DIVERSITY, ECOLOGY AND POPULATION DYNAMICS OF LEPIDOPTERAN STEM BORERS IN KENYA

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DEDICATION

I dedicate this thesis to my beloved wife, Esther Adhiambo Abonyo, and our son, Fidel Jones Ong'amo; for their understanding and patience during research period which subjected them to lonely family life

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ABBREVIATIONS AND ACRONYMS

ADC	Agricultural Development Co-operation
AMOVA	Analysis of Molecular Variance
BSU	Biosystematics Unit
Bt	*Bacillus thuringiensis*
CNRS	Centre National de la Recherche Scientifique
CYMMIT	International Maize and Wheat Improvement Center
Cyt. *b*	Cytochrome *b*
DNA	Deoxyribonucleic acid
DnaSP	Deoxyribonucleic acid Sequence Polymorphism
dNTP	Deoxyribonucleotide triphosphate
DSF	Departement Soutien et Formation des Communautes Scientific de Sud
GMO	Genetically Modified Organism
HWE	Hardy-Weinberg equilibrium
icipe	International Centre of Insect Physiology and Ecology
IRD	Institut de Recherche pour le Développement
KARI	Kenya Agricultural Research Institute
LEGS	Laboratoire Evolution, Génomes et Spéciation
MNHN	Museum National d'Histoire Naturelle
NSBBP	Noctuid Stem Borer Biodiversity Project
PCR	Polymerase chain reaction
SAS	Statistical Analysis Software

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ABSTRACT

Stem borers are important field insect pests of maize [*Zea mays* L.] and sorghum [*Sorghum bicolor* (L.) Moench] in Africa. They account for more than 30% yield losses depending on the composition of the pest community. A total of 21 pest species have been reported in sub-Saharan Africa all of which are indigenous to the continent except for *Chilo partellus* (Swinhoe), which was accidentally introduced from Asia. Stem borers are susceptible to environmental fluctuations and the pest species are thought to have experienced changes in physiology and behaviour after close association with highly nutritive crops. Recent studies indicate that in addition to the pest species, there are non-economic stem borer species among wild hosts in the uncultivated fragments. Owing to susceptibility of stem borer species, continued habitat fragmentation and degradation may ultimately result in host range expansion and eventual emergence of "new" pests. Unfortunately, previous studies have been geared towards reducing populations of pest species in the cultivated fields with few attempts to understand possible evolution of less known species to pest status. This research was therefore designed to gather information on stem borer species diversity, host range and ecology in selected agricultural landscapes in Kenya. Surveys were conducted during 2005/2007 growing seasons in and around selected cultivated fields in four localities, Muhaka, Mtito Andei, Kakamega and Suam, representing different altitudinal gradients across the country. A total of 29 stem borer species were identified from 9,771 larvae collected. The identified stem borer species were grouped into 10 different known genera (*Acrapex*, *Busseola*, *Carelis*, *Manga*, *Poecopa*, *Sciomesa*, *Sesamia*, *Eldana*, *Chilo* and *Ematheudes*) while the unknown species belonged to five different families (Crambidae, Peoriinae, Pyralidae, Schoenobiinae and Tortricidae). There was evidence of variation in both distribution and dominance among the surveyed localities with majority of the species belonging to the Noctuidae family found in Kakamega and Suam, while species belonging to Crambidae and Pyralidae were mainly found in Muhaka and Mtito Andei. The wild stem borer species were identified from 38 different plant species belonging to three different families (Cyperacea [27], Poaceae [10] and Typhaceae [1]), while pest species, *Busseola fusca* (Fuller), *Sesamia calamistis* Hampson, *Chilo partellus* Swinhoe, *Chilo orichalcociliellus* (Strand) and *Eldana saccharina* Walker were mainly found on maize and sorghum. *Sesamia calamistis* and *B. phaia* ssp. *phaia* occurred among both wild and cultivated hosts and provided good models for studying exchange of stem borer pest populations between the wild and cultivated habitats. Cytochrome *b* gene sequences, through the existence of strong genetic structuration, revealed evidence of limited exchange of *S. calamistis* populations between the habitats. However, genetic analyses of the same gene of *Busseola phaia* ssp. *phaia* Bowden populations revealed weak differentiation with respect to host use in different habitats (F_{ST} = 0.016; P = 0.015). Observed variations in the distribution of pest and non-pest stem borer species coupled with differences in genetic structure among model species (*S. calamistis* and *B. phaia* ssp. *phaia*) suggest two things; i) no single management strategy would apply across different landscapes and ii) continued habitat fragmentation / degradation would affect ecosystem stability resulting in host range expansion or local species extinctions. Similar intensive studies need to be extended to other areas as it will form the basis upon which different integrated pest management (IPM) packages could be developed.

CHAPTER ONE

1.1 General introduction

Tropical ecosystems support remarkably rich arthropod assemblages (Janz *et al*., 2001). Despite this wealth of biodiversity, our knowledge of these systems is based primarily on faunistic survey records and only little information is available on what determines arthropod abundance, community structure and species interactions (Erwin, 2004). The primary goal in ecology and evolutionary biology studies is to understand factors that explain patterns of arthropod diversity and associations among interacting species (Janz & Nylin, 1997; Janz *et al*., 2006). An approach to investigate such multi-species interactions requires focus on the dominant group or a "key assemblages" which is potentially critical to food-web dynamics of the local community. One suitable group for such studies is the herbivorous insects as insect-plant interactions are common and incredibly diverse.

The great diversity of insect-plant interactions reflects the fact that herbivores are highly host specific which leads to numerous questions regarding factors that generate, maintain and constrain these associations (Jaenike, 1990; Bernays & Chapman, 1994; Janz & Nylin, 1997; Janz *et al*., 2001). Herbivorous insects are remarkably species-rich, making up at least one-quarter of all described species (Jaenike, 1990). Explaining the mechanisms behind the diversification and interaction of these groups will thus go a long way towards the understanding of global biodiversity. The earliest possible link between insect diversification and feeding on plants was reported by Ehrlich & Raven (1964) in their paper on the co-evolution between butterflies and plants. Since then, it has been

clearly demonstrated that herbivory has repeatedly led to rapid diversification of insects, though mechanisms behind this diversification remain uncertain.

In an attempt to generate information in the diversification process, insect ecologists have been manipulating aspects of plant dispersion and patterning for decades, and recording impacts on herbivore diversity and densities. Reviews of progress in this area over the past two decades indicate that vegetation attributes such as diversity or "heterogeneity" sometimes have effects and other times do not (Andow, 1991). More recent attempts to quantify the effects of plant diversity using meta-analysis have indicated that diversity has at most a low to moderate effect on herbivores (Tonhasca & Byrne, 1994), highlighting the difficulties inherent in predicting how habitat transformations will affect herbivore communities. Although this does not bode well for the development of the general theory, it is important to note that these experiments have been carried out with a wide array of insects under a variety of field conditions. However, one unexplored dimension of these interactions is the influence of landscape structure at which plant diversity is manipulated.

The modern human-dominated landscapes are typically characterized by intensive land-use and high levels of habitat destruction. Most agricultural landscapes consist of a mosaic of wild and cultivated patches that provide the herbivore community with a large tapestry of habitats and resources (Janzen, 1994; Banks, 1998). Fragmentation of remaining habitats is a major threat to biodiversity and an important issue in landscape management (Banks, 1998). Several characteristics of habitat fragments such as size, isolation, proportion of edges, and habitat quality, as well as characteristics of the surrounding landscape are known to influence abundance of populations and diversity of

communities (Banks, 1998). The relative importance of each characteristic remains unknown since species are differentially affected by characteristics of habitat fragmentation such as isolation, area and habitat quality (Janzen, 1994). Nonetheless, significance of the resulting changes in community structure, interspecific interactions and ecological functions remain unknown.

Fragmentation is usually considered in the context of conservation, but is also related to the efficiency of biological control in the agricultural landscape (Manel *et al*., 2003). Species richness and the strength of interactions of populations between habitats often vary though it is still controversial as to which mechanisms create a positive relationship between biodiversity and ecological functions (Janz *et al.*, 2006). More information may be gathered through ecological studies based on model phytophagous insects of which lepidopterous stem borers forms the best candidate. Stem borers are among the most speciose and taxonomically tractable group (Manel *et al*., 2003), and due to their important functional roles as selective herbivores, they constitute an important fauna for understanding how changes in habitat quantity and quality interact to influence species richness and community structure.

Stem borers feed on a wide range of graminaceous plants including crops (Bowden, 1954; Khan *et al*., 1997; Gounou & Schulthess, 2004). Some of the species have narrowed their diet breadth and currently feed mainly on cultivated crops where they have attained pest status. Such species include *Busseola fusca* (Fuller), *Sesamia calamistis* Hampson, *Chilo partellus* (Swinhoe), *Chilo orichalcociliellus* (Strand) and *Eldana saccharina* Walker. These species however vary in their importance across the continent with *S. calamistis* dominating pest community in Western and Central Africa

(Bosque-Pérez & Schulthess, 1998), while *B. fusca* and *C. partellus* dominate the community in Eastern and Southern Africa (Overholt *et al*., 1997; De Groote, 2002). Despite variations in pest composition across the continent, severe yield losses have been reported from countries in Southern (van Den Berg *et al*., 2001), Western (Bosque-Pérez & Schulthess, 1998) and Eastern Africa (Songa *et al*., 1998; De Groote, 2002).

In addition to cultivated crops, the pest species have for along time been known to occur among the non-cultivated graminaceous plants hereafter referred to as "wild" host plants (Ingram, 1958; Nye, 1960). Persistence and build up of pest populations early in the season is thus blamed on the movement of individuals from wild hosts growing in the uncultivated fragments (Polaszek & Khan, 1998). In order to get a better insight in ecology and the way of controlling these pests, studies in wild environments have been recommended for a long time (Bowden, 1954). This is projected to provide understanding on the infestation dynamics, the possibilities of survival of both introduced and indigenous parasitoids as biological control agents (Gounou & Schulthess, 2004) and estimating the risk of species shift from wild host plants to cultivated crops (Le Ru *et al*., 2006a). An approach of this kind has been carried out in Eastern Africa (Polaszek & Khan, 1998; Le Ru *et al*., 2006a) and in Western and Central Africa (Schulthess *et al*., 1997) which led to a better knowledge of wild host plants, mainly for known pest species.

Studying stem borer species diversity and populations in the natural landscapes require proper identification of species which may be difficult because of morphological similarities between species, and intra-specific variability (Tams & Bowden, 1953; Holloway, 1998). However, stem borers other than the pest species have been reported from surveys carried out in natural habitats in Eastern and Southern Africa (Ingram,

1958; Nye, 1960; Khan *et al*., 1997; Haile & Hofsvang, 2001; Mazodze & Conlong, 2003; Le Ru *et al*., 2006a). Recent surveys in East and Southern Africa countries yielded 136 stem borer species from 75 wild host species (Le Ru *et al*., 2006b), exceeding stem borer species and host plants recorded in the earlier studies. Recorded increase in the number of borer species and host plants in this region suggest that these lists are far from being exhaustive. Unfortunately, habitats with some host plants of these stem borer species are currently undergoing destruction thus imposing potential danger of host range expansion or local extinction of some unknown species. It is therefore necessary to catalogue stem borer species diversity in less disturbed habitats as this would provide insight on the role of wild habitats in pest ecology and dynamics that could be useful in designing future management strategies.

1.2 Statement of the problem and justification

Indigenous African stem borers have been associated with a wide range of wild hosts belonging to Poaceae, Cyperaceae and Typhaceae families for millions of years (Harris, 1962; Harris & Nwanze, 1992). However, stem borers appear to be susceptible to environmental fluctuations and the pest species are thought to have experienced changes in physiology and behaviour after close association with highly nutritive crops (Haile and Hofsvang, 2001). Continued habitat fragmentation and degradation as demand for more agricultural land to feed the growing human population increases may ultimately result in host range expansion and eventual emergence of "new" stem borer pests, or local species extinction. Distribution of stem borers (economic and non-economic species), however varies with respect to their ecological requirements (Le Ru

et al., 2006a) suggesting that impacts of habitat degradation on species diversity and ecology may vary among different vegetation mosaics. This study was designed to gather information on stem borer species diversity, host range and ecology in selected agricultural landscapes, with a view to get insight on species diversity and exchange of stem borer pests between wild and cultivated habitats. The findings are projected to provide understanding on potential ecological consequences of changes in the spatial structure of uncultivated fragments on stem borer species diversity and ecology.

1.3 Research questions

a) How does the diversity and ecology of lepidopteran stem borers vary among different habitats?

b) How does stem borer pest community vary in agricultural systems within a season?

c) What is the role of wild habitats in stem borer pest build-up between the growing seasons?

1.4 Null hypothesis

a) Diversity and ecology of lepidopteran stem borers in both wild and cultivated habitats are the same in different vegetation mosaics.

b) Stem borer pest community in agricultural systems does not vary with seasons.

c) Wild habitats are not responsible for stem borer pest population build-up between the cropping and non-cropping seasons.

1.5 Objectives of the study

1.5.1 General objective

To assess the diversity, ecology and population dynamics of lepidopteran stem borers in the cultivated and wild habitats, and determine exchange of model species between the habitats.

1.5.2 Specific objectives

a) To estimate diversity and ecology of lepidopteran stem borers in both wild and cultivated habitats in different vegetation mosaics.

b) To evaluate stem borer pest community in the cultivated habitats and monitor seasonal variation in population dynamics.

c) To determine the genetic structure and movement of model pest species, *S. calamistis* and *B. phaia* ssp. *phaia*, between wild and cultivated habitats

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Evolution of stem borers as pests

Insects may become pests due to several factors. Some previously harmless insects became pests after their accidental or intentional introduction to areas outside their native range, where they escaped from their natural enemies (Strauss, 1997). Such range extensions have allowed many previously innocuous phytophagous insects to flourish as pests, usually following the deliberate spread of their hosts through human cultivation (Strauss, 1997). Additionally, some native insects may become pests if they move from native plants to introduced ones; such host switching is common among polyphagous and oligophagous insects (Magurran, 1988). Stem borer pests are among the phytophagous insects that remained among wild hosts before domestication of sorghum and introduction of maize in Africa (Harris, 1962; Harris & Nwanze, 1992). Their pest status has been elevated by the provision of abundant food resources that occur in simplified or virtually monocultural ecosystems in which maize and sorghum crops create dense aggregations of predictably available resources (Overholt *et al*., 2001).

2.2 Cultivation and economic importance of maize and sorghum

Maize originally domesticated in Central America is currently among the most widely cultivated cereal crop in the world (Seshu Reddy, 1998). Its successful distribution may be attributed to high productivity and availability of many varieties developed for diverse ecological conditions (Schulthess *et al*., 1997). In tropical Africa, it is used for many purposes such as human food, feed for animals and raw materials for

many industrial products. Similarly, sorghum is another widely grown cereal crop in tropical Africa. After initial domestication about 5000 years ago probably in the savannahs West of Ethiopia and East of Chad, cultivation of sorghum effectively spread to other parts of the world due to its good resistance to drought (Haile & Hofsvang, 2001). In Eastern Africa, sorghum is the staple food for millions of people and also is grown to feed livestock in form of grain, forage and fodder (Seshu Reddy, 1983). However, production of these crops has not kept pace with the ever-increasing human demand for food supply.

2.2.1 Domestication and introduction of maize in Africa

Domestication of maize is thought to have started from 7,500 to 12,000 years ago though it is not known what precipitated its domestication sine the edible portion of the wild variety is too small and hard to obtain to be eaten directly. However, studies of the hybrids readily made by intercrossing teosinte and modern maize suggest that this objection is not well-founded (Benz, 2001). Archaeological remains of early maize cobs, found at Guila Naquitz Cave in the Oaxaca Valley of Mexico, date back roughly to 6,250 years. Little change occurred in cob form until ca. 1100 BC when great changes appeared in cobs from Mexican caves: maize diversity rapidly increased and archaeological teosinte was first deposited (Benz, 2001).

Perhaps as early as 1500 BC, maize began to spread widely and rapidly (Iltis, 2006). Currently, maize is widely cultivated throughout the world, and a greater quantity is produced each year than any other grain (Benz, 2001). Its successful distribution may be attributed to high productivity and availability of many varieties developed for diverse

ecological conditions. As it was introduced to new cultures, new uses were developed and new varieties selected to better serve in those preparations.

2.2.2 Domestication and expansion of sorghum cultivation

Although wild species of sorghum were attested as early as 8000 years ago in the Nilotic regions of southern Egypt and Sudan, the location of true domestication within East Africa is still speculative (Harris & Nwanze, 1992). It is widely held that genetic separation of domesticated *S. bicolor* from the wild progenitor did not occur much before 2000 years ago somewhere in Eastern Africa, possibly the Ethiopian highlands (Seshu Reddy, 1989). The presence of true domesticated *S. bicolor* is claimed much earlier than this (3700-4900 years ago) in India, Oman, and Yemen, although the identity of the remains as full domesticated plants is still disputed (Haile & Hofsvang, 2001). Sorghum requires an average temperature of at least 25°C to produce maximum grain yields in a given year though it is well adapted to grow in hot, arid or semi-arid areas (Seshu Reddy, 1989).

2.3 Factors limiting production of maize and sorghum

Notable factors limiting production of maize and sorghum in tropical Africa include poor climatic conditions, low soil nutrients, weeds, diseases and insect pests (Bowden, 1976). Some of these limitations have been solved through improved farm management and development of suitable crop varieties (Overholt *et al*., 1997). Important among these limitations are the crop damages caused by field insect pests. Outstanding among field insect pests are larval stages of stem boring lepidopteran moths belonging to

Pyralidae and Noctuidae families (van den Berg *et al*., 1998). Over 17 stem borer species belonging to these two families have been reported to attack cultivated cereals in Africa, and account for about 20-50% yield losses in East Africa (Khan *et al*., 1997). In Kenya, *B. fusca* and *C. partellus* constitute the major proportion of the community and strongly limit yields of maize and sorghum crops from 20 – 80 % depending on region, borer population density and crop phenology during infestation (Seshu Reddy, 1983; Khan *et al*., 1997).

With the exception of *C. partellus*, which was accidentally introduced from Asia (Tams, 1932), all other pest species are indigenous to the African continent where they have been associated with wild hosts belonging to Poaceae, Cyperaceae and Typhaceae families for millions of years (Harris & Nwanze, 1992). Evolutionary changes that ended in host shifts may have involved both host plants and some indigenous stem borer pest species (Magurran, 1988). Cultivated crops probably lost their resistance because of long selection by agronomists that allowed for a better expression of stem borers' fitness (Polaszek & Khan, 1998). At the same time, economically important species are thought to have experienced changes in physiology and behaviour after close association with highly nutritive crops, including reduced resistance to plant secondary compounds (Haile & Hofsvang, 2001). Like other phytophagous insects, it is thought that there might have been some specific aspects in the ancestral population of species currently specific to cultivated crops that enhanced their ability to switch and / or to adapt genetically to cultivated crops (Nylin & Gotthard, 1998; Hawthorne & Via, 2001).

2.4 Biology and damage symptoms of stem borer pests

Stem borer species often associated with maize and sorghum in Kenya includes *B. fusca*, *S. calamistis*, *C. partellus*, *C. oricalchociliellus* and *E. sacharina* (Overholt *et al*., 2001). Other species such as *S. nonagrioides* and *S. cretica* are also present in some regions (Ingram, 1958; Overholt *et al*., 2001). All stem borer species characteristically undergo four development stages; egg, larva, pupa and adult. Life cycle begins with emergence of adults and mating follows immediately after emergence by males finding females with the help of pheromones released by the female moths (Overholt *et al*., 2001). Gravid moths oviposit on suitable young leaves that are later attacked by first instar larvae after hatching (Omwega *et al*., 2006). Larval attack on the meristematic tissue generally affects the translocation of nutrients in the plant resulting in reduced growth and in some cases death of the plant either through "dead heart" or breakage of the stem.

2.5 Management of stem borer pests

Different control methods have been used in Africa to reduce losses associated with field insect pests (Nwanze & Mueller, 1989; Saxena, 1990; Kfir, 1991). In Kenya, chemical, biological, cultural as well as planting of resistant crop varieties have been used in the management of stem borers.

2.5.1 Chemical control

Chemical control of stem borers is difficult as pesticides are expensive, often toxic and at times relatively ineffective since target larvae often burrow inside the

meristematic tissue (Kumar, 1984; Seshu Reddy, 1998). Despite these limitations, large-scale farmers in Trans-Nzoia district (Kenya) still apply insecticides such as carbofuran, carbaryl and endosulfan (Hassan *et al*., 1998) for the control of the first generation of stem borer population. In addition to economic constraints, other problems associated with this management option that may follow routine application include the need for reapplication, effect on non-target organisms, problems of residues and eventual development of resistance among the target pests (Kumar, 1984).

2.5.2 Cultural control

Habitat management, early planting, removal or destruction of crop residues are among the cultural practices associated with stem borer management as they reportedly disrupt stem borer population build-up (Randriamananoro, 1996; Khan *et al*., 1997).

2.5.2.1 Habitat management

Habitat management strategy has been developed in maize-based farming systems for small- and medium-scale farmers of eastern Africa (Khan *et al*., 1997; Polaszek and Khan, 1998). This strategy involved selection of plant species that could be employed as trap crops to attract (pull) stem borer colonization away from the cereal plants, or as intercrops to repel (push) the pests. The two most successful trap crops Napier grass, *Pennisetum purpureum* Schumaker (Plate 2.1), and Sudan grass, *Sorghum vulgare* var. *sudanensis* Hitchc attract greater oviposition by stem borers, than cultivated maize. Intercrops giving maximum repellent effect are molasses grass, *Melinis minutiflora* Beauv. and a legume species, silverleaf, *Desmodium uncinatum* (Jacq.). Adoption of this

management option has relatively been slow as proper groundwork to educate farmers on its potential has not been undertaken as this effort is still on experimental trials. However, recent field surveys indicate that majority of stem borer species found in *P. purpureum* are actually not pest species (Le Ru *et al*., 2006a) and thus it may not pull target pest like *B. fusca* in high potential areas in Kenya.

Plate 2.1: Wild grass habitats neighbouring the fields of cultivated Graminae; *Pennisetum pupureum* in Kakamega.

