parasitoids were 13.51% of the total *C. lameensis* stages collected. Parasitoid abundance depends on host stages abundance. That is the higher *C. lameensis* population, the higher the parasitoid species population in the oil palm community. This however does not affect the parasitoid species complex since no new parasitoid species was recovered apart from the four species that were collected from September, 2008 through to March, 2009. The high population level of *C. lameensis* and the low parasitism rate are indication of the fact that the parasitoid species complex is inadequate to effectively contain the *C. lameensis* populations.

More parasitoid species emerged from late stage larvae (Third and fourth stages) than early stage larvae (first and second stages) as also observed by Urbaneja *et al.*, (2000). This observation is collaborated by the fact that parasitoids tend to lay more eggs in large hosts where there is more food resources. In the situation where there is inadequate food store due to small body size (early stage larvae), competition between larvae in a host can be intense. This results in the emergence of smaller adult parasitoid species. Some parasitoid species collected from the study were collected mainly from third and fourth stage larvae and a few from the pupal stage of *C. lameensis* with none emerging from the first and second larval stages. This is consistent with observation made by Argov and Rossler (1998) and Mineo and Mineo (1999a; 1999b) who found out that the parasitoid *Semielacher petiolatus* attacked late stage larvae of the citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in the East Mediterranean region of Turkey.

Even those parasitoid species that emerged from all the stages including the first and second larval stages, they were minimal when compared with third and fourth larval stages.

5.3 Predatory ants

There was a representation of the three predatory ant species on the plots of the Twifo Oil Palm Plantations Limited. From the results obtained in this study, *Oecophylla* species (Plate 14) was most abundant on all the plots surveyed. *Crematogaster* species (Plate 13) was the next abundant after *Oecophylla* species. *Tetramorium* species (Plate 15) was the least abundant predatory ant species on the plots. Way and Khoo (1992); Hawkins *et al.* (1997); Queiroz, (2002); Philpott and Foster (2005) documented that ants (Formicidae) were the most frequently observed predators of the Coffee leafminer eggs, larvae and pupae and were the most frequently present predators on coffee plants. This supports the emphasis that ants are the most frequent important mortality factor affecting herbivore populations in tropical systems as they increase predation rates and reduce pest population densities. Some studies (Reimer *et al.*, 1993; Infante *et al.*, 2003) have however, suggested the elimination of ants to enhance biological control. Predatory ants effectively protect tropical tree crops as they actively patrol canopies and prey upon or deter a wide range of potential pests. They have therefore been employed in several biological control programmes.

5.4 Entomopathogens

The entomopathogens recovered from the survey yielded *S. racemosum* as the only fungi. Considering the low numbers of fungal infested *C. lameensis* developmental stages obtained from the fields and the fact that only one entomopathogenic fungal species was recovered, it makes it easy to speculate that pathogenic micro-organisms are not so well represented as to

contribute significantly to the reduction in *C. lameensis* population. A possible reason why low numbers of *C. lameensis* developmental stages were found to be infested by fungi could be due to the height of the oil palm trees that were used for the survey. Most of the oil palm trees sampled for the fungal infested *C. lameensis* stages were from ages 25 to 27 years and thus were very tall. When fronds were cut from such heights, it was possible that by the time they fell to the ground, the fungal infested *C. lameensis* adult would have dropped making it difficult for them to be collected. The 63 infested species were obtained from plants that were just about eight years old and were relatively short.

S. racemosum (Pitt and Hocking, 1985) showed that the colonies covered the entire petri dish with moderately dense, blackish grey mycelium with reverse side yellow brown in colour. With the increasing awareness of protecting the environment, use of chemicals is avoided and thereby, biocontrol measures are being promoted. Biocontrol measure of *S. racemosum* should be based on alteration of the optimum growth conditions, thereby promoting the growth of competitor micro-organisms (Garg and Prakash, 2006).

