



DIVERSITY AND DISTRIBUTION OF LEPIDOPTERAN STEM BORERS AND THEIR
NATURAL ENEMIES IN BOTSWANA

by

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A Dissertation/Thesis Submitted to the College of Science in Partial Fulfilment of the
Requirements for the Award of the Degree of Master of Science in **Biological Sciences** of
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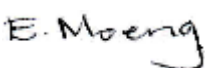
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CERTIFICATION

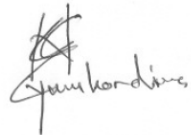
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Diversity and distribution of Lepidopteran stemborers and their natural enemies in

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(Entomology) of BIUST.



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Dr.

(Supervisor)

Date: 20 September 2017

Dedication

To my late mother, Kealesa Moeng, May your soul rest in eternal peace.

Acknowledgements

I would like to thank my advisor Dr Casper Nyamukondiwa who always had a door open for me whenever I had a question or needed advice for my research and writing. He allowed me to own the thesis but at the same time steering me in the right direction where there was need. Many thanks to other several anonymous referees for comments on an earlier version of my thesis. I am also indebted to the valuable support from Botswana International University of Science and Technology (BIUST), International Centre of Insect Physiology and Ecology (ICIPE), Kenya, South African Sugar Research Institute (SASRI), South Africa, Ministry of Agriculture and department of National Museum and Monuments, Herbarium unit, Botswana.

Furthermore, I am grateful to Reuben Kandji, Ministry of Agriculture staff for the active support in the survey and all the farmers who gave us permission to go into their fields and sample without any regrets. Finally I would like to extend gratitudes to my family and friends for providing me with continuous support and encouragement throughout my years of study. This would have not been possible without them.

Abstract

Botswana is a country which depends mainly on cereal agriculture, done mostly by rural farmers as their major income stream. However, lepidopteran stemborers are a major constraint in the rural farmers' cultivation of cereal crops. Species diversity and distribution of these insects of economic concern is currently unknown in Botswana. Through a survey, I catalogued the diversity of stemborers, host plant interactions and their associated parasitoids across Botswana. A total of 1597 stemborer larvae and 228 pupae were recorded; 63.1% and 36.9% larvae from cultivated versus wild hosts respectively and, 84.8% and 15.2% pupae from cultivated versus wild host plants respectively. In addition to *Chilo partellus* Swinhoe, *Sesamia calamistis* Hampson and *Eldana saccharina* Walker which were previously reported in Botswana, 15 'new' stemborer species were recorded for the first time, including 10 species which have never been described before. These species are: *Sesamia jansei* Tams & Bowden, *Sesamia perplexa*, *Sesamia nonagrioides* Lefebvre, *Sesamia perplexa* es nov sp 4, *Sesamia* sp nr *perplexa*, *Sesamia* nov sp 41 nr *nonagrioides*, *Sesamia* sp. *Mythimna loreyi*, *Conicofrontia* nov sp 1 nr *sesamoides*, Tortricidae sp1, Tortricidae sp2, Tortricidae sp3, Tortricidae sp. and a Schoenobiinae. These were collected from a variety of plants ranging from cultivated *Zea mays* L., *Sorghum bicolor* (sorghum), *Sorghum bicolor* L. Moench (sweet sorghum) to wild *Schoenoplectus corymbosus* Roth ex Roem. & Schult, *Chrysopogon nigritanus* (Vetiver), *Typha latifolia* L., *Cyperus papyrus* L., *C. dives* Delile, *Vossia cuspidata* (Roxb.) Griff., *Eriochloa fatmensis* (Hochst. & Steud.) Clayton, *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase and *Oryza longistaminata* A.Chev. & Roehr. *Busseola fusca* Fuller, previously reported to be one of the common stemborers in the country, was not recorded in this work. *Chilo partellus* had the highest relative abundance of 62.1% in *Z.mays*, grain sorghum, sweet sorghum, *E. pyramidalis* and *C. dives*; followed by *S. calamistis* at 11% in *Z. mays*, *E. pyramidalis*, grain sorghum, *P. macronium*, *S. corymbosus*, *C. papyrus* and *C. nigratinus*; with *Mythimna loreyi* (*Z. mays*) and *S.perplexa* es nov sp4 (*E. pyramidalis*) having the lowest abundance of 0.7%. Stemborer diversity index for the wild host plant species was found to be greater than that of the cultivated crops. In cultivated habitats, the most common larval parasitoids were *Cotesia flavipes* Cameron, *Cotesia sesamiae* Cameron (Hymenoptera: Braconidae), *Euvipio rufa* Szepligeti (Hymenoptera: Braconidae) and pupal parasitoids were *Pediobius furvus* Gahan (Hymenoptera: Eulophidae) and *Gambroides nimbipennis* Seyrig (Hymenoptera: Ichneumonidae). However, larval parasitoids *Chelonus curvimaculatus* Cameron (Hymenoptera: Braconidae), and *Goniozus indicus* Ashmead (Hymenoptera: Bethyridae) and a pupal parasitoid, *Dentichasmias busseolae* Heinrich

(Hymenoptera: Ichneumonidae) were common in natural habitats. Furthermore, larval and pupal predatory ants (*Linepithema humile* Mayr (Hymenoptera: Formicidae), *Crematogaster peringueyi* Emery (Hymenoptera: Formicidae) and *Aenictus* species) were identified in both cultivated and natural habitats. Cultivated Poaceae- *Zea mays* L., *Sorghum bicolor* (Sweet sorghum), *S. bicolor* (sorghum); wild Poaceae- *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase, *Sorghum bicolor* subsp *arundinaceum* (Desv.) de Wet & Harlan; wild Typhaceae- *Typha latifolia* L. and wild Cyperaceae- *Schoenoplectus corymbosus* (Roth ex Roem. & Schult.) J.Raynal, and *Cyperus dives* Delile were the major plants harbouring these natural enemies and their host insects. Stemborers *C. partellus* and *Sesamia* spp. were the major parasitoid hosts with *C. partellus* hosting most parasitoids in cultivated habitats and *S. jansei* in natural habitats. Larval parasitism ranged between 2.1-48.9% and 3.7-23.5% in cultivated and natural habitats respectively, with *C. flavipes* and *C. curvimaculatus* Cameron the predominant parasitoids respectively. Pupal parasitism ranged between 5.9-15% in cultivated and 6.7-11.1% in natural habitats with *P. furvus* Gahan and *D. busseolae* the predominant parasitoids respectively. Larval and pupal parasitoids relative abundance ranged between 1.1-47% with *C. flavipes* and *P. furvus* the dominant larval and pupal parasitoids respectively. Overall, this work reports Botswana landscapes host diverse stemborer species across wild and agro-ecosystems, and a significant diversity of natural enemy/parasitoid faunal diversity that may be worth conserving for future efficacy of biological control programmes.

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CHAPTER ONE

1.0 Introduction

Cereals represent the most important food crops grown in most of sub-Saharan Africa and have often been the focus of development policies. One of the challenges cereal farmers face is the control of stem and ear borer infestations which cause losses of up to about 10-70% in areas with severe stem and ear borer problems (BosquePérez & Mareck 1991; Cardwell *et al.*, 1997; Sétamou *et al.*, 2000). Major pests in cereal crops in sub-Saharan Africa are considered to be Lepidopteran stemborers (Harris & Nwanze, 1992, Kfir *et al.*, 2002). The four most significant species of stemborers belonging to the Order Lepidoptera (Conlong, 1994), families Noctuidae, Crambidae and Pyralidae are *Chilo partellus* Swinehoe (Lepidoptera: Crambidae), *Eldana saccharina* Walker (Lepidoptera: Pyralidae), *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) (Hearne *et al.*, 1994; Haile & Hofsvang, 2001; Assefa *et al.*, 2008). Stemborers can cause severe damage at different stages in the development of cereal crops; from seedling to maturity. The feeding habit of stemborers reduces the flow of water and nutrients throughout the plant, and can reduce grain weight and kernel number, therefore reducing yields (ISU, 2012). The distribution and abundance of the stemborers may be influenced by microclimatic factors such as temperature and precipitation, and biotic factors such as natural enemies and alternative host plants (Setamou *et al.*, 1993).

Different strategies have been implemented to control stemborers. These include the use of insecticides, cultural control, host plant resistance and biological control (Mugo *et al.*, 2001; Kfir *et al.*, 2002). The use of pesticides has been the main method of controlling the insect pests over the past decades (Aktar *et al.*, 2009). However, the high cost of pesticides is beyond the financial means of many small-scale farmers in sub-Saharan Africa who receive marginal profit from their produce. In addition, pesticides have a negative impact on the environment as they are a health hazard to humans and may not be compatible with other means of control such as biological control (Sylvian, 2015). The public health effects of pesticides have long been known and the undesired effects of chemical insecticides have been recognized as a serious public health concern during the past decades (Dey *et al.*, 2013). In humans, organophosphates and carbamates may affect the nervous system, others may irritate the skin, affect the hormonal and endocrine systems or may be carcinogenic (Aktar *et al.*, 2009). Since pesticides also have an effect on the environment and biodiversity, there is an increasing drive towards biologically based methods of pest management (Chidawanyika *et al.*, 2012).

Lepidopteran stemborer species relative abundance, distribution and composition are significant aspects to be considered in determining the stemborer species present in certain localities (Banwo *et al.*, 2001). In addition, knowledge of species diversity is important in planning any management strategies for these economic invertebrates. Several studies on diversity and distribution of stemborers have been conducted in Zimbabwe, South Africa, Ethiopia, Cameroon, Kenya, Togo, Mozambique, Mozambique, Uganda (Chinwada & Overholt, 2001; Kruger, 2005; Assefa *et al.*, 2006; Le Ru *et al.* 2006; Ndemah *et al.*, 2007; Matama-Kauma *et al.*, 2007; 2008; Mailafiya *et al.*, 2009; Kodjo *et al.*, 2013; also see Moolman *et al.*, 2014). Nevertheless, similar studies are still lagging behind in Botswana. Furthermore, the detection and characterization of genetic differentiation among insect populations is a key step for improvement of any pest management practice such as biotype ecology and evolution, biological control, resistance management and insect–plant interactions (Kourti, 2006, Margaritopoulos *et al.*, 2007, De La Poza *et al.*, 2008, Hoy, 2013, Agunbiade *et al.*, 2014). Several techniques such as mitochondrial DNA (MtDNA) comparisons have been used in population genetics studies of many insects (Kourti, 2006 & De La Poza *et al.*, 2008). MtDNA analysis is capable of revealing cryptic lineages representing distinct species within geographically widespread and apparently morphologically homogeneous organisms (Scheffer, 2000). It has been used to reconstruct the phylogenetic and phylogeographic relationships in indigenous stemborers (King *et al.*, 2002; Sezonlin *et al.*, 2006; Assefa *et al.*, 2006) and their associated parasitoids (Dittrich *et al.*, 2006; Muirhead *et al.*, 2006). It has proven powerful for genealogical and evolutionary studies of animal population and it is the most commonly employed marker for determining genetic relationships among populations (Sperling *et al.*, 1999; Scheffer, 2000). Similarly, no genetic diversity has been done for the ‘resident’ Botswana stemborer populations.

Although the diversity and distribution of stemborers and their parasitoids have been elucidated in other African countries, this critical information is lacking for Botswana. Through a scoping study, this work (1) catalogued the diversity and distribution of stemborers in Botswana in their wild and cultivated plants, and helped explain the potential role of wild habitats/hosts as a reservoir/source for stemborer pests; (2) catalogued the natural enemies (parasitoids) associated with the stemborers in the different habitats and (3) described the phylogeny of the important lepidopteran stemborer population (s) found in Botswana. The objectives of the research were to (1) identify the diversity and host plant associations of lepidopteran stemborers in Botswana, (2) identify parasitoids associated with these lepidopteran stemborers and host plants in Botswana and (3) to assess the genetic

relationship of the major stemborers in Botswana. I hypothesized that (1) Botswana hosts different species of lepidopteran stemborers in different graminaceous and cyperaceous host plants, (2) different hymenopteran parasitoids parasitize the diversity of stemborers found in the different host plant assemblages in Botswana and (3) lepidopteran stemborers found in Botswana are phylogenetically related.

1.1 Justification

Botswana is a country which depends mainly on agriculture-arable farming as its main source of food, and supporting rural incomes and livelihoods. Despite its dependence on crop production, there are some challenges that hinder economic transformation of local livelihoods through agriculture. Insect pests, economic lepidopteran stemborers are major challenges faced by cereal crop production farmers. Controlling the stemborers using chemical insecticides has of late, become a challenge because of low efficacy. Stemborers tunnel themselves in the stems making it difficult for the insecticides to reach them. Secondly, resistance development has been reported in different cereal growing areas, compounded by a shift in consumer's preference for the organic foods (Chidawanyika *et al.*, 2012). Hence, alternative and more sustainable methods that are safe to users and do not have any acute or chronic effects on consumers warrant further investigation. Biological control of stemborers using natural enemies may thus provide a more sustainable approach to the management of stemborers. It is therefore important to know (1) the diversity of the different stemborer host plants, both wild and cultivated species; (2) the diversity, abundance and phylogeny of the stemborers in Botswana, to better inform management plans; (3) the current natural enemies found in wild and agro-ecosystems and on different stemborers to better inform management plans. This work looked into the tritrophic interactions of stemborer host (plant), the stemborer and parasitoids and how they interact in both wild and cultivated habitats.

1.2 Literature Review

1.2.1 Major cereal stemborers

The spotted stemborer *Chilo partellus* Swinhoe, which is indigenous to Asia and was accidentally introduced into Southern Africa before the 1930s (Harris, 1990), has since then spread to many countries in eastern, western and southern Africa (Overholt *et al.*, 1994; Maes, 1998 reviewed in Mutamiswa *et al.*, 2017). The species is highly invasive and has

colonized many areas in eastern and southern Africa, displacing the indigenous stemborers (Kfir *et al.*, 2002; Mutamiswa *et al.*, 2017). *Chilo partellus* has a wide variety of hosts, both cultivated and wild (Harris, 1990; Khan *et al.*, 1997; Polaszek & Khan, 1998; Van den Berg *et al.*, 2001; Rebe *et al.*, 2004). Its ability to have a wide host range, and dominance over indigenous stemborer species makes it a highly economically important stemborer species (Mutamiswa *et al.* 2017). It is a significant pest of maize and sorghum in many parts of Africa (Seshu, 1983; Van den Berg *et al.*, 1990, Overholt *et al.*, 1994, Päts *et al.*, 1997) and also known as a pest of rice and sugarcane (Kfir 1992; 1993; Way & Kfir, 1997). Several studies documented the importance of *C. partellus* in sorghum and maize in the warm lowland regions (Gebre-Amlak, 1985; Getu *et al.*, 2001; Tefera, 2004). Generally, *C. partellus* has been known to occur in low to mid altitudes (< 1500m) and warmer areas (Warui & Kuria, 1983; Overholt *et al.*, 1997), however, a growing number of studies indicate that the pest is expanding its geographic distribution to higher elevations (Kfir, 1997; Getu *et al.*, 2001; Mutamiswa *et al.*, 2017). Tamiru *et al.* (2007) reported the pest at an altitude of 2088m above sea level in Ethiopia and Ong'amo *et al.* (2006) reported its presence in relatively high altitude zones in Kenya (moist high and moist mid-altitude zones) where the pest previously did not occur.

The sugarcane stalk borer *Eldana saccharina* Walker is also a serious pest of sugarcane in various African countries (Atkinson *et al.*, 1981). It is indigenous to Africa and feeds on cultivated graminaceous crops as well as on several wild grasses and sedges (Conlong, 1994). *Eldana* has a wide host plant range encompassing four families (Cyperaceae, Poaceae, Typhaceae, Juncaceae) but on a study by Conlong *et al.* (2007), *E. saccharina* larvae showed a distinct preference for *Cyperus* spp. over sugarcane and indigenous grasses. The insect is a key pest of sugarcane in western, eastern and southern Africa (Atkinson, 1980; Conlong, 2001). A large proportion of natural hosts of *E. saccharina* is made up of wetland sedges (Cyperaceae, Juncaceae, Typhaceae) (Atkinson, 1979; 1980; Conlong, 2001; Mazodze & Conlong, 2003) in addition to a number of grasses (Poaceae). The stemborer was first described from sugarcane in Sierra Leone over 100 years ago (Walker, 1865), has since spread and established throughout much of sub-Saharan Africa (Girling, 1972; Waiyaki, 1974; Atkinson, 1980; Betbeder-Matibet, 1981; Maes, 1998).

Maize stalk borer *Busseola fusca* Fuller, which is indigenous to Africa, is a serious pest of cereal crops and a major pest of maize and sorghum in all African countries south of the Sahara (Van Rensburg *et al.*, 1987; Harris, 1989; Abate *et al.*, 2000). It feeds on several other wild grasses and crops (Haile & Hofsvang 2002; Midega & Khan, 2003). The extent of

damage and associated crop losses due to *B. fusca* vary across different crops and agro-ecological zones (De Groote, 2002). In West Africa, *B. fusca* is of economic importance in the dry ecological zones and only thrives on sorghum (Schulthess *et al.*, 1997). According to Kfir *et al.* (2002) and Ong'amo *et al.* (2006), it is a pest at altitudes above 600m in South-Eastern Africa and above 1000m in East Africa. *B. fusca* is found in cool high altitude areas with an altitude of over 1140 meters above sea level (Kfir *et al.*, 2002; Ong'amo *et al.*, 2006). In Burundi, it was the only species detected in the high-altitude (2100m) location of Gisozi, with 80% infestation in maize and sorghum. It is a predominant borer species, accounting for more than 90% of all species in Cameroon and the Democratic Republic of Congo. *Busseola fusca* is dominant in Zimbabwe, in areas with an altitude above 900 meters (Cugala & Omwega, 2001), highvelds (>1200m) and middlevelds (600m -1200m) (CYMMT, 2011).

The pink stemborer *Sesamia calamistis* Tams & Bowden is the most widely distributed species (in Africa) and regarded as a potential pest in the sugarcane plantations in Ethiopia (Assefa *et al.*, 2006). Studies on *S. calamistis* showed that it has five to six continuous and overlapping generations per year, which are highly dependent on temperature (Carnegie & Leslie, 1991). Damage caused by *S. calamistis* larvae reduces yield by killing the growing point (dead heart), causing leaf senescence, reduced translocation and lodging due to weakened stems (Boseque-Perez & Mareck, 1990). It is a cosmopolitan species, mostly prevalent in medium and low elevation areas of about 700 to 900 (Kfir *et al.*, 2002; Ong'amo *et al.*, 2006). Ntiri *et al.*, 2016 also documented that *S. calamistis* is present in low numbers at different elevations.

1.2.2 Biology and General life cycle

The eggs of *Chilo partellus* are oval-shaped, flattened and laid in clusters, with total fecundity averaging 500-722 eggs/female (Plate 1a) and are usually found on the lower surfaces of the leaves, often near the midrib (Taneja & Nwanze, 1990; Sharma & Nwanze, 1997, Ofomata *et al.* 2000). The larvae have a cream to pink coloration, with dark spots along the dorsal surface and a brown head capsule (Hutchison *et al.*, 2008) (Plate 1b). The pupae is cylindrical in shape and dark brown (Plate 1c). The size of female pupa is slightly larger than that of the male. The adult moth has pale brown wingspan and forewings with dark brown scales forming a line on the tips of the wings and hind wings are usually pale yellow with females having much lighter wings than the males (Plate 1d) (Balaiji & Manvendra, 2013).