2.5.2.2 Timely planting as a stem borer management option

Stem borer management based on timely planting follows the principle of growing target crops when the pest is not present or when the pest is least abundant (Overholt *et al*., 1997). Field survey results indicate that infested young plants are slow in their recovery unlike older plants that recover fast and compensate for the attack (Kumar,

1984). Through field studies, flying periods of most species (*B. fusca* and *S. calamistis*) have been determined and this knowledge is being used in drawing planting schedule (Overholt *et al*., 1997). Most farmers plant early in the season to avoid early sorghum and maize infestations (Khan *et al*., 1997). Alternatively, maize or sorghum may be planted later in the cropping season after the flying period of most stem borer moths. This enables plants to escape infestation from the first generation and grow big enough to withstand infestations from populations of the second-generation. Utilization of this knowledge in stem borer management is very low particularly among subsistence farmers who depend on rainfed agriculture (Okech *et al*., 1994). Only few farmers may accept to delay planting during the beginning of growing seasons due to unreliable weather pattern.

2.5.2.3 Removal and destruction of crop residues

During the dry conditions, stem borers that are unable to complete their development in time enter diapause in stubbles / stalks of maize or sorghum left in the fields in anticipation of limited food resource or oviposition sites (Plate 2.2). This strategy enables various stem borer species to continue with normal development during favourable ecological conditions. Kumar (1984) recommended that burning or spreading residues / stalks in the field to expose larvae to the full effect of adverse weather conditions would limit stem borer carry-over between seasons. These recommendations are only practical in areas where crop residues / stubbles are neither used as animal feed nor fuel. In many parts of Kenya, stalks / residues are carried to different homes where they are used to feed animals and this encourages translocation of stem borers (Bonhof, 2000).

(a)

(b)

Plate 2.2: Old maize stalks left in the cultrivated fields (a) old maize stems stack on trees within the field (b) Old maize stalks left in the prepared field ready for planting.

2.5.3 Biological control

Biological control is often used to include any biologically based methods of pest suppression (Overholt *et al*., 1997). In the traditional sense, biological control means the manipulation of natural enemies of pests to reduce pest populations to levels where economic losses due to their attack are tolerable. The range of naturally occurring biological control agents, such as parasitoids, predators and diseases have been reported for different growth stages of stem borers (Overholt *et al*., 1997; Schulthess *et al*., 1997). Few studies on their effectiveness as well as host/parasite relationships have been made. Biological control agents of interest to many researchers particularly in Kenya include the egg parasitoids, such as *Telenomus* and *Trichogramma* species, larval parasitoids including *Cotesia sesamiae* (Cameron) and pupal parasitoids eg *Pediobius furvus* Gahan among others (Overholt *et al*., 1997).

Extensive distribution of the exotic *C. partellus* and the resultant losses associated with its infestations in Coastal low altitude areas evoked the search for effective classical biological control agent from their area of origin (Overholt *et al*., 2001). Natural enemies of *Chilo* were collected in different ecological zones in India, and cocoons of *C. flavipes* Cameron, pupae of *Sturmiopsis inferens* Townsend and *Xanthopimpla stemmator* were shipped to (International Centre of Insect physiology and ecology (*icipe*) Kenya for use in the management of *C. partellus* in East Africa. The braconid *C. flavipes* after release has established in most of the countries in East Africa where *C. partellus* dominates (Songa, 1999). There has been recorded reduction of losses by about 10% due to biological control of cereal stem borers by *C. flavipes*, which annually caused 10 to 40% loss in grain yield (Seshu Reddy, 1983; Zhou *et al*., 2002).

Despite the recorded impacts of *C. flavipes* on *C. partellus* population, research work on the management of other stem borer species are at their lowest (Omwega *et al*., 2006). *Cotesia flavipes* is only reported to be effective in *C. partellus* but do parasitise other species including certain biotypes of *B. fusca* which encapsulate *C. flavipes* rendering them ineffective, as they do not emerge (Gitau *et al*., 2006). *Busseola fusca* and *S. calamistis* that dominate high potential areas are given little attention though they cause higher maize losses compared to low potential areas (Eastern and Coastal) where *C. partellus* dominates (De Groote, 2002).

2.5.4 Plant resistance

Plant resistant strategy so far tried in the management of lepidopteran stem borers consists of introducing genetically engineered *Bt*-maize (*Bacillus thurigiensis* - *Bt*). The African pyralid stem borers are close relatives to the European corn borer *Ostrina nubilalis* Hubner against which the *Bt*-maize was constructed (Overholt *et al*., 2001). The Swiss company Novartis in co-operation with the Kenya Agricultural Research Institute (KARI) and the Latin-American CYMMIT introduced *Bt*-maize in Kenya: in 2000, in a 5-year program. This is still in pilot phase despite oppositions from anti-GMO crusaders.

2.6 Insect population carry-over between seasons

Chemical, biological and cultural control methods disrupt stem borer population build-up (Randriamananoro, 1996; Khan *et al*., 1997). However, these strategies only focus on the management of borer populations within the agricultural systems while ignoring the potential role of wild habitats in pest out breaks. Stem borers feed on one or

more closely related plant families in addition to cultivated host crops (Polaszek & Khan, 1998; Haile & Hofsvang, 2001). During cropping seasons, stem borers occur in large numbers in maize and sorghum plants (Songa *et al*., 1998). After harvest, gravid moths oviposit in alternative wild hosts where their populations survive during the crop free periods (Ingram, 1958; Nye, 1960). The presence of alternative hosts and crop residues in or near a field can increase survival of stem borers, thereby increasing the population that colonise maize and sorghum crops in subsequent growing season.

However, surveys in the forest zones of Cameroon, Côte d´Ivoire and Ghana showed that higher wild host abundance in the surrounding fields was correlated with a lower pest incidence on maize (Schulthess *et al*., 1997). Oviposition preference and life table studies revealed that some wild host species namely grasses, were highly attractive to ovipositing female moths, although survival of immature stages and adult moth fecundity were mostly close to 70 – 80% against 100% on maize (Shanower *et al*., 1993). In addition, relatively high parasitism of *S. calamistis* eggs by *Telenomus* spp (Hymenoptera: Scelionidae) was found during the dry season on wild hosts in Cameroon (Ndemah *et al*., 2001). Although Schulthess *et al*. (1997) showed that at the local scale, wild host plants can attract pests and reduce damage on cultivated plants, there is a possibility of increase in damage in the following season since stem borers have higher survival on some alternative host plants (Polaszek & Khan, 1998). These views appear to differ either because generated hypotheses have not been fully tested or because species in question are different.

2.7 Importance of wild habitat in pest dynamics

Stem borers occur in large numbers in maize and sorghum plants during cropping seasons (Songa *et al*., 1998) and their populations survive in wild hosts or in crop stubbles as diapausing larvae during long dry seasons (Ingram, 1958; Nye, 1960; Polaszek & Khan, 1998; Haile & Hofsvang, 2001). Alternative hosts in the vicinity of the crop fields and crop residues enhance survival of borers during off-seasons, and are thereby considered responsible for pest attacks on crops in the subsequent season (Polaszek and Khan, 1998). In contrast, oviposition preference studies showed certain wild grasses to be highly attractive to ovipositing moths, though larval survival and adult fecundity are generally low (Haile & Hofsvang, 2002). Based on these interactions, hypotheses were formulated which were validated with field and laboratory trials for *S. calamistis* and *E. saccharina* (Shanower *et al*., 1993; Schulthess *et al*., 1997). This was supported by surveys in the forest zones of Cameroon, Côte d'Ivoire and Ghana which showed that higher wild host abundance in the surrounding fields was correlated with a lower pest incidence on maize (Schulthess *et al*., 1997). However, evidence of successful development of pest borers among some host plants suggests that there might be different genetic structures among the pest populations between cultivated and wild host plants.

2.8 Genetic structure of pest population

Some stem borer species such as *Sesamia calamistis* are present in both wild and cultivated habitats (Le Ru *et al*., 2006a). However, it is not known if the polyphagous stem borers are composed of different host races adapted to specific plants and if there is any genetic relationship between stem borer pests in wild and cultivated habitats. This

information is necessary for biotechnological development of resistant crop varieties. Transgenic maize expressing Cry1Ab has been shown to be nearly immune to attack by pyralids (Koziel *et al*., 1993). However, a large number of insect species have already developed resistance to conventional insecticides, and to several *Bt* toxins under laboratory conditions or *Bt* sprays (Tabashnik *et al*., 1995). Unless appropriate management strategies are developed and implemented, the deployment of the proposed genetically engineered crops will certainly lead to the emergence of resistant stem borer populations (Tabashnik *et al*., 2008).

Most scientists agree that the high dose/refuge strategy is the best method to delay evolution of resistance (Brousseau *et al*., 1999). However, this can only be realised when the refuge component of the strategy have wild hosts or non *Bt* maize where resistant homozygous populations survive to mate heterozygous populations from the *Bt* – maize field. Currently, it is not clear whether stem borer pest species in both wild and cultivated habitats have similar genetic structure that would allow mating.

2.9 Diversity of non-economic stem borers

Management strategies earlier initiated to reduce losses associated with stem borer pest infestations are based on the previous knowledge on their biology, ecology and distribution (Overholt *et al*., 2001). However, pest species are among the many species that were earlier associated with wild hosts and only became pests after close association with domesticated graminaceous plants (Kaufmann, 1983). Efforts for a long time have been concentrated in the management of the pest species with little attention on the diversity and ecology of non-economic species. Though it is thought that there might

have been some specific aspects in the ancestral population of pest species that enhanced their ability to switch to cultivated crops, potentials of non pests species switching to cultivated crops cannot be ignored. Latest example being sugar cane borer *Eldana saccharina* in South Africa that moved from wild sedges onto sugar cane (Conlong, 2001).

Of the 190 known tropical species of noctuid stem borers, more than half are known from the African continent (Holloway, 1998). In East Africa, about thirty-two species have been reported in the Noctuidae family alone; 8 *Acrapex,* 2 *Busseola*, 2 *Manga*, 1 *Poecopa*, 1 *Poeonoma*, 5 *Sciomesa* and 13 *Sesamia*. Only 10 of these species have known hosts plants while the remaining 22 were described using few materials collected from light traps with no information on neither their ecology nor extent of their distribution (Fletcher, 1961; Rougeot, 1984).

Tropical Africa is considered as the centre of origin of many tropical grasses with Kenya alone having about 600 species in 145 genera (Ibrahim & Kabuye, 1987; Boonman, 1992). In such diverse ecosystems, surveys in less disturbed habitats are likely to reveal information on the diversity and ecology of associated arthropods. Similar surveys have been carried out in East Africa to generate detailed knowledge and understanding of the potential wild hosts of stem borers during the last 50 years (Ingram, 1958; Nye, 1960; Seshu Reddy, 1989; Polaszek & Khan, 1998). These surveys together reported about 34 wild host plant species and this has been used to support the assumption that most stem borers particularly the pest species are polyphagous. These have been used as part of argument to promote habitat management as a pest control strategy. However, host use and distribution of economic species may vary among

different regions and to support this assumption, the presence of non-economic stem borer species in both cultivated and wild habitats need to be investigated in different agricultural landscapes.

CHAPTER THREE

3 DIVERSITY AND ECOLOGY OF LEPIDOPTEROUS STEM BORERS IN WILD AND CULTIVATED HABITATS IN SELECTED VEGETATION MOSAICS IN KENYA

3.1 Introduction

Agro-ecosystems in tropical Africa are characterised by matrix of natural habitats interspersed with areas of degraded crop fields (Laurance and Bierregaard, 1997; Summerville & Crist, 2001). Relevant to agriculture is the role natural habitats play in the evolution of arthropod pests and conservation of natural enemies (Harris, 1962; Harris & Nwanze, 1992; Schulthess *et al*., 1997). The arthropod pests currently found in the cultivated fields originally colonised wild hosts where they remained less important (Polaszek & Khan, 1998). However, with increased agricultural activities, previously expansive natural habitats rich in wild hosts of arthropods became fragmented. These resulted in habitat reduction with some species expanding their diet breadth to include cultivated cereals (Magurran, 1988).

Lepidopterous stem borers are among the insect groups that adjusted their diet breadth some of which currently use mainly cultivated cereals (Polaszek and Khan, 1997). It is estimated that stem borers are responsible for yield losses ranging between 10 and 100% (Ingram, 1958; Nye, 1960; Bosque-Pérez & Schulthess, 1998). In East Africa, the noctuids, *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and, the crambid

Chilo partellus (Swinhoe) and *Chilo orichalcociliellus* (Strand) are the major constraints to maize and sorghum production (Overholt *et al*., 1994). In order to get a better insight in the ecology and the way of controlling stem borer pests, studies in wild environment have been recommended (Bowden, 1976), and the latest approach of this kind has been carried out in Central Africa (Ndemah *et al*., 2007) and in East Africa (Le Ru *et al*., 2006b; Matama-Kauma *et al*., 2007).

Surveys in Eastern Africa suggest that diversity of stem borer species in wild habitat has for along time been underestimated (Ingram, 1958; Nye, 1960; Bowden, 1976; Le Ru *et al*., 2006a). Le Ru *et al*. (2006a) reported a total of 136 stem borer species from 75 wild host species during their survey in East and Southern Africa. In addition to indigenous pests, identified species included a wide range of non-economic species some of which are new to science. Unfortunately, the natural habitats harbouring these species are currently undergoing fragmentation resulting in changes in spatial patterns (Bosque-Pérez & Schulthess, 1998; Gepts, 2004). This has resulted in the emergence of "new" pest species, *B. phaia* ssp. *phaia* and *S. piscator*, currently found among cultivated crops (Le Ru *et al*., 2006b). Though studies show that distribution of stem borer species varies with their respective ecological requirements (Gounou & Schulthess, 2004; Le Ru *et al*., 2006b), the likely impacts of habitat degradation on species diversity and ecology may vary among different agro-ecosystems. This study was designed to investigate stem borer species diversity and ecology in both wild and cultivated habitats with a view to generate a catalogue of stem borers and hosts. Findings are projected to provide understanding on pest infestation dynamics and estimate the risk of the shift of species from wild host plants to cultivated crops.

3.2 Materials and methods

3.2.1 Description of study areas

Surveys were conducted in four different sites (Muhaka, Mtito Andei, Kakamega and Suam; Fig. 3.1), occurring in different vegetation mosaics in Kenya. These sites were selected based on previous survey data which showed variation in stem borer pest community across the country (Le Ru *et al*., 2006b).

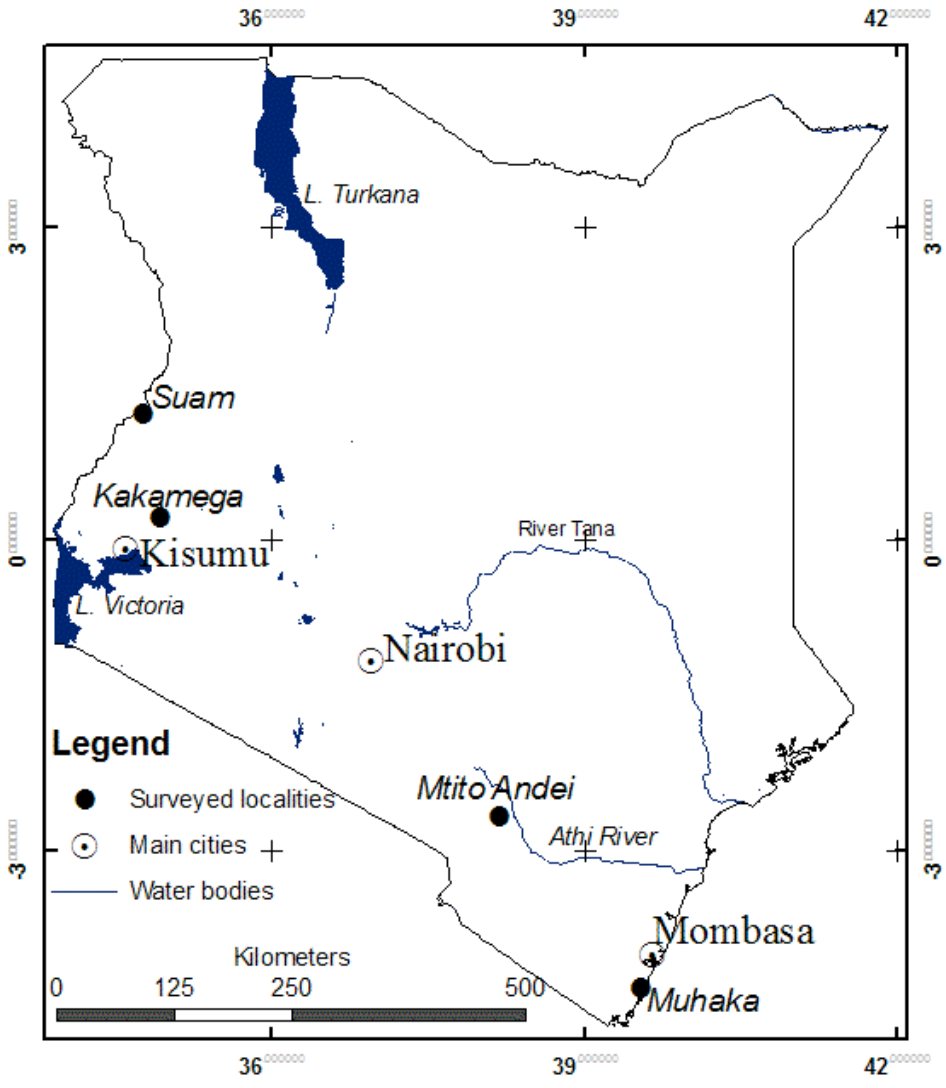

Figure 3.1 Map of Kenya showing distribution of the four sites surveyed during the study on stem borer species diversity and ecology.

High resolution satellite maps measuring 5 x 5 km of each of the four localities were used as basic spatial information to analyze vegetation mosaics and ground actualization to further describe the vegetation formations. The resultant land use map characterized respective study localities into various homogeneous vegetation structures containing natural (wild) and cultivated habitats, [Muhaka and Kakamega (Guiheneuf, 2004), Suam and Mtito Andei (Goux, 2006)].

3.2.1.1 Muhaka

Muhaka is located on the south of Mombasa in an area with secondary grassy and woody vegetation along the border of an undifferentiated forest with climatic condition of Inhambane type (minimum temperature, 22 °C; maximum, 30.4 °C; average rainfall, 1212 mm) (White, 1983). There is only one main cropping season from March to June, while the rest of the year is characterized by light showers that support growth of fast maturing maize varieties, cassava and cowpeas. The cultivated areas are characterized by scattered patches of cultivated maize and cassava fields (Guiheneuf, 2004) while uncultivated areas are occupied mainly by human settlements, palm trees and potential hosts of stem borers (e.g *Panicum maximum* and *Panicum infestum*).

3.2.1.2 Mtito Andei

Mtito Andei site is located on the border of Tsavo East National Park between the longitude 38.17 and 38.22^0 N and latitude 2.64 – 2.69^0 E (Goux, 2006). The landscape is composed of thick deciduous shrubby formations with *Acacia commiphora* and other riverine forest species of Somalia Maasai vegetation mosaic (White, 1983). The area

occurs in an altitude range varying from 680 to 770 m above sea level with an annual minimum temperature of 16.8^0 C, and maximum temperature of 29^0 C with an average rainfall of 680 mm (Corbett *et al*., 2001). The main cropping season falls between November and January, though maize and sorghum are grown under irrigation throughout the year. Some farmers grow tomatoes, kales, pepper and water melon under irrigation which they sell in the local markets.

3.2.1.3 Kakamega

Kakamega site covered an area of 21.2 km^2 along the border of transitional rain forest in a depression at the bottom of the Nandi escarpment (Kokwaro, 1988). Vegetation species within the forest are typical of planetary Guineo-Congolian rain forests (White, 1983). The location is characterized by a bimodal rainfall distribution that allows two cropping seasons, the long rains (*LR*) season lasting from March to mid-July and the short rains (*SR*) season from mid-August to November with a prolonged dry season from December to the end of February. High human density in the area (175 individuals / km^2) has led to considerable long-term human influence on the forest and its environs (Tsingalia, 1988). Due to ever increasing demand for more agricultural land coupled with favourable climatic conditions (temperature ranges from 12.7 to 27.1 °C and average rainfall is 1650 mm), parts of the forest have been opened for cultivation of maize and sorghum.

3.2.1.4 Suam

Suam site covered about 25 km^2 along the eastern slopes of Mt. Elgon between the longitudes 34.77 – 34.18^0 E, and latitude 1.17 – 1.18^0 N. The vegetation mosaic is Afromontane with the landscape consisting of lightly wooded grassy formations (White, 1983). Approximately 60% of this site is under maize cultivation while the wild habitat covers about 11% (Goux, 2006). The area is characterized by bimodal rainfall which allows only one annual cropping season (April to October). The dry season hereafter referred to as non-cropping season occur between the months of December and March (Corbett *et al*., 2001).

3.2.2 Sample size determination

3.2.2.1 The total number of plants (maize and sorghum) per locality in each sampling session

Each of the four study localities had different homogenous vegetation structures as described in the land use maps (Guiheneuf, 2004; Goux, 2006). Surveyed localities are characterised by small scale peasant farming with less than 2 ha per house hold except in Suam where expansive maize farms owned by Agricultural Development Co-operation (ADC) are used for the production of maize seeds. During the study design, cultivated fields were categorised as a single vegetation structure. The total number of plants inspected for stem borer infestation (sample size, *n*) in each locality was therefore determined with respect to the size of the cultivated structure from an equation earlier described by Webster & Oliver (1990), [Equation, 1]. However, in Muhaka and Mtito

Andei where areas under maize and/or sorghum were less than 20% of the total area, 0.5 was used as *p* value to limit errors associated with low sample sizes (table 2.1)

$$n = \left(\frac{Z_{\alpha/2}}{\delta}\right)^2 q / p \qquad \text{Equation 1}$$

where n is the number of maize fields required in each site, δ the reliability level expressed as a fixed proportion of the mean (0.05), $Z_{\alpha/2}$ is the standard normal deviate at 95% (1.96). p is the proportion of land under maize and or sorghum cultivation, while $q = 1 - p$

For example in Kakamega where about 43.3% of the total study area was under maize cultivation (Guiheneuf, 2004), the total number of maize plants required in each sampling session was estimated using the formula:

$$\left\{n = \left(\frac{1.96}{0.05}\right)^2 0.57/0.43 = 2036.94 \approx 2040\right\} \text{ maize plants.}$$

In Muhaka where maize cover was about 11% of the total area, 0.5 was used as *p* to limit errors associated with low sample size instead of 0.11. The number of maize plants required in each sampling session was thus estimated as

$$\left\{n = \left(\frac{1.96}{0.05}\right)^2 0.5/0.5 = 1536.64 \approx 1540\right\} \text{ maize plants}$$

3.2.3.2 Number of fields, and number of maize and / or sorghum plants per field

The number of maize plants inspected for stem borer infestation in each field was estimated from equation described by Zar (1999), [Equation 2].

$$n = \frac{Z^2_{\alpha(2)}}{4Dd^2} \qquad \text{Equation 2}$$

where $Z_{\alpha(2)}$ is the standard normal deviate (1.96), *d* permitted error (0.1) resulting in a uniform number plants in all farms, *D* is the design effect (1). The number has been rounded to 100 to correct for errors associated with attrition.

$$\left\{ n = \frac{1.96^2}{4 \times 1 \times 0.1^2} = 96.04 \approx 100 \right\} \text{ maize plants}$$

This equation did not consider previous data on stem borer infestation levels but ensured that uniform number of plants was inspected in each field during the surveys. This allowed for statistical comparison of species composition, infestation and density levels among different localities with fields as replicates. The number of maize fields surveyed in each locality (Table 3.1) was determined from the quotient of the total number of plants in each locality as estimated in Equation 1 divided by 100, maize plants inspected in each field, as estimated in Equation 2.

