A guide to biological control of maize stemborers, *Busseola fusca, Sesamia calamistis* and *Chilo partellus,* using the larval parasitoids, *Cotesia sesamiae* and *Cotesia flavipes*

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Cover photos:
Top left: ***Sesamiae calamistis*** larva
Top right: ***Sesamiae calamistis*** adult male
Bottom right: Stemborer eggs and larvae
Bottom left: *Cotesia sesamiae*

A Guide to biological control of maize stemborers, *Busseola fusca, Sesamia calamistis* and *Chilo partellus,* using the larval parasitoids, *Cotesia sesamiae* and *Cotesia flavipes*.

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Table of Contents

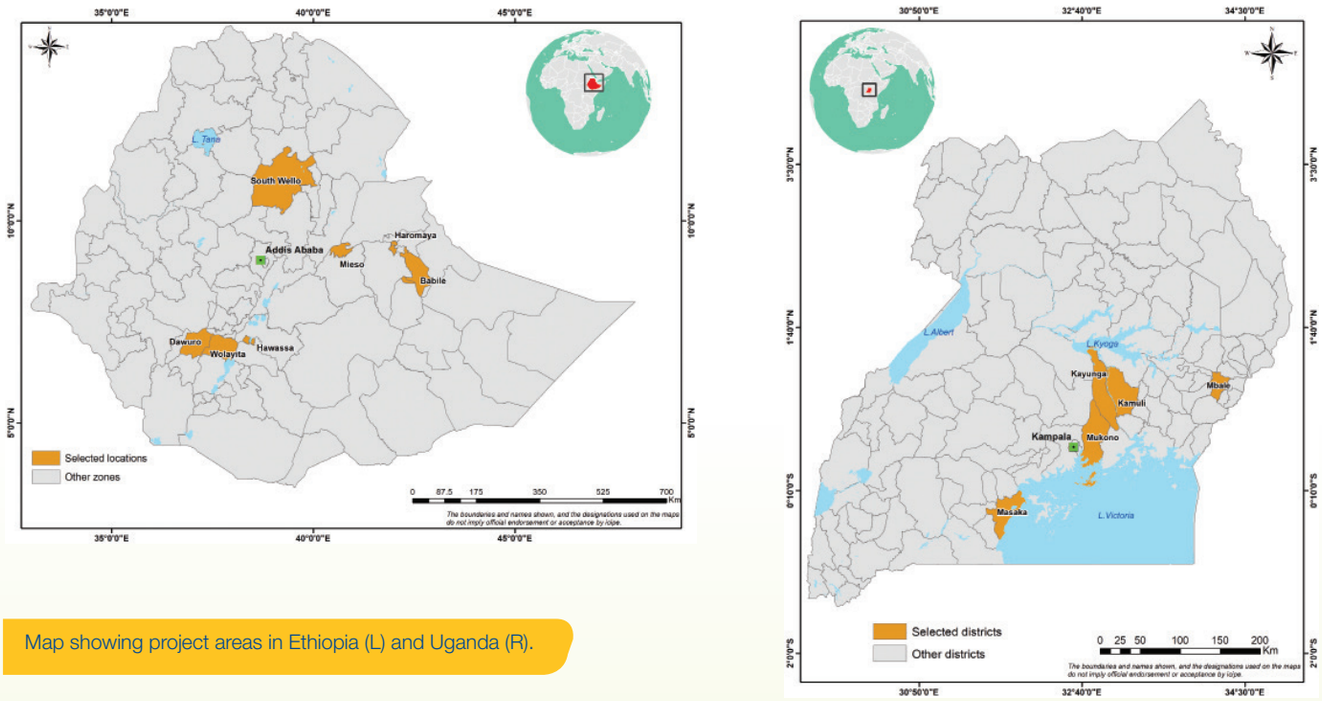
About SCLAMP-EA project

Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa (SCLAMP-EA) is a project funded by German Corporation for International Cooperation (GIZ). It is a 3-year project running from 2020 to 2022.

The purpose of the project is to facilitate the large-scale adoption of proven and piloted Climate Smart Pest Management (CSPM) technologies and practices by smallholder farmers to improve their food and nutrition security through mitigating yield losses due to key insect pests in maize and tomato.

The projects' target areas are:

- Ethiopia (Southern/SNNPR in Dawuro, Angacha and Shebedino; Northern/Amhara in South Wollo and Western Oromia Region in Sasiga and Diga); and
- Uganda (Central Uganda in Rakai and Kyotera; Eastern Uganda in Kamuli, Namutumba, Mbale and Kween; and Northern Uganda in Amuru, Nwoya, Adjumani and Pakwach/Southern West Nile).



Map showing project areas in Ethiopia (L) and Uganda (R).

Purpose of Manual

To provide a working document for rearing insect hosts (stemborers), and their larval parasitoids (Cotesia). Biological control using larval parasitoids will reduce damage caused maize stemborers, increase maize yield, protect the environment and safeguard human health – through reduced use of synthetic chemicals.

This manual describes: maize stemborers (*Busseola fusca, Sesamia calamistis* and *Chilo partellus*); larval parasitoids (*Cotesia sesamiae* and *Cotesia flavipes)*; their lifecycle, distribution, mass production, rearing procedure and colony establishment. It also describes storage, packing and field release of the larval parasitoids.

Objective of Manual

To strengthen the practice of biological control for maize stemborers. The challenge currently being faced by research institutions is the lack of adequate information on how to properly identify maize stemborers species, Cotesia species, and practical information on how to rear larval parasitoids.

Consequently, there is a low adoption rate of biological control by maize growers, and over-reliance on synthetic chemicals for pest and disease control.

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Abbreviations

AEZ	Agro-ecological zone
CIBC	Commonwealth Institute for Biological Control
cm	Centimetre
Cs	*Cotesia sesamiae*
CSPM	Climate Smart Pest Management
DNA	Deoxyribonucleic acid
FAO	Food and Agriculture Organization
FAW	Fall armyworm
g	Gram
GIZ	German Corporation for International Cooperation
Hr	Hour
icipe	International Centre of Insect Physiology and Ecology
km	Kilometre
KOH	Potassium hydroxide
L:D	Light:Darkness
M	Metre
Min	Minute
Mm	Millimetre
PCR	Polymerase chain reaction
RH	Relative Humidity
S	Second
SCLAMP-EA	Scaling-up Climate-Smart Pest Management Approaches for Enhanced Maize and Tomato Systems Productivity in Eastern Africa
TTU	Technology Transfer Unit
USD	United States Dollar

Introduction

African traditional crops (Poaceae), including sorghum (Sorghum bicolour (L.) Moench), pearl millet (Pennisetum glaucum (L.) R. Br.), finger millet (Eleusine coracana (L.) Gaertn) and African rice (Oryza glaberrima Steud), are the most widely cultivated cereal crops in Africa. However, maize (Zea mays L.) cultivation has exceeded the production of these traditional cereals, since its introduction into Africa during the 16th century (Macauley & Ramadjita, 2015). Today, maize is the main staple food crop grown in diverse African agro-ecological zones and farming systems (FAO, 2020). It is estimated that approximately 208 million people on the African continent depend on maize as a staple food (FAO, 2020). Despite the large production area and importance of maize and sorghum, the average grain yield of these crops inSaharan Africa is very low, with yields being generally <1.0 ton/ha, representing some of the lowest in the world (Cairns et al., 2013). This may be due to several abiotic and biotic constraints. Among the biotic constraints are the insect pests, especially Lepidopteran stemborers and the recent invasion of Fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) in maize fields in many parts of East Africa, which are considered the most crucial pests of maize in the region.

