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Exploration and Host Specificity Tests for Potential Natural Enemies of *Dioscorea bulbifera* L. (Dioscoreaceae) in Ghana.

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DECLARATION

I hereby declare that this research work presented in this thesis is my own work and that to the best of my knowledge; it contains no material previously published by any other person for the award of a degree in any other University, except where acknowledgement has been made in the text.

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Date   Date

DEDICATION

This piece of work is dedicated to Nana Ohene Mensah Abaampa Chief Technical Officer of Crops Research Institute and Chief Linguist of Kwahu Community of Ashanti and Brong Ahafo regions for urging me on despite all odds.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

ABBREVIATIONS/ACRONYMS

ACT	Almanac Characterization Tool
CBD	Conservation of Biodiversity
COP	Conference of Parties
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
DNA	Deoxyribonucleic acid
FDACS	Florida Department of Agriculture and Consumer Services
FDEP	Florida Department of Environmental Protection
FLEPPC	Florida Exotic Plant Pest Council
FORIG	Forestry Research Institute of Ghana
GDP	Gross Domestic Product
GPS	Global Positioning System
IUCN	International Union for the Conservation of Nature
KNUST	Kwame Nkrumah University of Science and Technology
NISC	National Invasive Species Council
OTA	Office of Technology Assessment
TAG	Technical Advisory Group
UCC	University of Cape Coast
UF	University of Florida
UG	University of Ghana
US	United States
USA	United States of America
USBC	United States Bureau of Census

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ABSTRACT

Dioscorea bulbifera L. (aerial yam) belongs to the yam family (Dioscoreaceae) and produces numerous aerial tubers in the leaf axils. *Dioscorea bulbifera* is considered the most serious environmental threat. Currently, it is the widespread weed throughout many parts of the southern United States of America where it threatens the stability and biodiversity of native communities. Control interventions such as chemical and manual methods have been ineffective, expensive and laborious, hence the decision to explore for natural enemies to control the weed. A survey to establish the presence of *D. bulbifera* and its associated phytophagous insects was conducted in Ghana in 2004 and 2005. Field exclusion and laboratory experiments were also carried out to assess the abundance, diversity and herbivory of insects in 2006 and 2007. Rearing and host range studies were undertaken on potential candidates in 2007 and 2008. A total of 40 phytophagous species in 9 orders were encountered on the plant with 24 species attacking *D. bulbifera* in two feeding guilds in Ghana; foliage and bulbil feeders. The impact of foliage feeding varied from species to species. The mean percent defoliation of aerial yam over all plots was 30%. Coleopterous species fed on only foliage and nearly all the leaves showed damage from these species. *Anomala* sp. and *Adoretus* sp. were the most important Coleoptera (Scarabaeidae) found feeding on the plant. *Anomala* species exhibited a very narrow host range, attacking only *D. alata* together with *D. bulbifera*. Even though the damage it caused to aerial yam was relatively small, it appears a potential biological control agent. Lepidopterous species, mainly the Arctiid moths *Diacrisia* and *Estigmene* species attack aerial yam leaves and bulbils. They caused considerable damage to the bulbils the principal planting material. They however exhibited a wide host range attacking other *Diocorea* species. In view of the poor representation of *Diocorea* species of economic importance in the United States of America, the results of the study will have favorable implications for research programs aimed at searching for long-term intervention of the ecological risks posed by *D. bulbifera* in the US.

CHAPTER ONE

1.0 Introduction

Dioscorea bulbifera L. is commonly called aerial yam, air potato or bitter yam. A member of the yam family (Dioscoreaceae), aerial yam produces large numbers of aerial tubers, potato-like growths from the leaf axils. The genus *Dioscorea* (true yam) is economically important worldwide as a food crop particularly in West Africa where their edible underground tubers are important commodities. According to Morisawa (1999), two-thirds of worldwide yam is grown in West Africa. *Dioscorea* varieties such as *D. mexicana*, containing the steroid diosgenin, are principal material for the manufacture of birth-control pills (Edwards *et al*., 2002).

The origin of *D. bulbifera* is, however, uncertain. Some believe that the plant is native to both Asia and Africa. Yayock *et al*. (1988), for example, reported that it originated from tropical Asia and West Africa, while others believe that it is native to Asia and was subsequently introduced into Africa (Hammer, 1998). *Dioscorea bulbifera* has been widely distributed through human activities and has become naturalized in many tropical and sub-tropical regions of the world (Martin, 1974).

Dioscorea bulbifera is characterized by its aggressively high-climbing annual twining stems, large ovate leaves with prominent veins, and potato-like aerial tubers in the leaf axils (Morton 1974; Long and Lakela, 1976). Production of large numbers of aerial tubers allows for rapid proliferation and colonization. The plants grow rapidly in full sun and they can overgrow and kill native flora (Schultz, 1993). According to Morisawa (1999), vines grow as rapidly as 20 cm per day, quickly spiralling up to tree tops to form dense masses that shade out trees and may eventually kill them (Plate 1).

Aerial yam was introduced to the Americas from Africa during the slave trade (Coursey, 1967), and specifically into Florida in 1905 for scientific study (Morton, 1976) and now constitutes one of the most aggressive weeds ever introduced to Florida (Hammer, 1998).

According to Langeland and Burks (1998), it is found throughout the state of Florida. It can now be found throughout the state and also in Mississippi, Louisiana, Texas, Hawaii and Puerto Rico (Overholt *et al.*, 2006).

Plate 1.1. Air potato engulfing cabbage palm in Glades County, Florida, United States of America (USA). Courtesy Overholt *et al.*, 2006.

Dioscorea bulbifera is considered the most serious environmental threat, described as a category I weed by the Florida Exotic Plant Pest Council (FLEPPC, 2003), it was listed earlier on in 1999 as a noxious weed by the Florida Department of Agricultural and Consumer Services (FDACS). The plant is currently a widespread weed throughout many parts of the southern United States where it threatens the stability and biodiversity of native communities (Wheeler and Pemberton, 2004). Presently, aerial yam is well established in Florida and probably throughout the Gulf states (Raz, 2002) where it has the potential to severely disrupt entire ecosystems (Hammer, 1998). According to the National Invasive Species Council (2001), invasive plants are species that after they have been moved from their native habitat to a new location, spread on their own. Some invasive plants reach high densities and cause economic or environmental losses and may even cause harm to humans. Invasive non-native plants are serious threat to native species, communities, and ecosystems in many areas around the world. They can compete with and displace native plants, animals, and other organisms that depend on them, alter ecosystem functions and cycles significantly, hybridize with native species, and promote other invaders (Hammer, 1998). Once an invasive plant species becomes established it is not easily suppressed or eliminated as these species often possess characteristics that favour their population increase, such as early maturation, profuse reproduction by seeds and/or vegetative structures, long life of seeds in the soil, adaptation for spread, and production of biological toxins that suppress the growth of other plants. In addition, many invasive plants are free of attack in their invaded range by natural enemy insects or plant pathogens, allowing plant resources to be shifted from defense to growth and reproduction (Klein, 1998). Similarly, Hajek (2004) asserted that many invaders become pestiferous largely due to the fact that they are no longer associated with the natural enemies with which they coevolved.

Integrated invasive plant management relies on a combination of control technologies. These include biological, mechanical, chemical, and cultural applications. Before the mid-1950s, chemical and mechanical applications were the main tactics used to suppress invasive plants in the continental United States. In the 1940s, classical biological plant control efforts were initiated and significantly increased in the United States and since then, biological control has become the most widely used tactic for weed suppression (van Driesche, 2002). Classical biological control of weeds is an increasingly prevalent practice of controlling alien invasive plant species (Julien and Griffiths, 1998; McEvoy and Coombs, 1999). The use of carefully chosen natural enemies has become a major tool for the protection of natural ecosystems, biodiversity and agricultural and urban environments (van Driesche *et al*., 2008). Also according to Hoddle (2004), one powerful technology for invasive species management in sensitive habitats is biological control, the use of carefully selected upper-trophic-level organisms that utilize the exotic pest as a resource, thereby reducing it to less harmful densities. The most appropriate means of controlling such a situation, therefore, is the application of classical biological control since other interventions such as chemical and manual methods have been ineffective, expensive and laborious (Schultz, 1993). Classical biological control, the science of reconnecting invasive plants with the specialized natural enemies that often limit their density in their native ranges, involves surveys in the pest's origin to discover potential natural enemies (van Driesche, 2002). Effective biological control provides the only guaranteed long-term effective solution to problems of invasion (Delfosse, 2005). Similarly, Tu *et al*. (2001) asserted that biological control is often viewed as a progressive and environmentally friendly way to control pest organisms because it leaves behind no chemical residues that might have harmful impacts on humans or other organisms, and when successful it can provide permanent widespread control with a very favourable cost-benefit ratio. Biological control obviously relies on collaboration with countries where the weed species originates, and this often necessitates collaborative contracts in these countries.

A high level of host specificity in the introduced natural enemies is desirable and should be sought during foreign exploration (Nechols *et al*., 1992). At present, there is no standard protocol to refer to when compiling a species test list for assessment of a biological control agent's host range. According to Kuhlmann *et al*. (2007), two categories of data can help in estimating the host ranges of insects: (1) host-natural enemy associations as seen in published literature or in specially conducted surveys; and (2) laboratory testing in which candidate species are presented in cages to natural enemies, whose oviposition and immature development are then observed. Because insect faunas are often very large, study of host-natural enemy associations by field surveys are often important in choosing species for testing in the laboratory. In laboratory trials, patterns of oviposition, feeding, or development in arthropod hosts are typically assessed in small containers (Kuhlmann *et al*., 2007). Sands and Papacek (1993) reported that restricted space often leads to an inaccurate assessment of host specificity by disrupting the processes governing host recognition and acceptance. For example, parasitoids in small cages may oviposit on hosts that normally do not support development of the parasitoids, or parasitoids may oviposit on hosts that normally are not accepted in the field. The physiological host range measured in the laboratory and the realized host range in the field thus might differ. The first in the host specificity process according to De Nardo and Hopper (2004) involves the collation of all recorded information on field hosts of not only the candidate biological control agent, but also of closely related species of the target pest, in this case, *D. bulbifera*.

1.1 Justification for biocontrol of aerial yam

Dioscorea bulbifera is an aggressive exotic weedy invader of native vegetation over much of Florida; it prefers to grow in hardwood forest and invades thickets and tropical hammocks. (Schultz, 1993; Wunderlin, 1982). Aerial yam has been listed by the Florida Exotic Pest Plant Council as one of Florida's most invasive plant species since 1993 and was added to the Florida Noxious Weed List (5b-57.007 FDACS) by the Florida Department of Agriculture and Consumer Services in 1999 (FLEPPC, 2003).

Aerial yam requires active management to prevent its spread and suppression of natural plant communities. It is difficult to eradicate because of its underground tuber and its abundant bulbils (aerial tubers) (Morton, 1982) and according to Duxbury *et al*. (2003), management of this vine is challenging, largely because of the plant's ability to grow from bulbils. Repeated herbicide application and mechanical methods (i.e. hand-pulling) are the most common control methods (Gordon *et al.*, 1996). Current practices of manual and herbicide control are very labour intensive, expensive and inefficient (Schultz, 1993). As the aerial yam was introduced to Florida and without its natural control agent, there is the need to institute classical biological control to bring it under check. However, such research should be approached cautiously because of the presence of closely related Florida natives (*D. floridana*) and five other taxa in the genus (Schultz, 1993). The use of biocontrol to weaken or eliminate exotic plants is based on a general, but untested, assumption that invasive exotics are successful because they have escaped intense consumer pressure in their native habitat (Callaway *et al*., 1999). Schultz (1993) intimated that there are no approved biological control agents for *D. bulbifera* in the U.S. and hence the need for research `needed to improve control methods of this weed pest. Earlier investigations by scientists at the Crops Research Institute, Kumasi, Ghana revealed that some coleopterans inflict feeding damages on the leaves of the plant. According to Overholt *et al.* (2004), using the chloroplast DNA technology it has been determined that Florida air potato is most likely to be of African origin. West Africa is also regarded as one of the origins of aerial yam (Yayock *et al*., 1988; Hammer, 1998; Morisawa, 1999). Ayensu and Coursey (1972) have noted the presence of wild and cultivated types in various parts of Ghana. The wild types are usually found at the peripheries of forest (Florida Department of Environmental Protection (FDEP) Report by CRI, 2004). Interestingly, however, it does not pose any threat to the vegetation here in Ghana. Preliminary investigations have revealed that insects attack both the bulbils and the leaves (FDEP, 2004). The probability, therefore, of finding a suitable control agent clearly increases when searching in the target weed's native range. It therefore sounds reasonable to initiate efforts to explore for natural enemies since the above revelations clearly suggest that a probable natural enemy can be found in Ghana.

1.2 Main Objective of the Study

The purpose of this study was to identify a good arthropod candidate for the control of this noxious weed. To achieve the above, the study seeks to address the following specific objectives.

1.3 Specific Objectives:

1. To determine locations of aerial yam in Ghana and associated insect pests.
2. To measure the impact of insect herbivores on the plant's performance.
3. To study the biology of potential biocontrol candidates.
4. To determine host specificity of the insect herbivores selected.

It is believed that the study will identify suitable biological control agents for the invasive *D. bulbifera* in the United States of America.

CHAPTER TWO

2.0 Literature Review

Exotic plants threaten the integrity of agricultural and natural systems throughout the world. Many invasive species are not dominant competitors in their natural systems, but competitively suppress their new neighbours. One leading theory for the exceptional success of invasive plants is that they have escaped the natural enemies that hold them in check, freeing them to utilize their full competitive potential (Wiedenmann, 2000). This perspective provides the theoretical framework for the widespread practice of introducing natural enemies as biological control agents which are exotic, to suppress invasive plants.

2.1 History of Biological Control of Weeds

The American, Asa Fitch, was the first to suggest biological control of weeds around 1855, when he observed that a European weed in New York pastures had no American insects feeding on it (Fitch, 1954). He suggested that importation of European insects feeding on this weed might solve the problem. The first practical attempt dates from 1863, when *Dactylopius ceylonicus* L. was distributed for cactus control in southern India after they had been observed to decimate cultivated plantings of the prickly pear cactus, *Opuntia vulgaris* (L), in northern India (Goeden, 1978). In 1865, the first successful international importation of insects for weed control took place, when this same insect was transferred from India to Sri Lanka, where in a few years widespread populations of the same cactus, *O. vulgaris* (L), was effectively controlled (Goeden, 1978) .

During the 19th century, taxonomy rapidly developed and many biological studies of natural enemies were made. Practical ideas and tests about application of biological control gradually advanced. Erasmus Darwin, the grandfather of Charles Darwin published *Phytologia*, a book on agriculture and gardening in 1800 and he stressed the role of natural enemies in reducing pests (Darwin, 1800).

