COTTON PESTS AND NATURAL ENEMY INTERACTION IN UGANDA: A BASIS FOR BIOSAFETY RISK ASSESSMENT

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DECLARATION

This thesis is my original work and has not been presented for any degree in any other university.

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This piece of work is dedicated to my children, Muchwezi Fahad, Kisakye Jeremiah, Hayyan Kisembo, Kabachwezi Husnah and Kasemire Rhaufa.

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ABSTRACT

Cotton in Uganda is attacked by many insect pests causing yield losses of between 30 – 80%. The biggest losses are caused by members of Lepidoptera. Use of chemical insecticides have been by been used as an approach to address the pest problem in cotton. Nevertheless, because of the cryptic nature of Lepidopteran pest on cotton, chemical pesticides have not been very effective in controlling the pests. Furthermore, pesticides are expensive and are associated with a number of problems. Consequently there has been a need to access new technologies for managing the pest of cotton and one such technology is genetic engineering. With the current advances in the use of genetic engineering in management of cotton pests, there is increasing pressure to adopt these technologies. However, before this technology can be introduced it is important to assess the biosafety risks associated with the technology. It is important to understand the danger and potential long term impacts the technology will have on the environment and associated ecosystem services. Article 16.2 of the Cartegena Protocol calls for assessments of the risk to the environment before introduction of geneticall modified organisms are introduced.

Understanding a good risk assessment requires significant amount of information on plant-natural-enemy interactions, a process that creates and maintains ecological processes. There is paucity of information on arthropod species and ecological processes in cotton growing systems which make it difficult to assess the risks that may be associated with the introduction of genetically modified cotton. And because there are very many species in and near the cotton cropping system, it is impossible to evaluate the risk to every species. It is therefore important to identify the different and most relevant species or ecological processs in the target region of release of the geneticall modified cotton. It's against this background that the work reported in this thesis was under taken to: document species and ecological processes of arthropods that occur in the cotton cropping system; establish and prioritize the potential likely exposure and adverse effect pathways of the transgene to non-target cotton arthropods; understand the dynamics of some selected cotton pests and their natural enemies in cotton as influenced by *Bt*-biopesticide and understand the influence of inherent plant factors on the searching behaviour and sensory ability of the ladybird beetles.

The study results identified that the arthropod community in cotton which is comprised of 67.0% beneficial arthropods and 33.0% herbivores (target and non-target) is categorized in three priority groups with 41% of the arthropods in the highest, 32% in the intermediate and 27% in the lowest priority category of being affected if GM cotton was to be introduced. The study further developed adverse-effect scenarios for the priority arthropod and process through bi- and multi-trophic exposure pathways. In the species assessment, an adverse-effect scenario was mainly through hypothetical changes in a population parameter, population density or with a behaviour resulting from the possible exposure. These changes alluded to a possible change in ecological functions or functions of the population which were then evaluated as adverse or beneficial. These potential adverse effects pathways and damage potential helped in the formulation of different generic hypotheses.

The proxy for GM-Cotton, XenTari (*Bt*-biopesticide) had very minimal control over aphids, but impacted the survival of the ladybird beetles. The results therefore revealed that, if XenTari which targets lepidopteran pest was introduced, the aphids will constantly be exposed. This exposure may cause increased fitness of the aphid and subsequently increasing their outbreak potential. Secondly, the limited control of aphids by XenTari may further lead to an increase in the aphid populations and subsequent continued use of chemicals. Ladybird behavioral studies highlighted that, the main events and sequence of behaviors exhibited were; searching, encountering and feeding on the prey. Whereas searching was the most frequent behavioral event for both the adults and larvae, more time was registered on feeding by the larvae. These findings lay a foundation to bridging some of the information gaps in preserving the plant-pest – natural enemy interactions and biodiversity in general as insect-resistant genetically modified cropping systems become a cornerstone tool of modern integrated pest management and provide a framework for biosafety risk assessment for cotton and other crops in Uganda.

CHAPTER ONE

1.0 Introduction

Cotton has been Uganda's major source of foreign exchange revenue since the beginning of the last century. It is produced in all regions of the country; most of the production being concentrated in the northern, eastern and south western regions (Walusimbi, 2002). Cotton was Uganda's most important export commodity Until 1950s, when it was over taken by coffee (Sekamatte and Heneidy, 1997). In 1969/70 with production of 465, 000 bales of lint, cotton contributed about 40 percent to foreign exchange earnings. However, in the 1980's, when the country experienced political and economic turmoil, production hit its lowest level of 11,000 bales (LMC – International, 2002, Sserunjogi *et al*., 2004). However, in the 1990's following strategic investments by the government of Uganda and the World Bank under the Cotton Sector Development Project, some recovery was registered (FAOSTAT, 2003). Cotton and cotton textile industries are important in the economy of both developed and developing countries. For many cotton farmers, it provides income for education, health housing, and transportation and often serves as a catalyst for industrialization and increasing welfare (Serunjogi *et al.,* 2001). Despite the strategic importance of cotton in national economic development, its productivity in most parts of the world is steadily declining (James, 2002). The production decline is attributed to several production constraints - which include: high pest infestation, low productivity mainly attributed to inefficient on-farm technologies such as hand hoe cultivation; limited availability of key inputs (fertilizer, seed and pesticides); insufficient research and extension services; limited access to credit for small farmers; low producer prices and low profitability compared with other prevalent crops (Walusimbi, 2002). However, primary among these production constraints is pest infestation which often times necessitate control through intensive use of synthetic chemicals and the use of high yielding cultivars geared at managing insect pests that are a major problem in cotton production (Naranjo *et al.,* 2008).

Several insect species mainly belonging to the order Lepidoptera are serious pests of cotton worldwide (Gutierrez et al 2005) with the cotton bollworm complex causing significant losses of between 30% - 80% in Uganda if not controlled (Horna *et al*., 2009). Control of these pests has traditionally depended on the use of insecticides, cultural methods, use of resistant varieties, and most recently, the use of transgenic *Bt* cotton. Apparently in Uganda, the most effective method of control is the use of broad-spectrum insecticides, which, however, is associated with pest resistance to insecticides and environmental problems. Secondly, resource-poor farmers often cannot afford pesticides, leading to decline in cotton production, and therefore there is a need to access new technologies and crop management techniques aimed at increasing crop productivity (Horna *et al*., 2009; Paarlberg 2008) through transferring insect resistance genes across the barriers to conventional plant breeding by use of plant genetic engineering (Abro *et al.*, 2004).

Genetic engineering is the one of the tools used in producing resistant varieties that are resistant to pests (Ania Wieczorek, 2003). It is the use of a series of techniques to transfer genes from one organism to another or to alter the expression of an organism's genes, focusing on producing transgenic resistant plants that will help in insect pest control.

In Africa, South Africa is leading in the use of *Bt* technology where it has had a substantial positive impact on the cotton industry (Hofs *et al.*, 2006). In general terms, it is estimated that between 1996 and 2005 the deployment of *Bt* cotton reduced the volume of insecticide active ingredients used for pest control in cotton by 94.5 million kilograms and increased farm income through reduced costs and improved yields by US$ 7.5 billion, with most of the benefits accrued by farmers in developing nations (Naranjo *et al*., 2008).

1.1 Problem Statement

Whereas the benefits of transgenic crops seem impressive, some regulators, consumers and environmentalists feel that inadequate effort has been made to understand their dangers and

potential long-term impacts (Qaim, 2009). Minimal amount of information or in some cases, misinformation has generated anxiety and caused concern about the impacts of the GM crops to the environment, ecological processes and biodiversity in general (Ania-Wieczorek, 2003; Andow *et al*., 2006). Therefore, responsible utilization of genetically modified crops requires that the associated potential biosafety concerns be addressed through a risk assessment (Songa *et al*., 2003), one of which is the effects of *Bt*-cotton on non-target arthropods. Assessing risk of GM crops to non-target biodiversity is challenging, both to the assessors and scientists interested in providing data relevant to a risk assessment. Because total diversity can be explained only in the field environments, the early stages of non-target assessments, prior to release in the environment, must rely on species based procedures. However, there are so many species that one of the main challenges is determining which species should be evaluated (Andow *et al.*, 2006). The objective of this study was therefore to identify and determine the relative abundance of the target and non-target arthropods of *Bt*-cotton in the cotton growing regions in Uganda and which of these could be tested for the potential impacts of GM crops.

1.2 Justification of the Study

Like any other new technology, transgenic cotton if introduced in Uganda might cause unpredictable consequences or risks while playing an important role in pest control. There is little or no knowledge regarding the potential impact on ecological environments, alternate trophic levels in food chain and their symbiotic relationships that favour the crop-natural enemy interactions which might be caused by extensive planting of genetically modified plants. The large-scale release of transgenic cotton may cause the materialization of potential dangers that are unlikely to happen when planting on small scales (Wu and Guo, 2005). The environmental risks caused by large-scale planting of transgenic cotton mainly involve changes in the populations of secondary pests of target insects such as aphids, spider mites and mirids, leading

to new problems in controlling cotton pests (Wu et al., 2002; Wilson *et al.,* 2007; Wu Kong-Ming, 2007). The toxic effects of transgenic cotton on lepidopteran insects may affect the food chain in the agro-ecosystem and lead to decreased biodiversity and imbalance of the ecosystem; and the occurrence of resistant target pests can lead to its ineffectiveness. With the transgenic crops being taken on as a new technology in pest management, their potential effect on ecosystem services need to be studied before they are approved for release. In Uganda, the last comprehensive profile of arthropods which has provided a useful basis for designing effective control strategies to avert the losses caused by pest infestations on cotton was conducted and documented in 1960s (Hill and Waller, 1988). However, it is possible that the pest profile documented over four decades ago has since changed due to excessive usage of pesticides and variability in the farming systems. Knowledge of the pest profile is necessary to support evidence-based approaches such as use of *Bt* cotton at farm level which would help reduce the dependence on pesticides with regard to recent approaches in pest management while at the same time taking environmental safety into consideration (Munduru, 2010; James, 2003). It is therefore necessary to establish the current profile of arthropods in cotton farming systems in Uganda. It's against this background that a study of cotton pests and natural enemy interactions – as a basis for biosafety risk assessment in Uganda was conducted.

1.3 Specific Objectives

1. To document species and ecological processes/functions of arthropods that occur in the cotton growing system in Uganda

2. To establish and prioritize potential likely exposure and adverse effect pathways of genetically modified cotton to the non-target arthropods

3. To understand the dynamics of cotton aphids and their ladybird natural enemies in cotton as Influenced by *Bacillus thuringiensis* (XenTari) Bio-pesticide – a surrogate for the *Bt*-cotton and dimethoate in the presence of Lepiosiota ants

4. To understand the influence of inherent plant factors on the searching behavior and olfactory orientation of ladybird beetle attacking cotton aphids

1.4 Hypotheses

1. Different cropping systems and agro-ecological zones in Uganda have varying species of cotton-associated arthropod that are likely to be affected by new pest management technologies
2. The non-target species that are not exposed directly or indirectly to the transgene products and their metabolites are less likely to be affected by the genetically modified crop.
3. The use of *Bacillus thuringiensis* (XenTari) biopesticide and black garden ants (*Lepiosiota* spp) has an influence on the population dynamics of the cotton aphid (*Aphis gossypii:* Homoptera: Aphididae) and its Coccinellidae beetle natural enemy.
4. The introduction of transgenic cotton will alter the inherent plant factors and therefore affect the efficiency of the foraging biological control agents in the different cropping systems and agro-ecological zones in Uganda.

1.5 Scope of the Study

The field survey studies were based in Kasese, Lira and Pallisa districts. These were chosen because they are some of the major cotton growing districts in Uganda producing above 13,000 bales per year for Lira and Palisa and between 7,000 – 13,000 for Kasese as per the Uganda

Household National Survey 1999 - 2000 (Liangzhi and Chamberlin, 2004). The experiments were carried out at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK). The content scope of the study was confined to documenting species and ecological processes of arthropods that occur in the cotton cropping system; establish and prioritize the potential exposure and adverse effect pathways of the transgene to non-target cotton arthropods; establishing the dynamics of some selected cotton pests and their natural enemies in cotton as influenced by *Bt*-biopesticide and investigating the influence of inherent plant factors on the searching behaviour and sensory ability of the ladybird beetles.

CHAPTER TWO

2.0 LITERATURE REVIEW

Genetically modified (GM) crops, also known as transgenic or genetically modified crops, are becoming an increasingly dominant feature of agriculture Worldwide (Hilbeck 2001; James 2007, O'Callaghan *et al*., 2005). Transnational corporations, the main proponents of genetically engineered crops, argue that carefully planned introduction of these crops should reduce crop losses due to weeds, insect pests and pathogens. They hold that the use of such crops will also benefit the environment by significantly reducing the use of agrochemicals (Abro *et al* 2004). It has been suggested that, 'if adequately tested', genetically engineered crops may promote a sustainable environment (Braun and Ammann 2003). This view is, however, not universal and environmental organizations and anti-GM lobby groups such as Greenpeace oppose genetically engineered crops (Qaim 2009). Scientists are intensely involved in investigating the possible adverse effects of genetically engineered crops and the literature in this field is large and growing explosively. The question whether we have learnt sufficiently from the past, particularly from the naive optimism with which pesticides were embraced in the mid 20th century is being raised (Herren, 2003; Tappeser, 2003). Experience since the mid-1990s, after which genetically engineered crops were widely grown and intensively investigated, suggests some significant advantages over conventional crops (O'Callaghan *et al*., 2005; Groot and Dickie, 2002; Lovei and Arpaia, 2005; Qaim 2009).

The government of Uganda is currently testing the performance of genetically modified cotton (insect resistant and herbicide tolerant) varieties. The government has recognized the potential of genetically modified (GM) cotton to improve cotton production and thus producers' livelihoods and the economy in general (Horna *et al*., 2009). The introduction of genetically

modified crops in Uganda is likely to generate a wide portfolio of concerns even if proven safe by scientists – as it has in other African countries (Kikulwe, 2010; Paarlberg 2008).

A number of ecological and economic issues need to be addressed while considering the production and deployment of transgenic crops globally and Uganda in particular (Hilbeck *et al.*, 2006; Horna et al 2009; Kikulwe, 2010). Concerns still exist with regard to their long term and potentially negative effects upon non target components of the food chain (O'Callaghan *et al*. 2005). Their role in integrated pest management is poorly understood (Groot and Dickie 2002; Lovei and Arpaia 2005).

2.1 Transgenic Crops and *Bt*-Formulations in Pest Management

The introduction of transgenic plants brings a new system of host plant resistance into play (Andow, 2008). The specificity of *Bt* is such that it was expected to have no direct effects on natural enemies, although indirect effects such as those from the sick and sub optimal prey might be expected (Obrist *et al.*, 2005, O'Callaghan *et al*., 2005, Sanvido *et al*., 2006, Torres *et al*., 2006). Adoption of sprays based on *Bt* formulations will be affected by their limited spectrum of activity as compared to chemical insecticides and because of their and lower efficacy compared to synthetic chemicals (Toprak *et al.*, 2007). In contrast, transgenic plants appear to be sufficiently effective to either displace chemicals or can be used in conjunction with insecticides or other methods of pest control, making such plants attractive from the standpoint of environmental protection (Carpenter *et al*., 2002; Kumar *et al*., 2008). Expenditure on insect control was reported to be marginally reduced using Bollgard II® technology for the years 2003–2005 (Brookes and Barfoot, 2005; Brookes and Barfoot, 2008b). In China, decrease in the use of pesticides has also been registered with an average of 6.6 applications in *Bt* cotton compared with 19.8 applications per season in non-*Bt* cotton (Huang *et al*., 2002; Huang *et al*., 2003; Wu and Guo, 2005). A further environmental benefit from reduced insecticide use on genetically

modified insect resistant cotton crops is the reduction in fuel use, and consequently lowered greenhouse gas emissions (Brookes and Barfoot, 2008a). However, it should be noted that transgenics are not a panacea for solving all the pest problems and related environmental aspects. With the development of transgenic crops, the secondary pests will no longer be controlled in the absence of sprays for the major pests. The cotton aphids, *Aphis gossypii*, which were once suppressed by early season sprays of endosulphan for *Helicoverpa* spp. in Australia, became more troublesome in transgenic cotton due to the absence of these insecticidal sprays (Wilson *et al.*, 2007) and so were the mirids in China (Wu *et al*., 2002; Wu Kong-Ming, 2007). This raises the need to control the secondary pests through chemical sprays which will kill the natural enemies and thus offset one of the major advantages of transgenics. The cost of producing and deployment of transgenics may be very high. Proximity of transgenic crops to sprayed fields and insect migration may reduce the effectiveness of transgenics and development of resistance in insect populations. This may subsequently limit the usefulness of transgenic crops for pest management (Sharma & Rodomiro, 2000) and hence all the above issues and challenges need to be addressed.

2.2 Assessing the Ecological Threat of Transgenic Crops

The potential for transgenic crops to threaten biodiversity conservation and sustainable agriculture is substantial (Altieri, 2005). Different studies have explored several aspects of transgenic crops, such as environmental impacts (Dale, 2002; Fontes *et al*., 2002; Hails, 2003; Jank and Gaugitsch, 2001), effects on ecosystem services (Lovei, 2001; Lovei *et al*., 2007), farm biodiversity (Firbank, 2003), invertebrate fauna (Brooks *et al*., 2003; Haughton *et al*., 2003; Hawes *et al*., 2003; Roy et al., 2003), development of Bt resistance insect strains (Cerda & Wright, 2002), effects on weed abundance and diversity (Heard et al., 2003a, 2003b), changes in plant community structure resulting from gene flow (Gildings, 2000; Pascher and Gollmann,

1999), ethical and social-economical considerations (Dale, 2002; Kikulwe, 2010). Meta-analyses of the extant literature on invertebrate non-target effects reveals that the pattern and extent of impact varies in relation to taxonomy, ecological or anthropomorphic guild, route of exposure and the non-Bt control against which effects are gauged. However, these meta-analyses point to gaps in knowledge of certain functional guilds, especially in field studies (Naranjo 2009).

The potential impacts of these crops are mainly associated with ecological processes operating in the agroecosystems through food chains (Lovei *et al*., 2007). Most if not all nontarget herbivores colonizing genetically modified crops in the field, although not lethally affected, ingest plant tissues containing Bt protein that they can pass on to their natural enemies (Andow and Hilbeck, 2004; Birch and Wheatley, 2005; Hilbeck *et al*, 2008). The fact that natural enemies can be affected directly through inter-trophic level effects of the toxin present in the genetically modified crops, raises concerns about the potential disruption of the crop – pest natural enemy interactions. The disrupted biocontrol mechanisms will likely result in increased crop losses due to pests or to increased use of pesticides by farmers, with consequent health and environmental hazards (Altieri, 2005; Naranjo, 2005; Marvier *et al*., 2007). Such fears have caused widespread public reservations that have led to a complex system of regulations which is a real threat for the further development and use of transgenic crops (Qaim, 2009; Kikulwe , 2010).

2.2.1 Effect of transgenic crops on tritrophic interactions of arthropods

The environmental advantages of pest control using formulations of the bacterium *Bacillus thuringiensis* (Bt) rather than synthetic insecticides are well known (Glare & O'Callaghan, 2000). However, field cultivation of Bt transgenic plants that continuously produce Bt protein throughout the growing season is relatively new and has evoked many concerns. Bt-proteins

present in transgenic corn have been detected in root exudates in soil (Saxena *et al.* , 1999), in pollen drifted to areas adjacent to fields (Jesse & Obrycki, 2000), in spider mites, thrips and leafhoppers fed Bt corn (Dutton *et al.* ,2004), in honeydew produced by plant hoppers fed on Bt rice (Bernal *et al.* , 2002) and in non target chewing herbivores (Howald *et al.* , 2003; Dutton *et al.* , 2003). The acquisition of Bt proteins by non target herbivores and by lepidopterans with low susceptibility to Bt transgenic crops indicates that Bt proteins can be transferred among trophic levels and may interfere with established food webs. However, each system is unique, being affected by the specific Bt protein, the promoter used to drive gene expression, plant species and tissues, genetic background, rainfall, soil type and soil fertility (Sachs *et al.* , 1998; Greenplate, 1999; Adamczyk & Sumerford, 2001). For example, Cry1Ab expression levels in corn are two-fold higher than Cry1Ac in cotton and the same trend occurs when both toxins are inserted in cotton plants (Perlak *et al.* , 1990; Sachs *et al.* , 1998). In addition, Cry1Ac present in Bt cotton terminal foliage can range from 19.1 _ g/g dry weight in cotton cultivated in Georgia to 125.6 _ g/g dry weight in cotton cultivated in Mississippi and vary between years and locations (Greenplate, 1999). Cry1Ac protein expression in Bt cotton is clearly influenced by environmental factors and these may differentially affect tritrophic associations (plant – herbivore – natural enemy). The cotton ecosystem supports a substantial complex of arthropod pests and natural enemies. Three major groups of predatory insects (heteropterans, coleopterans and neuropterans) are recognized as important natural enemies of key and secondary pests in cotton, and these predators are capable of consuming non pest arthropods to sustain their populations (López *et al.* , 1996). Herbivores in cotton may not be susceptible to Bt proteins but still may acquire Bt protein from the plant and convey it to higher trophic levels. Conveyance of Bt proteins in the prey/host body to predators and parasitoids has been investigated as a potential route for non target impact of Bt transgenic plants (Raps *et al.* , 2001; Head *et al.* , 2001; Bernal *et al.* , 2002; Dutton *et al.* , 2003; Schuler *et al.* , 1999 and 2001). The risk of Bt- protein

exposure to predators and parasitoids has been studied in transgenic corn under controlled conditions (Hilbeck *et al.* , 1999; Head *et al.* , 2001; Raps *et al.* , 2001; Dutton *et al.* , 2002). In the cotton ecosystem, it is possible that species moderately or not susceptible to Cry1Ac can acquire the protein from the plants and expose it to the third trophic level. Common species of lepidopterans in cotton fields only partially affected by Bt cotton (*Spodoptera* and *Pseudoplusia*) (Stewart *et al.* , 2001) could convey Cry1Ac to their predators. In addition, omnivorous predators that occasionally feed on plants may be directly exposed to Bt proteins. Although carnivory is the rule for coccinellids, chrysopids and predatory heteropterans, omnivory can occur in these species, and direct feeding on plants or their products, such as pollen and nectar, has been considered an important life history strategy (Coll & Guershon, 2002; Eubanks *et al.* , 2003). Therefore investigating if the Cry1Ac protein expressed in transgenic Bt cotton plants moves from plants to herbivores and subsequently to their predators in the cotton system i.e. potential exposure and adverse effect pathways becomes very imperative.

2.2.2 Effect of transgenic crops on aphids and their natural enemies

Due to the reduction of broad spectrum insecticides in *Bt* cotton, herbivores which are not targeted by the *Bt* protein survive and occasionally reach pest status (Naranjo *et al.,* 2008). To retain the biological control function provided by naturally occurring antagonists of herbivores – i.e. predators and parasitoids– and to prevent non-target pest outbreaks, the potential risk that GM crops may pose to natural enemies should be addressed as part of the environmental risk assessment prior to the commercial release of any novel GE crop(Garcia-Alanso *et al*., 2006; Romeis *et al*., 2008). Several studies have examined the effect of *Bt* crops on herbivores and arthropod natural enemies in recent years confirming the highly selective mode of action of the deployed *Bt* Cry proteins (Romeis *et al.,* 2006; Wolfebarger *et al.,* 2008).

Aphids generally play an important role in agricultural food webs since they serve as hosts or prey for a variety of parasitoids and predators. Consequently, the question whether aphids are affected by the *Bt* crop and whether they expose their natural enemies to the plant-expressed *Bt* protein is of high relevance. Studies available to date provide no evidence that *Bt* crops, expressing Cry1A proteins for the control of pest Lepidoptera, cause direct adverse effects on aphids (Schuller *et al.,* 2001; Dutton *et al*., 2002). This is not surprising, since the *Bt* protein does not appear to be ingested by aphids which feed on phloem-sap (Dutton *et al*., 2002; Torres *et al*., 2006; Romies & Meissel, 2010). Surprisingly, two studies have reported considerable amounts of *Bt* Cry proteins in aphid samples (Zhanga-F *et al.,* 2006; Obrist *et al.,* 2006; Schuller *et al* 2005). In addition, aphids might carry toxin containing faeces of other arthropods or plant parts (Romies & Meissel, 2010). It should also be noted that change in the use of promoters used for Cry protein expression in GM have an effect of the presence of the proteins in the phloem sap (Shi et al. 1994; Kanrar et al. 2002; Rahbe´et al. 2003; CERA 2010). For future GE crops, however, which may contain different promoters and/or different insecticidal proteins, the presence of the expressed protein in the phloem sap and hence in the aphids, cannot be ruled out (Kehr 2006). Further, aphids and other phloem feeders produce honeydew which is an important source of carbohydrates for sugar feeding arthropods, including hymenopteran parasitoids and aphid predators (Wackers, 2007). Sugars can enhance parasitoid reproductive fitness by increasing their longevity, fecundity and/or parasitism rate (Fadamiro & Heimpel, 2001; Wackers *et al*., 2008). However, honeydew can be a relatively unsuitable sugar source as a result of unfavorable sugar composition (Winkler *et al.,* 2006; Hogervost PAM *et al*., 2007; Wackers *et al*., 2008). Honeydew nutrient composition could also be altered as a result of plant transformation and this posses an exposure route. These results therefore suggest that aphids represent route of exposure of Cry proteins to natural enemies. The risk of natural enemies that exclusively or predominately feed on aphids becomes very important. Consequently, non-target

organisms' studies to support the environmental risk assessment of Bt crops need to focus on natural enemy species that are known to consume those host or prey species with Bt Cry protein.

2.2.3 Effect of transgenic crops on natural enemy habitat

Agro-ecosystems and adjacent habitats are the first environments to be exposed to GM crops and their products, and therefore they are correctly considered in risk assessments (Lovei 2001; Jorg *et al*., 2008) . Nevertheless, the spatial-temporal dispersion of GM products can influence other trophic chains that in specific cases might become relevant (Lovei 2001; Jorg *et al*., 2008). For example, pollen dispersal might bring other herbivore species in contact to newly-expressed toxins at distances from crop fields; pollen and plant residues may enter water bodies surrounding cultivated fields and therefore enlarge the possible exposure to expressed toxins (Arpaia *et al*., 2006). Trophic chains do not stop with predatory arthropods, such as spiders, but commonly involve other taxa, for instance, birds. It must be also noted that in some conditions non-arthropod herbivores (e.g. snails, rodents, wildlife) may commonly feed on cultivated plants. Agriculture depends on several ecosystem functions that are essential to soil fertility and agricultural productivity (e.g. microbial decomposition and nutrient cycling, crop pollination by animals, biological control of pests). Each of these ecosystem functions are mediated by several guilds of animal species. Therefore, in any given cropping system, many hundreds of arthropod species, thousands of microbial species, and scores of ecosystem functions can be found (Curtis *et al.*, 2002). It is therefore important to understand the impacts of GM technique on all potentially exposed species.

2.2.4 Effect of transgenic crops on altered plant phenology and chemistry

Genetic engineering is expected to play an important role in improving the quantity and quality of biomass and overall plant characteristics (Hisano et al., 2009; Li and Qu, 2011). Among the factors that influence pest-natural enemy interaction is the host plant characteristics and extensive host range. Leaf hairiness has been observed to cause some changes in the behaviour

of the parasitoid *Eretmocerus mundus* as it parasitizes *Bemisia tabaci* (Otim *et. al*., 2008). Reduction in the net searching speed of *Leptomastix* on dense trichomatous leaf lamina of Passiflora compared to citrus leaf lamina with no trichomes has also been observed (Mozaddedul and Copland, 2003). Parasitism rates have also been observed to vary with different vegetable species and other plants depending on hairlines (Greathead and Bennet, 1981; Headrick *et al*. 1995; Headrick *et al*. 1996).

The production of GM crops has grown from the cultivation of a few million hectares in three countries in 1996 to 42 million hectares in 20 countries as of 2007. GM crops represent an important tactic in the integrated pest management toolbox, providing effective control of certain key pests through host plant resistance, and contributing to the overall development of robust pest management systems. The assessment of environmental risk posed by GM crop growing continues to be a topic of research and debate and this study aims at addressing some of these concerns.

CHAPTER THREE

3.0 PROFILING OF ARTHROPOD SPECIES COMPOSITION, ARTHROPOD MEDIATED AND ECOSYSTEM SERVICES IN COTTON CROPPING SYSTEMS OF UGANDA

3.1 Introduction

More than 1000 insect species have been recorded in cotton agrosystems however, the number of economically important pests is relatively small (Sujii *et al*., 2006; Hilbeck *et al.,* 2008). A comprehensive profile indicated that cotton in Uganda, is attacked by a complex of insect pests in the families; Gelechiidae, Tortricidae, Tetranychydae, Miriadae, Aleyrodidae, Aphididae, Acaridae and Pyrrhocoridae (Hill and Waller, 1988). Although this classification, has for the last four decades provided a useful basis for designing control strategies to avert the losses by cotton pest infestations in Uganda (Munduru, 2008), it did not consider the ecological roles of other arthropod species. Consequently little or no work has been conducted to update or establish the current arthropod and their ecological function profile on cotton in Uganda. Furthermore, most of the work on cotton in Uganda has been breeding for resistance against Jassids (*Empoasca* spp) (Ogwal *et al.,* 2003; Sserunjogi *et al.,* 2004) without taking into consideration the impact of these actions on arthropod species composition. It is thus, possible that the pest profile documented over four decades ago has since changed (Hillocks 2005; Wu Kong-Ming 2007).

The need to have a proper profiling of the arthropod fauna in cotton is greater than ever before, because of the increased pressure to introduce new pest management technologies to reduce the increased yield losses due to pest pressure. Particularly, the potential of GM cotton in improving cotton production and producers` livelihoods in general has been recognized in Uganda (National Cotton Research Strategy, 2005; Horn *et al*., 2009).

However, for genetically modified cotton to be introduced there must be a comprehensive risk assessment and a clear understanding of interactions between genetically modified cotton, existing arthropod populations and their ecosystem services. As already observed, the introduction of genetically modified crops in any agricultural ecosystem may have a number of consequences on arthropod populations (Obrist *et al*., 2005; O'Callaghan *et al*., 2005; Sanvido *et al*., 2006; Torres *et al*., 2006; Wilson *et al.*, 2007 and Wu Kong-Ming, 2007). For example Hilbeck *et al*., (1998) reported a negative effect to lacewing larvae on the consumption of genetically modified corn pollen while Dutton *et al.,* (2003) and Romeis *et al.,* (2001) reported the opposite. Therefore, while looking at the prospects of introducing genetically modified crops, there is need to build baseline biodiversity data (Songa *et al*., 2003; Birch *et al*., 2004) through establishing the arthropod and their ecological profile to allow for proper risk assessment. It is against this background that this part of the study was conducted to document arthropod species composition in conventional cotton cropping systems and the ecological functions of the respective species in Uganda. The study was based on a hypothesis that different cropping systems and agro-ecological zones in Uganda have different cotton-associated arthropod species that are likely to be affected by new pest management technologies.

3.2 Materials and Methods

The study was carried out in two consecutive cotton growing seasons of 2006 and 2007 in the districts of Kasese (southwestern Uganda), Pallisa (eastern Uganda) and Lira (northern Uganda). These three districts were purposively chosen because they are some of the major cotton growing districts in Uganda producing above 13,000 bales per year as of 2000 (Liangzhi and Chamberlin, 2004).

3.2.1 Data collection

In each of the three districts, a multi staged approach was used in selecting the sample farmers. Two major cotton growing sub counties were selected from each of the districts for the survey. The sub counties selected were; Kisinga and Nyakiyumbu in Kasese; Apopong and Palisa Town Council in Palisa; Barr and Amach in Lira districts. In each of the sub counties, a parish was selected and in each parish two villages sampled. Four farmer fields in each were sampled five times; once in the vegetative stage (early stage), two times in each of the flowering (mid stage) and boll formation (Late stage) stages for all the pests that occur in the different cotton growth cycle. The different cropping systems under which cotton was managed by farmers and their farm sizes were established during the survey. A cotton field was considered to be intercropped if the population density of the component crop/crops was more than a third of the main component crop (Cotton). The kind and number of component crops were recorded. Size of the field was estimated in acres. Data on methods of pest control practices; chemical spraying and kind of chemicals used, frequency of spraying and source of pesticides was also collected.

3.2.1.1 Arthropod sampling techniques

3.2.1.1.1 Sweepnets

This method was used to capture air-borne adult active insects, especially the members of Orthoptera, Lepidoptera and Hemiptera (Ralph *et al*., 2007). In this study, sweepnet samples were taken between 9.30am – 11.30am. This is the time when the arthropods are very active and all the morning dew has evaporated (Kyamanywa, 1988; Ralph *et al*., 2007; Scott, 2007). Wet conditions were avoided and in intercropped fields, care was taken to allow for maneuverability around the component plants. In this study, a 15 inch diameter sweepnet was used and a sample consisted of 25 sweeps taken diagonally across the field. The contents of the sweepnet were

emptied in a bucket (45 cm height x 30 cm diameter) containing 40% ethanol to kill the insects,, sorted and identified.

3.2.1.1.2 Pitfall Traps

Pitfall traps have been used extensively to collect soil surface active arthropods (Kyamanywa, 1988; Bradley, 2005 & Rodrigo, 2003). The pitfalls used in this study consisted of a small plastic cups (12 cm height x 10 cm diameter) containing ethanol to kill and preserve the insects. The rims of the tins were in level with the ground so as not to obstruct activity of the potential catches. The whole set up was covered with a foil paper canopy to avoid water filling during the rainy periods. A set of nine pitfall traps was used and arranged along a rectangular transect according to a grid structure that allowed three traps placed (2 m from the boarder) in line along the border of each side and one in the centre of the field. The pitfall trap samples were emptied once in a week and processed in the laboratory using a mesh of 500-micro openings to separate soil from the arthropods trapped.

3.2.1.1.3 Beat bucket Technique

The beat bucket is probably the most effective method for sampling arthropods and other canopy-dwelling insects in crops. The method involves shaking the plant canopy parts to dislodge the arthropods into the container. However, the efficiency of this method is affected by wet conditions and the speed at which the plant canopy is shaken (Knutson and Wilson, 1999). In this study, a white clean bucket (45 cm height x 30 cm diameter) was used. Samples were taken between 9.30am – 11.30am when the arthropods are very active and all the morning dew has evaporated (Kyamanywa, 1988; Ralph *et al*., 2007; Scott, 2007). The sample plant was carefully approached, grasped at the stem near the base of the plant. While holding the bucket at a 45-degree angle to the ground, the plant was quickly bend into the bucket making sure that the terminal and as much of the plant as possible was inside the bucket. The plant was rapidly beat

against the side of the bucket 15 times for about 5 seconds. This dislodged the arthropods on the plant into the bottom of the bucket. The plant was then removed and the bucket held up right to prevent the arthropods from escaping. Quickly a second plant was sampled. Arthropods crawling up the side of the bucket while sampling, were kept inside by tapping the sides of the bucket. After the second plant was sampled, a record of the arthropods captured in the bucket was taken and this constituted a sample. Twenty plants were sampled along a diagonal transect per field. All the leaves and other parts of the crop that were dislodged in the bucket were examined to assess presence of any arthropods.

3.2.1.1.4 Visual Examination/Inspection

Visual examination is the most common, simplest, and fastest method of scouting insect. This technique, involves selecting a number of plants, leaves, stems, or flowers/fruiting structures and examining them for the presence of insects or damage caused by given arthropods. Visual inspection can be quantitative or presence/absence depending on the objective of inspection, and careful examination of the material sampled is necessary (Bradley, 2005 and Rodrigo, 2003). In this study, twenty plants were sampled along a diagonal transect per field. All the leaves, flowers, squares and bolls were examined for the presence of different arthropods. Special attention was given to; aphids, thrips, whiteflies and spidermites incidence; percentage lygus damage, percentage damaged squares/bolls by bollworms, and their natural enemies. Presence of black sunken spots on the leaves, fruiting structure and shriveled squares was an indication of lygus occurrence. In case of the bollworms, the incidence was determined through bollworm damage effects (population index) in which it was assumed that each hole on a boll, square or flower was equal to the presence of one larva.

3.3 Data analysis

The arthropods collected were sorted, identified to species level using entomological/morphological keys (Southwood and Henderson, 2000). Arthropod ecosystem services were identified through literature.