Apart from the exotic invasive pest FAW, the other most important pests are Lepidopteran stemborers such as the indigenous noctuids, *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson), and the exotic crambid, *Chilo partellus* (Swinhoe) (Kfir et al., 2002).

Different strategies, mainly comprising cultural and chemical controls, have been used to control stemborer infestation in Africa (Kfir et al., 2002). Cultural strategies include wild host plants, burning of crop residues, manipulating of planting dates, crop rotation, managing planting density and choice of varieties. Although cultural practices are very promising as strategies for reducing borer pests damage, they have not been adopted due to constraints in their use, making them impracticable and unattractive to farmers (Kfir et al., 2002). On the other hand, inconvenience inherent chemical control includes insect resistance to pesticides, adverse effects on non-target species, hazards of pesticides residues, direct hazard from pesticides, non-guaranteed results, high costs, and unavailability (Goftishu et al., 2017). Moreover, the use of pesticides requires a level of know-how about their efficiency, but this is usually limited. In addition, there is a general consensus that the use of synthetic pesticides in small-scale cereal production is unsafe and not profitable (Abate et al., 2000). Considering these constraints and the potentially negative impact of chemical control on human health and environment, biological control has been found to be an appropriate and efficient method to control Lepidopteran stemborers (Midingoyi et al., 2016). Because of its self-perpetuating characteristics when well established and the non-requirement for recurrent additional investment, biological control remains undoubtedly an appropriate tool for pest control by resource-poor farmers (Hajek, 2004; Kipkoech et al., 2006). The International Centre of Insect Physiology and Ecology (icipe), in partnership with the German Agency for International Cooperation (GIZ), is implementing stemborer biological controls in Ethiopia and Uganda. The larval parasitoids *Cotesia sesamiae* and *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) are considered excellent candidates among the biological control agents of stemborers for augmentative release. *Cotesia sesamiae* is one of the most important native parasitoids of stemborers in many countries of sub-Saharan Africa. This is an indigenous gregarious larval endoparasitoid that attacks mid to late instars of the stemborer larvae (Bonhof et al., 1997; Kaiser et al., 2015). *Cotesia flavipes* was introduced from Asia into Africa as a classical biological control agent and released in 1993 by icipe for the control of *C. partellus* the invasive exotic stemborer of maize and sorghum in Eastern and Southern African lowlands (Overholt et al., 1994a, b; Overholt et al., 1997).

Maize stemborers: *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus*

Lepidopteran stemborers are classified into five families, as follows: Noctuidae, Crambidae, Pyralidae, Tortricidae and Cossidae. In Kenya, for example, a total of 61 stemborer species belonging to families Noctuidae (25), Crambidae (14), Pyralidae (9), Tortricidae (11) and Cossidae (2) are reported to have been recovered from 42 wild plant species (Le Ru et al., 2006a & 2006b). However, two noctuid species, *B. fusca* and *S. calamistis*, and two crambids, *C. partellus* and Chilo orichalcociliellus Strand, are the four main borer species found in (and associated with) maize plants in East Africa (Le Ru et al., 2006a, b). Except for *C. partellus*, all the maize stemborer pests are believed to be indigenous. *Chilo partellus* invaded Africa from Asia, probably before 1930 (Overholt et al., 1994a). Since its introduction to Africa, *C. partellus* has spread to all countries in Eastern and southern Africa and has often become the most damaging stemborer of maize and sorghum especially in warmer lowland areas.

Figure 1: Typical damages on the leaves left by young stemborer larvae.

How to detect their presence in the field?

Since 2017, maize fields have generally become infested by both Fall Armyworm and Lepidopteran stemborers. The typical damages on the leaves left by young larvae (or caterpillars) of stemborers when they feed on the leaf surfaces are shown in Figure 1.

When the stemborer larvae are growing, they start to feed into the maize stems (Figure 2A), causing dead-heart (Figure 2B) if the maize plant is young (the centre of the young plant is drying) or visible holes (Figure 2C) along the stem of older maize plants.

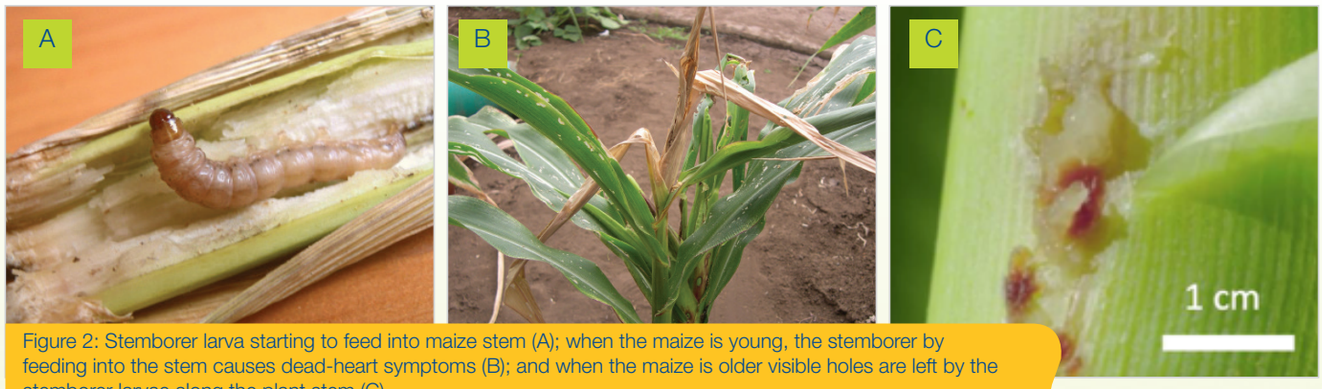


Figure 2: Stemborer larva starting to feed into maize stem (A); when the maize is young, the stemborer by feeding into the stem causes dead-heart symptoms (B); and when the maize is older visible holes are left by the stemborer larvae along the plant stem (C).

In contrast, the typical damages caused by young and older larvae of FAW are to the leaves, since they feed only on leaves and not inside the maize stems, as shown in Figure 3.

Figure 3: Typical damages on leaves left by FAW larvae.