5.5 Prospects for biological control

There are numerous successful examples of classic biological control (introduction of biologically active agents for the control of a native or foreign pest) with parasitoids for different species of leafminers, both in the open field (Dharmadhikari *et al.*, 1977; Johnson *et al.*, 2003; García-Marí *et al.*, 2004) and in greenhouses (van Lenteren and Woets, 1988; Heinz and Parrella, 1990; Abd- Rabou, 2006). In these cases, studies prior to introduction are important and include tolerance to humidity, ability to recognize previously parasitized hosts, existence of alternative

hosts, as well as synchronization with the host (Wang *et al.*, 1999; Grabenweger, 2004; Girardoz *et al.*, 2006b; Zappala and Hoy, 2004). Temperature constraints have also been shown to be an obstacle for a leafminer parasitoid to be successfully introduced in some regions (Klapwijk *et al.*, 2005; Ll acer *et al.*, 2006).

Three species of *Neochrysocharis* have been found attacking *Liriomyza huidobrensis* in large numbers in some samples from Southeast Asia: *N. beasleyi*, *N. okazakii* Kamijo and *N. formosa* (Westwood). These species appear to be contributing to fortuitous biological control in Southeast Asia (Sivapragasam *et al.*, 1999; Murphy and LaSalle, 1999; LaSalle, 1999; Thang, 1999; Rauf and Shepard, 1999), and have the potential for use in biological control programs of leafminers in Australia.

Some of these identified parasitoids genera have been found to parasitize on other insect species and also had been used in bio-control programmes. *Neochrysocharis* species had been used before in a trial control of the oil palm leafminer in Cote d'Ivoire (Lecoustre *et al.*, 1980). Species including *N. okazakii* have been found to parasitize the vegetable and ornamental leafminer *L. chinensis*, *L. huidobrensis*, *L. sativae* and *L. trifolii* (Greathead and Greathead, 1992; Johnson, 1993; Lynch and Johnson, 1987; Stegmaier, 1996). *Pleurotroppopsis* spp are also known to parasitize vegetable (tomato and watermelon) leafminers including *L. sativae*, *L. trifolii* and *L. huidobrensis* (Poe *et al.*, 1978; Getzin, 1960).

Oecophylla species have been reported to control a range of pests, including the citrus stinkbug *Rhynchocoris humeralis* (Thnb.) (Hemiptera: Pentatomidae), the aphids *Toxoptera*, leaf-feeding caterpillars *Papilio*, inflorescence eaters, coleopteran and various other pests (Yang, 1984; van Mele *et al.*, 2002). In a survey, van Mele and Cuc (2000), observed that Vietnamese citrus farmers who encouraged *Oecophylla* species on their farms spent about half the amount of

money on agrochemicals compared with those farmers who did not have *Oecophylla* in their orchard, yet obtained similar yields (van Mele and Cuc, 2000). Up to 20% of the orange growers produced their crop entirely without pesticide by making optimal use of *Oecophylla* species. In Australia, weaver ants controlled all major cashew pests (Peng *et al.*, 1999) and increased profits by at least 35%. Löhrr and Oswald (1989) found that colonization of palms by *O. longinoda* increased nut yield significantly, and that insecticide use could be significantly reduced. Predatory ants have been known to drive off a range of pests, including weevils *Pantorhytes* (Stapley, 1980b), coreid bugs *Amblypelta* and *Pseudotheraptus* (Lodos, 1967) and capsids and mirids (Leston, 1970; Way and Khoo, 1992, Ayenor *et al.*, 2004). The importance of the predatory ants triggered the recommendation to avoid spraying cocoa trees where weaver ants were abundant (Julia and Mariau, 1978; Majer, 1978) in major cocoa growing areas in Africa. This recommendation, however, was not given any serious consideration. Peng *et al.* (1997; 1999; 2001) document the effectiveness of predatory ants on Cashew. In mango the use of predatory ants are well documented by Peng and Christian (2004; 2005; 2007).