3.4 Results

3.4.1 Cropping system, Farm Size categorization and Pest Control Practices

All the cotton fields sampled in the study were intercropped. A total of fourteen different crop combinations were observed (Table 3.1). Lira and Palisa had the highest number, with over six different cotton crop combinations. Maize was the most companion crop of cotton consisting 72% of all crop combinations. This was followed by cassava and beans at 43%. In Kasese, cotton was predominantly combined with maize at 93.8%. In Pallisa cotton was predominantly planted with maize + cassava at 43.6%, and in Lira, the most predominant crop combinations were the maize + sunflower and maize each at 25% (Table. 3.1).

A total of 3 chemical pesticides all distributed by the Cotton Development Organization were utilized by the farmers. These pesticides were: Keshet Super 312 EC (Chlorpyrifos 300gl + Deltamelthrin 12 g/l); Polytrin (Profenophos 300g/l+Lamda Cyhalotrin 15g/l) and Agrothoate (Dimethoate – Organophosphate). Usage and frequency of pesticide application was highest in Kasese district at 57% as compared to 25% in Palisa and 22% in Lira.

3.4.2 Profile of Arthropod Species/Ecosystem Services in Cotton fields in Uganda

The arthropod community in cotton was comprised of 57 species belonging to 42 families in 12 orders (Table 3.2). Among these were beneficial (67.0%) and herbivores (33.0%) (Fig. 3.1). The beneficial arthropods consisted of predators (55%); pollinators (24%) and decomposers (21%). Abundance of arthropods collected varied with location with Lira district having the

highest arthropod abundance at 38.1%, Palisa district at 33.5% and Kasese district at 28.4% of the total arthropod population collected.

Table 3.1 Frequencies of the different crop combinations within the survey areas

	Crop Combinations	Lira	Palisa	Kasese	Overall
		Percent	Percent	Percent	Percent
1	Cotton + Maize	**25**	0	**93.8**	**39.6**
2	Cotton + Maize + cassava	12.5	**43.6**	0	**18.6**
3	Cotton + Maize + Sunflower	**25**	0	0	**8.3**
4	Cotton + Maize + Beans	6.3	0	0	2.1
5	Cotton + Maize + Banana	0	6.3	0	2.1
6	Cotton + Maize + Cassava + Beans	6.2	0	0	2.1
7	Cotton + Maize + Cassava + Banana	0	12.5	0	4.2
8	Cotton + Maize + Sunflower + Banana	6.3	0	0	2.1
9	Cotton + Cassava	0	**18.8**	0	**6.2**
10	Cotton + Cassava + Beans	0	12.5	0	4.2
11	Cotton + Cassava + Cowpeas	0	6.3	0	2.1
12	Cotton + Beans	0	0	6.2	2.1
13	Cotton + Sorghum + Cowpeas	12.5	0	0	4.2
14	Cotton + Millet + Cowpeas	6.2	0	0	2.1

Table 3.2 Profile of Arthropods and their Ecosystem Services in Uganda

Order	Family	Common Names	Species	Ecological Function
Orthoptera	Acrididae	Grass hopper	*Paracinema Fisher*	Defoliator/Pest
	Blattidae	Cockroach	*Supella Longipalpa*	Defoliator/Pest
	Gryllidae	Cricket	*Grlyllus* spp	Defoliator/Pest
	Tettigoniidae	Long horned grasshopper	*Melanoplus bivittatus*	Defoliator/Pest
Acarina	Tetranychidae	2- Spotted spider mite	*Tetranychus nuticae*	Pest
	Tetranychidae	Red Spider mite	*Tetranychus telarius*	Predator
	Agelenidae	Funnel Spider	*Aelvrillus* ssp	Predator
Homoptera	Aphididae	Aphid	*Aphis phaseolus*	Sucker/Vector/Pest
		Aphid	*Aphis gossypii*	Sucker/Vector/Pest
Hymenoptera	Apidae	Carpenter Bee	*Apoidea*	Pollinator
		Bamboo bees	*Xylocopa*	Pollinator
		Honey bees	*Apis mellifera*	Pollinator
	Formicidae	Black ants	*Lepiosiota* spp	Predators/Decomposers
		Black ants	*Monomorium pharaonis*	Predators/Decomposers
		Red Ants	*Solenopsis invicta*	Predators/Decomposers
		Red Ants	*Solenopsis. geminata*	Predators/Decomposers
		Black ants	*Pemphigus populivenae*	Predators/Decomposers
		Black/Sugar ants	*Dorylinae* spp	Predators/Decomposers
		Red ants	*Tetanops myopaeformis*	Predators/Decomposers
	Sphecidae	Small wasp	*Ammophillinae* spp	Pollinators/Predators
		Big wasp	*Diastrophus turgidus*	Pollinators/Predators
		Mud-nest building wasp	*Sphex atratus*	Predators/Parasitoids
Hemiptera	Miridae	Lygus	*Lygus* spp	Sucker/Pest
	Aleyrodidae	Whitefly	*Bemisia tabaci*	Sucker/Vector/Pest
	Pentatomidae	Stink bug	*Nezara viridula*	Sucker/Pest
	Coreidae	Leaf footed bug	*Anasa tristis*	Predator
	Tingidae	Lacebugs	*Gargaphia torresi*	Sucker/Pest
	Lygaeidae	Big eyed bug	*Geocoris* spp	Predator
	Berytidae	Stilt bug	*Jalysis* spp	Predator
	Phymatidae	Ambush bug	*Phymata* spp	Predator
	Reduviidae	Assissin bug	*Sinea* spp	Predator
	Nabidae	Damsel bugs	*Nabis* spp	Predator
	Anthocoridae	Minute pirate bugs	*Orius* spp	Predator
	Miridae	Plant bugs	*Spanagonicus* spp	Pest/Predators
	Pyrrhocoridae	Cotton stainer	*Dysdercus suturellus*	Sucker/Pest
Coleoptera	Carabidae	Ground beetle	*Stenolophus*	Predator
	Chrysomelidae	leaf beetle	*Epitrix hirtipennis*	Defoliator/Pest
	Chrysomelidae	Leaf Beetle	*Diabrotica longicornis*	Defoliator/Pest
	Coccinellidae	Ladybird	*Hippodamia sinvata*	Predator
	Curculionidae	Weevil	*Sphenophorus maidis*	Defoliator/Pest
	Meloidae	Blister beetle	*Epicauta pestifera*	Defoliator/Pest
	Scarabaeidae	Scarab Beetle	*Phyllophaga*	Decomposers
	Tenebrionidae	Darkling beetle	*Eleodes suturalis*	Defoliators/Pest
	Curculionidae	Boll weevil	*Anthonomus grandis*	Pest (Borer)
	Scarabaeidae	Dung beetle	*Phyllophaga fervida*	Decomposer
Diptera	Drosophilidae	Drosophila	*Fannia canicularis*	Pollinator
	Muscidae	Housefly	*Musca* spp	Pollinator
	Syrphidae	Hover fly	*Eristalis tenax*	Predator
Dermaptera	Forticulidae	Earwig	*Forficula auricularia L*	Predator
Lepidoptera	Gelechiidae	Bollworm	*Heliothis armigera*	Pest/Boll eater/Defoliator
	Noctuidae	Moth	*Pseudaletia unipuncta*	Pollinators/Defoliators
	Noctuidae	Semi-loopers	*Pseudoplusia* spp	Defoliators/Pest
	Noctuidae	Cut worms	*Agrotis* spp	Pest
	Papilionidae	Butterfly	*Eumorpha achemon*	Pollinators/Defoliators
Isoptera	Rhinotermitidae	Termite	*Incisitermes*	Decomposer/Predators
Odonota	Anisoptera	Dragon fly	*Pantala havescens*	Predator/pollinator
Thysanoptera	Thripidae	Thrips	*Thrips*	Sucker/Vector/Pest

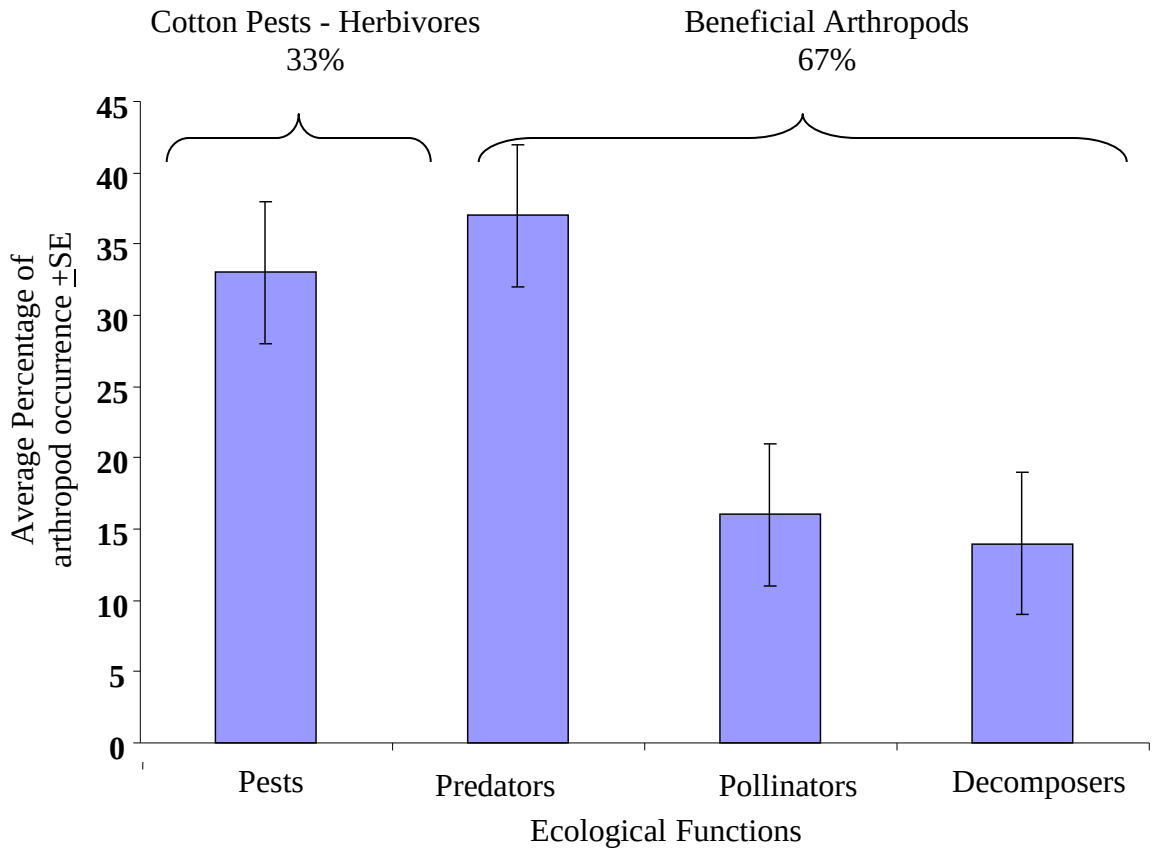


Fig. 3.1 Average percentage of arthropods and their ecological functions (± SE; $P<0.05$) observed in the sampled survey areas

3.5 Discussions

The results indicate that across the surveyed fields, cotton was grown as an intercrop in fourteen different crop combinations under very small production units with maize as the most common component crop; of all the arthropods collected 67% were beneficial and 33% herbivores.

The different crop combinations observed in the survey concurs with the tradition of multiple cropping common in many tropical developing countries and which is and will remain as insurance for meeting household incomes and daily food requirements (Jean-Philippe, 2007). However, in terms of genetically modified crops, risk assessment analyses show that the natural refuges derived from the mixed-planting system of cotton play an important function in delaying

evolution of cotton bollworm resistance and therefore making them more effective (Wu and Gou, 2005). The different intercrops being practiced by the farmers as observed in the current study seem therefore to indicate a positive trend in refugia presence in the management of the feared resistance development. In addition, the maize based cropping systems observed as the most frequent in the survey could have a major influence on bollworms' development of resistance to genetically modified cotton. Maize will not select for *Bt*-resistant bollworms but will act as a refuge and delay resistance (Hardee *et al* 2001). The more non-genetically maize and other non-genetically modified hosts grown in maize and cotton production area, the slower the *Bt* resistance develops (Hardee *et al* 2001). While multiple cropping is generally regarded as a good risk avoiding culture, if it is not well managed, it could increase pests' problems. In this particular study, maize, beans and sunflower which were amongst the crop components in the intercrops are also good hosts of key pest in cotton. All these factors have a great bearing on resistance management and hence a need to take a precautionary approach as genetically modified crops are introduce in a dominantly maize based cropping system.

The survey revealed that cotton production units were small. Farm size has been considered as one of the factors that has an influence on adoption and dissemination of technologies and its influence is dependent on time and space (Chaves and Riley, 2001; Sheikh et al., 2003). Theoretically, adoption of GM crops is size neutral since the technology is delivered in the seed, which is completely divisible and can be used in any amount (Manuel Gómez-Barbero *et al*, 2008), size of the area under cultivation of cotton may not be a major factor in the adoption of genetically modified cotton in Uganda. However, in assessing the effects of genetically modified crops and in this case cotton in Uganda, it should be noted that, farm size is a surrogate for other factors (Cost, ease of use, expected benefit and support of labour to the technology, pest pressure, wealth, education and aversion to risk) which affects the early phases of adoption, and

is very unlikely to have an impact on dissemination (Manuel Gómez-Barbero *et al*, 2008). With the small scale cropping systems as observed in this study, it is not likely that introduction of genetically modified cotton would pose a threat through gene flow (Katie and Jeremy, 2002). Therefore for policymakers, stakeholders and scientists, it is important to observe and assess other interacting factors in farm size and the role they might play in adoption of genetically modified cotton.

In this study four ecological functional categories were identified for cotton in Uganda: predators, non-target herbivores, pollinators and decomposers. The selected category group reflects a focus on non-target risks that could adversely affect cotton production. Secondary pests, pest resurgences, reduction of pollination, loss of biological control by natural enemies and loss of soil fertility are anticipated adverse effect that would require attention. These ecological functional groups and arthropod profile form the basis for chapter four, which gives methods for detailed assessments of the potential risks of GM cotton on non-target organisms.

In conclusion, this study has demonstrated that a number of crop combinations exist with the maize based component being the most popular in the cotton growing areas of Uganda. A diversity of arthropods (a new profiling), dominated by the beneficial arthropods existed within the various cropping systems. Since GM cotton will get into contact with surrounding ecosystems, there is need to understand and examine critically which of the species observed in the study and ecosystem functions are most associated with risk. Since identification of highest priority risk arthropods, potential exposure and adverse effects pathways is a major step in risk assessment, the next chapter dealt with categorizing and prioritizing of the non target arthropods and their ecosystem services, establishing and assessing the possible exposure and adverse

effects pathways that might occur to the species identified in the highest priority category, when genetically modified cotton is introduced.

CHAPTER FOUR

4.0 POTENTIAL EXPOSURE AND ADVERSE EFFECT PATHWAYS OF GENETICALLY MODIFIED COTTON TO THE NON-TARGET ARTHROPODS

4.1 Introduction

Results from the field surveys demonstrated the existence of a diversity of arthropods, dominated by the beneficial arthropods in the various cotton cropping systems in Uganda (Chapter Three). In the event that genetically modified cotton is introduced in Uganda, these arthropods will be exposed to the transgenic toxins and the effects of the toxin will depend on the level of exposure (Andow and Hilbeck, 2004; Birch and Wheatley, 2005). Exposure is the contact or co-occurrence of the modified attribute of the genetically modified crop with an environmental entity of value (Wolt *et al,* 2009). There must be a plausible pathway for exposure of the organism to the modified plant attribute and the description of this pathway represents an exposure scenario (Sears et al. 2001; Wolt *et al*., 2003). Management of the genetically modified plants including activities such as growing, propagating, breeding, producing, processing, importing, transporting, disposing, and using, will influence development of exposure scenarios (Wolt *et al.*, 2009). Exposure can occur through many pathways and is a function of concentration (how much), persistence (how long), frequency (how often) and movement (where to) (Hilbeck, 2002; Andow and Hilbeck, 2004; Birch and Wheatley, 2005). Transgenic plant materials and products can move in the agroecosystem by being transferred between organisms in the food web through bitrophic or/and higher trophic level exposure and these may have adverse effects to non-target organisms (Birch *et al*., 2002; Andow and Hilbeck, 2004; Hilbeck *et al.*, 2006; Hilbeck *et al.*, 2008).

For any given case of exposure, there is need to understand whether there is potential harm arising from it and the consequences there after. In case of lack of a reasonable exposure pathway, then in terms of risk assessment it would be negligible and therefore no opportunity for

harm (Wolt *et al.*, 2009). Effects of exposure are determined through exposure pathway analysis (Sears *et al*., 2001; Wolt *et al.*, 2003; Wolt *et al.*, 2009). Consequently a pathway may be described as a no adverse effect pathway if there is no significant exposure, or it may be an adverse effect pathway , if there is significant exposure and harm expected to occur (Hilbeck *et al.*, 2008). In a nutshell, exposure pathway analysis involves understanding the effects of the transgenic protein in the plant on the herbivores and predators and the resultant impact on crop yield. An adverse effect pathway is a causal chain that starts with an exposed entity and ends with an adverse effect. At a species level, an adverse effect pathway could begin with a change in a population parameter or behaviour of the species and ends with loss of crop production (Hilbeck *et al*., 2008). At an ecological level (herbivory, predation, decomposition, pollination), exposure could begin with a change in the timing, rate or magnitude of the process/function and end with a reduction in the ecological process/function (Hilbeck *et al*., 2008).

Assessing effects of adopting transgenic crops on natural ecosystems depends on obtaining information that can reliably detect changes in natural ecosystems and on comparing such information to existing baseline agricultural practices in a given environment (Wolfenbarger and Gonzalez, 2004). Knowledge of the environmental impacts of transgenic based technology on non-target organisms in the major cotton cropping systems is essential for the safe deployment of genetically modified cotton in Uganda. In Chapter Three, the general arthropod species composition in the cotton cropping system in Uganda was established and constituted 67% beneficial and 33% herbivores that are likely to be exposed to the transgenic toxins. Establishing possible exposure and adverse effect pathways for these non-target arthropods and their respective processes are an important factor in biosafety risk assessment (Snow *et al.*, 2005; Andow and Zwahlen, 2005; Andow *et al.*, 2006), and it is against this background that this study was conducted with the following objectives:

a) Prioritizing non-target arthropod species and ecological processes that should be included in risk assessment,

b) Establishing the high priority non-target arthropod potential exposure pathways and,

c) Establishing high priority non-target arthropod potential adverse effects pathways and damage potential.

The study hypotheses that non-target species that are not exposed directly or indirectly to the transgene products and their metabolites are less likely to be affected by the genetically modified crop.

4.2 Materials and Methods

4.2.1 Prioritizing Non-target arthropods for biodiversity risk assessment

In order to understand which species might be at a great risk if genetically modified cotton is introduced in Uganda, a methodology adopted from Andow *et al*., (2006); Sujii ER *et al.*, (2006) and Nguyen *et al.*, (2008) was used. The methodology has several important features: a process to select a restricted number of species and ecosystem processes for risk assessment, identifying appropriate risk endpoints and selecting those considered most at risk, a process relying on risk hypotheses to guide qualitative and quantitative exposure characterization, adverse effects/risk characterization, and a dynamic/adaptive tiered process. Additionally, this methodology takes into account the reliance on all available scientific information and information from local experts and literature, and a structure that overcomes the lack of information common in developing countries (Snow *et al*., 2005; Andow and Zwahlen, 2005; Andow *et al*., 2006).

The selection of risk endpoints starts by specifying the receiving ecosystem (crop environment) and listing relevant ecological functional groups into a smaller number of functional categories (Andow and Hilbeck, 2004). A comparison in geographical distribution, habitat specialization,

prevalence, abundance and crop phenology was done. A list of relevant species and ecosystem processes within each selected functional group (Birch *et al*., 2004; Hilbeck *et al*., 2006) was established. A series of qualitative ecological characteristics were used to rank the species in relation to the likelihood of the risk endpoint associated with the functional group (Sujii *et al*., 2006; Arpaia *et al*., 2006; Faria *et al*., 2006; Pallini *et al*., 2006; Mendonça *et al*., 2006) and this involved:

(i) identification of functional categories of biodiversity,

(ii) a species selection matrix to evaluate the likelihood association with the crop, location (geographical distribution) and crop habitat: i.e. association with crop: arthropod x crop plant coincidence; association with crop: arthropod x crop plant trophic relationship and functional significance of arthropod in the cropping system.

In the selection matrix, the following factors were used and accorded numbers as they corresponded with the arthropod in the survey.

1. Geographical distribution: occurrence in any of the three survey district: In this study geographic distribution was considered to be the occurrence of a given arthropod in space and time in any of the three survey district with the following score; Present in all the districts = 1; present in any of the two district (absent in one) = 2 and present in one district (absent in the two) = 3. The scores for each of the different areas were later summed to give the overall rank of a given species.

2. Habitat specialization: Habitat specialization answers the question "in which crop system does the species mostly occur" and is defined as the degree of association between the non-target species and the crop habitat (Hilbeck *et al*., 2008). In this study species were scored as generalist, specialists/restricted or no clear information on their habitat specialization as shown: Arthropod is a specialist/restricted = 1;

arthropod is a generalist = 2 and no information known (whether specialist or generalist) = 3.

3. Prevalence/abundance of arthropods: Prevalence brings out the aspects of the proportion of the crop habitat where species can be found reliably. The species was given a high rank if the species occurred in all fields of the crop and a low rank when it occurred in very few field in the survey area. The ranks were given the following scores that were finally summed up to give a final ranking of the species within the overall assessment ranking as; high = 1, medium = 2 and low = 3.

4. Abundance of arthropods (Quantity occurring): Abundance seeks to know how much of the species does occur on the crop. A species that is more abundant on a crop will mean more individuals exposed to the crop and greater functional significance (Nguyen *et al.,* 2008). The ranks were given the following scores that were finally summed up to give a final ranking of the species within the overall assessment ranking as; high = 1, medium = 2 and Low = 3. However, whereas abundance was considered in the assessment ranking, it should be noted that some times abundance may be difficult to compare across species because of the differences in sizes, behaviour, sampling methods and ecology that can cause vast differences in average densities (Duelli *et al*., 1999; Lang, 2000).

5. Crop phenology (Vegetative, Reproductive or Ripening/opening stage): The degree of phonological overlap was ranked from the perspective of the proportion of crop cycle during which the species was present and of the species' life cycle associated with the crop. However, it should be noted that any effect on the populations that are exposed to the crop throughout their life cycle are often greater than on ones that are exposed only as adults (Hilbeck *et al*., 2008). It is against this background that the score below were used; Present in all the growth stages (absent in

none) = 1, present in any of the two growth stages (absent in one) = 2 and present in one growth stage (absent in the two) = 3.

6. Prioritization of species through adaptive tiered process and selection matrix: A tiered risk assessment evaluates the potential adverse effects of a given plant in a given receiving environment to all elements of biological environment (Andow *et al*., 2008). Because there are thousands of species and hundreds of ecosystem processes that could be evaluated, it is not feasible to evaluate each one thus this methodology provided two "tiers" to assess risks to biological diversity in the cotton cropping system (Fig.3.2). Tier I is the determination of relevant arthropods and functional groups (first part of the identification of risk endpoints). The functional groups that are excluded are deemed irrelevant because there are no substantial risks associated with them.

Tier II was the selection of species and processes considered most associated with a risk endpoint. This tier further reduces the potential candidates for further risk assessment, because the unselected species and processes are judged to be at lower risk (and thus negligible) than the selected ones. Elements of tier I and II were established through a selection matrix using scores in geographical distribution, habitat specialization, prevalence/abundance, and crop phenology/pest life stage linkage with the crop.

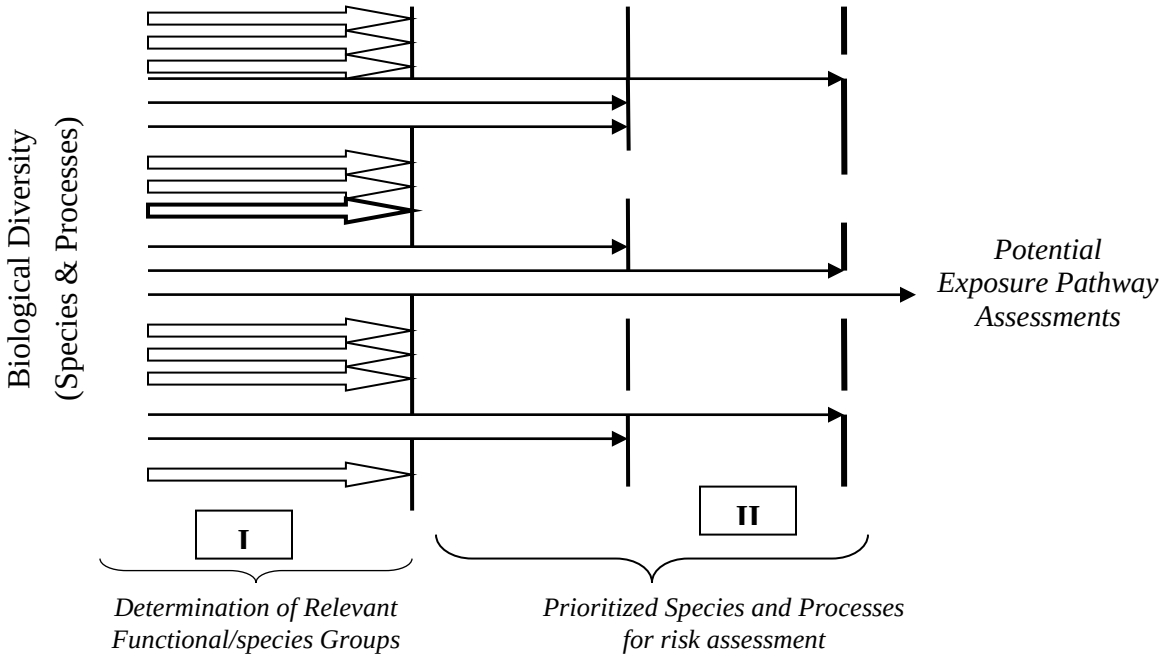


Fig 4.1 Risk assessment process through a two tiers {Source: *Andow et al.*, (2006)}.

A stepwise screening and sorting progressively reduced both the number of species or processes into a summation cluster; lowest range of summation cluster = highest priority, medium range of lowest summation cluster = intermediate priority and highest range of summation cluster = low Priority. The species/processes under a given cluster summation (priority level) were considered to have a significant role in the crop ecosystem (Nguyen Thi Thu Cuc *et al.*,2008).

4.2.2 Potential Exposure Pathways of Non-Target Pest to GM Cotton

Following the procedures of Birch *et al*, (2004); Hilbeck *et al.*, (2006) and Hilbeck *et al.*, (2008), the highest priority category arthropods observed were analyzed against bitrophic, tritrophic and higher trophic level exposure to transgene product and its metabolites. This assessment was based primarily on the literature of *Cry 1Ac* gene **{***Bt*-proteins are expressed in all growing tissues of GM cotton throughout the growing season, including flower and fruit tissues, roots and seed endosperm, and concentrations are generally highest in the leaves (Nguyen Thi Thu Cuc Hoa *et al.*,2008). Concentrations can vary considerably in different genetic backgrounds,

environmental conditions and over the growing season (Nguyen Thi Thu Cuc Hoa *et al.*,2008)**}** and information on the phenotypic pattern of transgene expression in the various parts of the transgenic plant over the whole growing season. A series of guiding questions (adopted from Nguyen Thi Thu Cuc *et al.*,2008) were used to assess potential routes of exposure to the transgene products and metabolites as shown in Table 4.1 (for Herbivores – Pests) and Table 4.2 (for the beneficial arthropods).

Table 4.1 Pest Potential Exposure assessment for BT Cotton

Selected Species	
Bitrophic Exposure	Which life cycle stage occurs in cotton?
	Growth stage of cotton when present (early, mid, reproductive)
	Does the species feed on plant tissues or products containing the transgene product?
	Is bitrophic exposure possible?
	Have the transgene product or metabolites been detected in the species after feeding?
	Does bitrophic exposure occur?
Tritrophic Exposure	Does the species feed on animal products or excretions that contain the transgene product?
	Is tritrophic exposure through animal products possible?
	Does tritrophic exposure through animal products occur?
Other Trophic Exposure	Is the species cannibalistic?
	Does it eat other species in its own feeding guild that are exposed?
	Is other trophic exposure through cannibalism possible?
Exposure by Changed Behaviour	Is the transgenic plant less attractive?
	Does it have a lower food value than the non-transgenic cultivar?
	Does the species avoid food containing the transgene product or metabolites?
	Are behaviors likely to increase or decrease exposure?
Summary	Is exposure possible and does exposure occur?

Source: Nguyen Thi Thu Cuc et al., 2008

Table 4.2 Beneficial Insect Potential Exposure assessment for Bt Cotton

Selected Species	
	List the plant tissues or secretions on which it feeds
Bitrophic Exposure	Which tissues or secretions fed upon express transgene products?
	Is the feeding important for the beneficial insect?
	Is bitrophic exposure possible?
	Are transgene products or metabolites detectable after feeding on plant tissue or secretions?
	Does bitrophic exposure occur?
Tritrophic Exposure Via Herbivore Products	Does the beneficial insect feed on prey products or excretions (honey dew, frass, feaces)
	Do any of these herbivores products have detectable transgene products or metabolites?
	Are herbivore products an important part of the beneficial insect diet?
	Is tritrophic exposure via feeding on herbivore products possible?
	Are transgene products or metabolites detectable in the beneficial insect after feeding on herbivore products?
	Does tritrophic exposure via feeding on herbivore products occur?
Tritrophic Exposure Via Prey	Does the beneficial insect feed on prey that feeds on the transgenic plant tissues?
	Is the prey likely to be exposed to transgene product or metabolites when eaten by the predator?
	Is the prey an important part of the predator's diet?
	Is trophic exposure via feeding on prey possible?
	Are transgene products or metabolites detectable in the natural enemy after feeding on prey?
Selected species	
	Does tritrophic exposure occur through prey?
Higher Trophic Level Exposure	Does the beneficial Insect cannibalize its own species or eat other intra guild foods (prey that are beneficial insects themselves)?
	Is this species possibly exposed?
	Are any of the intraguild foods significant food sources of the beneficial insects?
	Is higher trophic level exposure via cannibalism or intraguild feeding?
	Are transgene products or metabolites detectable in the natural enemy after cannibalism or intraguild feeding?
	Does higher trophic level exposure occur via cannibalism or intraguild feeding?
Behavioral Modification Of Exposure	What feeding preferences or other behaviour could increase or decrease exposure?
	Does beneficial insect avoid eating exposed prey or the Bt containing tissues?
	Are behaviours likely to increase or decrease exposure?
Summary	Is exposure possible?
	Does exposure occur?

Source: Nguyen Thi Thu Cuc et al.,2008

Furthermore, the high priority arthropod species (with lowest range of cluster summation - *Andow et al.,* 2006 & 2008) were evaluated for their likely exposure to transgenic proteins

considering exposure via multiple trophic levels. Using Pham Van Lam *et al.*(2008) approach, the evaluation considered the arthropod feeding habit, expression of the transgene product in the parts it feeds on and any documented information on the detection of the toxin in the herbivore (based on the literature on *Cry 1Ac* gene). The following factors were used in the assessment;

Yes — When exposure is possible (*Exposure can occur through many pathways. Transgenic plant materials and products can move in the agroecosystem by being transferred between organisms in the food web (Hilbeck et al., 2008) and this is a function of concentration (how much), persistence (how long), frequency (how often) and movement (where to) (Hilbeck, 2002; Andow and Hilbeck, 2004; Birch and Wheatley, 2005).*

No — When exposure is impossible/may never occur (*Exposure can never occur through any pathways*) (Hilbeck et al., 2008).

(?) — Where no information is available and uncertainty exists (*It is likely that there **will be significant** knowledge gaps for some of the candidate species that will create a degree of uncertainty in the assessment – **worse-case scenario** -* Hilbeck et al., 2008)

Yes? — Where contradiction/uncertainty exists (*It is likely that there **will be some** knowledge gaps for some of the candidate species that will create a degree of uncertainty in the assessment – **Best-case scenario** -* Hilbeck et al., 2008).

4.2.3 Non-Target Pest Potential Adverse Effects Pathways and Damage Potential

Following the procedures of Birch *et al.,* (2004); Hilbeck *et al.,* (2006) and Hilbeck *et al.,* (2008) in identifying potential exposure pathways, this assessment was based primarily on the literature of ecologically plausible adverse effects that may arise in the event of exposure of the ten highest priority category arthropods. The assessment was formulated (in accordance to the procedures by Hilbeck *et al.,* (2008) following the order below;

1. Identifying the adverse effect that might arise from the identified exposure pathways
2. Identification of pathways that result from indirect effects of genetically modified crops ('knock-on' pathways).
3. Developing plausible adverse effects that connect the exposure pathway to the adverse effect.

Using approaches adopted from Hayes *et al.,* (2004); Meier and Hilbeck (2005) and Institute of Engineering and Technology (2009), a fault-and event-tree analysis was used to systematically establish causal links between the genetically modified crop, adverse effect(s) and identifying multiple pathways by which an adverse effect could occur. A fault-tree analysis identifies possible pathways by tracing backwards from the adverse effect through suspected causal chains to the genetically modified crop. It also helps in clarifying events that cause the adverse effect, in what sequence they must occur and what assumptions must be made for the pathway to be likely to occur. Event-tree analysis uses a forward logic to identify pathways from initiating exposure events, through a casual chain to adverse effects.

4.4 Results

4.4.1 Non-target arthropods at risk of being affected by the transgenic toxin

From the assessment (Appendix 3), three priority range of summation clusters were developed: highest priority category 4 – 6 = at 41%; intermediate priority category 7 – 9 = at 32% and lowest priority category 10 – 12 = at 27% (Table 4.3).

The highest priority category 4 – 6 = was identified with 9 pests and 11 non-pest arthropods belonging to 17 families (Acrididae, Gryllidae, Aphididae, Apidae, Formicidae, Miridae, Aleyrodidae, Anthocoridae, Pyrrhocoridae, Coccinellidae, Drosophilidae, Muscidae, Gelechiidae, Noctuidae, Papilionidae, Rhinotermitidae and Thripidae) in 9 orders (Orthoptera, Homoptera, Hymenoptera, Hemiptera, Coleoptera, Diptera, Lepidoptera, Isoptera and Thysanoptera). The intermediate priority category 7 – 9 = had 9 pests and 9 non-pest arthropods belonging to 15 families (Blattidae, Tettigoniidae, Tetranychidae, Apidae, Formicidae, Sphecidae, Pentatomidae, Tingidae, Lygaeidae, Phymatidae, Reduviidae, Nabidae, Miridae, Curculionidae and Noctuidae) in 6 orders (Orthoptera, Acarina, Hymenoptera, Hemiptera, Coleoptera and Lepidoptera). The lowest priority category 10 – 12 = had 4 pests and 9 non –

pest arthropods belonging to 12 families (Agelenidae, Sphecidae, Coreidae, Berytidae, Carabidae, Chrysomelidae, Curculionidae, Meloidae, Scarabaeidae, Tenebrionidae, Syrphidae and Forticulidae) in 6 orders (Acarina, Hymenoptera, Hemiptera, Coleoptera, Diptera and Dermaptera).

4.4.2 Non-Target Herbivore Pest Potential Exposure Pathways

Through the evaluation process, it was noted that, all the nine non-target herbivore pests in the highest priority category arthropods (Appendix 4), have direct and indirect potential exposure to the transgene, toxins and secondary metabolites. The differences in the ecological functions, mode of feeding and other interacting forces of the high priority arthropods are expected to cause great variation in the posed risks (Table 4.4).

Direct tritrophic exposure was only registered for *Incisitermes* where no information was available and uncertainty exists. *Gryllus* spp, *Pseudaletia unipuncta, Thrips, Paracinema fisher* and *Lygus lineoralis* have been recorded to have some cannibalistic attributes and therefore there was a possibility of exposure through cannibalism though with some element of contradiction/uncertainty existing (Table 4.4). No information was available about exposure by changed behaviour (i.e. the transgenic plant being less attractive, lower in food value than the non-transgenic cultivar; does the species avoids food containing the transgene product or metabolites and are their any changes in the species behaviours towards the genetically modified crop) with all the species under evaluation (Table 4.4).