By 1850, biological control gained full attention in the United States, where imported pests were taking a large toll of both domestic imported crops. Entomologists, such as Asa Fitch, C. V. Riley and Benjamin D. Walsh suggested the importation of natural enemies from their homeland. It was C. V. Riley who organized the first intra-state parasitoid transportation when he sent parasitoids of the plum curculio, *Conotrachelus nenuphar* (Herbst), to different localities in Missouri. Riley was also the first to propose conservation of parasitoids of the rascal leafcrumpler of fruit trees, *Acrobasis indigenella* (Zeller), by collecting larvae in their cases in mid-winter and then placing them away from the tree far enough that the larvae could not reach the trees anymore, but the parasitoids emerging from parasitized individuals in springtime could easily do so. Also, in 1873, Riley (1893) stimulated the first international transfer of an arthropod predator by sending the predatory mite *Tyroglyphus phylloxerae* (Riley) to France for control of the grape *Daktulosphaira vitifolii* (Fitch). It established but did not result in effective control. The first international shipment of a predatory insect took place in 1874, when aphid predators, among which *Coccinella undecimpunctata* (Linn.), were shipped from England to New Zealand and became established. The first international transfer of parasitic insects was *Trichogramma evanescens* (Westwood) from the United States to Canada in 1882. The first intercontinental parasitoid shipment took place in 1883, when Riley organized the shipment of *Apanteles glomeratus* (Linn.) from England to the United States for control of cabbage white butterflies. It was just another six years before the spectacular success with *Rodolia cardinalis* (Mulsant) took place, again under the direction of Riley to control *Icerya purchasi* Maskel (Clausen, 1978). The first weed biological control program was established in Hawaii in 1902 for control of lantana, *Lantana camara* L. (Goeden, 1978).

2.2 Invasive Species

There are numerous definitions for invasive species. Any species occurring outside of its natural range, in a location that it could not get to without direct or indirect assistance by humans is considered alien (RNT Consulting Inc., 2002). According to the National Invasive Species Council (2001), invasive species are species that after they have been moved from their native habitat to a new location, spread on their own. However, an executive order 13112 issued in 1999 from the White House defines an invasive species as a species not native to the region or area whose introduction (by humans) causes or is likely to cause harm to the economy or the environment, or harms animal or human health (National Invasive Species Council, 2004). Coblentz (2002) defines an invasive species as one that has extensive recruitment into a population without human intervention. Alien species are considered to be invasive when they can become established in natural or semi-natural ecosystems or habitat, are agents of change, and threaten native biological diversity (International Union for the Conservation of Nature [IUCN] (1998). According to the Convention on Biodiversity (CBD), an "invasive alien species refers to an alien species whose introduction and spread threatens ecosystems, habitats or species with socio-cultural, economic and/or environmental harm, and/or harm to human health" (COP, 2002). Stirton (1979) defines plant invaders as 'alien plants that invade and oust native vegetation', while Mack (1989) classifies any new entrant to a territory as an invader. Joshi (2001) and Coblentz (2002) assert that invaders have also been referred to as exotics, colonisers, xenophytes, neophytes or simply weeds whereas invasive species can, therefore, be said to be exotic, often colonising organisms that exhibit weedy behaviour. This behaviour seldom manifests itself in their native environment, where they exist in competition or association with a complex of closely associated species (McFadyen, 1991). Natural movements of species into most areas are uncommon. Most exotics arrive in association with human activity such as transport, agriculture, tourism, trade etc (di Castri, 1989; Coblentz, 1990).

Since the UN Summit in Rio de Janeiro in 1992, invasive species have come to be regarded as one of the main reasons for the loss of biodiversity (Keane and Crawley, 2002). Invasive species are now considered a major problem worldwide due to the increasing human population, frequently moving organisms around the globe and thereby altering ecosystems at an increasing rate. Unfortunately, by all predictions, accidental introductions of invasive species will only continue with the increased global movement of humans and materials (Hajek, 2004). The good news according to Tu *et al*. (2001) is that many plant invasions can be reversed, halted or slowed, and in certain situations, even badly infested areas can be restored to healthy systems dominated by native species. In most instances this requires taking action to control and manage those invasive plants. As a result of human activities the spread of non-indigenous or "alien" organisms has today reached a scale that is well beyond what could be expected from natural processes. Some of these species have extensively colonised their new homes, causing great economic and ecological losses.

Factors affecting invasiveness have been invoked to account for differences in invasibility: evolutionary history, community structure, propagule pressure, disturbance and abiotic stress (Alpert *et al*., 2000). According to Schaffner (2005), there is a whole series of characteristics that correlate with successful establishment and subsequent build-up and/or spread of invasive species. Thus, lasting establishment is favoured by characteristics that enable a population to reach such a density that the danger of extinction is minimised. Among other things these characteristics include the reproductive system (e.g. asexual reproduction), the number of offspring and the length of the juvenile period. Among pests in agriculture, approximately 20–40% has been introduced from elsewhere. Most are accidental introductions, although a small percentage of these were purposeful introductions such as crop plants and honeybees. Some were purposeful introductions with unexpected side effects. For example, the weed 'kudzu' was introduced to the southeastern USA to control erosion and has since spread rampantly through most of the southeast, becoming a problematic weed (Hajek, 2004). According to Schultz (1993), samples of air potato (*Dioscorea bulbifera*) were sent to the horticulturalist Henry Nehrling for experimental cultivation in Gotha, Florida by the U.S. Bureau of Plant Industry in 1905 and have since become invasive, disturbing the state's ecology.

Impacts of invasive species on ecosystems are still a source of debate. di Castri, (1989) asserts that the central European flora have undergone an enrichment of diversity over historical time as a result of human induced plant invasions and McNeely (2000) cites Lake Nakuru's transformation from an ecosystem of very low diversity to one of much higher diversity by the introduction of *Tilapia grahami* (Boulenger). Moreover, many scientists have argued that alteration of relative abundance of native species cannot always be taken as deleterious. Nevertheless, numerous studies indicate that invasions, by altering biogeochemical cycles, hydrological cycles, fire regimes and the balance of competition, predation, parasitism and disease, by altering landscapes and entire ecosystems have resulted in thousands of extinctions of endemic species in the past few hundred years (Drake *et al*., 1989; Mooney and Drake, 1987). McNeely (2000) suggests that globally, in cases where the cause of species extinction is identifiable, biological invasions are the leading cause and that almost 20% of vertebrates considered to be in danger of extinction are threatened by invasive species. The overall picture, then, is one of global movement of species with unpredictable long-term effects.

According to Luwum (2003) invasions follow three phases - arrival, establishment and spread. Knowledge of the processes that take place within each stage, in terms of plant ecology and human activities is therefore of fundamental importance in management of plant invasions. Most exotics, once established are permanent. Eradication is possible in a few instances, but only at great expense and effort. Most others require control, which may be said to be successful when the plant no longer exceeds a threshold level determined by the objectives of the managers. Others may not be controllable by any practical means (Coblentz, 2002; Coblentz, 1990; Groves, 1989). Groves (1989) advocated that the promotion of policies aimed at preventing arrival of potentially troublesome species is one method used in controlling invasive species. He added that an allied method is the enactment of laws declaring certain plants 'noxious', stipulating methods for their management and giving management authorities legal powers to control them once they have 'arrived', established and spread. The Weed Science Society of America recognizes about 2,100 invasive plant species (i.e., noxious or weedy plants) in the United States and Canada. Currently, 94 kinds of invasive plant species are officially recognized as Federal Noxious Weeds and many more species are designated on State noxious weed lists. In the United States, invasive plant species comprise from 8 to 47 percent of the total flora of most States. In 1994, the economic impact of weeds on the United States economy was estimated to be $20 billion annually (Westbrooks, 1998).

2.3 Impact of Invasive Species

Invasive species threaten natural habitats worldwide, and active human management is required to prevent invasion, contain spread, or remediate ecosystems following habitat degradation. The economic value of an ecosystem or any segment thereof is difficult to calculate and very little agreement exists on how it should be done. Some aspects such as the decline in aesthetic and recreational values of property can be evaluated (RNT Consulting Inc., 2002). Others such as the loss of species are less easy to define. In principle, ecosystems have no “replacement value”. Thus, what is important is to assess the economic opportunity costs (e.g. livelihood, productivity and trade) to human society or “economies” from a spectrum of invasive species impacts that would disrupt existing economies while at the same time potentially destroy or disrupt the functioning of otherwise natural systems. The economic value one would put on biodiversity depends on the value framework of the human community towards the quality of life and economic well being on the ecosystem in question. There are approximately 50,000 foreign species and the number is increasing (Pimentel *et al*., 2005). About 42% of the species on the threatened or endangered species lists are at risk primarily because of alien invasive species (Pimentel *et al*., 2005). Approximately $4 billion in herbicides are applied to U.S. crops of which about 75% ($3 billion) is used for control of alien invasive weeds (Pimentel, 1997). Some invasive plants reach high densities and cause economic or environmental harm or harm to humans. Arial yam can quickly engulf native vegetation in natural areas, climbing high into mature tree canopies (Plate 1). It produces large numbers of bulbils, which facilitate its spread and make it extremely difficult to eliminate because new plants sprout from even very small bulbils. It invades a variety of habitats including pinelands and hammocks of natural areas (Langeland and Burks, 1998). In peninsular Florida, aerial yam is an aggressive weedy invader in many different habitats including thickets, disturbed areas, fence rows and hardwood hammocks (Wunderlin, 1982).

According to Westbrooks (1998), factors that can enhance the growth of invasive plants include overgrazing, land use changes, fertilization, and use of agricultural chemicals. Other human activities that can result in disturbed environments and encourage the establishment of invasive plant species include farming, highway and utility rights-of-way, clearing land for homes and recreation areas such as golf courses, as well as constructing ponds, reservoirs, and lakes. Consequently, the costs of controlling agricultural weeds are passed on to the consumers through higher prices and reduced quality of food according to a report by RNT Consulting Inc. (2002).

2.3.1 Environmental impact of invasive plant species

The invasion of natural ecosystems by alien plants is a serious environmental problem that threatens the sustainable use of benefits derived from such ecosystems (van Wilgen *et al*., 2001). According to Simberloff (1996) they can devastate farms and forests, impede waterways, foul lakes and ponds, affect human health, and invade natural areas and replace native species. Costs to natural systems, although not easily translated into dollars, are staggering and diverse. Perhaps, the greatest impacts are caused by plant species that come to dominate entire ecosystems. Melaleuca, for example, which is increasing its range in south Florida by some 35 acres each year, replaces cypress and other native plants and provides poorer habitat for numerous animals (Mazzotti *et al*., 1981).

In South Africa, for example, ten million hectares of land has been invaded by 180 alien species with undoubtedly significant impacts (van Wilgen *et al*., 2001). The development of an understanding of environmental impacts of invasive alien plants, and their consequences, would be extremely useful for the quantification of economic impacts. Unfortunately, no standard system exists for the objective quantification of the many and varied environmental impacts of invasive alien plants worldwide (Parker *et al*., 1999). *Hydrilla verticillata* (L. Fils) (hydrilla) is an aquatic weed that has spread throughout the country's waterways, clogging irrigation and drainage canals, degrading water quality, reducing productivity of recreational fisheries, and impeding navigation particularly in Florida (OTA, 1993). Invasive species have potential health market impacts and according to OTA (1993), *Schinus terebinthifolius* Raddi (Brazilian pepper tree), which has significantly invaded Florida, causes allergic reaction in many people, including respiratory difficulties and contact dermatitis. *Lythrum salicaria* L. (purple loosestrife) costs $45 million annually in forage losses and control costs. *Lythrum salicaria* is spreading at a rate of 115,000 ha/yr and has changed the basic structure of most of the wetlands it has invaded, resulting in biomass reduction of 44 native plants and the reduction of native wildlife that depend on the native plants (Pimentel *et al*., 1999).

2.3.2 Ecological impact of invasive plant species

Introduced invasive plants can also harm native ones by producing and releasing chemicals. The African crystalline ice plant, for example, has devastated native coastal vegetation in California. The ice plant is an annual that accumulates salt, which leaches from its leaves when the plant dies at the end of the season. The salt, not surprisingly, suppresses the growth and germination of native plants in these well-used but fragile habitats (Simberloff, 1996). Based on the estimate that about 73% of the weeds are alien (Pimentel, 1993), Pimentel (1997) noted that alien invasive weeds are more serious pests than native weeds. There are about 5800 vascular plant species in Canada. Of these plants, 25% are alien species that were either deliberately or inadvertently introduced. Fewer than 10% of these aliens are considered to be serious pests or invasive in natural habitats. However, this relatively small number has had a substantial ecological impact (Haber, 2002). Trammel and Butler (1995) reported that some introduced weeds such as leafy spurge, *Euphoria esula* (L) are toxic to cattle and wild ungulates. In addition, several alien thistles have replaced desirable native plant species in pastures, rangelands, and forests, thus reducing pasture quality for grazing cattle (Dewey, 1991). Non-indigenous plants are often also hosts for pathogens or pests that damage desirable plant species. *Berberis vulgaris* (barberry) hosts the wheat rust fungus which reduces wheat production. *Agropyron dertorum* L. (crested wheatgrass) carries the Russian wheat aphid which is an insect pest on wheat (OTA 1993). OTA (1993) also hinted that *verticillata* may provide habitat for disease-carrying mosquitoes.

2.3.3 Economic impact of invasive plant species

Invasive species can have major economic impacts which range from the loss of economically valuable species to the costs of controlling or managing infestations on public and private lands. The economic impacts of invasive plants are demonstrated best by their effects on agricultural production and cost of activities to undertake control, eradicate or remediate their damages. Biodiversity loss, ecosystem degradation, and aesthetic changes are important effects of invasive species and these effects are difficult to quantify in monetary terms. In the history of the United States, approximately 50,000 alien-invasive (non-native) species are estimated to have been introduced into the country (Pimentel *et al*., 2005). Other exotic species have been used for landscape restoration, biological pest control, sport, pets, and food processing. *Acacia* species in South Africa generate income through use as timber and firewood, while secondary industries involving, for example the employment of people on eradication programmes pose a considerable local income source (Turpie and Heydenrych, 2000; Wit *et al*., 2001). The cost of bringing invasive alien trees and shrubs under control in South Africa is estimated to be around US$ 1.2 billion, or roughly US$ 60 million per year for the estimated 20 years that it will take to deal with the problem (Versfeld *et al*., 1998)

Some non-indigenous species, however, have caused major economic losses in agriculture, forestry, and several other segments of the United Sates economy, in addition to harming the environment. Considerable crop production losses due to non-native weeds have a direct economic impact, such as reduced income in the agricultural sector (Tisdell, 1990). The loss of non-native species may also result in decreasing water supply and biodiversity, and implies indirect economic impacts (Wit *et al*., 2001). One recent study estimates that the total costs of invasive species in the United States amount to about $120 billion each year (Pimentel *et al*., 2005). Globally, invasive species are the second greatest threat to global biodiversity next to habitat; cost of damage caused by invasive species has been estimated to be £1.5 trillion per year which is close to 5% of global GDP in environmental and economic damage (Cabi, 2009).

In other countries, the costs caused by biological invasions are enormous. In New Zealand, for instance, the costs of invasive species' impacts are estimated to amount to about 1% of GDP (Bertram, 1999). According to van Wilgen *et al.* (2001), the economic consequences of invasions are huge in South Africa where invasions have reduced the value of fynbos (a Mediterranean-type shrubland) ecosystems by over US$ 11.75 billion. Similarly, Dawson (2002) quotes an estimate of the damage resulting from past introductions of harmful invasive plant pests on agricultural crops and forestry as $7.5 billion annually in Canada. Ranchers in the United Sates spend about $5 billion each year to control invasive alien weeds in pastures and rangelands, yet these weeds continue to spread (Babbitt, 1998). Templeton *et al*. (1998) estimated that $500 million is spent on residential exotic weed control and an additional $1 billion is invested in alien invasive weed control on golf courses. In the United Sates agriculture, weeds cause a reduction of 12% in crop yields. In economic terms according to USBC (2001), this represents about $33 billion in crop production loss annually, based on the crop potential value of all United Sates crops of more than $267 billion/year. The OTA (1993) estimated that $100 million was spent annually to control aquatic weeds. In other regions of the world, as many as 80% of the endangered species are threatened and at risk due to the pressures of non-native species (Armstrong, 1995). In Florida, of the approximately 25,000 alien plant species imported mainly as ornamentals for cultivation, more than 900 have escaped and become established in surrounding natural ecosystems (Frank and McCoy, 1995; Frank *et al*., 1997; Simberloff *et al*., 1997). According to Dowell and Krass (1992), more than 3000 plant species have been introduced into California.