S. racemosum has been used as an active biocontrol agent in many field crops. Its occurrence has been reported on cereals like rice (Pederes *et al.*, 1997), maize (Raybaudi and Martinez, 2000), wheat (Kunwar, 1989), oranges and lemons (Babu and Reddy, 1987, 1988, 1989), mango (Laxminarayana and Reddy, 1977), black pepper (Giridhar and Reddy, 2002). It has been shown that *S. racemosum* has been successfully used against various pests in Brazil (Ferron, 1978), Australia (Cullen, 1978), Europe (Hall, 1981; Hall and Burges, 1979; Hall and Papierok, 1982), Chile (Templeton *et al.*, 1979) and the United States (Templeton *et al.*, 1979). It is striking why documentation on the use of entomopathogens in Africa seems to be lacking.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Parasitoids are a major cause of mortality of oil palm leafminer, *C. lameensis*. The second, third and fourth larval stages are the most susceptible to parasitoid attack while first larval stage and the pupal stage were the least attacked. The parasitism due to parasitoid attack was 13.51%. The most dominant parasitoids were *Neochrysocharis* species and *Chrysocharis* species. *Pleurotroppopsis* species was least abundant after *Apleurotropis* species. These parasitoids can be used in biological control programme of the oil palm leafminer. Research into the exploration of more parasitoids must continue. The response of the parasitoids to evisect S must also be investigated. More importantly research into the development of an efficient rearing facility must commence to enable the mass production of parasitoids. It is also required to monitor the relationship between the parasitoid population and weather conditions. Studies that show the spread and distribution of oil palm leafminer throughout the oil palm growing areas across the regions of Ghana must be undertaken. Explanation as to why smallholder farms do not experience oil palm leafminer outbreak while it is endemic to plantations needs to be investigated.

Predatory ants were also present in the plantations of Twifo Oil palm Plantations. *Oecophylla* species was most dominant. *Crematogaster* species was more abundant than *Tetramorium* species. They also contribute to the mortality of oil palm leafminer and can be included in any biological control programme since they actively patrol tree canopies and actively pursue the pest. More surveys need to be conducted to discover other predators of the oil palm leafminer.

The entomopathogenic representation on the plantation was not encouraging since only a few of the *C. lameensis* developmental stages were found to be infested. Research into the isolation and

trial of *S. racemosum* on oil palm leafminer needs to be undertaken while work on the discovery of more entomopathogens needs to be considered. An explanation into the reason why few pathogenic fungal species were found at Twifo Oil Palm Plantations Limited needs to be sought and researched.

A combination of the identified parasitoids, predatory ants and the entomopathogens hold the key to a successful Integrated Pest Management programme.

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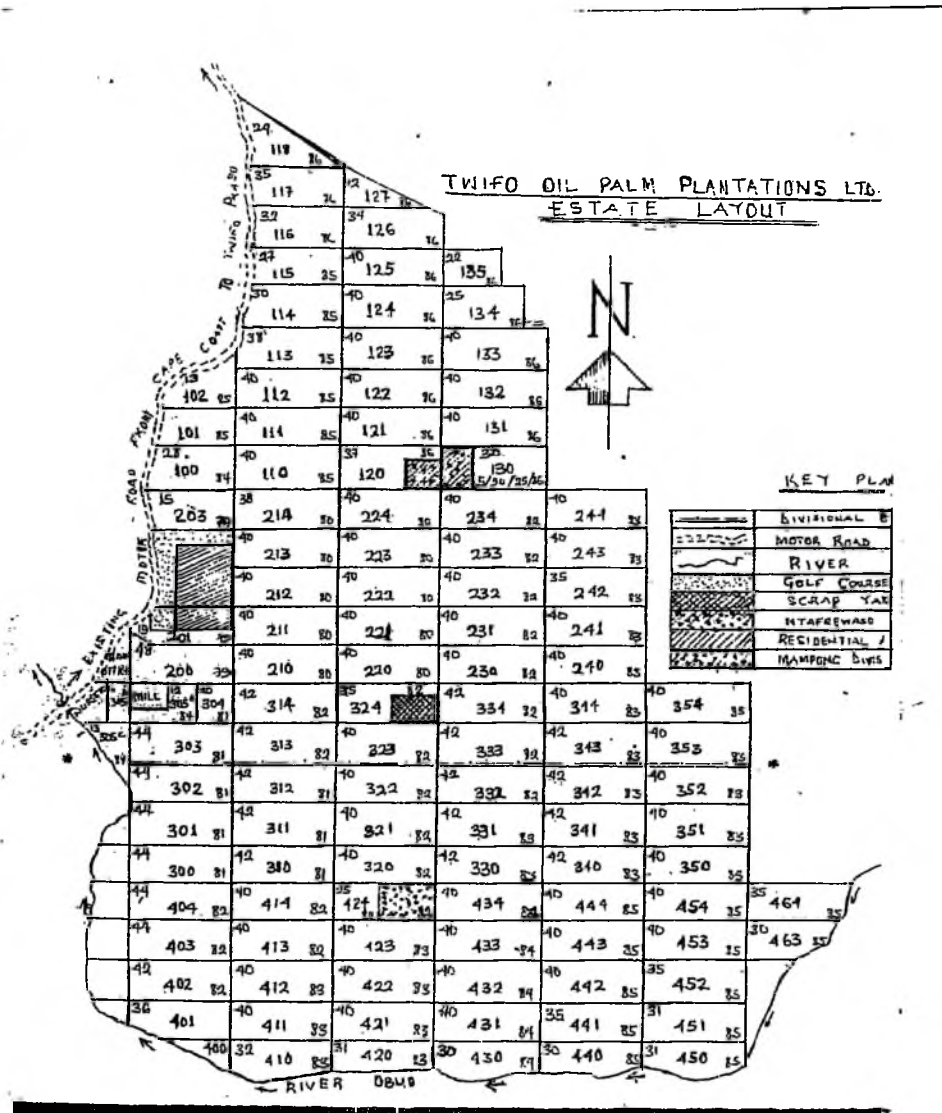
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APPENDICES