Table 4.3 Selection Matrix: Prioritized species

Order	Family	Common Names	Species	Prioritized rank for species[a] (*Summation Geog Distribution + Prevalence + Abundance + Phenology*)
Orthoptera	Acrididae	G.hopper	*Paracinema Fisher*	6 [a]
	Blattidae	Cockroach	*Supella Longipalpa*	7 [b]
	Gryllidae	Cricket	*Grlyllus* spp	4 [a]
	Tettigoniidae	Long horned grass hopper	*Melanoplus bivittanus*	9 [b]
Acarina	Tetranychidae	Spider mite	*Tetranychus nuticae*	8 [b]
	Tetranychidae	Spider mite	*Spider*	7 [b]
	Agelenidae	Funnel Spider	*Aelvrillus* spp	10 [c]
Homoptera	Aphididae	Aphid	*Aphis phaseolis*	5 [a]
		Aphid	*Aphis gossypii*	4 [a]
Hymenoptera	Apidae	Carpenter Bee	*Apoidea*	6 [a]
		Bamboo bees	*Xylocopa*	7 [b]
		Honey bees	*Apis mellifera*	4 [a]
	Formicidae	Black ants (Ngini ngini)	*Lepiosiota* spp	4 [a]
		Black ants (Nsolosozi)	*Monomorium pharaonis*	4 [a]
		Red Ants	*Solenopsis invicta*	5 [a]
		Red Ants	*Solenopsis. geminata*	6 [a]
		Black ants (Ebinyomo)	*Pemphigus populivenae*	6 [a]
		Sugar/Black ants (Ebikenembe)	*Dorylinae* spp	9 [b]
		Red ants (Kaasa)	*Tetanops myopaeformis*	8 [b]
	Sphecidae	Small wasp (Kararankoma)	*Ammophillinae* spp	12 [c]
		Big Wasp (Enwa - Enumba)	*Diastrophus turgidus*	11 [c]
		Mud-nest building wasp (Bumbuzi)	*Sphex atratus*	9 [b]
Hemiptera	Miridae	Lygus	*Lygus* spp	4 [a]
	Aleyrodidae	Whitefly	*Bemisia tabaci*	5 [a]
	Pentatomidae	Stink bug	*Nezara virindula*	8 [b]
	Coreidae	Leaf footed bug	*Anasa tristis*	10 [c]
	Tingidae	Lacebugs	*Gargaphia torresi*	9 [b]
	Lygaeidae	Big eyed bug	*Geocoris* spp	8 [b]
	Berytidae	Stilt bug	*Jalysis* spp	10 [c]
	Phymatidae	Ambush bug	*Phymata* spp	7 [b]
	Reduviidae	Assissin bug	*Sinea* spp	7 [b]
	Nabidae	Damsel bugs	*Nabis* spp	7 [b]
	Anthocoridae	Minute pirate bugs	*Orius* spp	6 [a]
	Miridae	Plant bugs	*Spanagonicus* spp	8 [b]
	Pyrrhocoridae	Cotton stainer	*Dysdercus suturellus*	6 [a]
Coleoptera	Carabidae	ground beetle	*Stenolophus*	11 [c]
	Chrysomelidae	leaf beetle	*Epitrix hirtipennis*	11 [c]
	Coccinellidae	Ladybird	*Hippodamia sinvata*	5 [a]
	Curculionidae	Weevil	*Sphenophorus maidis*	10 [c]
	Meloidae	Blister beetle	*Epicauta pestifera*	10 [c]
	Scarabaeidae	Scarab Beetle	*Phyllophaga*	10 [c]
	Tenebrionidae	Darkling beetle	*Eleodes suturalis*	12 [c]
	Curculionidae	Boll weevil	*Anthonomus grandis*	9 [b]
	Scarabaeidae	Dung beetle	*Phyllophaga fervida*	12 [c]
Diptera	Drosophilidae	Drosophila	*Fannia canicularis*	4 [a]
	Muscidae	Housefly	*Musca* spp	5 [a]
	Syrphidae	Syrphid fly	*Eristalis tenax*	11 [c]
Dermaptera	Forticulidae	Earwig	*Forficula auricularia L*	10 [c]
Lepidoptera	Gelechiidae	Bollworm	*Heliothis armigera*	4 [a]
	Noctuidae	Moth	*Pseudaletia unipuncta*	4 [a]
	Noctuidae	Semi-loopers	*Pseudoplusia* spp	7 [b]
	Noctuidae	Cut worms	*Agrotis* spp	8 [b]
	Papilionidae	Butterfly	*Eumorpha achemon*	6 [a]
Isoptera	Rhinotermitidae	Termite	*Incisitermes*	6 [a]
Odonota	Anisoptera	Dragon fly	*Pantala havescens*	10 [c]
Thysanoptera	Thripidae	Thrips	Thrips	5 [a]

Combination of associations with crop and functional significance in the cropping system.

[a]4 – 6 = highest priority (widely distributed, abundant, mostly present, closely linked, environmental effects significant); [b]7 – 9 = intermediate priority; [c]10 – 12 = low priority (distribution, abundance, and presence limited, closely linked, environmental effect not significant) adopted from Andow et al., 2006 and Nguyen Thi Thu Cuc et al., 2008

Table 4.4 Pest Potential Exposure assessment for Bt Cotton

Selected Species		***Gryllus* spp**	***Aphis (phaseolis/ gossypii)***	***Pseudaletia unipuncta***	***Dysdercus suturellus***	***Bemisia tabaci***	***Incisitermes***	***Thrips***	***Lygus lineoralis***	***Paracinema Fisher***
Bitrophic Exposure	Which life cycle stage occurs in cotton?	Adult/Nymphs	All	Larvae	Adult	All	Adults	All	Adults & Nymph	Adult/Nymphs
	Growth stage of cotton when present (Early, mid, reproductive)	Early	All	Early	All	All	Late	All	All	All
	Does the species feed on plant tissues or products containing the transgene product?	Yes	Yes ?	Yes	Yes ?	Yes ?	Yes ?	Yes	Yes ?	Yes
	Is bitrophic exposure possible	Yes	Yes ?	Yes	Yes ?	Yes ?	Yes ?	Yes	Yes ?	Yes
	Have the transgene product or metabolites been detected in the species after feeding	?	Yes ?	Yes ?	?	Yes ?	?	Yes	?	?
	Does bitrophic exposure occur	Yes ?	Yes?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?
Tritrophic Exposure	Does the species feed on animal products or excretions that contain the transgene product?	No	No	No	No	No	?	No	No	No
	Is tritrophic exposure through animal products possible?	No	No	No	No	No	?	No	No	No
	Does tritrophic exposure through animal products occur?	No	No	No	No	No	?	No	No	No
Other Trophic Exposure	Is the species cannibalistic?	Yes	No	Yes	No	No	No	Yes	Yes ?	Yes
	Does it eat other species in its own feeding guild that are exposed?	?	No	?	No	No	No	No	No	?
	Is other trophic exposure or exposure through cannibalism possible?	Yes	No	Yes	No	No	No	Yes ?	Yes ?	Yes ?
Exposure by Changed Behaviour	Is the transgenic plant less attractive?	?	?	?	?	?	?	?	?	?
	Does it have a lower food value than the non-transgenic cultivar?	?	?	?	?	?	?	?	?	?
	Does the species avoid food containing the transgene product or metabolites?	?	?	?	?	?	?	?	?	?
	Are behaviours likely to increase or decrease exposure	?	?	?	?	?	?	?	?	?
Summary	Is exposure possible?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?
	Does exposure occur?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?

Assessment is based primarily on the literature on Cry 1Ac cotton. ***?*** *Indicates uncertainty. As several Bt cottons use a similar promoter, their expression is expected to be similar.* Adopted from Andow et al., 2006 and Nguyen Thi Thu Cuc et al., 2008

4.4.3 Potential Adverse Effect Pathways and Damage Potential of high priority non-target pests

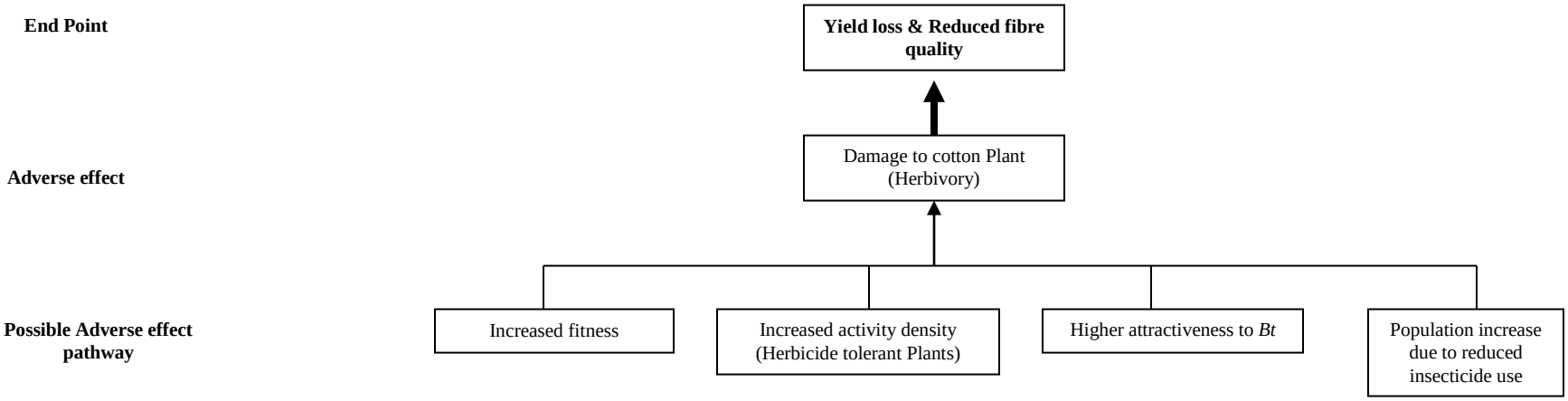
Yield loss and reduced fibre quality was anticipated to be the potential damage as a result of population outbreak of the selected species. Increased fitness of the species towards GM cotton, higher attractiveness of GM cotton to the species, species population increase due to reduced insecticide use and reduced natural enemies/biological control were the main possible adverse effect pathways catalogued. It was also observed that their might be increased disease transmission and prevalence for the species that have disease transmitting attributes (Fig. 4.2; a - d).

Increased damage of the cotton plant was the adverse effect anticipated for *Gryllus* spp, *Incisitermes and Paracinema fisher* which are currently not major cotton pests. It was also anticipated that where the insect resistance gene is combined with the herbicide tolerant gene, their might arise a risk of increased damage affects to cotton from the species in the Orthoptera order (*Gryllus* spp and *Paracinema fisher*), through the adverse pathway of increased activity of these species due to the weeds (Fig. 4.2; a).

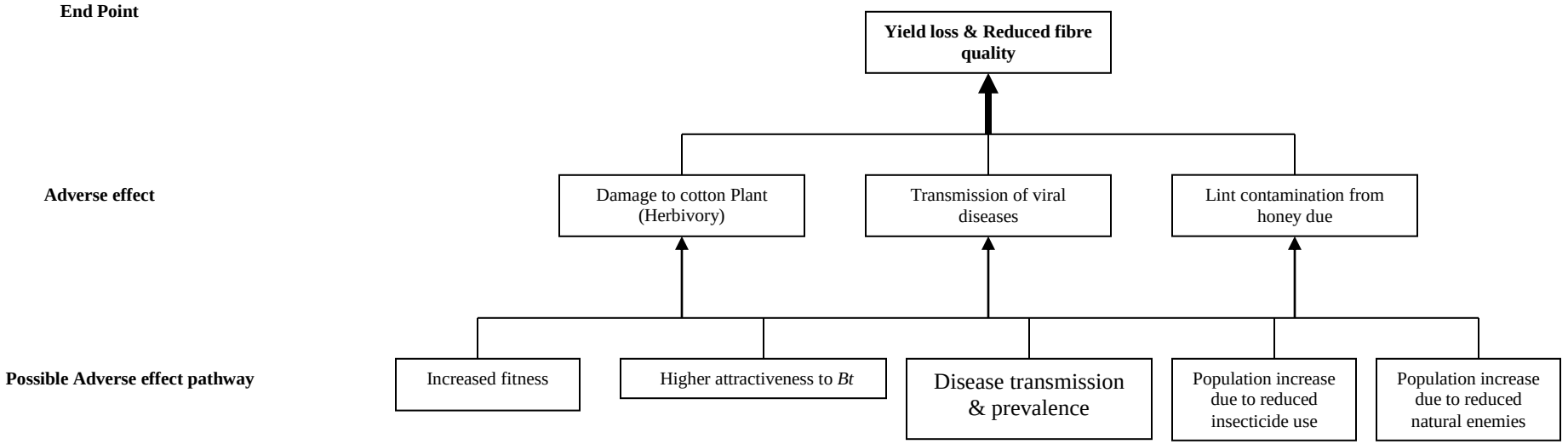
Aphis gossypii, Thrips spp *and Bemisia tabaci* which are important cotton pests are anticipated to cause more damage to the cotton crop through their sucking of plant sap, transmission of viral diseases and contamination of lint with honeydew (Fig. 4.2; b). *Pseudaletia unipuncta and Lygus lineoralis* are also important cotton pests and their adverse effects will be manifested through herbivory and the abortion of squares and flowers. *Dysdercus suturellus* adverse effects will be manifested through cotton sucking plant phloem sap to contamination of the lint and thereby reducing lint quality (Fig. 4.2; c and d).

Figure 4.2 Potential Adverse Effect Pathways and Damage Potential

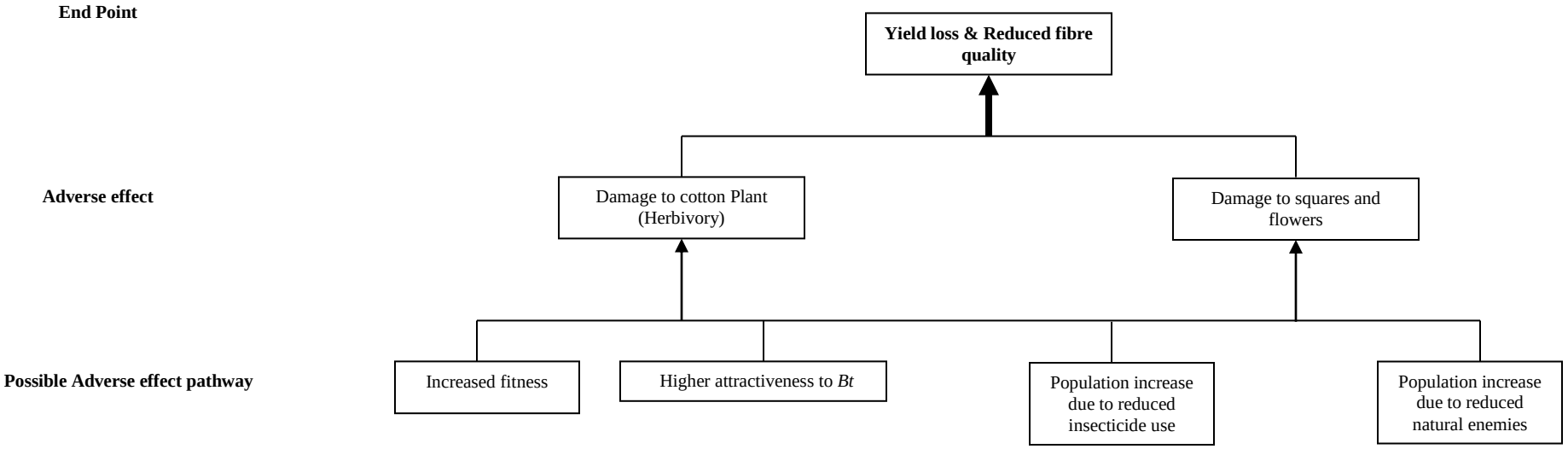
a) *Grlyllus* spp (Orthoptera: Gryllidae), Garden cricket; *Paracinema fisher* (Orthoptera: Acrididae), Grasshopper and *Incisitermes* spp (Isoptera: Rhinotermitidae), Termites



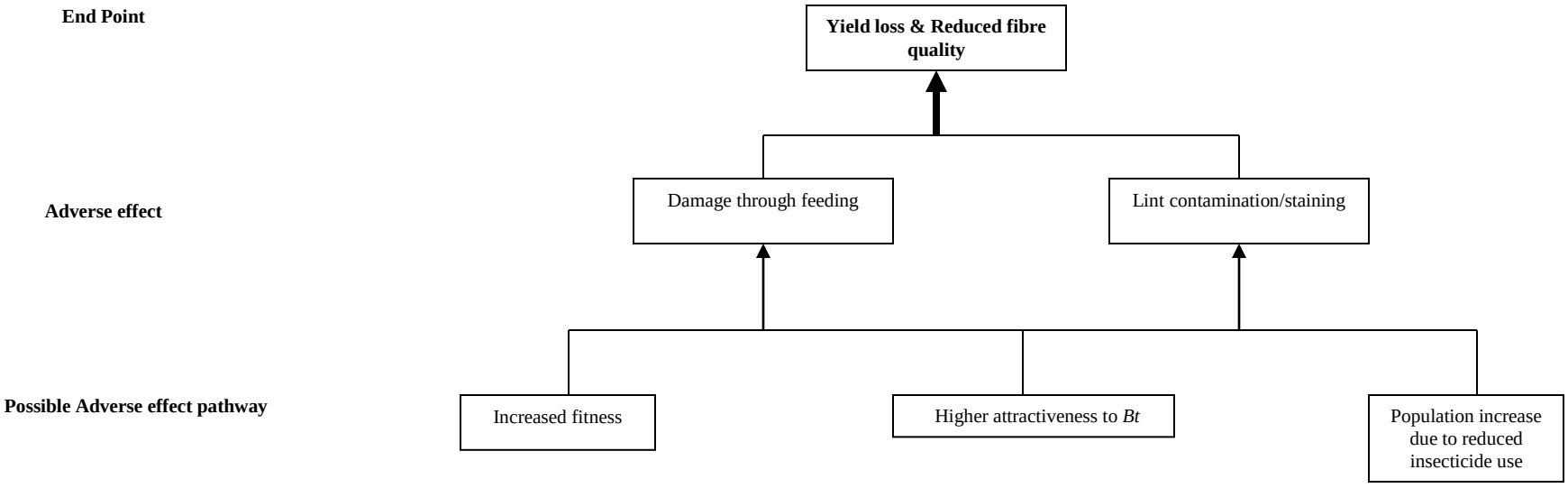
b) *Aphis gossypii* Glover (Homoptera: Aphididae), Cotton Aphid; *Bemisia tabaci* (Homoptera: Aleyrodidae), Whitefly; *Thrips (*Thysanoptera: Thripidae), Thrips and *Lygus lineoralis* (Hemiptera: Miridae), Lygus



c) *Pseudaletia unipuncta* (Lepidoptera: Noctuidae)



d) *Dysdercus suturellus* (Hemiptera: Pyrrhocoridae), Cotton Stainer



4.4.4 Potential exposure of beneficial insects to genetically modified cotton

All the twelve non-target beneficial arthropods in the highest priority category arthropods observed (Table 4.3) might be exposed to tissues and exudates from genetically modified cotton through multiple pathways (Table 4.5 below).

Bitrophic exposure through pollen/nectar feeding is possible for *Apis mellifera, Musca* spp, *Apoidea, Eumorpha achemon* and *Hippodamia sinvata*. For the other seven species *Fannia canicularis, Lepiosiota* spp, *Monomorium pharaonis, Solenopsis invicta, Solenopsis geminate* and *Pemphigus populivenae,* no information was available about potential bitrophic exposure and therefore uncertainty exists. However, Tritrophic exposure through herbivore products is possible for *Lepiosiota* spp, *Monomorium pharaonis, Hippodamia sinvata, Solenopsis invicta, Solenopsis geminate* and *Pemphigus populivenae*. Tritrophic exposure through prey was only suggested for *Hippodamia sinvata.* Key knowledge gaps about tritrophic exposure through herbivore products existed for *Apis mellifera, Musca* spp, *Apoidea, Eumorpha achemon* and *Fannia canicularis*. Key knowledge gaps about exposure through other higher trophic levels and behavioral modification of exposure existed for all the eleven species and this creates uncertainty in the assessment.

Selected Species		*Apis mellifera*	*Fannia canicularis*	*Lepiosiota* spp	*Monomorium pharaonis*	*Hippodamia sinvata*	*Solenopsis* (*invicta* & *geminate*)	*Musca* spp	*Apoidea*	*Pemphigus populivenae*	*Eumorpha achemon*	*Orius* spp
Bitrophic Exposure	List the plant tissues or secretions on which it feeds	Pollen	None	None	None	Pollen, nectar & anthers	None	Nectar?	Nectar Pollen ?	None	Nectar Pollen?	Pollen
	Which tissues or secretions fed upon express transgene products	Pollen	None	None	None	Pollen, nectar & anthers	None	Nectar?	Nectar Pollen ?	None	Nectar Pollen?	Pollen, nectar & anthers
	Is the feeding important for the beneficial insect?	Yes	No	No	No	No	No	Yes	Yes	No	Yes	Yes
	Is bitrophic exposure possible?	Yes	No	No	No	Yes	No	Yes	Yes	No	Yes	Yes
	Are transgene products or metabolites detectable after feeding on plant tissue or secretions	Yes	?	?	?	?	?	Yes	Yes	?	Yes	?
	Does bitrophic exposure occur?	Yes	?	?	?	?	?	Yes	Yes	?	Yes	?
Tritrophic Exposure Via Herbivore Products	Does the beneficial insect feed on prey products or excretions (honey dew, frass, feaces)	No	No	Yes	Yes	Yes	Yes	?	No	Yes	No	Yes
	Do any of these herbivores products have detectable transgene products or metabolites?	?	?	?	?	?	?	?	?	?	?	?
	Are herbivore products an important part of the beneficial insect diet?	No	No	Yes	Yes	Yes	Yes	?	No	Yes	No	Yes
	Is tritrophic exposure via feeding on herbivore products possible?	No	No	Yes	Yes	Yes	Yes	?	No	Yes	No	Yes
	Are transgene products or metabolites detectable in the beneficial insect after feeding on herbivore products?	?	?	?	?	?	?	?	?	?	?	?
	Does tritrophic exposure via feeding on herbivore products occur?	No	No	Yes	Yes	Yes	Yes	?	No	Yes	No	Yes
Tritrophic Exposure Via Prey	Does the beneficial insect feed on prey that feeds on the transgenic plant tissues?	No	No	Yes	No	Yes	Yes	No	No	No	No	Yes
	Is the prey likely to be exposed to transgene product or metabolites when eaten by the predator?	No	No	No	No	Yes	No	No	No	No	No	Yes
	Is the prey an important part of the predator's diet?	No	No	No	No	Yes	No	No	No	No	No	Yes
	Is trophic exposure via feeding on prey possible?	No	No	No	No	Yes	No	No	No	No	No	Yes
	Are transgene products or metabolites detectable in the	No	No	No	No	?	No	No	No	No	No	?

	natural enemy after feeding on prey?											
	Does tritrophic exposure occur through prey?	No	No	No	No	Yes	No	No	No	No	No	Yes
Higher Trophic Level Exposure	Does the beneficial Insect cannibalize its own species or eat other intra guild foods (prey that are beneficial insects themselves)?	No	No	No	No	Yes	No	No	No	No	No	Yes
	Is this species possibly exposed?	?	?	?	?	?	?	?	?	?	?	?
	Are any of the intraguild foods significant food sources of the beneficial insects?	Yes ?	Yes ?	Yes	Yes	Yes	Yes	Yes ?	Yes ?	Yes	Yes	Yes?
	Is higher trophic level exposure via cannibalism or intraguild feeding?	No	No	No	No	Yes	No	No	No	No	No	Yes?
	Are transgene products or metabolites detectable in the natural enemy after cannibalism or intraguild feeding?	?	?	?	?	?	?	?	?	?	?	?
	Does higher trophic level exposure occur via cannibalism or intraguild feeding?	?	?	?	?	?	?	?	?	?	?	?
Behavioral Modification Of Exposure	What feeding preferences or other behaviour could increase or decrease exposure?	Insect activity, Migration	Insect activity, Migration	Herbivore products & activity, Migration	Herbivore products & activity, Migration	Insect activity, Migration	Herbivore products & activity, Migration	None	None	Herbivore products & activity, Migration	None	Insect activity, Migration
	Does beneficial insect avoid eating exposed prey or the Bt containing tissues?	?	?	?	?	?	?	?	?	?	?	?
	Are behaviours likely to increase or decrease exposure?	?	?	?	?	?	?	?	?	?	?	?
Summary	Is exposure possible?	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Does exposure occur?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes ?	Yes?

Table 4.5 Beneficial Insect Potential Exposure assessment for Bt Cotton: Assessment is based primarily on the literature on Cry 1Ac cotton. ? Indicates uncertainty. As several Bt genes use a similar promoter, their expression is expected to be similar.

4.4.5 Potential adverse effects pathways of exposure of beneficial arthropods to GM cotton

A possible population decrease of beneficial insects and consequently reduction in biological control, pollination and altered decomposition efficiency was anticipated to occur. This would result in increased out outbreaks of the non-target herbivores, reduced pollinator/flower visitors' populations and subsequent reduction in flower pollination activities and altered soil organic matter decomposition (Figure 4.3: a - c).

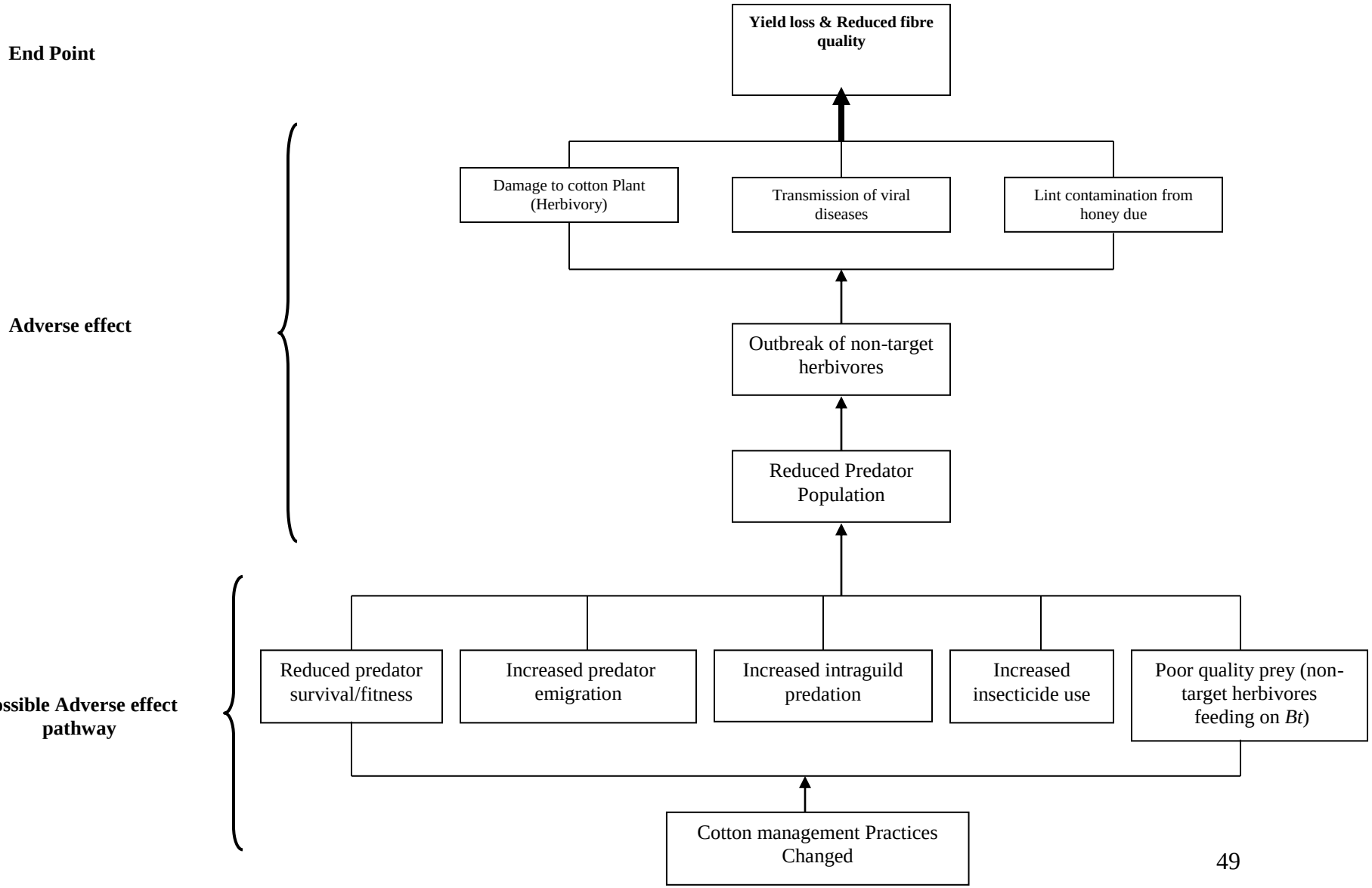
Changed cotton cropping systems, reduced predator survival/fitness, increased predator emigration, increased intraguild predation, increased insecticide use and poor quality prey (non-target herbivores feeding on genetically modified cotton) were the possible adverse effect pathways catalogued for the predators (Figure 4.3a).

The main possible adverse effect pathways catalogued for the pollinators/flower visitors were; changed cotton cropping systems, altered flower, and nectar or pollen composition in genetically modified cotton plant, transgenic toxin in flower, nectar or pollen ingested by pollinator, increased insecticide use and altered food chains (Figure 4.3b).

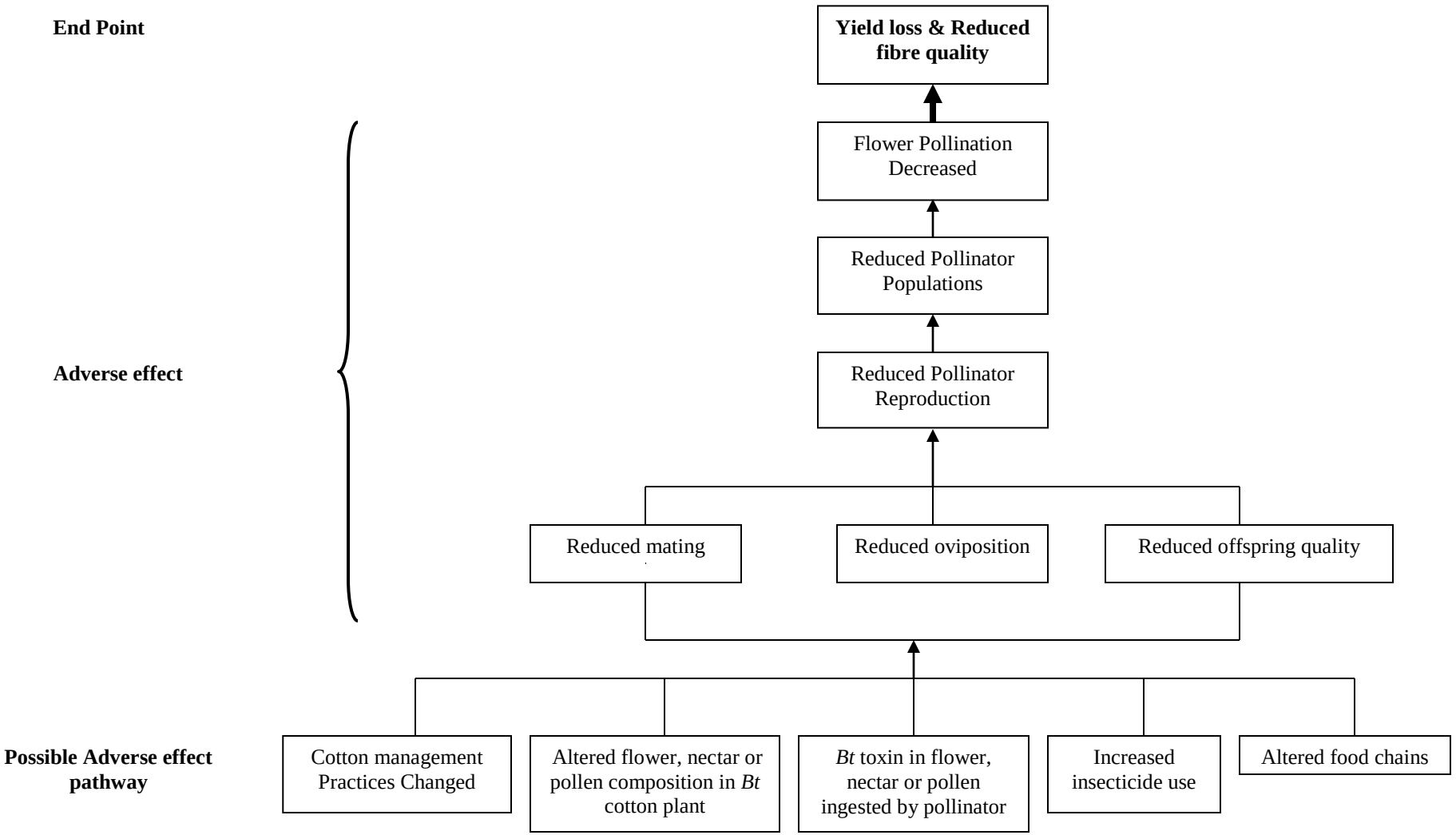
For the decomposer category of the selected species, the possible adverse effect pathways catalogued were; changed cotton cropping systems, altered composition in genetically modified cotton plant, increased insecticide use and altered food chains (Figure 4.3c).

Figure 4.3 Beneficial Insect Potential Adverse Effect Pathways

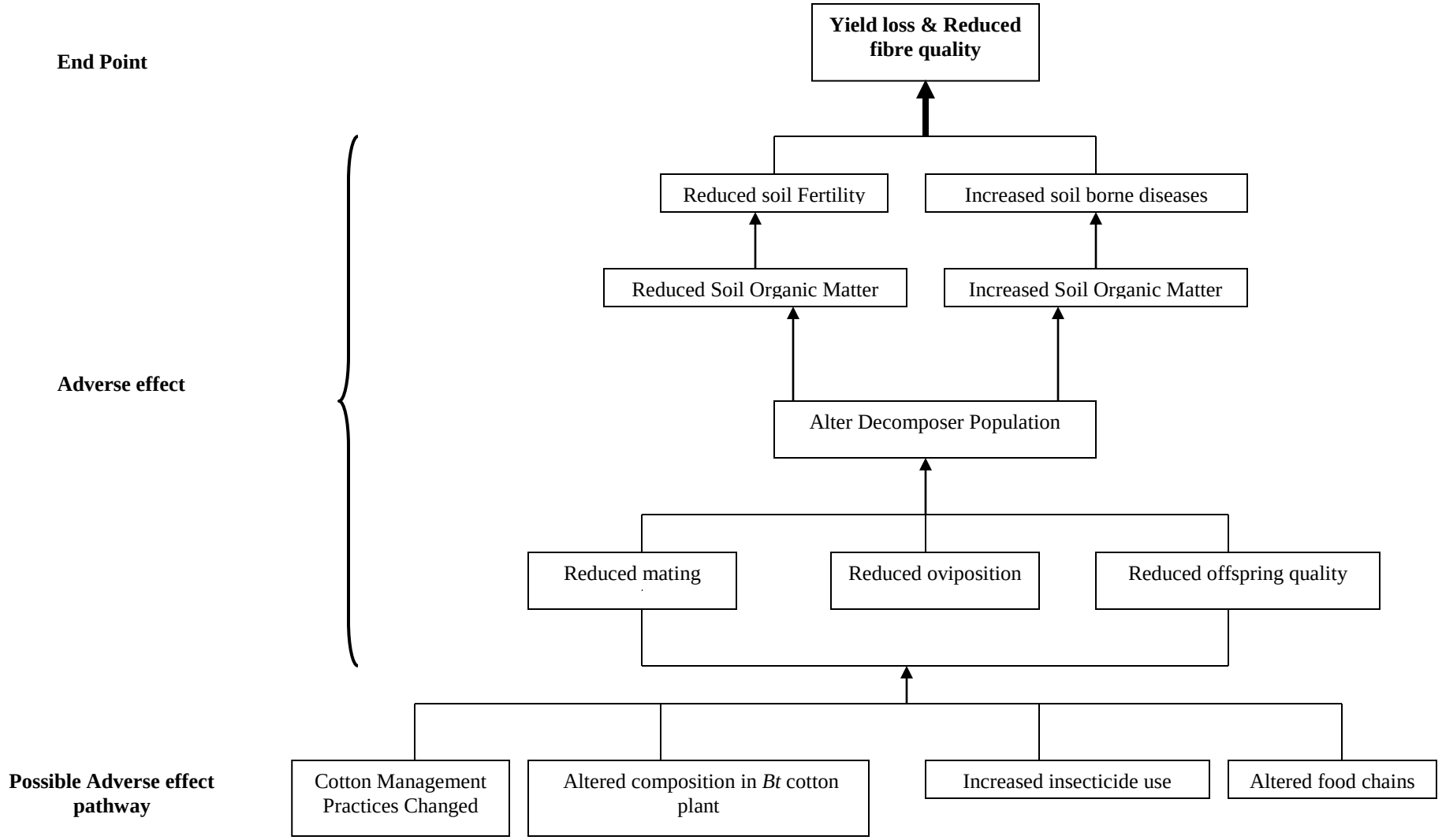
a) Predators: (*Hippodamia sinvata*), Ladybird beetle and *Orius* spp (Hemiptera: Anthocoridae), Minute Pirate Bugs



b) Pollinators/Flower vistors : (*Apidae*), *Apoidae* (Carpenter bee) and *Apis mellifera* (Honey bee)



c) Decomposers : (*Formicidae*), *Ants*



4.4 Discussions

Combining the potential exposure and adverse effect pathways identified in this study, it is noted that the cultivation of GM cotton will interact with different arthropods in various ways. Careful evaluation of the identified potential environmental risks associated with non target arthropods calls for strategies to prevent or ameliorate such effects. The information generated in this study will help in hazard identification and the development of testable assessment risk hypotheses for the different non target arthropods.