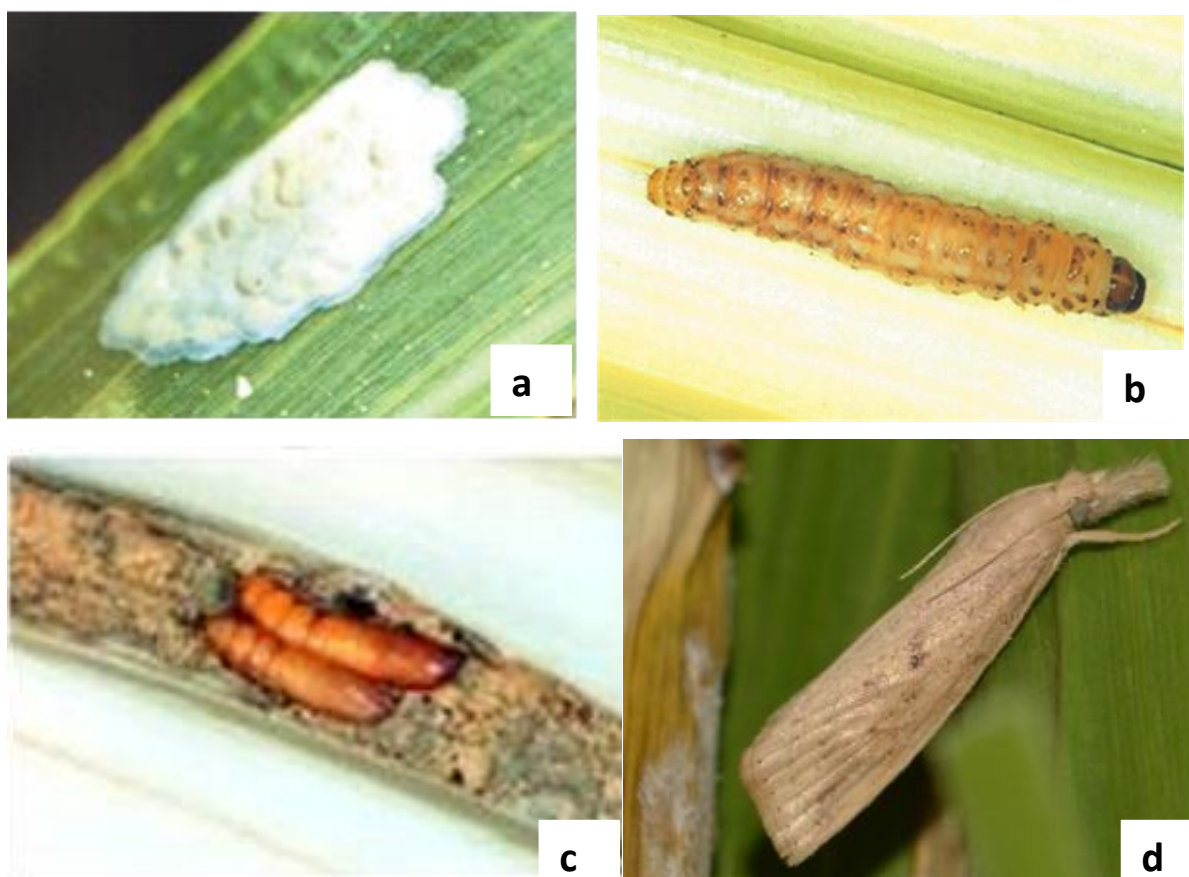


Plate 1: Different life stages of *Chilo partellus* a) Eggs b) Larva c) Pupa d) Adult moth

Eldana saccharina adult males mate with females, then females lay an average of 432, seldom up to 1000 eggs (Plate 2a) depending on temperature, nutritional status and biotype (Way, 1994). Since majority of the eggs are fertile, it confirms its potential as a crop pest, whose populations may increase if proper control is not effected (Walton & Conlong, 2016). Larvae (Plate 2b) hatching from the eggs disperse from the oviposition sites and feed initially on cane leaves and under leaf sheaths on organic matter and when the larvae are sufficiently robust, they start boring into the stalk and feed on the internal tissues (Atkinson, 1979 & Carnegie, 1974). The larvae is light brown to dark grey coloured with brown pinacula (Plate 2b), covered with very small dark-coloured spots and the head capsule is reddish brown to dark brown (Meijerman & Ulenberg, 1998). A pupae is brownish with a dotted abdomen (Plate 2c) and pupation period takes about 7-14 days, with up to six generations developing per year (Horton *et al.*, 2000). According to Maes (1998), the moths have longitudinal veins which are brown against a lighter background and there are two distinct dark spots in the anterior half of the fore wings (Plate 2d).

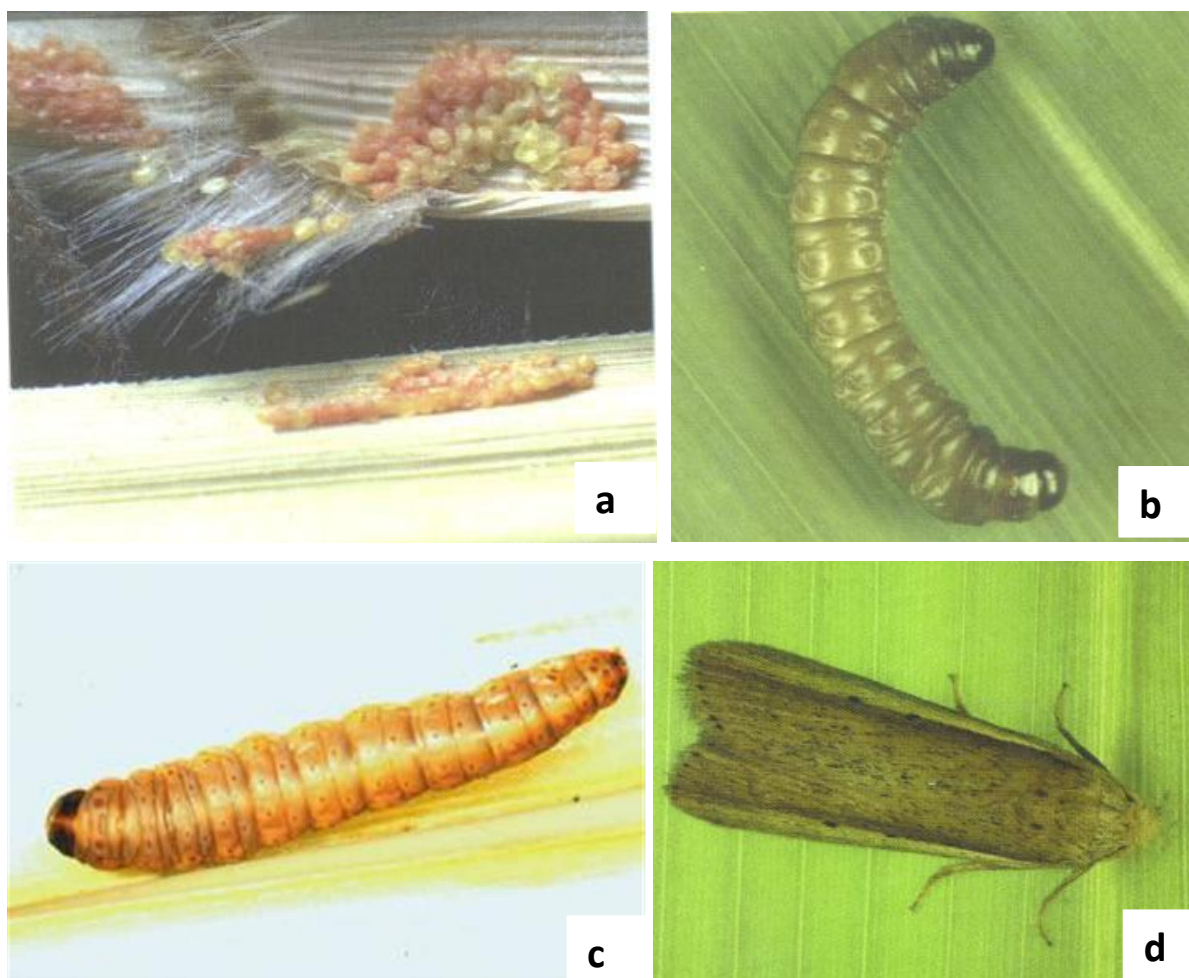


Plate 2: Different life stages of *Eldana saccharina* a) Eggs b) Larva c) Pupa d) Adult moth

Busseola fusca females oviposit a highly variable number (from 100 up to 800) of round and flattened eggs in batches (Plate 3a) (Unnithan, 1987; Van Rensburg, 1987; Kruger *et al.*, 2012). The batches are laid behind the vertical edges of leaf sheaths of pre-tasseling plants and also, but rarely, underneath the outer husk leaves of ears (Calatayud *et al.*, 2014). Larvae *B. fusca* (Plate 3b) are about 40 mm long when fully grown, normally with a creamy white colour and distinctive grey tinge. The heads have a brown head capsule with hooks on the prolegs (Harris & Nwanze, 1992). The adult moth emerges after a pupal period of 7–14 days from a hole that they produced before pupation (Plate 3c; d). Adults mate soon after emergence and under favorable conditions, the life cycle can be completed in 7–8 weeks (Ong'amo *et al.*, 2016).

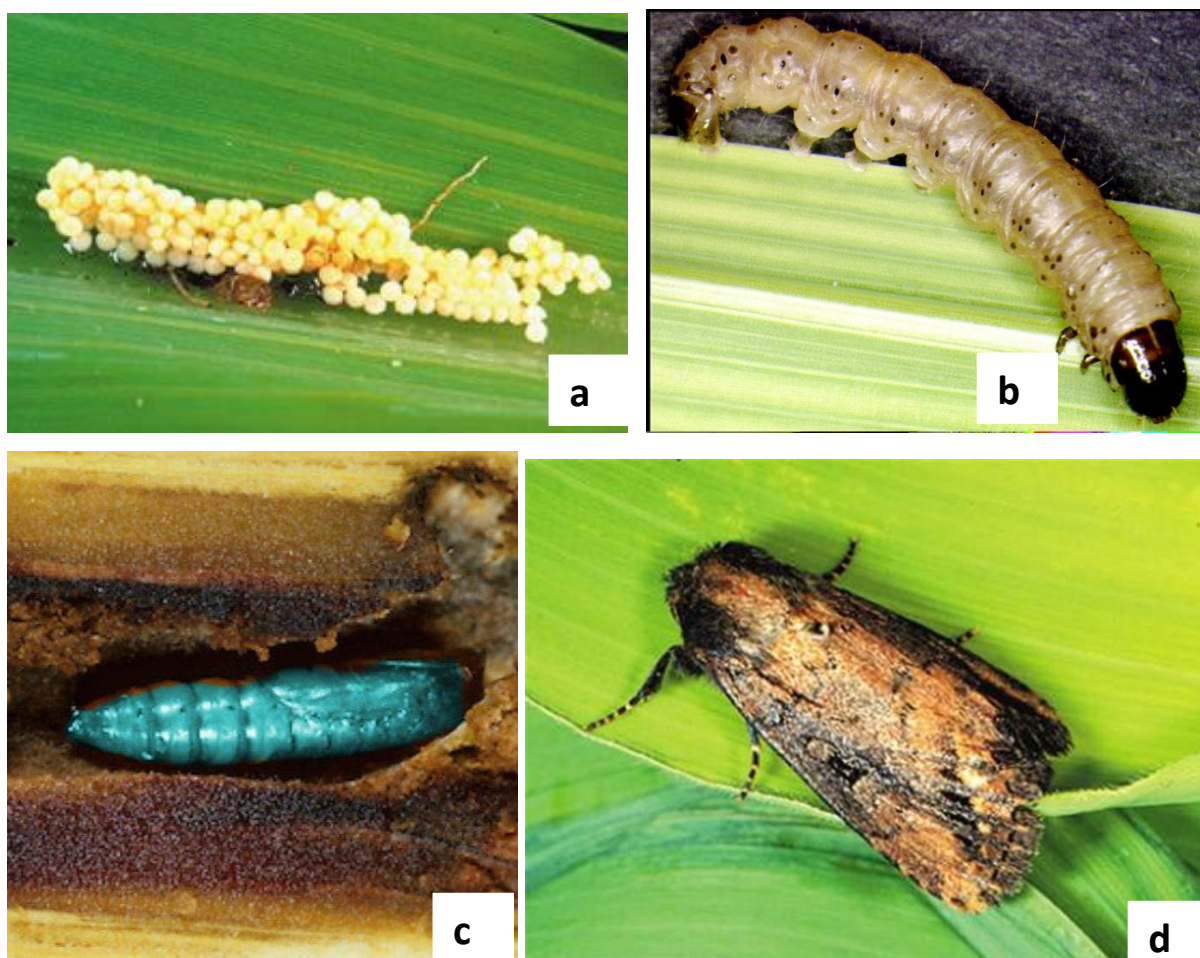


Plate 3: Different life stages of *Busseola fusca* a) Eggs b) Larva c) Pupa d) Adult moth

Sesamia calamistis adults have got pale-brownish forewings, variable but generally inconspicuous darker markings along the margin and an overall silky appearance (Ong'amo *et al.*, 2016). They lay eggs which are white at first but change to pale orange as they approach hatching. They have a flat top shape and are usually arranged in 2 to 4 contiguous rows (Plate 4a) between the lower leaf sheaths and stems (Mengistu *et al.*, 2009). According to Shanower *et al.* (1993), the number of days for eggs to hatch average 6.3 at a temperature of 30°C under field conditions. The larva of *S. calamistis* is a yellowish-pink colored caterpillar with more or less distinct black spots along the body representing the position of the spiracles (Plate 3b). The larvae, which are pinkish in color (Plate 4b), hence pink stemborer, take 4-6 weeks to complete their developmental stage (Ong'amo *et al.*, 2016). The pupa is pale brown (Plate 4c) and immobile but shows wiggling movement of the abdomen when touched. Eclosion takes 5-14 days at benign temperatures (Polaszek, 1998) to produce adult moths (Plate 4d)). Different authors have reported that the number of eggs laid per female could vary depending on host plant preference, larval food quality and climatic

conditions like temperature (Harris, 1962; Kaufmann, 1983; Bosque-Perez & Dabrowski, 1989; Shanower *et al.*, 1993).

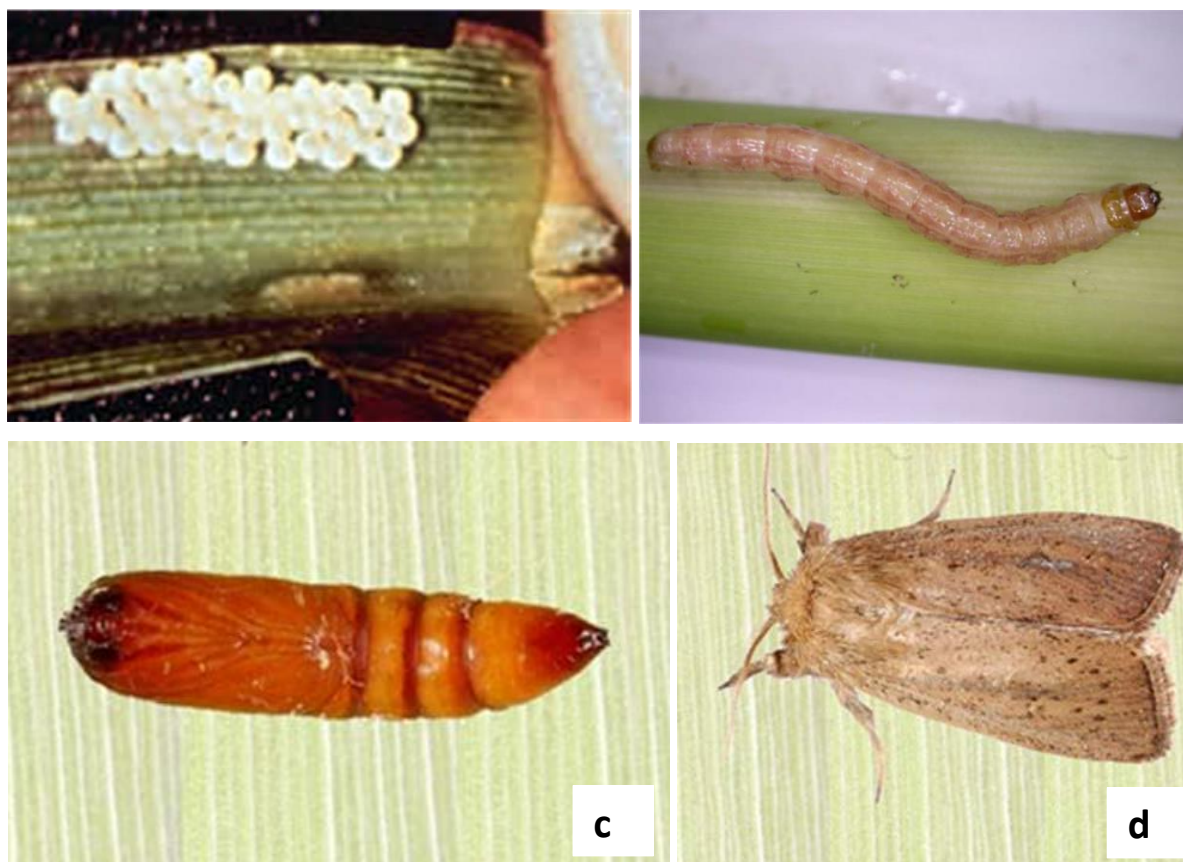


Plate 4: Different life stages of *Sesamia calamistis* a) Eggs b) Larva c) Pupa d) Adult moth

In about 2-3 days after emergence, adult males mate with females and a single moth may lay up to 800 eggs. The eggs hatch within 4-8 days after oviposition at benign temperatures. This highly contributes to increased propagule, pest pressure and consequent stemborer infestations. Generally, the first instar larvae will migrate from where it emerged to the leaf whorl where it will feed on young leaf tissue. *Sesamia calamistis* young instar larvae may also bore directly into the stem or initially feed on basal leaf sheaths for 2-3 days before stem tunneling. Older larvae for all stemborers tunnel into the stem tissue and feed internally. Depending on the species, larval stage may last 25-58 days with up to six instars. Pupation will normally take place 5-14 days after which adult moths eclose (Harris, 1990; Holloway, 1998; Maes, 1998; Polaszek, 1998).

During off seasons, natural habitats have been reported to play an important role in providing overwintering refugia for lepidopteran stemborers and their parasitoids. As a consequence, natural landscapes serve as sites of inoculum for stemborer re-infestations into field sites the next season. Likewise, these sites will also conserve stocks of natural enemies,

important in effecting control in the next season. For this reason parasitism of these borers was thought to occur mainly in natural habitats (Schulthess *et al.* 1997).

1.2.3 Damages and losses caused by stemborers

Larvae is the destructive stage in the stemborer life cycle. After hatching, the larvae tunnels itself into the stem of a host creating some holes. Young larvae also feed on lower palisade cells resulting in window panes (holes in the leaf surface). Leaf feeding by stemborers results in the reduction of plant photosynthetic tissue area. Larvae can feed on basal meristems of young plants resulting in the formation of 'dead hearts' which cause death of plants like maize while sorghum, millets, and rice compensate by tillering (Sithole, 1989). Stem tunnelling destroys the central pith and conductive tissues causing a reduction in nutrient uptake, with consequent interruption of grain filling. Secondary effects of stem tunnelling include peduncle breakage, poor pollen production, interruption of fertilization, stem breakage and ear drop. Injured plant parts also create avenues for entry by pathogens (Sithole, 1989; Seshu, 1989; De Groote, 2002; De Groote *et al.*, 2003).