Appendix 1: Estate layout of Twifo Oil Palm Plantations Limited



Appendix 2: Distribution of *C. lameensis* host stages during the study period

Months	Abundance (%)
September, 2008	3.81
October, 2008	2.85
November, 2008	15.33
December, 2008	19.45
January, 2009	10.41
February, 2009	5.77
March, 2009	42.38

Appendix 3: Distribution of *C. lameensis* stages sampled during the study period

<i>C.lameensis</i> stages	Abundance (%)	Abundance
First stage larvae	18.64	8467
Second stage larvae	16.46	7476
Third stage larvae	23.69	10756
Fourth stage larvae	23.79	10804
Pupae	17.42	7909

Appendix 4: Distribution of parasitized *C. lameensis* stages

<i>C. lameensis</i> stage	Abundance (%)
First stage larvae	10.01
Second stage larvae	16.12
Third stage larvae	27.45
Fourth stage larvae	27.60
Pupae	18.81

Appendix 5: *Neochrysocharis* species emergence from *C. lameensis* host stages

<i>C. lameensis</i> stages	Abundance (%)
First stage larvae	8.83
Second stage larvae	21.62
Third stage larvae	30.83
Fourth stage larvae	28.52
Pupae	10.20

Appendix 6: *Chrysocharis* species emergence from *C. lameensis* host stages

<i>C. lameensis</i> stages	Abundance (%)
First stage larvae	17.64
Second stage larvae	21.73
Third stage larvae	27.74
Fourth stage larvae	22.64
Pupae	10.25

Appendix 7: *Pleurotroppopsis* species emergence from *C. lameensis* host stages

<i>C. lameensis</i> stages	Abundance (%)
First stage larvae	0.00
Second stage larvae	0.00
Third stage larvae	33.94
Fourth stage larvae	41.41
Pupae	24.65

Appendix 8: *Apleurotropis* species emergence from *C. lameensis* host stages

<i>C. lameensis</i> stages	Abundance (%)
First stage larvae	0.00
Second stage larvae	0.00
Third stage larvae	35.50
Fourth stage larvae	36.33
Pupae	28.17

Appendix 9: Parasitoid abundance from *C. lameensis* host stages during the 2008-2009 study period

Month	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
September, 2008	812	543	44	40
October, 2008	722	487	34	23
November, 2008	1740	989	150	106
December, 2008	1978	1255	221	179
January, 2009	1034	463	151	127
February, 2009	760	608	96	104
March, 2009	2769	3105	294	500