2.4 Management of Invasive Species

Invasive species threaten natural habitats worldwide, and active human management is required to prevent invasion, contain spread, or remediate ecosystems following habitat degradation (Hoddle, 2004). The price society pays for invasive species is reflected not only in significant economic damage but also in high levels of environmental degradation, loss of recreational opportunities, and harm to animal, plant, and human health. Invasive species whether plants, animals, pathogens or parasites – are estimated to cost the U.S. economy of over $100 billion per year (Pimentel *et al*., 2005). They cause extensive environmental harm and are the second leading cause (after habitat loss) of species being listed as threatened or endangered and infest more than 100 million acres across the United States (National Invasive Species Council, 2006). Since 2000, the US Bureau of Land Management has taken inventory of over 567 million acres for invasive plants, treated invasive plants on 1.4 million acres while partnering to control invasive species with over $7.5 million (National Invasive Species Council, 2005). Methods of managing invasive plants have evolved with land use systems over time. Control methods used in agricultural systems usually differ from those used in natural systems because of different objectives. The former usually aims to simplify the system, while the latter aims to maintain diversity in the longer term (Groves, 1989). Mechanical removal of plants is widely used in both systems. It involves cutting, planned burning, ploughing or hand pulling. These methods are usually used in conjunction with other methods and have been known to have some success in controlling invasive species. However, except for fire, they are usually labour intensive and may be expensive for extensive and dense infestations (Groves, 1989; Zachariades *et al*., 1999). Treatments must typically be administered several times to prevent the weeds from re-establishing. One method used in controlling invasive species is the promotion of policies aimed at preventing arrival of potentially troublesome species. An allied method is the enactment of laws declaring certain plants 'noxious', stipulating methods for their management and giving management authorities legal powers to control them once they have 'arrived', established and spread (Groves, 1989). The spread of invasive exotic vegetation according to Langeland (1990) can be significantly reduced by public education. Individual methods of control are rarely effective in controlling invasive plants. The trend is, therefore, to adopt a combination of methods, usually chosen with the ecology of the plant as the major determinant. Other considerations include cost, environmental and social implications (Langeland, 1990).

2.4.1 Physical control

According to Tu *et al.* (2001), manual and mechanical techniques such as pulling, cutting, and otherwise damaging plants, may be used to control some invasive plants, particularly if the population is relatively small and/or where a large pool of volunteer labour is available. These techniques can be extremely specific, minimizing damage to desirable plants but they are generally labour and time intensive. Soil solarization is the technique of placing a cover (usually black or clear plastic) over the soil surface to trap solar radiation and cause an increase in soil temperatures to levels that kill plants. In addition, when black plastic or other opaque materials are used, sunlight is blocked which can kill existing plants (Katan *et al*., 1987). DeVay (1990) later noted that soil solarization is most effective during the summer months, and may be less effective in cooler climates. The higher the temperature, the more quickly a kill is achieved. Solarization is effective only if done in wet soil. Grinstein and Hetzroni (1991) also indicated that where soils are typically dry, they must first be irrigated until soil from the surface to 50 to 60 cm deep is at field capacity. Fire, according to Tu *et al*. (2001), can also sharply reduce the abundance of some species but the most effective fires for controlling invasive plant species are typically those administered just before flower or seed set, or at the young seedling/sapling stage. In contrast, however, Milberg and Lamont (1995) stated that in extensively disturbed areas of southwest Australia, fire actually enhanced the invasion of weeds along roadsides, and resulted in an overall decrease in the abundance of native species. According to Wiebe and Obrycki (2001), surface fire tends to consume dried aerial yam vines but does not generate sufficient heat to destroy the bulbils buried underground.

2.4.2 Chemical control

Chemical control has also been widely used with success, and can be cost effective and quick particularly in agricultural lands. However, specialized equipment and training of operators is needed. Weather and plant status can affect results of chemical treatment. In addition, aerial and foliar application may affect the environment. High costs also significantly reduce the suitability of this method. Chemical control is also rarely effective in the long term (Erasmus, 1988; Groves, 1989; Moore, 2002). Glyphosate, a nonselective herbicide which kills broadleaf plants and grasses, is effective at relatively low concentrations and has a low potential for bioaccumulation but hardly affect the numerous bulbils produced by the plant (Mullin, 1998).

2.4.3 Biological control

Biologically based control methods can provide cost-effective, sustainable means of limiting the adverse effects of invasive plants over extensive rangeland and natural areas. According to Julien and Griffiths (1998), biological control is seen as a long term, cost effective and environmentally friendly method in controlling invasive species, and has had some impressive successes in the long term. van Wilgen *et al*. (2001) emphasized that biological control of invasive alien plant species offers one of the best, and most cost-effective, interventions for addressing the problem. Similarly, Schultz (1993) noted that the most appropriate means of controlling the invasive aerial yam is classical biological control since other interventions such as chemical and manual methods have been found to be ineffective, expensive and laborious. When effective, the method provides low cost control with minimal disturbance, but it has a low level of predictability. Some of 'classical' biocontrol's greatest strengths are that once an agent is established, it will persist 'forever' and it may spread on its own to cover most or all of the area where the pest is present, generally with little or no additional cost. On the other hand, these strengths can become great liabilities if the agent also begins to attack desirable species (Pemberton, 1985; McEvoy and Coombs, 2000). According to Richardson *et al*. (1997), South Africa has been very successful in finding effective biological control solutions to many invasive weed problems.

Successful use of biological control requires a greater understanding of the biology of both the pest and its enemies and often, the results of using biological control are not as dramatic or quick as the results of pesticide use (Orr *et al*., 1997). The use of biological control agents have been highly successful in some cases, reducing the spread of invasive plants (Huffaker and Kennett, 1959; Cullen *et al*., 1973; McEvoy *et al*., 1991). However, biocontrol agents sometimes attack nontarget native species, compete with native species, and have unwanted community and ecosystem effects (Howarth, 1991; Simberloff and Stiling, 1996; Louda *et al*., 1997). Ecologists have also expressed alarm about the widespread use of biological control as a means of suppressing invasive weeds, because some agents may exert indirect effects that are not yet understood (Howarth, 1991). The main disadvantage of biological weed control, according to Johnson (2005), is that it often takes many years for the populations of the introduced agents to increase to levels that permanently decrease the pest plant populations.

CHAPTER THREE

3.0 Materials and Methods

3.1 Exploratory survey to determine locations of aerial yam and associated pests

Exploratory surveys were conducted in areas where aerial yams are cultivated in Ghana from May 2004 to August 2004. Wild types were also sampled along the major roads, specifically at the fringes of secondary forests. Information was sought on types or varieties grown and whether there were insect herbivores associated with the plant. The survey covered five regions of the country comprising the Ashanti, Brong Ahafo, Eastern, Central, and Upper West regions. Selected sites were based on herbarium records from the University of Ghana (UG), University of Cape Coast (UCC) and the Forestry Research Institute of Ghana (FORIG). In each selected region, twenty communities were explored both day and night and five farmers were interviewed per community by the administration of a questionnaire (Appendix 1). Additionally, the exact coordinates of every aerial yam plant sampled at each location was recorded using the etrex Garmin global positioning system (GPS). Data generated from the GPS was transferred onto a computer. A shape file was later created and with the help of the computer software known as the Almanac Characterization Tool (ACT), the various locations were captured on the map of Ghana as points (Figure 1).

A total of 500 respondents were involved in the study. Farmers were interviewed on their knowledge about cultivated and wild forms as well as pest problems associated with the plant. Insects found on the vegetative parts and bulbils were collected for further laboratory studies.

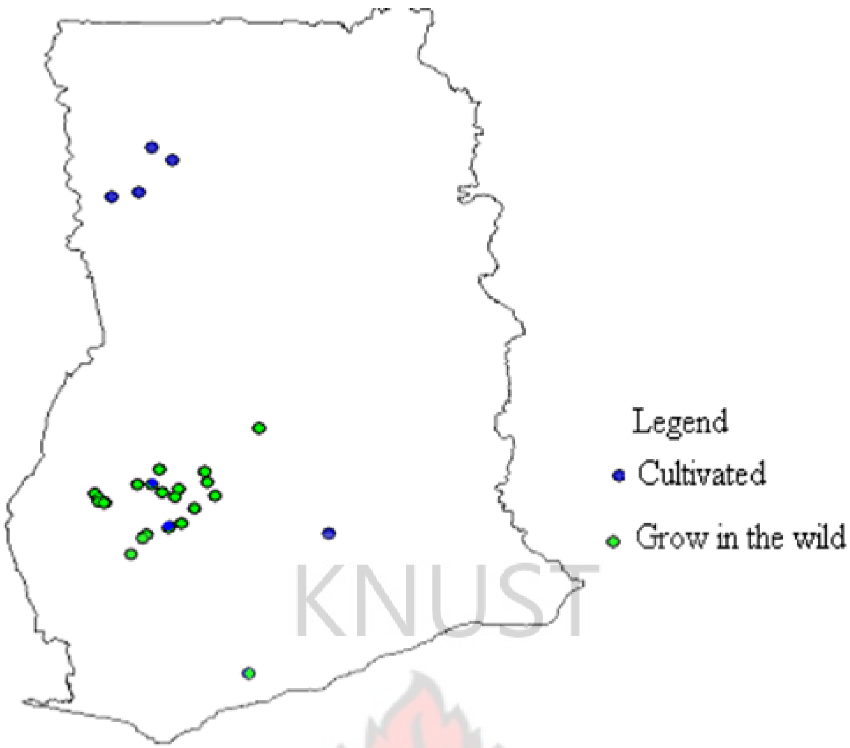

Figure 3.1 Distribution of *Dioscorea bulbifera* sampled areas in Ghana

3.2 Exclusion experiment to measure impact of herbivores on plant performance

This experiment began in May 2005 and ended in October 2005 at the experimental fields of the Crops Research Institute (CRI) at Kwadaso, Kumasi (Lat. 6º 42N; Long. 1º 40W; 262m above sea level). It was repeated from March 2006 to October 2006. The trial was set up with five accessions of cultivated types of aerial yams. Bulbils were collected from different locations and from some farmers during the survey. The experimental design was a two factor - factorial, with insecticide protection as Factor A and accession of aerial yam as factor B in three replications. Bulbils of similar sizes were planted individually in mounds of 50 cm in diameter and 40 cm high. Stakes were provided to direct and facilitate growth of vines in slanting fashion (Plate 2). Collection of agronomic data and chemical application started at 50% sprouting of bulbils. This continued on a weekly basis until harvest. No chemical was applied to the unprotected plots whilst a foliar insecticide Cymethoate Super EC 25 (combination of 36 g Cypermethrin and 400 g Dimethoate per litre) was applied weekly at a rate of 4 ml per litre of water using a knapsack sprayer to exclude herbivores from plants in the protected plots. On each Dioscorea accession, data was taken weekly on five plants in systematic sampling fashion until plant senesced (eight months). On each sampled plant, all leaves were counted as well as damaged ones. Vine length was measured from 10 cm above the mound with twine wrapped along the vine which was then measured with a measuring tape. The stem diameter was also measured at the 10 cm mark above the mound with veneer callipers. Percent defoliation was scored with a scoring scale (Appendix 2) at the upper, middle and lower portions of each plant. Number of bulbil damage was also recorded.

Plant biomass (dry weight of vines) was assessed at harvest. Performance of the plants under unprotected (control) and protected treatments were then compared.

Plate 3.1a. Field for insecticide exclusion studies at CRI, Kwadaso planted on 23rd March 2006.
Plate 3.1b. (Close up photograph) Slanted staking for easy measurement of vine length

3.3 Catalogue of insect herbivores

In all control programmes, an accurate identification of the pest must first be made in order to determine how to manage it. Therefore all invertebrate herbivores that visited the plants were documented and collected for identification and further investigation. Collection of the herbivores associated with the aerial yams started in 2005 and continued through the growing season in 2006. A rechargeable lamp was used to monitor insects at night and any insects found were collected with a pair of forceps or a camel hair brush into a one litre Kilner jar. Very small insects were collected into 30 ml glass vials containing 70% ethanol. Collection was made twice a week during the day and at night throughout the season i.e. from sprouting until the plants senesced. Known insect species were documented whilst unknown species were sent to the laboratory and preserved for identification later. Voucher specimens of arthropods from samples collected were curated according to standard procedures (Tripplehorn and Johnson, 2005). Specimens were identified by comparing species collected from the field with specimens in the insect museum of CRI to their respective family and feeding guilds were also determined. Voucher specimens of insects that could not be identified were sent to Professor W. A. Overholt of the University of Florida for identification.

3.4 Rearing of collected moth larvae (Lepidoptera: Arctiidae)

In August 2006, larvae found feeding on leaves and bulbils in the field were collected into Kilner jars and sent to the laboratory for rearing to adults. The larvae were regularly fed with detached young aerial yam leaves and were transferred to new one litre Kilner jars with fresh leaves every other day to avoid contamination with their faecal matter. The pupae in their cocoons were sexed based on the presence or absence of a suture on the eighth abdominal segment (Butt and Cantu, 1962; Genc, 2005). The sexed pupae were then transferred to separate one litre Kilner jars in pairs until emergence. The emerged adults which were in pairs (based on the previous sexing in the pupal stage) were allowed to mate and after oviposition the eggs were incubated in similar Kilner jars. Upon hatching, the larvae were transferred to net cages (30 x 40 x 50 cm) containing potted aerial yam plants. The adults that emerged were sent to University of Florida, USA, for identification.

3.5 Host specificity tests

3.5.1 No-Choice laboratory studies of *Estigmene* and *Diacrisia* species (Lepidoptera: Arctiidae) on the different *Dioscorea* species

The study was conducted to determine the host range of the larvae of the two herbivores in culture that were dominant and voracious feeders encountered. To determine their host range and pest status on the different *Dioscorea* species, newly hatched larvae of *Diacrisia* and *Estigmene* species were transferred to 9-cm plastic petri dishes individually using a small camel hair brush and supplied in turns with one detached young leaf of *D. bulbifera*, 'Akaba' and 'Matches' (*D. alata*), 'Nkanfo' (*D. dumenterum*), 'Pona', 'Lareboko', 'Dente' and 'Muchuumuduu' (*D. rotundata*). Thus leaves were changed daily and moist cotton wool was placed in each Petri dish every other day to maintain a moist environment in the Petri dishes. The larvae were monitored daily for feeding and development. There were four replicates of five petri dishes for each host plant with 1 larva per dish. The larvae were placed directly on the leaves, and leaves were subsequently replaced as needed. Mortality and developmental period from hatching to adult emergence were recorded.