The severity and nature of damage further depends on the stemborer species, crop growth stage, number of larvae feeding on the plant, plants reaction to borer feeding, prevailing environmental conditions and agronomic practices. First and second instar larvae of *B. fusca* and *Chilo* species causes foliar damage during plant whorl stage. The larvae scraps off epidermal and parenchyma cells on one side of the leaf leaving it intact but transparent. The resultant lesions become windows when the leaves unfold. Young larvae may also feed through the leaves of the intact whorl, which give an array of shot-holes when unfolded. Third to eighth instars tunnel into the stems through the midrib. *Sesamia calamistis* and *E. saccharina* larvae do not feed on plant leaves but instead penetrate plant stems shortly after egg hatch (Bosque-Pérez & Schulthess, 1998). Larvae may also attack and damage maize cobs, with direct consequences on yield quantity and quality. Losses have been reported to range from 5% to 75% of potential crop yield (De Groote, 2002; Kfir *et al.*, 2002; Kipkoech *et al.*, 2006; Moolman *et al.*, 2013) and yield losses in Africa could be as high as 80% for maize (Bhat & Baba, 2007).

1.2.4 Control of stemborers

Different methods have been used for the control of cereal stemborer species such as cultural, chemical, biological, host plant resistance and genetic engineering (Mailafiya, 2009). Cultural methods and practices that are used to control stemborers include appropriate crop residue disposal, planting date manipulation, host resistance, destruction of volunteer and alternative host plants, tillage practices, crop rotation, intercropping, stalk shredding and deep ploughing, which increase the mortality of diapaused larvae thereby reducing stemborer propagule populations in the following cropping season (Oloo & Ogedah, 1990; Skovgard & Päs, 1996; Gupta *et al.*, 2010). In large scale productions, cultural control is constrained by high labour demand and lack of management capabilities of some farmers (Nwanze & Mueller, 1989), thus renders the strategy logistically not feasible.

Chemical control on the other hand involves the use of pesticides to control stemborers. These chemical insecticides comprise methyl and dimethyl carbamates of heterocyclic compounds, organochlorines, organophosphates, and all have reportedly been used in some sub-Saharan countries for controlling lepidopterous stemborers (Sylvian *et al.*, 2015). Dusts and sprays are applied down the funnel of young plants to kill the emerging and first instar larva already feeding on host plants. Dusts of chemicals such as endosulfan, malathion and carbaryl have been documented to be efficacious against stemborer larvae (Van den Berg & Nur, 1998). However, insecticides have a limitation that they may only be effective against young but not older larvae, since the latter can penetrate the stalks thereby preventing insecticide contact, unless systemic pesticides are used. Overlapping *C. partellus* generations also create a complication when pesticides targeting stemborer larvae are used as there will be more than one breeding population. Coupled with stemborer high fecundity, this creates high overlapping stemborer infestations throughout the season thereby rendering insecticide application not that efficacious (Kfir *et al.*, 1989). Chemical control, however, gives a quick way of controlling pest populations in the event of outbreaks, compared to other alternative methods. The advantages of insecticides include increased food production, increased profits for farmers and the prevention of diseases (Sarwar, 2015). Insecticides protect buildings and other wooden structures from damage by termites and wood boring insects (Aktar *et al.*, 2009). However, pest management using pesticides also has some setbacks. Disadvantages of the use of chemicals include development of pesticide resistance, high costs, its costs to the environment and biodiversity at large and pesticide resistance among moths; especially those which are multivoltine and its inability to reach tunnelling stemborers (in the case of non-systemic pesticides) (Hill, 1987; Kfir, 1998; Overholt, 1998).

On the other hand, the general public is moving from synthetic towards organically produced products (Seshu, 1998; Chidawanyika *et al.*, 2012), probably because of health risks associated with pesticide residues in plants, plant based products and occupational exposure. Consequently, it is important to find sustainable alternative novel ways of controlling stemborer pest populations.

Biological control refers to any environmentally sound and effective means of reducing or mitigating insect pests and their effects through the use of living organisms/natural 'enemies'. It involves the use of control agents comprising predators, parasitoids and pathogens. Predators are entomophagous species that generally consume more than one prey individual to complete their development. Several indigenous stemborer natural enemies have been reported in Kenya, of which predators have been reported to cause high stemborer mortality in some regions, but their abundance is highly dependent on location and timing (seasonal fluctuations) (Bonhof *et al.*, 1997). Predators are an important component of Integrated Pest Management (IPM). The major predators of stemborers include ants (*Camponotus* spp., *Crematogaster* spp., *Tetramorium guineense* F., *Pheidole megacephala* F., *Cardio condyla badonei* Arnold and *C. emeryi* Forel.), earwigs (*Diaperasticus erythrocephala* Olivier), spiders (Thomisidae), coccinellids (*Cheilomenes propinqua* (Mulsant) and *Cheilomenes sulphurea* Olivier), cockroaches (*Blatella* spp.), praying mantis and staphylinids (*Paederus sabaeus* Erichson) (Bonhof *et al.*, 1997; Overholt, 1998). Ants (Hymenoptera: Formicidae) are considered the most important predators of stemborers in maize fields as they attack all stages of their life cycle, and are among the few predators preying on larvae and pupae (Bonhoff, 1998; 2000).

A parasitoid is an insect whose larval stage feeds exclusively on another insect, its host, and eventually kills it (Godfray, 1994). Parasitoids reduce overwintering refugia through parasitization of larvae in the late cropping season (Van den Berg & Ebenebe, 2001). Most parasitoids are Hymenopterans and Dipterans with most species belonging to Braconidae, Tachnidae and Ichneumonidae (Kfir, 1990, 1995; Polaszek, 1998 & Zhou *et al.*, 2003). The most commonly reported indigenous parasitoids of stemborers in Kenya are the egg parasitoids *Telenomus* species (Scelionidae) and *Trichogramma* species (Trichogrammatidae), the larval parasitoids *Cotesia sesamiae*, *Cotesia flavipes* and *Goniozus indicus* Ashmead, and pupal parasitoids *Pediobius furvus* (Gahan) (Hymenoptera: Eulophidae) and *Dentichasmias busseolae* Heinrich (Hymenoptera: Ichneumonidae) (Mohyuddin & Greathead, 1970; Mathez, 1972; Oloo & Ogedah, 1990; Bonhof *et al.*, 1997; Zhou *et al.*, 2003). The adult females lay their eggs either directly on the host or in the

vicinity of the host. Hymenopteran parasitoids such as *Cotesia* species lay eggs directly into the body of a host using their specialised ovipositors for stinging and depositing eggs (Godfray, 1994).

Pathogens can have an important influence on the natural control of insect populations in the field. Associations between microbes and insects range from mutualistic to pathogenic. The latter is thus used in the control of insect pest populations. Epizootics of insect pathogens may result in high mortality of insects, leading to a reduction in their numbers (Tanada, 1961; Entwistle *et al.*, 1977). A product based on *Bacillus* spp. was the first microbial control agent to be registered in 1948 (Dent, 1991). *Bacillus thuringiensis* was reported to significantly lower the population of stemborers in Kenya with a consequent increase in crop yield (Brownbridge, 1991). *Bacillus thuringiensis* is fast acting, acts as a stomach poison and is non-persistent (Dent, 1991). Entomopathogenic fungus *Beauveria bassiana* (Balsamo) (Fungi Imperfecti), microsporidian *Nosema partelli* Walters & Kfir (Protozoa: Microsporidia: Nosematidae) have also been used for *B. fusca* and *C. partellus* respectively (Walters & Kfir, 1993). Bacterial and fungal microorganisms have been reported to be highly prevalent in field populations of crop borers on sorghum, maize and cowpea in Kenya (Odindo *et al.*, 1989).

Host plant resistance is an important component of any strategy aimed at reducing the economic impact of crop pests and it is based on three factors: antibiosis, antixenosis, and tolerance (Dent, 1991). Antibiosis is based on the plant inhibiting the development of the feeding insect, while antixenosis acts by influencing adult and/or larval behaviour on the surface of the host plant (Sosa, 1988). Reduced preference for oviposition, reduced feeding due to the presence of some chemicals in the plant, reduced ability to be tunnelled and plant's tolerance to leaf damage, dead heart and stem tunnelling are some of the mechanisms of host plant resistance (Seshu, 1998).

Genetically modified (GM) plants with insecticidal properties are widely grown in other parts of the world and are now also prevalent in Africa (Bagnara, 2000; Van der Berg, 2013). In 1997, South Africa became the first country to commercially produce GM crops in Africa (Van den Berg, 2012). *Bt* maize is one of the GM plants which were initially used for the control of some stemborers in north America (Ostlie *et al.*, 1997 & Archer *et al.*, 2001). *Bt* maize contains a soil bacterium *Bacillus thuringiensis* which produces a Cry protein, hence *Bt* maize containing a Cry1 and Cry2 transgene are most effective against lepidopteran larvae (Van de Berg *et al.*, 2013 & James 2012). Commercialization of GM plants including *Bt* maize (MON810) and *Bt*11 in South Africa was later done in order to control *B. fusca*

(Van Rensburg, 1998, 2001). MON810 has also been reported to have a control efficiency of 100% on *C. partellus* (Van Rensburg, 1998 & Singh *et al.*, 2005) and *S. calamistis* (Van der Berg & Van Wyk, 2007). Adoption of GM over non-GM maize increases of farmers' income through savings on pesticides and protection from yield loss due to target pest species (Gouse *et al.*, 2005). According to Van der Berg (2012), farmers in South Africa indicated that they did not need to scout their fields for pests with the adoption of GM crop technology. Despite its advantages, continuous use of transgenics or GM plants producing the same insecticidal *Bt* toxin increases selection pressure resulting in an increase in the development of resistance to the *Bt* proteins in an insect population exposed to them (Ferre & Van Rie, 2002). The use of GM crops however remains controversial, especially in Africa, due to the issues on ethics, and especially the uncertainties about their harmful effects on human health and the environment (IUCN, 2004).

While stemborer diversity studies have been done extensively elsewhere, such data remains lacking in Botswana. With the increase in average temperature and variability with climate change (IPCC 2014), invasive species increase and their likelihood of invading new areas is also enhanced. Moreover, with the increase in movement of people and trade in plants and plant products, there is increased probability of new species being introduced in new non-native areas. In this thesis, I looked at the tritrophic interactions across plant host-stemborer and parasitoids. Looking into the diversity of plants hosting stemborers is important in planning management strategies. Similarly, the diversity of the stemborers themselves ought to be documented as this affects trade between different countries. Furthermore, the diversity of 'resident' natural enemies of stemborers has to be established as this is critical for pest management. This data is important in planning pest management programs, which are important in pest risk analysis and is critical in enforcing quarantine regulations.

1.2.5 References

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CHAPTER TWO

Diversity, distribution and relationship of lepidopteran stemborer species and their host plants in Botswana

2.1 Introduction

In sub-Saharan Africa, lepidopteran stemborers are the main insect pests attacking cereal crops (Harris & Nwanze, 1992; Kfir *et al.* 2002) for both small-scale and large-scale farmers. Losses have been reported to range from 5% to 75% of potential crop yield (De Groote, 2002; Kfir, 2002; Kipkoech *et al.*, 2006; Moolman *et al.*, 2013). Stemborers can inflict widespread damage at various developmental stages of cereal crops (Addo-Bediako & Thanguane, 2012). Damages to cereal crops have been reported to disrupt physiological growth, panicle emergence and grain development, resulting in decreased crop productivity (Lynch, 1980; Addo-Bediako & Thanguane, 2012). Despite being the major pests of cereal crops, stemborers have always lived in indigenous, or wild hosts of families Poaceae, Typhaceae and Cyperaceae (Le Ru *et al.*, 2006). Previous surveys on stemborer diversity in Africa have revealed contrasting results. For example, a survey by Le Ru *et al.* (2006) in Eritrea, Ethiopia, Kenya, Madagascar, Mozambique, Tanzania, Uganda and Zanzibar recorded higher stemborer species diversity in wild versus cultivated habitats, while Moolman *et al.*, (2013) reported otherwise.

The main lepidopteran stemborer species in Africa include spotted stemborer (*Chilo partellus* Swinhoe), African maize stemborer (*Busseola fusca* Fuller), pink stemborer (*Sesamia calamistis* Tams & Bowden), sugarcane stemborer (*Eldana saccharina* Walker) and coastal stemborer (*Chilo orichalcociliellus* Strand) (Kfir *et al.*, 2002; Assefa *et al.*, 2008; Addo-Bediako & Thanguane, 2012; Moolman *et al.*, 2014). Amongst these stemborers species, *B. fusca*, *S. calamistis*, *E. saccharina* and *C. orichalcociliellus* are indigenous to Africa whilst *C. partellus* is native to Asia. *Chilo partellus* was accidentally introduced into Southern Africa around 1930's and later spread to other countries in Eastern, Western and Southern Africa, thereby displacing indigenous stemborers (Van Rensburg *et al.* 1987; Harris 1990; Overholt *et al.* 1994; Maes 1998; Kfir *et al.* 2002; Mutamiswa *et al.* 2017).

Lepidopteran stemborer species relative abundance, distribution and composition are significant aspects to be considered in determining the borer species present in certain localities (Banwo *et al.*, 2001). Several studies on diversity and distribution of stemborers have been conducted in Zimbabwe (Chinwada & Overholt, 2001; Marveni *et al.*, 2013), South Africa (Kruger, 2005; Moolman *et al.*, 2014), Ethiopia (Assefa *et al.*, 2006), Cameroon (Ndemah *et al.*, 2007), Uganda (Matama-Kauma *et al.*, 2007; 2008), Kenya (Mailafiya *et al.*, 2009), Togo (Kodjo *et al.*, 2013), and Mozambique (Le Ru *et al.* 2006 also see Moolman *et al.* 2014). Nevertheless, such valuable data is still lacking for Botswana. Moreover, the detection and characterization of genetic differentiation among stemborer/insect populations

is a key step for improvement of any pest management practice such as biotype ecology and evolution, biological control, resistance management and insect–plant interactions (Kourti 2006, Margaritopoulos *et al.*, 2007, De La Poza *et al.*, 2008, Hoy, 2013, Agunbiade *et al.*, 2014). Several techniques such as mitochondrial DNA (MtDNA) comparisons have been used in population genetics studies of many insects (Kourti, 2006 & De La Poza *et al.*, 2008). MtDNA analysis is capable of revealing cryptic lineages representing distinct species within geographically widespread and apparently morphologically homogeneous organisms (Scheffer, 2000). It has been used to reconstruct the phylogenetic and phylogeographic relationships in indigenous stemborers (King *et al.*, 2002; Sezonlin *et al.*, 2006; Assefa *et al.*, 2006) and their associated parasitoids (Dittrich *et al.*, 2006; Muirhead *et al.*, 2006). It has proven powerful for genealogical and evolutionary studies of animal population and it is the most commonly employed marker for determining genetic relationships among populations (Sperling *et al.*, 1999; Scheffer, 2000). By contrast, such genetic and phylogenetic relatedness in local stemborer species is lacking in Botswana.

Little is known about the abundance, diversity, distribution of stemborers and associated host plants in Botswana. Moreover, genetic relatedness of the ‘resident’ stemborer species is currently unknown. This chapter catalogued the diversity and distribution of stemborers in Botswana in their wild and cultivated habitats, to help explain the potential role of wild habitats/hosts as a reservoir/source or an alternate habitat for stemborer pests in Botswana. The study also characterised the phylogenetic relationship across different stemborer species recorded in Botswana.

2.2 Materials and methods

2.2.1 Field collection

Lepidopteran stemborer species survey was conducted in all the 10 districts of Botswana (Fig. 1) during November to April for both 2014-2015 and 2015-2016 austral summer. Cultivated cereal crops of family Poaceae and wild host plants of families Typhaceae, Poaceae and Cyperaceae were sampled. Sampling was done in and around crops, open patches along forest roads, on banks of streams or rivers and in swamps. For cultivated crops; *Sorghum bicolor* (sorghum), *Sorghum bicolor* L. Moench (sweet sorghum), *Saccharum officinarum* L., *Pennisetum glaucum* (L.) R.Br. and *Zea mays* fields were sampled in each district. Crop fields were randomly selected with a total of 10 fields being sampled per district. Each field was divided into 4 quadrants and 10 plants randomly picked per quadrant.

In wild host plants, a biased sampling procedure was applied to increase the probability of finding stemborers as studies have shown a much lower density of borers in wild host plants than in cultivated crops (Gounou & Schulthess, 2004; Mailafiya *et al.*, 2009; Moolman *et al.* 2014). At each sampling sites, all selected hosts were inspected for symptoms of borer damage such as scarified or dried leaves and shoots (dead hearts), and/or frass or holes bored in the stems. Plants showing signs of stemborer damage were dissected and larvae or pupae found were collected. Host plant species from which the larva or pupa was collected from were recorded. Recovered larva or pupa were individually placed in 30mL plastic vials with perforated meshed lids containing artificial diet supplied by South African Sugarcane Research Institute (SASRI). Each locality where wild and cultivated hosts were sampled, was geo-referenced using a portable Garmin GPSMAP 62st Mapping Handheld Geographic Positioning System (GPS) meter.

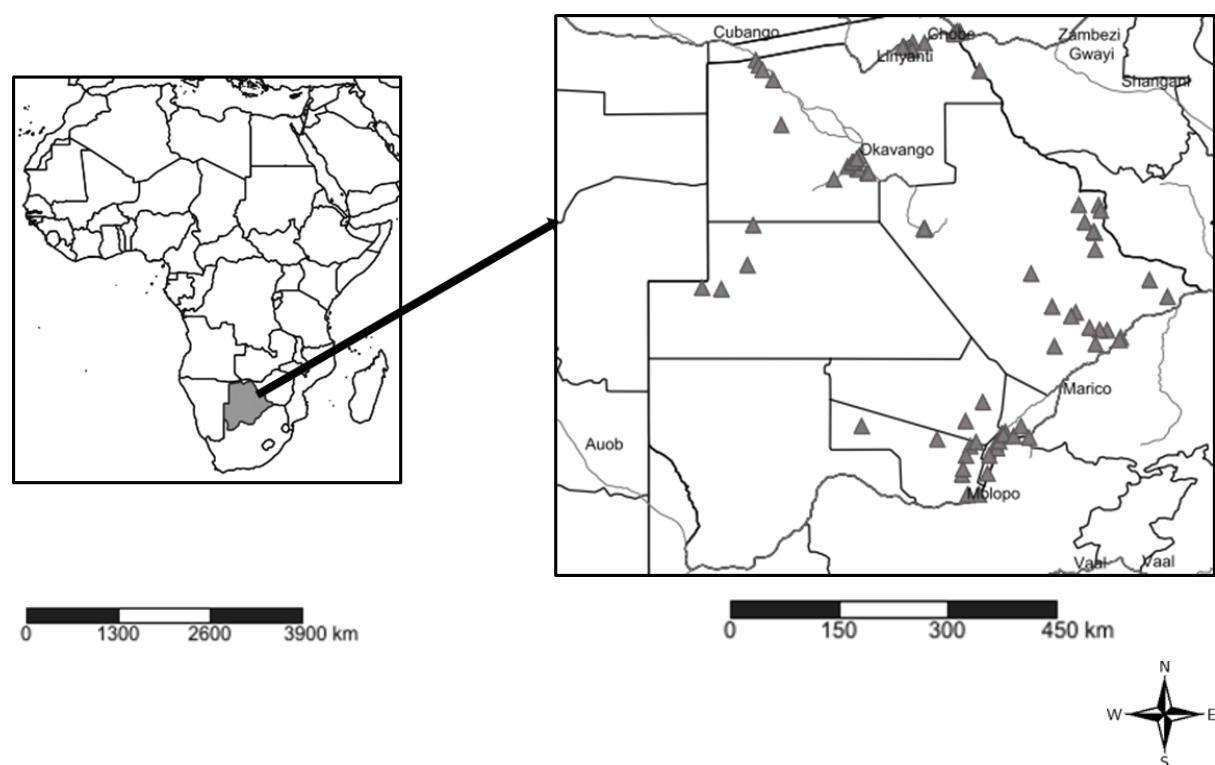


Figure 1: Map of Botswana showing cultivated and wild habitats surveyed for stemborers in the summers (November-April) of 2014/2015 and 2015/2016. The triangles represent the areas which were sampled.