Appendix 10: Parasitoids collected from *C. lameensis* host stages in September, 2008

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	41	28	0	0
Second stage larvae	163	87	0	0
Third stage larvae	251	142	11	8
Fourth stage larvae	244	187	10	15
Pupae	113	99	23	17

Appendix 11: Parasitoids collected from *C. lameensis* host stages in October, 2008

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	96	86	0	0
Second stage larvae	199	117	0	0
Third stage larvae	248	174	8	8
Fourth stage larvae	123	96	21	7
Pupae	56	14	5	8

Appendix 12: Parasitoids collected from *C. lameensis* host stages in November, 2008

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	38	124	0	0
Second stage larvae	893	350	0	0
Third stage larvae	310	149	83	59
Fourth stage larvae	388	172	43	33
Pupae	111	194	24	14

Appendix 13: Parasitoids collected from *C. lameensis* host stages in December, 2008.

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	81	41	0	0
Second stage larvae	62	73	0	0
Third stage larvae	1003	634	88	62
Fourth stage larvae	723	351	118	96
Pupae	109	156	15	21

Appendix 14: Parasitoids collected from *C. lameensis* host stages in January, 2009

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	2	9	0	0
Second stage larvae	20	24	0	0
Third stage larvae	288	136	22	16
Fourth stage larvae	315	178	49	32
Pupae	409	116	80	79

Appendix 15: Parasitoids collected from *C. lameensis* host stages in February, 2009

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	180	210	0	0
Second stage larvae	262	160	0	0
Third stage larvae	173	81	21	33
Fourth stage larvae	90	72	58	40
Pupae	55	85	17	31

Appendix 16: Parasitoids collected from *C. lameensis* host stages in March, 2009

<i>C. lameensis</i> stages	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
First stage larvae	429	782	0	0
Second stage larvae	523	776	0	0
Third stage larvae	753	697	103	197
Fourth stage larvae	916	584	111	169
Pupae	148	266	80	134

Appendix 17: Monthly parasitoid parasitism rate

Month	Parasitism rate (%)			
	<i>Neochrysocharis</i> species	<i>Chrysocharis</i> species	<i>Pleurotroppopsis</i> species	<i>Apleurotropis</i> species
September, 2008	46.91	31.37	2.54	2.31
October, 2008	55.8	37.64	2.63	1.78
November, 2008	25	14.21	2.15	1.52
December, 2008	11.07	14.21	2.5	2.03
January, 2009	21.87	9.79	3.19	2.69
February, 2009	29.02	23.21	3.67	3.97
March, 2009	14.39	16.13	1.53	2.6

Appendix 18: Parasitoid parasitism rate

Species	Parasitism rate (%)	Number
<i>Neochrysocharis</i> species	40.58	2489
<i>Chrysocharis</i> species	25.69	1576
<i>Pleurotroppopsis</i> species	16.14	990
<i>Apleurotropis</i> species	17.59	1079

Appendix 19: Predator abundance in September, 2008

<i>Tetramorium</i> species	<i>Crematogaster</i> species	<i>Oecophylla</i> species
0.39	0.19	0.54
0.39	0.25	0.38
0.48	0.18	0.35
0.33	0.21	0.24
0.10	0.08	0.51
0.50	0.15	0.24
0.26	0.05	0.20
0.32	0.13	0.12
0.13	0.10	0.08
0.27	0.24	0.35
0.41	0.23	0.21
0.17	0.13	0.53
0.35	0.12	0.29
0.24	0.14	0.44
0.46	0.09	0.21
0.38	0.14	0.36
0.21	0.05	0.26
0.09	0.64	0.45
0.28	0.23	0.23
0.56	0.17	0.22
0.29	0.19	0.37
0.26	0.15	0.47
0.60	0.00	0.36
0.36	0.17	0.45
0.23	0.27	0.52
0.40	0.19	0.28
0.25	0.31	0.65
0.28	0.14	0.27
0.43	0.23	0.84
0.17	0.29	0.33
0.28	0.09	0.72
0.27	0.23	0.46
0.18	0.15	0.31
0.23	0.32	0.37
0.42	0.18	0.36
0.41	0.21	0.56
0.40	0.17	0.26
0.50	0.14	0.22
0.31	0.21	0.49
0.21	0.12	0.30
0.19	0.14	0.27
0.31	0.08	0.56
0.27	0.18	0.45
0.17	0.27	0.47
0.33	0.20	0.47
0.38	0.14	0.29
0.32	0.20	0.60