3.5.2 Host specificity of *Anomala* species (Coleoptera: Scarabaeidae) on the different *Dioscorea* species

Adults of *Anomala* sp. (Coleoptera: Scarabaeidae) mostly found feeding on the leaves of *D. bulbifera* and *D. alata* in the night were collected from the field and kept in one litre kilner jars in the laboratory. This study was to determine their host preference among the different *Dioscorea* species used in the laboratory. The experiment was set up in three replications with 10 adults per host plant. Each adult insect was kept in one litre kilner jar with a fresh leaf of the different *Dioscorea* species and monitored daily for seven days. The number of leaves fed on (damaged) was recorded to determine their host preference and specificity status.

3.5.3 Field studies of preference and damage severity of *Anomala* sp. on eight *Dioscorea* spp.

All the *Dioscorea* species used for the study were planted on a separate block and monitored routinely twice a week to assess preference and damage capability of *Anomala* species. Number of insects and number of damaged leaves were recorded both at night and during the day. Data was taken throughout the growing season. To determine host preference in the field vines of *D. bulbifera, D. alata* and *D. rotundata* were allowed to climb a single stake and monitored.

3.6 Biology of *Estigmene sp and Diacrisia sp* (Lepidoptera: Arctiidae)

Larvae of *Estigmene* sp. *and Diacrisia* sp. (Lepidoptera: Arctiidae) were collected from an aerial yam field established at the Kwadaso station of the CRI in May 2008. The larvae were initially kept in one litre Kilner jars and fed with detached leaves and bulbils of aerial yam just as described in section 3.4 above until the third generation and used for the biological studies in November 2008. The idea was to attain a stable and adequate numbers for the biological study. The biological studies of the two insect species were carried out on aerial yam and seven other *Dioscorea* species, namely 'Akaba', 'Matches', 'Nkanfo', 'Pona', 'Lareboko', 'Dente' and 'Muchuumuduu'. All the experiments were conducted in the entomology laboratory of CRI where the temperature ranged from 22ºC to 26ºC and relative humidity ranged from 88% to 92%. The biological studies were conducted in three replications with the *Dioscorea* species as treatments.

3.6.1 Duration of developmental stages

To determine development time of the different life stages, a day old larvae were transferred to 9-cm plastic Petri dishes individually using a small camel hair brush. Each Petri dish contained one larva which was supplied with a freshly detached young leaf of the different *Dioscorea* species. A moist piece of cotton wool was replaced in each Petri dish every other day and leaves in the Petri dishes were also changed daily. Monitoring of growth stages was done daily, for presence of head capsules (which indicated larval moulting) pupation, and adult emergence. Temperature and relative humidity in the laboratory were recorded every hour using a Thermohygrograph.

3.6.2 Fecundity and longevity

To determine fecundity and longevity, five males and five females of each of *Diacrisia* and *Estigmene* species which emerged from each *Dioscorea* species used were randomly selected upon emergence (Wagner, 2005) and paired in small kilner jars (500 ml) fitted with net lids. Groups of females and males were also separately kept in kilner jars. Adults were provided with a feeding solution of one part sugar to two parts of water (Wagner, 2005), offered in a tiny ball of cotton wool which was changed every day. A sleeve made of plain paper (A4 sheet) was provided for each female as an oviposition site. The number of eggs laid by each female moth was recorded daily. Mortality of male and female moths was also recorded. Longevity was measured by recording the duration of adult period (Carey, 2001).

3.7 Data Analysis

Data from the exclusion experiment, biological studies of lepidopterous insects and host preference tests of the scarabs were analyzed using analysis of variance (ANOVA), SAS Institute (2007) computer software, followed by Student Newman Keul's (SNK) test for mean separation.

4.0 Results

4.1 Exploratory survey to determine locations of aerial yam and associated pests

The survey was conducted in the Ashanti, Brong Ahafo, Central, Eastern and Upper West regions. In the Central region, both wild and cultivated types of aerial yams were found but it was only in the Kakum forest that some insects were collected on the plant in the night. The coleopteran species collected from the Kakum forest did not feed on the detached leaves of aerial yam and other dioscorea species provided them until the insects died. The insects collected could probably be transient or were not pests of the plants tested. Other communities in the Central region visited are shown in Table 1.

In the Eastern region, efforts were concentrated around Bunso, where several cultivated types were encountered. At Bunso, the beetles *Anomala* sp. and *Adoretus* sp. which were also found at Kwadaso were observed feeding on the leaves of the plant as well as leaves of *D. alata*.

In the Ashanti and Brong Ahafo regions, both wild and cultivated types were present and were intercropped with cassava and plantain. The wild types were located along the fringes of the secondary forests. No insects were, however, found feeding or associated with both the wild and cultivated plants.

In the Upper West region, only cultivated types were encountered. The plant was also popular with the farmers since most of them emphasized that the crop was maintained to fight hunger during the lean seasons. No insect was found feeding on the plants during the survey in the Upper West region even though some plants showed damaged symptoms (perforated leaves and partly eaten bulbils).

Out of the 500 farmers interviewed only 10% were ignorant about the plant. About 60% of those who knew the plant admitted seeing damage holes on the leaves but could not attribute the damage to any animal. A few farmers (6%) in the Central and Ashanti regions admitted seeing rodents feeding on the bulbils. Results gathered from the exploratory survey suggested that several factors could account for the low incidence of the aerial yam in Ghana. Among these factors are insects as well as rodents which might consume fallen bulbils.

4.2 Exclusion experiment to measure impact of herbivory on plant performance

For both 2005 and 2006, no significant differences ($P \leq 0.05$) were observed among the five Dioscorea accessions for both the treated and untreated plots in all the parameters studied. Similarly, no significant differences ($P \leq 0.05$) were observed in the number of leaves of *D. bulbifera* for both the treated and untreated plots (Figure 4.1). There were, however, significant differences ($P \leq 0.05$) in the number of damaged leaves and number of damaged bulbils assessed (Figs. 4.2 and 4. 6) between treated and untreated plots and there were no significant differences in plant height, stem diameter and number of bulbils (Figs 4.3, 4.4 and 4.5). Damage to leaves was significantly ($P \leq 0.05$) greater in the untreated plots than the treated plots (Fig. 3). The differences observed between the treated and untreated crop for damaged leaves, however, could not be translated into the growth parameters; thus no differences were observed in plant height and stem diameter. Indeed, the crop was affected by drought which was taught to have affected the performance of the crop in the first year. Incidentally, when the experiment was repeated under more favourable rainfall conditions during the following year, similar results were obtained.

Table 4.1: Sampled locations of cultivated and wild types of *Dioscorea bulbifera* in five regions of Ghana

Region	**Town/village**	**Presence/Absence of *D. bulbifera***	**Other *Dioscorea* species found**	**Insect feeding on *D. bulbifera***	**Part of plant attacked**
Ashanti	Anyinamso	Present	Yes	No	-
	Mmoframfaadwene*	Present	Yes	No	-
	Amangoase	Present	Yes	Yes	Leaf
	Otaakrom*	Present	Yes	Yes	Leaf
	Nkansakrom	Absent	Yes	No	-
	Sakamukrom	Absent	No	No	-
	Ahenkro	Absent	No	No	-
	Anyinasuso	Present	No	Yes	Leaf
	Ejura/Hiawoanwu	Absent	Yes	No	-
	Mfensi	Absent	Yes	No	-
	Betinko*	Present	No	Yes	Bulbil
	Hwibaa	Absent	No	No	-
	Barniekrom	Present	No	No	-
	Ahwia Nkwanta	Absent	Yes	No	-
Brong Ahafo	Mmehame Nkwanta	Present	Yes	Yes	Leaf
	Goaso	Present	No	No	-
	Mim	Present	No	No	-
	Hiayeanimguase*	Present	Yes	Yes	Leaf
	Buokukruwa*	Present	No	No	-
Eastern	Bunso*	Present	Yes	Yes	Leaf
	Nkawkaw	Absent	Yes	No	-
	Pepease	Present	No	No	-
Central	Kakum	Present	No	Yes	Leaf
	Assin Manso	Present	No	Yes	Bulbil
	Hweremoase	Present	Yes	Yes	Bulbil
Upper West	Goyiri*	Present	Yes	No	-
	Tuna*	Present	Yes	No	-
	Dusie*	Present	Yes	Yes	Leaf
	Kolkpong*	Present	Yes	No	-

* Locations where cultivated types were planted

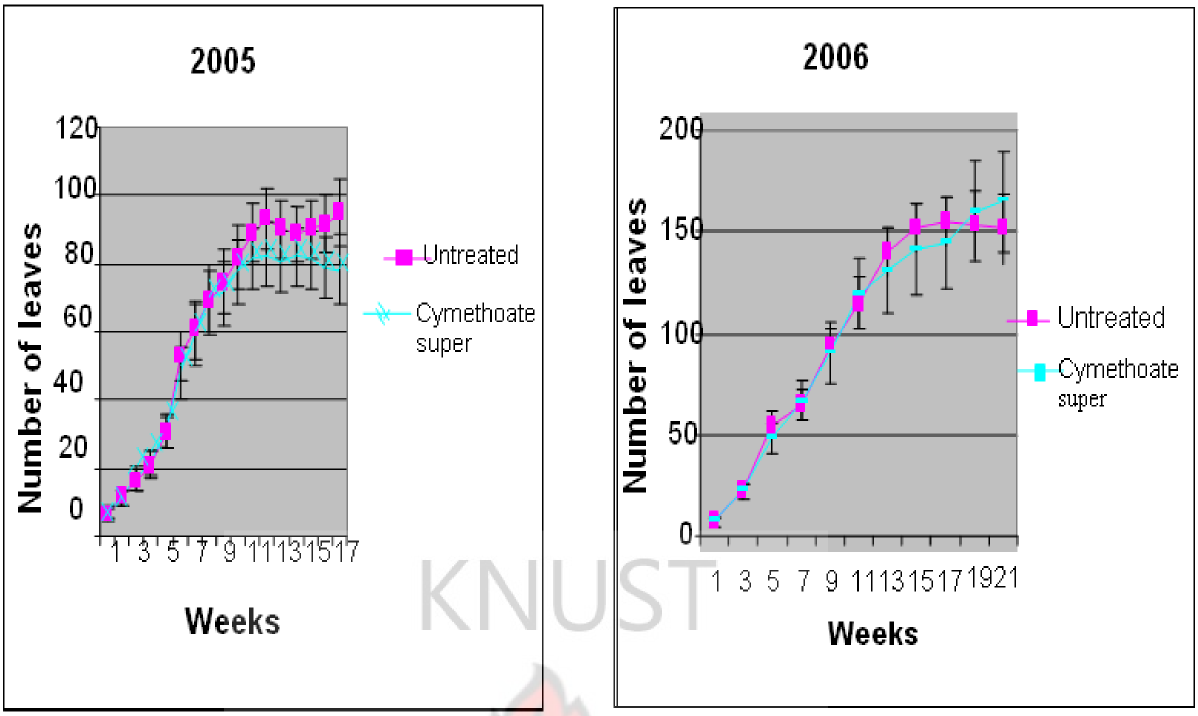

Figure 4.1. Effect of Cymethoate super on total number of leaves of *D. bulbifera* in the field, Kwadaso, Kumasi.

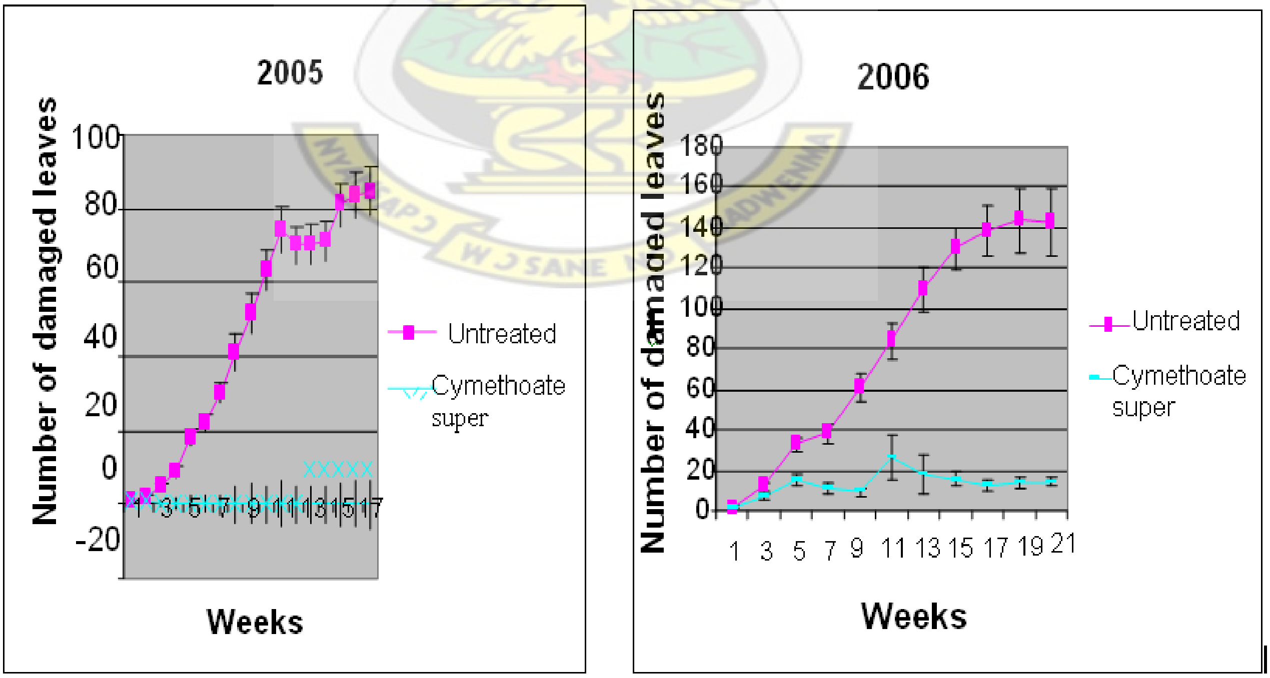

Figure 4.2. Effect of Cymethoate super on mean number of insect-damaged leaves of *D. bulbifera* in the field, Kwadaso, Kumasi.

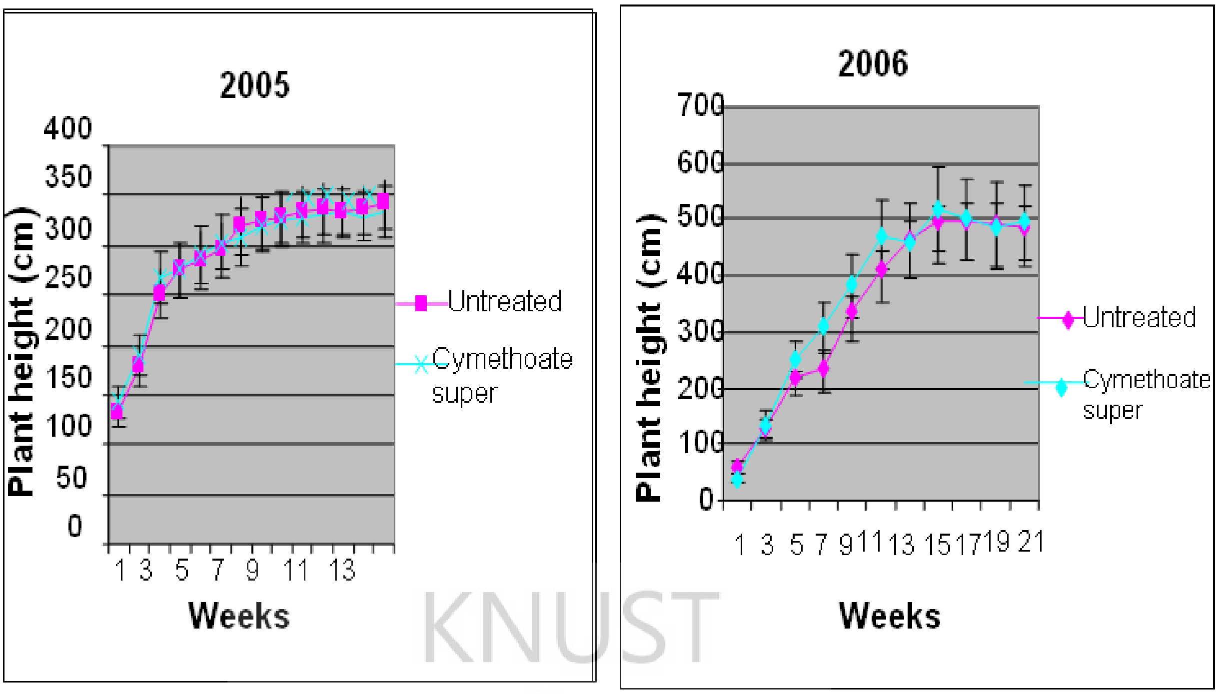

Figure 4.3. Effect of Cymethoate super on mean plant height (cm) of *D. bulbifera* in the field, Kwadaso, Kumasi.