Exposure is a necessary prerequisite for any subsequent effects associated directly with the transgene product. The absence of these products renders additional studies on the direct effects of the transgene products unnecessary. However, it is still possible that GM cotton has effects independent of or interacting with the transgene products. Like in Vietnam (Nguyen Thi Thu Cuc *et al* ., 2008) and considering the potential adverse effect pathways and damage potential in Fig. 4.2 a-c, about four generic risks hypotheses can be formulated to describe the ways GM cotton might result in an adverse effect via effects on non-target herbivores in Uganda.

Risk hypothesis 1: increased attractiveness and/or nutritive quality of GM cotton results in higher herbivore populations and greater damage to cotton. This risk hypothesizes that the GM cotton has increased nutritional value and/higher abundance of floral structure, providing better food resources for non-target herbivores. This could be as result of the lack of the feeding damage by the targeted Lepidoptera and/or because of differences between the GM cotton and the non-GM cotton varieties in phenology, morphology, or chemical composition due to its varietal background or the transformation and subsequent breeding process, as well as the expression of the Cry gene. Direct effects of the transgene, all interactions of the transgene in the GM cotton and other differences between the GM cotton and the non GM cotton varieties

that will be used as controls are all included in this hypothesis. The hypothesis could further be categorized into sub-hypotheses that differentiate survival and vigor (increased population fitness) and reproduction and immigration (increased population size). If the two sub hypotheses are unlikely, then one may conclude that attractiveness and food quality in GM cotton are unlikely to cause greater damage from non-target pests. The sub hypotheses are unlikely for any particular herbivore if the GM cotton suppresses its population. Therefore the following experiments are proposed for the eleven herbivores: Experiment 1. The absolute fitness of non-target herbivores feeding on GM cotton is increased (Risk hypothesis for survival and vigor – to test if insects feeding on GM cotton have similar growth, fecundity, survival and lifespan to those on conventional plants); Experiment 2: Non-target herbivores prefer(avoid) feeding or ovipositing on GM cotton (Risk hypothesis for reproduction and immigration – to establish if non-target herbivores show preference for GM cotton plants for feeding or oviposition compared with conventional cotton, as this may increase population and cotton damage); and Experiment 3: The non-target herbivores contain GM proteins in their bodies during some/any developmental stages – to test if insects feeding on GM cotton contain GM proteins in their bodies – any exposure pathways (risk hypothesis 1 and 4).

Risk hypothesis 2: Effects of GM cotton disease transmitting arthropods results in higher transmission of vectored plant pathogens. This risk hypothesis requires that the arthropod associated with GM cotton transmits the diseases more rapidly and thereby causes greater damage. Pathogen transmission is higher if there are more vectors, a larger reservoir, or faster transmission. If GM cotton results in higher population of e.g *A. gossypii* (Sub Hypothesis 1 and 2 and the increased release due to reduces pesticide use), the following hypothesis may apply. This hypothesis will apply for all the disease transmitting arthropods - if the various diseases are

observed in Uganda. The proposed experiments for this hypothesis would be susceptibility of GM cotton to diseases - to determine if GM cotton is more susceptible than non-GM cotton to diseases transmitted by different arthropods (e.g. CBD and *A.gossypi*).

Risk hypothesis 3: The reduction of insecticide sprays in GM cotton will result in higher populations of non target herbivores. In many of the countries where GM cotton has been grown, there has been a decrease in the use of insecticides (Wilson *et al*., 2007; Nguyen Thi Thu Cuc *et al*., 2008) which might be the case in Uganda and this might lead to the release of herbivores that were previously controlled by these insecticides. Under this hypothesis, the GM cotton itself has no effect on the non-target herbivore, but he herbivore population increases because they are no longer killed by the insecticides. The hypothesis assumes that the herbivore was originally controlled and will now cause damage. It is also assumed that there will be an increase in the beneficial populations that are sufficient to control these pests.

Risk hypothesis 4: Indirect effects via natural enemies. Three sub hypotheses are proposed:

4.1: The natural enemies are affected sub- lethally by GM cotton and have a longer development time, lower larval survival and body weight and decreased pupation rate. This altered fitness may affect negatively the predators feeding on the targeted arthropod, and the decreased predator population would lead to the release of a different pest from biological control.

4.2: The non target herbivores are not affected by the GM cotton, but accumulate significant amounts of the Cry toxins in their bodies. Predators consuming large quantities of these herbivores may be affected negatively by the Cry toxins, leading to the release of a different pest from biological control.

4.3: Predators may be affected by the lack of the lepidoptera on the GM cotton which are killed by the Cry toxin. As a consequence, different pest that were controlled by these predators are released. The proposed experiment is: Evaluation of target herbivore population on GM cotton in the field – to determine if GM cotton increases the abundance of non-target herbivore pest species, due to: (i) increased food quantity or quality from reduced competition with the target species (hypothesis 1); (ii)release from insecticide applied against the target species (risk hypothesis 3); and (iii) release from biological control by natural enemies (risk hypothesis 4). This will require experimental comparisons under sprayed and unsprayed conditions.

Cultivation of GM cotton can negatively interact with predator populations in various ways as noted in this study Fig 4.3a. A possible population decrease of predators and consequently reduction in biological control was anticipated to occur – leading to an outbreak of secondary pests and more cotton damage. The increased pest population could prompt farmers to change insecticide regimes, which might stimulate resurgence of other pests. These adverse-effect and damage scenarios are a guide to developing testable hypotheses which will serve as a basis for developing experiments for assessing the GM cotton. For the predators in this study the following risk hypotheses are developed:

Risk hypothesis 1: feeding on GM cotton material and GM cotton fed prey has an adverse effect on predator abundance, leading to an outbreak of aphid and mirid populations and increased incidence of cotton diseases; Risk hypothesis 2: early season reduction of prey due to GM cotton affects predators negatively, thus leading to an outbreak of aphid and mirid populations and increased disease incidence; and Risk hypothesis 3: due to high intraguild predation in Gm cotton aphids and Mirids escape biological control, leading to an increased occurrence of CBD.

Laboratory feeding trials using purified *Bt* toxins are proposed – trials should be compared between individuals fed on diets with and without Cry toxins. The purified toxin should be verified to be equivalent to that which is expressed in the plant. Stage or age-specific mortality of larvae, pupae and adults should be assessed; other factors that should be assessed include: age specific fecundity, size/weight of adult males and females, adult life span and egg-hatching rate/viability. Other crops and predators could also be evaluated with similar test that are tailored to the biology of the predator and crop in question. Species should be identified using either morphological or molecular methods. The experiments should be separated into three categories: (i) evaluations of bitrophic exposure and its effects on predators; (ii) evaluations of multi-trophic exposure and its effects on predators; and (iii) field-level studies that investigate the relative effects of GM cotton to predator populations and predation in the field. The experiments should consider the toxicity of a specific GM cotton event (i.e. what proportion of the predator are killed when they ingest the GM toxin from the GM cotton), the degree of exposure that is likely (i.e. what proportion of the predator is exposed to cotton material, and what dose do they likely ingest under natural conditions?), or both of these. Using this framework it will be possible to elucidate the mechanisms that underlie the potential adverse effects to predators, rather than simply document the effects of transgenic crops on predator populations. These mechanisms will allow for more focused experimentation and precise estimation of the actual risk to predators in the field.

Exposure of flower visitors to stressors associated with GM cotton may occur as indicated in this study directly via consumption of floral parts or products, or indirectly via the consumption of other flower visitors (Le Thi Thu Hong *et al*., 2008). Considering these exposure pathways and adverse effect pathways, five generic hypotheses in concurrence with Le Thi Thu Hong *et al*.,

(2008) are formulated to describe the ways in which GM cotton might lead to adverse effects via pollinators and flower visitors. The risk hypotheses should be assessed for their relevancy to each of the species identified. The risk hypotheses developed involves a reduction in the population density of the flower visitors and they are caused by any of the following: (i) Direct effects of the GM toxin in pollen and/or nectar and/or decreased in the quality of cotton nectar and pollen in GM cotton; (ii) Changes in the attractiveness of GM cotton flowers for flower visitors, due either to changes in volatile patterns and/or changes in the quality (and therefore attractiveness) of cotton nectar and pollen in GM cotton; and (iii) Alterations in landscape structure as a consequence of growing GM cotton. The risk hypotheses:

Risk hypothesis 1: GM cotton causes a reduction in bee population density and/or colony quality, resulting in reduced pollination effects, production of honey and other bee products; Risk hypothesis 2: GM cotton causes a reduction in pollinator populations density, resulting in reduced genetic diversity of the species; Risk hypothesis 3: GM cotton causes a reduction in bee population density, resulting in adverse effects on species associated with the bees; Risk hypothesis 4: GM cotton causes a reduction in pollinator forager quality or density of feral bee colony density, resulting in reduced pollination and yield of nearby crops; and Risk hypothesis 5: GM cotton causes a reduction in population density of important flower visitors other than bees. The possible experiments to test these hypotheses are: Laboratory experiments to measure the effect on flower visitors/pollinators of Cry toxin either purified in Vitro (Brodsgaard *et al*., 2003) or in pollen (Hanley *et al.*, 2003). Colonies of pollinators could also be released in greenhouses during the flowering stages of GM cotton - and comb occupation, reproduction rates, growth rates, adult size, adult lifespan and age structure can be measured for each colony.

For the decomposer category of the selected species, the possible adverse effect pathways catalogued were; changed cotton cropping systems, altered tissue composition in genetically modified cotton plant, increased insecticide use and altered food chains. Successful recycling of plant nutrients is a major governing force in crop production (Pham Van Toan *et al*., 2008). Such nutrient cycling is a function of microbial and macrofauna functional activities, which in turn obtain their energy requirements from the plants (Pham Van Toan *et al*., 2008). The cultivation of GM cotton might have significant implications to the functional dynamics in the soil ecosystem, which are dependent on the kind of plant residues and other inputs entering the soil (Pham Van Toan *et al*., 2008). Biomass decomposition, cellulose and lignin breakdown, nutrient uptake, soil particle aggregation and water holding capacity are some of the processes and indicators most critical for assessment ((Pham Van Toan *et al*., 2008). The risk hypotheses developed are: Risk hypothesis 1: Decomposition rates of GM cotton plant residues are faster than those for conventional cotton plant residues and Risk hypothesis 2: Symbiotic and mutualistic mycorrhizal fungi is reduced in GM cotton, resulting in lower nutrient uptake. Litterbag and living plant experiments could be set to test the hypotheses.

In conclusion it is necessary to assess the potential environmental risks and benefits of GM cotton on non-target arthropods before introducing them on a large scale in Uganda in order to support cotton production. The study through a selection process ranked out the high priority non-target arthropods to be assessed against potential risk by the introduction of Gm cotton. Different hypotheses have been have been developed and various experiments suggested to test the propose risk hypotheses.

CHAPTER FIVE

5.0 INFLUENCE OF BACILLUS THURINGIENSIS & ANTS (Lepiosiota spp) ON COTTON APHIDS (Aphis gossypii Glover: Homoptera: Aphididae) CONTROL BY THE LADYBIRD BEETLE (Cheilomenes: Coleoptera: Coccinellidae)

5.1 Introduction

Results from the field surveys (Chapter Three) demonstrated the existence of a diversity of arthropods, dominated by the beneficial arthropods in the various cotton cropping systems in Uganda such as the ladybird beetles and black ants (Lepiosiota spp). Prioritization of these arthropods at risk of being exposed to the transgenic toxin highlighted three priority categories with 41% of the arthropods falling in the highest priority category where the ladybird beetles and black ants (Lepiosiota spp) fall, it was also shown that all the non targets were likely to be exposed to the toxin in transgenic cotton and this might have negative impacts on cotton crop yield.

Numerous arthropods have been recorded in cotton fields' worldwide (Hilbeck *et al.,* 2006) and Uganda has a diversity of these dominated by the beneficial arthropods (Chapter Three: 3.4.2). While genetically modified cotton is targeting a few lepidopteran species, the dynamics of other species may be indirectly affected and these effects may be positive due to the removal of disruptive pesticides, or negative due to the effective removal of prey (Biradar & Vennila, 2008; Birch and Wheatley, 2005; Nguyen *et al*., 2008; Wilson *et al*., 2007). Previously in chapter four of this study, it was indicated that increased fitness of the species and higher attractiveness of the species to the genetically modified cotton may additionally influence the dynamics of the arthropod community and the introduction of genetically modified crops in any agricultural ecosystem may also alter the dynamics of several non target arthropods (Nagrare, *et al*., 2009;

Biradar & Vennila, 2008; Wilson *et al.,* 2007; Wu *et al*., 2002; Wu Kong-Ming, 2007; Obrist *et al*., 2005; O'Callaghan *et al*., 2005; Sanvido *et al*., 2006; Torres *et al*., 2006). For example, the cotton aphids, *Aphis gossypii*, which were once suppressed by early season sprays of endosulphan for *Helicoverpa* spp. in Australia, became more troublesome in transgenic cotton due to the absence of these insecticidal sprays (Wilson *et al.,* 2007) and so were the mirids in China (Wu *et al*., 2002; Wu Kong-Ming, 2007). It should be noted that aphids and the scale insects have mutualistic relationships with ants (Eubanks *et al.*, 2002; Kaplan and Eubanks, 2005). Ants are attracted to honeydew, a sugar-rich solution excreted by many hemipterans, and subsequently ''tend'' the hemipterans, providing protection from their natural enemies thus influencing plant-based food webs (Eubanks *et al.*, 2002). As a result, honeydew-producing insects have the potential for generating strong indirect effects on arthropod communities by manipulating the behavior and abundance of ants foraging on plants. An increase in the aphids and other hemipteran arthropods, and may be the change in the quality of the cotton crop as a result of the introduction of genetically modified cotton as observed in Australia and China (Wilson *et al.,* 2007; Wu *et al*., 2002; Wu Kong-Ming, 2007) might therefore exacerbated or curtailed the mutualistic relationship between the aphids and ants which might in the long run have an influence on the biological control of aphids by the ladybird beetle.

Therefore, while looking at the prospects of introducing genetically modified crops, there is need to understand the changes in interactions of predators and insect pests associated with genetically modified cotton in Uganda especially those observed in the high priority category (Chapter four). This part of the study was conducted with an objective of understanding the influence of *Bacillus thuringiensis* (XenTari) biopesticide and black garden ants (*Lepiosiota* spp) on the population

dynamics of a high priority category cotton pest (cotton aphid (*Aphis gossypii:* Homoptera: Aphididae) and their natural enemy (*Cheilomenes*: Coleoptera: Coccinellidae) in Uganda.

5.2 Materials and Methods

5.2.1 Effect of Dimethoate, *Bt*-spray and *Coccinellidae* Beetle on Aphid Population

Experiments were conducted at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) located in Wakiso District, 16Km West of Kampala in Central Uganda in 2008. 36 buckets were arranged in groups of four and used to grow the BPA 2002 cotton plants. The germinated cotton plants were kept free from any pest infestations using cypermethrin chemical sprays. At four weeks, the cotton plants were infested with 20 aphids per plant obtained from infested cotton plants in the field. One week later, 5 adult ladybird beetles obtained from milk weeds and black jack were introduced in each of the bucket. Predator conditioning was done by introducing the beetles to non-experimental cotton plants for a week and thereafter introduced to the caged experimental plants. A week later after beetle establishment, a randomized complete block design (RCBD) with three treatments (XenTari: *Bacillus thruringiensis.* Aizawai, Lepidopteran a.i. 10.3% w/w; dimethoate: 400g/L EC; 0.5L/Ha and water) applied onto the cotton plants was set. The treatments were coded as follows: Treatment (T_1) = (*Bacillus*); Treatment (T_2) = (dimethoate) and Treatment (T_3) = (Water). The experiment had three replicates (blocks) with four buckets each and a plant in each bucket. The distance of separation between experimental units was 1 metre. Populations of the beetles and aphids under the different treatments were monitored for five consecutive weeks. Aphid scoring was used based on the number of aphids observed on the infested leaf**.** A leaf was scored 0 (when 0 aphids were present); 1 (1 – 50 aphids); 2 (51 – 100 aphids); 3 (101 – 150) and 4 (> 150 aphids). Pale brown bloated aphids were discounted as these were assumed parasitized. A summation of the scores

made from the 36 plants, was calculated to get the average aphid score. The ladybird beetles were enumerated by counting how many were present on the plants. The data collected from all the six cages was subjected to analysis of variance (ANOVA) test using Genstat computer package. The significantly different treatment means were separated using Least Significant Difference (LSD) test at 5% probability level.

Plate 5.1 Screenhouse used for Aphid and Beetle trials under different chemical treatments.

5.2.2 Effect of ants (*Lepiosiota* spp) on *Coccinellidae* control of cotton aphid

A randomized complete block design (RCBD) experiment with three treatments (Aphids without any control strategy; Aphids + Beetles, and Aphids + Beetles + Black Garden Ants) was set. The experiment setup was as in 5.2.1. At four weeks, using Kaplan & Eubanks (2005) approach, the cotton plants were infested with 20 aphids per plant obtained from infested cotton plants in the field. Four aphids were placed on each of the five marked leaves per plant. At seven days after aphid infestation, 20 black garden ants (*Lepiosiota* spp) were introduced. On day 10, ladybird beetles were introduced. The beetles (as in 5.2.1) were obtained from milk weeds and black jack growing in the field. Aphid populations under the different treatments were monitored by counting the aphids present on the crop for twenty consecutive days. The data collected were then analyzed as in 5.2.1 above.

5.3 Results

5.3.1 Effect of Dimethoate, *Bt*-spray and *Coccinellidae* Beetle on Aphid Population

There were significant ($p<0.05$) variations in the mean aphid scores with respect to the three different treatments. Dimethoate treatment had the least aphid population density, followed by the Bacillus treatment and the highest density was registered in the Water treatment. The mean scores were highest in the first sampling date and decreased progressively with each sampling date.

Table 5.1 Effect of Control Strategies on aphids and beetles density on cotton

Sampling dates	**Treatments**					
	Surviving Aphids (means ±SE)/36 plants			Surviving Ladybird Beetles (means ±SE)/36 plants		
	Bacillus	**Dimethoate**	**Water**	***Bacillus***	**Dimethoate**	**Water**
Week 1	2.8 ± 0.7 a	2.4± 0.5a	3.0 ± 0.7a	1.8 ± 0.1a	1.6 ± 0.1a	1.8 ± 0.3a
Week 2	2.3 ± 0.3 a	2.1 ± 0.4b	2.7 ± 0.7ab	0.8 ± 0.2b	0.7 ± 0.1b	0.8 ± 0.2b
Week 3	2.1 ±0.7bc	1.4 ± 0.2c	2.5 ± 0.4 b	0.3 ± 0.0c	0.2 ± 0.1c	0.7 ± 0.3bc
Week 4	1.6 ± 0.3 c	1.1 ± 0.3d	1.9 ± 0.2 c	0.3± 0.1c	0.0 ± 0.0c	0.3 ± 0.0c
Week 5	1.7 ± 0.4c	1.0 ± 0.3d	1.8 ± 0.1c	0.0 ± 0.0c	0.0 ± 0.0c	0.3 ± 0.0c

Different letters indicate significant differences between treatments (P = 0.05).

Significant ($p<0.05$) variations occurred in the ladybird counts between the sampling dates in each of the treatments. Ladybird counts were highest in the water treatment followed by the *Bacillus* treatment and least in the dimethoate treatment. On average the population of ladybirds in all the treatment was highest in the first sampling date and decreased progressively with each sampling date (Table. 5.1).

At the end of week 1, ladybird beetle population in all the three treatments had fallen to below 40% of the initial population introduced. At the end of week 2, the **B**acillus treatment was at

17% population density as compared to the water and dimethoate treatment that were at 15% and 13% respectively (Figure 5.1). The population density in all the three treatments continued decreasing progressively through week 3 to week 5, with the water treatment having the highest population density, followed by the *Bacillus* treatment. It should be noted that by week 4, all the beetles in the dimethoate treatment were dead (Figure 5.1).

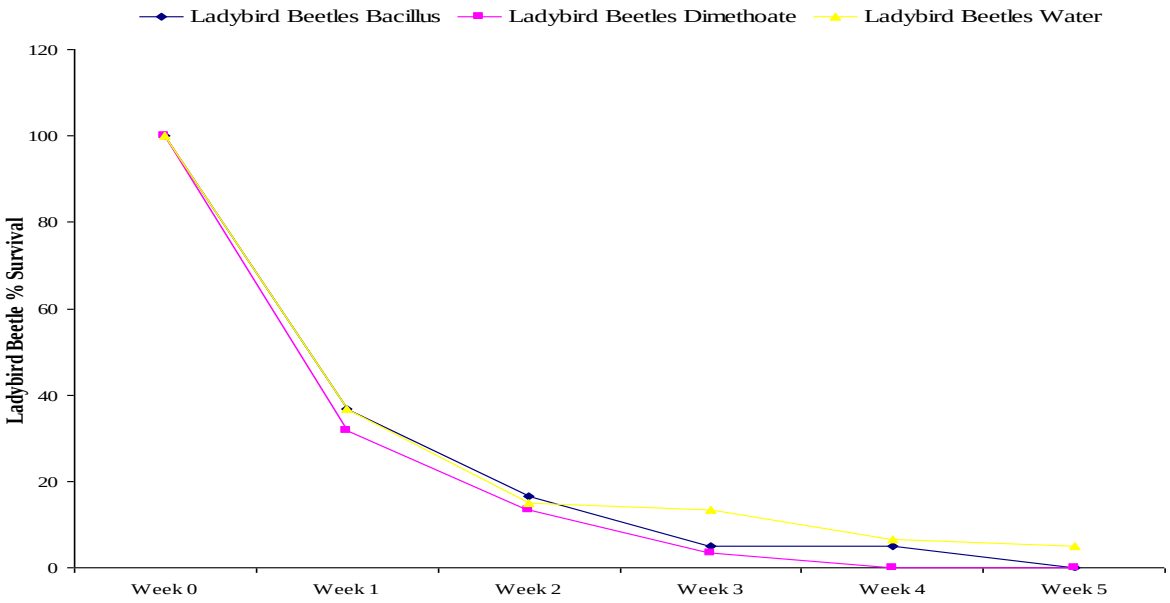


Figure 5.1 Effect of control strategies on Ladybird Beetle Population Density

5.3.2 Effect of ants (*Lepiosiota* spp) on *Coccinellidae* control of cotton aphids

Significant variations in the mean aphid populations were observed. The Lady Bird Beetles treatment had the least mean aphid population density at 17.44 aphids per leaf, followed by the Black garden ants + Ladybird beetles treatment at 25.73 aphids per leaf and the highest mean aphid population were registered in the control treatment as shown in table 5.2 below.

Table 5.2 Aphid population as influenced by the treatments

Treatment	Cotton Aphid Counts
Control (No Ladybirds/ Black garden ants)	45.12 ± 6.7 a
Ladybird beetles	17.44 ± 5.3 c
Black garden ants + Ladybird beetles	25.73 ± 3.9 b

Different letters indicate significant differences between treatments (P = 0.05).

Significant (P<0.05) differences occurred in the mean aphid populations (Fig 5.2). Highest mean aphid populations were registered in the control treatment, followed by the Black garden ants + Ladybird beetles treatment with aphid mean population highest (63.0 aphids per plant) at the eighth sampling time and lowest (10.2 aphids per plant) at the nineteenth sampling time. Overall the Ladybird beetle treatment had the lowest aphid population. It should also be observed that at the tenth sampling when the control treatment (Aphid uncontrolled) had the highest population mean (102.0 aphids – five times the introduced population), the other two treatments; Ladybird beetle treatment and Black garden ants + Ladybird beetles treatment had 11.4 and 16.6 aphids per plant, which population was below the introduced one as shown in graph 5.2 below.

5.3.3 Effect of ants (*Lepiosiota* spp) on *Coccinellidae* beetle percentage survival

Overall, ladybird beetles suffered the highest mortality in the first two days of about 40% in the presence and absence of black garden ants. Mortality was most registered in the black garden ants' treatment as compared to the black garden ant free treatment. Generally ladybird beetle survival was about 50% lower in the presence of black garden ants than in the absence of the black garden ants (Fig. 5.2). However, in both the treatments (in the presence and absence of the black garden ants), the survival rates for the ladybird beetles continued decreasing progressively through the sampling period (Fig. 5.2).

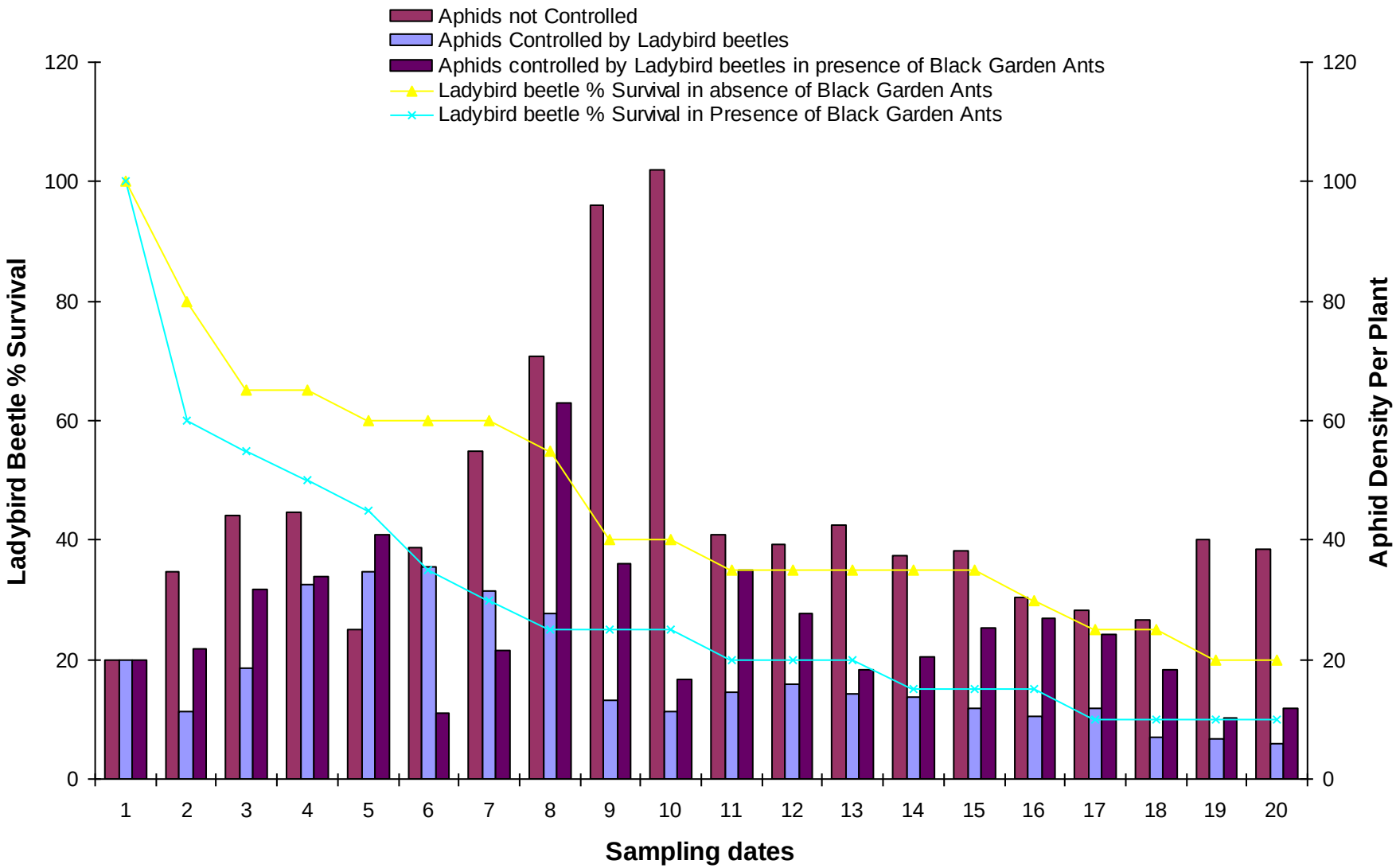


Figure 5.2 Effects of Biological Agents on Aphid Population Dynamics

5.5 Discussions

The results indicated that dimethoate which is a broad-spectrum chemical caused the highest mortality of all arthropods (aphids and beneficial ladybird beetles) compared to *Bacillus* and water. These finds concur with other many studies that have alluded to broad-spectrum chemicals causing mortality of both beneficial and destructive spp (Kennedy, 2008; Vodouhe, 2007 & Fontes *et al*., 2006; Fadare & Amusa, 2003). However, it should be noted that in the management of pests especially when transgenic crops are used, threats to biodiversity conservation and sustainable agriculture is substantial (Altieri, 2005). In addition, Bollgard II® crops which is a transgenic cotton variety, the management of the non target pest is biased toward dimethoate (Wilson *et al*., 2007). From the results in this study and other earlier findings (Wilson *et al*., 2007; Kennedy, 2008; Vodouhe, 2007 & Fontes *et al*., 2006; Fadare & Amusa, 2003), dimethoate which is a broad spectrum pesticide is detrimental to beneficial populations and may increase the risk of other pest outbreaks (Wilson *et al*., 2007). Therefore as cotton pests control strategies are being developed in the wake of genetically modified cotton in Uganda, consideration of how the emerging secondary pests are controlled should consider biosafety implications that come up with the dimethoate packaged strategies.

Whereas there was some decline with in the aphid levels, their control by XenTari (*Bt*-biopesticide) was slightly different from that offered by dimethoate. This might have been due to the fact that, aphids are not a major target for the XenTari biopesticide. However, if XenTari was introduced to control the target lepidopteran pest, this will put the aphid into constant exposure to the protein toxin. This exposure may cause increased fitness of the aphid and subsequently increasing their outbreak potential (Hilbeck *et al.,* 2006). Secondly, the limited control of aphids by XenTari may further lead to an increase in the aphid populations and

subsequent continued use of chemicals. This therefore calls for judicious integration of *Bacillus thuringiensis*, dimethoate and other chemicals in a better integrated pest control system in the management of non-*Bt* target pests in *Bt*-cotton.

XenTari biopesticide, though slightly different from dimethoate in managing the aphids in this study, it was observed to have affected the survival rates of the ladybirds. However, results on the effects of the *Bt*-toxin (in biopesticides or incorporated in the plant) on non-targets are inconsistent. Some of them reported no effects (Dogan et al. 1996, Pilcher et al. 1997), while others demonstrated some effects of *Bt*-crops on non-target insects and natural enemies (Hilbeck et al. 1998, Dutton et al.2002).

From the results it was observed that, the presence of the ants reduced the efficiency of ladybird beetles. This concurs with the findings of Kaplan & Eubank (2005); Hemipitinne & Dixon (2000) and Stadler and Dixon (2005) who noted a lower survival rate of herbivores (caterpillars) and predators (ladybird beetles and lacewings) in the presence of fire ants and aphids. Ants are known to limit alate aphid dispersal by physically removing wings and through chemical manipulation of the alate developmental pathway (Eubanks *et al.*, 2002; Kaplan and Eubanks, 2005). This results in reduced dispersal and higher local densities of aphids, which benefit ants in terms of increased honeydew and prey availability (Oliver *et al*., 2007). Since introduction of genetically modified cotton may have an effect of increasing secondary pest especially the sucking pests which include the aphids, the protection of the aphids by the ants may be detrimental to biological control (Kaplan & Eubank (2005). It has also been highlighted that when both aphids and other herbivores are at outbreak densities, the ant–aphid association may benefit cotton plants by enhancing predation on other, potentially more damaging herbivores (Lach 2003; Styrsky & Eubanks, 2007). On the other hand, Renault *et al* 2005 observed that

relationships among plants, aphids and ants are complex, depending on when and where they are studied. Mutualisms (ants-Homoptera) are common in nature and are one of the "great forces in the ecology and evolution of species" affecting populations, communities, and ecosystems (Katherine *et al.*, 2008). Therefore, while seeking strategies of cotton pest control, especially the secondary pests that may come up as result of the introduction of genetically modified cotton, the aphid/ant mutualistic interactions need to be given due consideration so as have a better management package of cotton pests.

Ladybird beetle survived less in the presence than in the absence of the black garden ants. Kaplan and Eubanks (2002; 2005) highlighted the mutualistic relationship between fire ants and aphids as a key interaction that altered community structure of arthropod communities. Through tending of the aphids, Grover *et al* (2008) noted that, ants significantly decreased the population of herbivores and aphid-predators on cotton plants and black nightshade, *Solanum nigrum* L. Therefore the study findings on the aphid – ants – beetle interaction seem to imply that if genetically modified cotton is introduced, and aphids become a major cotton pest (Biradar & Vennila, 2008; Birch and Wheatley, 2005; Nguyen *et al*., 2008; Wilson *et al*., 2007), then ants and their corresponding effects might be manifested (Kaplan and Eubanks, 2002; 2005; Grover *et al.*, 2008). Mutualisms between ants and aphids vary with abiotic and biotic conditions, including the species involved and the needs of each partner, with more aggressive ant species protecting more aphids from predation, parasitism and hyperparasitism than less aggressive ant species (Kaplan and Eubanks, 2002; 2005; Kaneko 2003; Katherine *et al.*, 2008). Therefore as the introduction of genetically modified cotton becomes a reality in Uganda, mutualistic interactions between ants and aphis/other honey dew producing arthropods as highlighted by the

study findings need to be given due consideration in biosafety risk assessment and subsequent development of cotton pest management strategies.

In conclusion therefore, XenTari biopesticide showed some effectiveness in the control of aphids. However, like the dimethoate treatment, *Bacillus* biopesticide had some significant impact on ladybird beetle counts highlighting some effect on biological control. Results from tri-trophic interactions between aphids, black garden ants and ladybird beetle indicated that, the presence of ants reduced survival rates of ladybird beetles and their efficiency in the control of aphids. Therefore in this era of biotechnology, integrated pest management systems of cotton pests will call for a strategy that incorporates the judicious use of *Bacillus thuringiensis* (as a bio pesticide or incorporated in the plant), dimethoate and natural biological agents. This will be necessary and important as long as biosafety issues that relate to; environmental impacts, effects on ecosystem services, farm biodiversity, invertebrate fauna, changes in plant community structure, ethical and social-economical considerations are catered for.

However, bio-control aspects of integrated pest management have not worked as well as they should have because of the lack of knowledge of the several variables involved in the biology of the crop, interacting natural enemies and the pest under consideration. Results from the biological control experiments (Chapter five: sections 5.3.1; 5.3.2 and 5.3.3) highlighted the importance of ladybird beetles in the management of aphids through the use of inorganic chemicals/biopesticides (dimethoate and *Bacillus thuringiensis*) and in the presence/absence of ants. Therefore, establishment of the different variables in the ecosystem is important so as to strengthen the actual ecological models that can be used in the biological control of pests. Knowledge and understanding of particular foraging behaviours and how they happen is

important in developing a successful biological control approach. The next chapter (chapter six) therefore examined the searching behaviour and olfactory orientation of the ladybird beetle (*Cheilomenes*: Coleoptera: Coccinellidae) on cotton aphid (*Aphis gossypii:* Homoptera: Aphididae).