Appendix 20: Predator abundance in October, 2008

<i>Tetramorium</i> species	<i>Crematogaster</i> species	<i>Oecophylla</i> species
0.00	0.21	0.25
0.00	0.29	0.07
0.07	0.61	0.15
0.19	0.36	0.18
0.19	0.40	0.26
0.00	0.23	0.12
0.27	0.53	0.42
0.26	0.44	0.32
0.04	0.23	0.13
0.23	0.42	0.26
0.13	0.50	0.17
0.00	0.29	0.19
0.06	0.24	0.33
0.19	0.38	0.28
0.46	0.18	0.22
0.18	0.24	0.38
0.12	0.36	0.42
0.23	0.55	0.09
0.14	0.41	0.20
0.23	0.35	0.30
0.00	0.27	0.21
0.26	0.46	0.14
0.22	0.60	0.29
0.00	0.19	0.26
0.06	0.49	0.35
0.24	0.31	0.43
0.00	0.19	0.28
0.15	0.36	0.24
0.00	0.64	0.50
0.00	0.24	0.38
0.06	0.69	0.32
0.14	0.33	0.17
0.09	0.37	0.14
0.28	0.46	0.21
0.24	0.36	0.31
0.09	0.47	0.31
0.35	0.49	0.24
0.04	0.24	0.38
0.15	0.27	0.22
0.26	0.40	0.15
0.25	0.44	0.17
0.00	0.22	0.42
0.17	0.40	0.21
0.23	0.47	0.28
0.14	0.48	0.27
0.04	0.18	0.14
0.08	0.34	0.08

Appendix 20: Predator abundance in October, 2008

<i>Tetramorium</i> species	<i>Crematogaster</i> species	<i>Oecophylla</i> species
0.00	0.21	0.25
0.00	0.29	0.07
0.07	0.61	0.15
0.19	0.36	0.18
0.19	0.40	0.26
0.00	0.23	0.12
0.27	0.53	0.42
0.26	0.44	0.32
0.04	0.23	0.13
0.23	0.42	0.26
0.13	0.50	0.17
0.00	0.29	0.19
0.06	0.24	0.33
0.19	0.38	0.28
0.46	0.18	0.22
0.18	0.24	0.38
0.12	0.36	0.42
0.23	0.55	0.09
0.14	0.41	0.20
0.23	0.35	0.30
0.00	0.27	0.21
0.26	0.46	0.14
0.22	0.60	0.29
0.00	0.19	0.26
0.06	0.49	0.35
0.24	0.31	0.43
0.00	0.19	0.28
0.15	0.36	0.24
0.00	0.64	0.50
0.00	0.24	0.38
0.06	0.69	0.32
0.14	0.33	0.17
0.09	0.37	0.14
0.28	0.46	0.21
0.24	0.36	0.31
0.09	0.47	0.31
0.35	0.49	0.24
0.04	0.24	0.38
0.15	0.27	0.22
0.26	0.40	0.15
0.25	0.44	0.17
0.00	0.22	0.42
0.17	0.40	0.21
0.23	0.47	0.28
0.14	0.48	0.27
0.04	0.18	0.14
0.08	0.34	0.08