The lifecycle of *Busseola fusca*, *Sesamia calamistis* and *Chilo partellus*

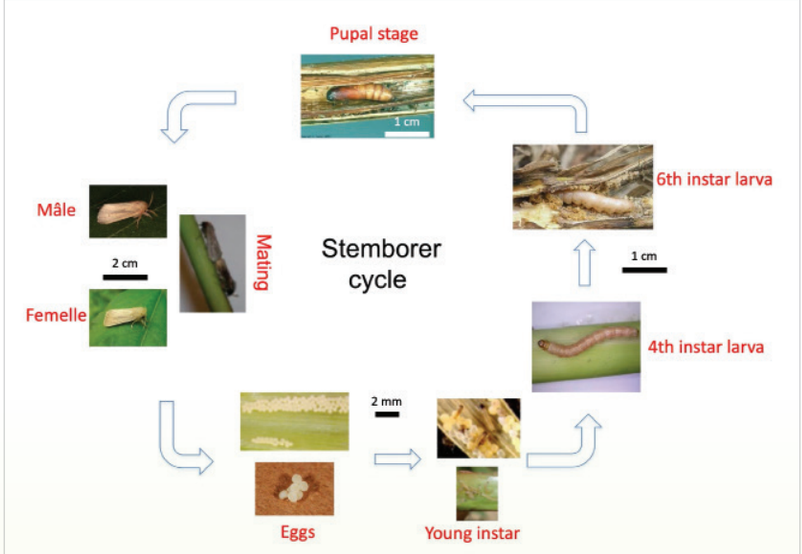


Figure 4. General scheme of stemborer's life cycle.

The general scheme of the stemborer's life cycle (Figure 4) encompasses the development from eggs to adults (both males and females). Stemborers generally have six larval stages. At young larval stages, the small larvae (or caterpillars) feed on leaves, while they grow older,, they bore into the stem and feed inside the plant stems, up to the pupal stage (generally pupating into the stem).

Busseola fusca (Figure 5) takes about 60 days to develop from egg to the adult stage, although this duration varies greatly depending on climatic conditions, which include variations in temperature, humidity and atmospheric pressure (Calatayud et al., 2014).

Figure 5: Developmental stages of *Busseola fusca*: (A) eggs laid between a leaf sheath and stem, (B) larvae, (C) pupa, and (D) male and (E) female adults.

Sesamia calamistis (Figure 6) completes its life cycle within 44 to 56 days (Khadioli et al., 2014).

Figure 6: Developmental stages of *Sesamia calamistis*: (A) eggs batch, (B) larva, (C) pupae, and (D) male (above) and female (below) adults.

The complete life cycle of *C. partellus* (Figure 7) ranges between 38 and 55 days.

Figure 7: Developmental stages of *Chilo partellus*: (A) eggs laid on leaf surface, (B) larva, (C) pupa, and (D) male (left) and female (right) adults.

How to distinguish the three stemborer species?

The last instar larvae, the pupae and adults (both males and females) exhibit morphological characteristics specific to each stemborer species, allowing us to distinguish them as follows:

Busseola fusca

Size in last instar: 40mm

Colour: Yellowish white with dark longitudinal band sideways.

Head with prothoracic shield and anal plate brown.

Abdominal prologs with crochets in a linear pattern.

Length: 18 - 20mm

Abdominal segment without spines.

Cremaster with a small rounded lobe with two short and widely apart diverging spines.

Sesamia calamistis

Crochets on ventral prologs linear pattern with spines of the same length.

Size of the last instar: 35mm

Colour: Pinkish with brown pinaculae.

Head and prothoracic plate brown.

Anal plate yellow brown.

Length: 18 - 20mm

Anterior tip of head rough with stone like swellings.

Cremaster with two short, robust and widely apart spines dorsally, two small ones close to each other vertically, spines sharp in side view.

Chilo partellus

Size in last instar: 12 - 17mm

Colour: cream white with large brown sports.

Body with four purplish or reddish brown longitudinal stripes.

Abdominal prologs with crochets in a circular pattern.

Head capsule brown prothoracic shield and anal plate brown.

Three small asetose tubercles in the mesothorax and one big dorsal asetose in the metathorax.

Lateral asetose tubercles on abdominal segments between two spiracles present and clearly visible.

Colour: Yellowish brown to reddish brown.

Length: 12 - 14mm

Abdominal segment 4 - 8 with interia marginal spines.

Cremaster with 2 short ventral and 4 dorsal spines presented in lateral view of the cremaster.

Busseola fusca

Size: wing span 24 - 42 mm

Robust body with thick hairs in the head and thorax.

Fore wings relatively wide.

Colour: Forewing grayish brown to coppery brown and edge with small black spots, two faint transverse lines distinct with two spots in between, dark arrow head markings slightly present.

Hind wings whitish cream in males and light brown in females.

Genitalia: Male

Aedeagus (or penis) with two lateral chitinized processes.

Hollow cornuti.

Sacculus with a strong chitinized process asymmetrically formed.

Uncus narrow, long and pointed apically.

Broad valves with two large sacculus overlapping.

Sesamia calamistis

Size: wing span 24 - 42 mm

Robust body with thick hairs in the head and thorax.

The fore wings are a streaked straw colour, the pattern more uniform, smooth in appearance and straight distal margin.

Hind wings creamy white in male, light brown in females.

Genitalia: Male

Juxta is diagnostically flask-shaped, broad, long with a dorsal notch.

Aedeagus with two lateral chitinized processes.

Long valves with two narrow sacculus overlapping.

Chilo partellus

Size: wing span 19 - 28 mm

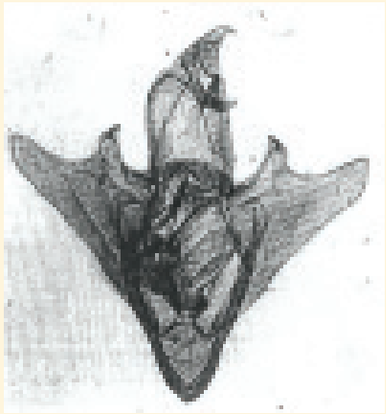
Fore wing long and narrow with discal spots.

Fore wing ground colour varying from yellow to brown, variability dusted with scales.

Hind wing dirty white to grey in colour.

Abdomen broad in females and slender in males.

Genitalia: Male



Coastal projection in the costa valve.

The juxta plate is symmetrical with a large central part.

Oval sacculus with two notches at the base.

Aedeagus with bulbous base projection and ventral arm.

Busseola fusca	*Sesamia calamistis*	*Chilo partellus*
Aedeagus: 	Aedeagus: 	Aedeagus: 
Female	Female	Female
Ductus bursae is diagonally attached between lateral plates. Corpus bursae with two lateral signum. 	Sterigma clearly surrounding the opening or ostium of the ductus bursae is unsclerotized. Ductus bursae is diagonally inserted between the lateral lobes. Signum absent at the corpus bursae. 	Ostium heavily sclerotized, longitudinally wrinkled. Ostium well separated from the ductus bursae. Ductus bursae narrowly allocated, bulging at corpus bursae. Corpus bursae with lanelate signum at the central ridge. 