2.2.2 Stemborer lab inoculation

Larvae and pupae collected from the fields were placed in perforated ziplock bags and placed in a cooler box and then taken to the laboratory where they were incubated at $25\pm1^{\circ}\text{C}$, $65\pm10\%$ relative humidity and natural photoperiodic conditions of L12:D12 (Onyango & Ochieng'-Odero, 1994, Mailafiya *et al.*, 2009) in Memmert climate chambers (Memmert HPP 260, Memmert GmbH + Co.KG, Germany) until eclosion. Moth emergence was recorded daily until all the pupae eclosed. Abundance of stemborers was calculated using the formula below; as outlined in Dejen *et al.* (2013) and Rahaman *et al.* (2014):

Relative abundance of each stemborer sp = $\frac{\text{Total number of individuals of each sp}}{\text{Total number of individuals of all stemborers}} \times 100$

Stemborer diversity index was calculated using $H' = -\sum_{i=1}^S p_i \ln p_i$, where H is the Shannon's diversity index, S is the total number of species in the community (richness) and P_i is the proportion of S made up of the i^{th} species.

2.2.3 Morphological identification of stemborers and their host plants

All collected life stages of stemborers were reared to adult stage, pinned and sent to the Division of African Insect Science for Food and Health, International Centre for Insect Physiology and Ecology [ICIPE], Nairobi, Kenya for morphological identification. Moths were identified using male genitalia morphology. Voucher specimens were deposited at ICIPE museum and at Botswana International University of Science and Technology. Host plants were identified morphologically in the field, and for others which the author failed to identify, they were collected, packed and labelled before sending to the Department of National Museum & Monuments, Botswana for further identification. Identified stemborers were grouped with their respective host plants from each vegetation type and geographic region for analysis.

2.2.4 Molecular identification and evolutionary relationship of stemborers

Genomic DNA was extracted from adult and larval thoracic tissue using the KAPA Express DNA Extraction kit (KapaBiosystems, South Africa) (as in Hunt, 1997). Polymerase Chain Reaction (PCR) amplification for cytochrome oxidase I (COI) was performed in 25 μl

volume containing 5µl 5X KAPA2G buffer, 5µl 5X KAPA enhancer 1, 0.5µl 10mM dNTP, 0.25µl of each PCR primer, 0.1µl KAPA2G Robust DNA polymerase and 1µl of genomic DNA. A pair of primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and CO1R (C1-2353) (5'-GCTCGTGTATCAACGTCTATTCC-3') were used in this study. PCR was performed using a Perkin Elmer GeneAmp PCR System 2400, under the following conditions: 94°C for 2 minutes (min), 35 cycles of (94°C for 30 seconds (s), 50-55°C for 50 s, 72°C for 90 s) and 72°C for 10 min. Amplified DNA was purified using the DNA Clean and Concentrator kit (Zymo Research, USA). Resulting PCR products were then cycle sequenced using an ABI PRISM BigDye Terminator version 3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, CA) following the manufacturer's recommended specifications. Completed reactions were cleaned using ethanol/ethylenediaminetetraacetic acid (EDTA) precipitation and sequences were visualized on an ABI 3500 Genetic Analyzer (Applied Biosystems). Editing and assembling DNA sequence chromatograms was completed using Staden package (Staden, 1996). The partial mtDNA COI sequences for the stemborer specimens from our surveys were then aligned with sequences of positively identified stemborers using ClustalX (Thompson *et al.*, 1997). All datasets were manually corrected and trimmed by deleting the flanking regions and abnormal sequences to achieve a final data set using BioEdit sequence alignment (Hall, 1999). A phylogenetic tree was generated using the neighbour-joining method (Saitou & Nei 1987) in MEGA 6 (Tamura *et al.*, 2013). Clade support was estimated using 10,000 bootstrap replicates. The genetic relationship between the 28 sample sequences and their relationship was then assessed by reconstructing a phylogenetic tree.

2.3 Results

2.3.1 Stemborer diversity, distribution and abundance

A total of 1497 larvae and 228 pupae were collected from five cultivated host plant habitats (sorghum, sweet sorghum, *Saccharum officinarum*, *Pennisetum glaucum* and maize) and nine wild hosts (*Cyperus dives*, *Chrysopogon nigrifolius* (Vetiver), *Schoenoplectus corymbosus*, *Cyperus papyrus*, *Vossia cuspidata*, *Eriochloa fatmensis*, *Typha latifolia*, *Echinochloa pyramidalis* and *Oryza longistaminata*) during this survey (Table 1 & 2). In both seasons, no eggs and adults were recorded, apart from larvae and pupae which were taken for incubation. Interestingly, I found at least one species of stemborer in all districts except for Kgalagadi district. The stemborer families recorded in this survey

included Noctuidae, Crambidae, Pyralidae, Tortricidae and Schoenobiinae with Noctuidae having the largest number of species (Table 1 & 2). Fifteen stemborer species were found infesting the above mentioned hosts. Of the collected specimens, six were identified to species level, four to genera and five to family level. Species collected included *C. partellus*, *S. calamistis*, and *E. saccharina* which were have been previously reported in Botswana. However, I also report, for the first time, a new *Tortricidae* sp1, *Schoenobiinae*, *Sesamia* nov sp 41 nr *nonagrioides*, *Sesamia nonagrioides*, *Sesamia jansei*, *Sesamia perplexa* es nov sp 4, *Sesamia* sp., *Sesamia* sp nr *perplexa*, *Sesamia perplexa* and *Tortricidae* sp., *Conicofrontia* nov sp 1 nr *sesamoides*, *Mythimna loreyi* and *Tortricidae* sp 3, *Sesamia calamistis* and *Tortricidae* sp2 that were found in both wild and cultivated hosts (see Table 1 & 2). Stemborer diversity index for the wild host plant species was found to be higher than that of the cultivated crops ($H= 1.83$ and $H= 0.46$ respectively). *Chilo partellus* and *S. calamistis* were the most abundant stemborer species (at 62.1% and 11%) respectively, compared to other species e.g. *M. loreyi* and *Sesamia perplexa* es nov sp4 that were the least, at 0.7% each (Fig. 2).

Table 1: Stemborer species and their cultivated host plants in Botswana.

District	Location	GPS coordinates	Stemborer species	Family	Host plant
Chobe	Kasane	S 17°79.144'; E 25°20.573' 924masl	<i>Sesamia calamistis</i>	Noctuidae	Grain sorghum
		S 17°79.144'; E 25°20.573' 924masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Mabele	S 17°98.124'; E 24°64.452' 935masl	<i>Sesamia calamistis</i>	Noctuidae	Sweet sorghum
		S 17°98.124'; E 24°64.452' 935masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
		S 17°98.124'; E 24°64.452' 935masl	<i>Conicofrontia nov sp1 nr sesamoides</i>	Noctuidae	Sweet sorghum
	Ndumba	S 17°97.402'; E 24°44.734' 932masl	<i>Sesamia calamistis</i>	Noctuidae	Sweet sorghum
		S 17°97.402'; E 24°44.734' 932masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Chobe farms Pandamatenga	S 17°79.332'; E 25°18.863' 944masl S 18°44.968'; E 25°57.470' 1066masl	<i>Chilo partellus</i> <i>Chilo partellus</i>	Crambidae Crambidae	<i>Zea mays</i> <i>Zea mays</i>
Ngamiland	Maun	S 19°99.686'; E 23°43.182' 931masl	<i>Sesamia calamistis</i>	Noctuidae	Sweet sorghum
		S 19°99.686'; E 23°43.182' 931masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Matlapana	S 19°94.073'; E 23°50.739' 937masl	<i>Sesamia calamistis</i>	Noctuidae	<i>Zea mays</i>
		S 19°94.073'; E 23°50.739' 937masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Samedupi	S 20°11.391'; E 23°50.235' 937masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Makgalo Chanoga	S 20°28.219'; E 23°50.235' 937masl S 20°17.411'; E 23°69.703' 935masl	<i>Chilo partellus</i> <i>Chilo partellus</i>	Crambidae Crambidae	Grain sorghum <i>Zea mays</i>
North-East	Botlalaote Ditladi	S 20°71.455'; E 27°25.186' 1130masl	<i>Sesamia calamistis</i>	Noctuidae	<i>Zea mays</i>
		S 21°47.007'; E 27°52.998' 953masl	<i>Sesamia calamistis</i>	Noctuidae	<i>Zea mays</i>
		S 21°47.007'; E 27°52.998' 953masl	Tortricidae sp3	Tortricidae	<i>Zea mays</i>
	Mathangwane	S 21°00.702'; E 27°35.363' 1028masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Tshesebe	S 20°72.042'; E 27°58.715' 1191masl	<i>Chilo partellus</i>	Crambidae	Sweet sorghum
	Mowana	S 20°80.890'; E 27°62.159' 1149masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
Central	Serowe	S 22°43.226'; E 26°80.107' 1083masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Bookane	S 23°06.528'; E 27°53.493' 881masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Boteti	S 22°12.674'; E 24°62.705' 911masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Jubui	S 21°11.645'; E 24°65.262' 921masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Masame	S 22°43.226'; E 26°80.105' 1080masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Taukome	S 22°83.163'; E 27°73.073' 844masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
		S 22°83.163'; E 27°73.073' 844masl	Tortricidae sp2	Tortricidae	<i>Zea mays</i>
	Dithojane	S 22°45.031'; E 26°76.353' 1076masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Palapye	S 22°59.409'; E 27°12.268' 986masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Boratapula	S 22°82.683'; E 27°60.450' 871masl	<i>Chilo partellus</i>	Crambidae	<i>Sorghum bicolor</i>
	Bobonong	S 21°98.344'; E 28°44.528' 707masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>

	Ramokgonami	S 22°78.944'; E 27°43.199' 912masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Seleka	S 22°95.112'; E 27°96.191' 783masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Mmashoro	S 21°87.684'; E 26°44.524' 1089masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Mathathane	S 22°26.688'; E 28°75.075' 681masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Mahibitswane	S 22°53.008'; E 27°19.947' 931masl	<i>Mythimne loreyi</i>		<i>Zea mays</i>
Okavango	Gumare	S 19°36.463'; E 22°22.206' 961masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
Kweneng	Kumakwane	S 24°72.081'; E 25°51.619' 1059masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Hatsalatsadi	S 24°04.468'; E 25°62.869' 1107masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Dikgonyane	S 24°36.434'; E 25°33.758' 1141masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
South-East	Sebele	S 24°57.707'; E 25°94.781' 986masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Glen Valley	S 24°60.213'; E 25°97.820' 953masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Ramotswa	S 24°82.081'; E 25°86.776' 1003masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
		S 24°82.081'; E 25°86.776' 1003masl	<i>Chilo partellus</i>	Crambidae	Sweet sorghum
	Mogobane	S 24°94.659'; E 25°73.672' 1048masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Tlokweng	S 24°62.217'; E 25°97.400' 945masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
Kgatleng	Oodi	S 24°54.710'; E 26°00.949' 966masl	Tortricidae sp2		<i>Zea mays</i>
	Modipane	S 24°61.832'; E 26°15.096' 1020masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Dinogeng	S 24°44.908'; E 26°27.554' 950masl	<i>Chilo partellus</i>	Crambidae	Sweet sorghum
		S 24°44.908'; E 26°27.554' 950masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
Southern	Gatsatsa	S 25°26.881'; E 25°27.763' 1254masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Logagane	S 25°61.017'; E 25°37.130' 1251masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Ramonnedi	S 25°17.388'; E 25°29.437' 1270masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Ramatlabama	S 25°60.480'; E 25°56.388' 1301masl	<i>Chilo partellus</i>	Crambidae	Grain sorghum
	Lobatse	S 25°25.092'; E 25°70.840' 1200masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
	Mabutsane	S 24°94.807'; E 23°58.030' 1091masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>
		S 24°94.807'; E 23°58.030' 1091masl	<i>Chilo partellus</i>	Crambidae	Sweet sorghum
		S 24°94.807'; E 23°58.030' 1091masl	<i>Sesamia calamistis</i>	Noctuidae	Sweet sorghum
Ghanzi	Kalkfontein	S 22°11.466'; E 20°88.564' 1190masl	<i>Chilo partellus</i>	Crambidae	Sweet sorghum
		S 22°11.466'; E 20°88.564' 1190masl	<i>Eldana saccharina</i>	Pyalidae	Sweet sorghum
	Ghanzi	S 27°73.196'; E 21°65.119' 1119masl	<i>Chilo partellus</i>	Crambidae	<i>Zea mays</i>

Table 2: Stemborer species and their associated wild host plants.

District	Location	GPS coordinates	Stemborer species	Family	Host plant
Chobe	Kasane	S 17°79.144'; E 25°20.573' 924masl	<i>Sesamia calamistis</i>	Noctuidae	<i>Chrysopogon nigritanus</i> (Vetiver)
		S 17°79.144'; E 25°20.573' 924masl	<i>Sesamia</i> sp nr <i>perplexa</i>	Noctuidae	<i>Vossia cuspidata</i>
		S 17°79.144'; E 25°20.573' 924masl	<i>Sesamia perplexa</i> es nov sp4	Noctuidae	<i>Echinochloa pyramidalis</i>
		S 17°79.144'; E 25°20.573' 924masl	<i>Chilo partellus</i>	Crambidae	<i>Echinochloa pyramidalis</i>
		S 17°79.144'; E 25°20.573' 924masl	<i>Sesamia</i> sp.	Noctuidae	<i>Cyperus papyrus</i>
		S 17°79.144'; E 25°20.573' 924masl	<i>Sesamia nonagrioides</i>	Noctuidae	<i>Cyperus papyrus</i>
	Parakarungu	S 17°79.338'; E 25°15.445' 944masl	<i>Sesamia calamistis</i>	Noctuidae	<i>Pennisetum macronium</i>
		S 18°03.961'; E 24°29.545' 938masl	<i>Eldana saccharina</i>	Pyralidae	<i>Schoenoplectus corymbosus</i>
	Satau	S 18°03.961'; E 24°29.545' 938masl	Schoenobiinae	Schoenobiinae	<i>Typha latifolia</i>
		S 18°09.776'; E 24°43.822' 932masl	<i>Eldana saccharina</i>	Pyralidae	<i>Vossia cuspidata</i>
		S 18°09.776'; E 24°43.822' 932masl	<i>Sesamia nonagrioides</i>	Noctuidae	<i>Oryza longistaminata</i>
		S 18°09.776'; E 24°43.822' 932masl	<i>Sesamia nonagrioides</i>	Noctuidae	<i>Vossia cuspidata</i>
		S 18°09.776'; E 24°43.822' 932masl	<i>Sesamia</i> sp nr <i>perplexa</i>	Noctuidae	<i>Vossia cuspidata</i>
		S 18°09.776'; E 24°43.822' 932masl	<i>Sesamia</i> nov sp41 nr <i>nonagrioides</i>	Noctuidae	Unknown
	Mabele	S 17°98.124'; E 24°64.452' 935masl	<i>Eldana saccharina</i>	Pyralidae	<i>Vossia cuspidata</i>
		S 17°98.124'; E 24°64.452' 935masl	<i>Sesamia</i> sp nr <i>perplexa</i>	Noctuidae	<i>Vossia cuspidata</i>
		S 17°98.124'; E 24°64.452' 935masl	<i>Sesamia nonagrioides</i>	Noctuidae	<i>Oryza longistaminata</i>
	Kazungula	S 17°80.556'; E 25°24.944' 937masl	<i>Sesamia nonagrioides</i>	Noctuidae	<i>Oryza longistaminata</i>
	Kalundu	S 18°05.375'; E 24°27.251' 930masl	<i>Sesamia nonagrioides</i>	Noctuidae	<i>Oryza longistaminata</i>
Ngamiland	Maun	S 19°99.686'; E 23°43.182' 931masl	<i>Sesamia jansei</i>	Noctuidae	<i>Typha latifolia</i>
		S 19°99.686'; E 23°43.182' 931masl	<i>Sesamia jansei</i>	Noctuidae	<i>Eriochloa fatmensis</i>
		S 19°99.686'; E 23°43.182' 931masl	<i>Sesamia jansei</i>	Noctuidae	<i>Schoenoplectus corymbosus</i>
		S 19°99.686'; E 23°43.182' 931masl	Tortricidae sp2	Tortricidae	<i>Schoenoplectus corymbosus</i>
		S 19°99.686'; E 23°43.182' 931masl	Tortricidae sp	Tortricidae	<i>Eriochloa fatmensis</i>
	Sexaxa	S 19°88.568'; E 23°54.620' 931masl	<i>Sesamia jansei</i>	Noctuidae	<i>Eriochloa fatmensis</i>
		S 19°88.568'; E 23°54.620' 931masl	<i>Sesamia jansei</i>	Noctuidae	<i>Schoenoplectus corymbosus</i>
		S 19°88.568'; E 23°54.620' 931masl	Tortricidae sp1	Tortricidae	<i>Schoenoplectus corymbosus</i>
		S 19°88.568'; E 23°54.620' 931masl	Tortricidae sp2	Tortricidae	<i>Schoenoplectus corymbosus</i>
	Samedupi	S 20°11.391'; E 23°50.235' 937masl	<i>Sesamia jansei</i>	Noctuidae	<i>Echinochloa pyramidalis</i>
		S 20°11.391'; E 23°50.235' 937masl	Tortricidae sp	Tortricidae	<i>Cyperus dives</i>
		S 20°11.391'; E 23°50.235' 937masl	<i>Sesamia jansei</i>	Noctuidae	<i>Typha latifolia</i>
Okavango	Shakawe	S 18°35.927'; E 21°84.229' 956masl	<i>Eldana saccharina</i>	Pyralidae	<i>Cyperus papyrus</i>
		S 18°35.927'; E 21°84.229' 956masl	<i>Sesamia perplexa</i>	Noctuidae	<i>Cyperus papyrus</i>
		S 18°35.927'; E 21°84.229' 956masl	Schoenobiinae	Schoenobiinae	<i>Cyperus papyrus</i>