Appendix 21: Predator abundance in November, 2008

<i>Tetramorium</i> species	<i>Crematogaster</i> species	<i>Oecophylla</i> species
0.10	0.13	0.54
0.21	0.09	0.27
0.28	0.21	0.32
0.18	0.15	0.54
0.22	0.14	0.21
0.40	0.17	0.43
0.14	0.19	0.24
0.18	0.23	0.32
0.12	0.58	0.36
0.18	0.22	0.46
0.38	0.19	0.26
0.15	0.23	0.17
0.33	0.15	0.46
0.29	0.14	0.45
0.10	0.24	0.59
0.44	0.37	0.28
0.18	0.08	0.15
0.27	0.27	0.56
0.13	0.26	0.58
0.29	0.26	0.31
0.08	0.17	0.46
0.09	0.11	0.33
0.21	0.03	0.46
0.14	0.18	0.28
0.17	0.21	0.58
0.31	0.32	0.13
0.42	0.17	0.38
0.21	0.21	0.42
0.14	0.10	0.21
0.12	0.10	0.57
0.14	0.10	0.45
0.33	0.22	0.53
0.12	0.09	0.55
0.19	0.26	0.56
0.19	0.15	0.50
0.14	0.10	0.33
0.26	0.12	0.58
0.19	0.32	0.38
0.31	0.35	0.42
0.23	0.19	0.62
0.13	0.24	0.46
0.19	0.19	0.51
0.26	0.19	0.42
0.18	0.23	0.24
0.19	0.08	0.32
0.45	0.05	0.29
0.28	0.26	0.26
0.33	0.25	0.59
0.23	0.29	0.24
0.21	0.25	0.49

Appendix 22: Predator abundance in December, 2008

<i>Tetramorium</i> species	<i>Crematogaster</i> species	<i>Oecophylla</i> species
0.19	0.32	0.21
0.15	0.23	0.24
0.03	0.55	0.21
0.17	0.26	0.17
0.43	0.28	0.26
0.21	0.22	0.18
0.24	0.28	0.32
0.15	0.24	0.19
0.24	0.27	0.40
0.24	0.13	0.26
0.21	0.21	0.24
0.00	0.15	0.18
0.10	0.14	0.46
0.12	0.29	0.21
0.00	0.31	0.17
0.15	0.21	0.31
0.13	0.22	0.28
0.00	0.17	0.55
0.10	0.45	0.28
0.23	0.24	0.33
0.09	0.41	0.05
0.12	0.51	0.19
0.44	0.17	0.35
0.21	0.50	0.28
0.23	0.19	0.31
0.19	0.18	0.48
0.51	0.26	0.35
0.41	0.13	0.24
0.37	0.19	0.25
0.20	0.35	0.29
0.09	0.16	0.25
0.20	0.36	0.25
0.22	0.13	0.38
0.14	0.17	0.37
0.59	0.13	0.17
0.44	0.19	0.13
0.53	0.23	0.13
0.29	0.18	0.24
0.24	0.20	0.44
0.26	0.23	0.31
0.31	0.18	0.38
0.24	0.17	0.31
0.29	0.13	0.41
0.46	0.10	0.19
0.37	0.15	0.31
0.23	0.22	0.29
0.23	0.36	0.15
0.46	0.16	0.34
0.29	0.17	0.36
0.29	0.28	0.48

Appendix 23: Predator abundance in January, 2009

<i>Tetramorium</i> species	<i>Oecophylla</i> species	<i>Crematogaster</i> species
0.17	0.59	0.05
0.22	0.50	0.23
0.13	0.49	0.26
0.18	0.57	0.19
0.38	0.36	0.29
0.22	0.43	0.40
0.15	0.19	0.62
0.15	0.46	0.24
0.28	0.37	0.27
0.10	0.44	0.28
0.23	0.41	0.24
0.16	0.40	0.26
0.14	0.41	0.15
0.24	0.46	0.15
0.13	0.36	0.26
0.15	0.36	0.26
0.18	0.32	0.17
0.21	0.40	0.23
0.23	0.17	0.28
0.15	0.24	0.19
0.16	0.52	0.20
0.24	0.46	0.27
0.19	0.50	0.16
0.15	0.40	0.31
0.08	0.23	0.46
0.06	0.25	0.37
0.16	0.27	0.15
0.18	0.44	0.21
0.24	0.39	0.07
0.00	0.39	0.18
0.12	0.52	0.21
0.04	0.36	0.11
0.10	0.36	0.24
0.22	0.46	0.24
0.29	0.37	0.26
0.15	0.31	0.15
0.24	0.42	0.21
0.14	0.45	0.21
0.10	0.41	0.23
0.23	0.31	0.22
0.19	0.23	0.26
0.23	0.37	0.24
0.22	0.22	0.28
0.17	0.42	0.14
0.19	0.32	0.15
0.45	0.15	0.23
0.29	0.24	0.13
0.18	0.25	0.28
0.16	0.20	0.33
0.15	0.25	0.12