CHAPTER SIX

6.0 SEARCHING BEHAVIOUR AND OLFACTORY ORIENTATION OF LADYBIRD BEETLE, *Cheilomenes* (Coleoptera: Coccinellidae) ATTACKING COTTON APHID, *Aphis gossypii* (Homoptera: Aphididae)

6.1 Introduction

Results from the field surveys indicated that beneficial arthropods were the most abundant arthropods in the various cropping systems in Uganda (chapter three: section 3.4.2). Additionally, prioritization studies of arthropods at risk of being affected by transgenic toxins catalogued ladybird beetles (Coleoptera: Coccinellidae), *Lepiosiota* spp (Formicidae) as beneficial arthropods and *Aphis gossypii* (Aphididae) as cotton pest, in the highest priority category (chapter four: section 4.4.1). Ladybird beetles are widely distributed predators of several aphid species in diverse habitats (Schaller & Nentwig, 2000) whose efficiency is sometimes affected by the presence of ants spp (Formicidae) (Kaplan and Eubanks, 2005; Lach 2003; Styrsky and Eubanks 2007; Oliver *et al* 2007). Results from biological control experiments (Chapter five: section 5.3.2 and 5.3.3) highlighted the importance of ladybird beetles in the management of aphids in the presence and absence of ants. However, Walter, (2003), Upanisakorn *et al*., (2007) and Brodt *et al*., (2007) observed that bio-control strategies have not worked as well as they should have, because of the lack of knowledge of the several variables involved in the biology of the crop, interacting natural enemies and the pest under consideration.

Different plant features have been noted to alter the availability of herbivores to natural enemies. For example, semio-chemicals are known to play a major role as cues to aid natural enemies in locating and recognizing their hosts or prey (Cook *et al*, 2007; Almohamad *et al.*, 2009). In

addition to semio chemicals and plant chemical factor, structures such as trichomes and cuticle thickness have also been observed to directly affect natural enemies (Lucas *et* al 2004; Otim 2007; Otim *et al* 2008). Similarly, plant architecture has been documented to affect predators indirectly by influencing the availability of spatial refuges for prey, the spatial distribution of prey, and the abundance and diversity of herbivorous prey (Lessando *et al* 2008). Genetic engineering is expected to play an important role in improving the quantity and quality of biomass and overall plant characteristics (Hisano et al., 2009; Li and Qu, 2011). Use of these direct and indirect defenses of the plant in combination with engineered crop plants could enhance biological control (Guy *et al* 2004).

Whereas ladybird beetle have been widely used in biological control, quantitative assessments of their efficacy has not been done in most agricultural crops. Within crops, the ladybird beetle will undertake a wide range of behaviours, including locating of food, mates, oviposition sites and refugia to escape adverse conditions. These behaviours are mediated by diurnal activity patterns and sensory perception, internal factors, such as hunger and reproductive state (Dixon, 1982; Rhamhalinghan, 1987) and external environmental factors, such as climatic conditions (Nakamuta, 1987), habitat quality (Honek 1983; Carter & Dixon, 1982) and possibly exposure to pesticides (Tank *et al.,* 2007). It has also been noted that, if the ladybird beetle picks sub-lethal doses of pesticides, some behavioural changes, such as altered foraging patterns, disrupted sexual communication or host recognition and/or physiological changes, such as altered reproduction, reduced longevity, egg viability or fitness might occur (Wang *et al* 2003; Thornham *et al* 2008; Zhou *et a*l 2005). Genetically modified cotton might have altered chemical composition, plant morphological and architectural modifications and all these might affect the potential and efficiency of biological control agents.

Thus, knowledge and understanding of a particular foraging behaviour and how it happens are important in developing a successful biological control approach (Otim, 2007; Otim *et al.*, 2008). Leaf hair density, waxiness and sensory abilities (Lucas *et al*., 2004; Verheggen *et al* 2007); different pesticides (Wang *et al* 2003; Thornham *et al* 2008; Zhou *et a*l 2005), antagonistic/mutualistic effects of ants (Kaplan & Eubanks, 2005; Lach 2003; Styrsky and Eubanks 2007; Oliver *et al* 2007) and different volatiles from different cotton varieties (Loughrin *et al* 1995) have been found to influence search behaviour. This part of the study investigates the influence of leaf pubescence and sensory abilities on the searching and orientation behaviour of Coccinellidae beetle a principal predator of aphids in the cotton cropping system was studied. Genetic engineering is expected to play an important role in improving the quantity and quality of biomass and overall plant characteristics (Hisano et al., 2009; Li and Qu, 2011) and it is against this that this study hypothesed that the introduction of transgenic cotton will further alter the inherent plant factors and therefore affect the efficiency of the foraging biological control agents in the different cropping systems and agro-ecological zones in Uganda.

6.2 Materials and Methods

6.2.1 Influence of leaf pubescence on searching and predation behaviour

6.2.1.1 Leaf Hair characteristics on the different cotton varieties

Three cotton varieties, BPA 1999, BPA 2000 BPA 2002 and a filter paper (as a control) were used in studying influence of leaf pubescence on searching and predation behaviour of the ladybird beetle, *Cheilomenes* spp. The study was conducted in a screen house at Makerere University Agricultural Research Institute Kabanyolo (MUARIK). To determine leaf hair characteristics on the different cotton varieties, 1cm leaf discs were obtained using a cork borer

(No. 5). The number of trichomes on the upper and lower sides of the leaf disc was counted under a microscope, for each cotton variety. Leaf hair density was significantly lower ($p< 0.05$) on BPA 1999 (6.4 ± 0.35 mm^2) (range, 5.8 – 13 mm^2) than on the other two varieties BPA 2000 (11.20 ± 0.68 mm^2) and BPA 2002 (11.20 ± 0.72 mm^2).

6.2.1.2 Searching and predation behaviour of the Coccinellidae ladybird beetle

Cheilomenes ladybird beetles and aphids used in this experiment were obtained as described in chapter five (Section: 5.2.1). However, before being used, the ladybird beetles were examined for the presence of all appendages (antenna, legs, and other mouth parts) and thereafter starved for 24Hrs. The foraging behaviour was studied using the approaches used by Otim, (2007) as adopted from Headrick *et al.,* (1995). In this approach, one month old cotton plant leaves infested with aphids were placed, with a daxial side up, in a petri dish of 10cm diameter. Cello tape was used to keep the leaves firm and flat in position. Individual adult ladybird beetle or larvae were placed in the middle of an aphid infested leaf in a petri dish. The behaviour of the adult ladybird beetle or larva including searching for a prey, feeding on the prey, antennation- (feeling the prey using antennae), Standing still - (standing on the leaf or prey), encountering – (making contact with the prey), cleaning – (grooming itself) and flying/walking away- (leaving the prey or leaf area) were recorded using "The Observer®" computer software: (Program 5.0, Noldus Information), for a period of 1 hr. The observation was terminated whenever the adult ladybird beetle flew away or the larva left the searching area respectively for over 35 seconds. The observations were carried under room temperature (laboratory conditions). In order to input the behavioural data for the ladybird beetle in the computer, a code was allocated to a given button for a particular activity on the computer keyboard. This button was pressed whenever the activity was initiated. The duration of the following behavioural events were recorded:

a. Searching – W – (probing or looking around to find prey),
b. Feeding – F – (Feeding on the prey)
c. Antennation – (A) (feeling the prey using antennae),
d. Standing still - T (standing on the leaf or prey),
e. Encountering – (E) (making contact with the prey),
f. Cleaning – (C) (cleaning or grooming itself) and
g. Flying/Walking away- (L) (leaving the prey unattended to or leaf area).

There were twenty replications for each variety.

6.2.1.3 Data analysis

Behavioural data were entered into Microsoft Excel spreadsheets and collated for statistical analysis. Means were based on total frequencies of the behaviours summed over all trials for each ladybird beetle on the different cotton varieties and filter paper. Behavioural frequencies were used to create ethograms for the ladybird beetle on the different cotton varieties/filter paper and time spent on different behaviours was used to create time budget graphs (Otim, 2007; Otim *et al*., 2008).

6.2.2 Olfactory orientation of the Coccinellidae ladybird beetle to the cotton aphid

In mimicking the influence of the odour that might be given off by genetically modified cotton on biological control of cotton pests, olfactory orientation of the ladybird beetle to/away from cotton aphids in the presence/absence of ants under different treatments was studied at Makerere University Agricultural Research Institute Kabanyolo (MUARIK) using an olfactometer.

6.2.2.1 Plant, Insect management and odour sources

Three local cotton varieties, BPA 1999, BPA 2000 and BPA 2002 were used in studying the olfactory orientation of ladybird beetle, *Cheilomenes* spp (Coleoptera: Coccinellidae) towards *Aphis gossypii* (Homoptera: Aphididae). Ladybird beetles, cotton aphids and ants used in this experiment were obtained as described in chapter five (Section 5.2).

To achieve the different odours, BPA 1999, BPA 2000 and BPA 2002 cotton leaves infested with aphids were sprayed with dimethoate (dimethoate: 400g/L EC; 0.5L/Ha) and XenTari (XenTari: *Bacillus thruringiensis.* Aizawai, Lepidopteran a.i. 10.3% w/w) in the presence and absence of ants (*Lepiosiota* spp: Hymenoptera: Formicidae).

The odours sources were selected because:

a. Dimethoate – insecticides use in *Bt* cotton (Bollgard II® crops) is biased toward Dimethoate (Pyke and Doyle 2006; Wilson *et al.* 2007).
b. XenTari – in some countries where *Bt*-Cotton and other genetically modified crops have not yet been approved for trial/commercialization, *Bt*-biological insecticides (XenTari: *Bacillus thruringiensis.* Aizawai, Lepidopteran a.i. 10.3% w/w) are being used (Goodell, 2004; Aggarwal *et al.,* 2006; Maxwell and Fadamiro, 2006).
c. Ants (*Lepiosiota* spp: Hymenoptera: Formicidae) - The presence of antagonistic/mutualistic ants have been observed to affect biological control of aphids (Kaplan & Eubanks, 2005; Lach 2003; Styrsky and Eubanks 2007; Oliver *et al* 2007). In addition, ants are also known to limit aphid dispersal through direct physical manipulation or through chemical influence (Cook *et al,* 2007).

6.2.2.2 Four-arm olfactory settings and Behavioural (Orientation) observations

Ladybird beetles were introduced to BPA 1999, BPA 2000 and BPA 2002 cotton leaves under the different odours in a four arm olfactometer which was a modifications of the design of using Veronica (2003) and Godfried (2006).

Setup Category One (With no Ants)

***Cheilomenes* (Coleoptera: Coccinellidae)**	**Odour Source**
a) Beetles (Adult/Larvae)	Dimethoate
b) Beetles (Adult/Larvae)	XenTari – *Bt* Spray
c) Beetles (Adult/Larvae)	Distilled Water)
d) Beetles (Adult/Larvae)	NIL – **Control**

Setup Category Two (With Ants)

***Cheilomenes* (Coleoptera: Coccinellidae)**	**Odour Source**
e) Beetles (Adult/Larvae)	Ants X Dimethoate
f) Beetles (Adult/Larvae)	Ants X Xen Tari – *Bt* Spray
g) Beetles (Adult/Larvae)	Ants X Distilled Water
h) Beetles (Adult/Larvae)	Ants X NIL – **Control**

The olfactometer used in this study was of a square shape with four arms constructed from glassy percepts. The four arms were made from plastic specimen bottles whose bottoms were cut so as to be fitted on the rectangular glass percepts. The arena of the olfactometer was made of: a clear glassy 300 × 300 × 5 mm perforated square (top) and a 300 × 300 × 5 mm square (bottom) containing four-pointed plastic bottles (specimen bottles whose bottoms were cut so as to be fitted on the square glassy percepts) as exposure chamber (90° arc, radius 80 mm) (Plate 6.1).

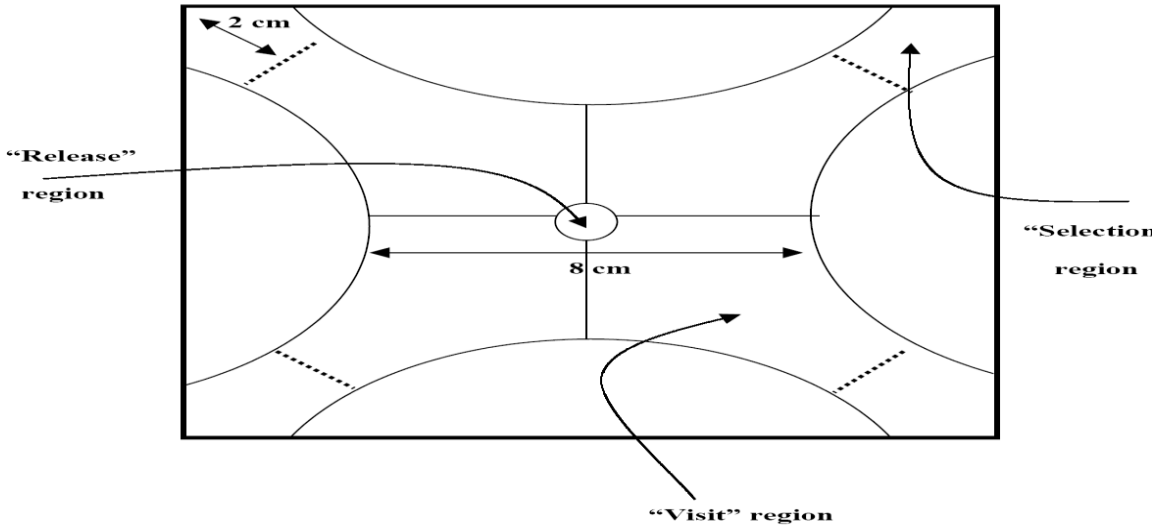


Figure 6.1 Four-arm olfactometer arena for behavioral responses

Plate 6.1 Olfactometer setup for the ladybird odour response experiment on cotton

Each olfactometer arm was divided into three regions: the "visit" region nearest to the center, the "selection" region nearest to the odour source, and the "release" region at the center (Fig. 6.1). Airflow/ventilation inside the arena was equalized through the perforation in the top cover and occasional opening of the green seals of the plastic bottles (exposure chamber) (Plate 6.1). To

provide a uniform light in the arena and reduce biases due to other distractions, a fluorescent lamp was used.

In the release region, a filter paper was placed onto which the ladybird beetles to be tested were introduced to assess and consequently respond to the various odours. After insect introduction into the olfactometer, movement towards a given odour sources was observed. Final behavioural (orientation) choice, was registered whenever the beetle entered the selection region (Fig. 6.1). All experiments were run between 10:00 am and 5:00 pm. An observation was terminated if the beetles did not enter any odour source (selection area) after 5 min. A total of six groups of four beetles were tested during a 2-hour period that constituted a replication.

6.2.2.3 Data analysis

Using Narayanan and Nadarajan (2005) and Prasuna *et al* (2008) approach but with some modifications, the three test chambers constituting dimethoate, XenTari and water odour were considered as the main treatments and the fourth test chamber (with no dimethoate, XenTari and water) served as control. For each experiment "activity" and "preference/attraction" of the beetles was assessed. Activity was the number of beetles that showed response (moved to the odour chambers) in the olfactometer compared to the beetles that were not attracted to any odour and stayed in the release/visit region and the percentage activity was computed as;

$$\left\{ \frac{\text{No. of beetles released} - \text{No. of beetles not responding to any odour}}{\text{Total number of beetles released}} \right\} \text{X } 100$$

Preference/attraction was the number of active beetles choosing any of the test odours in the olfactometer. It was recorded as an 'attraction index (AI)'and computed as;

$$\frac{\text{No. of beetles responding to a given odour} - \text{No. of beetles responding to control}}{\text{No. of beetles released} - \text{No. of beetles responding to control}}$$

Thereafter the calculation of the "activity" and "preference/attraction", data was subjected to analysis of variance (ANOVA) using Genstat computer package. The significantly different treatment means were separated using Least Significant Difference (LSD) test at 1% probability level.

6.3 Results

6.3.1 Search Behaviour

When the beetle larvae were placed in the middle of the leaf infested with aphids, they immediately begun searching for aphids. The adults kept walking away or flying off but with some intermittent searching. Generally for both the adults and larvae,; searching for the prey, encountering the host and feeding on the prey were the main events and sequence of behaviors exhibited.

Encountering between larvae and aphids was recorded when the larva made contact with the aphid, which in most cases led to feeding. Feeding involved the larvae using its fore legs to grab the aphid to its mouth, chewing and finally swallowing it. All the feeding was done when the larva was standing still at the same position. In the adults, encountering was recorded when the adult made contact with the prey. This was followed sometimes with antennation and cleaning. The adults would sometimes fly or walk away from the leaf. The adult feeding was not as frequent as with the larval stage.

6.3.2 Behavioural Pathways

Ethograms of adult and larvae of beetles were developed from the recorded sequences. Transition frequencies (Fig 6.1 – 6.8) are reported as a percent of the total number of times a particular behaviour was recorded.

6.3.2.1 Behavioural Pathways on Filter Paper

When placed on the filter paper, the adult beetle began searching (Fig. 6.1) which was followed by prey encounter, standing still, flying, cleaning and antennation which represented (44%),(33%), (11%) (9%) and (3%) of the total search frequencies respectively. Prey encounter led to feeding (93%), searching (4%) and standing still (2%) of the time. Feeding led to antennation (2%), encounter (2%), cleaning (7%), standing still (19%) and searching (69%) of the time. Antennation led to resumption of searching (77%) of the time, encountering (15%) and standing still (8%) of the time. Cleaning led to standing still (52%) of the time.

Searching by the larvae was followed by prey encountering (60%), standing still (36%) and cleaning (4%) of the time (Fig. 6.2). Prey encounter led to feeding (86%) of the time, standing still (11%) and renewed searching (3%) of the time. Feeding led to renewed searching (76%) of the time, standing still (22%), prey encountering (2%) and cleaning (1%) of the time. Cleaning was followed by searching (90% of the time) and prey encounter (10% of the time). Standing still was followed by searching (61% of the time), prey encounter (31% of the time) and cleaning (8% of the time).

6.3.2.2 Behavioural Pathways on BPA 1999

When place on BPA 1999, the adult ladybird beetle began by searching for prey and this was followed by encountering, standing still, cleaning, flying and antennation which represented

(49%), (32%), (11%),(5%) and (3%) of the total activity time respectively (Fig. 6.3). Adult ladybird prey encounter led to feeding (88%), standing still (6%), cleaning (4%) and renewed searching (1%) of the time. Feeding led to resume searching (70%), standing still (27%), prey encounter (2%) and cleaning (1%) of the time. Antennation led to resume searching (71%), standing still (17%) and prey encountering (13%) of the time. Cleaning led to resume searching (41%), standing still (46%), antennation (5%), encountering (4%) and fling off (3%) of the time. Standing still led to cleaning (66%), resume searching (25%), antennation (5%), flying (2%) and encountering (1%) of the time. Flying off led to resumed searching (71%) and antennation (29%) of the time.

Larvae's searching was followed by encountering (73%), standing still (15%) and cleaning (12%) of the total search time. Encountering led to feeding (93%), resuming searching (3%), cleaning (2%) and standing still (2%) of the time. Feeding led to resume searching (49%), standing still (39%), cleaning (9%) and encountering (3%) of the time. Cleaning led to resumption of searching (64%), standing still (23%) and encountering (13%) of the time. Standing still led to searching (39%), encountering (47%) and cleaning (14%) of the time (Fig 6.4).

6.3.2.3 Behavioural Pathways on BPA 2000

When placed on BPA 2000 leaflets, the adult ladybird began searching (Fig. 6.5) which was followed by encountering, standing still, cleaning, flying and antennation which represented 30%, 26%, 25%, 18% and 1% of the total search frequencies respectively. Prey encounter led to feeding (83%), standing still (13%) and resumed searching (3%). Feeding led to standing still (52%), cleaning (28%), resumed searching (16%) and antennation (4%) of the time. Cleaning led to standing still (47%), resumed searching (45%), encountering (4%), antennation and

cleaning both at 2% o f the time. Standing still led to resumed searching (44%), cleaning (40%), encountering and antennation (each at 7%) and flying (2%) of the time. Flying led to antennation (12%) of the time. Antennation and flying led to renewed searching 100% and 88% of the time respectively.

Searching by the larvae was followed by prey encountering 90%, standing still 8% and cleaning 2% of the time (Fig. 6.6). Prey encounter led to feeding 90% of the time, standing still 7% and renewed searching 3% of the time. Feeding led to renewed searching 32% of the time, standing still 50%, prey encountering 4% and cleaning 14% of the time. Cleaning was followed by searching (28% of the time), prey encounter (22% of the time) and standing still 50% of the time. Standing still was followed by searching (41% of the time), prey encounter (24% of the time) and cleaning (35% of the time).

6.3.2.4 Behavioural Pathways on BPA 2002

When placed on BPA 2002, the adult ladybird beetle began by searching and this was followed by encountering, cleaning, standing still, flying and antennation which represented 39%, 26%, 21%, 12% and 2% of the total search time respectively (Fig. 6.7). Adult ladybird prey encounter led to feeding (93%), standing still (4%), cleaning (2%) and renewed searching (1%) of the time. Feeding led to resume searching (40%), standing still (46%), prey encounter (2%), cleaning (8%), flying and antennation (at 2% each) of the time. Antennation led to resume searching 100% of the time. Cleaning led to resume searching (53%), standing still (40%), antennation (3%) and encountering (4%) of the time. Standing still led to resumed searching (41%), cleaning (32%), encountering (22%), antennation (3%) and flying (2%) of the time. Antennation led to resumed searching 100% of the time. Flying off led to resumed searching (88%) and antennation (12%) of the time.

Searching by the larvae was followed by prey encountering 80%, standing still 15% and cleaning 5% of the time (Fig. 6.8). Prey encounter led to feeding 96% of the time, standing still 3% and renewed searching 1% of the time. Feeding led to renewed searching 49% of the time, standing still 45%, prey encountering 3% and cleaning 3% of the time. Cleaning was followed prey encounter (62%) and standing still (38%) of the time. Standing still was followed by searching (35% of the time), prey encounter (57% of the time) and cleaning (8% of the time).

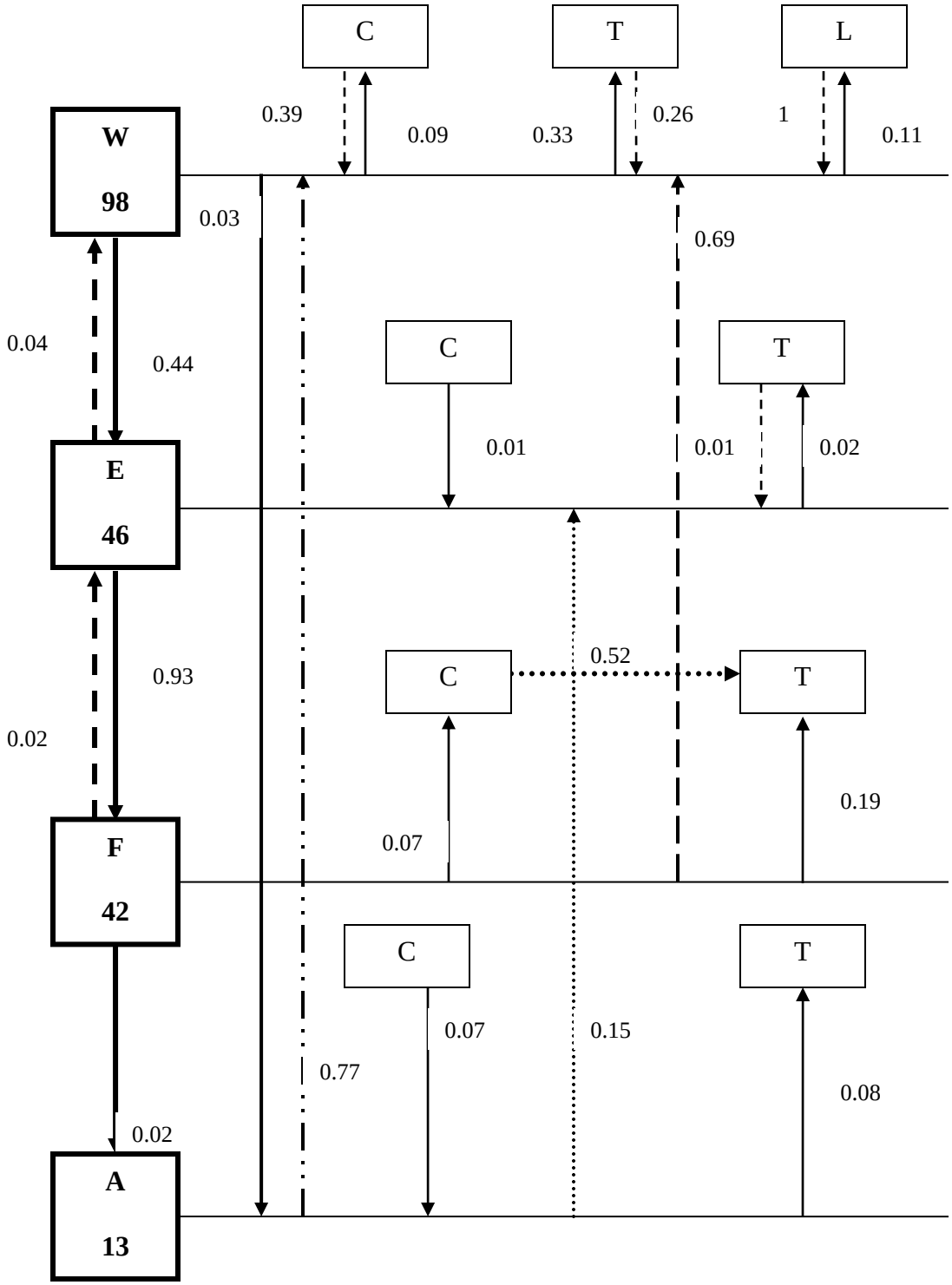


Figure 6.1 Ethogram for behaviours of the adult beetle on filter paper.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation, L- Flying. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

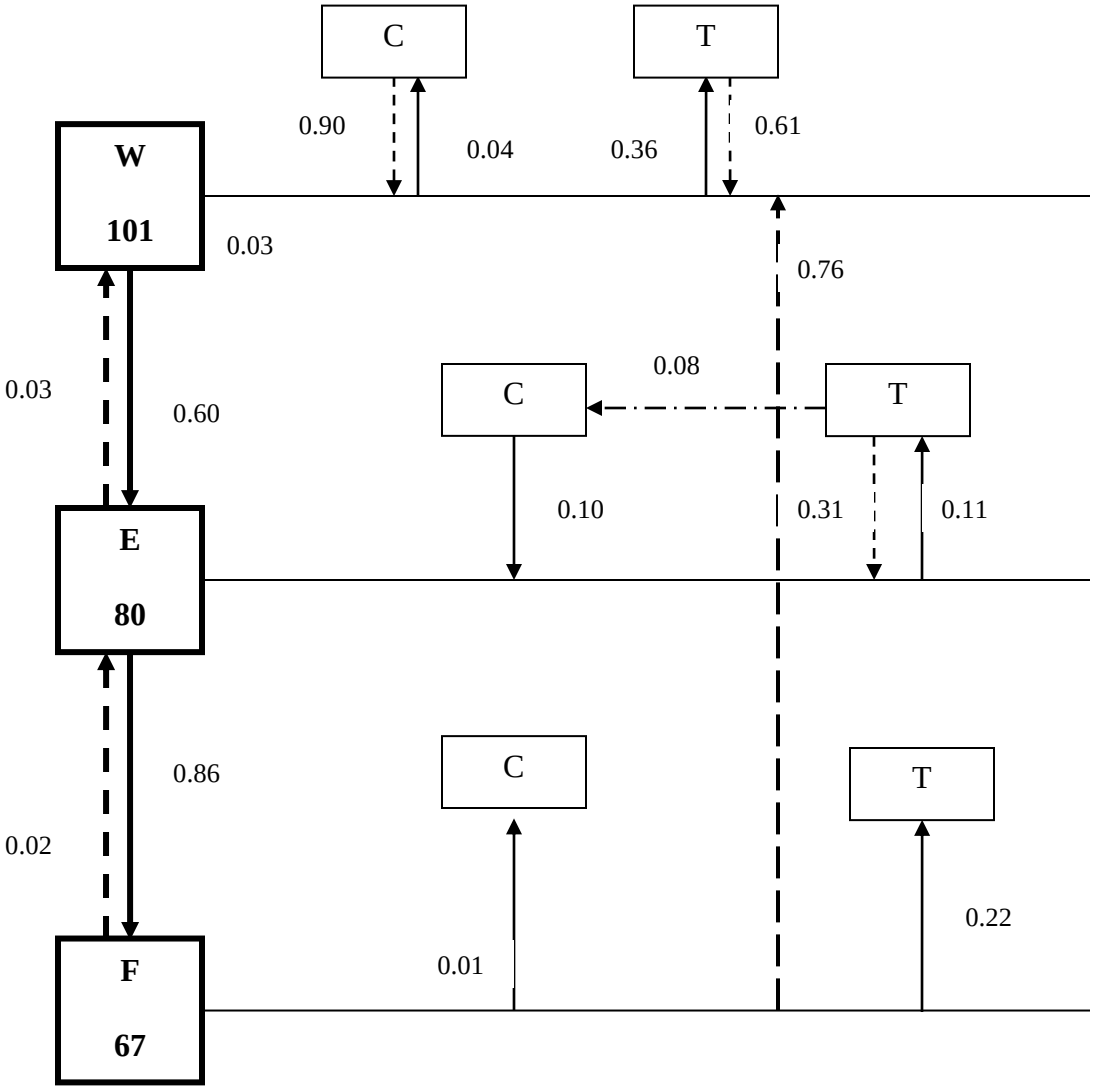


Figure 6.2 Ethogram for behaviours of the larvae beetle on filter paper.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

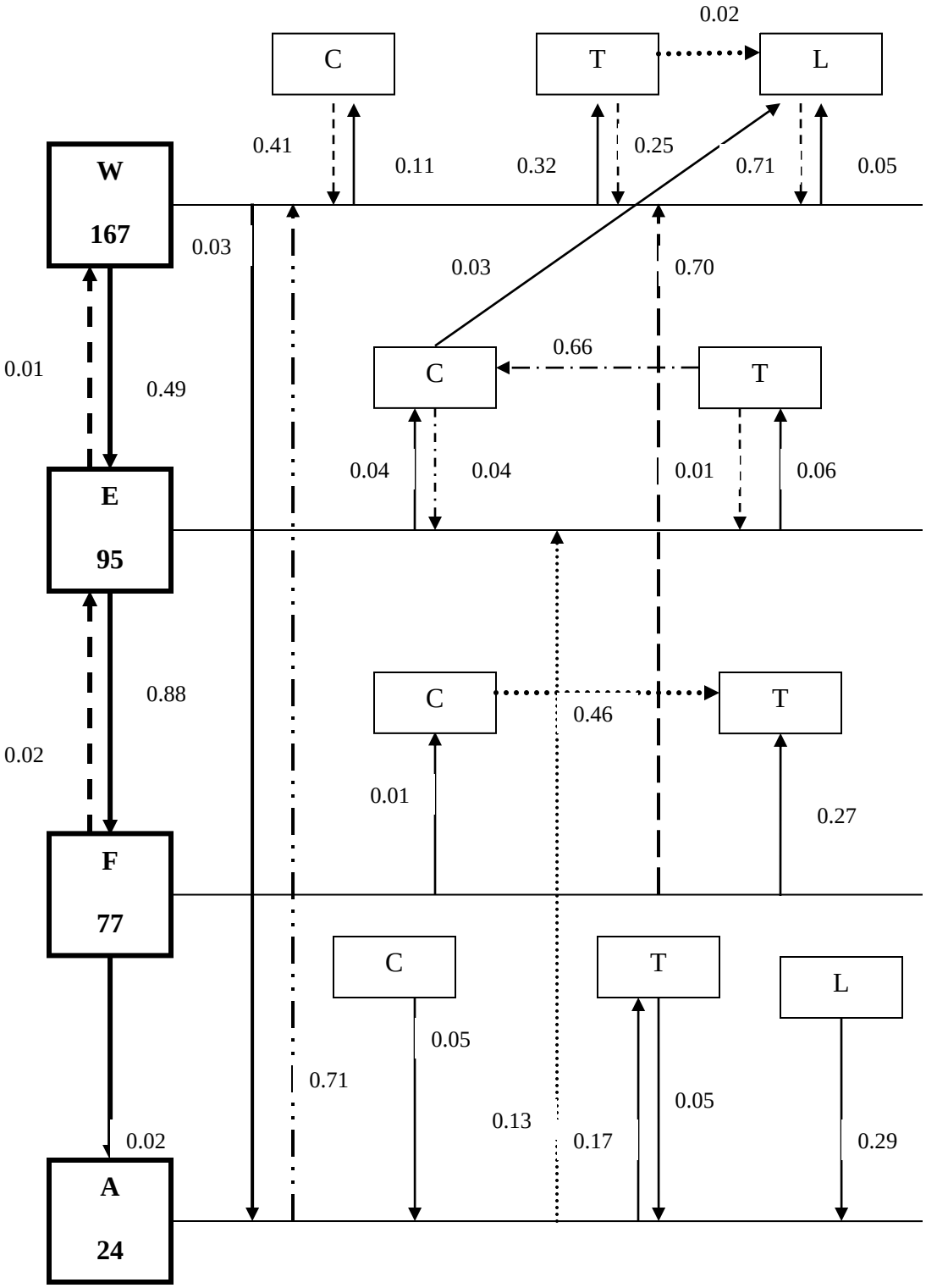


Figure 6.3 Ethogram for behaviours of the adult beetle on BPA 1999.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation, L- Flying. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

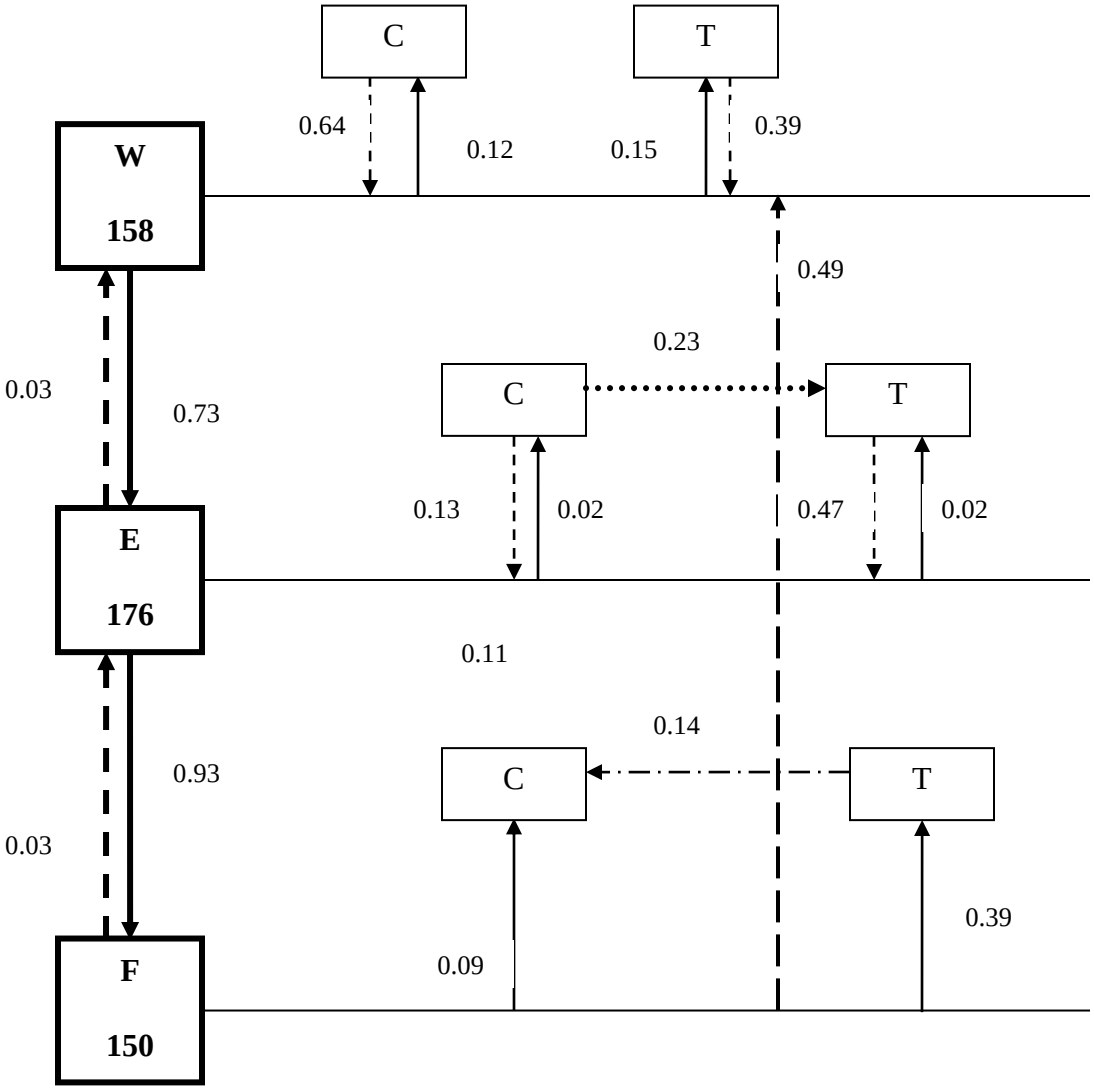


Figure 6.4 Ethogram for behaviours of the larvae beetle on BPA 1999.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