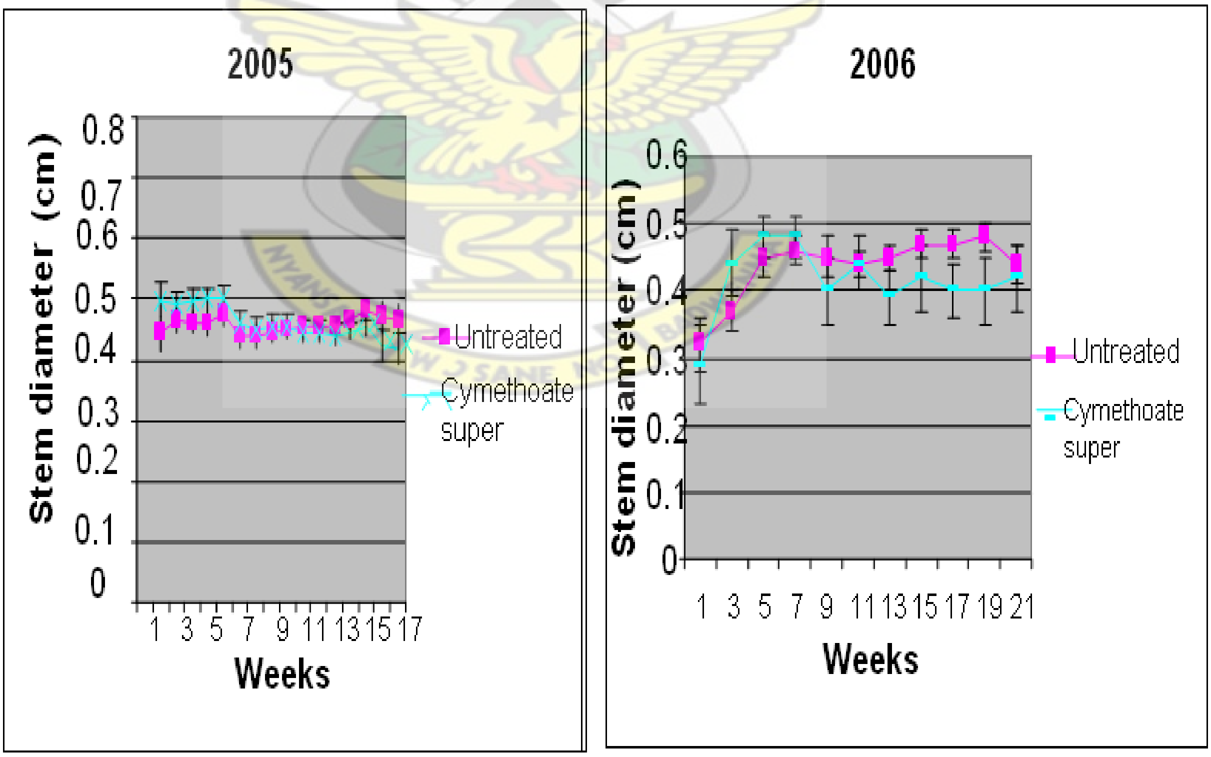

Figure 4.4. Effect of Cymethoate super on mean stem diameter (cm) of *D. bulbifera* in the field, Kwadaso, Kumasi.

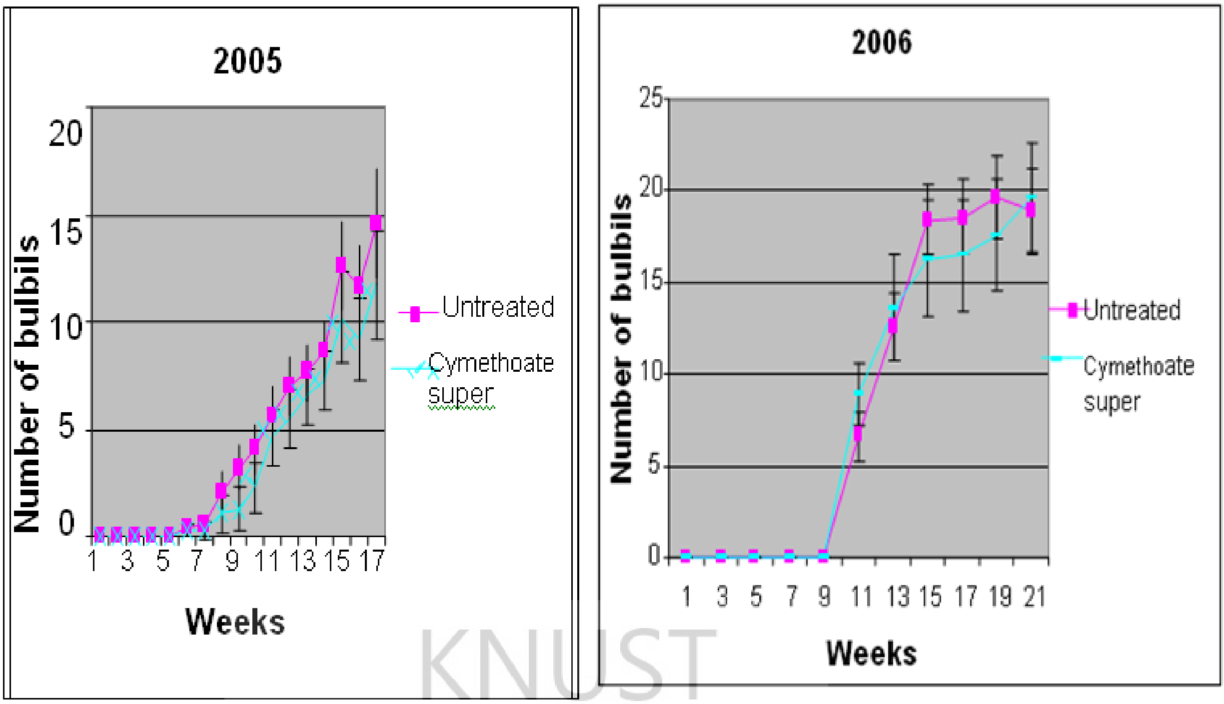

Figure 4.5. Effect of Cymethoate super on mean number of bulbils of *D. bulbifera* in the field, Kwadaso, Kumasi.

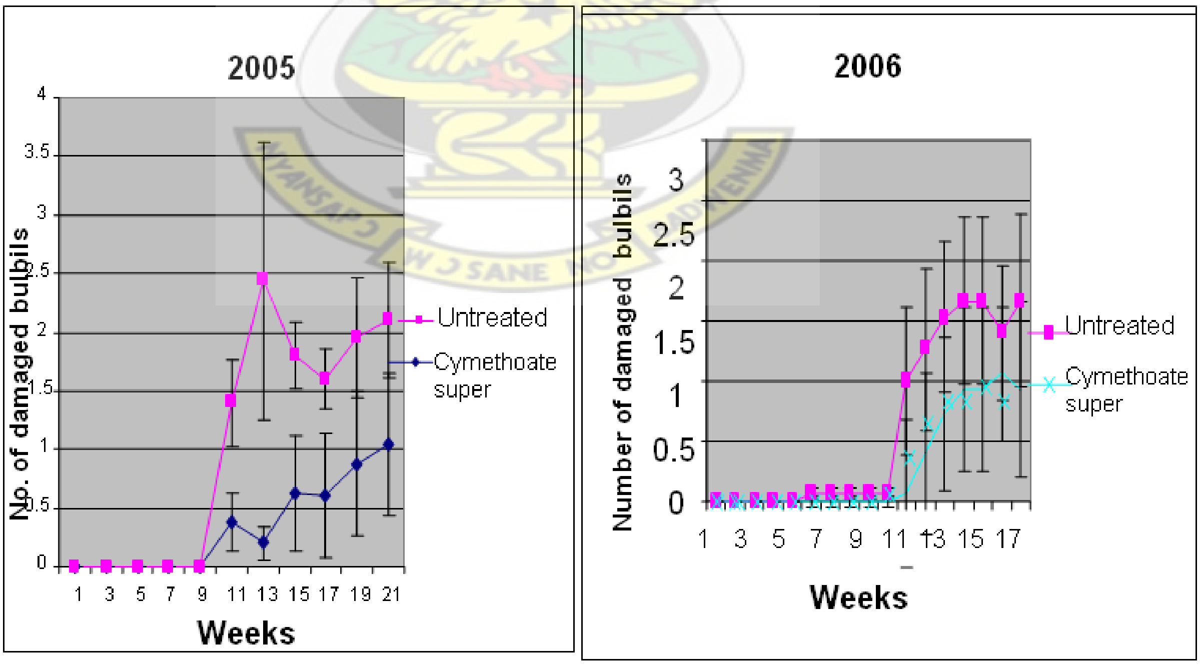

Figure 4.6. Effect of Cymethoate super on mean number of damaged bulbils of *D. bulbifera*.

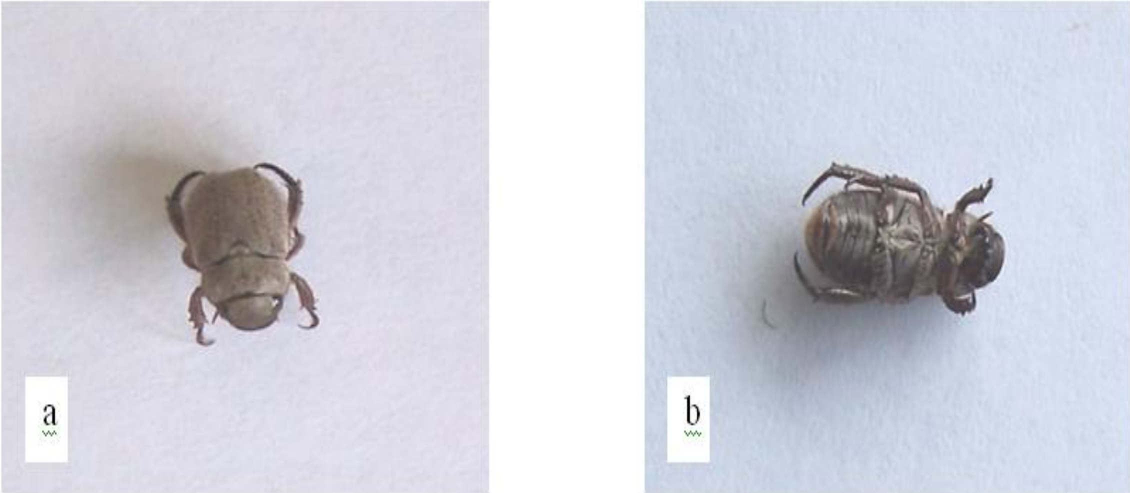

Plate 4.1. Dorsal view (a) and ventral view (b) of *Ar ala* sp. observed on leaves of *D. bulbifera* and *D. alata* at Kwadaso, Kumasi.

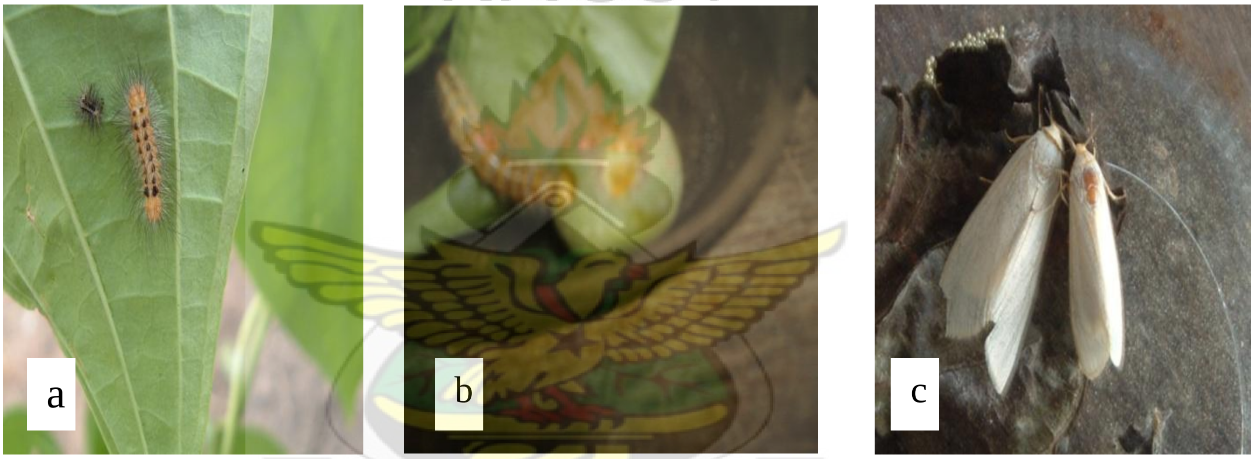

Plate 4.2. Larva of *Estigmene* sp. feeding on (a) leaves, (b) bulbils of *D. bulbifera* and (c) male (right) and female (left) adults of *Estigmene* sp.

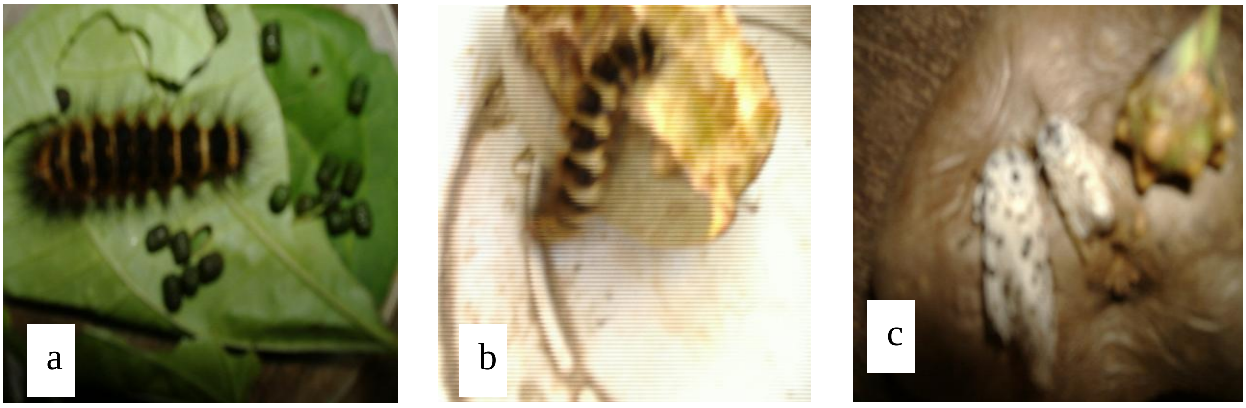

Plate 4.3. Larva of *Diacrisia* sp. feeding on (a) leaves, (b) bulbils of *D. bulbifera* and (c) male (right) and female (left) adults of *Diacrisia* sp.