Protocol to prepare male and female genitalia for stemborer species identification

- label the pinned specimen from which the abdomen is removed. The label should include sex of the specimen, identifying number, and the name of the genitalia, using symbols male and female, country, locality, host plant, GPS position and date of collection. Preferably use a coloured paper so that it can be easily detected in the curated collections. The dissection number label is duplicated and is carried with the abdomen through the various reagents.
- the abdomen is detached from the insect moth using a Irish dissecting scissors. These scissors have spring-return handles and straight, sharp, smooth cutting edges (10 mm) and are used to cut closely to the thorax, especially for females which have long bursae through the abdomen.
- insert the cut abdomen into a Pyrex tube.
- add 10% of potassium hydroxide (KOH) to the Pyrex tube with the abdomen.
- submerge the Pyrex tube into a 250 ml glass beaker, with water ready for boiling.
- place the beaker onto a hot plate for boiling.
- regulate the heat of the hot plate and boil for about five minutes or to boiling point.
- once you notice the popping sound of the KOH, remove the beaker and dispense the specimen in a 70% alcohol solution in a disk cavity ready for dissection. (NB: in case you are not heating the specimen you can leave it in the Pyrex vial with 10% KOH overnight to soften. However, it is preferable to heat the specimen in order to soften the body tissues and to liquefy the fatty contents in the abdomen).
- place the disc cavity in a dissecting microscope and magnify to the required magnification.

- gently clean hair scales and the debris from within the abdomen with a fine soft camel hair brush, using a pair of Rubis forceps to hold the abdomen in place while brushing.
- transfer the abdomen to another disc cavity with 70% alcohol for further cleaning, three times.
- from the disc cavity with 70% alcohol separate the genitalia from the abdomen (NB: avoid using a higher concentration above 70% alcohol, as this will make the specimen more brittle and subject to tearing while preparing it).
- hold the genitalia with a pair of Rubis forceps and then use the Irish dissecting scissors to pierce the segmental membrane between the last segment of the abdomen for the male genitalia, while for the female genitalia, you start cutting from the upper side of the abdomen to avoid damaging of the bursae.
- gently open the valves of the male genitalia using insect pins and start cleaning the genitalia with a fine forceps to remove any extra debris and cuticles, and in case the valves are hairy, pluck them with the use of fine forceps or a soft camel hair brush.
- transfer the genitalia to a clean disk cavity with 70% alcohol, place it upside down and keenly detach the aedeagus from the genitalia, carefully without breaking the juxta where it is attached.
- rinse the genitalia with absolute alcohol and dispense it into a disc cavity with clove oil for 25 minutes for dehydration.
- use any of the following as your mountant for preparing permanent slides: Euparal, Canadian balsam, or Eukitt, on a clean microscopic slide.
- place your specimen in a microscopic slide with a few drops of the mountant and carefully spread the valves, unicus and the teguman. Place the aedeagus below the saccus of the specimen genitalia in the right position.
- add one drop a your mountant to the specimen, then place the cover clip on top and allow the mountant to flow, covering fully the cover slip on the microscopic slide (NB: avoid any air bubbles between the slide and the cover slip).
- label your slide, place it on filter paper, and put it into an oven for drying. Adjust the temperatures to 40 °C for 48 hours for drying.
- Transfer your slide to insect slide boxes and retain your box in insect cabinets for further reference.

Equipment required for genitalia preparation:

- Irish dissection scissors
- Rubis fine forceps
- Pyrex vials (tubes)
- Beaker
- Disc cavity
- Hot plate
- Insect pins
- Soft fine camel hair brush
- Dissecting microscope
- Oven
- Microscopic slides
- Cover slips
- Slide boxes

Chemicals needed for genitalia preparation:

- Potassium hydroxide (KOH)
- Ethanol
- Mountant: Canadian balsam, Euparal, or Eukitt.
- Xylene
- Clove oil

Distribution of cereal stemborers in Africa

Busseola fusca is distributed widely throughout sub-Saharan Africa (Figure 8A), with the populations in East and Southern Africa differing in environmental adaptability to that exhibited by the West African populations. In the continent's eastern and southern parts, *B. fusca* occurs mostly in mid- and high-altitude areas (>600 m) where it is often the most serious pest of maize. Nye (1960) found that in the cool and high altitude zones above 1050 m, *B. fusca* was predominant. However, low altitude areas were also reported to harbour some (Overholt et al., 2001; Le Ru et al., 2006a, b). *Busseola fusca* is an oligophagous species, feeding mostly on maize as well as cultivated and wild sorghum (Calatayud et al., 2014).

On the other hand, *S. calamistis,* is mainly found in sub-Saharan Africa and some of the Indian Ocean islands commonly occurring in wetter localities at all altitudes (Tams & Bowden, 1953) (Figure 8B). This species is more polyphagous than *B. fusca* is, being able to infest maize, sorghum, pearl millet, wheat, rice and sugarcane, and it is a major, important economic pest on maize in West Africa (Bosque-Pérez & Mareck, 1990).

Chilo partellus, or the spotted stemborer, is native to Asia where it is a pest of maize and sorghum. It was first reported in Malawi in the 1930s and spread in the 1950s to most East Africa countries. Ingram (1958) reported that *C. partellus* did not occur above 1200 m, particularly in the highland areas of southern and western Uganda. Since then, it has become widespread throughout eastern and southern Africa (Kfir et al., 2002) and several West African countries (Overholt et al., 2000) (Figure 8C). *Chilo partellus* is considered to be the most important stemborer species in most low to medium elevated areas of eastern and southern Africa.

However, in some countries such as Uganda, the increasing importance of cereal crops such as maize, which are now widely grown, has influenced the current distribution of cereal stemborers. Presently, *B. fusca*, *S. calamistis* and *C. partellus* occur virtually in all maize-growing regions, with the co-occurrence of the different species. In field surveys conducted during 1997 and 1998 in the Southeastern agro ecological zone (AEZ) of areas where the various stemborer species occurred, it was found that *B. fusca* was the dominant species, representing 58% of the stemborers, while *C. partellus* accounted for 38% inafter the 1998 second rains (Matama-Kauma et al., 2001). However, in the subsequent seasons of 1999, *C. partellus* was abundant (range: 53–85%), while *B. fusca* populations decreased. Meanwhile, *S. calamistis* occurred in very low numbers, accounting for less than 15% of the total stemborer population in any season in this AEZ (Matama-Kauma et al., 2001). Later surveys conducted in 4 major maize growing AEZs during 2003 and 2004 showed that *C. partellus* was the dominant stemborer species in the Eastern AEZ, representing 77.2% of the stemborers, while *B. fusca* was predominant in Lake Albert Crescent, Lake Victoria Crescent and Southeastern AEZs, accounting for 60–79% of the population, across seasons (Matama-Kauma et al., 2007). *Sesamia calamistis* occurred in all the AEZs, but the population was low, ranging from 1.3 to 4.1% with the highest population recorded in the Eastern, and the lowest in Midwestern, AEZs (Matama-Kauma et al., 2007).

Field data generated from monitoring surveys in low- to mid-altitude AEZs from 2003 to 2011 showed that the various stemborer species were overlapping in occurrence in various altitudinal ranges. For instance, while the highest population of *B. fusca* (80–100%) occurred in altitude ranges from 996 to 1084 m in Southern, Southeastern and Lake Albert Crescent, the highest population for *C. partellus* (80–100%) occurred in altitudinal ranges from 989 to 1147 m in Southern, Southeastern and Eastern AEZs (Molo et al., 2014). Meanwhile, low populations of *S. calamistis* (0.9–4.1%) occurred at altitudes ranging from 960 to1147 m in the various AEZs. Later surveys conducted in high-altitude AEZs from 2014 to 2016 showed that *B. fusca* extended its distribution to 2614 m in Mount Elgon Farmlands, while the upper limit for *C. partellus* was up to 1626 m (Molo et al., submitted). However, in the Western Medium High Farmlands, *C. partellus* was common at high altitudes up to 1600m, while *B. fusca* was predominant at both low and high altitudes, (Molo et al., submitted). On the other hand, *S. calamistis* did not occur at altitudes above 1086 m in Mount Elgon Farmlands and above 1147 m in the Western Medium High Farmlands.