3.2.4 Sampling of stem borers

3.2.4.1 Cultivated fields

Surveys were carried out in the cultivated habitats during the 2005/2007 study period. Field infestations were estimated by inspecting 100 randomly selected maize or sorghum plants in each field as estimated using equation by Zar (1999). Infested stems were dissected for recovery of stem borer larvae and pupae (Plate 3.1 a). The same fields were visited at regular intervals after every two months throughout the study period. During the non-cropping periods, the abundance of stem borer larvae and pupae were assessed on the dry stalks in old maize fields.

Table 3.1: Number of cultivated fields sampled during different sampling sessions

Locality	*p*	*q*	No. of plants	No. of fields
Muhaka	0.5	0.5	1540	16
Mtito Andei	0.5	0.5	1540	16
Kakamega	0.43	0.57	2040	21
Suam	0.62	0.38	942	10

(**a**) (**b**)

Plate 3.1: Stem borer sampling process in Kakamega (**a**) Sampling of young maize plants (**b**) Sampling wild plants, *Euclaena mexicana* along the banks of river Yala

3.2.4.2 Uncultivated habitats

Plant species belonging to Poaceae, Cyperaceae and Typhaceae families growing within 50 m around the cultivated fields were randomly selected and inspected for stem borer infestation symptoms or damage charcaterised by scarified leaves, dry leaves and shoots (dead hearts), frass and also holes bored. The number of stems inspected within this margin varied between 20 and 100 depending on the plant species, cover density and the infestation levels. Infested plants were cut and dissected in the field. All the stem borer larvae and pupae from different host plant type and field were placed in labelled 1 litre plastic jars with ventilated lids and later brought to the laboratory for rearing.

In addition to the uncultivated fragments within 50 m around the cultivated fields, inspection for stem borer infestation among wild host plants was extended to other habitats beyond the 50 m margins, including the riverbanks and swamps (Plate 3.1 b). Stem borer densities among wild host plants are exceedingly low, and a biased sampling procedure was adopted in these habitats to increase chances of finding borers. All infested plants were dissected in the field and collected larvae and pupae placed in labelled 1 litre plastic jars with ventilated lids, and later brought to the laboratory for rearing. Infested plants were identified in the field, and where identification was not possible, the voucher specimen (whole plant; roots, stems, leaves and flowers) were collected and taken to the East Africa herbarium (Nairobi, Kenya) for expert identification.

3.2.5 Rearing and identification of stem borers

Recovered larvae were all reared until pupation on fresh maize stems (10 cm long) kept in plastic vials (16 x 10 cm) with perforated plastic lids or artificial diet prepared from maize stalk (Onyango & Ochieng-Odero, 1994). A prepared artificial diet was kept in glass vials (7.5 x 2.5 cm) closed with cotton wool (Plate 3.2 a). Pupae taken out of the artificial diet / maize stems were kept separately in plastic vials (16 x 10 cm) closed with perforated plastic lids until adult emergence (Plate 3.2 b).

(**a**) (**b**)

Plate 3.2: Insect rearing set up in the laboratory, (**a**) Glass vials with artificial diet in which larvae were maintained until pupal formation (**b**) Wild plants in which larvae were maintained and plastic vials with soft paper towel where the pupae are maintained until emergence.

Upon emergence, the moths were divided into two portions. One portion of female and/or male was used for species identification and morphological description, and the other portion was used for molecular analysis. Adult moths were identified to species level by Pascal Moyal (Laboratoire Evolution, Génomes et speciation, Gif-sur-

Yvette, France) and voucher specimens deposited in Museum National d'Histoire Naturelle (MNHN, Paris, France) and at the ICIPE Museum (Nairobi, Kenya). The identified borers were grouped in their respective hosts according to localities. The remaining proportion of the insects were preserved in absolute ethanol (>99%) upon emergence for DNA extraction.

3.2.6 Stem borer species diversity

Identified stem borer species were recorded with respect to host plants and localities. Species recorded from different host plants and localities during the study period (2005/2007) were analyzed for species richness (*S*) and diversity using Shannon's diversity index (*H'*) (McAleece, 1997)). The hypothesis that stem borer species vary in distribution with respect to their ecological requirements was tested by calculating faunistic similarities. Similarities were compared using Kulczynski coefficient (KC), which measures the percentage similarity among communities between two areas (Price, 1982). This index was chosen because of its advantage over other similarity indices as it takes into account the disproportionate number of species. The Kulczynski coefficient is given by the following formula,

$$KC = \frac{1}{2}\left[\frac{s}{(s+u)} + \frac{s}{(s+v)}\right] \times 100$$

Where *s* is the number of species common to area A and B; *u* is the number of species found in area A and absent from B and, *v* is the number of species found in area B but absent in area A.

3.3 Results

3.3.1 Stem borer species diversity and composition in the cultivated fields

Seven stem borer species were identified from the collections made in the cultivated fields (Table 3.2; See Plates 3.3 a and b). Distribution of these species varied among localities with *Busseola* species occurring in Kakamega and Suam, while *Chilo* species occurred in Muhaka and Mtito Andei. The *Chilo* species (*C. partellus* and *C. orichalcociliellus*) co-existed with *S. calamistis* in Muhaka, a community dominated by *C. partellus* (65%). The dominance of *C. partellus* was also observed in Mtito Andei (78%) where it co-existed with only *S. calamistis*. In Suam, only two pest species, *B. fusca* and *S. calamistis* were identified with *B. fusca* constituting about 97% of the community. Both species (*B. fusca* and *S. calamistis*) were found in Kakamega where they co-existed with three other species, *B. phaia* ssp. *phaia*, *S. piscator* and *E. saccharina*. Pest community in Kakamega was generally dominated by *B. fusca* (40%) followed by *B. phaia* ssp. *phaia* (30%) with species richness enhanced by the presence of less known pest species, *B. phaia* ssp. *phaia* and *S. piscator*.

Table 3.2: Average composition of stem borer pest community among cultivated host plants in different localities

Stem borer species	Composition of the stem borer community (%)			
	Muhaka	Mtito Andei	Kakamega	Suam
Busseola fusca (Fuller)			40	97
Busseola phaia Bowden			30	
Sciomesa piscator Fletcher			2	
Sesamia calamistis Hampson	22	22	26	3
Chilo orichalcociliellus (Strand)	13			
Chilo partellus (Swinhoe)	65	78		
Eldana saccharina Walker			2	

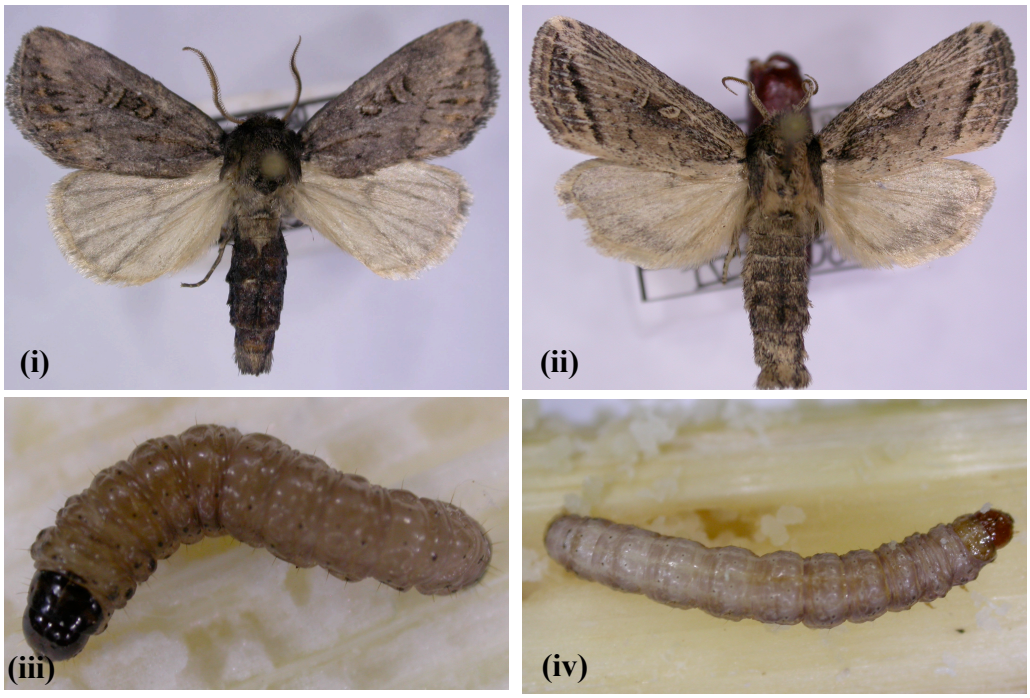

Plate 3.3 a: Stem borer species in the *Busseola* genus; *Busseola fusca*, **i** [Male], **iii** [Larva]; *Busseola phaia*, **ii** [Male], **iv** [Larva]

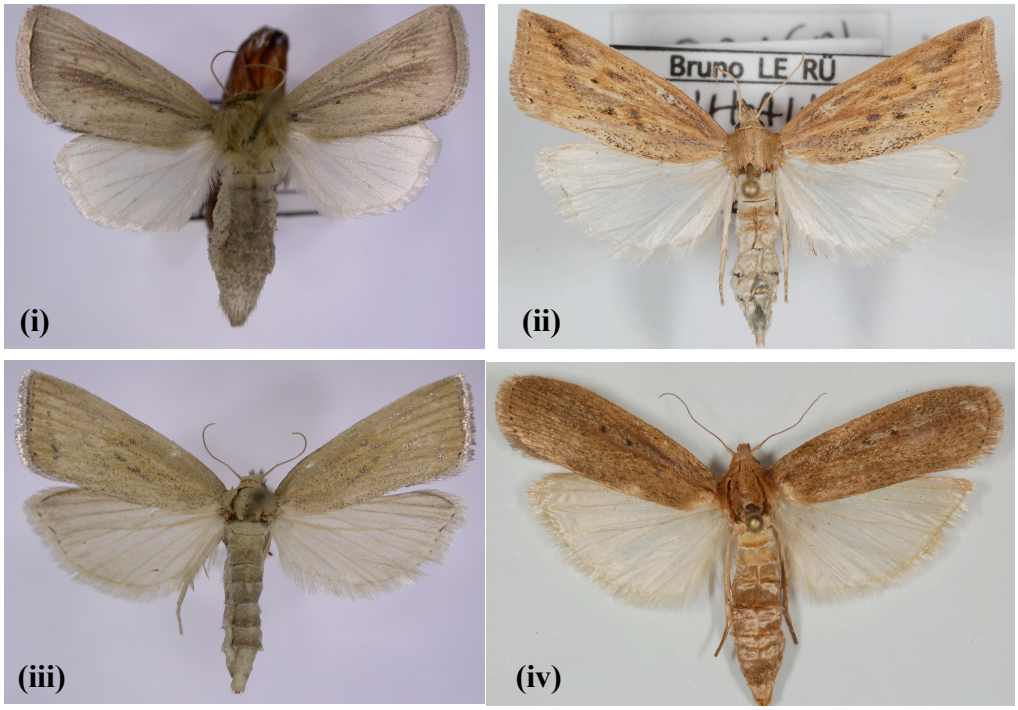

Plate 3.3 b: Stem borer pest species (**i**) *Sesamia calamistis* (**ii**) *Chilo partellus* (**iii**) *Chilo orichalcociliellus* (**iv**) *Eldana saccharina*.

Distribution and the importance of host plant species

A total of 34 plant species were found infested during this study. These plants however, cover different areas in the surveyed localities as summarized in table 3.3, a table based on the average seasonal plant species cover as established by Otieno *et al*. (2006 & 2008). Ten of these host plant species were identified in Muhaka with *P. maximum* covering the largest proportion of the study area (1.61%) followed *C. dactylon* that covered 0.32%. *Cynodon aethiopicus* covered the largest area (2.31%) in Mtito Andei, followed by *P. maximum* (0.67%) and *R. cochinchinensis* (0.32%), dominating the other 11 host plants in the area. In Kakamega where there were a total of 20 plant species, the largest area was covered by *C. dactylon* (5.49%) followed by *S. megaphylla* (2.04%) and *S. arundinaceum* (0.73%). In Suam, the largest area was covered by *C. nardus* (7.34%), followed by *C. dactylon* (7.22%), *S. incrissata* (1.06%) and *C. dives* (1.53%).

Stem borer host plants generally varied in their importance (Table 3.3). The highest number of stem borer species were found on *P. purpureum* (10) followed by *P. maximum* (9), *S. arundinaceum* (6), *C. involucratus* (6), *P. macrourum* (5) and *E. haploclada* (5). The other host plants were infested by between one and four stem borer species. However, importance of these hosts varied among the localities. *Pennisetum purpureum* which was infested by 7 stem borer species in Kakamega, was only infested by two stem borer species in both Mtito Andei and Suam, and only one in Muhaka. The other important host, *P. maximum*, was infested by 5 different stem borer species in Muhaka, three in both Mtito Andei and Suam, and two in Kakamega. Infestation of some hosts were however limited to certain localities. For example infestation in *S. arundinaceum* was observed only in Muhaka, Mtito Andei and Suam, while *C.*

aethiopicus and *S. megaphylla* were infested only in Mtito Andei and in Kakamega respectively.

Table 3.3: Relative annual cover (%) of different host species in different localities.

	Host plant species	Average area cover (%)			
		Muhaka	Mtito Andei	Kakamega	Suam
1	*Cymbopogon nardus* (L.) Rendle [3]	00.00	00.00	00.51 [2]	07.34 [1]
2	*Cynodon aethiopicus* Clayton & J.R. Harlan [3]	00.00	02.31 [3]	00.00	00.00
3	*Cynodon dactylon* (L.) Pers. [2]	00.32	00.00	05.49	07.22 [2]
4	*Digitaria milinjiana* (Endle) Staff [1]	00.01 [1]	00.00	00.00	00.00
5	*Echinochloa colonum* (L.) Link [1]	00.00	00.01 [1]	00.00	00.00
6	*Echinochloa haploclada* (Stapf) Stapf [5]	00.06 [5]	00.13	00.00	00.00
7	*Echinochloa pyramidalis* (Lamark) Hitchcock & Chase [1]	00.00	00.00	00.03 [1]	00.00
8	*Eleusine corocana* L.* [3]	00.00	00.00 [2]	00.01 [2]	00.01 [2]
9	*Eleusine jaegeri* Pilg. [1]	00.00	00.02 [1]	00.00	00.20
10	*Euclaena mexicana* Schrader [2]	00.00	00.00	00.01 [2]	00.00
11	*Panicum deustum* Thunb. [2]	00.00	00.01 [2]	00.00	00.00
12	*Panicum infestum* Andersson. [4]	00.01 [4]	00.00	00.00	00.00
13	*Panicum maximum* Jacquin [9]	01.61 [5]	00.67 [3]	00.61 [2]	00.26 [3]
14	*Pennisetum cladestinum* Hochst ex Chiov. [1]	00.00	00.00	00.00	00.01 [1]
15	*Pennisetum macrourum* Trinius [5]	00.00	00.00	00.32 [3]	00.17 [2]
16	*Pennisetum purpureum* Schumach§.[10]	00.00 [1]	00.04 [2]	00.63 [7]	00.50 [2]
17	*Pennisetum trachyphyllum* Pilg.[2]	00.00	00.00	00.35	00.66 [1]
18	*Pennisetum unisetum* (Nees) Benth. [1]	00.00	00.00	00.00 [1]	00.52
19	*Rottboella cochinchinensis* (Lour.) Clayton [4]	00.14 [4]	00.32 [2]	00.00	00.39
20	*Saccharum officinarum* L.*[2]	00.00	00.00	00.01 [2]	00.00
21	*Setaria megaphylla* (Steud.) T. Durand & Schinz [3]	00.00	00.00	02.04 [3]	00.09
22	*Setaria incrassata* (Hochst.) Hack. [2]	00.00	00.00	00.00	01.01 [3]
23	*Setaria verticillata* (L.) P. Beauv. [3]	00.00	00.00 [2]	00.00	00.30
24	*Sorghum arundinaceum* (Desv.) Stapf [6]	00.02 [4]	00.09 [3]	00.73	01.06 [2]
25	*Cyperus articulatus* L.[1]	00.00	00.00	00.00	00.00
26	*Cyperus distans* L. [3]	00.00	00.02 [3]	00.19	00.22
27	*Cyperus dives* Delile [4]	00.02	00.00	00.06 [2]	01.53 [3]
28	*Cyperus involucratus* Rottb. [6]	00.00	00.01 [6]	00.00	00.00
29	*Cyperus papyrus* L. [1]	00.00	00.00	00.00 [1]	00.00
30	*Cyperus rotundus* L. [3]	00.01 [3]	00.00	00.01	00.00
31	*Scirpus inclinatus* (Delile ex Barbey) Boiss. [1]	00.00	00.00	00.01 [1]	00.00
32	*Schoenoplectus confusus* (NEBr.) Lye [3]	00.00	00.00	00.00	00.01 [3]
33	*Scleria racemosa* Poiret [1]	00.00	00.00	00.23 [1]	00.00
34	*Typha domingensis* Pers. [2]	00.00	00.14 [2]	00.15	00.01

Cultivated crops included in the list are marked with stars (*) while *P. purpureum* as a domesticated grass is marked §. In bracket [S] is the number of borer species recovered from different host plants in corresponding localities.

Diversity and distribution of stem borer species

A total of 29 stem borer species were identified from the 3,203 larvae collected. These species however varied in distribution among the localities as reflected in the diversity indices (Table 3.4). The highest Alpha (α) diversity was recorded in Suam (α = 3.0), followed by Mtito Andei (α = 2.77) and Kakamega (α = 2.5). In contrast, more larvae were collected in Muhaka (1551) followed by Kakamega (683), Mtito Andei (627) with least materials collected in Suam (342). The highest number of species were recorded in Kakamega (15) followed by both Mtito Andei and Suam with 13 species each. The least number of species was recorded in Muhaka where only 11 were identified. The total larvae and diversity indices recorded in different localities suggested variations in composition of stem borer communities.

Stem borer species constituting communities in different localities could be grouped in different categories but species constituting less that 1% of the total collection in respective localities are hereafter referred to as rare. Stem borer community in Muhaka was dominated by *C. orichalcociliellus* (Berger-Parker Dominance, d% = 46.68) followed by *C. partellus* and *Ematheudes* spp, with *Acrapex* sp as a rare species. In Mtito Andei, stem borer community was dominated by *S. nonagrioides* (d% = 33.81) followed by *S. calamistis* and *C. partellus*. Several species were rare in Mtito Andei and these included *S. piscator*, *C. orichalcociliellus*, *Chilo* sp. nr *orichalcociliellus* and *E. saccharina*. Stem borer communities in Kakamega and Suam were dominated by *Sesamia* nov sp 9 (d% = 20.80) and *S. nyei* (d% = 32.75) respectively. Several rare species including *M. melanodonta*, *P. mediopuncta*, *S. peniseti* and *E. saccharina*, were found in Kakamega, while *Busseola* sl nov sp 3 and *S. poephaga* were found in Suam.

Table 3.4: Stem borer species diversity in different localities.

Stem borer species	Muhaka	M. Andei	Kakamega	Suam
Acrapex sp	001 [6]	000	000	000
Busseola fusca	000	000	000	018 [15,17,24]
Busseola phaia	000	000	135 [1,10,12,13,15,16,20]	001 [14]
Busseola sl nov sp 3	000	000	000	005 [1,3,16]
Busseola sl nov sp1	000	000	111 [21]	000
Caleris nov sp 3	000	000	058 [16,27,31,33]	000
Manga melanodonta	000	000	003 [13]	098 [13]
Manga nubifera	089 [13]	000	000	000
Poecapa mediopuncta	000	000	008 [21]	000
Sciomesa nov sp 3	000	075[2]	000	000
Sciomesa nyei	000	000	010 [15,16]	112 [13,17]
Sciomesa piscator	000	003 [2,28]	022 [1,7,10,13,15,16,27]	028 [3,15,16,27,32]
Sciomesa venata	000	000	000	026 [15,25,32]
Sesamia calamistis	011 [6,13,24]	110 [5,8,9,11,13,16,23,24,26]	010 [8,16]	003 [24,27]
Sesamia peniseti	000	000	005 [16]	000
Sesamia nonagrioides	000	330 [2,5,23,26,28,34]	000	000
Sesamia nov sp 5	051 [6]	000	000	000
Sesamia nov sp 9	000	000	142 [29]	000
Sesamia poephaga	019 [4,12,13]	066 [13]	000	010 [13]
Noctuid (unknown spp)	133 [10,11,16]	000	138 [21]	000
Chilo orichalcociliellus	724 [6,12,13,16,19,24]	001 [13]	000	000
Chilo partellus	390 [12,13,19,24]	108 [8,16,24]	000	000
Chilo sp.nr *orichalcociliellus*	000	001 [11]	000	000
Crambidae (unknown)	000	000	001 [1]	004 [8]
Eldana saccharina	000	002 [28]	005 [16,20]	000
Emautheudes spp	253 [6,12,19,20,24]	023 [19]	000	001 [22]
Pyralidae	000	002 [24,28]	023 [6,24,29]	004 [22,27]
Schoenobinus spp	004 [27]	013 [28,34]	000	000
Tortricidae sp.	011 [30]	007 [26,28]	006 [24]	003 [32]
Total Individuals	1551	627	683	342
Total Species (S)	11	13	12	13
Alpha (α)	1.43	2.77	2.50	3.00

Berger-Parker Dominance (d%)	46.68	33.81	20.80	32.75

In superscript are numbers for host plant codes from which different borer species were collected [refer to Table 3.3]

Stem borer species distribution and host range

Stem borer species generally varied in distribution among the four localities, with species in the family Noctuidae occurring mainly in Kakamega and Suam, while the non-noctuids were found mainly in Muhaka and Mtito Andei (Table 3.4). Species in the *Busseola* genera (*B. fusca*, *B. phaia* ssp. *phaia*, *Busseola* sl nov sp 1 and *Busseola* sl nov sp 3) were found in both Kakamega and Suam. However, among the *Busseola* species, only *B. phaia* ssp. *phaia* was common to the two localities as *B. fusca* and *Busseola* sl nov sp 3 were found only in Suam, and *Busseola* sl nov sp 1 only in Kakamega. Species in the *Sciomesa* genus were found in three localities (Mtito Andei, Kakamega and Suam) and like the *Busseola* species, they varied in distribution among these localities. *Sciomesa* nov sp 3 was found only in Mtito Andei where it infested *C. aethiopicus* while *S. venata* was found only in Suam where it infested *P. macrourum*, *C. articulatus* and *S. confusus*. Among the *Sciomesa* species, only *S. piscator* was common to the three localities infesting a total of 11 plant species with seven of them occurring in Kakamega.