During both 2005 and 2006, some species of scarabs, *Anomala* sp. (Plate 4.1) and *Adoretus* sp. that caused damage to the leaves of *D. bulbifera*, previously reported in earlier studies by CRI scientists (FDEP Report, 2004) were encountered. The activities of these beetles were observed shortly after dusk. Many of the beetles were seen and collected during routine visits to the field between 1900 and 2000 hrs GMT. These beetles were not encountered at all during the day. Even though *Anomala* sp. and *Adoretus* sp. were seen feeding on leaves in the field and caused most of the foliar damage, the beetles did not feed on the bulbils and leaves of *D. rotundata* yam varieties in the laboratory.

In addition to the beetles, larvae of the two Lepidopterous insects (*Estigmene* sp. and *Diacrisia* sp.) belonging to the family Arctiidae were found causing damage to both the leaves and bulbils. (Plates 4.2 and 4.3). They fed voraciously on leaves and bulbils particularly at night.

4.3 Catalogue of insect herbivores of *D. bulbifera*

The phytophagous herbivores associated with aerial yam during 2005 and 2006 are summarized in Table 2. A total of 40 species were collected, out of which twenty-nine were familiar and known species. The insects belonged to the following Orders/Families; Coleoptera, Hemiptera, Dermaptera, Mantodea, Diptera, Orthoptera, Lepidoptera, Hymenoptera and Isoptera. A few millipedes and molluscs were also found. Majority of the insect species belonged to the Coleoptera, Lepidoptera and Hemiptera orders.

Table 4.2. Invertebrate herbivores collected from *D. bulbifera* in Ghana during the study period of 2004 – 2006

Insects

Order	**Family**	**Scientific name**	**Stage**	**Part Damage**	**Specificity**	**Remarks**
Coleoptera	Scarabaeidae	*Anomala* sp.	Adult	foliage	y	Agent 1
	Scarabaeidae	*Adoretus pullus*	Adult	foliage	y	Agent 2
	Scarabaeidae	*Pachnoda cordata*	Adult	bulbils	x	
	Bruchidae	*Callosobruchus maculatus*	Adult	unknown	b	
	Chrysomelidae	*Ootheca mutabilis*	Adult	foliage	b	
	Chrysomelidae	*Podagrica uniformis*	Adult	foliage	b	
	Coccinellidae	*Coccinella septempunctata*	Adult	beneficial	c	
	Lagriidae	*Lagria villosa*	Adult	unknown	b	
	Lagriidae	*Lagria cuspida*	Adult	unknown	b	
	Unidentified		Adult	unknown	b	
Hemiptera	Pentatomidae	*Nezara viridula*	Adult	unknown	b	
	Alydidae	*Riptortus dentipes*	Adult	unknown	b	
	Pyrrhocoridae	*Dysdercus superstitious*	Adult	unknown	b	
	Alydidae	*Anoplocnemis curvipes*	Adult/Nymph	unknown	b	
	Aphididae	*Aphis craccivora*	Adult	foliage	x	
	Aphididae	*Brevicoryne brassicae*	Adult	foliage	x	
	Aleyrodidae	*Bemisia tabaci*	Adult	foliage	x	
	Aleyrodidae	*Aleurodicus dispersus* (Rus.)	Adult	foliage	x	
	Cicadellidae	*Empoasca fabae*	Adult	unknown	b	
	Pseudococcidae	*Phenacoccus manihoti*	Adult	foliage	x	
Dermaptera	Forficulidae	*Forficula auricularia*	Adult	unknown	b	
Mantodea	Mantidae	*Sphodromantis viridis*	Adult & Nymph	beneficial	c	
	Mantidae	*Mantis religiosa*	Adult & Nymph	beneficial	c	
Diptera	Muscidae	*Musca domestica*	Adult	unknown	b	
Orthoptera	Pyrgomorphidae	*Zonocerus variegatus*	Adult	foliage	x	
	Acrididae	Not identified	Adult	unknown	b	
	Tetrigidae	Not identified	Adult	unknown	b	
	Tettigonidae	Not identified	Adult	foliage	x	

Table 4.3 Invertebrate herbivores collected from *D. bulbifera* in Ghana during the study period of 2004 – 2006 (contd)

Order	**Family**	**Scientific name**	**Stage**	**Part Damage**	**Specificity**	**Remarks**
Orthopthera		Not identified	Adult	unknown	b	
	Gryllidae	Not identified	Adult	foliage	x	
Lepidoptera	Arctiidae	*Estigmene* sp.	Larva	Leaves & bulbils	x	Agent 3
	Arctiidae	*Diacrisia* sp.	Larva	Leaves & bulbils	x	Agent 4
	Arctiidae	Not identified	Adult	unknown	b	
	Arctiidae	Not identified	Adult	unknown	b	
	Noctuidae	*Spodoptera litoralis*	Larva	Leaves & bulbils	x	
	Noctuidae	*Spodoptera exempta*	Larva	Leaves & bulbils	x	
	Noctuidae	Not identified	Larva	foliage & bulbils	x	
	Noctuidae	Not identified	Larva	foliage & bulbils	x	
	Plutellidae	*Plutella xylostella*	Adult	unknown	b	
	Pyralidae	*Maruca vitrata*	Adult	unknown	b	
Hymenoptera	Apidae	*Apis melifera*	Adult	unknown	b	
	Formicidae	Not identified	Adult	unknown	b	
	Formicidae	Not identified	Adult	unknown	b	
Isoptera	Macrotermitidae	*Macrotermes* sp.	Adult	unknown	b	

Note: a, Specificity index: x - not specific to *D. bulbifera* attack other Dioscoreaceae; y- only known to attack *D. bulbifera and D. alata* but not *D. rotundata*
b, Species possibly accidental or transient; not found feeding on *D. bulbifera*
c, Beneficial insect feeding on Aphids

4.4 Description of developmental stages of *Estigmene* sp. (Lepidoptera: Arctiidae)

All the experiments were conducted in the entomology laboratory of CRI where the temperature ranged from 22º to 26ºC and relative humidity ranged from 88% to 92%.

4.4.1 Egg: The eggs were spherical in shape and initially were yellowish but turned greyish in colour as they matured. The eggs were laid in two or more clusters with an average of 60 eggs per cluster. The eggs hatched between 4 and 5 days.

4.4.2 Larva: Upon hatching, the first instar larvae fed gregariously on the lower leaf surface, scraping but not eating entirely through the leaf. The larva was brown in colour and remained so in later instars. First instar larvae produced silken thread when brushed off the host. Succeeding instar larvae did not produce the silken threads but moved or crawled fast away when disturbed. Young larvae (first two instars) had a pair of black spots on each larval segment and fed gregariously on the lower surface of leaves. They dispersed as they matured and moved onto bulbils. The body of the larva was covered with light, plumose setae which become more abundant as the instar matured. They crawled rapidly and fed voraciously at night. Third instars exhibited longitudinal stripes, usually yellowish and white. Fourth and fifth instars maintain the same general appearance except that they grew bigger at each moult. Larval feeding, as observed from the damage done to aerial yam host plants, increased with each instar. It was also observed that the larvae at the end of each instar entered a quiescent and dormant period before each moult. Feeding by fifth instar larvae was the most damaging to the plant as they devoured the leaves and bulbils. The larval stage consisted of five instars. The duration of larval development averaged 25 days.

4.4.3 Pre-pupa and pupa: Prior to pre-pupation, the fifth instar larvae were observed to be restless and moved about in the rearing cage. The fifth instar larvae spun a light silken cocoon dotted with larval setae during pre-pupation. Matured larvae usually pupate between layers of leaves at the bottom of the rearing cage/ jar. The duration of the pupal stage on the average was 11 days.

4.4.4 Adult: The insects finally emerged into pale moths identified as *Estigmene* sp. (courtesy: Prof. Overholt, UF). The adult is a medium sized cream-coloured moth very active at night. Emergence mostly occurred at night. The females were found to be larger than males. Mating occurred at dusk, usually less than 24 hrs after emergence and could last for several hours. Adults were not seen to be feeding even though food was provided. Females began to oviposit the day after copulation and could oviposit during each of the next three days, but most of the eggs were laid during the first two days after mating.

4.5 Description of developmental stages of *Diacrisia* sp. (Lepidoptera: Arctiidae)

4.5.1 Egg: The eggs initially were yellowish and became greyish in colour as they matured. The eggs were spherical in shape and laid in two or more clusters. The eggs hatched between 5 and 7 days.

4.5.2 Larva: Larvae initially were brown in colour but grew darker after each moult. Young larvae (first two instars) feed gregariously on the lower surface of leaves and disperse as they matured moving onto bulbils. The body of the larva was densely covered with light dark, plumose setae and were more abundant in relation to those present in the previous instar. They crawled rapidly and fed voraciously at night. Only the first instar larvae produced silken threads. Older larvae did not. When disturbed the larva curled into a tight loop and dropped from the leaf. Third instars displayed a pronounced black lateral stripes (Plate 5a). Fourth and fifth instars maintain the same general appearance except that they grew bigger at each moult. Larval feeding increased with each instar, and the fifth instar larvae were the most damaging to the plant as they devoured the leaves and bulbils. Larval development period ranged between 25 and 30 days.

4.5.3 Pre-pupa and pupa: Prior to pre-pupation, the fifth instar larvae moved about restlessly in the rearing cage. The fifth instar larva spun a light silken cocoon sprinkled with larval setae during pre-pupation. Matured larvae usually pupate between layers of leaves at the bottom of the rearing cage/ jar. With *Diacrisia* species pupation also took place on the walls and in the corners of the cage or kilner jars. The larval stage consisted of five to six instars. The duration of the pupal stage ranged between 10 and 14 days.

4.5.4 Adult: The adult insect emerged as a medium sized moth, white coloured with scattered black spots on the forewings and identified as *Diacrisia* sp. (courtesy: Prof. Overholt, UF). Emergence mostly occurred at night and the adults were nocturnal. Mating occurred at dusk and could last for several hours. Adults were not seen to be feeding even though food was provided. Females began to oviposit one day after copulation and oviposited during each of the next three days, but most of the eggs were laid during the first two days after mating.

4.6 Host Specificity Tests

4.6.1. (Lepidoptera: Arctiidae)

During the no-choice tests in the laboratory the larvae of both *Estigmene* and *Diacrisia* species successfully fed and completed their development on the leaves of all yam varieties tested. Both arctiid moths, thus exhibited a wide host range status.

4.6.2 (Coleoptera: Scarabaeidae)

The results revealed that the *Anomala* sp. had a narrow host range as shown in Plate 4.4 and Figure 4.7 below. There were significant differences ($P \leq 0.05$) between the feeding or damage caused to the dioscorean species. The scarabs significantly ($P \leq 0.05$) caused damage to aerial yam and water yam leaves as compared to the other dioscorean species (Figure 4.7). The scarabs also preferred aerial yam to *D. rotundata* yam varieties as evidenced in Plate 4.4c.

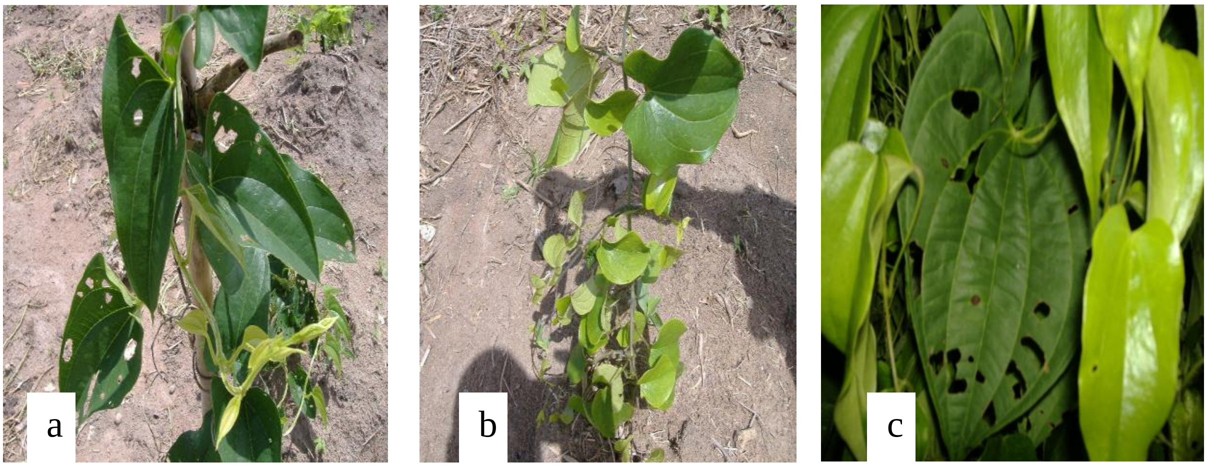

Plate 4.4. (a.) Leaves of *D. alata* damaged by some species of scarabs; (b.) undamaged entire leaves of *D. rotundata*; (c) selective damage of *D. bulbifera* leaves within *D. rotundata* leaves on a common stake.