Other factors have been found to play key roles in determining the distribution of stemborers. The role of crop phenology was reported in central Uganda in influencing the distribution and abundance of *C. partellus* and *B. fusca* (Kalule et al., 1998). In Masaka district, they found that from 2 to 8 weeks after crop emergence, *B. fusca* was the dominant stemborer species, followed by *C. partellus*. However, the population declined after eight weeks. In Wakiso district, the incidences of *B. fusca* and *C. partellus* were similar in both seasons, from 2 to 4 weeks of maize growth, but *B. fusca* population declined thereafter, relative to *C. partellus*. In another study, evidence was also found of the existence of a number of wild plant species, including *Pennisetum purpureum* Shumach, *Sorghum arundinaceum* (Desv.), *Echinochloa pyramidalis* (Lam.), *Euchlaena mexicana Schrader*, *Hyparrhenia papillides* (A. Rich.), *Panicum deustum Thunb* and *Panicum maximum* Jacq), which are potential hosts to stemborers (Calatayud et al., 2014). The importance of host plants was highlighted in the Lake Victoria Crescent and Eastern AEZs, as their occurrence in the vicinity of maize fields has also been associated with reduced incidences of stemborers on maize crops (Matama-Kauma et al., 2006).

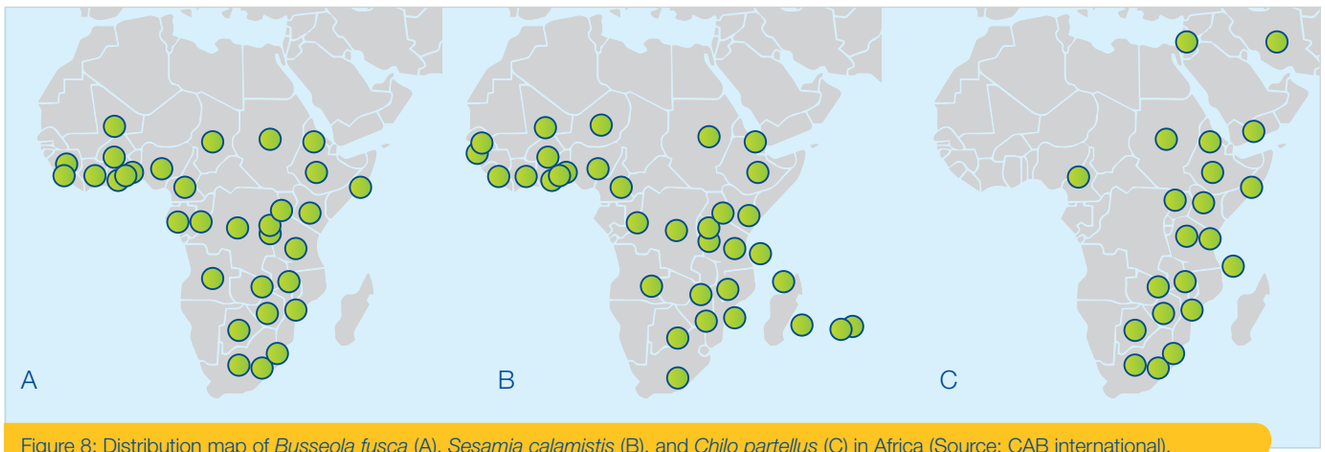


Figure 8: Distribution map of *Busseola fusca* (A), *Sesamia calamistis* (B), and *Chilo partellus* (C) in Africa (Source: CAB international).

Economic impact of cereal stemborers in Africa

Estimates of yield losses caused by these borer species vary greatly among regions, depending on the agroecology, density of borer population, season, fertility status of the soil, crop type and phenology during infestation (De Groote, 2002; Kfir et al., 2002; Wale et al., 2006; Demissie et al., 2011). Accordingly, yield reductions ranging between 15 and 50% were estimated in East Africa (Wale et al., 2006; Demissie et al., 2011), while in South Africa, losses exceeded 50% (Kfir et al., 2002), indicating the importance of stemborers as a major yield-limiting factor for cereal crops in the region. Infestation by stemborers caused significant losses, ranging from 11% in the highlands to 21% in the dry areas in Kenya (Odendo et al., 2003). In Ethiopia, the crop losses due to stemborers generally range from 10 to 50% (Getu et al., 2003; Wale et al., 2006; Demissie et al., 2011).

In Uganda, studies conducted on the contribution of larvae from different species of stemborers showed that *B. fusca* caused the highest stem damage in Lake Albert Crescent, Lake Victoria Crescent and the Southeasten AEZs, while *C. partellus* was more important in the Eastern AEZ (Matama-Kauma et al., 2007). In another field study conducted during 1992 in Lake Victoria Crescent on maize protected against stemborers by different insecticide treatments, versus unprotected maize, the mean grain yield increased in all protected plots over that of the unprotected maize in the two seasons. In the long rains of 1992, yield increases ranged from 3.4 to 12.4% while in the short rains, the yield increases ranged from 15.7 to 13.3% (Kalule et al., 1998), indicating the significance

of stemborers. Recently, Otim et al. (unpublished) found that treatment with beta-cyfluthrin insecticide against stemborers resulted in a 119.2 percent yield increase over that of the untreated control. At household level, this value suggests that at least 1–3 bags of maize grains are lost to stemborers for every 10 bags of maize. On the other hand, perceptions of farmers on losses due to stemborers in Tororo, Bugiri and Kapchorwa districts in the Southern and Eastern AEZs indicate that losses range from 25 to 50 kilograms of maize per acre, and these losses are consistent with the yield loss values reported in the field (Kalule et al., 2006). Stemborer larva can cause considerable cereal yield loss, especially on maize, due to its inability to compensate for stem damage by formation of tillers.

Biological control of cereal stemborers

Introduction to biological control

Biological control, the oldest form of managing insect pests, is defined as the pest management tactic in which the manipulation of natural enemies leads to maintenance of pest populations to below damaging levels. Different from natural control, biological control, or bio-control, relies heavily on natural enemies of pests for their control (Altieri et al., 2005; Mahr et al., 2008). Natural enemies are living organisms that kill or weaken insects, thereby reducing their numbers. Insects' natural enemies are diverse and include insects themselves, other invertebrates, vertebrates, nematodes and microorganisms. These natural enemies have been effectively divided into parasites, parasitoids, predators or pathogens (*Altieri et al., 2005; Mahr et al., 2008)*.

There are three basic methods for the biological control of pests:

- classical biological control(importation of natural enemies) where the natural enemy is introduced in a new environment to achieve control;
- augmentative biological control(augmentation of natural enemies), in which a large population of natural enemies already present is regularly administered to increase their numbers for control;
- conservation biological control (conservation of natural enemies), in which measures are taken to maintain natural enemies through regular re-establishment.