The majority of the noctuid species identified belonged to the *Sesamia* genus. These species however showed a wide range of distribution, from the low altitude (Muhaka) to high altitude locality (Suam). Of these species, *S. calamistis* was found in all the four localities infesting a total of 11 plant species. The majority of infested plants (9) were found in Mtito Andei (9) followed by Suam (5). The second *Sesamia* species with a relatively wide distributional range was *S. poephaga* which was found in Muhaka, Mtito Andei and Suam. Unlike *S. calamistis* that was found on several plants species, *S. poephaga* was found on only 3 plant species with *P. maximum* being the most important

host in all the three localities. The other two host plants included *D. milinjiana* and *P. infestum*, which were only infested in Muhaka. Other *Sesamia* species appeared localized in their distribution using varied hosts in respective localities. Both *S. peniseti* and *Sesamia* nov sp 9 were found only in Kakamega infesting *P. purpureum* and *C. papyrus* respectively. *Sesamia nonagrioides* on the other hand was found only in Mtito Andei where it infested six different plant species though majority of the larvae were found on *T. domingensis*, while *Sesamia* nov sp 5 was found only in Muhaka where it infested *E. haploclada*.

The non-noctuids species belonged to Crambidae, Pyralidae, Schoenobinae and Torticidae families with species in the *Chilo* genera forming bulk of the materials. The *Chilo* species, *C. partellus*, *C. orichalcociliellus* and *Chilo* sp. nr *orichalcociliellus* were found only in Muhaka and Mtito Andei. These species infested a wide range of plants though the majority of *C. partellus* and *C. orichalcociliellus* were found on *P. maximum* and *S. arundinaceum* respectively. The pyralid, *E. saccharina* was found in both Mtito Andei where it infested *C. involucratus*, and Kakamega where it infested *P. purpureum* and *S. officinarum*. The only non-noctuid species found in four localities was the unknown species in the Tortricidae family. Though it occurred in low numbers, this species was found on *C. rotundus* in Muhaka, *C. distans* and *C. involucratus* in Mtito Andei, *S. arundinaceum* in Kakamega and *S. confusus* in Suam.

3.3.3 Stem borer faunistic similarities among wild host plants

Cross-wise comparison of faunistic similarity (expressed as the second Kulczynski coefficient) yielded a matrix reflecting variation in distribution of species

(Table 3.5). For instance when Mtito Andei was compared with Muhaka, a Kulczynski coefficient ($KC_{Mtito\ Andei/Muhaka}$) of 60.0 was found, similarly the $KC_{Mtito\ Andei/Suam}$ and $KC_{Suam/Kakamega}$ remained high (48.7 and 42.0 respectively) indicating that these localities share considerably high number of species. However, the results of $KC_{Kakamega/Muhaka}$ = 17.7, $KC_{Suam/Muhaka}$ = 38.2 and $KC_{Kakamega/Mtito\ Andei}$ = 29.7 suggested that there was considerable variation in species composition among these pairs.

Table 3.5: Kulczynski coefficient (KC) as calculated between the different localities surveyed in Kenya.

KC	Muhaka (11)	Mtito Andei (13)	Kakamega (12)	Suam (13)
Muhaka	-	-	-	-
Mtito Andei	60.0	-	-	-
Kakamega	17.7	29.7	-	-
Suam	38.2	48.7	42.0	-

The numbers between brackets in the leading row represent species counts in different localities.

3.4 Discussion

This study reveals variation in species diversity and distribution among different localities. Despite variations in species diversity, *B. fusca*, *S. calamistis*, *C. partellus* and *C. orichalcociliellus* were the main pest species, corroborating earlier reports by Seshu Reddy (1983), Overholt *et al*. (2001) and Zhou *et al*. (2002). *Busseola fusca* dominated high altitude locality, Kakamega (planetary Guineo-Congolian rain forest) and Suam (Afromontane vegetation mosaic), while *C. partellus* dominated low altitude localities, Muhaka (Zanzibar Inhambane vegetation type). Though this study focused on species composition in different localities, the observed distribution pattern corroborates findings of previous studies conducted along different agro-ecological zones in Kenya (Overholt *et al*., 2001; Zhou *et al*., 2002).

In addition to the four widely reported pest species in Kenya (*B. fusca*, *S. calamistis*, *C. partellus* and *C. orichalcociliellus*), other stem borer species, *B. phaia* ssp. *phaia* and *S. piscator* were found in cultivated crops in Kakamega, along the Guineo-Congolian rain forest. For a long time, these species have been associated with only wild plants in East Africa (Nye, 1960). This was however contradicted by results of the recent surveys in Kenya in which Le Ru *et al*. (2006b) found these species among cultivated crops. They indicated that the presence of these species in the cultivated fields could be as a result of accidental oviposition or the gradual shift in response to habitat modification. Continued presence of these species among the cultivated crops suggests the emergence of "new" stem borer pests in Kenya. This however is not the first time host shift among stem borers is reported in Africa as similar shifts have been reported of *E. saccharina* from sedges to sugarcane in South Africa (Atkinson, 1980).

In addition to host range expansion and eventual emergence of "new" pest species, habitat transformation may affect the general species richness and composition. There have thus been increasing interest to understand stem borer species diversity among wild habitats with an aim of averting potential consequence of habitat transformation (Le Ru *et al*., 2006a; Matama-Kauma *et al*., 2008). In this study, 29 stem borer species were collected. Out of the 29 species collected, 15 of them are known, while the rest are unknown and could only be identified to either genus or family level. The identified species varied in distribution among the surveyed localities with the highest diversity recorded in Kakamega (wet and hot guineo-congolian mosaic). This corroborates the findings of a study conducted by Le Ru *et al*. (2006b) in which they found higher diversity of noctuids in wet and hot guineo-congolian mosaic in western Kenya. In Suam, Mtito Andei and Muhaka, stem borers were found mainly among the hosts growing along the riverines and swamps. Similar distribution pattern was observed by Nye (1960) when he found stem borer larvae mainly from wetter parts of different vegetation mosaics.

The stem borer pests, *B. fusca*, *S. calamistis*, *C. partellus*, *C. oricholcociliellus* and *E. saccharina* were among the known species collected though their populations were lower compared to the collections made in the cultivated fields. This however is not the first time such a low pest population has been reported in the uncultivated fragments within agro-ecosystems. Similar results were observed by Le Ru *et al*. (2006a) during their surveys in Kenya and Eastern Africa respectively, and Gounou & Schulthess, (2004) in western Africa. Laboratory studies have shown low larval survival and development on several wild grasses (Shanower *et al.*, 1993; Ofamata *et al*., 2000; van den Berg *et al*.,

2001), which may be attributed to high silica contents in the epidermis of leaves (McNaughton *et al.*, 1985; Sétamou *et al.*, 1993). The other species collected; *S. penniseti*, *S. poephaga*, *S. nonagrioides*, *S. venata*, *S. piscator*, *S. nyei*, *P. mediopuncta*, *M. nubifera*, *M. melanodonta* and *B. phaia* ssp. *phaia*, varied in distribution among the surveyed localities.

Each locality harbored dominant borer species accounting at least for 20% of the specimens collected. Populations of rare or unknown species in these localities were generally low, though they exerted a strong influence in the overall species assemblage. However, their role and importance in structuring broad community patterns among regions is not well understood (Price *et al*., 1995; Novotny & Basset, 2000). Some of the rare or unknown species, *Sesamia* nov sp 9, *Sciomesa* nov sp 3 and *P. mediopuncta*, appeared localised in their distribution, results that could be attributed to seasonality and ecological preference. Species like *Sciomesa* nov sp 3 and *P. mediopuncta* were earlier reported from *C. aethiopicus* and *S. megaphylla* in Mtito Andei and Kakamega respectively (Le Ru *et al*., 2006b). The presence of unknown and some rare species confirms earlier assertions that stem borer species and host list in East Africa is far from complete (Le Ru *et al*., 2006a). Stem borer species generally vary in their annual flying periods (Holloway, 1998) and thus the single or few sampling sessions that characterised previous studies may not have captured the seasonal species. The presence of unknown and some rare species in this collection can therefore be attributed to regular sampling intervals that allowed recovery of seasonal and conspicuous stem borer species.

Majority of the rare and unknown stem borer species were found on isolated host batches growing along wetlands and riverines. However, these habitats are targeted for

agricultural expansions as they constitute suitable areas for both irrigation and horticultural farming. Transformation of wetlands as well as other habitats poses a major threat to stem borer species diversity. Of immediate concern to agriculture is the resultant effect of these transformations to stem borer pest dynamics. Will these have direct or indirect effect on pest populations in the cultivated fields? Will some non-economic species expand their host range and adapt to cultivated crops?

CHAPTER FOUR

4 DYNAMICS AND MANAGEMENT OF STEM BORERS IN KENYA

4.1 Introduction

Busseola fusca, *Sesamia calamistis*, *Chilo orichalcociliellus* and *Chilo partellus* are the most important stem borer pests of maize and sorghum in Kenya. The distribution and economic importance of these species vary among different regions across the country depending on their respective ecological requirements (Le Ru *et al*., 2006a). *Busseola fusca* and *C. partellus* are found mainly in high and low altitude areas respectively with *S. calamistis* occurring in all altitudinal gradients as a minor pest species. Based on the knowledge of their distribution, several control techniques have been developed. Among the tried management strategies are late planting, application of insecticides, planting of border rows with grasses serving as trap plants and/or refugia for pests and natural enemies (Khan *et al*., 1997) and biocontrol using *Cotesia flavipes* against *C. partellus* in low altitude areas.

Despite these management initiatives, stem borer pests persist in the cultivated fields where they account for up to 14% cereal losses annually (Songa *et al*., 1998). There is therefore need to introduce other strategies to augment the existing stem borer management practices to further reduce losses associated with their infestations. One of such strategies is the proposed introduction of transgenic maize expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (*Bt*). Transgenic plants were first commercialised in 1996 amid concern from some scientists, regulators and environmentalists that the widespread use of *Bt* crops would inevitably lead to resistance and the loss of a public good, especially, the susceptibility of insect pests to *Bt* proteins

(Gould *et al*., 2002). However, proponents of transgenic crops argued that the refuge approach in the agro-ecosystems would adequately contain rapid evolution of resistance. The theory underlying the refuge strategy for delaying insect resistance is that most of the rare resistant individuals surviving on *Bt* crops will mate with abundant susceptible individuals from refuges of host plants without *Bt* toxins (Bourguet *et al.,* 2000a). If inheritance of resistance is recessive, the hybrid offspring produced by such matings will be killed by *Bt* crops, markedly slowing the evolution of resistance.

Stem borers, the target pests are thought to be polyphagous and their persistence in crop fields is blamed on the influx of diaspore population from wild hosts growing in the uncultivated fragments (Polaszek & Khan, 1998). Based on this assumption, wild habitats fit well in the management of resistance as a refuge should the proposed transgenic maize get into hands of small scale farmers. However, this assumption has been contradicted by the results of recent surveys in Kenya and Eastern Africa in which stem borer pests were found to be more specialized infesting limited host plant species (Le Ru *et al*., 2006a). *Busseola fusca* which is an important pest in high altitude areas was only reported from *S. arundinaceum* while *C. partellus* was reported mainly from *S. arundinaceum* and *P. maximum* in low altitude areas. With this revelation, it appears that there is no generally agreed source of stem borer pests found in the cultivated fields as these may vary among pest species and regions. Does stem borer pest community vary in agricultural systems within a season? Could these variations be attributed to the influx of the diaspore populations from the wild? How does this fit in the context of the proposed introduction of transgenic maize? Thus, the study was initiated to identify stem borer pests in the cultivated habitats and monitor seasonal variation in population dynamics in

an attempt to establish the role wild host plants play in population dynamics and relate this finding to the proposed introduction of transgenic maize in Kenya.

4.2.0 Materials and methods

This study was carried out in the four localities, Muhaka, Mtito Andei, Kakamega and Suam, occurring in different agro-ecological zones. Description of the study areas, sampling of stem borers in both wild and cultivated fields, rearing and identification of stem borers are presented in Chapter 3.

4.2.1 Data management and analysis

The average infestation was estimated in each field from the number of plants infested against the number of plants checked for infestation. The total number of stem borer larvea collected in each field (both wild and cultivated fields) was divided by corresponding number of plants checked for infestation to estimate larval densities. The effects of growing seasons on the general stem borer infestations (in the cultivated fields) and species densities (in both wild and cultivated fields) were analysed for different localities. Infestation data (%) was arcsine transformed and subjected to one way analysis of variance (ANOVA) to compare the general variation stem borer pest infestations (%) in different localities. Means were later separated using Student-Newman-Keuls (SNK) multiple range test (SAS, 1997). Average infestations (%) per season for different localities were compared separately for each season using Students' *t* test.

The average proportions of different stem borer species in the pest community (%) and their respective densities in different growing seasons were compared for each locality using One-way ANOVA. The percentage data was arcsine

transformed while the density data was log transformed ($1 + \log_{10} x$). Means were separated using Student-Newman-Keuls (SNK) multiple range test (SAS, 1997). Seasonal variation in species densities for both wild and cultivated fields were compared separately for each locality using Students' *t* test.

4.3 Results

4.3.1 Stem borer pest infestations and the associated seasonal variations

There was evidence of variation in the mean annual stem borer pest infestations among the surveyed localities ($P < 0.05$; Table 4.1). The highest infestation was recorded in Muhaka and Mtito Andei while the lowest levels were recorded in Kakamega and Suam. Variations were also observed within seasons in respective localities ($P < 0.05$). The highest infestation in the *LR* growing season was observed in Muhaka (19.16 ± 2.89 %) followed by Mtito Andei (12.00 ± 5.51 %) while low infestations were recorded in Kakamega and Suam (4.07 ± 0.67 and 9.71 ± 1.09 % respectively). Similar trends were observed in the *SR* growing season during which the highest infestation was observed in Muhaka (35.57 ± 3.81%) followed by Mtito Andei (30.67 ± 7.54%). Despite the differences in infestation levels among the localities, there was no evidence of variation in infestation levels between the growing seasons except in Muhaka where there was significantly higher infestation during *SR* growing season ($t_{83} = 3.26$; $P = 0.002$).

Table 4.1: Stem borer annual and seasonal infestations (%) in different cultivated fields

Locality		Mean ± SE (%)	Seasonal average infestation (Mean ± SE) %				
			Long rain	Short rain	*t*	*Df*	*P*
Muhaka		28.43 ± 2.63[a]	19.16 ± 2.89[a]	35.57 ± 3.81[a]	3.263	83	0.002*
Mtito Andei		26.00 ± 6.04[a]	12.00 ± 5.51[ab]	30.67 ± 7.54[a]	1.369	18	0.188
Kakamega		05.43 ± 0.63[b]	04.07 ± 0.67[b]	06.17 ± 0.89[b]	1.606	121	0.111
Suam		09.71 ± 1.09[b]	09.71 ± 1.09[b]	-	-	-	-
Statistics	*F*	39.54	13.56	40.02			
	Df	3, 376	3, 133	2, 140			
	P	< 0.0001	< 0.0001	< 0.0001			

Means (± SE) within columns followed by the same letters are not significantly different (Student-Newman-Keuls multiple range tests, $P \leq 0.05$).

4.3.2 Stem borer species composition and seasonal density fluctuations

Stem borer pest species varied in distribution among the surveyed localities (Table 4.2). See chapter 2 for an overview of stem borer pest composition and distribution in the cultivated fields. In addition to the variation in distribution and composition of pest communities, there was evidence of variation in densities of different species within seasons ($P < 0.05$). The highest density was recorded during the *LR* growing season in Muhaka (0.64 ± 0.08) followed by Mtito Andei (0.25 ± 0.07). *Chilo partellus* dominated the larval population in both localities where its density was recorded as 0.46 ± 0.06 and 0.18 ± 0.07 per plant respectively. The other species with relatively higher density was *B. fusca* though this was only observed in Suam where it co-existed with *S. calamistis*. Variations in larval densities were also observed during the *SR* growing season with relatively higher densities in Mtito Andei (0.35 ± 0.07) and Muhaka (0.34 ± 0.05).

Student's *t* test on species numbers in different localities revealed significant variations in numbers among some species between *LR* and *SR* growing seasons ($P < 0.05$; Table 4.2). In Muhaka, significant variations in larval densities were observed among *S. calamistis* and *C. partellus* with higher densities recorded during the *LR* for both species. Variation in species numbers was also observed in Kakamega among the populations of *B. fusca* and *B. phaia*. High *B. fusca* density was recorded during the *LR* growing season while *B. phaia* density was higher during the *SR* growing season. The other species *S. calamistis S. piscator*, *E. saccharina*, found in Kakamega generally had low densities with no evidence of variation between the growing seasons ($P > 0.05$). Similar results were observed in Mtito Andei where no seasonal variation in densities was recorded among the two pest species, *S. calamistis* and *C. partellus*.

Table 4.2: Stem borer pest composition (%) and seasonal average densities (mean number per stem ± SE) in the different cultivated fields and localities

Stem borer pest species		% Composition (Mean ± SE)	Seasonal average density (Mean ± SE)			
			Long rains	Short rains	*t*	*P*
Muhaka						
Sesamia calamistis		18.1 ± 3.3[b]	0.116 ± 0.027[b]	0.045 ± 0.012[b]	2.28	0.025*
Chilo partellus		67.0 ± 5.8[a]	0.461 ± 0.063[a]	0.131 ± 0.023[a]	4.68	0.000*
Chilo orichalcociliellus		19.8 ± 2.8[b]	0.082 ± 0.020[b]	0.141 ± 0.033[a]	1.55	0.123
	F	43.76	34.61	5.41		
	Df	2, 234	2, 170	2, 149		
	P	< 0.0001	< 0.0001	0.005		
Mtito Andei						
Sesamia calamistis		25.6 ± 6.6[b]	0.073 ± 0.024[a]	0.046 ± 0.022[b]	0.80	0.431
Chilo partellus		74.4 ± 6.6[a]	0.177 ± 0.066[a]	0.306 ± 0.073[a]	1.19	0.243
	F	27.04	2.32	16.26		
	Df	1, 51	1, 21	1, 37		
	P	< 0.0001	0.143	0.0003		
Kakamega						
Busseola fusca		41.0 ± 5.2[a]	0.038 ± 0.008[a]	0.015 ± 0.004[b]	2.13	0.036*
Busseola phaia		26.9 ± 4.5[ab]	0.015 ± 0.004[b]	0.043 ± 0.010[a]	2.93	0.004*
Sesamia calamistis		26.4 ± 4.6[ab]	0.018 ± 0.006[b]	0.035 ± 0.010[a]	1.49	0.139
Sciomesa piscator		07.0 ± 5.4[b]	0.003 ± 0.002[b]	0.001 ± 0.001[b]	0.62	0.535
Eldana saccharina		11.0 ± 5.9[b]	0.001 ± 0.001[b]	0.002 ± 0.001[b]	0.49	0.624
	F	4.76	8.86	8.57		
	Df	4, 245	4, 279	4, 164		
	P	0.001	< 0.0001	< 0.0001		
Suam						
Busseola fusca		99.8 ± 0.3[a]	0.206 ± 0.023[a]			
Sesamia calamistis		00.5 ± 0.5[b]	0.004 ± 0.002[b]			
	F	2.21	92.34			
	Df	1, 43	1, 119			
	P	< 0.0001	< 0.0001			

Means (± SE) within columns followed by the same lowercase letters are not significantly different (Student-Newman-Keuls multiple range tests, $P \leq 0.05$)

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4.3.3 Seasonal variation in densities of stem borers among wild plants

Seven stem borer pest species, *B. fusca*, *S. calamistis*, *B. phaia*, *S. piscator*, *E. saccharina*, *C. partellus* and *C. orichalcociliellus*, identified from the cultivated fields were found among wild plants. These species, however, varied in distribution and densities among the localities (Table 4.3). Four of these pest species, *S. calamistis*, *B. phaia*, *S. piscator* and *E. saccharina* were identified in Kakamega, and *S. calamistis*, *C. partellus* and *C. orichalcociliellus*, in Muhaka. Only two pest species were found in both Mtito Andei (*S. calamistis* and *C. partellus*) and Suam (*B. fusca* and *S. calamistis*). *Busseola fusca* was only found among wild plants in Suam where it infested *S. arundinaceum*.