	Samochima	S 18°35.927'; E 21°84.229' 956masl S 18°43.617'; E 21°90.683' 967masl	<i>Sesamia calamistis</i> <i>Eldana saccharina</i>	Noctuidae Pyralidae	<i>Cyperus papyrus</i> <i>Cyperus papyrus</i>
	Mohembo	S 18°27.111'; E 21°79.267' 944masl S 18°27.111'; E 21°79.267' 944masl S 18°27.111'; E 21°79.267' 944masl	<i>Eldana saccharina</i> <i>Eldana saccharina</i> <i>Sesamia calamistis</i>	Pyralidae Pyralidae Noctuidae	<i>Echinochloa pyramidalis</i> <i>Cyperus papyrus</i> <i>Cyperus papyrus</i>
Southern	Kanye Moshupa	S 24°94.807'; E 25°34.476' 1239masl S 24°78.787'; E 25°41.477' 1096masl S 24°78.787'; E 25°41.477' 1096masl	<i>Sesamia jansei</i> <i>Chilo partellus</i> Tortricidae sp2	Noctuidae Crambidae Tortricidae	<i>Schoenoplectus corymbosus</i> <i>Cyperus dives</i> <i>Schoenoplectus corymbosus</i>
Central	Martins drift Mahalapye	S 22°99.554'; E 27°99.224' 974masl S 22°99.554'; E 27°99.224' 974masl S 23°10.200'; E 26°83.948' 1022masl	<i>Eldana saccharina</i> <i>Eldana saccharina</i> Tortricidae sp2	Pyralidae Pyralidae Tortricidae	<i>Cyperus digitatus</i> subsp <i>auricomus</i> <i>Schoenoplectus corymbosus</i> <i>Schoenoplectus corymbosus</i>
North-East	Botalaote	S 20°71.455'; E 27°25.186' 1130masl S 20°71.455'; E 27°25.186' 1130masl	<i>Sesamia calamistis</i> Tortricidae sp2	Noctuidae Tortricidae	<i>Schoenoplectus corymbosus</i> <i>Schoenoplectus corymbosus</i>
South-East	Notwane Gaborone dam	S 24°60.480'; E 25°56.388' 1301masl S 24°70.752'; E 25°90.632' 992masl	Tortricidae sp2 <i>Sesamia jansei</i> <i>Sesamia jansei</i>	Tortricidae Noctuidae Noctuidae	<i>Cyperus dives</i> <i>Cyperus dives</i> <i>Cyperus dives</i>

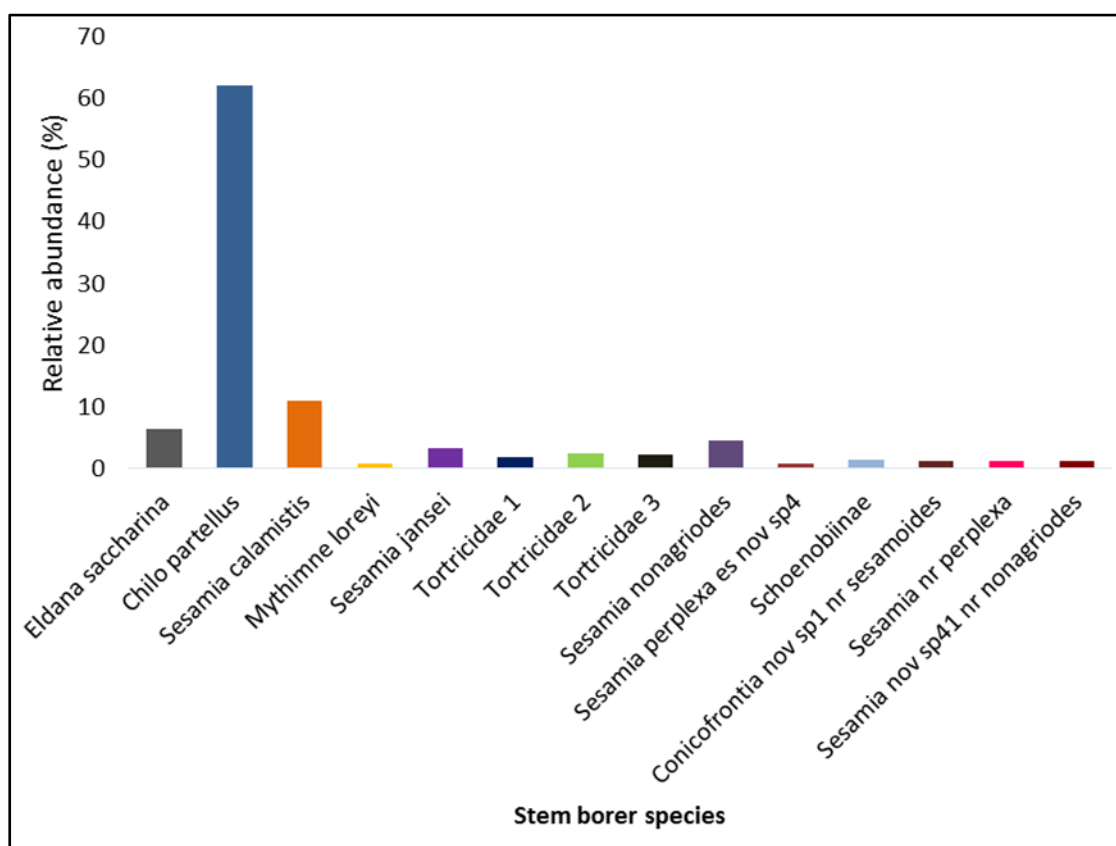


Figure 2: Relative abundance of stemborer species collected from cultivated and wild hosts in Botswana.

2.3.2 Diversity of host plants of stemborer

From the 13 host plant species found infested by stemborers, the highest number of stemborers were found attacking cultivated habitats, especially maize. *Schoenoplectus corymbosus* was the wild host most attacked by stemborers. *Sesamia calamistis* and *C. partellus* were found to be common stemborers in *Z. mays*, *S. bicolor* and *S. bicolor* L. Moench. In addition to *S. calamistis* and *C. partellus*, *M. loreyi*, *Tortricidae* sp1 and *Tortricidae* sp3 were found attacking *Z. mays* and *Conicofrontia nov sp 1 nr sesamoides* was found infesting sweet sorghum, *Schoenoplectus corymbosus* was found infested with *E. saccharina*, *S. jansei*, *Tortricidae* sp1 and *Tortricidae* sp2; *C. nigratinus* (Vetiver) infested with *S. calamistis*; *C. papyrus* infested with *E. saccharina*, *Sesamia sp nr perplexa*, *Sesamia* sp and *Schoenobiinae*; *T. latifolia* hosting *Schoenobiinae* and *S. jansei*; *E. pyramidalis* hosting *S. jansei* and *Sesamia perplexa es nov sp4*; *C. dives* hosting *C. partellus* and *Tortricidae* sp; *E. fatmensis* hosting *Totricidae* sp and *V. cuspidata* hosting *E. saccharina* and *Sesamia sp nr perplexa*. I report here, for the first time *C. dives* as a host of *C. partellus* in Botswana (see Table 1 & 2).

2.3.3 Stemborer evolutionary relationships

Different stemborer species which whose genome was sequenced were used in the construction of a phylogenetic tree (Table 3; Fig. 3). Seven (*Sesamia perplexa* (ex sp 4/14), 4 *Sesamia mabela?* and 2 *S. perplexa*) of the samples were grouped with another *S. nr perplexa* species; 2 (*Conicofrontia mabela* and *Conicofrontia mabela ex nov sp 1*) were grouped with *Conicofrontia mabela*; 2 *S. jansei* species were grouped with another *S. jansei*; 10 (2 *Sesamia satau* (ex sp 41), 7 *Sesamia nonagrioides* and *Sesamia* sp) grouped with another *Sesamia nonagrioides* and 2 *Eldana saccharina* species were grouped together into groups 1, 2, 3, 4 and 5 respectively. Group 5 was distantly related to the other 4 groups, while group 4 was found to be highly diverse (Fig. 3).

Table 3: Location, host plants and coordinates of the sample sequences used in construction of a phylogenetic tree.

Sample ID	Stemborer species	Host plant	Location	GPS coordinates
XXBOG8696	<i>Eldana saccharina</i>	<i>Cyperus papyrus</i>	Shakawe	18°35.927'; 21°84.229' 956m
XXBOG8703	<i>Eldana saccharina</i>	<i>Cyperus papyrus</i>	Mohembo	18°27.111'; 21°79.267' 956m
SEBOG8744	<i>Sesamia perplexa</i>	<i>Echinochloa pyramidalis</i>	Kasane	17°79.144'; 25°20.573' 924m
SEBOG8745	<i>Sesamia perplexa</i>	<i>Echinochloa pyramidalis</i>	Kasane	17°79.144'; 25°20.573' 924m
SEBOG8747	<i>Sesamia nonagrioides</i>	<i>Vossia cuspidate</i>	Satau	18°09.776'; 24°43.822' 932m
SEBOG8748	<i>Sesamia nonagrioides</i>	<i>Vossia cuspidate</i>	Satau	18°09.776'; 24°43.822' 932m
SEBOG8749	<i>Sesamia nonagrioides</i>	<i>Vossia cuspidate</i>	Satau	18°09.776'; 24°43.822' 932m
COBOG8750	<i>Conicofrontia mabela</i>	<i>C.nigritanus</i>	Shakawe	18°35.927'; 21°84.229' 956m
COBOG8751	<i>Conicofrontia mabela</i>	<i>C.nigritanus</i>	Shakawe	18°35.927'; 21°84.229' 956m
SEBOG8752	<i>Sesamia nonagrioides</i>	<i>Vossia cuspidate</i>	Shakawe Village	18°35.927'; 21°84.229' 956m
SEBOG8753	<i>Sesamia sp</i>	<i>Echinochloa pyramidalis</i>	Samedupi	20°11.391'; 23°50.235' 937m
SEBOG8754	<i>Sesamia perplexa</i>	<i>Piège lumineux</i>	Satau	18°09.776'; 24°43.822' 932m
SEBOG8755	<i>Sesamia jansei</i>	<i>Echinochloa pyramidalis</i>	Samedupi	20°11.391'; 23°50.235' 937m
SEBOG8756	<i>Sesamia jansei</i>	<i>Echinochloa pyramidalis</i>	Samedupi	20°11.391'; 23°50.235' 937m
SEBOG8758	<i>Sesamia mabela?</i>	<i>Vossia cuspidate</i>	Mabele	17°98.124'; 24°64.452' 935m
SEBOG8759	<i>Sesamia mabela?</i>	<i>Vossia cuspidate</i>	Mabele	17°98.124'; 24°64.452' 935m
SEBOG8760	<i>Sesamia mabela?</i>	<i>Vossia cuspidate</i>	Mabele	17°98.124'; 24°64.452' 935m
SEBOG8761	<i>Sesamia mabela?</i>	<i>Vossia cuspidate</i>	Mabele	17°98.124'; 24°64.452' 935m
COBOG8381	<i>Conicofrontia mabela ex nov sp 1</i>	<i>Sorghum bicolor</i>	Mabele	17°98.124'; 24°64.452' 935m
SEBOG8382	<i>Sesamia nonagrioides</i>	<i>Oriza longistamina</i>	Kazungula	17°80.556'; 25°24.944' 937m
SEBOG8390	<i>Sesamia jansei</i>	<i>Schoenoplectus corymbosus</i>	Maun	19°99.686'; 23°43.182' 931m
SEBOG8393	<i>Sesamia perplexa</i> (ex sp 4/14)	<i>Echinochloa pyramidalis</i>	Kasane	17°79.144'; 25°20.573' 924m
SEBOG8394	<i>Sesamia nonagrioides</i>	<i>Echinochloa pyramidalis</i>	Kalundu	18°05.375'; 24°27.251' 930m
SEBOG8441	<i>Sesamia sataua</i> (ex sp 41)	Unknown	Satau	18°09.776'; 24°43.822' 932m
SEBOG8531	<i>Sesamia nonagrioides</i>	<i>Vossia cuspidate</i>	Shakawe	18°35.927'; 21°84.229' 956m
SEBOG8391	<i>Sesamia sataua</i> (ex sp 41)	Unknown	Satau	18°09.776'; 24°43.822' 932m
SEBOG8762	<i>Sesamia nonagrioides</i>	<i>Vossia cuspidate</i>	Mabele	17°98.124'; 24°64.452' 935m

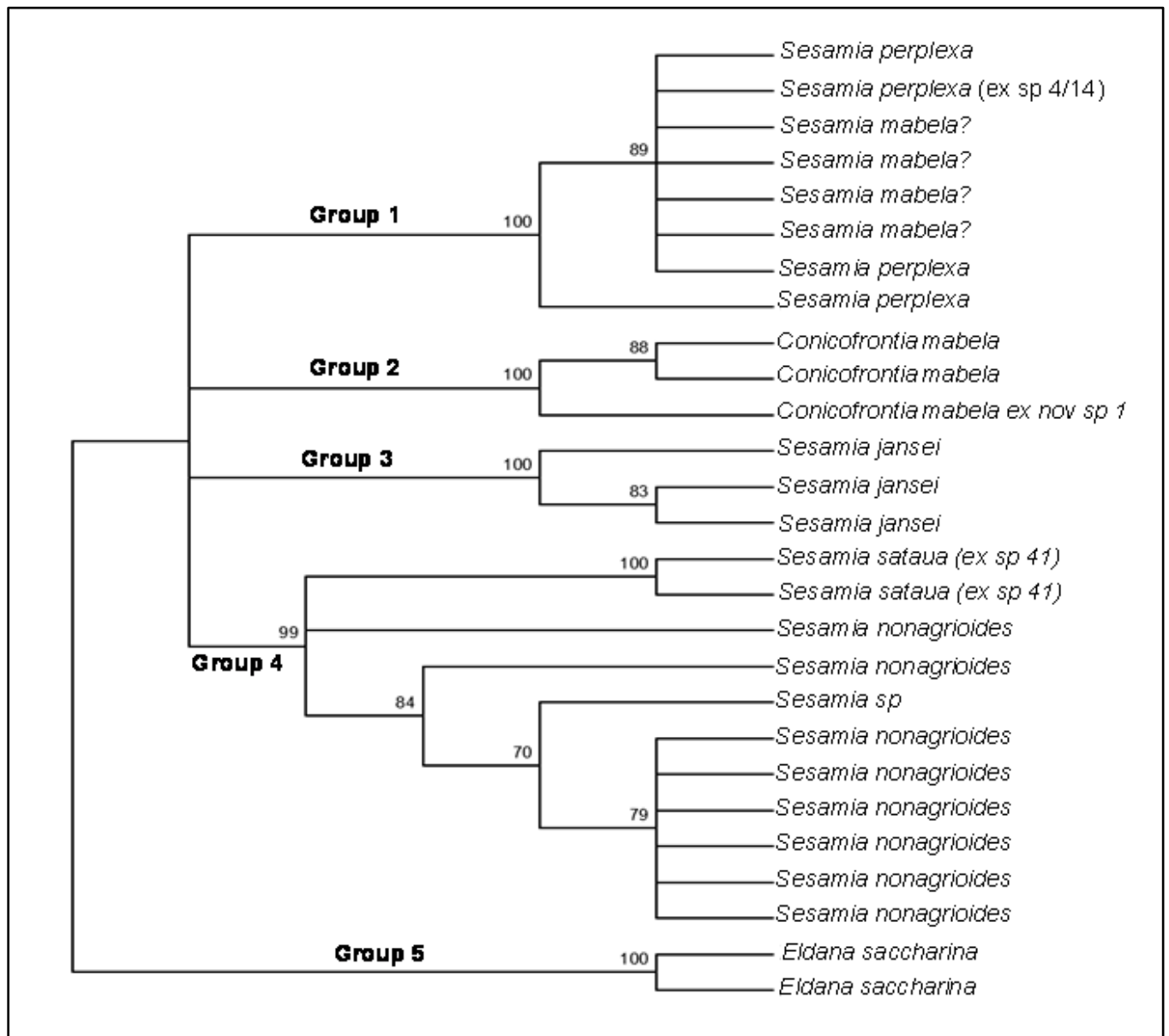


Figure 3: Neighbour-Joining tree showing the genetic relationships between stemborer specimens collected in Botswana. Bootstrap values (10000 replicates) are shown next to the branches. Only values more than 70% are indicated.

2.4 Discussion

2.4.1 Stemborer diversity, distribution and abundance

With the exception of *E. saccharina* (Conlong, 1988), *S. calamistis* and *C. partellus* (Obopile & Mosenkie, 2003), this current study is the first to record the abundance and distribution of *Sesamia* sp, *S. nonagrioides*, *S. perplexa*, *S. sp nr perplexa*, *S. perplexa es nov sp4*, *S. jansei*, *S. nov sp41 nr nonagrioides*, *Totricidae* sp1, *Totricidae* sp2, *Totricidae* sp3, *Tortricidae* sp, *Schoenobiinae*, *Conicofrontia nov sp 1 nr sesamoides* and *Mythimne loreyi* in Botswana. Moreover, this study is the first to date, documenting stemborer diversity and their hosts both under natural and agro-ecosystem setups.

Chilo partellus was found in all the districts except in Kgalagadi where no stemborer was collected during the study. *Chilo partellus* had a relative abundance of 62.1% followed by *S. calamistis* at 11%. According to Kfir *et al.* (2002), *C. partellus* has become the highly competitive colonizer in different areas where it replaced the indigenous stemborers. A study by Seshu (1983) and Kfir (1997) described *C. partellus* as a highly competitive colonizer in eastern and southern Africa, thereby becoming the most injurious stemborer. *Chilo partellus* has been documented in several studies as an important pest of maize and sorghum in warm lowland regions (Gebre-Amlak 1985; Getu *et al.*, 2001; Tefera, 2004). Variations in dominance among *C. partellus* indicate that it has specific adaptations in its ecological requirements (Ong'amo *et al.*, 2006; Mutamiswa *et al.* 2017). Its successful establishment in areas originally colonised by other indigenous stemborer species can be attributed to the plasticity of its biology that gives it an advantage over the indigenous species (Ofomata *et al.*, 1999; Mutamiswa *et al.*, 2017). These attributes include resource utilization efficiency (indicating its high fitness potential), occupation of appropriate feeding habitats earlier than the native borer species (thereby outcompeting the population of competitive stemborers that successfully colonize the same habitats), emission of chemical odours by females upon oviposition (which deter other female stemborers from ovipositing on the same plants), larvae dispersal for longer distances and increased basal and plasticity of temperature tolerance (Kfir, 1997; Ofomata, 1997; Ofomata *et al.*, 2000; Niyibigira *et al.*, 2001 & Ntiri *et al.*, 2016; Mutamiswa *et al.*, 2017). Evidence from laboratory studies conducted to examine the displacement of the indigenous stemborer has revealed that *C. partellus* had a higher fecundity at 25°C and 28°C (Ofomata *et al.*, 2000).

Obhiokhenan *et al.* (2002) reported higher percentage of *S. calamistis* in the mangrove and rain forest zones in Nigeria partially displacing *B. fusca*. Over a period of 7 years in the eastern veld region of South Africa, *S. calamistis* constituted 90% of the total stemborer population within 2 years. Hence an indication of a potential threat by *S. calamistis* in future. The possible reasons for the replacement of the indigenous species is because of its hibernating larval populations which terminate diapause and emerge earlier. Its life cycle is also shorter, giving it a further competitive advantage due to higher potential rate of population increase (Emana *et al.*, 2008 & Dejen *et al.*, 2014).

Although Conlong (1988) reported the presence of *E. saccharina* in Parakarungu (Chobe district), its distribution in space has not been reported. In the current study, I document this borer species in Satau and Mabele (Chobe district) and Shakawe (Okavango district), revealing the extent of distribution or its extension of geographical range.

The stemborer diversity index for this survey was similar to a study which was carried out by Mailafya *et al.* (2009) in Kenya, where he found stemborer diversity to be high in the wild as compared to cultivated crops. Similarly, results also conform to a study in Cameroon (Ndemah *et al.*, 2007) and Uganda Matama-Kauma *et al.* (2008) that documented higher insect diversity in the wild relative to cultivated host plants. The reason for the higher stemborer diversity in wild host plants is unknown but may likely be a result of higher host plant diversity, which likely sustains higher stemborer populations and attracts higher diversity. The northern part of Botswana had a wider range of stemborer species relative to all other places. This maybe because of many water bodies with a wide variety of wild host plant diversity. According to Ong'amo *et al.* (2006), diversity and abundance of stemborer species are favored by the presence of suitable hosts.