Insect parasitoids and predators comprise the major biological control agents, and these control insect populations in the wild at certain thresholds, below which the environment can sustain itself. All insect families and many spiders are hosts for insect parasitoids, thus constituting major natural factors limiting arthropod populations. The use of parasitoids has received increased attention because they have been proven to be specific as a biological control component, cost effective, and offer a pest control strategy that safeguards human health and the environment (*Calatayud et al., 2020).*

Biological control agents mostly used, parasitoids

Parasitoids are a group of insects that parasitize other insects or arthropods at any host stage (eggs called eggs parasitoids; larvae called larval parasitoids and pupae called pupal parasitoids). A parasitoid is only parasitic in its immature stage. The free-living adult parasitoids lay their eggs inside the host or attach them outside (Godfray, 1994). Parasitoids occur in 86 families belonging to Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera and Strepsiptera. The most important of these parasitoids include the small parasitic wasps in the families of Ichoneumonidae, Braconidae and Chalcidoidae (Pedigo & Rice, 2009).

Among insect parasitoids, Cotesia is one of the most diverse genera of the subfamily Microgastrinae (Hymenoptera, Braconidae), with almost 300 species already described and probably over 1,000 species existing world-wide. Many Cotesia species may appear generalists, but careful ecological studies may reveal a hidden complexity with an assemblage of populations having a more restricted host range (see Kaiser et al. [2017]).

Cotesia sesamiae

Cotesia sesamiae (Cameron) (Hymenoptera: Braconidae) (Figure 9) is one of the most important native parasitoids of stemborers in many countries of sub-Saharan Africa. This is an indigenous, gregarious larval endoparasitoid that attacks mid to late instars of the stemborer larvae (Bonhof et al., 1997, Kaiser et al., 2015). *Cotesia sesamiae* attacks several stemborer species, including *S. calamistis*, *B. fusca*, *C. partellus* and C. orichalcociliellus (Mohyuddin, 1971; Polaszek & Walker, 1991). Although the parasitoid is distributed widely in Africa, not all local populations of the species appear to be equally effective in controlling stemborer pests (Mohyuddin, 1990; Polaszek & Walker, 1991). In Kenya, in regions of elevations higher than 600 m above sea level, *C. sesamiae* is the main larval parasitoid attacking *B. fusca* (Overholt et al., 1994, Getu et al., 2003).

There are two biotypes of *C. sesamiae* in Kenya that differ in their ability to parasitize *B. fusca* (Ngi-Song et al., 1995; 1998): *C. sesamiae* inland (Cs-inland) from western Kenya and *C. sesamiae* coast (Cs-coast) from Mombasa (coastal Kenya). Whereas the Cs-inland population is virulent and completely develops in *B. fusca* (Mochiah et al., 2002b), the Cs-coast population is regarded as avirulent and not able to completely develop in *B. fusca* as its oviposited eggs get encapsulated in *B. fusca* larvae (Ngi-Song et al., 1995; Mochiah et al., 2002b). This encapsulation mechanism, where oviposited eggs are melanized in *B. fusca* (Ngi-Song et al., 1995; 1998; Mochiah et al., 2002b), reduces the efficiency of the parasitoid, especially in regions where the predominant pest species is the unsuitable host (Ngi-Song et al., 1995). Conversely, both biotypes develop effectively in *S. calamistis* larvae (Ngi-Song et al., 1995; Mochiah et al., 2002b).

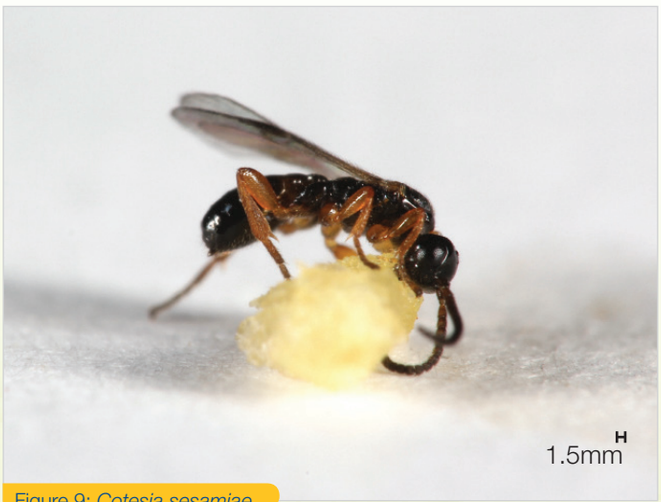


Figure 9: *Cotesia sesamiae*.

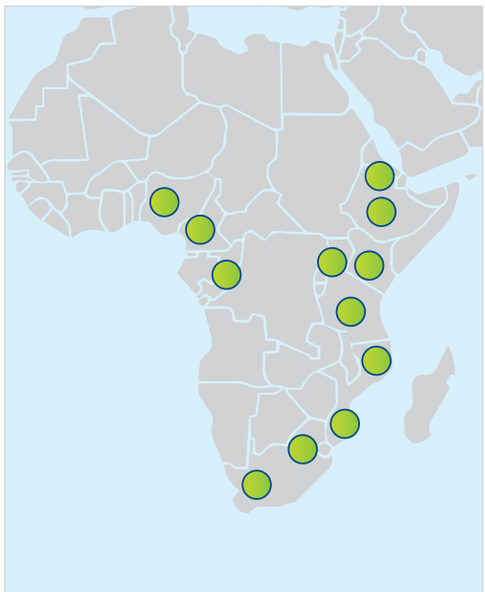


Figure 10: Distribution map of *Cotesia sesamiae* in Africa (Source: Branca et al., 2019).

Distribution of *Cotesia sesamiae* in Africa

Cotesia sesamiae is a common indigenous larval parasitoid that is widespread in various AEZs in Eastern, Southern, central and some countries of West Africa (Figure 10). This species is, for example, widespread in various AEZs in Uganda. In surveys conducted in Southeastern AEZ during 1998 and 1999, the highest parasitism rate was 13% on *B. fusca*, 9.2% on *C. partellus* and 100 % on *S. calamistis* (Matama-Kauma et al., 2001). During surveys conducted in 2015 and 2016, *C. sesamiae* mean parasitism rates ranged from 17 to 29% on *B. fusca* with the highest parasitism recorded in Western Mid-Altitude Farmlands, while in Lake Victoria Crescent and Southern AEZs, mean parasitism rates ranged from 7 to 22%, with the highest parasitism recorded in Lake Victoria Crescent on *B. fusca* (Molo et al., submitted).

Cotesia flavipes

Cotesia flavipes Cameron (Hymenoptera: Braconidae) (Figure 11), from Pakistan and India was introduced into Africa as a classical biological control agent. It was released in 1993 by icipe for the control of *C. partellus* – the invasive exotic stemborer of maize and sorghum in Eastern and Southern African lowlands (Overholt et al., 1994a, b; Overholt et al., 1997). Due to its successful history in its aboriginal home in Asia (Overholt et al., 1994a), *C. flavipes* was chosen as the best candidate to complement the activity of *C. sesamiae*, which was linked initially with indigenous borer species such as *B. fusca* (Mohyuddin & Greathead, 1970; Overholt et al., 1994a, b; Zhou et al., 2001b; Songa et al., 2002).