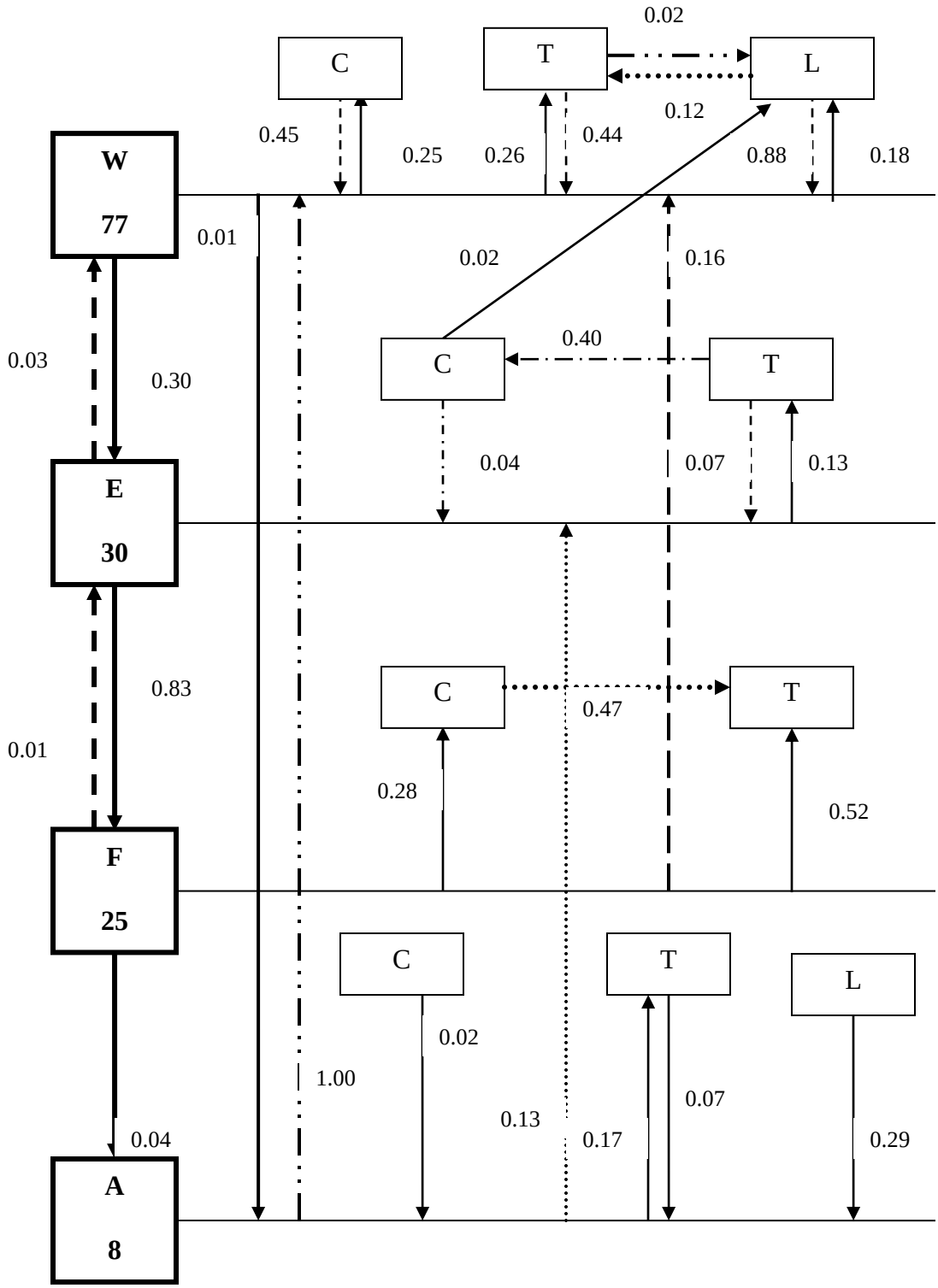


Figure 6.5 Ethogram for behaviours of the adult beetle on BPA 2000.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation, L- Flying. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

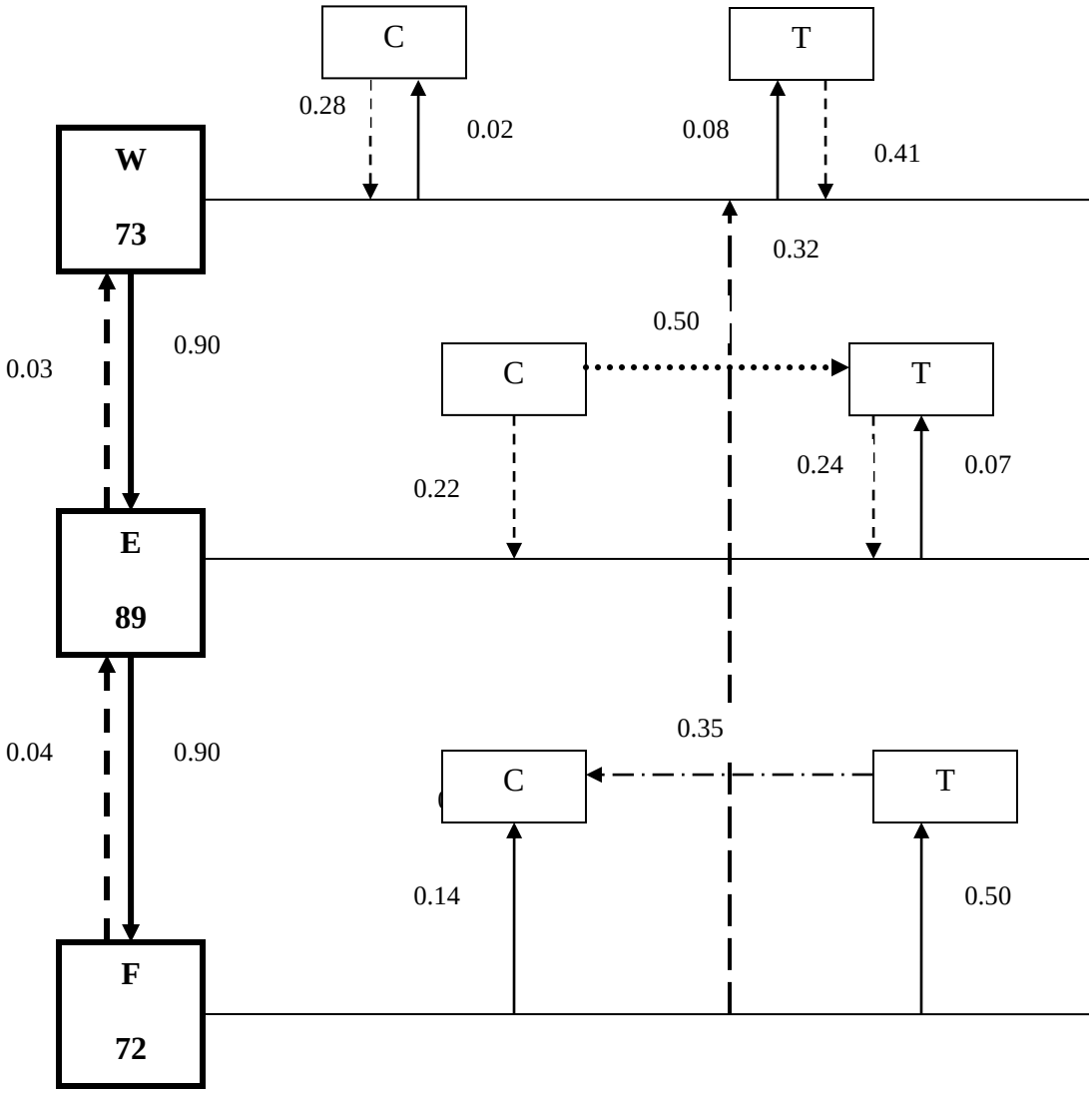


Figure 6.6 Ethogram for behaviours of the larvae beetle on BPA 2000.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

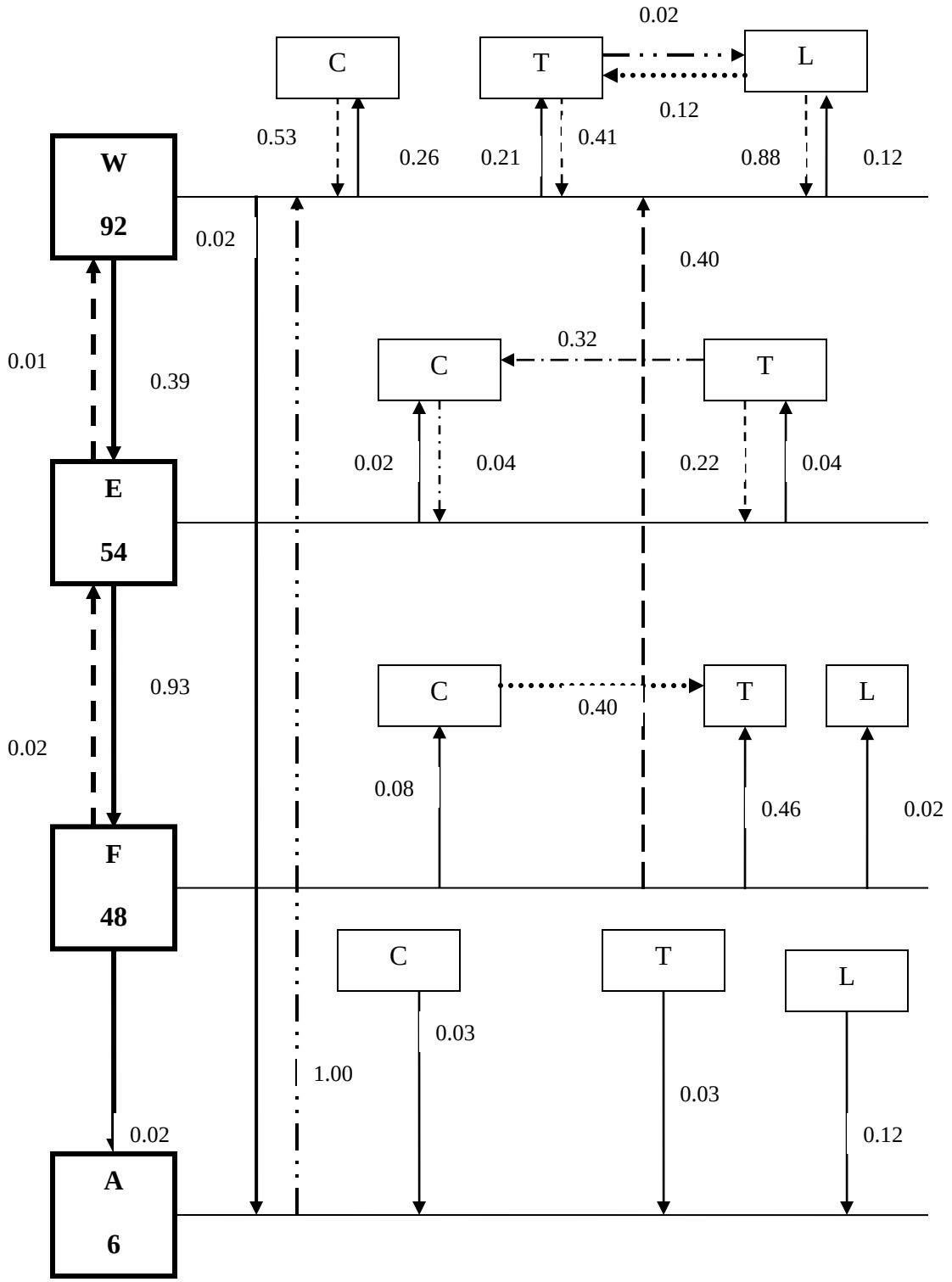


Figure 6.7 Ethogram for behaviours of the adult beetle on BPA 2002.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation, L- Flying. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

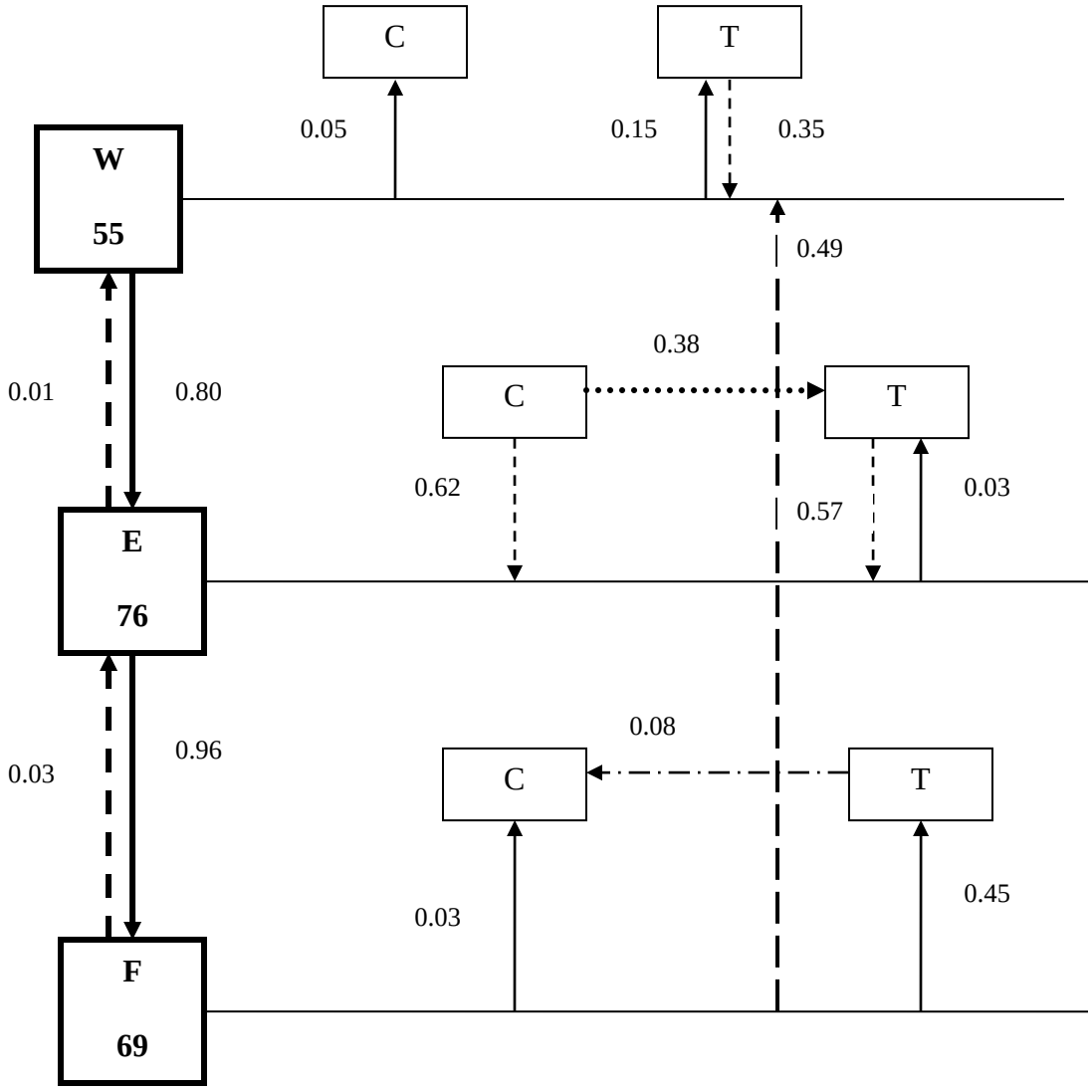


Figure 6.8 Ethogram for behaviours of the larvae beetle on BPA 2002.
Arrows indicate subsequent behavioral events and the associated numbers indicate the transition frequencies of each behavioural sequence. The figures in the rectangles represent the total number of times a particular behaviour occurred. The main behavioral pathway begins at the top left and moves down the left margin as indicated by the arrows. T- Standing still on leaf, F- feeding, E- encountering, W- searching, C- cleaning, A-Antennation. Encountering has no transition frequency because the adult encounters the prey and starts feeding on it immediately.

6.3.3 Frequency and duration of events

Generally, BPA 1999 for both the adults and larvae had the highest behavioural events (Fig. 6.9). In total, 349 and 317 behavioral events were recorded for the adult and larvae ladybirds on the filter paper, 634 and 632 behavioral events for the adult and larvae ladybirds on BPA 1999, 263 and 324 behavioral events for the adult and larvae ladybirds on BPA 2000 and 324 and 254 behavioral events adult and larvae ladybirds on BPA 2002, respectively.

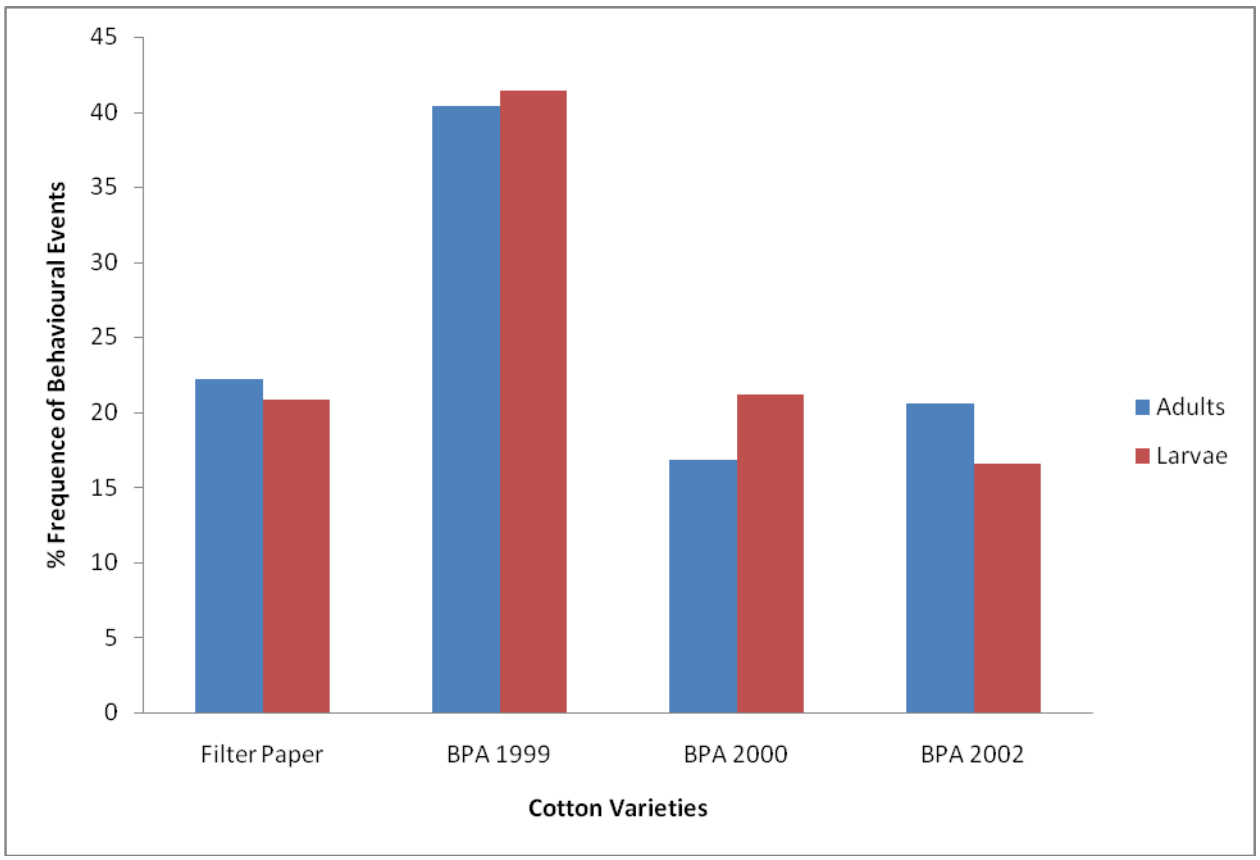


Figure 6.9 Frequency of Behavioural events of Beetles on different cotton varieties

Searching was the most frequent behavioral event on the different cotton varieties except for larvae on varieties BPA 1999, BPA 2000 and BPA 2002 where encountering was the most frequent. Whereas antennation and flying were the least frequent (in the adults), flying was higher than antennation in BPA 2000 and BPA 2002 (Table 6.1).

Table 6.1 Percentage Frequency of Behavioural events of ladybird beetles

`	Adult				Larvae			
	Filter Paper	**BPA 1999**	**BPA 2000**	**BPA 2002**	**Filter Paper**	**BPA 1999**	**BPA 2000**	**BPA 2002**
Searching	28.1	26.3	29.3	28.4	31.9	25.0	22.5	21.6
Encountering	13.2	15.0	11.4	16.7	25.2	27.9	27.8	29.9
Feeding	12.0	12.2	9.5	14.8	21.1	23.8	22.2	27.2
Cleaning	19.2	18.1	18.6	14.5	3.2	7.5	11.1	3.2
Standing Still	21.2	22.4	21.7	19.4	18.6	15.9	16.7	18.1
Antennation	3.7	3.8	3.1	1.9	0.00	0.00	0.00	0.00
Flying	2.6	2.2	6.5	4.3	0.00	0.00	0.00	0.00

On all the different cotton varieties, mean duration was highest in feeding at 49.52%. Ladybird beetle larvae on BPA 2002 had the highest feeding duration (59.55%). Generally the adults spent more time in searching on varieties BPA 1999, filter paper and more time on standing still in varieties BPA 2000 and BPA 2002. Antennation was allocated the least time in all the varieties (Table 6.2). It should also be noted that whereas BPA 1999 had the highest percentage frequency of behavioural events (fig. 6.9), it (BPA 1999) registered the lowest percentage duration of behavioral event for feeding at 40.04% as compared to the other varieties BPA 2000 and BPA 2002 which had lower frequency behavioural events but higher percentage durations for feeding at 55.94% and 59.55% respectively (Table 6.2).

Table 6.2 Percentage duration (in seconds) of behavioural events of ladybird beetles

`	Adult				Larvae			
	Filter Paper (23353)	**BPA 1999 (43200)**	**BPA 2000 (19094)**	**BPA 2002 (23347)**	**Filter Paper (35489)**	**BPA 1999 (37710)**	**BPA 2000 (20085)**	**BPA 2002 (16183)**
Searching	29.5	34.4	20.4	20.7	26.5	24.9	14.7	9.9
Encountering	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Feeding	13.8	18.0	7.1	24.5	42.5	40.0	55.9	59.6
Cleaning	28.6	17.9	18.2	14.2	1.1	6.9	5.4	4.0
Standing Still	27.0	28.2	29.4	29.4	29.9	28.1	23.9	26.5
Antennation	0.2	0.3	0.1	0.1	0.0	0.0	0.0	0.00
Flying	1.1	1.2	24.8	11.2	0.0	0.0	0.0	0.00

The figures in brackets associated with the different ladybird stages is the total duration in seconds per behavioural activity.

6.3.4 Time budget for the different adult and larvae ladybirds

Generally time budgets for each of the ladybird adult and larvae differed markedly between the cotton varieties. The adults spent more than half of their time (54.69%) - standing still (28.47%), and searching (26.22%) on the cotton leaves. Only 15.84% of the adult time was spent on feeding on all the different cotton varieties as compared to the larvae that spend almost half of their time (49.52%) feeding and (29.10%) standing still. The larvae on varieties BPA 2002 and BPA 2000 spent 59.55% and 55.94% respectively of their time feeding which was more than double the highest time spent by the adults (BPA 2002 at 24.48%) (Fig. 6.10, 6.11 and 6.12).

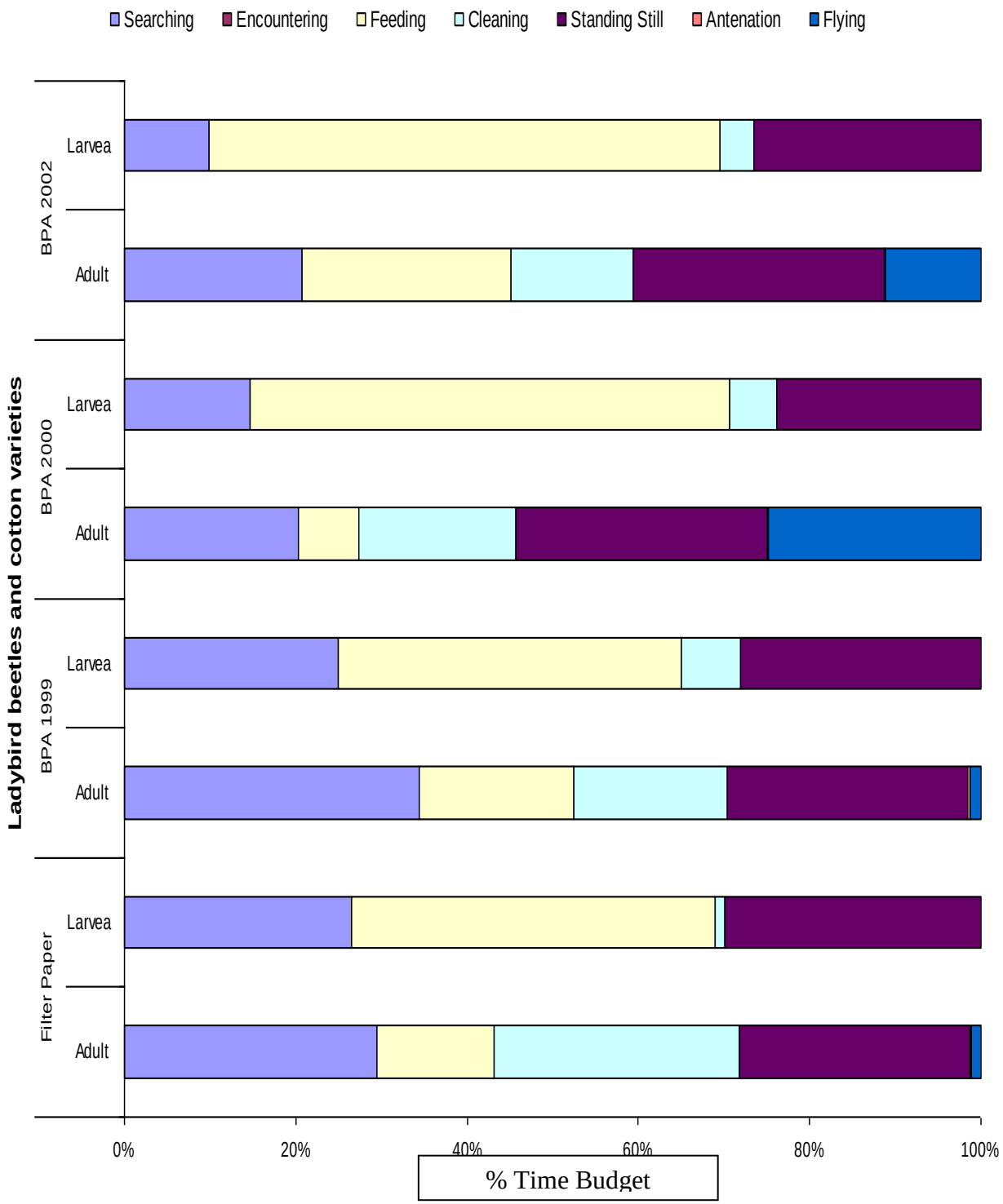


Figure 6.10. Time budgets of ladybird beetles attacking aphids on different cotton varieties

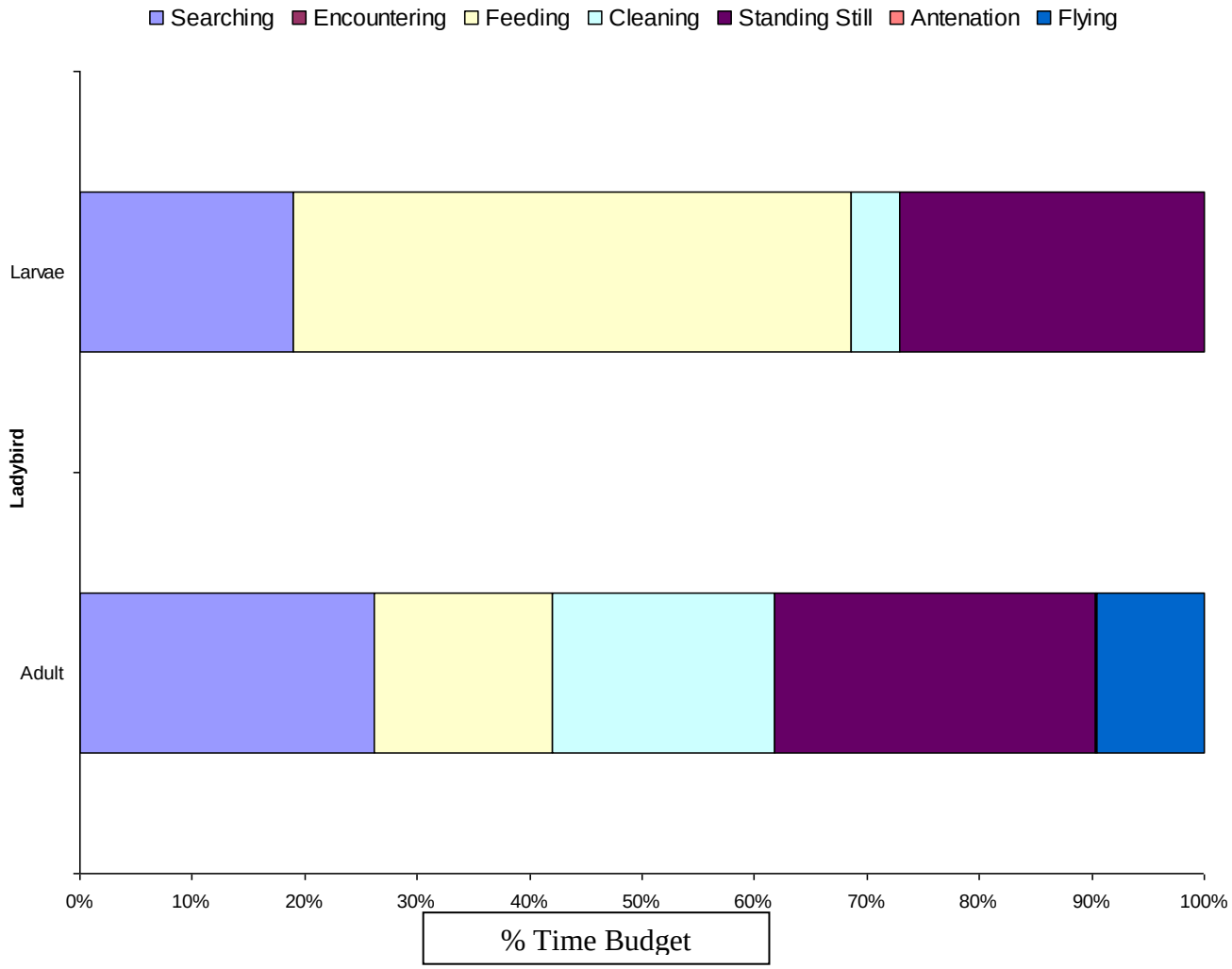


Figure 6.11. Average time budgets of ladybird beetles attacking aphids on cotton

Comparison of the major behavioural events (searching, standing still and feeding) and their time budgets further reaffirmed the fact that the adult beetles were more in searching and invested little time in feeding as compared to the larvae that invested a lot of time in feeding despite the fact that the percentage behavioural event of feeding and searching were not significantly different. In all the cotton varieties, the adults and larvae had relatively invested the same amount of time in standing still (Fig. 6.12).

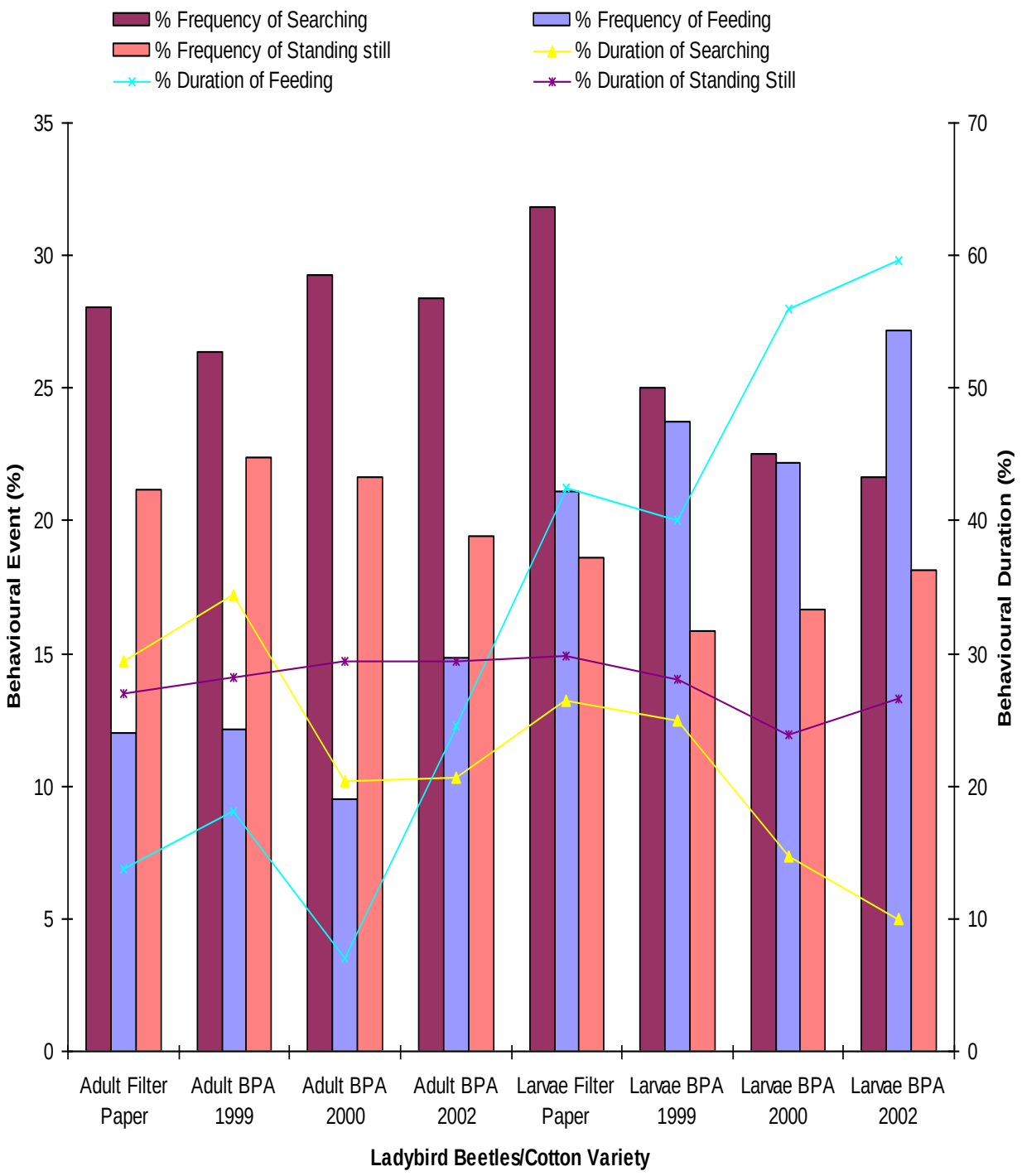


Figure 6.12 Comparison of the most common Behavioural events and Duration

6.3.5 Olfactory Orientation Behaviour

Whereas there were no significant differences($P = 0.01$) in ladybird beetle activity in the presence and absence of ants, the adults had highest activity in the presence of black garden ants as compared to the larvae whose activity was highest in the absence of the black garden ants. BPA 1999 had the highest activity for the larvae in both the presence and absence of black garden ants. However, for the adults, BPA 2002 and BPA 2000 had the highest activity in the absence and presence of black garden ants respectively. Generally the number of active adult and larvae ladybird beetles that moved towards the test chamber (chemical odour sources) was smaller than those that moved towards the control/reference and therefore, the attractive index (percent preference) resulted in negative values. The highest orientation away from the odour source was registered with the dimethoate treatment in both the adult and the larvae ladybird beetles in all the three cotton varieties.