2.4.2 Diversity of stemborer - host plant interactions

Prior to the current study, little information was available on natural hosts for different stemborers in Botswana. Previous records by Obopile & Mosenkie (2003) only documented different stemborer species in cultivated habitats. Nevertheless, there are different stemborer hosts, and some of them are wild plants which serve as significant overwintering sites for both stemborers and their natural enemies. Results of the present study therefore report novel findings for stemborer records in Botswana, both in cultivated and natural habitats. *Chilo partellus* was

found more on cultivated host plants as compared to natural hosts. This result corroborate well with a study by Moolman *et al.* (2014) in Mozambique and Le Ru *et al.* (2006) who documented the same trend. In this study, *S. calamistis* was recorded only in natural host plants. However, several reports have documented this stemborer species on several other host plant species, including corn, sorghum, millet, rice and sugar cane (Askarianzadeh *et al.*, 2008 & Spigno *et al.*, 2008). Though this host polyphagy was not reported here, it represents a potential threat to future pest management and cultivated plants invasion. This may imply a future potential threat to the farmers as the stemborer may later shift to the cropping fields resulting in losses due to its damages.

Current results also reported that *E. saccharina* attacks *S. corymbosus*, *V. cuspidata*, *C. digitatus* subsp *auricomus*, *E. pyramidalis*, *C. papyrus* and sweet sorghum in Botswana. Previous reports of this species was documented only on *C. papyrus* (Conlong & Graham, 1988). Thus, we report four more novel host plant species for *E. saccharina* in Botswana. Elsewhere, e.g. South Africa, *E. saccharina* is contrastingly common in sugarcane, maize, *Prionium serratum* (L.f.) Dre`ge ex E.Mey, while in Zimbabwe, the borer species feeds on sugarcane, maize, sorghum, *T. latifolia* and *C. digitatus*, and West Africa, its predominant on sugarcane and maize (Shanower *et al.*, 1983; Conlong, 2001 & Anon 2003). It has also been recorded on a number of alternate host plants including millet, rice, cassava, sedges, large grasses and pigweed (*Amaranthus dubius*) (Carnegie, 1974; Conlong, 1997). Thus, the current results are in keeping with previous results to date, documenting the diversity of *E. saccharina* hosts in space. This host switching implies that the species may thrive upon introduction to new habitats and with climate change, it is more likely to invade and thrive in new habitats comprising different host plant species. Indeed, host plant switching is one of many factors contributing to the invasion success of some invasive stemborer species (see e.g. Mutamiswa *et al.*, 2017).

Mythimne loreyi has previously been documented attacking sugarcane and other graminaceous crops in Africa, Australia, Philippines and Southern Europe (Box, 1953; Chandler & Benson, 1991; Calora, 1966; Edwards, 1992). Here, I reported the same species on *Z. mays*, in keeping with the above studies. However, these results still remain novel, since it is the first time this species is being reported on maize, in a Botswana context. According to Conlong (2000) and Moolman *et al.* (2014), *C. sesamoides* only attacks predominantly wild host plants. Nevertheless, another novel finding of the current study is that this species was recorded, for the

first time, in a Botswana context and attacking sweet sorghum. Similarly, Assefa (2015) also recorded *C. sesamoides* from sugarcane in South Africa, likely indicating some degree of host plant switching between wild and cultivated plants.

Interestingly, *C. partellus* was recorded on *C. dives*, and this has never been reported before. This may imply that the borer species may be expanding its host range, and probably geographic ranges as a result of pressures likely shaped by climate change (see discussions in Mutamiswa *et al.*, 2017). This represents high potential to colonize novel environments and an ability to utilize a diverse range of hosts (Kfir, 1997; Overholt *et al.*, 1997) and may likely contribute to its invasion success when faced with climate change. Besides surviving in cultivated habitats, previous studies have also revealed that *C. partellus* can survive in wild hosts more than other indigenous stemborer species (Ofomata *et al.*, 2000; Songa *et al.*, 2002) but none of them have documented it in *C. dives*.

Of the 5 stemborer clades found in Botswana, clade 4 comprising of 7 *S. nonagrioides* species, 2 *Sesamia sataua* species and *Sesamia* sp was the most diverse. This could be due to the variation in host range or geographic isolation of the stemborer species. In a study carried out by Moyal *et al.* (2011) in East Africa, *S. nonagrioides* was found only in wet areas and was collected from several wild host plants belonging to Poaceae, Cyperaceae and Typhaceae. This study also shows an alternative hypothesis for the relationship of different stemborers in Botswana through molecular phylogenetics. A known relationship between different stemborers in an area can be a key in the development of future management plans for the control of stemborers. The control of previously known stemborer species may be incorporated in the control of the unknown species with regard to their relationship as they may be attacked by the same natural enemies.

Results of the present study document several novel findings. I document, for the first time in Botswana, several stemborer host plants, both wild and cultivated, that have implications on pest management. I also document several species of stemborers for the first time in Botswana, though some of them have been reported elsewhere in Southern Africa. Of utmost importance, some results reported here, need significant followup. For example, stemborers identified only to family and genus levels warrants further identification to species level. This may likely add new species to the list of stemborers attacking cultivated and wild host plants.

In conclusion, this study documents the diversity and distribution of stem borers and associated wild and cultivated host plants in Botswana. Results document significant diversity of lepidopteran stem borer species feeding on diverse cultivated and wild host plants/habitats. Current results highlight the significance of wild, especially wetland habitats for their ecological functions; and particularly conservation of lepidopteran stem borer and plant host biodiversity.

2.5 References

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CHAPTER THREE

Species diversity and abundance of lepidopteran stemborer natural enemies in wild and cultivated habitats in Botswana

3.1 Introduction

Cereal crops such as maize, sorghum and millet are mostly grown by small-scale farmers at subsistence level in sub-Saharan Africa, with the majority of cereal fields usually surrounded by patches of natural habitats harbouring stem borer natural host plants (e.g. grasses and sedges) (Mailafiya *et al.*, 2009). These natural habitats often serve as propagule refugia, sustaining the diversity of stem borer natural enemy populations within the agroecosystem (Mailafiya *et al.*, 2009; Moolman *et al.*, 2013). Lepidopteran stem borers are the main destructive insect pests of cereal crops in sub-Saharan Africa, accounting for ~5-75% of potential crop yield losses (De Groote, 2002; Kfir *et al.*, 2002; Kipkoech *et al.*, 2006; Moolman *et al.*, 2013). In Africa, this represents a significant household food insecurity burden since ~70-80% of the populations depend on agriculture for sustenance (FAO, 2002). The major cereal stem borer species accounting for damage in Africa include the indigenous pyralid *Eldana saccharina* Walker, the crambid *Chilo orichalcociliellus* (Strand), the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* Hampson and the exotic crambid *Chilo partellus* Swinhoe (Kfir *et al.*, 2002; Obonyo *et al.*, 2010; Addo-Bediako & Thanguane, 2012).

The abundance and distribution of cereal stem borers may be influenced by abiotic factors such as temperature and precipitation, and biotic factors such as natural enemies and alternative host plants (Mailafiya *et al.*, 2009; Addo-Bediako & Thanguane, 2012). The diversity of parasitoids and parasitism levels have been reported to be higher in arable fields intermixed with natural habitats than in arable fields only (Thies *et al.*, 2003). Although natural habitats have been reported to provide refuge for some parasitoid species, low stem borer parasitism levels (<8%) were recorded in East and Austral Africa in natural host plants (Mailafiya *et al.*, 2011; Moolman *et al.*, 2013). Gramineous plants have been reported to produce secondary metabolites that recruit parasitoids of stem borers (Potting *et al.*, 1995; Arab & Bento, 2006). Indeed, this has been the case for *B. fusca* and *C. partellus* infested maize and sorghum plants (Mutymbai *et al.*, 2015). Isolated volatiles from these plants are effective in recruiting hymenopteran braconid parasitoids *Cotesia sesamiae* Cameron, *Cotesia flavipes* Cameron and the ichneumonid *Dentichasmias busseolae* Heinrich (Arab & Bento, 2006).

Understanding the diversity, spatial distribution, relative abundance and ecological contribution of stem borer natural enemies in natural and agroecosystem environments is significant in ensuring ecosystem stability and putting in place the integrated pest management

approach (Conlong & Rutherford, 2009). Natural enemies play an important role in the control of lepidopteran stem borers (Sylvain *et al.*, 2015; Midingoyi *et al.*, 2016) and are known to increase stability both in natural and agroecosystems by responding to host upsurges, thus preventing potential outbreaks (Muyekho *et al.*, 2005; Mailafiya *et al.*, 2010a, 2013; Kodjo *et al.*, 2013). In eastern and southern Africa, cereal stem borer populations have been reported to be regulated by a wide range of native and exotic parasitoid species (Conlong, 2000; Zhou *et al.*, 2003; Moolman *et al.*, 2013). Most of these parasitoids belong to the orders Hymenoptera and Diptera (Polaszek, 1998; Conlong, 2000; Zhou *et al.*, 2003; Moolman *et al.*, 2013). There have been numerous attempts over the past decades to introduce exotic parasitoids into Africa for biological control of indigenous and exotic stem borers, but only a few species managed to successfully establish (Conlong, 1994, 1997, 2001; Kfir *et al.*, 2002). Nevertheless, a *C. partellus* larval endoparasitoid, *C. flavipes* was introduced from Pakistan into Kenya in 1993 where it got fully established (Overholt *et al.*, 1997; Kfir *et al.*, 2002; Kipkoech *et al.*, 2006; Dejen *et al.*, 2013), while releases have subsequently been successful in nine other countries in east and southern Africa (Kipkoech *et al.*, 2006; Omwega *et al.*, 2006; Chinwada *et al.*, 2008). Different life stages of stem borers have been reported to be attacked by predators such as ants, spiders, earwigs, nematodes and microbial pathogens [*Beauveria bassiana* (Balsamo), *Bacillus thuringiensis* Berliner, *Serratia marcescens* Bizio] in various landscapes (Oloo, 1989; Bonhof *et al.*, 1997; Assefa *et al.*, 2006). However, research data has shown that indigenous natural enemies may be unable to keep stem borer populations below economic injury levels (Overholt *et al.*, 1994; Bonhof *et al.*, 1997), unless their potential efficacy is improved through conservation.

Stem borer natural enemy species diversity and abundance can vary across different landscapes, and diversity and distribution may be under threat due to global climate change (Batalden *et al.*, 2007; Kodjo *et al.*, 2013). While previous studies have documented lepidopteran stem borers, their parasitoids and associated host plants in some parts of East Africa; e.g. Ethiopia (Assefa *et al.*, 2006), Uganda (Conlong & Mugalula, 2001; Matama-Kauma *et al.*, 2007; 2008), Kenya (Mailafiya *et al.*, 2009), Central Africa; e.g. DRC (Kankonda *et al.*, 2017), West Africa; e.g. Cameroon (Ndemah *et al.*, 2007), Togo (Kodjo *et al.*, 2013) and Southern Africa; e.g. Zimbabwe (Chinwada & Overholt, 2001; Mazodze & Conlong, 2003), Mozambique (Conlong & Goebel, 2002, 2006; Moolman *et al.*, 2013) and South Africa (Moolman *et al.*, 2013), to our knowledge, except for a survey in *Cyperus papyrus* L. for parasitoids of *E.*

saccharina (Conlong *et al.*, 1988), such information is lacking for Botswana. Through extensive surveys, we assessed the diversity and abundance of stem borer natural enemies and their associated hosts in both cultivated and natural landscapes in Botswana. Such information is crucial in explaining the potential roles of natural enemies in regulating insect pest populations, and may help in developing pest management systems. We hypothesise that natural enemy species diversity is higher in natural compared to cultivated habitats in Botswana.

3.2 Materials and Methods

3.2.1 Field Surveys

Field surveys on cereal crops and natural host plants were conducted in all the ten districts of Botswana during 2014/15 and 2015/16 austral summers. A total of 84 sites were sampled in ten districts during the two seasons (Fig. 1).

3.2.1.1 Sampling in Natural Habitats

Natural lepidopteran stem borer host plants belonging to the families, Poaceae, Cyperaceae and Typhaceae were sampled (i) around cereal crops, (ii) in open patches along forest roads, (iii) on banks of streams or rivers (iv) in swamps and (v) in national parks. A biased sampling procedure was employed to increase the chances of finding stem borers, as previous studies have reported a considerable lower density of borers in natural host plants than in cultivated crops (Gounou & Schulthess, 2004; Mailafiya *et al.*, 2009; Moolman *et al.*, 2014). Potential host plants were inspected for stem borer damage symptoms, such as dry leaves and shoots (dead hearts), scarified leaves (window panes and pin/shot holes), larval entry and exit holes in stems and the presence of frass. Stems that exhibited damage symptoms were dissected in the field and borer life stages found were placed individually in 30 ml plastic vials containing artificial diet sealed with aerated screw-cap lids obtained from South African Sugarcane Research Institute (SASRI), South Africa. The diet for noctuids was as formulated by Onyango & Ochieng'-Odero (1994), and for pyralids and crambids as outlined by Walton & Conlong (2016). However, predatory insects, parasitoid cocoons or dead larvae were placed individually in labelled plastic vials without diet, while pupae found were placed in the same plastic vials with a water-soaked cotton wool to avoid desiccation related mortality.

3.2.1.2 Sampling in Cultivated Habitats

For cereal crops, sweet sorghum, maize, millet and sorghum fields were selected every 10–15 km along minor and major roads in each respective district. In consequence, a total of 10–15 fields were sampled per village in each district. Each field was divided into four quadrants and 10 plants/stalks per quadrant were randomly sampled along a transect. Each plant was dissected and searched for lepidopteran stem borer larvae, pupae, mummified larvae and pupae as well as parasitoid cocoons. Life stages found were collected as described above for those collected from natural host plants.

For each of the vials, we recorded (1) the date of collection, (2) name of host plant (if it could be identified in the field), (3) host developmental stage and (4) borer and/or parasitoid species (if it could be identified in the field). Geo-referencing (latitude, longitude and altitude) using a portable Geographic Positioning System (GPS) (Garmin GPSMAP 62st) was done for each field where natural and cultivated hosts were sampled. All the vials were placed in cooler boxes and taken to the laboratory for subsequent incubation.

3.2.2 Laboratory Incubation

In the laboratory, larvae, pupae and parasitoid cocoons were incubated at $25 \pm 1^{\circ}\text{C}$ and $65 \pm 10\%$ RH with natural photoperiodic conditions of about L12:D12 (Onyango & Ochieng'-Odero, 1994; Mailafiya *et al.*, 2009) in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) until moth or parasitoid adult emergence. Collected predatory insects and emerged adult parasitoids were preserved in 70% alcohol before morphological identification. Adult stem borers and parasitoids were identified up to family, genera or species level by the third author. However, where identification to species level was impossible, genus and family levels were used. Natural host plant species were identified at the department of National Museum and Monuments, Herbarium unit, Botswana.

3.2.3 Data Analysis

Host plant - parasitoid and stemborer - parasitoid interaction networks were analysed in R using 'BIPARTITE' package (Dormann *et al.*, 2009). In all interaction network analyses, the number of parasitised hosts against host plants and stemborer species in all habitats were used.

Parasitoid species diversity in natural and cultivated habitats was computed using Simpson's diversity index (Simpson, 1949):

$$D = \frac{\sum n(n-1)}{N(N-1)}$$

Where D is the Simpson diversity index, N is total number of organisms of all species found and n is number of individuals in each species. Parasitism rate for (1) larvae and (2) pupae and, (3) relative abundance of each parasitoid were calculated as outlined by Zhou *et al* (2003), Dejen *et al.* (2013) and Rahaman *et al.* (2014) as follows:

$$(1) \text{ Larval parasitism} = \frac{\text{Number of parasitised larvae} \times 100}{\text{Number of healthy larvae} + \text{Number of parasitised larvae}}$$

$$(2) \text{ Pupal parasitism} = \frac{\text{Number of parasitised pupae} \times 100}{\text{Number of healthy pupae} + \text{Number of parasitised pupae}}$$

$$(3) \text{ Relative abundance of each parasitoid sp} = \frac{\text{Total of individuals of each sp} \times 100}{\text{Total number of individuals of all parasitoids}}$$

3.3 Results

3.3.1 Stemborers, their natural enemies and host plants

As in chapter 2, a total of 1497 lepidopteran stem borer larvae and 228 pupae were collected from three cultivated and 11 natural host plants (Tables 1 & 2) in the ten districts sampled in Botswana. From these collections, 30 cereal stem borer natural enemy species (27 parasitoids and 3 predators) belonging to 13 families and two orders of insects were recovered. Parasitoid species diversity was significantly higher in cultivated (D = 0.23) than natural habitats (D = 0.14). Nineteen (16 larval, 3 pupal) of the 30 parasitoid species collected were recovered from three stem borer species in cultivated hosts while 11 parasitoid species (9 larval, 2 pupal) from 7 stem borer species in natural hosts. Furthermore three predator species were recovered from both cultivated and natural habitats (Table 4). These parasitoids identified belonged to the orders Diptera and Hymenoptera, with the hymenopterans dominating in all the sampled districts. The hymenopteran appeared dominant to the dipteran parasitoids in all the sampled districts, with Braconidae and Tachinidae the most common families represented in the two

orders, respectively. The stem borer – parasitoid interaction network showed a moderate Shannon interaction diversity ($H' = 2.56$) and a low generality ($G_{qw} = 1.23$). Similarly, the host plant – parasitoid interaction showed moderate Shannon interaction diversity ($H' = 2.93$) and generality (G_{qw}) of 1.81. A total of 19 different parasitoid species were recovered from *C. partellus* in both cultivated and natural habitats (Fig. 4). In addition, 11 maize and 10 sweet sorghum parasitoid species were recovered from stem borer species in cultivated habitats (Fig. 5). Furthermore, 4 parasitoid species were recorded parasitising stem borers attacking *S. corymbosus* in natural habitats (Fig. 5). The most common larval parasitoids recorded comprised the gregarious *C. flavipes*, and *C. sesamiae*, and the solitary *C. curvimaculatus* all recovered from *C. partellus* (Fig. 4). Similarly, the main pupal parasitoids included the gregarious *P. furvus* and the solitary *G. nimbipennis* isolated from the same host stem borer (Table 4; Fig. 4). The larval parasitoids *G. indicus*, *C. curvimaculatus* and pupal parasitoid *G. nimbipennis* were recovered in both cultivated and natural host plants infested with *C. partellus* Swinhoe, *Sesamia nonagrioides* Lefèbvre and *S. jansei* Tams & Bowdwn (Table 4). Nevertheless, the exotic *C. flavipes* and the indigenous larval parasitoid, *C. sesamiae* were more specific to *C. partellus* in cultivated habitats relative to other stem borer species (Table 4; Fig. 4). Stem borers, *C. partellus* and *Sesamia calamistis* Hampson were the most common in the cultivated host plants whilst *E. saccharina*, *S. jansei* and *S. nonagrioides* were common in natural host plants. As for predators, *L. humile*, *C. peringueyi* and *Aenictus* sp were common in both cultivated and natural host plants where they attacked larvae and pupae of *E. saccharina*, *C. partellus*, *S. jansei* and *S. calamistis* in their borings (Table 4). Hymenopteran parasitoids were recovered from borers on Poaceae, Cyperaceae and Typhaceae plant families whilst Dipterans were recovered from borers on Poaceae and Cyperaceae plant species only (Tables 5 & 6). The host plant genus, *Sorghum* occurred in both cultivated and natural habitats (Tables 5 & 6). In cultivated habitats, maize and sorghum were the most common whilst *S. corymbosus* was the most common stem borer host plant in natural habitats (Tables 5 & 6; Fig. 5).