Figure 11: *Cotesia flavipes*.

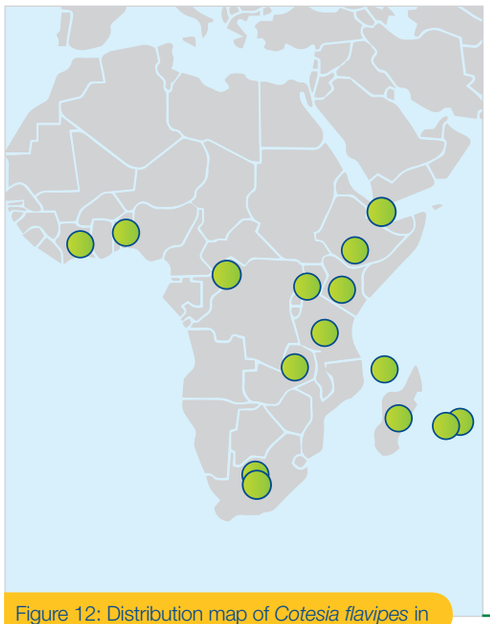


Figure 12: Distribution map of *Cotesia flavipes* in Africa (Source: CAB international).

Distribution of *Cotesia flavipes* in Africa

Since its release in 1993, this species has become well established in East and Southern Africa. It is also present in West Africa, as well as in Madagascar (Figure 12).

In Uganda, for example, the first attempt at classical biological control of cereal stemborers was made from 1968 to 1972, when the Commonwealth Institute for Biological Control (CIBC) introduced *C. flavipes* from Pakistan to control *C. partellus*. In spite of multiple releases, the parasitoid did not become established (CIBC1968–1972).

Later in 1998, a total of 11,300 adult *C. flavipes* was imported from the International Centre for Insect Physiology and Ecology (icipe) into Uganda. They were released in maize and sorghum fields in the Southeastern AEZ to control *C. partellus* (Matama-Kauma et al., 2001). Since then, the parasitoid has spread widely and become established in various AEZs. By 2003, the larval parasitoid had parasitism levels up to 30% on *C. partellus* and 12% on *B. fusca* (Matama-Kauma et al., 2007). In surveys conducted during 2016, the parasitoids were recovered from various locations in Uganda, including Northern Moist Farmlands, Northwestern Farmlands, Lake Victoria Crescent, and the Southern and Eastern AEZs. The mean parasitism rates on *C. partellus* ranged from 25 to 44% with highest recorded in Lake Victoria Crescent and the lowest in the Southern and Eastern AEZs (Molo et al., submitted). The mean parasitoid numbers ranged from 10 to 36.7 per cocoon, with the highest in Northwestern Farmlands and the lowest in Southern and Eastern AEZs

Their lifecycle

Their biology was initially studied and recorded by Gifford and Mann (1967), and later by Mohyuddin (1971). Briefly, the adult is a small wasp, about 2–4 mm in length, and lives for only a few (3–4) days. Females lay about 15–65 eggs inside the host larva, and the eggs hatch after about 3 days inside the host larva (Figure 13A). The parasitoid larvae develop through three instars within the stemborer larvae, feeding on the larva body fluids. The egg-larval period takes about 10–15 days at 25 °C, 50–80% Relative Humidity (RH), and a photoperiod of 12:12 (L:D) hr. The last instars of the parasitoid emerge from the host body by chewing through the stemborer larva integument (Figure 13B) and immediately spin cocoons and pupate (Figure 13C). Adult parasitoids emerge 5–6 days later, at 25 °C, 50–80% RH, and a photoperiod of 12:12 (L:D) hr (Figure 13D). Usually, adult parasitoids emerge in the morning hours of the day, with males emerging first, followed immediately by the females, and mating begins soon afterwards.

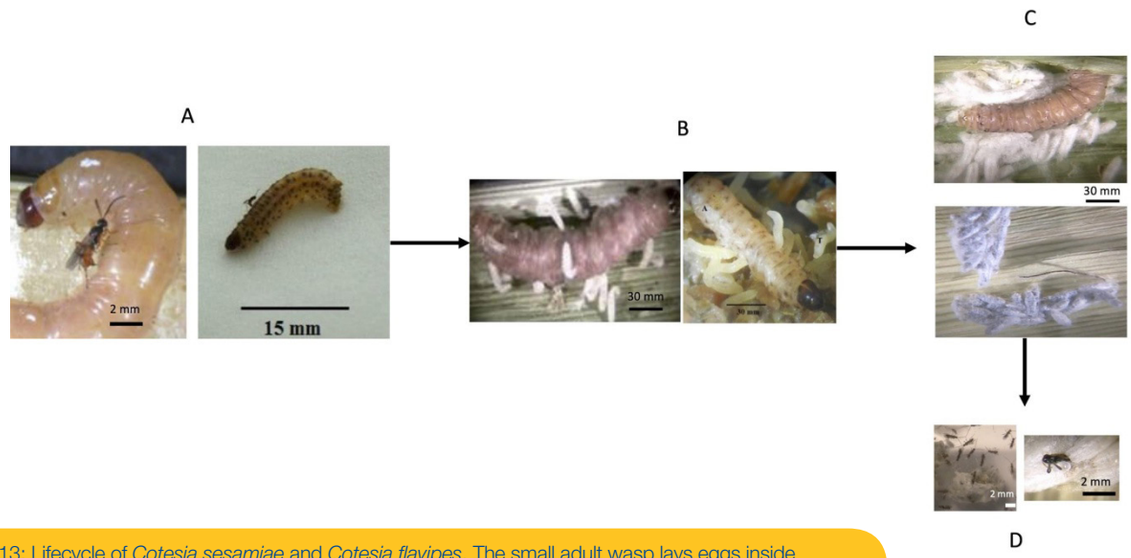


Figure 13: Lifecycle of *Cotesia sesamiae* and *Cotesia flavipes*. The small adult wasp lays eggs inside the stemborer larva (A). After 10–15 days, the final stages of the parasitoid emerge from the host body (B) and immediately spin cocoons and pupate (C). Five to six days later, the adult parasitoids emerge (D), to mate and to find a new larval host.

How to distinguish *Cotesia sesamiae* to *Cotesia flavipes*?

Weak morphological differences between *C. sesamiae* and *C. flavipes* can allow us to identify the two species: the length of the wings tend to be longer for *C. sesamiae*, as compared with *C. flavipes*, and the abdomen is more flattened dorsally for *C. sesamiae*, as compared with *C. flavipes* (Figure 14). Furthermore, the coxa III is usually black or dark reddish brown for *C. sesamiae*, whereas it is usually light brown to brownish yellow for *C. flavipes*. The mandibles of the mouth parts are rounded and closed for *C. sesamiae*, and are slightly pointed and opened for *C. flavipes*. The scutellin (propodium) is rugose or rough for *C. sesamiae* and smooth for *C. partellus*. Under suspension in absolute ethanol, *C. sesamiae* specimens appear slightly dark black in colour, whereas they appear slightly brown for *C. flavipes*.