6.3.5.1 Olfactory orientation behaviour of Ladybird Beetles

6.3.5.1.1 Activity of Ladybird Beetles in absence of ants

Activity for the ladybird beetles in response to the different odour in the absence of ants was not significantly different with a mean of ($p<0.001$; 94.63 ± 0.58) for larvae and ($p<0.001$; 94.54 ± 0.71) for the adults on the different cotton varieties.

Table 6.3 Activity of Ladybird Beetles on different cotton varieties in absence of ants.

Cotton Varieties	Percentage Activity	
	Adults	**Larvae**
BPA 1999	94.1 ± 0.7a	94.7 ± 0.6a
BPA 2000	94.5 ± 0.5a	94.7 ± 0.4a
BPA 2002	95.0 ± 0.5a	94.5 ± 0.6a

Similar letters indicate no significant differences between treatments (P = 0.01).

6.3.5.1.2 Preference/attraction of Ladybird Beetles on the different cotton varieties

Overall, significant (P<0.01) variations were observed with the different odour sources. Grand mean attractive index for the adults and larvae were (p<0.01; -0.1984 ± -0.0229) and (p<0.01; -0.1764 ± -0.0212) respectively. Whereas Xen Tari-*Bt* spray and water odour sources were not significantly different, in their influence of the orientation of the beetles, the adults were slightly oriented towards Xen Tari-*Bt* spray than water and the larvae were oriented to water than Xen Tari-*Bt* spray (Table 6.4).

Odour Source	**Adults**	**Larvae**
Aphids X Cotton X Dimethoate	- 0.4 ± 0.1 a	- 0.3249 a
Aphids X Cotton X XenTari – *Bt* Spray	- 0.1 ± 0.2 b	- 0.0640 b
Aphids X Cotton X Water	- 0.1 ± 0.3 b	- 0.0210 b
LSD (0.01)	**0.1**	**0.1**
CV (%)	**46.9**	**30**

Different letters indicate significant differences between treatments (P = 0.001).
Table 6.4 Attractive Index of ladybird beetles in the absence of ants *Lepiosiota* spp

BPA 2000 cotton variety had the highest mean attractive index (p<0.001; - 0.193 ± -0.0) for the adults and BPA 1999 (p<0.001; - 0.2 ± -0.0) for the larvae. BPA 2002 had the lowest mean attractive index amongst the three varieties (p<0.001; - 0.2 ± -0.0) for the adults and (p<0.001; - 0.2 ± -0.0) for the larvae. Both ladybird beetle adults and larvae least oriented towards where dimethoate was the odour source (Fig. 6.14)

6.3.5.2 Olfactory orientation behaviour Beetles in the presence of black garden ants

6.3.5.2.1 Activity of Ladybird Beetles in the presence of ants

Activity for the ladybird beetles in response to the different odour in the presence of ants was not significantly different, however the adults had a higher mean activity percentage (p<0.01; 94.9 ± 0.8) as compared to the larvae (p<0.01; 92.6 ± 2.1). Generally, adult activity was highest in the

BPA 2000 and lowest in BPA 2002. For the larvae, activity was highest in BPA 1999 and lowest in BPA 2000 (Table 6.5).

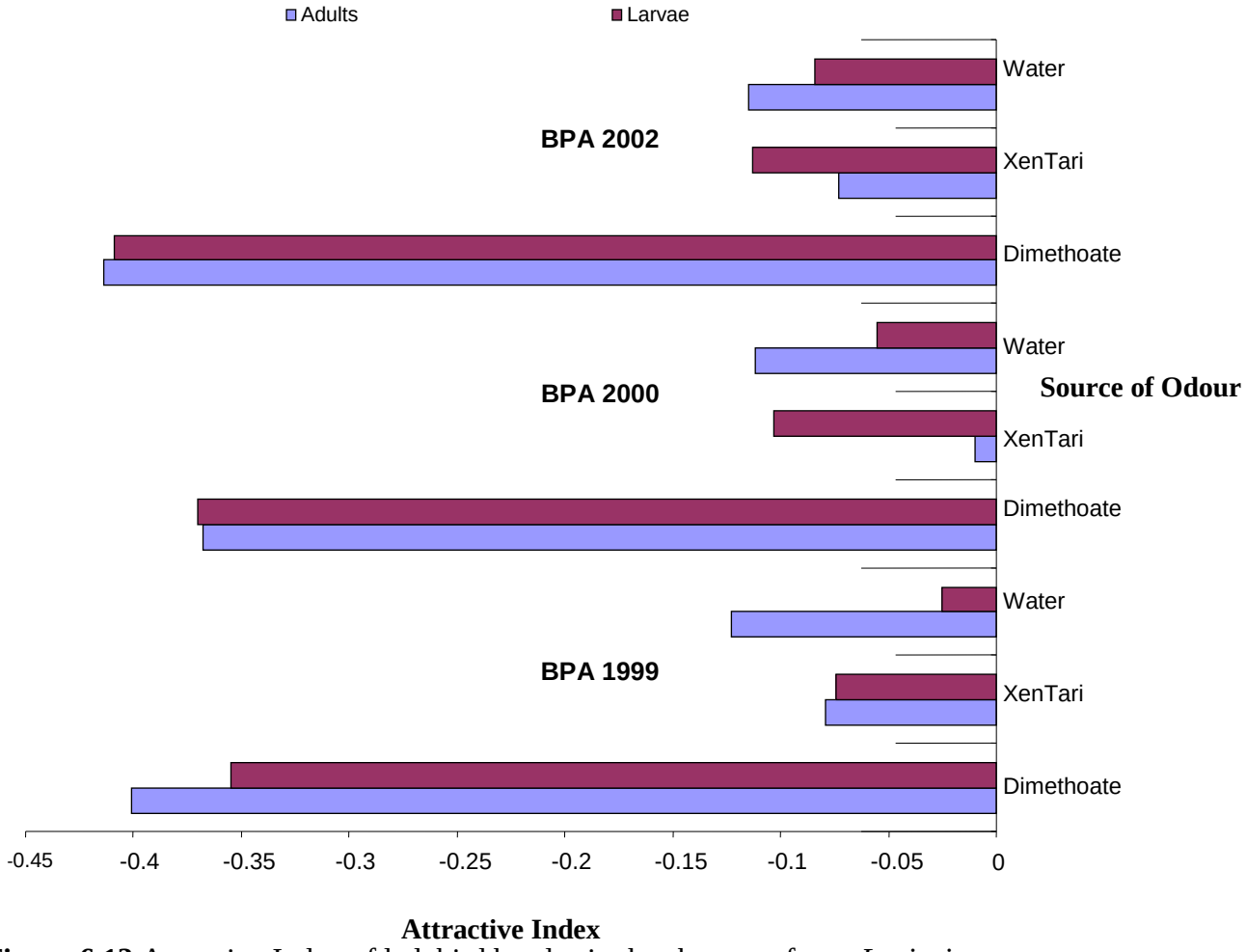


Figure 6.13 Attractive Index of ladybird beetles in the absence of ants *Lepiosiota* spp

Table 6.5 Activity of Beetles in the presence of ants on different cotton varieties.

Cotton Varieties	Percentage Activity	
	Adults	**Larvae**
BPA 1999	94.9 ± 2.1 a	94.7 ± 4.2 a
BPA 2000	95.2 ± 0.9 a	90.4 ± 0.9 a
BPA 2002	94.8 ± 3.1 a	92.6 ± 5.4 a

Similar letters indicate no significant differences between treatments (P = 0.01).

Significant ($p<0.01$) variations were noted with the different odour sources with dimethoate having the least attractive index. Grand mean attractive index for the adults was ($p<0.001$; - 0.240 $\pm$ -0.03) and for the larvae ($p<0.01$; - 0.240 $\pm$ -0.04). Whereas Xen Tari-*Bt* spray and water odour sources were not significantly different, in their influence towards the orientation of the beetles, the adults and larvae beetles were slightly oriented towards water than Xen Tari-*Bt* spray (Table 6.6).

Odour Source	**Adults**	**Larvae**
Aphids X Ants X Cotton X Dimethoate	- 0.4 $\pm$ 0.0 a	- 0.3 $\pm$ 0.0 a
Aphids X Ants X Cotton X XenTari – *Bt* Spray	- 0.1 $\pm$ 0.0 b	- 0.03 $\pm$ 0.0 b
Aphids X Ants X Cotton X Water	- 0.1 $\pm$ 0.0 b	- 0.04 $\pm$ 0.0 b
LSD (0.01)	**0.07**	**0.04**
CV (%)	**72.1**	**75.2**

Different letters indicate significant differences between treatments (P = 0.01).
Table 6.6 Attractive Index of beetles in the presence of ants *Lepiosiota* spp.

In the presence of black garden ants, BPA 2002 cotton variety had the highest mean attractive index ($p<0.001$; - 0.202 $\pm$ -0.026) for the adults and BPA 1999 ($p<0.001$; - 0.1657 $\pm$ -0.018) for the larvae. BPA 2000 had the lowest mean attractive index amongst the three varieties ($p<0.001$; - 0.265 $\pm$ -0.02) for the adults and ($p<0.001$; - 0.28 $\pm$ -0.03) for the larvae. In all the varieties dimethoate was the least orientated to odour source by both the adult and larvae ladybird beetles amongst the three varieties (Fig. 6.16)

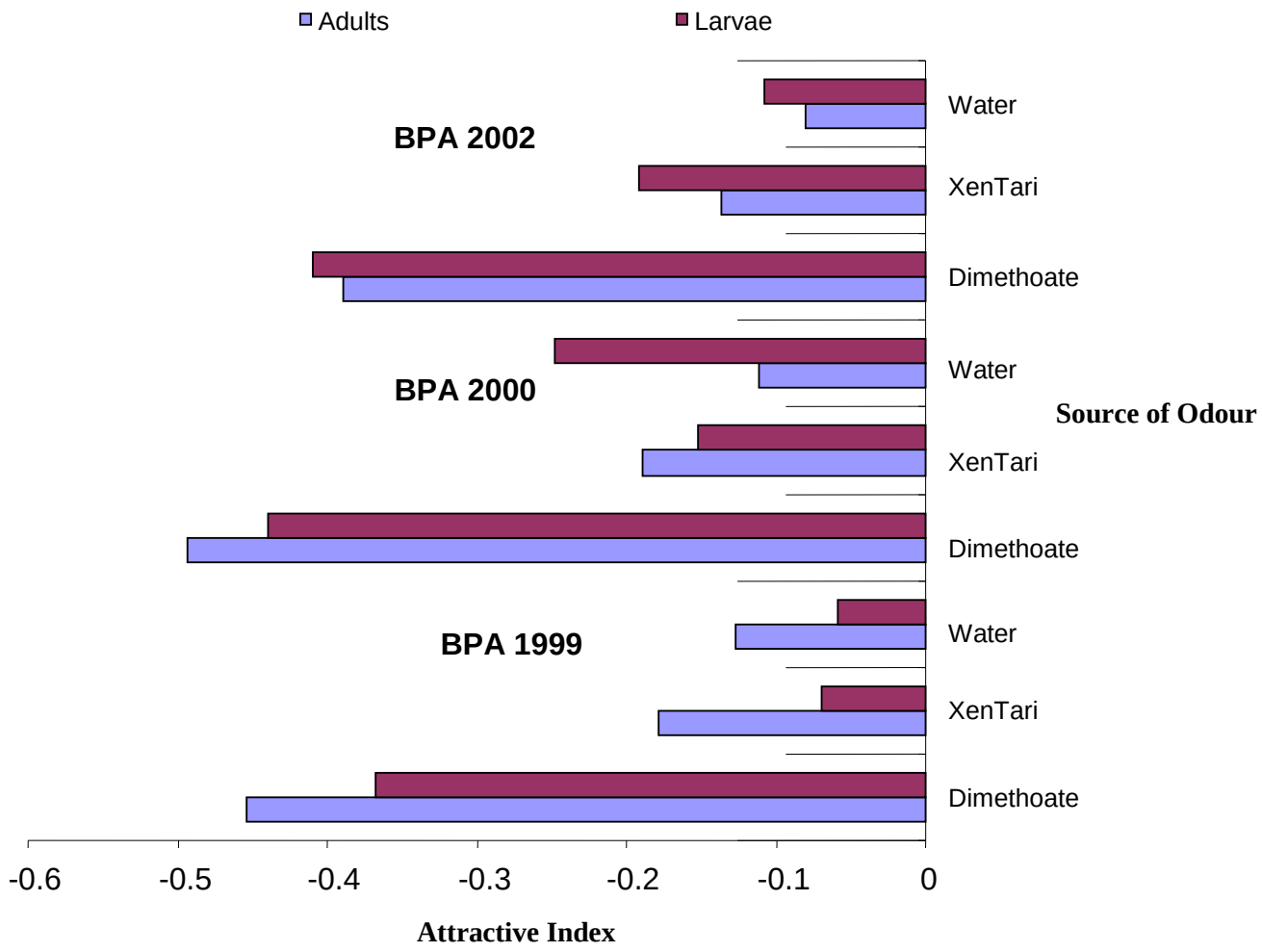


Figure. 6.14 Attractive Index of beetles in the presence of ants *Lepiosiota* spp

6.4 Discussions

Generally the main events and sequence of behaviors exhibited were; searching, encountering and feeding on the prey. These events varied amongst the different varieties. BPA 1999 cotton variety which had the lowest leaf hair density had the highest behavioural events (in the searching behaviour experiments). Similarly in the olfactory orientation experiments, highest ladybird beetle activity especially for the larvae was registered with BPA 1999 cotton variety. Whereas searching was the most frequent behavioral event on all the different cotton varieties for the adults and larvae, more time was spent on feeding by the larvae especially in varieties BPA

2000 and BPA 2002, highlighting the importance of the larvae beetle in the management of aphids (Schaller & Nentwig, 2000; Kindelmann and Dixon, 1993). Olfactory orientation behaviour results highlighted fewer numbers of active adult and larvae ladybird beetles moving towards the test chamber (odour sources) compared to those that moved towards the control/reference resulting in negative values.

The study findings indicated that, searching, encountering and feeding are functionally important behaviours in the predatory repertoire in the ladybird beetle larvae populations as compared to searching, standing still, encountering, feeding and antennation in the adult ladybird beetle populations. This general trend concurs with the descriptions of MacNulty *et al*., (2007) that highlighted predatory behaviour being composed of 3 major phases of search, pursue and capture. The difference in the functionally important behavioural events in the larvae and adult behavioural pathways could be explained by the studies of Kindelmann and Dixon (1993) who noted that, the foraging behaviour of the ladybird larvae and the adult differ with theory indicating that it is an adult's ability to select suitable patches for oviposition that is likely to be the most important in the determining its fitness and orientation. However, the foraging behaviour of the larvae is hunger dependent. Large body size hinders locomotor performance in ways that may lead to trade-offs in predator foraging ability that limit the net predatory benefit of larger size (.MacNulty *et al*., 2009). Adult ladybird beetles are bigger than the larvae beetles and therefore these behavioural pathways support the generalization that bigger predators are overall better hunters, but they also indicate that increasing size ultimately limits elements of predatory behaviour that require superior locomotor performance and this potentially narrows the dietary niche of larger predators especially if prey are substantially more difficult to pursue than to

handle. All these factors seem to qualify the larvae as better candidates for the management of aphids as compared to the adult beetles.

Searching behaviour pathways were different for both the adult and larvae ladybird beetles on the different varieties of cotton and filter paper used and this might have been due to effects of leaf trichomes. Encountering – feeding and renewed feeding which was one of the major functional pathway was observed to be most pronounced in the filter paper (with no leaf hairs) and BPA 1999 (with the least leaf hair density) as compared to the percentage frequencies of the same in BPA 2000 and BPA 2002 (with higher leaf density). This seems to imply that after the first search, the effects of leaf pubescence tend to come in and influence the subsequent search behaviour. However, observations on time spent on feeding indicated that, the adult ladybird percentage time spent on feeding was highest in the least trichome density and least in the highest trichome density cotton variety. With the ladybird larvae, the reverse occurred with the net percentage time spent on feeding being lowest in the least trichome density and highest in the highest trichome density cotton variety. Leaf trichomes and hairiness have been demonstrated to interfere with the searching efficiency of generalist predators including the coccinellidae *Delphastus pusillus* on poinsettia *Euphorbia pulcherrima* (Cortesero *et al*., 2000; Bottrell *et al*., 1998; Norton *et al*., 2001); search and oviposition behavioral pathways in whitefly parasitoids *Eretmocerus mundus* and *Encarsia sophia* (Otim, 2007 and Otim *et al.*,2008); reduced net speed of searching of *Leptomastix nr. epona on Passiflora* (Mozaddedul and Copland, 2003). The difference in the larvae beetles with the net percentage time spent on feeding being least in the least trichome density and highest in the highest trichome density cotton variety, besides hairiness, might be due exudates of glandular trachoma's as well as leaf hair size (Augustin, 2002).

On the other hand, Xue *et al* (2008) observed that the more trichomes on a cotton leaf, the more difficulties for aphids to live (get attached to) on the leaf and this might have made the aphids easily accessible to the hunger driven beetle larvae. Therefore, the results observed in the study and earlier research suggests that any genetic modification in the cotton that may have an effect on leaf trichomes and hairiness might have an impact on natural enemy interactions. However, it has been reported that behaviour in insects is often a response to a particular stimulus and is therefore reactionary (Giebultowicz *et al*, 2001). Several foraging traits (behaviours) are genetically determined in insects, such as circadian rhythms, the time for switching from extensive to intensive search, sensory and scanning abilities, dispersal tendencies, food and habitat preferences although in the coccinellids, little is known about their generic variability in searching behaviour (Ferran and Dixon, 1993). Therefore, these discrepancies between the ethograms accounts for the importance of the different varieties, beetle development state and their influence in hunting techniques and overall behaviour and if further exploited may offer biosafety precautionary protocols in the management of the introduction of genetically modified cotton. Furthermore, the difference in the time spent on feeding could be explained by the fact that the adult ladybird are known to have a wide dietary range compared to the larvae that are only found developing on a fewer species of prey and mainly aphids (Hemipitinne *et al.,* 2000; Perdikis *et al.,* 2008). These results strengthen the fact that ladybird beetle larvae are a better candidate to exploit in pest management in this era of genetically modified cotton.

Whereas in this study prey defense aspects, quality, alternate diet of the predator and other interacting competitors were not considered, the ethograms in the study provide a framework for examining aspects of predator and prey behaviour that might be sensitive to different phases of the predation process, such as predator diet (Sih and Christensen 2001; Harwood *et al.* 2001;

2003; Creel and Creel 2002; MacNulty *et al*., 2007). The framework provided may also be particularly useful in optimal diet/foraging studies as it may allow for future assessment of divisions between attacked-group (prey clusters) and attacking individual allowing one to measure up to three decision variables: which group to attack, how long to spend searching and which individuals to attack within the cluster. And if biological control is to be well effected within the confines of predator behaviour, the decision variables that fit in the observed trends in the ethograms need to be integrated with factors that relate to prey (aphids) stages are more vulnerable to lady beetle attack and effect of other competitors (intra & inter guild) and prey quality.

Mean frequency of behavioural events in this study varied between the adult and larvae beetle in the three cotton varieties and filter paper. BPA 1999 had the highest behavioural events amongst all the varieties in both the adult and larvae ladybird beetles and this might have been due to the fact that it had lower leaf hair counts (Otim, 2007; Otim *et al*. 2008). However, the filter paper (which was the control and had no leaf hairs at all had the least behavioural events with especially the larvae ladybird beetles. This trend of event may be attributed to the fact that difference between the attraction of coccinellids to infested and uninfected leaves could be a factor of qualitative differences in the volatile compound blend emitted in both cases (Ninkovic *et al.* 2001; Dudareva *et al,* 2006). Although less attention has been paid to the use of infochemicals by generalist insect predators, there is scattered evidence that some prey-associated infochemicals modify coccinellidae foraging behaviour and therefore further understanding of such factors might act as reliable indicators of herbivore infestation (which are likely to increase in the invent of the introduction of genetically modified crops). Therefore as genetically modified cotton and other such crops are being developed, due consideration must be

accorded to changes that might alter infochemical systems and subsequently affect natural enemy interactions. Therefore, the information about behavioural frequencies on the different varieties generated in this study could provide a foundation for more studies on biosafety risk assessments on the alterations that might occur in the crop architecture, leaf hairlines and infochemical systems with genetically modified cotton.

Study results form predatory orientation indicated that ladybird beetle activity was highest in the BPA 2002 for the adult and BPA 1999 for the larvae and lowest in BPA 1999 for the adults and BPA 2002 for the larvae in the absence of ants and highest in the BPA 2000 for the adult and BPA 1999 for the larvae and lowest in BPA 2002 for both the adults and larvae in the presence of ants. Different factors such as; sensory perception, internal factors, such as hunger and reproductive state (Kindelmann and Dixon, 1993; Dixon 2000), external environmental factors (Nakamuta, 1987), habitat quality (Honek, 1983; Carter and Dixon, 1982) and possible exposure to pesticides (Tank *et al*., 2007) have been reported to influence activity and behaviour of coccinellid beetles within crops some of which may explain the variations observed in the different varieties of cotton in the study. The study results seems to suggest that, while looking at the introduction of genetically modified cotton, consideration to such factors that might have an influence on quality of cotton, field management practices including pesticide application (Wolt *et al*, 2009; Pyke and Doyle 2006) all of which affect the activity of natural enemies in the management of cotton pests is important. Therefore an approach that blends well biotechnology, behavioural manipulation and enhancement of biological control agents in the overall pest management is imperative and the findings in this study could be useful in providing a framework in the developing such approaches and protocols.

The number of active adult and larvae ladybird beetles that moved towards the test chamber (odour sources) was smaller than those that moved towards the control/reference and therefore, the attractive index (per cent preference) resulted in negative values. The highest orientation away from the odour source was registered with the dimethoate treatment in both the adult and the larvae ladybird beetles in all the three cotton varieties. These results reveal the repelling effect insecticides might have on biological agents in the management of pests. This concur with studies by Martin and Jepson, 1996 who observed a greater tendency of female *Aphidius* parasitoids of the cereal aphids *Sitobion avenea* dispersing away from aphid-infested plants when insecticide treated plants were brought in the near vicinity and thereby affecting the parasitoid efficiency. Behavioral changes have been observed in natural enemies exposed to insecticides Tank *et al*., 2007; Fernandes *et al.* 2010). In general, the effect of insecticides on behavior is a syndrome that affects motility, orientation, feeding, oviposition and learning. In many cases, insecticides act as repellents that are associated to the behavior of food searching (Fernandes *et al.* 2010). The orientation and changes in arthropod behavior (Salerno *et al.* 2002), may lead to the reduction in the detection of kairomones (Delpuech *et al.* 2005), generating an increase in the speed in which the stimuli of the attractive or repellent substances are noticed and this has an impact on the overall predator efficiency. Therefore the above factors (Fernandes *et al.* 2010; Salerno *et al.* 2002 and Delpuech *et al.* 2005) may be some of the reasons for orientation away from the odour source registered especially with the dimethoate treatment in both the adult and the larvae ladybird beetles. Thus as genetically modified cotton is being introduced, its anticipated effect through the use of dimethoate in the management of sucking pests (Pyke and Doyle 2006; Wilson *et al.*, 2007) need to be addressed. Therefore the results and observation in these experiments highlight the importance of a precautionary approach as biotechnology and

integrated pests management are developing better cotton pests' management systems especially the use of pesticides in the management of the outbreak of other pests not originally a problem.

Attractive index in the presence of black garden ants was lower than where ants were excluded in all the three varieties. Studies have shown that the presence of ant semiochemicals change the behavior of arthropods (Kaplan and Eubanks 2005; Oliver et al. 2007). Aphids and ants are two abundant and highly successful insect groups, which often live in the same habitat and therefore are likely to interact with one another (Stadler and Dixon, 2005). Also from prioritization studies of arthropods, aphids and ants were catalogued in the highest priority category (chapter four: 4.4.1 of this study). However, the indirect interactions between aphids and other honey-producing insects and host plants mediated by direct interactions with ants are potentially important but often overlooked interactions in terrestrial arthropod communities and pest management in general (Styrsky, 2006). Therefore the observations made in this study about the slightly higher repellency level of the ladybird predator in the black ant present over the non black ant absent treatments in the presence of the different pesticide odours, seem to call for more investigations in these indirect interactions (mutualistic interaction between aphids and ants – plants – applied pesticides) that have a bearing on the overall pest management strategy especially in this era of genetically engineered crops. Therefore while looking at the management of the anticipated sucking pest out breaks in the wake of introduction and subsequent adoption of genetically modified crops, the integration of the different factors such as; repulsion effects of the predator by the pesticides, seemingly further enhanced repulsion by the ants and varietal factors highlighted in this study will provide a good benchmark for the management of terrestrial arthropod communities, pest management strategies and further research in biosafety aspects.

Accruing from the study it is evident that varietal difference might have an influence on arthropod activity in general and biological activity in particular. BPA 1999 cotton variety that the lowest trichome density had the highest behavioural and activity events amongst all the varieties therefore revealing the influence of leaf hairiness might have on predatory activity. Ethogram and time budget results indicated that the ladybird larvae which did more feeding (which from literature is a function of hunger dependent factors, and consequently consuming more prey than the adult's whose foraging behaviour are governed by the ability to select suitable patches for oviposition) put the larvae as better choice in the biological control process. Further, the results in the ethograms and time budgets developed in this study, give a framework of units that can be used to quantify a number of foraging characteristics, such as encounter rates, foraging efficiency and final predation, which are important factors in understanding how behaviour combines in higher order, animal behaviour and ecological processes. Therefore as genetically modified cotton technologies are being developed, there will be a need to take into consideration some of these factors (crop – hairiness, architectural arrangements; biological control agents – life cycle stages and their feeding habits/processes/responses, generic factors, reproductive responses and other environmental factors) that might have an impact in integrated pest management. However as consideration is given to the most effective ladybird beetle larval stages in controlling aphids and other sucking pests, the challenges as highlighted by Lommen *et al*., 2008, of developing large numbers of larvae required for population buildup and delayed dispersion or breeding should addressed.

Dimethoate had the highest repulsive effect on both the adult and the larvae ladybird beetles in all the three cotton varieties in the (with ants and without ants) experiments. The adult beetles

were slightly oriented towards Xen Tari-*Bt* spray than water and the larvae were oriented to water than Xen Tari-*Bt* spray. These results seem to imply that the biological agents' attraction to Xen Tari-*Bt* spray and water highlights the less risk factor as compared to the dimethoate that is already known to be detrimental to biological pest control. Therefore, the results in this study tend to indicated the opportunities that might be offered by genetically modified crops through increased understanding/use of morphological traits in plants (Way & van Emden 2000; Martha 2008; Davis *et al* 2006 and Xue Kun *et* al 2008) and management of the threats of falling back to the use of chemical in the control of other emerging pests problems (Pyke and Doyle 2006; Wilson *et al.* 2007). However, more studies that integrate the framework of the behavioral pathways and time budgets observed in the behavioural pathway experiments and the orientation of the beetles with respect to the chemicals that might be used with the introduction of genetically modified cotton should be conducted so as to have better risk assessment protocol in line with better biosafety guidelines.

As genetically modified cotton is being taken on as a pest management strategy, the ladybird beetle foraging process through the behavioural pathways, behavioural frequencies, time budgets frameworks and repulsive effects of different chemicals observed in this study need to be given due consideration. Any morphological and architectural modifications that might occur on cotton as a crop will be of paramount importance to incorporate in biotechnology pest management systems. This could help in enhancing and harnessing the plant, pest and biological (mutualistic/antagonistic relations) control strategic decisions in the foraging process and biological control of pests in general and sucking pest in particular that might become a major threat as genetically modified cotton is introduced.

CHAPTER SEVEN

7.0 GENERAL DISCUSSIONS AND CONCLUSIONS

7.1 Overview

Like any other new technology, transgenic cotton if introduced in Uganda might cause unpredictable consequences or risks while playing an important role in pest control. There is little or no knowledge regarding the potential impact on ecological environments, alternate trophic levels in food chain and their symbiotic relationships that favour the crop-natural enemy interactions which might be caused by extensive planting of genetically modified plants. Knowledge of the arthropod and their ecological function profile on cotton is very important in pest management. It's against this that a study of cotton pests and natural enemy interactions – as a basis for biosafety risk assessment in Uganda was conducted to: i) document species and ecological processes/functions of arthropods that occur in the cotton growing system in Uganda; ii) establish and prioritize potential likely exposure and adverse effect pathways of genetically modified cotton to the non-target arthropods; iii) understand the dynamics of cotton aphids and their ladybird natural enemies in cotton as Influenced by *Bacillus thuringiensis* (XenTari) Bio-pesticide – a surrogate for the *Bt*-cotton and dimethoate in the presence of Lepiosiota ants; and iv) understand the influence of inherent plant factors on the searching behavior and olfactory orientation of ladybird beetle attacking cotton aphids. The study results revealed that there is substantial heterogeneity in the cotton-crop combinations with the cotton-maize combination the most popular. Overall the arthropod community in cotton is comprised of 57 species belonging to 42 families in 12 orders with 67% being beneficial and 33% herbivores. Three priority categories with 41% of the arthropods in the highest, 32% in the intermediate and 27% in the lowest priority category were observed to be at risk of being affected by the transgenic toxin with

yield loss and reduced fibre quality anticipated to be the potential damage as a result of pest population outbreak. The results also indicated that aphids control was most effective through dimethoate use as compared to the *Bt*-biopesticide and Ladybird beetles efficiency in the control of aphids was impacted by the presence of *Lepiosiota* ants. Ladybird behavioral studies highlighted that, the main events and sequence of behaviors exhibited were; searching, encountering and feeding on the prey and whereas searching was the most frequent behavioral event for both the adults and larvae, more time was registered on feeding by the larvae.

7.2 General Discussions

In this study a diversity of arthropods giving an updated cotton arthropod profiling in Uganda, dominated by the beneficial arthropods existed within the various crop combinations. Four ecological functional categories: predators, non-target herbivores, pollinators and decomposers were established. The diversity of arthropod can be attributed to the inherent differences in agro-ecological zones in Uganda with key features that greatly influence the population dynamics of pest and insect populations (Kamanyire, 2000). Factors such as farming systems and practices, cropping history and different microclimatic conditions between locations are known to influence the activity density of arthropods (Kamanyire, 2000). The dominance of the beneficial arthropods could be explained by the existence of diverse cotton-crop combinations that were observed in the study. Comparison studies of have reveled reduced numbers of herbivores in polycultures or diverse habitats than in monocultures and this is attributed to several factors such as increased parasitoid and predator populations, availability of alternative food for natural enemies, decreased colonization and reproduction of pests, chemical repellency and masking and/ or emigration and optimum synchrony between pests and natural enemies (Andow, 1983; Matteson *et al*., 1984; Munduru, 2010). The 67% beneficial and 33% herbivores arthropod

species composition in the cotton cropping system is likely to be exposed to the transgenic toxins. Establishing possible exposure and adverse effect pathways for these non-target arthropods and their respective processes are an important factor in biosafety risk assessment (Snow *et al.*, 2005; Andow and Zwahlen, 2005; Andow *et al.*, 2006). The 41% of the high priority category group reflects a focus on non-target risks that could adversely affect cotton production. Secondary pests, pest resurgences, reduction of pollination, loss of biological control by natural enemies and loss of soil fertility are anticipated adverse effect that would require attention like in many other places where GM cotton has been introduced (Hilbeck *et al*., 2006; 2008; Birch and Wheatley, 2005). Identification and understanding these ecological functional groups and arthropod profile is a very important factor in the assessments of the potential risks of GM cotton on non-target organisms. Since GM cotton will get into contact with surrounding ecosystems and the different cotton-crop combinations as identified in the study, the 41% species and ecosystem functions high risk prioritized category offers a basis for assessment against potential exposure and adverse effects pathways which is a major step in risk assessment. Exposure is a necessary prerequisite for any subsequent effects associated directly with the transgene product in GM cotton. The absence of these products renders additional studies on the direct effects of the transgene products unnecessary. However, it is still possible that GM cotton has effects independent of or interacting with the transgene products (Nguyen Thi Thu Cuc *et al*., 2008). Combining the prioritization process, potential exposure and adverse effect pathways identified in this study, it is noted that the cultivation of GM cotton will interact with different arthropods in various ways and exposure is inevitable. Therefore careful evaluation of the identified potential environmental risks associated with non target arthropods calls for strategies to prevent or ameliorate such effects. Considering the potential adverse effect

pathways and damage potential formulated in this study and in comparison with those developed elsewhere (Hilbeck *et al*., 2006; 2008; Nguyen Thi Thu Cuc *et al*., 2008), different generic risks hypotheses can be formulated to describe the ways GM cotton might result in an adverse effect via effects on non-target herbivores in Uganda.

Prioritization, exposure and adverse effect pathway analysis in this study revealed increased fitness of the species and higher attractiveness of the species to the genetically modified cotton may additionally influence the dynamics of the arthropod community and the introduction of genetically modified crops in any agricultural ecosystem may also alter the dynamics of several non target arthropods like in other places where GM cotton has been introduced (Nagrare, *et al*., 2009; Biradar & Vennila, 2008; Wilson *et al.,* 2007; Wu *et al*., 2002; Wu Kong-Ming, 2007; Obrist *et al*., 2005; O'Callaghan *et al*., 2005; Sanvido *et al*., 2006; Torres *et al*., 2006) most especially aphids (Wilson *et al.,* 2007) and mirids (Wu *et al*., 2002; Wu Kong-Ming, 2007). Management of aphids by use of dimethoate as is the case in other areas where aphids have become a problem as a result of the introduction of GM cotton (Wilson *et al.,* 2007), caused the highest mortality of all arthropods (aphids and beneficial ladybird beetles) which is detrimental and this concurs with other earlier studies (Wilson *et al*., 2007; Kennedy, 2008; Vodouhe, 2007 & Fontes *et al*., 2006; Fadare & Amusa, 2003). Therefore as cotton pests control strategies are being developed in the wake of genetically modified cotton in Uganda, consideration of how the emerging secondary pests are controlled should consider biosafety implications that come up with the dimethoate packaged strategies. XenTari (*Bt*-biopesticide) expressed some minimal control of aphids and this is due to the fact that, aphids are not a major target for the XenTari biopesticide. However, in controlling the target lepidopteran pest, the aphids will continuously be exposed to the protein toxin. This exposure may cause increased fitness of the aphid and

subsequently increasing their outbreak potential (Hilbeck *et al.*, 2006) and subsequent continued use of chemicals, thus undermining the importance of GM cotton. It should also be noted that aphids have mutualistic relationships with ants (Eubanks *et al.*, 2002; Kaplan and Eubanks, 2005) through honeydew, a sugar-rich solution excreted by many hemipterans, and subsequently ‘‘tend’’ the hemipterans, providing protection from their natural enemies thus influencing plant-based food webs (Eubanks *et al.*, 2002). The findings of this study revealed that Ladybird beetle survived less in the presence than in the absence of the black garden ants – thus affecting the control of aphids. These findings concur with earlier studies (Eubanks *et al.*, 2002; Kaplan and Eubanks, 2005; Oliver *et al*., 2007), that suggested the influencing factors of reduced dispersal and higher local densities of aphids, which benefit ants in terms of increased honeydew and prey availability. Mutualistic systems are common in nature and are one of the “great forces in the ecology and evolution of species” affecting populations, communities, and ecosystems (Katherine *et al.*, 2008). On the other hand, Renault *et al* 2005 observed that relationships among plants, aphids and ants are complex, depending on when and where they are studied. Therefore, while seeking strategies of cotton pest control, especially the secondary pests that may come up as result of the introduction of genetically modified cotton, risk assessment protocols should give due consideration to aphid/ant mutualistic interactions.

The results revealed varying predatory repertoire in the ladybird larvae and adult beetle populations. This concurs with other study findings elsewhere on predatory behaviour (Kindelmann and Dixon, 1993; MacNulty *et al*., 2007; MacNulty *et al*., 2009). The foraging behaviour of the ladybird larvae is hunger dependent and the adults’ fitness and orientation is hinged on selecting suitable patches for oviposition and this may be the reason for the difference in the functional important behavioural events in the larvae and adult behavioural pathways.