The importance of these species varied within localities with some species showing variations in average densities between *LR* and *SR* growing seasons. Despite the changes in density levels, none of the species had statistically significant difference between the growing seasons as revealed by *t* test ($P > 0.05$; Table 4.3). *Busseola phaia* ssp. *phaia* had the highest density level among the species in Kakamega during both *LR* and *SR* growing seasons followed by *E. saccharina* and *S. piscator*. The average density of *B. phaia* increased from 0.03 to 0.05 between *LR* and *SR* growing seasons contrary to both *E. saccharina* and *S. piscator* that showed a general reduction in density between *LR* and *SR* growing seasons. *Chilo orichalcociliellus* was the most important pest species followed by *C. partellus* in Muhaka. During the *LR* growing season, *C. orichalcociliellus*' density averaged 0.04 and increased to 0.06 larvae per plant during the *SR* growing season. In contrast, the density of *C. partellus* decreased slightly from 0.021 to 0.018 larvae per plant between *LR* and *SR* growing seasons.

Table 4.3: Seasonal pest densities (number per stem ± SE) in the wild habitats in different localities

Stem borer pest species	Seasonal average density (Mean ± SE)			
	Long rain	Short rain	*t*	*P*
Muhaka				
Sesamia calamistis	0.008 ± 0.005[b]	0.001 ± 0.001[b]	1.471	0.1443
Chilo partellus	0.021 ± 0.008[ab]	0.018 ± 0.007[b]	0.275	0.7834
Chilo orichalcociliellus	0.040 ± 0.010[a]	0.064 ± 0.011[a]	1.627	0.1058
F	3.82	17.67		
Df	2, 203	2, 183		
P	0.024	< 0.0001		
Mtito Andei				
Sesamia calamistis	0.003 ± 0.001[a]	0.034 ± 0.018[a]	1.158	0.2543
Chilo partellus	0.023 ± 0.013[a]	0.031 ± 0.015[a]	0.343	0.7341
F	3.17	0.01		
Df	1, 21	1, 47		
P	0.09	0.94		
Kakamega				
Busseola fusca	0.000 ± 0.000[b]	0.000 ± 0.000[b]	-	-
Busseola phaia	0.031 ± 0.006[a]	0.045 ± 0.010[a]	1.32	0.191
Sesamia calamistis	0.001 ± 0.001[b]	0.001 ± 0.001[b]	0	1.000
Sciomesa piscator	0.014 ± 0.004[ab]	0.005 ± 0.004[b]	1.158	0.251
Eldana saccharina	0.026 ± 0.029[a]	0.003 ± 0.004[b]	0.572	0.583
F	9.86	12.79		
Df	4, 196	4, 90		
P	< 0.0001	< 0.0001		
Suam				
Busseola fusca	0.014 ± 0.014[a]	-	-	-
Sesamia calamistis	0.006 ± 0.002[a]	-	-	-
F	0.94			
Df	1, 22			
P	0.344			

Means (± SE) within columns followed by the same letters are not significantly different (Student-Newman-Keuls multiple range tests, $P \leq 0.05$).

The pest densities were generally low in Mtito Andei and Suam, with *C. partellus* dominating the community in Mtito Andei, and *B. fusca* in Suam where there was only one growing season. The importance of *C. partellus* in Mtito Andei varied between the growing seasons with densities increasing from 0.02 during the *LR* to 0.03 in the *SR* growing season. Similar increase in density between the growing seasons was observed among *S. calamistis* larvae in Mtito Andei where its density increased from 0.003 to 0.034 larvae per plant.

4.3.4 Stem borer pest management practices

Stem borer pest management practices initiated by local farmers varied among localities depending on the economic importance associated with stem borer infestation. Management efforts were observed in Muhaka, Mtito Andei and Suam with no observed attempt in Kakamega. Conventional pesticide applications were observed in Muhaka and Suam. In Muhaka, few farmers applied Bulldock granules on maize leaf whorls (Plate 4.1a) while in Suam all farms designated for maize seed production were sprayed with Bulldock suspension (Plate 4.1b).

Plate 4.1a: Bulldock granules in a container during field application and granules as seen on the leaf whorl immediately after application in Muhaka.

Plate 4.1b: Tractor mounted sprayer applying Bulldock suspension on young maize plants in Suam

Bulldock in granule or suspension formulations are applied early in the season, two or three weeks after germination, an application meant to reduce populations of the first generation. However, non-conventional management attempts were observed in Mtito Andei, where the farmers applied fine ash or soil dust on the infested plants. These applications were meant to inhibit the larval movement and eventually suffocate them to death. Unlike the Bulldock application that is done once early in the season, ash or soil is applied any time infestation is observed, an approach that could effectively reduce pest populations in any generation. Despite these management attempts, stem borer pests still persist in the cultivated fields and generations found early in the season are thought to have come from crop residues.

4.3.5 Management of crop residues

Management of crop residues varied among the surveyed localities. In Muhaka, old stalks were cut and used to mulch the intercropped cassava plants (Plate 4.2a), or left in the fields after harvest until the beginning of the subsequent growing season (Plate 4.2 b). In other localities, Suam, Kakamega and Mtito Andei, old maize stalks are grazed on after harvest or cut and used to feed cattle. In Suam, the lower parts of the stack remained in the field until the beginning of the next growing season when they are burnt during land preparation (Plate 4.3a). Some portions of these stalks however remain unburnt and are later buried in the soil when the land is ploughed (Plate 4.3b). On different occasions, dissection of the unburnt old stalks in Suam revealed presence of diapausing stem borer larvae, which later were identified as *B. fusca*.

(a) **(b)**

Plate 4.2: Management of old maize stalks in Muhaka (a) Old stalks cut and used for mulching (b) Stalks left in the field after harvest for natural decomposition.

(a) **(b)**

Plate 4.3: Management of old maize stalks in Suam (a) Stalks cut and burnt in the old fields after harvest (b) Remaining stalks buried in the soil during land preparation.

4.4 Discussion

Stem borer pest populations were mainly composed of *B. fusca*, *S. calamistis*, *C. partellus* and *C. orichalcociliellus*. The distribution of these species, nonetheless, varied among the surveyed localities with *B. fusca* occurring in Kakamega and Suam, and *C. partellus* occurring in Muhaka. The results of this study corroborate previous findings in which Le Ru *et al*. (2006a) reported similar composition in high and low altitude agro-ecological zones corresponding to Suam and Muhaka respectively. The observed variation in distribution and composition of stem borer pests could be attributed to differences in species ecological requirements. *Chilo partellus* prefers warm/hot and humid areas, climate condition found mainly in low altitude areas, while *B. fusca* prefers cool and wet/dry areas, a condition found mainly in high altitude areas.

In this study, *C. partellus* is evidently important pest in Muhaka and Mtito Andei localities, corresponding to low and mid-altitude zones, where it constituted more than 67% of the stem borer community in the cultivated fields. These corroborate previous findings in which Le Ru *et al*. (2006a) found this species constituting about 65% of the stem borer community in low and mid-altitude zones in Kenya. In the wild habitats, *C. partellus* was found mainly on *S. arundinaceum* unlike its low altitude homologue, *C. orichalcociliellus* that was found in both *S. arundinaceum* and *P. maximum*. This finding indicates host use differentiation among wild hosts with *C. orichalcociliellus* as a dominating stem borer species. This however contradicts previous findings in which *C. partellus* was reported to have displaced *C. orichalcociliellus* from its original wild host plants at the Kenya Coast (Zhou *et al*., 2002).

In addition to the four pest species, three other species *Sciomesa piscator*, *Busseola phaia* and *Eldana saccharina* were found among cultivated crops in Kakamega. This however is not the first time *B. phaia* and *S. piscator* are reported from maize in Kakamega. Similar observation was made by Le Ru *et al*. (2006b) who attributed their presence to host range expansion or accidental oviposition. The continued presence of these species among cultivated crops suggests that they may become important pests in future unless factors responsible for their establishment are understood and addressed. Habitat fragmentation currently experienced in this area (Le Ru *et al*., 2006a) could be one of the factors responsible for their persistence in cultivated fields. Native polyphagous and oligophagous insects respond to changes in host availability by diversifying their diet breadth (Magurran, 1988). These species like other native Africa stem borers are known mainly from wild plants (Nye, 1960; Khan *et al*., 1997; Le Ru *et al*., 2006a) and changes in plant diversity and abundance, a consequence of habitat fragmentation, is likely to affect their host use pattern. This might explain the gradual host shift and subsequent establishment of *S. piscator* and *B. phaia* as important pests of cultivated crops in Kakamega.

The presence of "new" pests, *S. piscator* and *B. phaia*, in Kakamega increased the general pest community in the area with no resultant increase in infestation. Infestations were high in Muhaka and low in Kakamega, corresponding to values earlier reported by Le Ru *et al*. (2006b) in low and high altitude agro-ecological zones. These variations may be attributed to the effects of environmental factors on the biology and ecology of dominant stem borer species (Overholt *et al*., 2001). Knowledge on the distribution of pest species has been used in the designing of stem borer management plans for different

regions (Overholt *et al*., 1997). However, among the conventional management options, chemical application is used by few farmers on a local scale except in Suam where maize fields under ADC management are sprayed with Bulldock suspension. In addition to chemical application, cultural practices aimed at disrupting stem borer population build-up were observed in some fields. This included habitat management, early planting, removal or destruction of crop residues. These practices are however carried out on small scale depending on the farmers' perception on the economic importance of stem borer pests. Despite these practices, pests persist in the cultivated fields resulting in reduced cereal yields.

Stem borers are thought to feed on one or more closely related plant families in addition to cultivated host crops (Polaszek & Khan, 1998; Haile & Hofsvang, 2001) and their persistence in the cultivated fields is attributed to the influx of the wild populations early in the season (Ingram, 1958; Nye, 1960). On this background, the presence of alternative hosts and crop residues in or near a field can increase the survival of stem borers, thereby increasing the population that colonise maize and sorghum crops in a subsequent growing season. This long held argument (Khan *et al*., 1997) has limitations as it is based on the assumption that stem borer pest species are polyphagous, information contradicted by recent studies which suggest high specialisation among stem borer species (Le Ru *et al*., 2006a). This section however does not give clear evidence on the relationship between stem borer pest abundance and the neighbouring wild hosts upon which the management option could be proposed. This is because stem borers like other phytophagous insects may undergo local genetic adaptation to survive on the available suitable host plants (Shanower *et al*., 1993). The role of wild host plants in stem borer

pest exchange and carry-over between the seasons was thus addressed through genetic characterisation of the model pest species, *S. calamistis* and *B. phaia* ssp. *phaia*.

CHAPTER FIVE

5 HOST-PLANT DIVERSITY OF *SESAMIA CALAMISTIS* HAMPSON (LEPIDOPTERA: NOCTUIDAE): CYTOCHROME *B* GENE SEQUENCES REVEAL LOCAL GENETIC DIFFERENTIATION

5.1 Introduction

Sesamia calamistis Hampson (Lepidoptera: Noctuidae) is one of the indigenous stem borer pests associated with maize (*Zea mays* L.) and sorghum [*Sorghum bicolor* (L.) Moench] in Africa (Ingram, 1958; Bowden, 1976). However, its economic importance varies across the continent. It is a major pest in West Africa but remains a minor pest in Eastern and Southern Africa (Moyal, 1988; Bosque-Pérez & Schulthess, 1998). Differences in its pest status may be attributed to variations in the evolution of diet breadth and ecological preferences among different populations (Seshu Reddy, 1998; Kfir, 1997). *Sesamia calamistis* is reported to have remained among non-cultivated hosts belonging to Gramineae and Cyperaceae families (Harris, 1962) and presumably switched to cultivated crops after domestication of sorghum and introduction of maize in Africa (Polaszek & Khan, 1998). In an attempt to reduce losses associated with its infestation, efforts have been made to understand its ecology and the importance of wild host plants in its population build-up (Gounou & Schulthess, 2004; Le Ru *et al*., 2006a; Le Ru *et al*., 2006b). Like other phytophagous insects, adaptation to feed on sorghum and maize may have been accompanied by physiological and behavioural changes through a process of natural selection (Futuyma & Moreno, 1988). Since host choice and

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oviposition behaviour among phytophagous insects can be genetically mediated (Jaenike, 1990), natural selection may have favoured the choice of oviposition sites (hosts) that facilitated growth and survival of offspring (Gassmann *et al*., 2006).

Host selection may result in evolution of genotypes suited for different host plants (Futuyma & Moreno, 1988; Jaenike, 1990), which may in turn affect economic importance and spatial distribution of phytophagous pests. Currently, knowledge on *S. calamistis* variation in terms of economic importance in Africa is being used in an attempt to reduce losses associated with its infestation in maize and sorghum through exchange of biological control agents (Schulthess *et al*., 1997). In the Kenyan context, similar variations in economic importance are observed along varying altitudinal gradients among cultivated crops (Le Ru *et al*., 2006b). High densities are observed in low altitude areas and low densities in high altitude areas. However, the *S. calamistis* management initiatives in Africa generally ignore the possible existence of genetic variability among populations in different regions. In addition to this limitation, knowledge on factors that govern variation in its densities across different regions that would necessitate understanding of its ecology is lacking. In an attempt to understand factors that govern variation in *S. calamistis* populations across Africa, this study was initiated in Kenya to establish the possible existence of genetic variability among populations in sites with different insect densities. Cytochrome *b* (Cyt. *b*) was chosen as an appropriate mitochondrial marker since it had previously been used during phylogeographic study of the noctuid *Busseola fusca* (Fuller) in Africa (Sezonlin *et al*., 2006).

5.2 Materials and methods

5.2.1 Survey sites and processing of specimens

Stem borers were collected from four sites (Suam, Kakamega, Mtito Andei and Muhaka; Fig. 5.1) across different vegetation mosaics in Kenya (White, 1983). These sites represent regions characterised by both different growing seasons and stem borer pest composition (Le Ru *et al*., 2006a). To capture these variations, two surveys were undertaken in each of the sites during the cropping and the non-cropping seasons and collection was later pooled in respective localities (Table 5.1). Each study site measured 25 km^2 within which the number of cultivated fields was randomly chosen proportionate to the total area under cultivation. Plants growing in the cultivated fields (*Z. mays*, *S. bicolor* and *Eleusine corocana* L.) and wild habitats (wild host plants) in the neighbourhood were inspected for stem borer infestations. During each survey, plants with stem borer infestation symptoms were dissected in-situ for larval recovery. Survey in the wild habitats involved inspection of both known and unknown hosts during which all infested plants were identified and the recovered larvae were maintained separately with respect to different host plants and sites. *Sesamia calamistis* larvae are morphologically similar to some other *Sesamia* and *Sciomesa* species and their identity can only be confirmed by observations of the genitalia at the adult stage (see Plate 5.1). Based on this knowledge, all recovered larvae were reared to pupae on artificial diet according to the method described by Onyango & Ochieng'-Odero (1994). Pupae were kept in separate plastic vials until emergence of the moths. Upon emergence, the moths were identified and preserved in absolute ethanol (>99%) ready for DNA extraction.

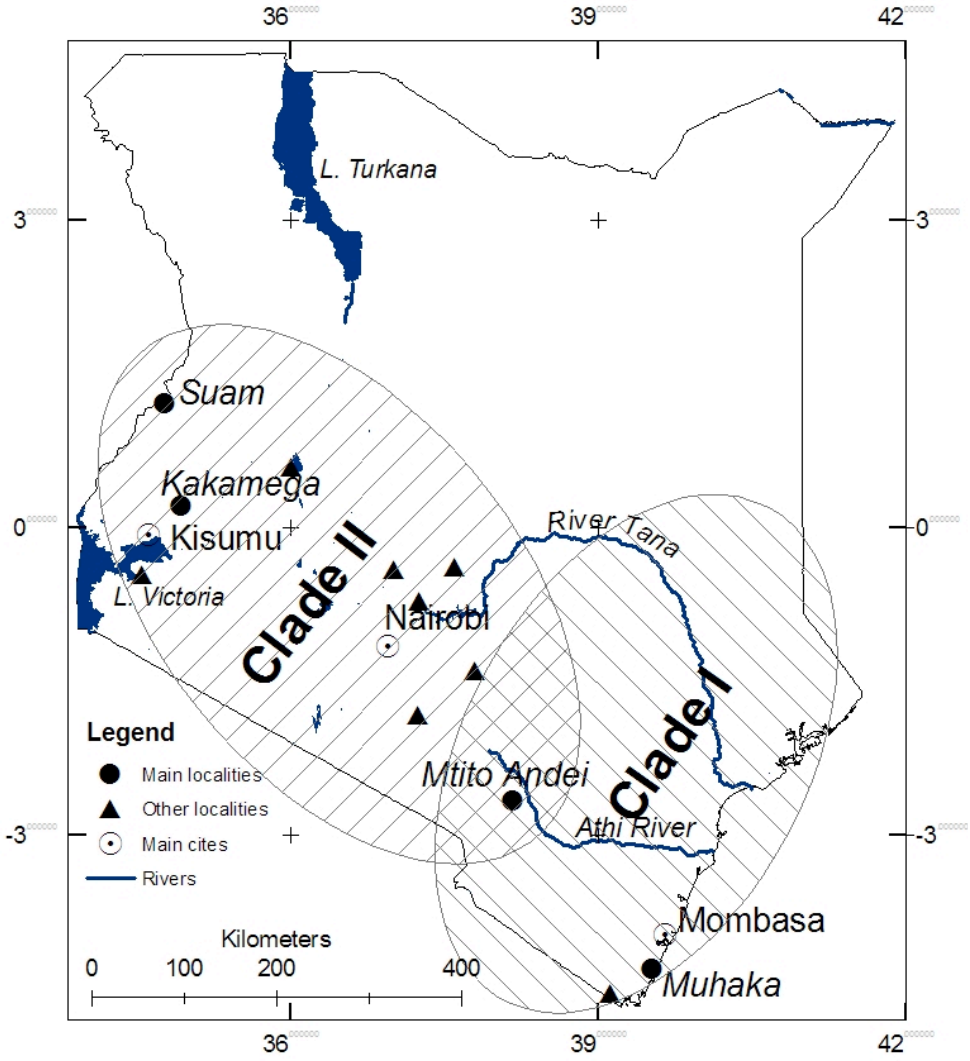

Figure 5.1: Map of Kenya showing the four surveyed sites and the estimated spatial distribution of *Sesamia calamistis*, Clades I and II. Estimates on spatial distribution of the two clades were based on findings of the current study and results of the ongoing phylogeography study in Africa.

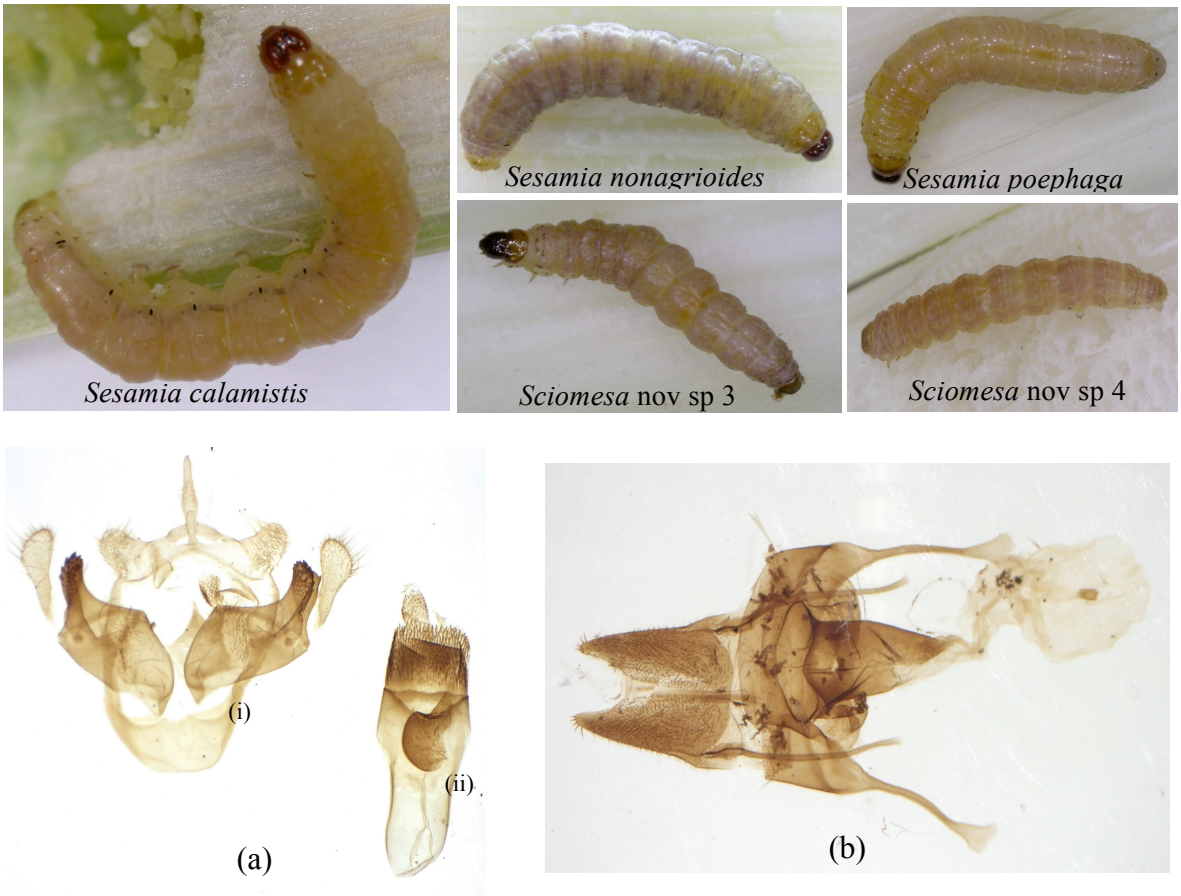

Plate 5.1: Pictorial comparison of different larvae belonging to *Sesamia* and *Sciomesa* genera and dismembered genetalia of *S. calamistis* used during identification; (a) Male genitalia (i) characteristic centrally placed flask-shaped juxta (ii) aedeagus (b) female genitalia.

5.2.2 DNA extraction and sequence analysis

The total genomic DNA was extracted from the thoracic muscles using commercial kit (DNeasy^{TM} Tissue Kit, Qiagen GmbH, Germany) protocol with Proteinase K digestion as recommended for animal tissues. The extracted DNA was stored at -20 °C until required for amplification. Voucher specimens are housed at the International Centre of Insect Physiology and Ecology (*icipe*) Biosystematic unit, Kenya.