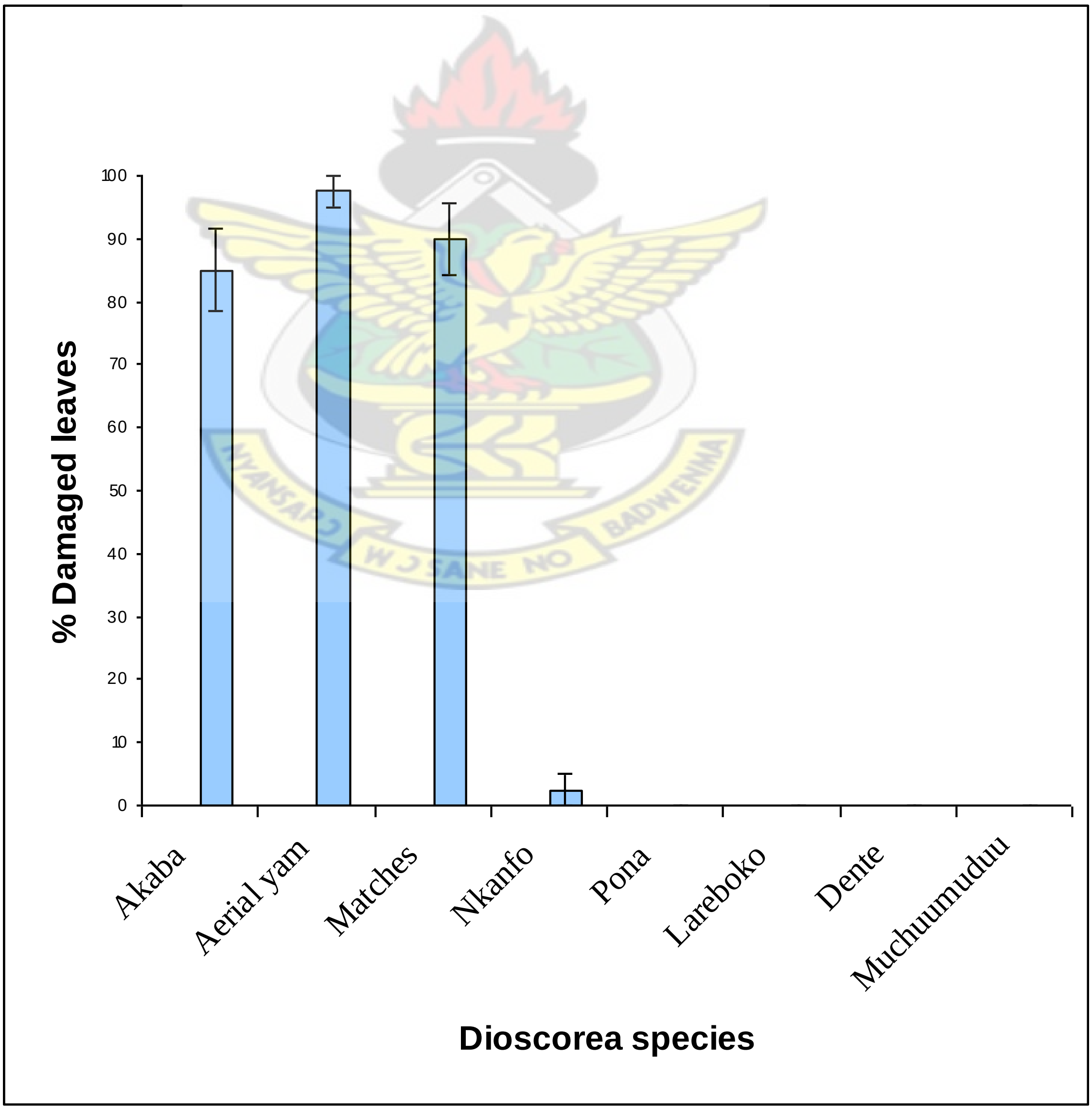

Figure 4.7. Host preference of *Anomala* sp. within the Dioscoreaceae

4.7 Biology of *Estigmene* sp. (Lepidoptera: Arctiidae)

4.7.1 Egg: The eggs were spherical in shape, and measured about 0.6 mm in diameter. Initially they were yellowish but become greyish in colour as they matured. The eggs were always laid in clusters with an incubation period of 5.0 ± 0.6 days (Table 4.4).

4.7.2 Larva: The larvae fed on and developed to the pupal stage on all yam varieties tested. In this process they caused 100% damage to all the different host plant species of *Dioscorea* used. The first instar took 10 – 11 days (Table 4.4). The 2nd, 3rd, 4th and 5th larval instars took between 3 and 4 days for all the *Dioscorea* species used in the study. There were no significant differences ($P \leq 0.05$) in the developmental periods of the larval stages on the *Dioscorea* species tested. Duration of the larval development was 22 to 26 days (Table 4.4).

4.7.3 Pre-pupa: In the pre-pupal stage the matured larvae stopped feeding and began to search for a site to pupate. It then shrank and enclosed itself with silken substance and detached the setae and began the construction of a cocoon. Pre-pupation ranged between 2 and 3 days without significant differences ($P \leq 0.05$) between the eight *Dioscorea* species tested.

4.7.4 Pupa: Pupation occurs among leaf debris, in a thin cocoon formed from silken hairs interwoven with caterpillar body hairs. The pupa measured about 25 - 30 mm in length and had a shinny reddish brown colour. Duration of the pupal stage ranged from 10 to 11 days (Table 4.4).

4.7.5 Adult: Adults were fairly large moths, measuring 3.5 to 5 cm in wingspan, and were cream coloured. All the abdominal segments were yellow and bore a series of large black spots dorsally. Mating occurred on the evening following emergence, and egg deposition took place the next evening. Females usually were larger and also lived longer than males. Adults were also observed to be ‘capital breeders’ and thus did not feed at all. A generation of the *Estigmene* species was completed between 35 and 40 days in the laboratory where the temperature ranged from 22º to 26ºC and relative humidity from 88% to 92%.

4.7.6 Fecundity and longevity

Mean fecundity of females reared on the entire *Dioscorea* host treatments were not significantly different ($P \leq 0.05$). Females produced an average of 167 eggs after mating in more than one cluster. There were no significant differences in mean longevity of female moths either mated or unmated between the eight yam varieties. The females lived 3 days more than males when mated and between 3 and 5 days when unmated (Table 4.5).

4.8 Biology of *Diacrisia* sp. (Lepidoptera: Arctiidae)

4.8.1 Egg: The eggs were spherical in shape, and measured about 0.6 mm in diameter. Initially they were yellowish and a greyish in colour as they matured. The eggs were always laid in clusters. Incubation period of eggs was 7 to 8 days (Table 4.6).

4.8.2 Larva: The larvae fed on and developed to the pupal stage on all yam varieties tested. In this process they caused 100% damage to all the different host plant species of Dioscorea used. The first instar took 13 days (Table 4.6). The 2nd, 3rd and 4th larval instar stages ranged between 2 and 4 days for all the dioscorea species used in the study. In the 2nd instar, however, there were significant differences ($P \leq 0.05$) in larval developmental period between 'Lareboko' and 'Dente', 'Muchuumuduu' 'Aerial yam' and 'Akaba'. In the 3rd instar, there were again significant differences ($P \leq 0.05$) between 'Akaba' and aerial yam on one hand and all the Dioscorea species on the other for larval development time. In other words, the period of the 3rd larval instar was shorter for 'Akaba' and aerial yam than the other *Dioscorea* species. No significant differences ($P \leq 0.05$) occurred in the 4th instar. The 5th instar lasted between 4 and 6 days with significant differences ($P \leq 0.05$) between 'Muchuumuduu' and 'Matches' but no significant differences were observed between either 'Muchuumuduu' or 'Matches' and the other Dioscorea species (Table

4.6). In the 5th instar larvae, larval development was longer on ‘Muchuumuduu’ than it was on ‘Matches’. Duration of larval development was from 25 to 30 days (Table 4.6).

4.8.3 Pre-pupa: In the pre-pupal stage the matured larvae stopped feeding and began to search for a site to pupate. It then shrank and enclosed itself with silken substance and detached the setae and began the construction of cocoon. Pre-pupation period ranged between 3 and 4 days. ‘Lareboko’ was significantly different from Dente’, Aerial yam and ‘Matches’. No significant differences ($P \leq 0.05$) were observed on the duration of pre-pupation between ‘Lareboko’ and ‘Dente’, Aerial yam as well as ‘Matches’ on one hand and the other Dioscorean species on the other. In effect the duration for pre pupation was longer in ‘Lareboko’ for *Diacrisia* larvae.

4.8.4 Pupa: Pupation occurred among leaf debris, in a thin cocoon formed from silken hairs interwoven with caterpillar body hairs. The pupa measured about 25 - 30 mm in length and had a dark brown colour. Duration of the pupal stage was about 11 to 13 days (Table 4.6).

4.8.5 Adult: The adults were medium sized moths, measuring 3.5 to 4.5 cm in wingspan, and were white in colour, although the forewings had numerous small, irregular black spots. The hind wing was plain white. The abdominal segments were white with dark brown spots. Mating occurred the evening following emergence, and egg deposition the next evening. Females usually were larger and also lived longer than males. Adults were also observed to be ‘capital breeders’ thus did not feed at all. A generation of the *Diacrisia* species was completed from 39 to 45 days in the laboratory where the temperature ranged from 22º to 26ºC and relative humidity from 88% to 92%.

4.8.6 Fecundity and Longevity

Mean fecundity of females reared on all the *Dioscorea* host leaves was not significantly different. Females lived five to six days and produced an average of 95 eggs after mating, in more than one cluster. There were no significant differences in mean longevity of female moths either mated or unmated between the eight yam species. Significant differences ($P \leq 0.05$) however occurred only in the unmated males of *Diacrisia* species between 'Muchuumuduu', 'Pona', 'Akaba' and 'Matches' and 'Nkanfo' (Table 4.7). The longevity of the males was found to be shorter in 'Nkanfo' than in 'Akaba', 'Matches' 'Muchuumuduu', 'Pona'.

Table 4.4. Duration of life stages (mean ± SEM) of *Estigmene* sp. fed on different dioscorean species in the Laboratory

Dioscorean species /Variety	Incubation period (days)	Larval Instars 1	2	3	4	5	Pre-pupation	Pupation	Total
		Duration (days)							
Aerial yam (*D. bulbifera*)	5.0 ±0.6	10.7 ± 0.3	3.7 ± 0.3	4.0 ± 0.5	3.7 ± 0.3	3.7 ± 0.3	3.3±0.3	11.3 ± 0.3	40.3±0.3
Akaba (*D. alata*)	5.0 ±0.0	10.3 ± 0.9	4.0 ± 0.0	4.3 ± 0.3	3.7 ± 0.3	4.0 ± 0.5	3.3±0.7	11.3 ± 0.8	41.0±0.6
Matches (*D. alata*)	5.0 ±1.0	10.3 ± 0.3	4.0 ± 0.6	4.0 ± 1.0	3.7 ± 0.7	4.3 ± 0.3	3.3±0.6	11.0 ± 0.6	40.7±0.3
Nkanfoɔ (*D. dumenterum*)	5.0 ±0.6	10.7 ± 0.3	4.0 ± 0.6	4.0 ± 1.0	3.7 ± 0.8	4.1 ± 0.3	3.3±0.3	11.0 ± 1.0	40.7±0.3
Pona (*D. rotundata*)	5.0 ±0.6	10.3 ± 0.9	4.0 ± 0.6	3.3 ± 0.3	3.7 ± 0.9	4.7 ± 0.3	3.3±0.3	11.3 ± 0.9	40.7±1.7
Larebako (*D. rotundata*)	5.3 ±0.3	11.0 ± 0.6	4.3 ± 0.3	4.0 ± 0.6	3.7 ± 0.3	4.0 ± 0.0	3.7±0.3	10.3 ± 0.7	41.0±0.6
Dente (*D. rotundata*)	5.0 ±0.6	11.0 ± 0.6	3.7 ± 0.3	3.3 ± 0.3	3.7 ± 0.3	3.7 ± 0.7	3.7 ± 0.7	10.7 ± 0.3	40.3±1.2
Muchuumuduu (*D. rotundata*)	5.0 ±1.2	10.3 ± 0.9	4.0 ± 0.6	3.3 ± 0.3	3.7 ± 0.8	4.7 ± 0.3	3.3±0.3	11.3 ± 0.9	40.7±1.7
F value	0.03	0.21	0.27	0.33	0.04	0.49	0.09	0.31	0.10
P	1.0000	0.9782	0.9553	0.9306	0.9998	0.8277	0.9981	0.9402	0.9973
	N S	N S	N S	N S	N S	N S	N S	N S	N S

Table 4.5 Fecundity and longevity of *Estigmene* sp. fed on different dioscorea species in the Laboratory

Dioscorean species /Variety	**Fecundity No. of eggs laid / Female**	**Longevity mated**		**Longevity unmated**	
		Female	**Male**	**Female**	**Male**
Aerial yam *D. bulbifera*	167.3 ± 25.7	6.3 ± 0.7	3.0± 0.6	9.7 ± 0.9	5.3 ± 0.7
Akaba (*D. alata*)	167. 7 ± 27.1	6.0 ± 0.6	3.3 ± 0.9	8.3± 0.7	5.7± 0.1
Matches (*D. alata*)	164.8 ±18.4	6.0 ± 1.0	3.0 ± 0.6	8.3 ± 0.3	5.7 ± 0.9
Nkanfoɔ (*D. dumenterum*)	167.0± 24.6	6.3 ± 0.3	3.3 ± 0.3	8.7± 0.6	5.3 ± 0.7
Pona (*D. rotundata*)	166.7 ± 35.6	6.0 ± 0.6	3.0 ± 0.6	8.0± 0.6	5.0 ± 0.6
Larebako (*D. rotundata*)	167.3 ± 33.8	5.7 ± 0.7	3.0 ± 0.6	8.7 ± 0.3	5.0 ± 0.6
Dente (*D. rotundata*)	167.3 ± 36.1	6.0 ± 0.6	3.3 ± 0.3	9.7 ± 0.3	5.3± 0.3
Muchuumuduu (*D. rotundata*)	167.7± 32.0	6.0 ± 1.2	3.3 ± 0.9	10.7± 0.9	5.3 ± 0.7
F value	0.82	0.08	1.53	0.15	0.00
P	0.5843	0.9987	0.2269	0.9912	1.000
	N.S	N.S	N.S	N.S	N.S

Table 4.6. Duration of life stages (mean ± SEM) of *Diacrisia* sp. fed on different dioscorean species in the Laboratory

Dioscorean species /variety	**Incubation period (days)**	**Larval Instars** **1**	**2**	**3** **Duration (days)**	**4**	**5**	**Pre-pupation**	**Pupation**	**Total**
Aerial yam (*D. bulbifera*)	7.0 ±0.0	13.0 ± 0.0	3.0 ± 0.0 b	2.0 ± 0.0ab	2.8 ± 0.3	4.5 ± 0.3ab	3.0±0.0b	11.3 ± 0.3	39.5± 0.3c
Akaba (*D. alata*)	7. 5 ±0.8	13.0 ± 0.0	3.0 ± 0.0 b	1.5 ± 0.3 b	3.0 ± 0.4	4.0 ± 0.0ab	3.3±0.3ab	12.0 ± 0.4	40.8 ±1.2bc
Matches (*D. alata*)	7.0 ±0.4	13.2 ± 0.2	3.2 ± 0.2ab	3.5 ± 0.3 a	3. 5 ± 0.5	3.5 ± 0.3b	3.0±0.0b	11.5 ± 0.29	41.0±0.7bc
Nkanfoɔ (*D. dumenterum*)	7.0 ±0.5	13.0 ± 0.0	3.3 ± 0.3ab	3. 5 ± 0.3 a	4.0 ± 0.0	5.0 ± 0.0ab	3.2±0.2ab	11.5 ± 0.6	43.5±0.6ab
Pona (*D. rotundata*)	7.5 ±0.6	13.2 ± 0.2	3.2 ± 0.25b	3.0 ± 0.4a	3.5 ± 0.3	4.0 ± 0.4ab	3.5±0.9ab	11.2 ± 0.2	44.1±0.8ab
Larebako (*D. rotundata*)	7.5 ±0.6	13.5 ± 0.2	4.0± 0.0a	3.5 ± 0.5 a	4.0 ± 0.4	5.0 ± 0.8ab	4.3±0.3a	11.0 ± 0.2	45.0±1.2a
Dente (*D. rotundata*)	8.0 ±0.4	13.0 ± 0.0	2.7 ± 0.2b	3.7 ± 0.6 a	4.0 ± 0.0	4.7 ± 0.4ab	3.0±0.0b	13.0 ± 0.7	44.2±0.6ab
Muchuumuduu (*D. rotundata*)	8.00 ±0.41	13.0 ± 0.0	2.7 ± 0.5b	3.5 ± 0.5 a	3.5 ± 0.2	5.7 ± 0.6a	3.2±0.2ab	11.2 ± 0.6	42.5±0.6abc
F value	0.55	1.37	4.09	4.43	2.91	2.35	2.81	1.61	5.85
P	0.7915	0.2622	0.0044	0.0028	0.0	0.0235	0.0274	0.1799	0.0005

Means within a column followed by the same letter are not statistically different ($P \leq 0.05$) ANOVA, Student Newman Keul's test [SNK], SAS Institute 2007).