Table 4: Details of natural enemy species recovered from stemborers on cultivated and wild host plants in Botswana, and information on associated locality, parasitoid family, behaviour, stemborer host and the developmental stage from which the parasitoid was isolated.

Location	Coordinates	Parasitoid species	Family	Habitat	Stemborer	Host stage	Behaviour
Hymenoptera							
Chanoga	S20.17703; E23.67496; 939m	<i>Pediobius furvus</i> Gahan	Eulophidae	C	Cp	P	G
Mogobane	S24.94659; E25.73672; 1048m						
Modipane	S24.61832; E26.15096; 1020m						
Tsootsha	S22.11460; E20.88564; 1190m						
Oodi	S24.54710; E26.00949; 966m	<i>Euvipio rufa</i> Szepligeti	Braconiadae	C	Cp	L	S
Mabele	S17.98124; E24.64453; 935m			C	Con	L	S
Maun	S19.99686; E23.43182; 937m	<i>Gambroides nimbipennis</i> Seyrig	Ichneumonidae	N	Tot	L	G
Mabele	S17.98124; E24.64453; 935m			C	Cp	L	G
		<i>Goniozus indicus</i> Ashmead	Bethylidae	C	Cp	L	G
Maun	S19.99686; E23.43182; 937m			N	Sj	L	G
Kazungula	S17.80556; E25.24944; 937m			N	Sn	L	G
Oodi	S24.54710; E26.00949; 966m	<i>Cotesia flavipes</i> Cameron	Braconiadae	C	Cp	L	G
Seleka	S22.95112; E27.96191; 783m						
Bobonong	S21.98344; E28.44528; 707m						
Ramotswa	S24.82314; E25.86776; 1003m						
Ramatlabama	S25.60480; E25.56388; 1301m						
Hatsalatladi	S24.04468; E25.62869; 1107m						
Glen valley	S24.59256; E25.97819; 960m						
Ramotswa	S24.82314; E25.86776; 1003m	<i>Cotesia sesamiae</i> Cameron	Braconiadae	C	Cp	L	G
Tlokweng	S24.62219; E25.97400; 945m						
Glen valley	S24.59256; E25.97819; 960m						
Dithojane	S22.45031; E26.76353; 1076m	<i>Chelonus curvimaculatus</i> Cameron	Braconidae	C	Cp	L	S
Moshupa	S24.78787; E25.41477; 1096m			N	Cp	L	S
Maun	S20.00484; E23.42598; 924m			N	Sj	L	S
Gaborone Dam	S24.70752; E25.90632; 992m			N	Sj, Cp	L	S

Glen valley	S24.59256; E25.97819; 960m	<i>Dolichogenidea polaszeki</i> Walker	Braconidae	C	Cp	L	S
Qabo	S21.05622; E21.74792; 1082m	<i>Stenobracon rufus</i> Szepligeti	Braconidae	C	Cp	P	S
Lobatse	S25.25092; E25.70840; 1200m	<i>Temelucha picta</i> Holmgren	Ichneumonidae	C	Cp	L	S
Chanoga	S20.17411; E23.69703; 935m	<i>Chalcidoidea</i> sp	Encyrtidae	C	Cp	L	S
Bookane	S23.06528; E27.53493; 881m	<i>Ceraphronidae</i> sp	Ceraphronidae	C	Cp	L	S
Ditladi	S21.47007; E27.52998; 952m	<i>Eichneumonidea</i> sp	Braconidae	C	Sc, Cp	L	S
Mabutsane	S24.44993; E23.58053; 1091m	<i>Rhaconotus</i> sp	Braconidae	C	Cp	L	G
		<i>Norbanus</i> sp	Pteromalidae	C	Sc, Cp	L	G
Kumakwane	S24.72081; E25.51619; 1059m	<i>Linepithema humile</i> Mayr	Formicidae	C	Cp	L	G
Ndumba	S17.97402; E24.44734; 932m						
Parakarungu	S18.03961; E24.29545; 938m			N	Es	P	G
Maun	S20.00484; E23.42598; 924m			N	Es	L	G
Nxamaseri	S18.60587; E22.08834; 1008m			N	Es	L	G
Kasane,	S24.61832; E26.15096; 1020m	<i>Aenictus</i> sp	Formicidae	C	Sc	L	G
Mochudi	S17.98124; E24.64452; 935m			C	Cp	P	G
Matlapana	S 19.94073; E 23.50739; 937m						
Martins Drift	S 22.99681; E 27.94028; 783m			N	Es	L	G
Hatsalatladi	S24.04468; E25.62869; 1107m	<i>Crematogaster peringueyi</i> Emery	Formicidae	C	Sc	L	G
Ramotswa	S24.82314; E25.86776; 1003m						
Maun	S20.00484; E23.42598; 924m			N	Sj	L	G
Kasane	S17.793325; E25.18863; 944m	<i>Bracon testaceorufatus</i> Granger	Braconidae	N	Sc	L	G
Kanye	S24.94807; E25.34476; 1239m	<i>Dentichasmias busseolae</i> Heinrich	Ichneumonidae	N	Tot	P	S
Kasane	S17.79203; E25.19751; 930m	<i>Cremastinae</i> sp	Ichneumonidae	N	Sc	L	S
Maun	S20.00484; E23.42598; 924m	<i>Cercerinae</i> sp	Philantenidae	N	Sj	L	S
Nxamaseri	S18.60587; E22.08834; 1008m	<i>Formicidae</i> sp	Formicidae	N	Es	L	S
Samedupi	S 20.11207; E 23.52979; 935m	<i>Syzeuctus</i> sp	Ichneumonidae	N	Tot, Sj	L	S
Diptera							
Hatsalatladi	S24.04468; E25.62869; 1107m	<i>Descampsina sesamiae</i> Mesnil	Tachnidae	C	Cp	L	G
Qabo	S21.05622; E21.74792; 1082m						
Mabutsane	S24.44993; E23.58053; 1091m	<i>Sturmiopsis parasitica</i> Curran	Tachnidae	C	Cp	L	S/G
Oodi	S24.54710; E26.00949; 966m	<i>Sepsidae</i> sp	Sepsidae	C	Cp	L	G

Hatsalatladi	S24.04468; E25.62869; 1107m	<i>Chloropidae</i> sp	Chloropidae	C	Cp	L	S
Gatsatsa	S25.26881; E25.27763; 1254m						
Satau	S18.09805; E24.43839; 944m	<i>Siphona murina</i> Mesnil	Tachinidae	N	Sn, Sp	L	G
Shakawe	S 18.35927; E 21.84229; 956m	<i>Phoridae</i> sp	Phoridae	N	Es	L	G

C, cultivated; N, natural; Cp, *Chilo partellus*; Con, *Conicofrontia* sp; Sj, *Sesamia jansei*; Sn, *Sesamia nonagrioides*, Tot, *Totricidae* sp; Sc, *Sesamia calamistis*; Es, *Eldana saccharina*; Sp, *Sesamia perplexa*; L, larva; P, pupa; G, gregarious; S, solitary

Table 5: Natural enemies and their associated cultivated host plants recovered in Botswana.

Natural Enemy Species	Poaceae		
	Maize	Sorghum	Sweet sorghum
Bethylidae			
<i>Goniozus indicus</i>			X
Braconidae			
<i>Euvipio rufa</i>		X	X
<i>Cotesia flavipes</i>	X	X	X
<i>Cotesia sesamiae</i>	X	X	X
<i>Chelonus curvimaculatus</i>			X
<i>Dolichogenidea polaszeki</i>	X		
<i>Stenobracon rufus</i>	X		
<i>Eichneumonidea</i> sp			X
<i>Bracon testaceorufatus</i>			
<i>Rhaconotus</i> sp			X
Ceraphronidae			
<i>Ceraphronidae</i>		X	
Chloropidae			
<i>Chloropidae</i> sp		X	
Encyrtidae			
<i>Chalcidoidea</i> sp	X		
Eulophidae			
<i>Pediobius furvus</i>	X	X	
Formicidae			
<i>Aenictus</i> sp	X	X	
<i>Linepithema humile</i>	X		X
<i>Crematogaster peringueyi</i>	X	X	
<i>Formicidae</i> sp			
Ichneumonidae			
<i>Telemucha picta</i>	X		
<i>Gambroides nimbipennis</i>		X	
<i>Cremastinae</i> sp			
<i>Dentichasmias busseolae</i>			

<i>Syzeuctus</i> sp			
Philantenidae			
<i>Cercerinae</i> sp			
Phoridae			
<i>Phoridae</i> sp			
Pteromalidae			
<i>Norbanus</i> sp			X
Sepsidae			
<i>Sepsidae</i> sp	X		
Tachnidae			
<i>Descampsina sesamiae</i>	X		X
<i>Sturmiopsis parasitica</i>			X
<i>Siphona murina</i>			

Table 6: Natural enemies and their associated natural host plants recovered in Botswana.

Natural Enemy Species	Poaceae				Cyperaceae					Typhaceae	
	<i>Sorghum bicolor</i> subsp <i>arundinaceum</i>	<i>Echinochloa</i> <i>pyramidalis</i>	<i>Hyparrhenia</i> sp	<i>Vossia</i> <i>cuspidata</i>	<i>Oryza</i> <i>longistaminata</i>	<i>Chrysopogon</i> <i>nigritanus</i>	<i>Cyperus dives</i>	<i>Schoenoplectus</i> <i>corymbosus</i>	<i>Cyperus</i> <i>papyrus</i>	<i>Cyperus</i> <i>digitatus</i> subsp <i>auricomus</i>	<i>Typha latifolia</i>
Bethylidae											
<i>Goniozus indicus</i>					X			X			
Braconidae											
<i>Euvipio rufa</i>											
<i>Cotesia flavipes</i>											
<i>Cotesia sesamiae</i>											
<i>Chelonus curvimaculatus</i>							X				
<i>Dolichogenidea polaszeki</i>											
<i>Stenobracon rufus</i>											
<i>Eichneumonidea</i> sp											
<i>Bracon testaceorufatus</i>		X									
<i>Rhaconotus</i> sp											
Ceraphronidae											
<i>Ceraphronidae</i>											
Chloropidae											
<i>Chloropidae</i> sp											
Encyrtidae											
<i>Chalcidoidea</i> sp											
Eulophidae											
<i>Pediobius furvus</i>											
Formicidae											
<i>Aenictus</i> sp										X	
<i>Linepithema humile</i>								X	X		
<i>Crematogaster peringueyi</i>			X					X			
<i>Formicidae</i> sp									X		
Ichneumonidae											

3.3.2 Rate of Parasitism

The highest level of parasitism was recorded in cultivated habitats compared to natural habitats. Larval parasitism ranged between 2.1- 34.7% and 3.3- 14.3% in cultivated (Table 7) and natural (Table 8) habitats respectively. In addition, pupal parasitism ranged between 6.1- 10.6% and 6.7- 9.1% in cultivated and natural habitats respectively (Tables 7 & 8). In cultivated habitats, the parasitoids *C. flavipes* and *C. sesamiae* had the highest larval parasitism levels of 34.7% and 29% respectively, whilst *G. nimbipennis* had the highest pupal parasitism of 10.6% (Table 7). However in natural habitats, *C. curvimaculatus* had the highest larval parasitism (14.3%) whilst *D. busseolae* had highest pupal parasitism (9.1%) (Table 8).

Table 7: Larval and pupal parasitism in cultivated host plants in Botswana.

Host Plant Species	Host species	Parasitoid species	% Parasitism
Maize	<i>Chilo partellus</i>	<i>Cotesia flavipes</i>	34.7 (33)
		<i>Cotesia sesamiae</i>	18.8 (13)
		<i>Euvipio rufa</i>	4.3 (2)
		<i>Sepsidae</i> sp	2.1 (1)
		<i>Pediobius furvus</i>	8.5 (5)
		<i>Descampsina sesamiae</i>	5.9 (1)
		<i>Chalcidoidea</i> sp	7.7 (1)
		<i>Telemucha picta</i>	2.9 (1)
		<i>Dolichogenidea polaszeki</i>	6.3 (1)
		<i>Stenobracon rufus</i>	6.1 (2)
Sorghum	<i>Chilo partellus</i>	<i>Cotesia flavipes</i>	5.7 (2)
		<i>Cotesia sesamiae</i>	12.5 (2)
		<i>Chloropidae</i> sp	4.5 (2)
		<i>Descampsina sesamiae</i>	6.3 (1)
		<i>Ceraphonidae</i> sp	7.1 (1)
Sweet sorghum	<i>Concofrontia</i> sp	<i>Euvipio rufa</i>	5.9 (1)
	<i>Sesamia calamistis</i>	<i>Echneumonidea</i> sp	2.8 (1)
	<i>Chilo partellus</i>	<i>Cotesia flavipes</i>	17.6 (3)
		<i>Cotesia sesamiae</i>	29 (9)
		<i>Goniozus indicus</i>	5.9 (1)
		<i>Rhaconotus</i> sp	4.3 (1)
		<i>Norbanus</i> sp	4.3 (1)
		<i>Sturmiopsis parasitica</i>	4.3 (1)
		<i>Chelonus curvimaculatus</i>	9.1 (1)
		<i>Echneumonidea</i> sp	3.2 (1)
		<i>Gambroides nimbipennis</i>	10.6 (3)

() number of parasitised hosts

Table 8: Larval and pupal parasitism in natural host plants in Botswana.

Host Plant Species	Host species	Parasitoid species	% Parasitism
<i>Sorghum bicolor</i> subsp <i>arundinaceum</i>	<i>Sesamia calamistis</i>	<i>Bracon testaceorufatus</i>	4 (1)
	<i>Sesamia nonagrioides</i>	<i>Siphona murina</i>	3.3 (1)
<i>Schoenoplectus</i> <i>corymbosus</i>	<i>Sesamia jansei</i>	<i>Goniozus indicus</i>	4.8 (1)
		<i>Cercerinae</i> sp	9.5 (2)
	<i>Tortricidae</i> sp	<i>Chelonus curvimaculatus</i>	14.3 (3)
		<i>Dentichasmias busseolae</i>	9.1 (2)
<i>Cyperus dives</i>	<i>Chilo partellus</i>	<i>Chelonus curvimaculatus</i>	7.8 (5)
<i>Oryza longistaminata</i>	<i>Sesamia nonagrioides</i>	<i>Goniozus indicus</i>	3.5 (1)
<i>Typha latifolia</i>	<i>Sesamia jansei</i>	<i>Gambroides nimbipennis</i>	6.7 (1)
	<i>Tortricidae</i> sp	<i>Syzeuctus</i> sp	4.2 (1)
<i>Chrysopogon</i> <i>nigritanus</i>	<i>Sesamia calamistis</i>	<i>Cremastinae</i> sp	4 (1)
<i>Cyperus papyrus</i>	<i>Eldana saccharina</i>	<i>Formicidae</i> sp	3.8 (1)
<i>Vossia cuspidata</i>	<i>Sesamia perplexa</i>	<i>Siphona murina</i>	4.5 (1)
<i>Echinochloa</i> <i>pyramidalis</i>	<i>Sesamia jansei</i>	<i>Syzeuctus</i> sp	5.2 (1)
<i>Cyperus papyrus</i>	<i>Eldana saccharina</i>	<i>Phoridae</i> sp	3.7 (1)

() number of parasitised hosts

3.3.3 Relative Abundance of Parasitoid Species

Larval and pupal parasitoids relative abundance ranged between 1.1- 41.6% in cultivated and 4.8-38.1% in natural habitats (Fig. 6 & 7). Hymenopteran parasitoids were more abundant than Dipteran parasitoids in both habitats accounting for 21 and 6 respectively. The hymenopteran larval parasitoids *C. flavipes* and *C. sesamiae* were the most abundant in cultivated habitats, constituting 41.6% and 24.7% of the parasitoids found respectively (Fig. 6). However, in natural habitats *C. curvimaculatus* dominated in abundance constituting 38.1% of the parasitoids found there (Fig. 7). Similarly, hymenopteran pupal parasitoid *P. furvus* was the most abundant in cultivated habitats, making up 7.9% of the parasitoid population, whilst *D. busseolae* dominated in natural habitats, contributing 9.5% of the parasitoids' abundance (Fig. 6 & 7).

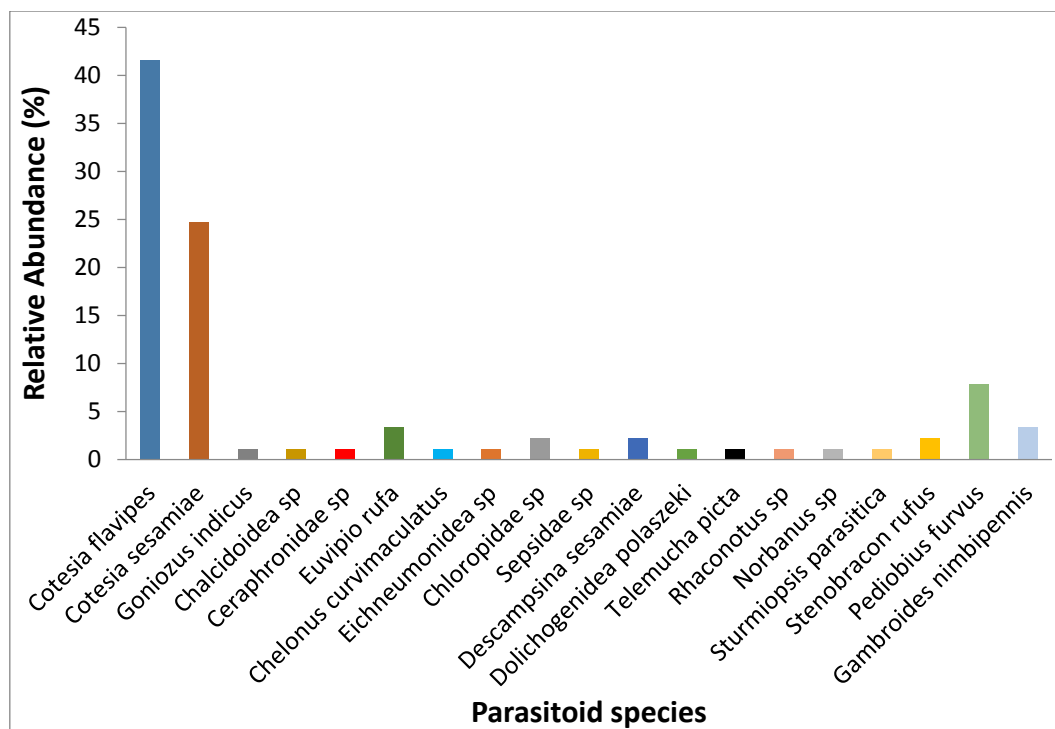


Figure 6: Relative abundance of cereal stemborer larval parasitoids in Botswana.