Polymerase chain reaction (PCR) was used to amplify the 873 bp Cyt. *b* mitochondrial fragment using the primers CP1 (5′-GATGATGAAATTTTGGATC-3′) (modified from Harry *et al*., 1998) and Tser (5′-TATTTCTTTATTATGTTTTCAAAAC-3′) (Simon *et al*., 1994). The PCR was performed on a Biometra GeneAmp PCR System in a 25 µl reaction mixture containing 1 µl of the genomic DNA, 5X Green GoTaq® Flexi Buffer, 0.24 mM dNTPs, 3 mM $MgCl_2$, 0.4 µM of each primer and 1 unit of Taq polymerase (GoTaq, Promega). After initial denaturation at 94°C for 5 min, PCR condition was 40 cycles of 94°C for 1 min of denaturation, 46°C for 1 min 30 s of annealing, 72°C for 1 min 30 s of extension and a final extension period of 10 min at 72°C. The PCR products were visualised by means of electrophoresis in 1% agarose gel previously stained with ethidium bromide to verify amplification. Amplified products were purified with the Promega Wizard SV Gel and PCR Clean up System following the manufacturer's protocol. DNA sequencing reactions were performed using the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems), cleaned using ethanol/EDTA precipitation. Sequences were visualized on an ABI 3130 automated sequencer using Big-Dye fluorescent terminators. The consensus sequences obtained were aligned manually using Mac Clade 4.05 (Maddison and Maddison, 2001). Additional sequences of individuals from maize were obtained from P. Moyal of Laboratoire Evolution, Génomes et spéciation - France, to enable comparison with collection made in other localities in Kenya and some African countries (South Africa, Uganda, Benin, Ghana, Nigeria and Togo) (P. Moyal, Unpublished data). All the sequences were deposited in the Genebank (Accession numbers EU305065-EU305228)

5.2.3 Evaluation of reproductive parameters

Fourth and fifth larval instars of *S. calamistis* were collected from sorghum plants in Kisumu (western part of Kenya) and from plant species belong to Cyperaceae family in Shimba Hills (eastern part of Kenya, Coastal region). Both populations were reared separately on artificial diet as described by Onyango and Ochieng'-Odero (1994) until pupae formation. Some individuals from each reared population were randomly sequenced for Cyt. *b* to establish their genetic status in relation with the genetic population analysis. Male and female pupae were kept in separate plastic boxes (30 cm long, 12 cm wide, 10 cm high) containing a moist cotton pad to maintain a relative humidity (r.h) at about 80 %, and monitored for adult emergence. Pupae and adults were maintained in a controlled chamber at 25.3 ± 0.9 °C, 68.6 ± 12.8% r.h (means ± SE) under reversed L12:D12 photoperiod with scotophase lasting from 7.00 to 19.00 h, hereafter referred to as night. The reversed photoperiod allowed all experiments to be carried out during the day.

In each population, one-day old males and females (minimum 10 individuals of each sex per night) were released in a mosquito-net cage (40 x 40 x 63 cm) at the onset of scotophase. They were provided with a diluted honey solution impregnated in a piece of cotton. Mating behaviour was observed and recorded after every 30 min during the dark period. Mated pairs were transferred from the cage to a transparent plastic jar (16 cm high, 9 cm diameter), to facilitate measurement of copulation duration. The plastic jar contained a wet piece of cotton wool that maintained relative humidity at around 80%. One cylindrical surrogate stem made from a rectangular piece of nylon cloth (15 long, 5cm wide) rolled helicoidally from top to bottom was placed in each jar. This support had

earlier been found to elicit good ovipositional response in *S. calamistis* (P-A. Calatayud, personal observation). The total number of eggs laid by each female was counted each night, renewing the surrogate stem every night throughout the female life. The life duration of each female was finally recorded and similarly, the time of emergence of the first neonate after egg laying for each female was recorded.

5.2.4 Statistical analysis

Basic sequence statistics were calculated using DnaSP (Rozas *et al*., 2003). The following parameters were used to estimate genetic variability among populations between sites (Muhaka, Mtito Andei, Kakamega and Suam) and between host plants in Mtito Andei (wild and cultivated hosts): number of haplotypes (*h*), number of polymorphic sites (*S*), haplotype diversity (*d*) (Nei, 1987), nucleotide diversity (*Pi*) (Lynch and Crease, 1990) using the Jukes and Cantor correction (Jukes and Cantor, 1969), mean number of nucleotide differences (K) (Tajima, 1983). The extent of genetic differentiation between the populations (F_{ST}) (Hudson *et al*., 1992) was performed with the Arlequin 2.000 software (Schneider *et al*., 2000). A maximum parsimony network was drawn using TCS 1.21 software (Clement *et al*., 2000). For reproductive parameters, means were computed and separated by Mann-Whitney U-test (rank analysis for a two-sample test).

5.3 Results

5.3.1 Diet breadth of *Sesamia calamistis*

Sesamia calamistis larvae were found on twelve different plant species (Table 5.1). For the purpose of this study, all plant species from which *S. calamistis* larvae were recovered have been considered as host plants without quantifying their relative contribution to *S. calamistis* population dynamics. The host list (see Table 1) contains both known and unknown hosts of *S. calamistis* as given by Khan *et al*. (1997), Gounou & Schulthess (2004) and Le Ru *et al*. (2006b) with their importance varying among surveyed geographic sites. This species had a limited number of host plants in Suam and Kakamega. In addition to the cultivated cereals (maize, sorghum and finger millet), its larvae were found on *Cyperus dives* Delile and *Sorghum arundinaceum* (Desvaux) Stapf in Suam and from *Pennisetum purpureum* Schumacher in Kakamega. Together with the cultivated cereals, the larvae were recovered from seven more host plants in Mtito Andei (*Cyperus distans* L., *Eleusine jaegeri* Pilg., *Panicum deustum* Thunb, *Panicum maximum* Jacquin, *P. purpureum*, *Setaria verticillata* (L.) P. Beauv. and *S. arundinaceum*) and three in Muhaka (*Echinochloa haploclada* (Stapf) Stapf, *P. maximum* and *S. arundinaceum*).

Table 5.1: List of plant species from which *Sesamia calamistis* larvae were collected in different

Infested plants species	No. of moths processed			
	Muhaka	M. Andei	Kakamega	Suam
Cyperus distans L.	–	2	–	–
Cyperus dives Delile	–	–	–	2
Echinochloa haploclada (Stapf) Stapf,	1	–	–	–
Eleusine corocana L.	1	6	2	4
Eleusine jaegeri Pilg.*	–	2	–	–
Panicum deustum Th unb	–	1	–	–
Panicum maximum Jacquin	2	1	–	–
Pennisetum purpureum Schumacher	–	3	1	–
Setaria verticillata (L.) P. Beauv.*	–	1	–	–
Sorghum arundinaceum (Desvaux) Stapf	3	11	–	–
Sorghum bicolor (L.) Moench	14	4	2	–
Zea mays L.	24	47	31	1

Marked with stars (*) are “new plants” (previously not recorded as host plants)

5.3.2 Differentiation of *S. calamistis* populations in Kenya

Qualitative TCS maximum parsimony network of 194 sequences revealed 68 haplotypes. These haplotypes separated into two clades with an average divergence of 1.89 ± 0.24 % (Fig. 5.2). Clade 1 containing 33 haplotypes showed an average divergence of 0.36 ± 0.19 % while Clade II containing 35 haplotypes showed an average divergence of 0.44 ± 0.20 %. Except for three specimens (EU305074, EU305088 and EU305112), all other individuals from Muhaka and a part of collection from Mtito Andei grouped in Clade I together with individuals from South Africa, while individuals from Suam, Kakamega and the remaining part of the collection from Mtito Andei grouped together in Clade II with individuals from Uganda, Benin, Togo, Ghana and Nigeria.

There was evidence of variation in the spatial distribution of the two clades in Kenya. Clade I was mainly found in Muhaka and Mtito Andei while Clade II was found in Mtito Andei, Kakamega and Suam. However, partial distribution of individuals from Mtito Andei into Clade I and II suggested greater genetic variability in that area. This was further reflected in genetic diversity parameters (*S*, *h*, *d*, *Pi* and K) that revealed higher variability in Mtito Andei relative to Muhaka, Kakamega and Suam (Table 5.2a).

Table 5.2a: Genetic diversity of the Cytochrome b gene in *Sesamia calamistis* populations from four localities in Kenya (Diversity values ± SD)

Genetic diversity parameters	Sampled sites			
	Muhaka	M. Andei	Kakamega	Suam
Number of sequences, *n*	43	78	35	8
Number of segregating sites, *S*	30	35	23	11
Number of haplotypes, *h*	15	24	12	5
Haplotype diversity, *d*	0.792 ± 0.060	0.834 ± 0.034	0.677 ± 0.082	0.786 ± 0.151
Nucleotide diversity, *Pi*	0.004 ± 0.001	0.009 ± 0.001	0.003 ± 0.001	0.004 ± 0.001
Mean number of nucleotide differences, K	3.508 ± 1.822	7.740 ± 3.644	2.927 ± 1.563	3.214 ± 1.852

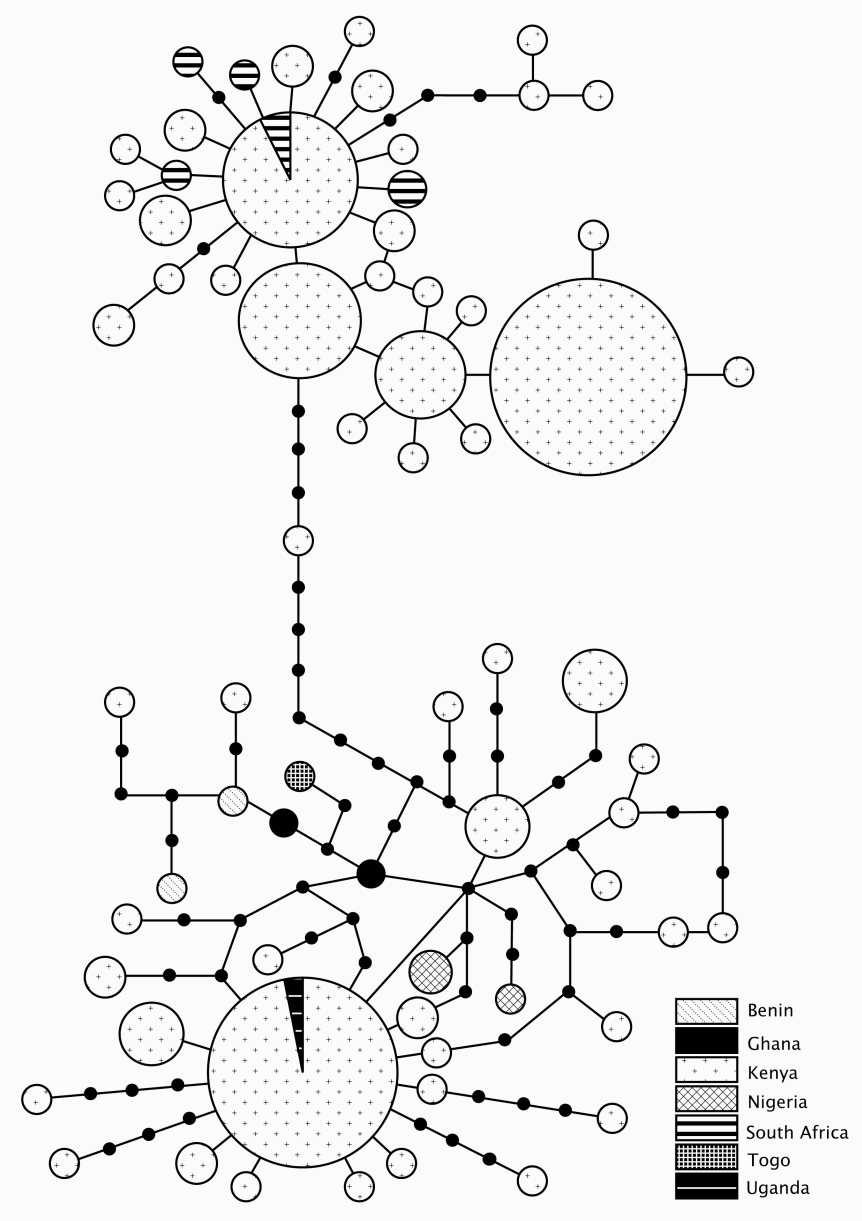

Figure 5.2: TCS mitochondrial haplotype network of 195 *Sesamia calamistis* specimens processed from seven African countries. The area of the circle is proportional to the number of samples sharing each haplotype. Lines represent single nucleotide mutations and shaded circles represent haplotypes, which are not observed in the sample while different shading patterns represent different countries.

5.3.3 Genetic differentiation in host utilization (Mtito Andei)

Sesamia calamistis was found on a wide range of cultivated and wild host plants in Mtito Andei. Values obtained from genetic diversity parameters (*d*, *Pi* and K; Table 5.2b) indicate that individuals from the wild plants are more genetically variable compared to individuals from the cultivated fields. However, there were more haplotypes among the cultivated hosts (*h*, 16) compared to the wild host plants (*h*, 13). Excerpt of haplotypes in Mtito Andei from the global network (Fig. 5.2) indicated that individuals in respective clades varied in terms of host plant preference suggesting differentiation with respect to host plants (F_{ST} = 0.4008; P < 0.001). The first group with 14 haplotypes corresponded to Clade I, while the second group with 10 haplotypes corresponded to Clade II (Fig. 5.3). The majority of individuals in Clade I (87.5%) came from the cultivated host plants (*Z. mays*, 78.6%; *E. corocana*, 5.4%; *S. bicolor*, 3.5%), while Clade II was dominated by individuals mainly from wild host plants (63.6%). However, haplotypes of individuals from *S. arundinaceum* appeared in both Clade I and II accounting for 7 and 32% respectively.

Table 5.2b: Genetic diversity of the Cytochrome b gene in *Sesamia calamistis* populations from wild and cultivated hosts in Mtito Andei (Diversity values ± SD).

Genetic diversity parameters	Mtito Andei	
	Cultivated host	Wild hosts
Number of sequences, *n*	57	21
Number of segregating sites, *S*	30	25
Number of haplotypes, *h*	16	13
Haplotype diversity, *d*	0.723 ± 0.076	0.891 ± 0.049
Nucleotide diversity *Pi*	0.006 ± 0.001	0.010 ± 0.001
Mean number of nucleotide differences, K	5.312 ± 2.603	8.381 ± 4.073

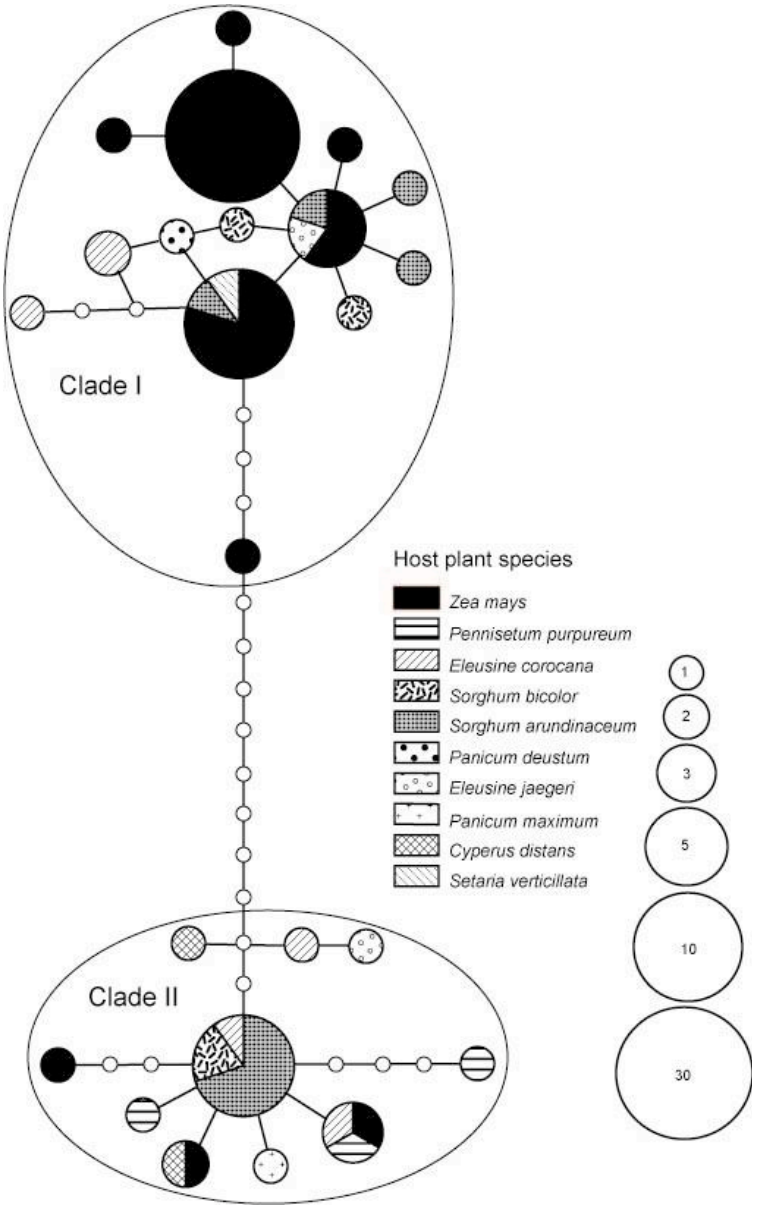

Figure 5.3: TCS mitochondrial haplotype network of *Sesamia calamistis* individuals collected from different host plants in Mtito Andei. The area of each circle is proportional to the number of samples in each haplotype. Lines represent single nucleotide mutations and small white circles represent haplotypes that are not observed in the sample. Different shading patterns represent the different sampled host plants.

5.3.4 Reproductive and life trait parameters

Clade I and II populations appeared to vary in their mating times (hour) after the onset of scotophase (Table 5.3; Mann-Whitney's U-test, $P < 0.0001$). The mean mating times of Clade I and II populations were 5:30 and 7:12 respectively. In addition to variations in mating time, there were also differences in the duration of mating (Mann-Whitney's U-test, $P = 0.04$) with Clade I taking relatively longer time (129.6 min) compared to Clade II (99.6 min). Despite variations in both mating time and duration, all females oviposited only during the scotophase and no eggs were deposited during the photophase. In addition, there were no differences among the two populations in terms of the total number of eggs laid by each female (Mann- Whitney's U-test $P = 0.79$) and the time of first eclosion (Mann-Whitney's U-test $P = 0.64$). Each female laid about 680 eggs during their life and the laid eggs took about 8 days before eclosion. On average however, the females varied in their life durations (Mann-Whitney's U-test $P = 0.009$), with Clade II living slightly longer (7.7 days) compared to Clade I (6.9 days).

Table 5.3: Reproductive parameters of the Clade I and II populations of *Sesamia calamistis* as recorded under laboratory conditions

Biological parameters		Hour of mating (h after the onset of night)	Mating duration (min)	Total number of eggs laid per female	Female life duration (days)	Time of eclosion after egg laying (days)
Populations	Clade I	5.4 ± 0.2 (37) b	129.6 ± 8.5 (16) a	685.0 ± 47.8 (21)	6.9 ± 0.3 (23) b	8.1 ± 0.2 (23)
	Clade II	7.1 ± 0.1 (32) a	99.6 ± 13.8 (12) b	679.5 ± 39.2 (17)	7.7 ± 0.2 (19) a	8.1 ± 0.1 (19)
Statistics	U (values)	150.0	51.5	169.5	87.5	182.0
	P (values)	<0.0001	0.0376	0.7916	0.0085	0.6382

Statistics: Mann Whitney U test (rank analysis for two-sample test). Means ± SE (number of replicates)

5.4 Discussion

This study shows that populations of *S. calamistis* in Kenya are divided into two clades (Clade I and II); Clade I dominant in South East, Clade II in the South West and that the two clades co-exist in Central Kenya. The genetic distance between both clades (about 1.8%) suggests an ancient fragmentation. According to an arthropod $2.3x10^{-8}$ mitochondrial substitution/site/year rate (Brower, 1994), fragmentation may have occurred about one million years ago. Individuals collected from other localities in Kenya confirmed the observed geographic differentiation. This was further supported by the results of collection made in other African countries (Uganda, Ghana, Benin, Togo, Nigeria and South Africa) which showed a similar pattern with Clade I found only in South Africa while clade II was found only in countries on the west of Kenya (Uganda, Nigeria, Benin, Togo and Ghana). From a more general African point of view, these clades can be classified as East clade (Clade I) and West clade (Clade II), both of which meet in central Kenya.

Currently, *S. calamistis* appears to favor maize and sorghum as preferred hosts though it appears to have retained the close association with the original host plants. Irrespective of the dominant clade, *S. calamistis* larvae were found in both cultivated and wild host plants in all localities (Muhaka, Mtito Andei, Kakamega and Suam). Preference for maize and cultivated sorghum as suitable hosts is reflected in the relatively higher densities observed in the cultivated fields, results that confirm earlier observations (Le Ru *et al*., 2006b). Shanower *et al*. (1993) noted the same phenomenon under experimental conditions and attributed low numbers of larvae among wild host plants to the poor nutritive value of the latter. Evolution of host and/or oviposition choice by *S. calamistis*

like other phytophagous insects may have included allelochemicals, quantity and/or quality of plant resources (Jaenike, 1990). Interactions between these factors may favour oviposition dispersion and egg laying on sub-optimal host plants. This may ultimately lead to variation in performance and survival of progeny for eggs deposited on different hosts as observed by Gassmann *et al*. (2006) during their study on adaptation of *Ophraella notulata* (Fabricius) to feed on *Ambrosia artemisiifolia* (Heliantheae: Ambrosiinae).

Unlike in other localities that were dominated by individuals from either Clade I or II (Muhaka – Clade I; Kakamega and Suam – Clade II), there was evidence of genetic differentiation with respect to host plant use in Mtito Andei where both clades co-existed. These clades differentiated in Mtito Andei with almost all individuals belonging to Clade I found on maize, while larger proportion of individuals in Clade II were found on wild plants. However, some individuals belonging to Clade I were found on wild plants and this explained the high genetic variability observed in this clade. Differentiation of these populations with respect to host plants is not specific to *S. calamistis* alone since similar results have been recorded on another noctuid species, the fall armyworm *Spodoptera frugiperda* (J. E. Smith). Two populations of *S. frugiperda* inhabited the same geographical area and showed such patterns of differentiation including host races (Prowell *et al*., 2004). The observed separation among *S. calamistis* population in host use in Mtito Andei could be attributed to either low attraction of maize to Clade II or competitive advantage of Clade I on that host plant.