Table 4.7. Fecundity and longevity of *Diacrisia* species fed on different dioscorean species in the Laboratory

Dioscorean species/ Variety	Fecundity No. of eggs laid / Female	Longevity mated		Longevity unmated	
		Female	Male	Female	Male
Aerial yam (*D. bulbifera*)	104.0 ±12.2	5.0± 0.4	2.7 ± 0.3	9.5 ± 0.5	4.2 ± 0.4ab
Akaba (*D. alata*)	92.5 ± 8.0	5.5 ± 0.3	3.3 ± 0.3	10.5 ± 0.7	5.3 ± 0.5a
Matches (*D. alata*)	84.8 ± 8.4	5.5 ± 0.3	3.5 ± 0.5	10.5 ± 0.7	5.3 ± 0.3a
Nkanfoɔ (*D. dumenterum*)	94.3 ± 2.8	4.5 ± 0.3	3.3 ± 0.3	11.3± 0.3	3.3 ± 0.3b
Pona (*D. rotundata*)	92.8 ± 4.9	5.5 ± 0.7	3.8± 0.5	11.0 ± 0.4	5.0 ± 0.4a
Larebako (*D. rotundata*)	92.8 ± 4.9	5.5 ± 0.3	3.8± 0.5	11.5 ± 0.7	3.5 ± 0.3ab
Dente (*D. rotundata*)	93.0 ± 7.1	5.0 ± 0.4	3.0 ± 0.4	10.8 ± 0.3	4.8 ± 0.5ab
Muchuumuduu (*D. rotundata*)	84.3 ± 8.9	4.8 ± 0.5	3.0 ± 0.4	9.8± 0.5	5.3 ± 0.5a
F value	0.67	0.97	0.86	1.90	4.06
P	0.694	0.4730	0.5477	0.1132	0.045

Means within a column followed by the same letter are not statistically different ($P \leq 0.05$) ANOVA, Student Newman Keul's test [SNK], SAS Institute 2007).

CHAPTER FIVE

5.0 Discussion

Insect surveys on aerial yam in Ghana confirmed an earlier assertion by Ayensu and Coursey (1972) that both cultivated and wild forms of aerial yam are found in several places in Ghana. The survey also revealed a considerable number (40) of phytophagous insect fauna associated with *D. bulbifera*, with 24 species attacking the foliage and bulbils. Similar surveys by R. Pemberton and M. Rayamahji discovered several highly damaging herbivore species on *D. bulbifera* in Kathmandu valley in Nepal (Wheeler and Pemberton, 2004).

In order to successfully implement a classical biological control program, it is critical that basic information about the ecology and biology of the target species and its associated fauna be established both in its area of origin and newly invaded area (van Driesche and Bellows, 1996). This study indicated that some arthropods are potential biological control agents of aerial yam in Ghana. In the exclusion study for example, about 94% herbivory by native insects were recorded although the growth performance of aerial yam was not hampered. My observations in this study have shown that two members of the family Scarabaeidae, *Anomala* sp. (Plate 4.1) and *Adoretus pullus* caused damage to the leaves of *D. bulbifera* and *D. alata* (Plate 4.4a and 4.4c), however, there was no infestation on leaves of *D. rotundata* (Plate 4.4b). Laboratory investigations confirmed the results obtained from the field as regards the non-pestiferous nature of the beetles on the rotundata yams. Both insect species consumed significant amount of leaves of aerial yam and water yam. Neither the stem diameter, plant height, number of leaves nor damage to bulbils as indicators for changes in plant development after herbivore attack showed a relation to beetle density.

Wheeler and Pemberton (2004) intimated that no *Dioscorea* species are cultivated commercially as food crops in Florida and the southern U. S. A. However, several species of *Dioscorea* have significant medicinal value as sources of a steroidal sapogenin diosgenin, a precursor in the industrial synthesis of human steroid hormones. According to Edwards *et al.*

(2002) diosgenin is also used in the manufacture of oral contraceptives. The ecological and economic effects of aerial yam to the economy of the United States require the consideration of the lepidopteran insect pests of aerial yam identified from this study.

From an ecological perspective, it seems likely that the abundant and diverse insect fauna and others such as rodents that were observed on aerial yam, constitute an important check on the growth and spread of aerial yam in Ghana, while the relatively sparse fauna on the yam in the USA contributes to its invasive habit and pest status there. From a biological control perspective, the defoliators and bulbil feeders could all be considered as potential control agents. In this regard, *Diacrisia* sp. and *Estigmene* sp. (Lepidoptera: Arctiidae) were found to significantly defoliate aerial yam in Ghana and were also able to feed and develop on other dioscorea yam species in confinement. Since this did not occur in the wild, indicates an agreement with current theory that the physiological range, delineated in no-choice laboratory experiments, is broader than the ecological range realized in the field (Briese, 2005; Sheppard *et al.*, 2005). It became evident that there is the need to use alternative testing procedures that could reflect what really happens in nature. Furthermore, during cage tests, if an insect is confined with a non host plant species it may be forced to accept the plant as survival instinct and may become habituated to inherent feeding deterrents (Jermy *et al.*, 1982; Marohasy, 1998). The larval host range in the laboratory was broader than the field host range for both Lepidoptera species. This situation according to Harris (1998) arises when the field host range is determined by adult habit and host finding requirements. According to Loan and Holdaway (1961), in laboratory tests, insects often accepted a broader range of hosts than in nature. In most insects the adult is responsible for host selection since its larvae lack the necessary mobility. The female mostly, is under strong selection pressure to oviposit on plants that optimize the survival of its progeny (Harris, 1998). The larva, on the other hand, has to stay on the plant, which may involve distinguishing it from intermingled vegetation, and feed. In a situation where the larva finds itself on a wrong plant, its best survival option is usually to try and feed on it.

Consequently, the adult host preference tends to be narrower and more firmly held than that of the larva (Harris, 1998). The data indicated that all *Dioscorea* species tested, including *D. bulbifera* and *D. alata,* were acceptable hosts for the arctiid moths, but it was observed in the field that *D .bulbifera* was the preferred hosts.

The life history described in this study for the arctiid moths reared in the laboratory is similar to its biology described under natural conditions (Ojala *et al*., 2005). The observation that most eggs were laid on the sides of the rearing cages and kilner jars rather than on leaves in this study corroborates the findings that it is common for moths in confinement to lay a large proportion of their eggs on the walls, even when the cages are relatively large (Ramaswamy, 1988; Eigenbrode and Bernays, 1997). In the field, it was observed that *Diacrisia and Estigmene* species laid their eggs on the underside of *D. bulbifera* leaves only. It was observed in the field that *Diacrisia and Estigmene* species laid their eggs on the leaves of *D. bulbifera* which most likely reflected the preferred choices of the ovipositing adults. Eggs laid in captivity were found to be far more than what was observed in the field. This is in contrast to the belief that Arctiid moths could lay between 400 and 1000 eggs (Capinera *et al*., 1987), although other geometrid moth species have been reported to lay eggs when collected in the field and then confined in 50-ml plastic vials (Tammaru and Javois, 2000). In this study, the arctiid moths laid fewer eggs when kept in pairs in small containers, possibly because of failure to mate, lack of appropriate substrate or space. This finding agrees with that of Joy *et al*. (1993), when evaluating three parental sex-ratios of *Pareuchaetes pseudoinsulata* in the family Arctiidae, showed that maximum fecundity of 214.7 eggs were realized for 1:1 female-male ratio as opposed to 161.3 for 1: 2. Most larvae developed through 5 instars, although there were individuals with 6 instars. In each larval moult the setae abundance increased and the larva coloration also changed from the third instar. The number of larval instars for arctiids has been stated to be as low as 5 and as high as 7 for different species (Otazo *et al*., 1984; Betzholtz, 2003; Gomi *et al*., 2003), In this study, although food was made available to adult insects, they were not observed to feed and this underscores the fact that many adult moths (capital breeders) do not feed at all and rely completely on reserves accumulated during the larval stages (Tammaru and Haukioja, 1996).

The principles underlying the success of biological control agents rest on the possession of ecological attributes such as high reproductive capacity or the rate of reproduction, host specificity and high fecundity. Crawley (1989) found that characteristics that predicted ability of natural enemies to establish also broadly predicted success, including small size, high voltinism and high fecundity. The short development time of 39 to 45 days of the lepidopterous species found indicate that several generations could be produced during the growing period of aerial yam and could enhance their ability as biological control agents. The best growing period of Aerial yam according to this study was found to be about nine months (March - October). The growing period therefore is long enough to support about 4 to 5 generations of the lepidopterous species identified from the study. This finding corroborate with that of Symondson *et al*. (2002) who indicated that good biocontrol agents produce large numbers of offspring which can complete more than one generation during the life cycle of the pest.

In the biological control of pests, the most important attribute of a potential biological control agent is its host specificity. The first step according to De Nardo and Hopper (2004) involves the collation of all recorded information on field hosts of not only the candidate biological control agent, but also of closely related species. The scarabs identified in the study exhibited a narrow host range in that close relative of aerial yam of economic significance were not attacked. The validity of the Technical Advisory Group (TAG) guidelines approach is supported by the historical data that indicates that close relatives are most likely to suffer damage (Pemberton, 2000; Sheppard *et al*., 2005). Field and laboratory investigations have revealed that the lepidopterans found in 2005 voraciously fed very well on both leaves and bulbils of aerial yam. Unfortunately, they defoliated other dioscorean species and therefore exhibited broad host status questioning their candidacy for biological control of aerial yam. Natural enemies with broad host ranges are more apt to utilize alternative sources of nutrition and remain in the habitat when hosts are scarce (Wiedenmann and Smith Jr. 1997). The *Anomala* sp. even though fed on *D. alata* is worth considering since the crop, according to FLEPPC (2003) is considered a category I invasive species in the United States. Similarly, Wunderlin and Hansen (2003) reported that like *D. bulbifera, D. alata* is widely naturalized in Florida where it has been reported from nine counties. According to Pemberton (2000) and Sheppard *et al*. (2005), a few *Dioscorea* species other than aerial yam are present on the continental USA. However, the family *Dioscoreaceae* is poorly represented in North America, north of Mexico. The two native species that are sympatric with aerial yam are from a different subgeneric taxon than the weed. As herbivore host range may be limited by taxonomic affinities and lack of sympatry with potential host species, Wheeler *et al*. (2007) proposed that this weed will be a relatively safe target because of taxonomic and geographic isolation from desirable native and economic plant species. Insect species under investigation showed a distinct host preference for fresh foliage and bulbils, whereas the observed impact on plant vigour appeared negligible. The host range of potential biocontrol agents is a critical factor in biological control. The arctiid moths, *Diacrisia and Estigmene* species did not demonstrate preference for any one of the dioscorean species in the laboratory studies. Caterpillars of these species have been described by Capinera *et al*., (1987) as polyphagous and therefore attack all host species and possibly other plant genera; therefore, they may not be suitable as biological control agents. The negative impact of the scarabs *Anomala* sp. and *Adoretus pullus* on the weed was small. This study suggests that mature aerial plants can tolerate rather high herbivore (scarabs) loads. However, the impact exhibited by the lepidopterans can be described as highly devastating since leaves as well as bulbils were consumed although some preference was shown for bulbils.

Bulbils that had any feeding damage to the primary meristematic region do not sprout. The ability of the larvae to feed on the bulbils is also important because the bulbils are the primary means of persistence and spread of the plant. However, when larvae were transferred onto fresh bulbils as neonates, high mortality was recorded. This could probably be as a result of their undeveloped mouth parts to chew the bulbils.

The fecundity of the two species of arctiid moths did not differ much. The phenotype of eggs laid by both species was similar but *Estigmene* species laid more eggs as compared to that of *Diacrisia* species. The high fecundity trait exhibited by the moth species indicate that their intrinsic rate of increase as natural enemy will prove to the task of controlling the invasive aerial yam weed. Agent fecundity offer great influence on the success of biological control, (Lane et al., 1998) The longevity for both moth species followed the same pattern. Generally, the females lived longer than the males either mated or unmated in the study, and, according to Arakawa *et al.* (2004) females live longer than males emerging from the same hosts in most insects. The unmated male however showed significant differences between the different yam species for *Diacrisia* species possibly because of the food source or as a result of environmental effects. According to Raupp and Denno (1983), seasonal phenologically related changes in the nutritional quality and secondary substances of host plants have been reported to affect basic biology (e.g., reproductive capacity and longevity) of herbivorous insects. Although not evaluated in this study, such factors may account for the observed differences between the *Dioscorea* species tested.

6.0 Conclusion and recommendation

6.1 Conclusion

In the studies, it was found that both cultivated and wild forms of aerial yam exist in Ghana. A considerable number of phytophagous insect fauna were observed attacking the foliage and bulbils of *D. bulbifera*.

Two lepidopteran species (*Estigmene* and *Diacrisia*) were easily reared on aerial yam. No-choice tests in the laboratory demonstrated that all selected non-target species were attacked and were largely suitable for insect development. Larvae of both lepidopteran species completely devoured all the *Dioscorea* species provided them and successfully completed their development on leaves and bulbils. Generally, *Estigmene* and *Diacrisia* species do not appear to be good biological control candidates but their consumption preference for bulbils, the main source of propagation and spread, and considering the poor representation of *Dioscorea* species of economic importance in the United States of America, they could be valuable.

Two species of beetles were however, found to cause considerable damage to the plant. Although most of the foliar damage observed in the field was caused by the *Anomala* sp. and *Adoretus* sp. the damage caused could not affect the performance of the plants.

Anomala species, consumed *D. bulbifera* and *D. alata* but not rotundata yam species. However, the damage they caused to aerial yam was rather small. This species even though exhibited a very narrow host range, attacking only *D. alata* together with *D. bulbifera* may not be suitable candidate for the control of *D. bulbifera* because they fed only on the leaves and also their feeding could not impact negatively on the performance of the plant.

6.2 Recommendation

It is however recommended that further studies be carried out to study the biology and evaluate the level of predation of the *Anomala* species to support their candidacy for biological control of aerial yam.

Ecological and economic impact of *D. bulbifera* in USA call for further evaluation of the moth species identified during the study for the control of the weed.

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Appendix 1.

QUESTIONNAIRE

Farmers' indigenous knowledge of *Dioscorea bulbifera*
(Aerial Yam)

A:

Location ……………………………………………… Date ………………………
Geoposition: Latitude …………………………….. Longitude ………………….
Elevation ………………………………………….
Name of Farmer …………………………………… Sex …………………………
Years of farming ……………………………………
Yam cultivars grown …………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………

Time of planting ………………………………………………………………………
Acreage ………………………………………………………………………………….

B:

Usage
Quantity used ……………………………………… Quantity sold ……………………….
Types of dishes ……………………………………………………………………………
…………………………………………………………………………………………

C:

Awareness of aerial yam
Cultivated ………………………………………… Grown in the wild ………………….
Region …………………………………………… Town ………………………………
Ecology …………………………………………………………………………………….

Uses: (Medicinal, Famine food etc) ……………………………………………..

Problems associated with crop …………………………………………………………….

Insect Pest

a) Field
b) Storage

Other pests (if any) ……………………………………………………………………..
Popularity …………………………………………….. Why? ……………………….
Problems created by crop (if any) ……………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………

Appendix 2.

SCORING SCALE FOR PERCENT DEFOLIATION

Score	Description
1	0 – 25 % of leaves damaged
2	25 – 50% of leaves damaged
3	50 – 75 % of leaves damaged
4	75 – 100 % of leaves damaged