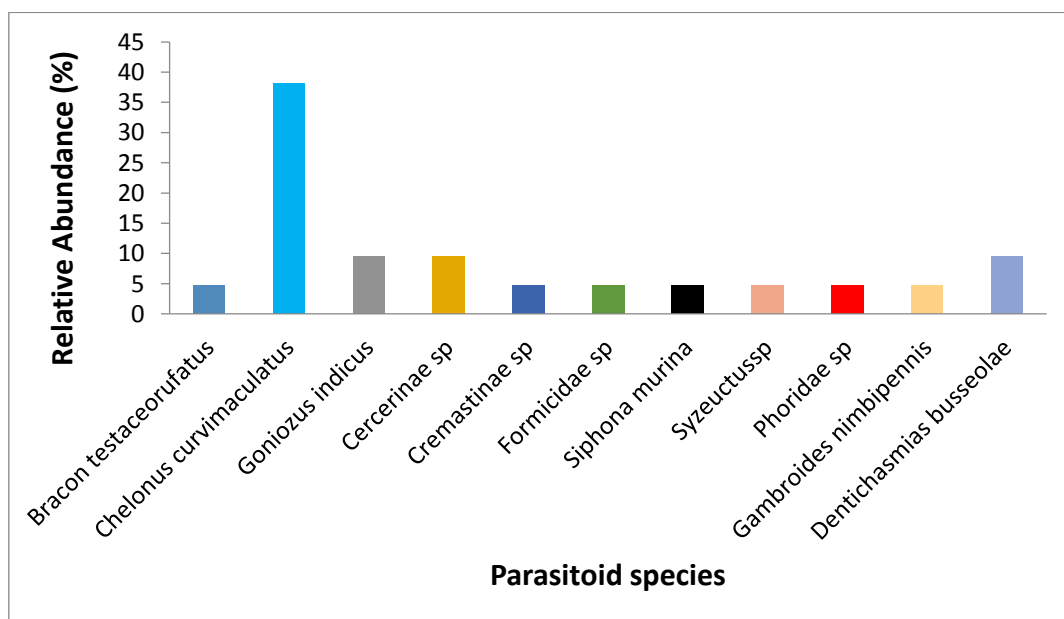


Figure 7: Relative abundance of cereal stemborer pupal parasitoids in Botswana.

3.4 Discussion

Understanding the interactions between pest species (herbivore), the plant (cultivated and natural hosts) and their natural enemies are important in developing sustainable pest management options (Hance *et al.*, 2007). To my knowledge, this is the first report detailing the tritrophic relationships and diversity of lepidopteran stem borer natural enemies, from both natural and cultivated habitats in Botswana.

3.4.1 Natural Enemy Diversity, Associated Stemborer Species and Host plants

The current research reports 30 natural enemy species of lepidopteran stem borers in both cultivated and natural habitats in Botswana. Prior to this study, only nine parasitoid species had been reported in sorghum, maize and *Cyperus papyrus* L. (Conlong *et al.*, 1988; Obopile & Mosinkie, 2003). Similarly, no predatory species had been reported attacking lepidopteran stem borers in the country. Nevertheless, the current study reports predators *L. humile*, *C. peringueyi* and *Aenictus* sp in both cultivated and natural host plants. Conlong *et al.* (1988) reported the presence of *Goniozus natalensis* Gordh (= *G. indicus*) on *C. papyrus* along the Chobe river in Botswana, in keeping with the current study reporting the occurrence of this parasitoid in natural habitats. In addition, Obopile & Mosinkie (2003) reported the presence of *C. sesamiae*, *P. furvus* and *S. rufus* in agreement with the current study.

My results showed a higher parasitoid diversity in cultivated than natural habitats, contrary to previous surveys in Cameroon (Ndemah *et al.*, 2007), Uganda (Matama-Kauma *et al.*, 2008) and Kenya (Mailafiya *et al.*, 2009) which reported a high stem borer parasitoid diversity on natural host plants. Nevertheless, the results are in keeping with a previous study by Zhou *et al.* (2003) that reported a high parasitoid diversity in cultivated habitats in the coastal region of Kenya. Thus, my results do not support the hypothesis that there is higher parasitoid diversity in natural compared to cultivated habitats. The current study also recorded a moderate Shannon interaction diversity and low generality for both stem borer–parasitoid and host plant–parasitoid interaction, indicating on average that each stem borer species was attacked by one to two parasitoid species (see Helena *et al.*, 2016). This also implies that every host plant hosted stem borers that were attacked by one to two parasitoid species. Therefore, this parasitoid overlap may optimize stem borer parasitism and hence improve biological control efficacy.

Chilo partellus was the most commonly targeted stem borer species by parasitoids and predators in cultivated habitats whilst in natural habitats *Sesamia* species were the most commonly targeted may be due to abundance of *C. partellus* and *Sesamia* species in cultivated and natural habitats respectively. However, the sugarcane stem borer, *E. saccharina* was the least attacked by natural enemies in natural habitats. This may be because *E. saccharina* spins defensive silk cocoons around its body and host stem tunnels. Furthermore, *E. saccharina* secretes exudates upon disturbance and these may have repellent effects on natural enemies (Sampson & Kumar, 1986; Hordzi & Botchey, 2012). The larval parasitoids *C. flavipes* and *C. sesamiae* and pupal *P. furvus* were the most prevalent species, in keeping with previous results from eastern Africa (Zhou *et al.*, 2003; Mailafiya *et al.*, 2009). While *C. sesamiae* is considered a generalist parasitoid (Branca *et al.*, 2011) and *C. flavipes* more specific to *C. partellus* (Mailafiya *et al.*, 2010b), in the current study, these two parasitoids were recovered on *C. partellus* in cultivated habitats may be due to high abundance and wide distribution of the host *C. partellus* in these habitats relative to other species. Although *C. partellus* was recovered on *Cyperus dives*, there were no *C. flavipes* individuals recorded in natural habitats, in agreement with results by Moolman *et al.* (2013). A gregarious larval parasitoid *Bracon testaceorufatus* Granger, previously known to attack rice stem borers (Van Achterberg & Walker, 1998) was recorded parasitising *S. calamistis* and not *S. jansei* as previously reported by Moolman *et al.* (2013) in the natural habitats, suggesting probable host diversity. In natural habitats, the Cyperaceous host plant, *S. corymbosus* had the highest stem borer parasitoid diversity, in keeping with results by Moolman *et al.* (2013). The graminaceous crops maize and sorghum had the highest stem borer parasitoid diversity in cultivated hosts. This may be because cultivated plants constitute an important resource with higher levels of nutrients and low allelochemical toxicity relative to natural host plants (Ofomata *et al.*, 2000; Mailafiya *et al.*, 2009). These attributes promote higher survival and stem borer parasitoid richness in the cultivated relative to the natural hosts. To my knowledge, no study to date has reported *C. partellus* parasitism on host *Cyperus dives*. Therefore, I report for the first time, *C. curvimaculatus* parasitizing *C. partellus* on *C. dives*.

3.4.2 Parasitisation Levels in Cultivated and Wild Host Plants

In cultivated habitats larval and pupal parasitism ranged between 2.1- 34.7% and 6.1- 10.6% respectively, in keeping with previous reports in East Africa for both larval

(Matama-Kauma *et al.*, 2008; Mailafiya *et al.*, 2009) and pupal (Zhou *et al.*, 2003) parasitism. Larval and pupal parasitism levels were also higher in cultivated than natural habitats, in agreement with results by Mailafiya (2011). Low parasitism levels in natural habitats may be caused by lower densities of stem borers relative to cultivated habitats (Mailafiya *et al.*, 2009; Moolman *et al.*, 2014) and this has negative consequences on parasitoid host searching efficiency (as in e.g. Udayagiri & Walter, 2000). Obipile & Mosinkie (2003) reported highest parasitism levels of 13.3% for *C. sesamiae*, 10.3% for *P. furvus* and 3.2% for *S. rufus* on *C. partellus* on maize and sorghum in Botswana. However, the current study reports generally higher *C. partellus* parasitism levels in cultivated habitats ranging 18.8% for *C. sesamiae*, 6.1% for *S. rufus* and of 8.5% for *P. furvus*. This may again, be due to higher densities of *C. partellus* relative to other stem borer species in these habitats. *Cotesia flavipes* recorded the highest larval parasitism levels in cultivated habitats, in corroboration with (Sétamou *et al.*, 2005), who reported higher larval parasitism by *C. flavipes* in cultivated relative to natural habitats. High parasitism level for *C. flavipes* (34.7%) also suggests efficiency of the parasitoid in host utilization. Indeed, research has shown *C. flavipes*' high host searching capability that permits it to exploit habitats with low stem borer host densities (Wiedenmann & Smith, 1993; Mailafiya *et al.*, 2009). While no releases have been done for the exotic *C. flavipes* in Botswana, field releases of this parasitoid have been done in South Africa, (Skoroszewski & Van Hamburg, 1987), Mozambique, (Cugala & Omwega, 2001), Malawi, Zambia (Sohati *et al.*, 2001) and Zimbabwe 1999 (Chinwada *et al.*, 2001). Parasitoid releases in these neighboring countries may account for the presence of this exotic species in Botswana. More so, *C. flavipes* has been reported to parasitise stem borers >2000km away from their release sites (Assefa *et al.*, 2008) suggesting high migration potential, spread and establishment in novel environments. Therefore, host availability and spread potential may partly account for the presence of exotic *C. flavipes* in Botswana. *Gambroides nimbipennis* recorded higher pupal parasitism levels in cultivated habitats relative to natural habitats, suggesting high host density and resource availability in cultivated habitats.

3.4.3 Relative Abundance of Parasitoid Species

Larval parasitoids *C. flavipes* and *C. sesamiae* and pupal parasitoid *P. furvus* had a higher relative abundance compared to other parasitoid species in cultivated habitats. Similarly, the larval parasitoid *C. curvimaculatus* and pupal parasitoid *D. busseolae* had

higher relative abundance in natural habitats. Previous studies have reported ecological relatedness between *C. sesamiae* and *C. flavipes*, suggesting niche overlaps between the two larval parasitoids. Indeed, competition between the two congeners has resulted in the displacement of *C. sesamiae* in some regions (see Ngi-song & Overholt, 1997; Sallam *et al.*, 1999; Zhou *et al.*, 2003). However, in the current study, the two parasitoid species dominated in both abundance and parasitism of the same host, *C. partellus*. This may imply that *C. flavipes* and *C. sesamiae* may potentially share the same habitat regardless of whether they are attacking the same host or not (Overholt *et al.*, 2000). In addition, this result also support previous research by Mailafiya *et al.* (2011), that reported a significant interaction between *C. flavipes* and *C. sesamiae* in regulating cereal stem borer populations in cultivated habitats. The presence and dominance of *C. partellus* in most of the districts in Botswana makes *C. flavipes* a suitable exotic larval parasitoid for augmentative biocontrol field releases (Overholt *et al.*, 2000). *Chelonus curvimaculatus* was recovered in both habitats, and had a higher abundance in natural than cultivated habitats. This implies that this parasitoid can switch habitats, hence can be used in augmentative biological control, in addition to *C. flavipes* and *C. sesamiae*. Moreover, since *P. furvus* and *D. busseolae* are indigenous, and also predominant in Botswana, these pupal parasitoids can be integrated with other key larval parasitoids such as *C. flavipes* and *C. sesamiae* in managing cereal stem borers (Conlong & Cugala, 2008). Although some studies reported the presence of hyperparasitoids (Polaszek & LaSalle, 1995; Rwomushana *et al.*, 2005; Wale *et al.*, 2006; Moolman *et al.*, 2013), the current study did not find any.

In conclusion, the current study documents novel diversity of stem borer natural enemy species in Botswana, and their interactions with host stem borers and host plants in both natural and agroecosystems. I recorded 30 cereal stem borer natural enemy species in both cultivated and natural habitats with 24 of the parasitoids and three predatory species recorded for the first time in Botswana. From these species, larval parasitoids *C. curvimaculatus*, *G. indicus* and pupal parasitoid, *D. busseolae* were common in all habitats. Our results showed that stem borer natural enemy species diversity and parasitism were higher in cultivated than natural habitats probably due to higher availability of host stem borer host species in cultivated than natural habitats. In addition, larval parasitoids were abundantly distributed in both habitats compared to pupal parasitoids. Overall, Botswana landscapes host parasitoid faunal diversity that are worth conserving for future efficacy of biological control programmes which can be useful in intergrated pest management, and an equally diverse fauna and flora hosting parasitoid-host stem borer interactions.

3.5 References

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CHAPTER FOUR

4.1 General discussion

In this study, a total of 15 stemborer species were recorded in both wild and agro-ecosystem habitats. Six species were recorded from agro-ecosystem habitats while 11 were collected from wild habitats. However, there was also an overlap in species inhabiting both natural and agro-ecosystem habitats, for example, *S. calamistis*, *C. partellus* and *Tortricidae* sp2 were recorded in both habitats. Only six species were identified to species level while five were identified up to family level. This calls for more work in identifying the family level stemborers up to species level, followed by the taxonomical description of any new species. Overall, the study is the first to document the stemborer – host – natural enemy tritrophic interactions, and the first to report the diversity of these borer species in a Botswana context. Such similar work has been confined to other parts of Africa other than Botswana (Chinwada & Overholt, 2001; Kruger, 2005; Assefa *et al.*, 2006; Le Ru *et al.*, 2006; Ndemah *et al.*, 2007; Matama-Kauma *et al.*, 2007; 2008; Mailafya *et al.*, 2009; Kodjo *et al.*, 2013; Moolman *et al.*, 2014).

My results showed higher stemborer species diversity in wild habitats compared to agro-ecosystem habitats. Most of the natural habitats were sampled in the northern part of Botswana which has conducive climatic and environmental conditions for a wide range of natural habitats. The seasonal dry conditions in the southern part of Botswana on the other hand were not conducive for survival of a wide range of natural hosts. *C. partellus* and *Sesamia* sp were found to be the most abundant stemborer species in both cultivated and wild habitats respectively in all the districts, except Kgalagadi. This may be due to the lack of rainfall and plant hosts in this area. Furthermore, the temperatures associated with this area may be higher than the optimum needed for the stemborers to thrive. Compounded by the average temperature and variability with climate change, it seems likely that stemborers may not inhabit Kgalagadi.

Identification of stemborers on an array of wild hosts suggest stemborer polyphagy and this may have implications on its management. Stemborers often move from agro-ecosystem to natural habitats and the latter is exploited during off-season when cultivated plants are not available. This means wild hosts may serve as refugia for stemborer propagules for re-infestations later in the season when planting season ‘opens’. I recorded for the first time, *C. partellus* feeding on *Cyperus dives*. This may mean stemborers in general and especially *C. partellus* are broadening their host range. Indeed, this may explain why it has a high invasion potential. Mutamiswa *et al.* (2017) reported that *C. partellus* has higher invasion potential relative to other stemborer species, and is invading new areas where it is,

often, displacing indigenous species. Botswana is an agro-dependent country, mainly arable farming, and especially rural livelihoods for household and economic level sustenance. With an increase in stemborer host range, and increase in invasion potential, there is a possibility of insect pest upsurge thereby threatening household and national food security and consequently livelihoods. The increase in host range for some stemborers remains largely unknown but may be due to diminishing preferred host plants, intensive monoculture in cereal production and climate change.

A total of 30 natural enemy species were recovered in wild and cultivated habitats, of which 27 were parasitoids and three were predator species. Previous study by Obopile & Mosinkie (2003), confirmed the presence of parasitoids *C. sesamiae*, *P. furvus* and *S. rufus* in cultivated habitats. Indeed their findings are corroborating with my findings as the three species were also recorded here. However, Obopile & Mosinkie (2003) only worked in agro-ecosystems and did not consider the natural habitats. Here, I report the collection and identification of 19 parasitoid species from cultivated habitats and 11 from natural habitats with the majority of parasitoids being recovered from *C. partellus*, suggesting a low host and habitat specificity. This may be a survival strategy to enable parasitoids to switch between hosts if need arises (Askew 1994, Hofferster & Vidal, 1994).

Studies done in, Zimbabwe (Chinwada & Overholt 2001), Mozambique and South Africa (Moolman *et al.*, 2013) have reported high parasitoid diversity in wild habitats as compared to cultivated habitats. However, my study reports higher parasitoid diversity in cultivated than wild habitats. *Chilo partellus* was the most preferred stemborer host in cultivated habitats whilst *Sesamia species* hosted most parasitoids in the wild habitats. Common larval parasitoids recorded included *C. flavipes*, *C. sesamiae* and pupal parasitoid, *P. furvus*. According to Overholt (2000), Mailafiya *et al.* (2010) and Kfir *et al.* (2002), *C. sesamiae* is a generalist species, and is the main larval parasitoid of *B. fusca* (Fuller), and was introduced into Madagascar and Mauritius to control cereal stemborers *S. calamistis* and *C. partellus*. Nevertheless, *C. flavipes* is a specific natural enemy, parasitizing especially *C. partellus* (Kfir *et al.*, 2002). A wide diversity of natural enemies reported here suggest Botswana habitats still host biological diversity that ought to be conserved. For pest management, these results present a possibility to harness these parasitoids in future for biological control of stemborer species.

Stemborer species collected during the survey showed a phylogenetic relationship with a common ancestor and *Sesamia jansei*, *S. nonagrioides* and *S. perplexa* appeared to have a common putative ancestor. The stemborers were closely related except *E. saccharina*

which was distantly related to the other groups. Species on the 4th group (*Sesamia* sp, *Sesamia sataui* and majority being *Sesamia nonagrioides*) in the phylogenetic tree were the most diverse. This variation may have been caused in part, by the variation in host or geographic isolation. Knowledge of this phylogenetic relationship is highly critical in managing these economic insects. For like/related borer species, it means control measures may overlap since the genetic makeup is largely the same.

Stemborers were collected from both the natural and cultivated hosts. In the cultivated hosts, *Zea mays* hosted more stemborer species (*S. calamistis*, *C. partellus*, *Mythimne loreyi*, *Tortricidae* sp2 and *Tortricidae* sp3) as compared to the two sorghum species. For the natural hosts, *S. corymbosus* and *C. papyrus* were found to host more stemborer species as compared to the other hosts. In a study conducted by Moolman (2014) in South Africa, Schoenobiinae were recovered from Cyperaceae and Typhaceae, and Tortricidae were collected from Poaceae, Cyperaceae, Juncaceae and Typhaceae. This is in consistant with our study where *Typha latifolia* and *Cyperus papyrus* were found hosting Schoenobiinae while *Z. mays*, *Cyperus dives* and *Schoenoplectus corymbosus* hosted different Tortricidae species. *Chilo partellus* was mostly found in the cultivated hosts than the natural hosts, which is in line with a study conducted by Ong'amo *et al.* (2006) in Kenya.

Overall, the results of this study are important in understanding host plant-stemborer-natural enemies tritrophic interactions in order to develop and implement appropriate pest management practices. Though the study was the second to focus on cultivated host plant-stemborer-parasitoid interaction in Botswana, it also presents the first comprehensive findings on stemborer tri-trophic interactions in both agro- and wild ecosystems. Such data is important in planning pest management strategies, especially biological control, conservation of natural enemies and integrated pest management. Our results also reveal the important role of natural habitats in the population ecology of natural enemies and their effect on levels of parasitism of stemborers in cereal crops in both wild and agro-ecosystem setups.

4.2 Conclusions

Botswana hosts a wide range of stemborer species in both wild host plants and cultivated host plants. The natural habitats have a higher stemborer diversity than the cultivated hosts. On the other hand, stemborer parasitoid diversity was higher in cultivated than in wild habitats. Wild hosts surrounding cereal crops appeared to serve as refugia for sustaining a wide range of parasitoids. Knowledge on stemborer-parasitoid interaction is

important in the development and implementation of appropriate pest management practices in the future. We recommend further identification of the stemborer species, which we only identified to family and genus levels, as there are possibilities of identifying new stemborer species. Furthermore, the role of natural habitats as refugia for stemborer parasitoids needs to be investigated to understand the suitability of wild stemborer species to parasitoids of cultivated cereal stemborer pests. These results suggest a wide diversity of stemborer host plants, stemborers and their natural enemies. Diversity of the latter (natural enemies) shows that conservation biological control may be a viable option for pest management of stemborers in Botswana.

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