Appendix 24: Predator abundance in February, 2009

<i>Crematogaster</i> species	<i>Oecophylla</i> species	<i>Tetramorium</i> species
0.18	0.49	0.15
0.15	0.55	0.00
0.12	0.50	0.08
0.21	0.60	0.00
0.00	0.17	0.64
0.21	0.32	0.29
0.14	0.41	0.12
0.15	0.55	0.21
0.19	0.42	0.05
0.00	0.37	0.00
0.13	0.40	0.18
0.13	0.48	0.00
0.24	0.36	0.29
0.19	0.38	0.29
0.14	0.40	0.22
0.12	0.60	0.14
0.19	0.38	0.26
0.23	0.33	0.15
0.32	0.50	0.15
0.27	0.44	0.10
0.07	0.44	0.07
0.31	0.61	0.15
0.15	0.53	0.10
0.09	0.41	0.21
0.10	0.51	0.13
0.07	0.54	0.17
0.13	0.60	0.13
0.31	0.51	0.11
0.26	0.26	0.18
0.12	0.35	0.22
0.24	0.41	0.24
0.16	0.22	0.24
0.25	0.25	0.05
0.15	0.24	0.09
0.17	0.37	0.14
0.10	0.40	0.18
0.12	0.50	0.22
0.15	0.50	0.05
0.09	0.47	0.05
0.08	0.31	0.08
0.18	0.31	0.23
0.22	0.44	0.19
0.24	0.35	0.22
0.24	0.21	0.29
0.17	0.42	0.22
0.13	0.24	0.14
0.23	0.21	0.03
0.19	0.45	0.13
0.24	0.29	0.19
0.20	0.30	0.24

Appendix 25: Predator abundance in March, 2009

<i>Crematogaster</i> species	<i>Oecophylla</i> species	<i>Tetramorium</i> species
0.21	0.44	0.35
0.17	0.45	0.28
0.14	0.44	0.40
0.22	0.39	0.53
0.19	0.38	0.40
0.38	0.26	0.42
0.23	0.32	0.36
0.15	0.29	0.44
0.10	0.60	0.28
0.18	0.32	0.50
0.33	0.29	0.33
0.22	0.25	0.29
0.15	0.26	0.49
0.18	0.33	0.38
0.08	0.47	0.35
0.22	0.38	0.17
0.29	0.22	0.19
0.23	0.44	0.40
0.33	0.33	0.15
0.22	0.24	0.36
0.28	0.40	0.08
0.25	0.48	0.38
0.42	0.39	0.38
0.13	0.19	0.23
0.18	0.42	0.29
0.21	0.54	0.39
0.13	0.45	0.21
0.10	0.42	0.29
0.21	0.46	0.29
0.00	0.24	0.24
0.07	0.52	0.27
0.07	0.39	0.04
0.00	0.21	0.00
0.00	0.30	0.22
0.13	0.59	0.19
0.17	0.62	0.12
0.18	0.41	0.21
0.15	0.50	0.22
0.09	0.36	0.43
0.23	0.50	0.37
0.19	0.38	0.26
0.23	0.37	0.28
0.19	0.42	0.24
0.14	0.40	0.19
0.13	0.49	0.24
0.19	0.19	0.38
0.26	0.38	0.35
0.26	0.52	0.28
0.16	0.56	0.15
0.14	0.46	0.26

Appendix 26: Mean monthly predator abundance

Month	<i>Crematogaster</i> species	<i>Oecophylla</i> species	<i>Tetramorium</i> species
Septembe, 2008r	0.18	0.38	0.31
October, 2008	0.37	0.25	0.14
November, 2008	0.2	0.4	0.2
December, 2008	0.24	0.28	0.24
January, 2009	0.23	0.37	0.23
February, 2009	0.17	0.41	0.16
March, 2009	0.18	0.39	0.29