Whereas the larvae were a better candidates for the management of aphids as compared to the adult beetles, searching behaviour pathways were different for both the adult and larvae ladybird beetles on the different varieties of cotton. Encountering – feeding and renewed feeding the major functional pathway was most pronounced in the varieties with the least leaf hair density. Genetic engineering is expected to play an important role in improving the quantity and quality of biomass and overall plant characteristics (Hisano et al., 2009; Li and Qu, 2011), which factors influence pest-natural enemy interaction. Leaf hairiness has been observed to influence biological control of pests (Otim *et. al*., 2008; Otim, 2007; Mozaddedul and Copland, 2003; Greathead and Bennet, 1981; Headrick *et al*. 1995; Headrick *et al*. 1996). On the other hand, Xue *et al* (2008) observed that the more the trichomes on a cotton leaf, the more difficulties for aphids to stay attached to the leaf and this might make the aphids easily accessible to the hunger driven larvae beetles. Therefore, the study findings and earlier research seems to suggest that any genetic modification in the cotton that may have an effect on leaf trichomes and hairiness might have an impact on natural enemy interactions. Whereas the findings do qualify the larvae ladybird beetle as a better candidate to exploit in pest management in this era of genetically modified cotton, the alterations in leaf hairiness or trichome density that might come as result of genetic modification need to be given attention. The discrepancies between the ethograms accounting for the importance of the different varieties, beetle development state and their influence in hunting techniques and overall behaviour observed in this study may offer biosafety precautionary protocols in the management of genetically modified cotton.

7.3 Conclusions

In conclusion, this study has demonstrated that 14 different crop combinations exist with the maize based component accounting for about 65% being the most popular in the cotton growing

areas of Uganda, with a diversity of arthropods dominated by the beneficial arthropods at 67%. Prioritization of these arthropods at risk of being exposed to the transgenic toxin highlighted three priority categories with 41% of the arthropods falling in the highest priority. It was also shown that all the non targets were likely to be exposed to the toxin in transgenic cotton and this might have negative impacts on cotton crop yield. Through potential exposure and adverse effect pathways identified in this study and through careful evaluation of the identified potential environmental risks associated with non target arthropods calls for strategies to prevent or ameliorate such effects through hazard identification and the development of testable assessment risk hypotheses for the different non target arthropods. Control of aphids one of the pest that might become a serious pest with the introduction of GM was most effective with dimethoate. XenTari biopesticide also showed some effectiveness in the control of aphids. However, like the dimethoate treatment, *Bacillus* biopesticide had some significant impact on ladybird beetle. Results from tri-trophic interactions between aphids, black garden ants and ladybird beetle indicated that, the presence of ants reduced survival rates of ladybird beetles and their efficiency in the control of aphids. Accruing from the study it is evident that varietal difference might have an influence on arthropod activity in general and biological activity in particular. Varietal difference and subsequent variation in trichome density was observed to influence ladybird beetle behavioural and activity events and consequently predatory activity. The ladybird larvae whose behaviour is a function of hunger dependent factors consumed more prey than the adults whose foraging behaviour are governed by the ability to select suitable patches for oviposition. This qualifies the larvae as better choice in the biological control process. Furthermore, the results in the ethograms and time budgets developed in this study, give a framework of units that can be used to quantify a number of foraging characteristics, such as encounter rates, foraging

efficiency and final predation, which are important factors in understanding how behaviour combines in higher order, animal behaviour and ecological processes.

7.4 Recommendations

Given the potential and significant prospects of the introduction of genetically modified cotton, this study has revealed a number of issues about cotton pests and natural enemy interactions – as a basis for biosafety risk assessment in Uganda that need to be further investigated. In accordance with the findings obtained in this study, the following recommendations are suggested:

i) The arthropod profile in the cotton cropping system in Uganda should be updated to - 57 species belonging to 42 families in 12 orders of which arthropods 67.0% are were beneficial arthropods and 33.0% herbivores (target and non-target);

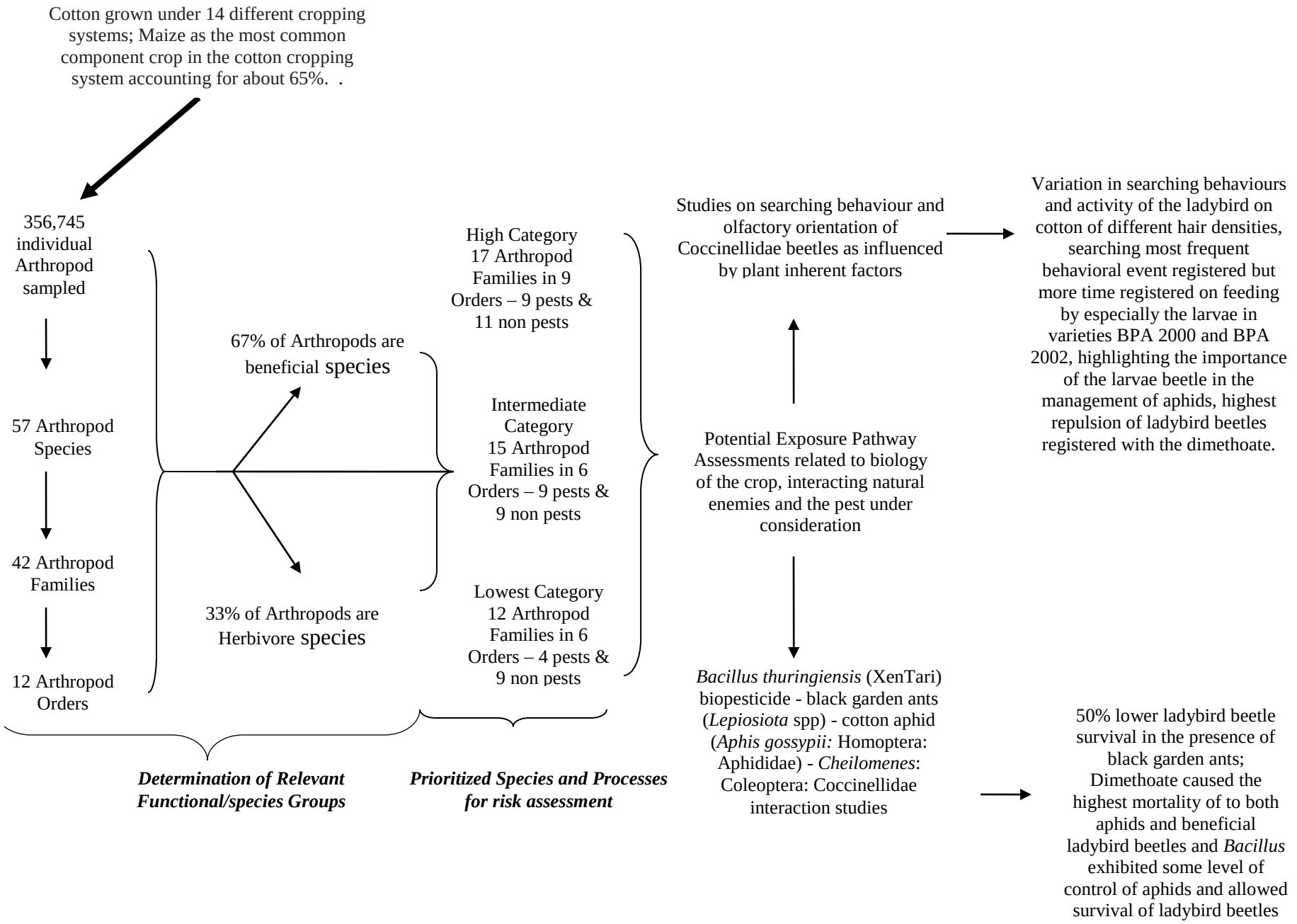
ii) It is anticipated that GM cotton has effects independent of or interacting with the arthropod populations and considering the potential adverse effect pathways and damage potential developed in this study different generic risks hypotheses have been formulated to describe the ways GM cotton might result in an adverse effect via effects on non-target herbivores in Uganda and should be used in hazard identification risk assessment of the different non target arthropods (herbivores, predators, pollinators and decomposers).

iii) Assessment of mutualistic relationship in pest management – especially *Lepiosiota* ants in the management of aphids by the ladybird beetle – how do we take advantage of these with GM technologies

iv) More studies need to be conducted on morphological and architectural modifications that might occur on cotton as a crop (use dummy structures) as this is of paramount importance to incorporate in biotechnology pest management systems.

v) More studies on mutualistic/antagonistic relations on control strategic decisions in the foraging process and biological control of pests in general and sucking pest in particular that might become a major threat as genetically modified cotton is introduced should be conducted.

vi) There is need to further investigate the ladybird beetle foraging process through the behavioural pathways, behavioural frequencies, time budgets frameworks and repulsive effects of different chemicals observed in this study.


Cotton grown under 14 different cropping systems; Maize as the most common component crop in the cotton cropping system accounting for about 65%. .
356,745 individual Arthropod sampled
57 Arthropod Species
42 Arthropod Families
12 Arthropod Orders
67% of Arthropods are beneficial species
33% of Arthropods are Herbivore species
High Category 17 Arthropod Families in 9 Orders – 9 pests & 11 non pests
Intermediate Category 15 Arthropod Families in 6 Orders – 9 pests & 9 non pests
Lowest Category 12 Arthropod Families in 6 Orders – 4 pests & 9 non pests
Determination of Relevant Functional/species Groups
Prioritized Species and Processes for risk assessment
Studies on searching behaviour and olfactory orientation of Coccinellidae beetles as influenced by plant inherent factors
Variation in searching behaviours and activity of the ladybird on cotton of different hair densities, searching most frequent behavioral event registered but more time registered on feeding by especially the larvae in varieties BPA 2000 and BPA 2002, highlighting the importance of the larvae beetle in the management of aphids, highest repulsion of ladybird beetles registered with the dimethoate.
Potential Exposure Pathway Assessments related to biology of the crop, interacting natural enemies and the pest under consideration
Bacillus thuringiensis (XenTari) biopesticide - black garden ants (Lepiosiota spp) - cotton aphid (Aphis gossypii: Homoptera: Aphididae) - Cheilomenes: Coleoptera: Coccinellidae interaction studies
50% lower ladybird beetle survival in the presence of black garden ants; Dimethoate caused the highest mortality of to both aphids and beneficial ladybird beetles and Bacillus exhibited some level of control of aphids and allowed survival of ladybird beetles

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APPENDIX

Appendix 1. Mean number of individuals of listed arthropod family/species recovered from sweepnet, pitfall and beat bucket traps, in farmers' cotton fields in Lira, Palisa and Kasese in 2006

Family	Species	Growing Season 2006								
		Early Stage			Mid Stage			Late Stage		
		Lira	Palisa	Kasese	Lira	Palisa	Kasese	Lira	Palisa	Kasese
Acrididae	*Paracinema Fisher*	56.4	59.9	50.81	35.7	33.19	33.19	17.3	13.19	52.06
Blattidae	*Supella Longipalpa*	70.7	68.3	48.81	31.7	19.31	19.31	21.4	14.31	44.06
Gryllidae	*Grlyllus* spp	76.5	86.7	50.81	18.9	22.44	22.44	21.3	14.69	36.12
Tettigoniidae	*Melanoplus bivittatus*	10.1	17.5	0	7.6	27.56	0	13.9	6.56	0
Tetranychidae	*Tetranychus nuticae*	16.9	0	0	15.3	0	0	9.44	0	0
Tetranychidae	*Spider*	16.1	16.5	6.5	17.1	21.62	21.62	27.8	9.88	13.69
Agelenidae	*Aelvrillus* spp	8.4	0	0	10.8	0	0	23.9	0	0
Aphididae	*Aphis phaseolus*	41.9	0	52.44	52.2	0	32.44	29.2	0	35.62
Aphididae	*Aphis gossypii*	42.8	90.4	80	98.5	42.12	42.12	29.7	26.13	53.56
Apidae	*Apoidea*	0	23.1	16.25	0	39.75	39.75	0	22.69	25.62
Apidae	*Xylocopa*	16.7	22.5	19.5	12.1	23.31	23.31	15.5	11.81	15.69
Apidae	*Apis mellifera*	13.4	11.2	20	47.1	20.94	20.94	12.9	10.75	12.44
Formicidae	*Lepiosiota* spp	236.6	256.2	100.94	96.9	18.94	18.94	11.6	11.44	85.37
Formicidae	*Monomorium pharaonis*	124.1	95.6	52.38	81.1	19.25	19.25	36.6	18.88	28.5
Formicidae	*Solenopsis invicta*	184.3	133.1	74.62	70.9	44.12	44.12	34.8	24.56	36.5
Formicidae	*Solenopsis. geminata*	78.2	87.6	63.19	53.1	37.81	37.81	19.2	32.25	28.87
Formicidae	*Pemphigus populivenae*	74	77.1	52.13	19.3	11.31	11.31	18	16.62	27.56
Formicidae	*Dorylinae* spp	34.3	40.1	27.56	25.7	24.62	24.62	29.8	15	26.44
Formicidae	*Tetanops myopaeformis*	23.7	0	40.31	29.4	0	39.19	27.6	0	21
Sphecidae	*Ammophillinae* spp	5.9	9.3	0	11.3	67.5	0	23.5	17.19	0
Sphecidae	*Diastrophus turgidus*	12.2	0	10.44	7.8	0	49.88	38.1	0	11.37
Sphecidae	*Sphex atratus*	9.6	0	10.44	14.6	0	61.31	26.5	0	9.37
Miridae	*Lygus* spp	6.4	72.19	42.94	64.1	19.84	22.19	17.3	23.14	18.69
Aleyrodidae	*Bemisia tabaci*	219.2	158.2	85.87	115.4	31	31	30.5	32.69	49.75
Pentatomidae	*Nezara viridula*	12.9	14.1	0	23.1	21.19	0	21.8	14.44	0
Coreidae	*Anasa tristis*	5.2	0	0	11.1	0	0	6.88	0	0
Tingidae	*Gargaphia torresi*	8.4	7.6	5.25	10	3.31	3.31	6.06	3.25	7.5
Lygaeidae	*Geocoris* spp	3.1	4.6	0	7.5	8.5	0	9.31	5.75	0
Berytidae	*Jalysis* spp	0	2.4	0	0	14	0	0	3.87	0
Phymatidae	*Phymata* spp	8.9	8.1	5.5	16.1	41.13	41.12	17.8	20.75	9
Reduviidae	*Sinea* spp	9.4	10.3	13.81	10.9	59.75	59.75	16.3	17.56	10.5
Nabidae	*Nabis* spp	16.1	17.5	20.31	21.3	8.25	8.25	16.9	8.88	17.19
Anthocoridae	*Orius* spp	11.4	0	9.56	25.2	0	11	14.5	0	11.75
Miridae b	*Spanagonicus* spp	0	68.4	0	0	22.19	0	0	20.09	0
Pyrrhocoridae	*Dysdercus suturellus*	1.9	2.4	1.44	7.7	2.19	2.19	7.88	1.75	2
Carabidae	*Stenolophus*	0	0	10	0	0	7.56	0	0	10.62
Chrysomelidae	*Epitrix hirtipennis*	3.6	0	0	11.2	0	0	7.66	0	0
Coccinellidae	*Hippodamia sinvata*	14	13.7	14.31	42.8	9.37	9.37	21.7	4.44	12.06
Curculionidae	*Sphenophorus maidis*	0	2.2	0.81	0	10.22	10.22	0	5.94	2.28
Meloidae	*Epicauta pestifera*	0	2.9	2.13	0	10.94	10.94	0	4.87	2.44
Scarabaeidae	*Phyllophaga*	0	2.4	0	0	6.5	0	0	5.44	0
Tenebrionidae	*Eleodes suturalis*	0	2.2	0	0	2.38	0	0	0.88	0

Curculionidae	*Anthonomus grandis*	3.4	1.9	0	9.8	9.34	0	12.5	3.46	0
Scarabaeidae	*Phyllophaga fervida*	0	2.2	0	0	3.37	0	0	1.19	0
Drosophilidae	*Fannia canicularis*	11.7	16.3	23.62	38.9	14.62	14.62	13.6	9.19	12.87
Muscidae	*Musca spp*	19.9	29.2	37.63	48.5	7.81	7.81	13.8	5.31	20.38
Chrysomelidae	*Diabrotica longicornis*	7.1	0	0	15.4	0	0	9.62	0	0
Syrphidae	*Eristalis tenax*	6.4	0	0	6.2	0	0	5.06	0	0
Forticulidae	*Forficula auricularia L*	13.4	0	0	19.5	0	0	5.31	0	0
Gelechiidae	*Heliothis armigera*	0.5	0.4	0.19	44.8	38.12	33.25	8.88	0.69	0.38
Noctuidae	*Pseudaletia unipuncta*	12	9.1	7.69	30.6	6.12	6.56	19.4	6.06	10.81
Noctuidae	*Pseudoplusia spp*	9.7	8.6	9.88	17.8	17.69	17.75	13.4	8.69	8.94
Noctuidae	*Agrotis spp*	16.2	13	8.44	19.4	24.31	23.94	8.31	15.25	14.87
Papilionidae	*Eumorpha achemon*	8.8	7.7	6.63	24.8	15.12	15.81	9.13	5.62	8.37
Rhinotermitidae	*Incisitermes*	39.8	42.9	34.75	18.2	11.37	11.44	21.1	9.44	36.88
Anisoptera	*Pantala havescens*	5.8	0	0	7.2	0	10.06	19.6	0	5.31
Thripidae	*Thrips*	47.6	51.6	41.31	45.2	10.69	11.5	19.6	10.62	40.75

Appendix 2. Mean number of individuals of listed arthropod family/species recovered from sweepnet, pitfall and beat bucket traps, in farmers' cotton fields in Lira, Palisa and Kasese in 2007

Family	Species	Growing Season 2007								
		Early Stage			Mid Stage			Late Stage		
		Lira	Palisa	Kasese	Lira	Palisa	Kasese	Lira	Palisa	Kasese
Acrididae	*Paracinema Fisher*	62.4	85.31	0	0	11.12	13.44	44.94	34.31	0
Blattidae	*Supella Longipalpa*	19.6	18.75	8.38	34.69	0	35.25	0	33	0
Gryllidae	*Grlyllus* spp	0	16.19	26.62	43.88	50	0	58.87	27.31	31.06
Tettigoniidae	*Melanoplus bivittatus*	13.8	0	4.88	45	50.37	44.38	14.12	8.69	0
Tetranychidae	*Tetranychus nuticae*	12.6	12.81	5.25	32.06	0	44.25	16.81	27	15.5
Tetranychidae	*Spider*	24.9	46.62	0	0	16	0	16.12	5.62	6.69
Agelenidae	*Aelvrillus* spp	33.3	56.69	84.81	31.44	26.56	24.94	11.5	0	7.69
Aphididae	*Aphis phaseolus*	0	33.62	13.44	14.75	36.44	13.94	0	35.69	31.69
Aphididae	*Aphis gossypii*	24.4	0	17.5	24.12	0	0	49.19	46.12	0
Apidae	*Apoidea*	18.4	14.63	8	20.56	59.62	40.25	26.38	0	14.69
Apidae	*Xylocopa*	120.1	81.19	99.31	49.5	0	74.75	0	18.56	14.19
Apidae	*Apis mellifera*	48.6	83	39.19	40.88	20.81	40.12	12.12	8.38	6.5
Formicidae	*Lepiosiota* spp	69.7	69.56	0	22.62	22.12	21.44	96.69	40.94	79.13
Formicidae	*Monomorium pharaonis*	41.8	73.25	62	0	28.25	0	31.25	0	34.88
Formicidae	*Solenopsis invicta*	43.1	0	50.94	20.62	54	0	34.62	15.38	47.69
Formicidae	*Solenopsis. geminata*	0	43.69	23	17.12	40.06	65.5	0	34.25	0
Formicidae	*Pemphigus populivenae*	20.2	43.25	41.25	6.56	0	64.88	41.12	23.81	0
Formicidae	*Dorylinae* spp	19	11.19	3.38	19.62	54.12	68.13	21.69	0	0
Formicidae	*Tetanops myopaeformis*	11.6	0	9.31	29.19	0	52.38	30.94	0	20.81
Sphecidae	*Ammophillinae* spp	12.9	11.75	0	0	0	28	0	3.12	4.13
Sphecidae	*Diastrophus turgidus*	55.7	101.81	50.25	24.62	17.56	0	14.31	9.69	8.81
Sphecidae	*Sphex atratus*	65.4	88.62	95.38	31.44	3.62	0	10.31	6.94	9.31
Miridae	*Lygus* spp	24	14.12	8.37	18.16	0	18.84	26.41	0	23.59
Aleyrodidae	*Bemisia tabaci*	44.4	0	3.25	18.37	9.87	16.88	0	30.31	69.09
Pentatomidae	*Nezara viridula*	13.7	9.88	5	7.87	0	0	14.96	8.69	8.88
Coreidae	*Anasa tristis*	0	5.63	0	12.25	54.88	70.06	5.25	0	4.69
Tingidae	*Gargaphia torresi*	5.1	2.69	0	0	10.62	35.06	8.5	5.38	0
Lygaeidae	*Geocoris* spp	8.9	10.75	4.69	4.94	9.25	5.19	0	0.87	0
Berytidae	*Jalysis* spp	6.9	0	12.56	0	0	0	2.5	0	1.25
Phymatidae	*Phymata* spp	15.9	16.81	19.38	12.38	0	2.13	8.06	4.69	5.13
Reduviidae	*Sinea* spp	11.4	10.13	7.06	17.62	4.12	1.56	9.25	12.69	8.56
Nabidae	*Nabis* spp	16.3	12.81	21.12	5.69	5.56	4.13	0	0	12.19
Anthocoridae	*Orius* spp	0	0	1.5	17.88	13.88	12.06	9.12	7.44	0
Miridae b	*Spanagonicus* spp	18	16.53	9.46	14.34	0	16.79	21.34	22.16	17.69
Pyrrhocoridae	*Dysdercus suturellus*	5.8	3.69	0	0	9.81	0	2.31	1.37	2.25
Carabidae	*Stenolophus*	9.6	17.5	13.06	8.75	6.87	19.12	0	9.19	6.75
Chrysomelidae	*Epitrix hirtipennis*	9	11.72	7.52	17.53	8.53	11.19	4.31	0	4.62
Coccinellidae	*Hippodamia sinvata*	3.8	0	2.12	4.37	0	6.06	15.69	14.48	9.06
Curculionidae	*Sphenophorus maidis*	5.6	8.28	12	0	3.59	2.84	0	0	0
Meloidae	*Epicauta pestifera*	8.5	1.06	0	7.69	15.75	9.31	2.81	0	0
Scarabaeidae	*Phyllophaga*	0	2.06	0	6.12	10.06	4.06	2.38	2.06	0
Tenebrionidae	*Eleodes suturalis*	2.5	2.06	1.25	3.94	3.12	1.75	1.06	0.25	0.69
Curculionidae	*Anthonomus grandis*	5.6	6.95	3.24	9.62	0	0	7.86	1.12	1.44
Scarabaeidae	*Phyllophaga fervida*	10.1	0	36.25	4.12	3.06	0	2.25	1	1.69

Drosophilidae	*Fannia canicularis*	11.6	5.81	3.13	26	4.56	2.56	15.19	0	14.25
Muscidae	*Musca spp*	11.3	5.63	0	0	6.37	4.63	0	37.62	20.5
Chrysomelidae	*Diabrotica longicornis*	12.9	11.72	8.96	12.06	0	11.19	8.67	0	6.98
Syrphidae	*Eristalis tenax*	4.1	0.63	0	23.12	18.56	31.44	4.44	1.37	0
Forticulidae	*Forficula auricularia L*	0	12.25	6.19	7.5	10	11.5	17.56	10.63	8.13
Gelechiidae	*Heliothis armigera*	6.4	11.56	4.25	26.75	23.69	0	0	0	0.5
Noctuidae	*Pseudaletia unipuncta*	11.3	18.56	0	7.37	0	9.44	10.25	6.94	6.38
Noctuidae	*Pseudoplusia spp*	6	0	5	12.75	20.25	3.38	0	0	8.94
Noctuidae	*Agrotis spp*	21.8	51.56	31.44	32.44	25.62	5.25	14.31	7.69	6.75
Papilionidae	*Eumorpha achemon*	8.3	7.31	0	0	24.75	10	8.12	5.31	6
Rhinotermitidae	*Incisitermes*	34.6	64.5	38	61.31	0	0	39.62	34.5	0
Anisoptera	*Pantala havescens*	48.8	0	0	11.5	14.56	8.19	0	2.44	4.19
Thripidae	*Thrips*	41.2	81.37	44.94	11.56	25.88	31.31	34.25	0	25.31

Appendix 3 *Selection Matrix – Association with the Crop, Crop habitat and Crop Phenology*

Herbivore/Pest species X crop coincidence X Crop Stage

Geographical Distribution: Degree of overlap of cotton crop and pest taxon

Habitat Specialization: Degree of association between taxon and cotton habitat.

Prevalence: Proportion of cotton habitat occupied by taxon.

Abundance on cotton ~~crop and on other plants occurring in the cotton habitat: average~~ or typical densities

Crop Phenology: Cotton crop growth stages

Order	Family	Common Names	Species	Feeding Guild	Association with Crop – coincidence			Crop Phenology	Summation of code Number
					Geographical Distribution	Prevalence	Abundance		
Orthoptera	Acrididae	Grass hopper	*Paracinema Fisher*	Defoliator	1	2	2	1	6
	Blattidae	Cockroach	*Supella Longipalpa*	Defoliator	1	2	3	1	7
	Gryllidae	Cricket	*Grlyllus* spp	Defoliator	1	1	1	1	4
	Tettigoniidae	Long horned grass hopper	*Melanoplus bivittanus*	Defoliator	2	3	3	1	9
Acarina	Tetranychidae	Spider mite	*Tetranychus nuticae*	Pest	3	2	2	1	8
	Tetranychidae	Spider mite	*Spider*	Predator	1	2	3	1	7
	Agelenidae	Funnel Spider	*Aelvrillus* spp	Predator	3	3	3	1	10
Homoptera	Aphididae	Aphid	*Aphis phaseolis*	Sucker/Vector	2	1	1	1	5
		Aphid	*Aphis gossypii*	Sucker/Vector	1	1	1	1	4
Hymenoptera	Apidae	Carpenter Bee	*Apoidea*	Pollinator	1	2	2	1	4
		Bamboo bees	*Xylocopa*	Pollinator	1	3	2	1	7
		Honey bees	*Apis mellifera*	Pollinator	1	1	1	1	4
	Formicidae	Black ants (Ngini ngini)	*Lepiosiota* spp	Predators/Decomposers	1	1	1	1	4
		Black ants (Nsolosozi)	*Monomorium pharaonis*	Predators/Decomposers	1	1	1	1	4
		Red Ants	*Solenopsis invicta*	Predators/Decomposers	1	2	1	1	5
		Red Ants	*Solenopsis. Geminate*	Predators/Decomposers	1	2	1	1	5
		Black ants (Ebinyomo)	*Pemphigus populivenae*	Predators/Decomposers	1	2	2	1	5
		Black/Sugar ants (Ebikenembe)	*Dorylinae*	Predators/Decomposers	1	3	3	2	9
		Red ants (Kaasa)	*Tetanops myopaeformis*	Predators/Decomposers	2	3	2	1	8
	Sphecidae	Kararankoma		Pollinators/Predators	3	3	3	3	12
		Enwa (Enumba)	*Diastrophus turgidus*	Pollinators/Predators	2	3	3	3	11
		Bumbuzi	*Sphex atratus*	Predators/Parasitoids	2	2	3	1	8
Hemiptera	Miridae	Lygus	*Lygus* spp	Sucker	1	1	1	1	4
	Aleyrodidae	Whitefly	*Bemisia tabaci*	Sucker/Vector	1	2	1	1	5
	Pentatomidae	Stink bug	*Nezara virindula*	Sucker	2	2	2	2	8
	Coreidae	Leaf footed bug	*Anasa tristis*	Predator	3	2	3	3	11

	Tingidae	Lacebugs	*Gargaphia torresi*	Sucker	1	3	3	2	9
	Lygaeidae	Big eyed bug	*Geocoris* spp	Predator	2	2	3	1	8
	Berytidae	Stilt bug	*Jalysis* spp	Predator	3	3	3	3	12
	Phymatidae	Ambush bug	*Phymata* spp	Predator	1	2	2	2	7
	Reduviidae	Assissin bug	*Sinea* spp	Predator	1	2	2	2	7
	Nabidae	Damsel bugs	*Nabis* spp	Predator	1	2	2	2	7
	Anthocoridae	Minute pirate bugs	*Orius* spp	Predator	2	2	1	1	6
	Miridae	Plant bugs	*Spanagonicus* spp	Pest/Predators	3	2	2	1	8
	Pyrrhocoridae	Cotton stainer	*Dysdercus suturellus*	Sucker	1	1	1	3	6
Coleoptera	Carabidae	ground beetle	*Stenolophus*	Predator	3	3	3	2	11
	Chrysomelidae	leaf beetle	*Epitrix hirtipennis*	Defoliator	3	3	3	2	11
	Coccinellidae	ladybird	*Hippodamia sinvata*	Predator	1	1	1	1	5
	Curculionidae	weevil	*Sphenophorus maidis*	Defoliator	2	3	3	2	10
	Meloidae	Blister beetle	*Epicauta pestifera*	Defoliator	2	3	3	2	10
	Scarabaeidae	Scarab Beetle	*Phyllophaga*	Decomposers	3	3	3	1	10
	Tenebrionidae	Darkling beetle	*Eleodes suturalis*	Defoliators	3	3	3	3	12
	Curculionidae	Boll weevil	*Anthonomus grandis*	Pest (Borer)	2	2	3	2	9
	Scarabaeidae	Dung beetle	*Phyllophaga fervida*	Decomposer	3	3	3	3	12
Diptera	Drosophilidae	drosophilla	*Fannia canicularis*	Pollinator	1	1	1	1	4
	Muscidae	Housefly	*Musca* spp	Pollinator	1	1	2	1	5
	Syrphidae	syrphid fly	*Eristalis tenax*	Predator	3	3	3	2	11
Dermaptera	Forticulidae	Earwig	*Forficula auricularia L*	Predator	3	3	3	1	10
Lepidoptera	Gelechiidae	Bollworm	*Heliothis armigera*	Target Pest/Defoliator	1	1	1	1	4
	Noctuidae	Moth	*Pseudaletia unipuncta*	Pollinators/Defoliators	1	1	1	1	4
	Noctuidae	Semi-loopers	*Pseudoplusia* spp	Defoliators	1	2	2	2	7
	Noctuidae	Cut worms	*Agrotis* spp	Pest	1	2	3	2	8
	Papilionidae	Butterfly	*Eumorpha achemon*	Pollinators/Defoliators	1	2	2	1	6
Isoptera	Rhinotermitidae	Termite	*Incisitermes*	Decomposer/Predators	1	2	2	1	6
Odonota	Anisoptera	Dragon fly	*Pantala havescens*	Predator/pollinator	2	3	3	2	10
Thysanoptera	Thripidae	Thrips	Thrips	Sucker/Vector	1	1	1	1	4

Geographical Distribution: 1 = Present in all districts; 2 = Present in any two districts; 3 = Present in only one district
Habitat Specialization: Arthropod is a specialist/restricted = 1; Arthropod is a generalist = 2: No information about specialization = 3
Prevalence: High = 1; Medium = 2; Low = 3
Abundance (Quantity Occurring): High = 1; Medium = 2; Low = 3
Crop Phenology: 1 = Present in all growth stages; 2 = Present in any two growth stages; 3 = Present in only one growth stages
Prioritization of species: Lowest summation cluster = Highest priority; Medium summation cluster = Intermediate priority; Highest summation cluster = Lowest priority

Order	Family	Common Names	Species	Prioritized rank for species[a] (*Summation Geog Distribution + Prevalence + Abundance + Phenology*)
Orthoptera	Acrididae	G.hopper	*Paracinema Fisher*	6 [a]
	Blattidae	Cockroach	*Supella Longipalpa*	7 [b]
	Gryllidae	Cricket	*Grlyllus* spp	4 [a]
	Tettigoniidae	Long horned grass hopper	*Melanoplus bivittanus*	9 [b]
Acarina	Tetranychidae	Spider mite	*Tetranychus nuticae*	8 [b]
	Tetranychidae	Spider mite	*Spider*	7 [b]
	Agelenidae	Funnel Spider	*Aelvrillus* spp	10 [c]
Homoptera	Aphididae	Aphid	*Aphis phaseolis*	5 [a]
		Aphid	*Aphis gossypii*	4 [a]
Hymenoptera	Apidae	Carpenter Bee	*Apoidea*	6 [a]
		Bamboo bees	*Xylocopa*	7 [b]
		Honey bees	*Apis mellifera*	4 [a]
	Formicidae	Black ants (Ngini ngini)	*Lepiosiota* spp	4 [a]
		Black ants (Nsolosozi)	*Monomorium pharaonis*	4 [a]
		Red Ants	*Solenopsis invicta*	5 [a]
		Red Ants	*Solenopsis. geminata*	6 [a]
		Black ants (Ebinyomo)	*Pemphigus populivenae*	6 [a]
		Sugar/Black ants (Ebikenembe)	*Dorylinae* spp	9 [b]
		Red ants (Kaasa)	*Tetanops myopaeformis*	8 [b]
	Sphecidae	Small wasp (Kararankoma)	*Ammophillinae* spp	12 [c]
		Big Wasp (Enwa - Enumba)	*Diastrophus turgidus*	11 [c]
		Mud-nest building wasp (Bumbuzi)	*Sphex atratus*	9 [b]
Hemiptera	Miridae	Lygus	*Lygus* spp	4 [a]
	Aleyrodidae	Whitefly	*Bemisia tabaci*	5 [a]
	Pentatomidae	Stink bug	*Nezara virindula*	8 [b]
	Coreidae	Leaf footed bug	*Anasa tristis*	10 [c]
	Tingidae	Lacebugs	*Gargaphia torresi*	9 [b]
	Lygaeidae	Big eyed bug	*Geocoris* spp	8 [b]
	Berytidae	Stilt bug	*Jalysis* spp	10 [c]
	Phymatidae	Ambush bug	*Phymata* spp	7 [b]
	Reduviidae	Assissin bug	*Sinea* spp	7 [b]
	Nabidae	Damsel bugs	*Nabis* spp	7 [b]
	Anthocoridae	Minute pirate bugs	*Orius* spp	6 [a]
	Miridae	Plant bugs	*Spanagonicus* spp	8 [b]
	Pyrrhocoridae	Cotton stainer	*Dysdercus suturellus*	6 [a]

Order	Family	Common Names	Species	Prioritized rank for species[a] (*Summation Geog Distribution + Prevalence + Abundance + Phenology*)
Coleoptera	Carabidae	ground beetle	*Stenolophus*	11 [c]
	Chrysomelidae	leaf beetle	*Epitrix hirtipennis*	11 [c]
	Coccinellidae	Ladybird	*Hippodamia sinvata*	5 [a]
	Curculionidae	Weevil	*Sphenophorus maidis*	10 [c]
	Meloidae	Blister beetle	*Epicauta pestifera*	10 [c]
	Scarabaeidae	Scarab Beetle	*Phyllophaga*	10 [c]
	Tenebrionidae	Darkling beetle	*Eleodes suturalis*	12 [c]
	Curculionidae	Boll weevil	*Anthonomus grandis*	9 [b]
	Scarabaeidae	Dung beetle	*Phyllophaga fervida*	12 [c]
Diptera	Drosophilidae	Drosophila	*Fannia canicularis*	4 [a]
	Muscidae	Housefly	*Musca* spp	5 [a]
	Syrphidae	Syrphid fly	*Eristalis tenax*	11 [c]
Dermaptera	Forticulidae	Earwig	*Forficula auricularia L*	10 [c]
Lepidoptera	Gelechiidae	Bollworm	*Heliothis armigera*	4 [a]
	Noctuidae	Moth	*Pseudaletia unipuncta*	4 [a]
	Noctuidae	Semi-loopers	*Pseudoplusia* spp	7 [b]
	Noctuidae	Cut worms	*Agrotis* spp	8 [b]
	Papilionidae	Butterfly	*Eumorpha achemon*	6 [a]
Isoptera	Rhinotermitidae	Termite	*Incisitermes*	6 [a]
Odonota	Anisoptera	Dragon fly	*Pantala havescens*	10 [c]
Thysanoptera	Thripidae	Thrips	Thrips	5 [a]