Clade II could be considered as a recent population gradually invading cultivated fields after retaining wild graminaceous plants as preferred hosts in the expansive

Kakamega (Kenya) and Mabira (Uganda) forests for a long time. Even though there is no evidence to support this hypothesis, retaining wild plants as their preferred hosts may have been facilitated by low agricultural activities around Mabira forest (Uganda) and Kakamega forest (Kenya). These forests and their environs are colonised by a wide range of natural enemies associated with diverse noctuid stem borers that inhabit the forests (B. P. Le Ru, unpublished data) and this may have limited rapid population build-up and subsequent expansion in this area. In addition to acting as a reservoir of natural enemies, other noctuid stem borers such as *Busseola fusca* (Fuller) out competes *S. calamistis* in cultivated fields since they oviposit early in the season while *S. calamistis* moths which arrive later in the season show little preference for infested host plants (Seshu Reddy, 1983). This may explain the observed low *S. calamistis* densities in Kakamega (Clade II) compared to the observation made in Mtito Andei (Clade I). Similar competition may have excluded Clade II from the maize fields in Mtito Andei where both clades exist. Though Shanower *et al*. (1993) did not test the performance of the two *S. calamistis* clades on different host plants, they observed variations in the development time among different hosts. Because of the good nutritive value of maize plants, stem borers reared on the latter complete their development faster compared to the individuals reared on wild host plants. In Mtito Andei where the two clades exist, Clade I which is mainly found on maize plants probably completes its development well before Clade II and re-infests the available hosts (both maize and wild plants) before the emergence of moths belonging to Clade II. This therefore excludes Clade II from the cultivated fields and limits its population to few available wild host plants. Coupled with the observed biological differences, this structuration may strongly reduce interbreeding between the two clades.

Applied entomologists across Africa are presently concerned with *S. calamistis* because of its pest status (Bosque-Pérez &Schulthess, 1998; Gounou &Schulthess, 2004; Le Ru *et al*., 2006a). Current revelation of differentiation of two clades with respect to different geographic regions brings in fresh knowledge that may radically influence management initiatives. The level of differences among these clades suggests that the evolutionary mechanism that separated them may have taken place one million years ago explaining variations in pest status observed across Africa (Seshu Reddy, 1983; Bosque-Pérez &Schulthess, 1998). For sustainable management of *S. calamistis*, there is need to adopt a region specific management approach based on the knowledge of the dominant clade. However, questions with practical implications may still be asked about evolutionary shift and subsequent host preference of *S. calamistis* clades before designing a sustainable management strategy. For example, has Clade I, which constitutes an important stem borer proportion in low altitude areas in Kenya, adapted fully to the new host plants (maize and sorghum)? Is the observed preference (rather than an expanded host range) evolutionarily favoured because of trade-offs in fitness on different plants, with adaptations to the new host reducing fitness on the original host (Futuyma & Moreno, 1988)? Further laboratory experiments as well as intensive field studies need to be done at other sites and in different geographical locations. This is the only way to unravel the relationships between host-plant colonization, particularly the attractivity of both clades to the different host plants, and spatial distribution.

CHAPTER SIX

6 GENETIC DIVERSITY AND POPULATION STRUCTURE OF *BUSSEOLA PHAIA* SSP. *PHAIA* BOWDEN (LEPIDOPTERA; NOCTUIDAE) IN WILD AND CULTIVATED HABITATS

6.1 Introduction

Natural habitats in Africa have been subjected to diverse forms of modification over the past half century resulting in novel spatial patterns of organisms and resources (Bosque-Pérez & Schulthess, 1998; Gepts, 2004). This, coupled with increased human pressure, has severed connections between once-continuous expanses of native habitats resulting in native habitat fragments interspersed with areas of degraded agricultural landscapes (Forman, 1995; Fahrig, 2003). Habitat loss and fragmentations are currently widespread and are likely the most serious threats to the earth's biological diversity (Laurance & Bierregaard, 1997; Summerville & Crist, 2001). In response to natural habitat loss and fragmentation, and the subsequent increase in contact areas between wild and cultivated habitats, some indigenous phytophagous insects that initially remained among indigenous plants in the natural habitats expanded their host ranges and are currently taking advantage of these human induced resource changes (Futuyma & Moreno, 1988; Thies *et al*., 2003; Gassmann *et al*., 2006). But depending on the degree of landscape heterogeneity and stability, organisms can either specialize on a particular host plant or successively or even simultaneously exploit a wide range of host plant species (Jonsen & Fahrig, 1997; Kennedy & Storer, 2000; Tischendorf *et al*., 2003). However, the extent of ecological specialization remains poorly known in many species,

and the reciprocal influences of populations from different crops and/or from wild and cultivated areas through the exchanges of individuals or genes is little studied.

Lepidopteran stem borers are among the indigenous phytophagous insects that expanded their host ranges, some of which later specialised and are currently dependent on cultivated plants where they remain important insect pests (Bosque-Pérez & Schulthess, 1998; Polaszek & Khan, 1998). Like other phytophagous insects, stem borer pests regularly experience sudden destruction of their habitats that force them to migrate to other favorable hosts or they get locally extinct (Ingram, 1958; Nye, 1960; Khan *et al*., 1997; Schulthess *et al*., 1997; Haile & Hofsvang, 2001; Mazodze & Conlong, 2003). For example, Harris (1962) argued that the distribution of *Busseola fusca* (Fuller) was closely linked with human population density and the intensity of cultivations of cereal crops. Similarly, in South Africa the pyralid *Eldana saccharina* (Walker) switched from *Cyperus papyrus* to sugar cane, where it became the economically most important pest (Carnegie, 1974). Due to the temporary character of many crops (e.g., maize and sorghum), migration frequently implies a temporal shift between different host plant species. As a consequence, stem borer pests evolved complex life cycles that could involve the exploitation of different plant species more or less phylogeneticaly related in cultivated or non-cultivated habitats (Gounou & Schulthess, 2004; Le Ru *et al.*, 2006a and b). Although alternative host plants may constitute a temporary or a permanent source of pest migrants as well as their natural enemies (Thies *et al*., 2003), the source-sink role of cultivated and non-cultivated habitats in the life cycle of crop pests has received little attention (Manel *et al*., 2003; Vialatte *et al*., 2005). This is largely due to

the difficulty of tracking movements of small organisms in agricultural landscapes (Lushai & Loxdale, 2004).

Cultivated fields where maize [*Zea mays* L.] and sorghum [*Sorghum bicolor* (L.) Moench] are currently grown were initially natural habitats (Schulthess *et al*., 1997) and remain surrounded by non-cultivated fragments. Attempts to reduce losses associated with stem borer pest infestation have been one of the strategies to increase maize and sorghum production in Africa (Harris, 1962). These efforts have been concentrated on reducing stem borer pest populations in the cultivated habitats (Overholt *et al*., 1994; Schulthess *et al*., 1997) with few studies focusing on the diversity of non-pest species, diet breadth and potential role of native hosts on pest population build-up (Gounou & Schulthess, 2004; Ndemah *et al*., 2000; Le Ru *et al*., 2006a). The recent recovery of *Busseola phaia* ssp. *phaia* Bowden on maize (Le Ru B., unpublished data), formerly known to exist among the non-cultivated plants, supports earlier reports that the list of borers and host plant species in eastern Africa is far from being exhaustive (Polaszek & Khan, 1998). The presence of *B. phaia* ssp. *phaia* on several hosts in both Kisii and Kakamega in Kenya (Le Rü *et al*., 2006b), brings into question its potential to shift and become an important pest of cultivated cereals since the non-cultivated fragments that accommodate its alternative host plants may not persist for long due to human population pressure. The analysis of relationships between populations living in wild and cultivated habitats is thus important for pest management at the landscape scale, through enhancing naturally occurring control or resistance management in transgenic crops using wild host plants (Gould *et al*., 2002). This study was initiated to examine the genetic relationships of *B. phaia* ssp. *phaia* populations living in the wild and cultivated fragments within the

landscape, in order to assess the extent of historical adaptation and the role of non-cultivated fragments in population build-up between growing seasons.

Different genetic markers have been used to analyse relationships among populations of different insect groups (Gaete-Eastman *et al*., 2004; Angelone *et al*., 2007). Among these are mitochondrial markers particularly cytochrome *b* (Cyt. *b*) gene which has been used mainly in phylogeographic studies as well as for estimation of long-standing historical host use differentiation (Lushai & Loxdale, 2004; Sezonlin *et al*., 2006). Cytochrome *b* gene was used in this study to address the aforementioned objectives.

6.2 Materials and Methods

6.2.1 Description of the study area

The Kakamega Forest is located in western Kenya about 40 km North West of Lake Victoria. It is the only remnant of Guineo-Congolian rainforest in Kenya and was thus gazetted in 1933 by the Forest Department to enhance its protection (Cords & Tsingalia, 1982; Kokwaro, 1988; Tsingalia, 1988). However, the high human population density in the area, 175 individuals/km^2, has led to considerable long-term human influence on the forest and its environs (Tsingalia, 1988). Surveyed agricultural landscape that covered 21.2 km^2 along the forest edge was originally part of the main forest block but was opened for cultivation of maize and sorghum due to human population pressure (Kokwaro, 1988). The area is characterized by a bimodal rainfall distribution that allows for two cropping seasons. The first season lasts from March to mid-July (long rain growing season, *LR*) and the second from mid-August to November

(short rain growing season, *SR*). There is a prolonged dry spell from the beginning of December to the end of February, hereafter referred to as non-cropping season.

6.2.2 Sampling, rearing and identification of stem borers

Surveys were carried out in both wild and cultivated habitats in 2005-2007 growing seasons (*SR*, 2005 & 2006; *LR*, 2006 & 2007). A total of 32 maize fields were surveyed for stem borer infestation during each sampling session. In each field, 100 randomly selected maize plants were inspected for stem borer infestation symptoms and only infested stems were dissected for larval recovery. In the wild habitats, potential hosts growing around crop fields, along the riverbanks and in swamps were checked for stem borer infestation. Since stem borer densities in wild hosts are exceedingly low (Nye, 1960; Gounou & Schulthess, 2004), a biased rather than a random sampling procedure was used to increase the chances of finding borers. In all habitats, plant species belonging to Poaceae, Cyperaceae and Typhaceae families were carefully inspected for stem borer infestation symptoms or damage (scarified leaves, dry leaves and shoots, frass, dead hearts, holes bored). Infested plants were cut and dissected in the field and any larvae recovered were reared until pupation on artificial diet according to the method described by Onyango & Ochieng-Odero (1994). Pupae were taken out of the diet and kept separately in plastic vials until adult emergence. Identified *B*. *phaia* ssp. *phaia* moths were preserved in absolute ethanol for DNA extraction. Voucher specimens were deposited in the Muséum National d'Histoire Naturelle (MNHN, Paris, France) and in ICIPE Biosystematics Unit – BSU (Nairobi, Kenya).

6.2.3 DNA extraction and sequence analysis

At least two individuals were randomly selected where possible to represent each host plant sampled at a given time, location/field and season. This was meant to avoid processing individuals belonging to the same parents as *B. phaia* ssp. *phaia* is gregarious in its early instars.

A total genomic DNA was extracted from the thoracic muscles using commercial kit (DNeasy™ Tissue Kit, Qiagen GmbH, Germany) protocol with Proteinase K digestion as recommended for animal tissues. The extracted DNA was stored at -20 °C until required for amplification. Polymerase chain reaction (PCR) was used to amplify a 709 bp Cyt. *b* fragment using the primers CP1 (5′-GATGATGAAATTTTGGATC-3′) (modified from Harry *et al*., 1998) and Tser (5′-TATTTCTTTATTATGTTTTCAAAAC-3′) (Simon *et al*., 1994). The PCR was performed on a Biometra GeneAmp PCR System in a 25 µl reaction mixture containing 1 µl of the genomic DNA, 1X Green GoTaq® Flexi Buffer, 0.24 mM dNTPs, 3 mM $MgCl_2$, 0.4 µM of each primer and 1 unit of Taq polymerase (GoTaq®, Promega). After initial denaturation at 94°C for 5 min, PCR condition was 40 cycles of 94°C for 1 min of denaturation, 46°C for 1 min 30 s of annealing, 72°C for 1 min 30 s of extension and a final extension period of 10 min at 72°C. The PCR products were visualised by means of electrophoresis in 1% agarose gel previously stained with ethidium bromide before UV exposure to verify amplification. Amplified products were purified with the Promega Wizard SV Gel and PCR Clean up System following the manufacturer's protocol. DNA sequencing reactions were performed using the ABI PRISM® BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems), cleaned using ethanol/EDTA precipitation.

Sequences were visualized on an ABI 3130 automated sequencer using Big-Dye fluorescent terminators. The consensus sequences were obtained after aligning respective forward and reverse sequences manually using Mac Clade 4.05 (Maddison & Maddison, 2001). All consensus sequences were deposited in the Genebank (Accession numbers EU526412-EU526556).

6.2.4 Data management and statistical analysis

Sequences of individuals from different host plant species were grouped with respect to habitats (wild and cultivated) and seasons (*LR* and *SR*) for estimation of genetic diversities and the rates of population exchange. Basic sequence statistics were calculated using DnaSP (Rozas *et al*., 2003) and haplotype parsimony networks drawn using TCS 1.21 software (Clement *et al*., 2000). The following parameters were used to estimate genetic variability among populations in wild and cultivated habitats, and among *LR* and *SR* growing seasons: number of haplotypes (*h*), number of polymorphic sites (*S*), haplotype diversity (*Hd*) (Nei, 1987), nucleotide diversity (*Pi*) (Lynch & Crease, 1990) using the Jukes and Cantor correction (Jukes & Cantor, 1969), mean number of nucleotide differences (*K*) (Tajima, 1983). The extent of genetic differentiation between the populations (F_{ST}) (Hudson *et al*., 1992) was performed with the Arlequin ver. 2.0 Software (Schneider *et al*., 2000). Analysis of molecular variance (AMOVA) was used in Arlequin to indirectly assess the exchange and carryover of stem borer populations between habitats by comparing differences in haplotype composition in different habitats according to the growing seasons.

6.3 Results

6.3.1 Distribution and utilization of potential host plants

Busseola phaia ssp. *phaia* larvae were found on nine different plant species, belonging to the Panicoideae grass sub-family which were *Zea mays*, *Cymbopogon nardus*, *Euchlaena mexicana*, *Panicum maximum*, *Pennisetum macrourum*, *Pennisetum purpureum*, *Pennisetum unisetum*, *Saccharum officinarum* and *Sorghum bicolor* (Table 6.1). Apart from maize, sorghum and *P. purpureum*, which occupied large areas in the cultivated habitats, other host plant species were localised in the less disturbed patches and along the riverines with infestations occurring mainly in patches along the streams and edges of the cultivated fields.

Table 6.1: Infested plant species in the surveyed agricultural landscape in Kakamega during long and short rain growing seasons. Asterisks (*) indicate the cultivated host plants.

Host plant species	Total number of larvae recovered	
	Long rains season	*Short rains season*
Cymbopogon nardus (L.) Rendle	1	-
Euclaena mexicana Schrader	2	-
Panicum maximum Jacquin	28	29
Pennisetum macrourum Trinius	9	1
Pennisetum purpureum Schumach*	18	25
Pennisetum unisetum (Nees) Benth.	-	12
Saccharum officinarum L.*	2	2
Sorghum bicolor Delile*	18	-
Zea mays L*	65	115

6.3.2 Genetic diversity and differentiation in host utilization

6.3.2.1 Diversity between habitats

TCS parsimony network (0.95 parsimony limit) built from 147 *B*. *phaia* ssp. *phaia* sequences revealed 40 haplotypes (*h*) (Fig. 6.1). Thirty of these haplotypes were found from the collections made on maize, sorghum and sugar cane in the cultivated fields, and 25 of them were found in the collection made from wild host plants (Table 6.1). Twenty-three of all the haplotypes were common to both wild and cultivated hosts. However, both the average number of nucleotide differences (K) and haplotype diversity (*Hd*) were relatively higher among the wild host plants compared to the cultivated ones. This may explain the low differentiation observed between the two habitats (F_{ST} = 0.016; P = 0.015).

6.3.2.2 Seasonal variations in haplotype composition

A total of 28 haplotypes were found during the *SR* season of which 20 were found among the cultivated host plants and 19 on the wild host plants (Table 6.2 and Fig. 6.1). Though only 11 haplotypes were common to both habitats, there was no evidence of differentiation between them (F_{ST} = 0.017; P = 0.102). Similarly, there was no differentiation between the cultivated and wild habitats during the *LR* season (F_{ST} = 0.019; P = 0.092). However, there was strong evidence of variation in genetic composition between growing seasons in the wild habitat (F_{ST} = 0.060; $P < 0.001$), with more haplotypes found during the *SR* season. Out of the 25 haplotypes identified from the wild host plants, only 8 of them, mainly from *P*. *purpureum* were common in both seasons.

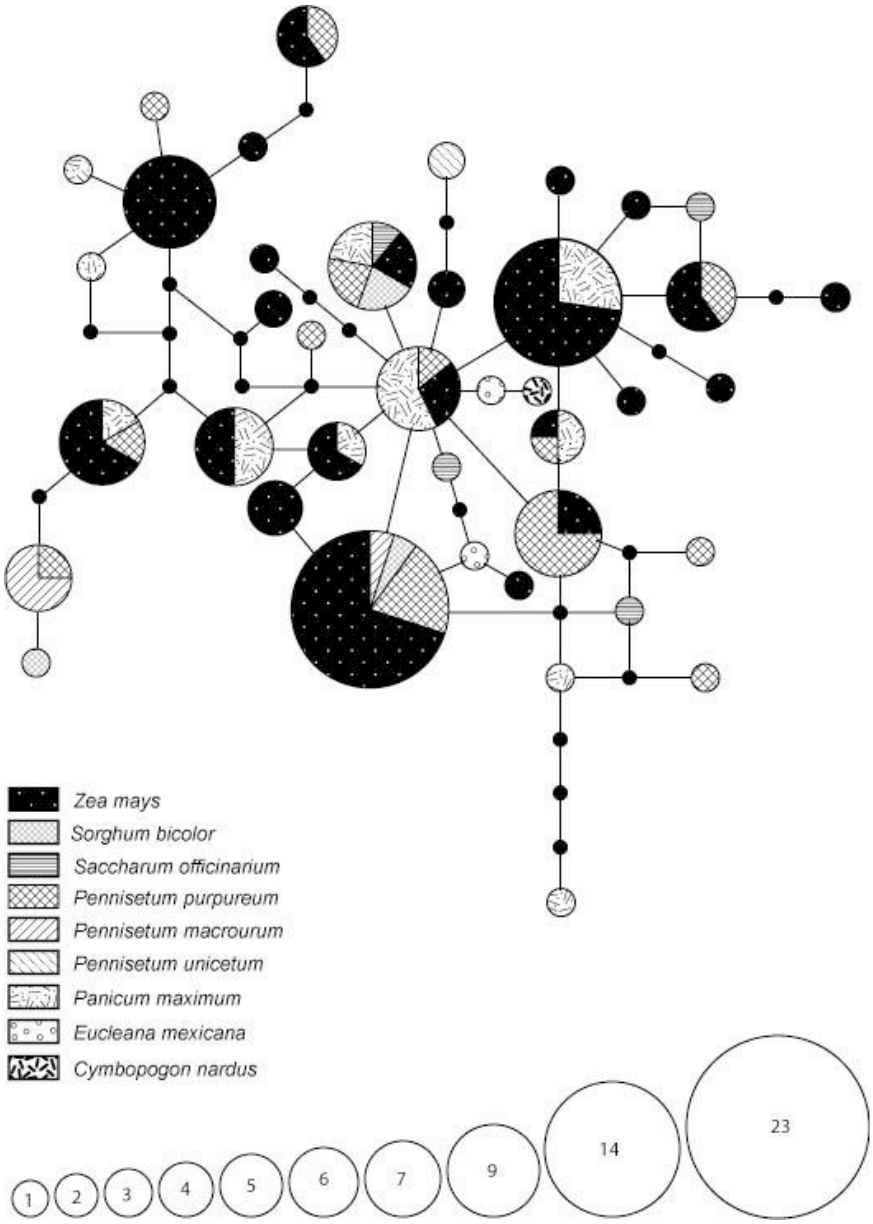

Figure 6.1: TCS mitochondrial haplotype network of *Busseola phaia* ssp. *phaia* individuals collected from different host plants in Kakamega. The area of each circle is proportional to the number of samples in each haplotype. Lines represent single nucleotide mutations and black circles represent haplotypes that are not observed in the sample. Different shading patterns represent the different sampled host plants

Table 6.2: Genetic diversity of the Cytochrome b gene in *Busseola phaia* ssp. *phaia* populations from different habitats across seasons in Kakamega (Kenya).

Genetic parameters		Cultivated host plants			Wild host plants		
		LR	**SR**	Total	**LR**	**SR**	Total
Number of sequences		42	36	78	35	34	69
Number of segregating sites, *S*		20	28	35	25	20	28
Number of haplotypes, *h*		16	20	30	17	19	25
Haplotype diversity, *Hd*		0.904	0.948	0.932	0.946	0.950	0.951
Average number of differences, *K*		3.113	3.632	3.375	3.832	3.403	3.641
Nucleotide diversity, *Pi*		0.004	0.005	0.005	0.005	0.005	0.005
AMOVA results	F_{ST}	0.015			0.060		
	P	0.118			0.001		

LR = Long rains; **SR** = Short rains

6.3.3 Stem borer carry-over between habitats and seasons

Wild and cultivated habitats, and two growing seasons (*LR* and *SR*) may be considered theoretically as four independent units (cultivated habitat *LR* and *SR*, and wild habitat *LR* and *SR*). The stem borer populations captured in any of the four units at a given time of the season can therefore be considered as products of either carry-over or exchange from the other three units (Fig. 6.2). Though host plants deep inside the forest could be one source of *B. phaia* ssp. *phaia* migrants, this analysis was limited to comparison of populations in the four units mentioned above. The results revealed the existence of free exchange of haplotypes between seasons and habitats except in isolated cases where there was evidence of variation in haplotype composition. Significant variation was observed between the wild *SR* against both the wild *LR* (F_{ST} = 0.060; P < 0.001) and cultivated *LR* (F_{ST} = 0.071; P < 0.001). Variation was also observed between the wild *LR* and the cultivated *SR* (F_{ST} = 0.027; P = 0.028) despite the high number of haplotypes (12) shared between the units.

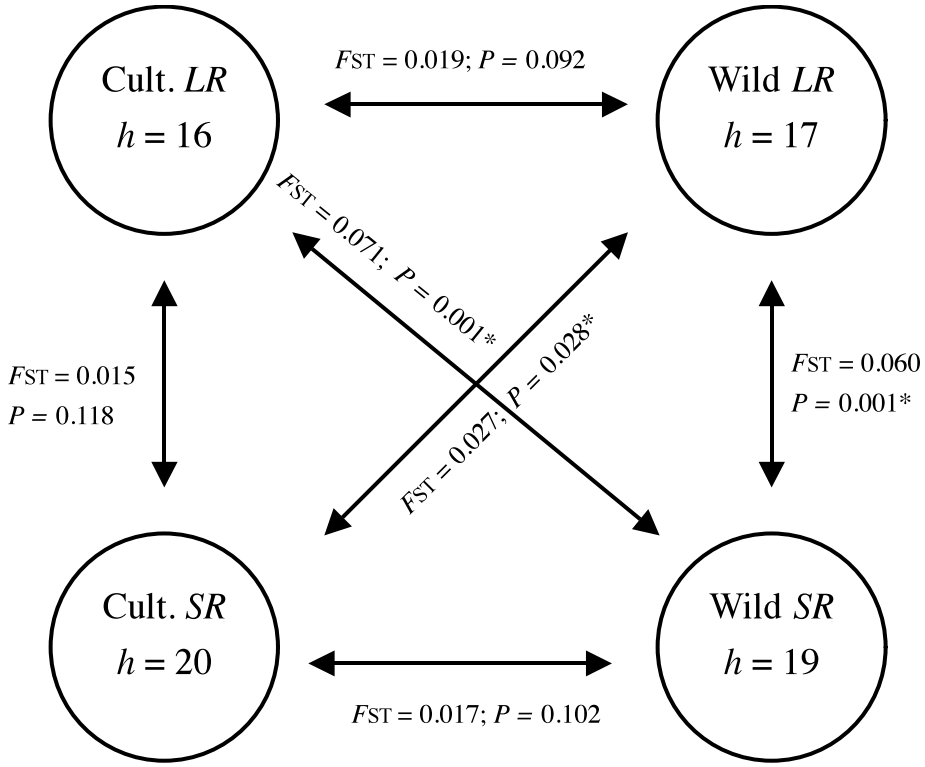

Figure 6.2: Summary of assumed population movement between habitats and seasons (indicated by arrows). Each unit is assumed to receive and give immigrants to each of the three units. *h* represents the number of haplotypes found in each unit while F_{ST} and *P* values are the AMOVA results computed between respective units. Asterisks (*) indicate where haplotype compositions between respective units varied significantly ($P < 0.05$).

6.4 Discussion

This study confirms the establishment of *B*. *phaia* ssp. *phaia* as a pest on a small agricultural landscape along the edge of the Guineo-congolian rain forest relict in Kakamega (Le Ru *et al*., 2006a). The presence of larvae in both wild and cultivated plants suggests plasticity in its host range with no evidence of long historical host use adaptation. This is contrary to the observed long historical host use adaptation among *S. calamistis* in Kenya as shown in the previous chapter. The ability of *B*. *phaia* ssp. *phaia* to use a wide range of plant species in this landscape enables it to co-exist with other species as population exchange ensures its persistence among cultivated plants. The evidence of population exchange is confirmed by the general lack of genetic structure among individuals found in different habitats and growing seasons. The cultivated fields surrounded by small non-cultivated habitat fragments on the other hand function as mainland–island metapopulation system (Bourguet *et al*., 2000b), where populations in the small non-cultivated habitat fragments persist because of the rescue effect (Kennedy & Storer, 2000).

Busseola phaia ssp. *phaia* was earlier reported from maize in the highland areas of Kisii (Kenya) where its population was low compared to *B*. *fusca*, the dominant pest species (Le Ru B., personal observations). Both Kakamega and Kisii, where it infests maize, share proximity to a less disturbed natural forest as one common characteristic. It thus appears that populations currently found in the cultivated fields originally infested wild hosts growing in the adjacent forest. This is not the first time an indigenous stem borer species expanded its diet breadth to include cultivated crops where it becomes an important pest (Polaszek & Khan, 1998). *Eldana saccharina* is the most recent species

that initially colonized mainly sedges and now have expand its diet breadth to include sugarcane in both Western and Southern Africa countries (Mazodze & Conlong, 2003). *Busseola phaia* ssp. *phaia* population in Kakamega may have expanded its host range to include maize and sorghum in response to habitat loss. However, it appears to have retained the old association with native hosts. This might explain the wide host range, which has allowed for its persistence in both wild and cultivated habitats and subsequent carry-over between the seasons. However, *B*. *phaia* ssp. *phaia* populations were generally higher in the cultivated fields than in the wild habitat, with the majority of larvae (60%) found on maize and sorghum. Observed distribution in the two habitats conforms to the ideal free distribution theory by Fretwell & Lucas (1970). The theory states that organisms aggregate in different patches with respect to the amount of resources available. Maize and sorghum are very nutritious and are readily available for stem borers as they are grown under mono-cropping system along the edge of Kakamega forest. This is contrary to wild host plants that are sparsely distributed and are poor in nutrient content (Shanower *et al*., 1993).

The movement of *B*. *phaia* ssp. *phaia* moths between host plants in different habitats could be one strategy to adapt and avoid competitive exclusion by dominant borer species in respective habitats (Jonsen & Fahrig, 1997; Hawthorne & Via, 2001). For example, duringthe non-cropping season, moths appear to favour oviposition on the alternative hosts growing in the adjacent wild habitats where they remain until the beginning of the season. However, this persistence strategy may not be successful for all seasons particularly in the wild habitat where there was evidence of limited carry-over between the seasons. One of the factors that may affect success of this strategy and limit

the stem borer carry-over is the use of *P. purpureum* by local farmers as animal feed. During the end of *LR* growing season until the beginning of *SR* growing season, *P. purpureum* is cut and transported to respective homes where it is used to feed cattle. Several individuals (haplotypes) are very likely killed through this practice thus limiting the population carried to the next season. This could explain the observed variation in haplotype diversity between *LR* and *SR* growing seasons in wild habitat. The majority of haplotypes recovered in the wild habitats during the *SR* growing season are assumed to have originated from the cultivated fields (*LR* population).

Another possible explanation for such wide host range and persistence is what could be called the 'plasticity theory' in which phytophagous insects are thought to be carrying genotypes for plasticity that allow them to broaden their resource use when new resources become available (Nylin & Gotthard, 1998; Hawthorne & Via, 2001). Janz *et al.* (2001) used this theory to explain the increased likelihood of nymphalid butterfly tribe Nymphalini colonizing ancestral host *Urtica dioica* or related plants during their study. Such plasticity can function as pre-adaptation to novel environments and allow organisms to respond differently depending on the environmental conditions (Kennedy & Storer, 2000; West-Eberhard, 2003). *Busseola phaia* ssp. *phaia* is mainly associated with hosts belonging to the subfamily Panicoideae, both cultivated and wild host plants. A possible contributing mechanism for the host range expansion might be that *B. phaia* ssp. *phaia* larvae like other insects have kept chemical 'memory' of some plants belonging to subfamily Panicoideae used earlier in the history of their lineage (Moczek, 2007). They are therefore pre-adapted to utilize any representative of this subfamily not presently used by the females for oviposition. However, this mechanism may not apply to all stem

borers since species like *B*. *fusca* is specialized and is currently found on a limited number of hosts (Le Ru *et al*., 2006a).

The management of stem borer pests has been one of the priority areas among agricultural entomologists in Africa (Overholt *et al*., 1994; Schulthess *et al*., 1997; Le Ru *et al*., 2006a). Like other field insect pests, inclusion of a new species in a pest community would affect the existing management practices as most approaches appear to be species specific. On this background, movement and subsequent utilization of maize plants by *B*. *phaia* ssp. *phaia* is an issue of concern among entomologists as well as farmers (Le Ru *et al*., 2006a). *Busseola phaia* ssp. *phaia* is currently not yet considered as an important pest probably due to the presence of a wide range of host plants within the non-cultivated fragments in the landscape. This might be attributed to the fitness of its population, which remains suboptimal on maize probably until selection processes that favour suitable genotypes take place. *Busseola phaia* ssp. *phaia* is just one example of the little known insect species that is in process of becoming an important pest. However, as demand for more land and food increases, the non-cultivated fragments, an important ecosystem of both known and unknown insect groups, are threatened and are likely to be cleared. These may ultimately result in host range expansion with some little known insect species becoming important pests of the cultivated crops. Host range expansion to include crops is not new as it happened in the recent past when *E. saccharina* expanded its host range to include sugar cane in South Africa (Mazodze & Conlong, 2003). Based on these findings, there is need to protect the uncultivated fragments around crop fields in order to maintain the species diversity and ecosystem services realized from the agricultural landscape.

CHAPTER SEVEN

7 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

This study presents higher stem borer species diversity among wild plants contradicting previous studies in which fewer stem borer species were reported from wild plants (Ingram, 1958; Nye, 1960; Overholt *et al*., 1997; Khan *et al*, 1997). Differences in the number of stem borer species between previous and present studies may be attributed to variations in the rigors of study and identification tools. The majority of previous studies were designed to generate knowledge that would be used in the management of pest species with little interest on the general stem borer species diversity (Khan *et al.*, 1997). With this objective, previous studies were conducted on a limited time frame and thus failed to capture rare and seasonal stem borer species. The present study was designed to catalogue stem borer species among wild habitats with a view to establish the role of wild host plants in stem borer pest dynamics. It thus covered both the sampling scale and the frequency aspect, and these together explain the higher stem borer species diversity observed.

The stem borer species belonging to *Sesamia*, *Sciomesa, Busseola* genera as well as Crambids, Pyralids and Tortricids constituted an important proportion of the total collection. However, distribution of some wild stem borers was found restricted to either some plant species or localities. For example, species in the *Manga* genus (*M. nubifera* in Muhaka; *M. melanondonta* in Suam) were commonly found on *P. maximum*, while species in *Busseola* genus were found mainly in the high altitude localities (*B. phaia* and *Busseola* sl nov sp 1 in Kakamega; *Busseola* sl nov sp 3 in Suam). The presence and

abundance of the wild stem borer species appear to be affected by the availability of suitable host plants with the majority of stem borer species recovered from a limited number of host species. According to Hermsmeier *et al*. (2001), such specialised species are more likely to adapt to the toxic compounds which they encounter. In this regard, distribution and abundance of some stem borer species may be attributed to their adaptations to overcome defences of localized host plant species. Nonetheless, some of the wild stem borer species (*B. phaia* and *S. piscator*) develop easily on maize stems thus exhibiting a potential to shift and become pests of cultivated cereals. The recent host switch of *E. saccharina* from sedges to sugar cane in South Africa where it became the key pest (Atkinson, 1980) confirms the possiblities of host range expansion among *B. phaia* and *S. piscator*.

Recent surveys in eastern Africa revealed higher stem borer diversity with 17 unknown species (Le Ru *et al*., 2006b). Five among the unknown species identified in this study included *Busseola* sl nov sp. 1, *Busseola* sl nov sp. 3, *Carelis* nov sp. 3, *Sciomesa* nov sp. 3 and *Sesamia* nov sp. 4. Another species, *Sesamia* nov sp. 9, was found on *C. papyrus* in Kakamega. It was initially thought that these species were missed in the previous studies because they were either rare or cryptic. However, the ease with which they were recovered in this study suggests that more undescribed stem borer species and host plants could be found if such studies are extended to other areas.

Even with the observed variation in distribution among localities, the importance of wild stem borers as alternative hosts of natural enemies during both cropping and non cropping periods cannot be ignored. Bonhof (2000) reported high diversity and abundance of natural enemies in the maize fields at the Kenyan coast during the

beginning of the long rainy season. She ascribed this diversity to the possible movement of natural enemies from wild habitats where they attack alternative host stem borer species. Schulthess *et al.* (2001) reported relatively high parasitism rate of *S. calamistis* eggs by *Telenomous* spp (Hymenoptera: Scelionidae) during the dry season in the Inland valley in Benin and Cameroon. Wild habitats rich in alternative stem borer species may attract and sustain populations of natural enemies that would eventually move to the cultivated fields and suppress pest populations.

In the cultivated fields, stem borers recovered during this study included both known and unknown pest species. The known pest species included *B. fusca*, *S. calaimistis*, *C. partellus*, *C. orichalcociliellus* and *E. saccharina* while the unknown species were identified and classified as *S. piscator* and *B. phaia*. The pest species however varied in distribution among the surveyed localities, results that corroborate recent studies in which stem borers were found to vary in species composition and distribution among different vegetation mosaics (Le Ru *et al*., 2006a) and agro-ecological zones. *Busseola fusca* and *C. partellus* dominated the pest community in high (Suam and Kakamega) and low (Muhaka and Mtito Andei) altitude localities respectively. These findings support earlier reports in which Seshu Reddy (1983), Songa *et al*. (1998) and Zhou *et al*. (2002) reported variations in the distribution of *B. fusca* and *Chilo partellus* according to altitude. Nye (1960) ascribed differences in distribution among these species to climatic variations especially the temperature.

Busseola fusca was found in maize in Kakamega and Suam, but only on *S. arundinaceum* among wild plants contrary to earlier reports in which it was reported from several wild hosts (Polaszek & Khan, 1998; Overholt *et al*., 2001). Laboratory studies

demonstrated that *B. fusca* larvae were reluctant to bore in *P. purpureum* stems and that adult moths did not oviposit on that host plant (Wilkinson, 1936). The likely reports of *B. fusca* on *P. purpureum* in Kenya may have been a result of the larval movement from maize or sorghum onto *P. purpureum*, or misidentification. *Busseola fusca* was reported to be common on *P. purpureum* in Central Africa (Cameroon) (Ndemah *et al*., 2001) though recent review of these collections indicated that previous reports were based misidentified materials (Le Ru *pers. com*). However, unlike *B. fusca*, *S. calamistis* was recovered from many plant species confirming its polyphagy corroborating reports from West and Central Africa (Ndemah *et al*., 2001). Though wild plants are attractive to ovipositing moths, larval survival and adult fecundity are generally low (Shanower *et al*., 1993), which may explain the low populations observed in the maize fields surrounded by wild hosts in Benin and Cameroon (Schulthess *et al*., 1997; Schulthess *et al*., 2001; Ndemah *et al*., 2002).

Chilo partellus was found restricted to Muhaka and Mtito Andei with high populations in maize and low population in the wild habitats. *Chilo partellus* populations were higher than that of other borers supporting earlier studies by Le Ru *et al*. (2006a) which suggested that low altitude areas are ecologically suitable for its establishment. According to Zhou *et al*., (2002), suitable climatic conditions coupled with available alternative hosts are thought to have favoured *C. partellus*. This can explain its rapid population build up that resulted in the displacement of the indigenous *C. orichalcociliellus* (Seshu Reddy, 1983). However, there was evidence of variation in niche occupation among these two species in the wild as most of the *C. partellus* larvae

were found on *S. arundinaceum* while the *C. orichalcociliellus* group larvae were found mainly on *P. maximum*.

Busseola phaia ssp. *phaia* and *S. piscator* were found in maize plants and could therefore qualify as minor pests. These species were however limited in distribution and occurred mainly in Kakamega where they infested maize fields and wild plants growing in the adjacent uncultivated fragments. In Kenya, these species have for along time been associated with wild plants (Nye, 1960) until recently when they were reported from maize plants (Le Ru *et al*., 2006a). *Busseola phaia* is known to infest a wide range of wild plants particularly *P. purpureum* and *P. maximum* in Eastern Africa (Le Ru *et al*., 2006a). Together with *S. piscator*, their presence in maize plants in Kenya was first reported by Ong'amo *et al*. (2006) from field survey along the Kakamega forest. Since the first report of their incidence, these species have continuously been found among maize plants along Kakamega forest. There are several probable reasons that could be used to explain their continued presence among the maize plants;

i) Kakamega area is characterised by high human population density. Some parts of the forest have been cleared to open more land for farming as demand for extra agricultural land increases. These have resulted in habitat fragmentation and loss of important migration corridors. As expected among other phytophagous species (Gaete-Eastman *et al*., 2004), *B. phaia* and *S. piscator* that earlier colonised these habitats may have responded by diversifying their diet breadth to include easily available hosts (eg maize plants).

ii) *Busseola fusca* and *S. calamistis* have for along time been known to dominate stem borer pest community in this area (Seshu Reddy, 1983). However, in view of the fact that several previous stem borer studies were marred with taxonomic errors (Le Ru *et al*., 2006b), some materials earlier identified as *B. fusca* or *S. calamistis* may have been misidentified as they can respectively be confused for *B. phaia* or *S. piscator* particularly at the larval stage.

Stem borer pests occurred in both wild and cultivated fields though they infested limited number of plant families. Similar host use pattern was reported by Le Ru *et al*. (2006a) contradicting previous reports in which stem borer pests were considered as polyphagous (Polaszek & Khan, 1998). For example, *B. fusca* was only found in *S. arundinaceum* contrary to reports from previous studies in which it was reported from *P. purpureum*, *S. arundinaceum* and *P. maximum*. Nevertheless, populations of other pest species were generally high in the maize fields compared to the wild habitats except for *B. phaia* and *S. piscator* which were mainly found on *P. purpureum* and *E. mexicana*. High pest populations in maize fields indicates better suitability of cultivated crops to support stem borers compared to wild grasses. Shanower *et al*. (1993) showed that survival of *S. calamistis* and *E. saccharina* larvae was less than 10% in *P. maximum*, *S. arundinaceum*, *P. purpureum* and *P. polystachion* (L.) Schultes, while larval survival in maize was between 19 and 30%. The observed difference in pest populations between the habitats could thus be attributed to low stem borer survival among wild plants.

Despite the fact that wild habitats support low stem borer populations, they are still responsible for pest build-up early in the season. However, this role varies among

stem borer pest species and regions. In Kenya for example, *B. fusca* rarely infests wild plants and populations found in the cultivated fields early in the season come from crop residues (Seshu Reddy, 1983). In contrast, *S. calamistis*, *C. partellus*, *C. orichalcociliellus* and *B. phaia* exhibit continuous exchange of populations between cultivated crops and wild plants. This role however may vary among stem borer species depending on the host use adaptation and diet breadth, of which the majority of these species are specialised (Le Ru *et al*., 2006a). Host specialization among phytophagous insects takes place through the action of many factors that ultimately affect diet breadth (Bernays & Graham, 1988; Jermy, 1988; Rausher, 1992; Bernays & Chapman, 1994). A long-standing hypothesis on this issue is that phytophagous insects with a restricted diet breadth (i.e. specialists) should be more prone to genetic differentiation than insects with a greater diet breadth (Price, 1980; Futuyma & Moreno, 1988).

Sesamia calamistis and *B. phaia* were evaluated as model species in this study as they infested both wild and cultivated plants. High genetic differentiation was observed among *S. calamistis* populations and can be based upon (i) reduced population size which can increase the effects of genetic drift, (ii) the patchier distribution of host plants, which can reduce cohesion between populations, reducing gene flow (Peterson & Denno, 1998). In contrast, there was no clear genetic differentiation among *B. phaia* populations, results that could be attributed to spatial overlap in the distributions of host-plants in Kakamega. Since suitable hosts are patchier for *S. calamistis* than *B. phaia*, gene flow should be relatively less among populations of *S. calamistis* (Price, 1980; Futuyma & Moreno, 1988), which due to their reduced effective population size may also suffer genetic erosion at species level (Ingvarsson & Olsson, 1997).

7.2 Conclusions and recommendations

1) Stem borer species were more diverse among wild plants than in the cultivated fields. The wild species however occurred on limited number of host plants suggesting that they are monophagous contrary to earlier reports in which they were presented as polyphagous. This study further confirmed variation in host use pattern and distribution among different stem borer species. Wild habitats may however accommodate more unknown stem borer species and there is need to extend similar surveys to other areas, particularly in the wetlands, to generate more information on the general species diversity and host use pattern.

2) Variation in distribution of wild stem borers as alternative hosts of natural enemies during both cropping and non cropping periods cannot be ignored. High diversity and abundance of natural enemies in maize fields has been reported at the Kenyan coast during the beginning of long rainy season. This was ascribed to possible movement of natural enemies from wild habitats where they attack *C. partellus* and *C. orichalcociliellus* infesting wild host plants. There is therefore need to conserve wild habitats rich in alternative stem borers as they sustain populations of natural enemies that would eventually move to cultivated fields and control pest populations.

3) The majority of the unknown stem borer species were recovered from host plants growing along the streams and other wetlands. Unfortunately, most of wetlands

are earmarked for rehabilitation as demand for agricultural activities increases, and these will ultimately result in habitat loss. Conservation of ecologically important habitats is the only sustainable way of maintaining stem borer species and their respective host plants. There is therefore need to educate the local communities on the ecological benefits of wetlands and involve them in planning and execution of conservation programmes.

7.3 Future prospects

Natural ecosystems in East Africa are endowed by diverse stem borer species which include both wild and pests species. Unfortunately, most of these ecosystems are currently threatened by urbanization and agricultural activities, which may ultimately compromise the provision of essential ecosystem services. Threats on the ecosystem and provision of ecosystem services may worsen with the anticipated climate change. Climate change is already having impacts on the dynamics of the African biomes and its rich biodiversity, although species composition and diversity is expected to change due to individual species response to climate change conditions (IPCC, 2007).

The projected rapid rise in temperature and rainfall changes (IPCC, 2007) combined with other stresses, such as the destruction of habitats from land use could easily disrupt the connectedness among species and transform the existing communities. This together with varied movements among species through ecosystems could result in the reduction of species diversity or localized extinctions (IPCC, 2007). However, most work on climate change in Africa to date has concentrated largely on prediction and

modeling without real data on these changes and their potential impacts on the provision of ecosystem services.

Climate has a direct influence on insect development, reproduction and survival and any increase in temperature will have a direct effect in various aspects of their life cycle and ecology. Impacts of climate change on insects could be complex as it may lead to competition and subsequent displacement among interacting species or local extinction. There is thus need to assess diversity and trends of stem borers and the associated natural enemies, and predict how climate change will affect their interaction and the general ecosystem processes.

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