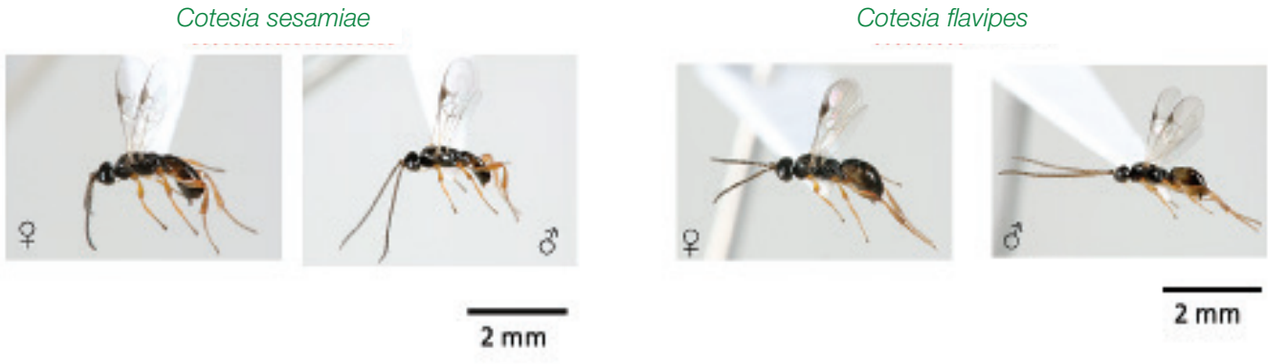


Figure 14: Comparisons between *Cotesia sesamiae* and *Cotesia flavipes*.

In contrast, for both species it is easier to distinguish the males and the females. The length of male antennae is longer (almost twice in length) than the female antennae.

For a better distinction between the two species, it is advised to compare the males' genitalia. For *C. flavipes*, the aedeagus is elongated toward the apex, whereas that of the *C. sesamiae* is rounded toward the apex (Figure 15).

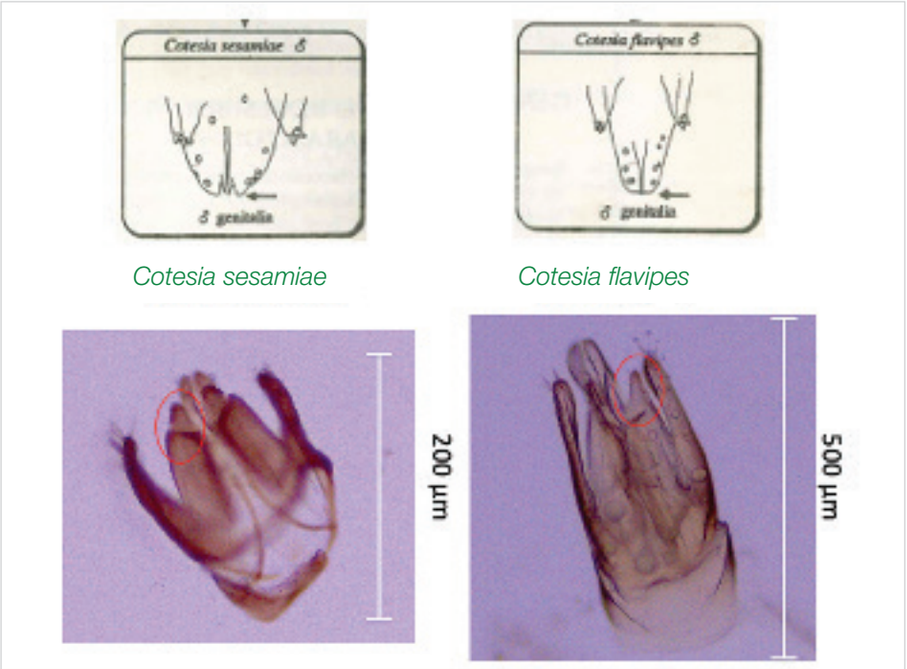


Figure 15: Comparison of male genitalia between *Cotesia sesamiae* and *Cotesia flavipes*.

Protocol to prepare male genitalia for Cotesia species identification:

- submerge your male specimen into a Pyrex tube with 10% of KOH.
- put the Pyrex tube with the specimen into a beaker with water.
- place the beaker on top of a hot plate for boiling.
- boil for about 5 minutes or until you hear the popping sound of the KOH in the vial. (NB: close the vial with cotton wool to avoid the specimen being pumped out, because KOH, when heated, burns with popping sound, spilling some of the chemical into to the boiling water in the beaker).
- transfer your specimen into a disk cavity with 70% of alcohol for rinsing.
- mount your microscopic slide to the microscope.
- add a drop of your mountant into the microscopic slide.
- place your specimen in the mountant for dissection.
- carefully hold your specimen with a sharp fine forceps and then with an insect pin or another sharp forceps, press the abdomen from the last third segment of the abdomen for the genitalia to come out.
- separate the genitalia from the insect and mount it upwardly.
- add one drop of mountant to the specimen and place cover slip.
- label your specimen and dry it in an oven for 48 hours.
- remove your slide and put it into a slide box for reference.

Equipment required for genitalia preparation:

- aspirator for sucking your specimen from a cage or vial into the Pyrex tube
- Pyrex tubes for heating the specimen
- beaker for boiling the specimen tube
- hot plate
- sharp-pointed forceps
- insect pins
- disk cavity
- microscopic slides
- cover slips
- dissecting microscope
- slide boxes

Chemicals needed for genitalia preparation:

- ethanol
- KOH
- mountant: Akutt, Euparal, or Canadian balsam

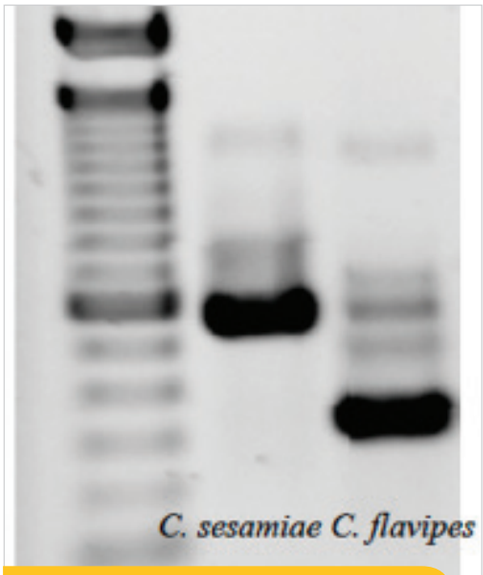


Figure 16: Results of PCR tests distinguishing *Cotesia sesamiae* from *Cotesia flavipes* (Source: Branca, 2009).

The definitive identity of the parasitoid species can be done by PCR, using the protocol described by Dupas et al. (2006) (Figure 16). Individuals are previously extracted using the Chelex® (BioRad) protocol.

The PCR cycling conditions are as follows: initial denaturation 5 min at 94 °C, 40 cycles of 50 s at 94 °C, 1 min 20 s at 60 °C, and 1 min 20 s at 72 °C. The final extension is 10 min at 72 °C. The reaction mixture contains 0.4 µM primers, 0.24 µM dNTP, and 1 µl DNA plus 1 Taq T4 DNA polymerase (Promega) per 25 µl of reaction. The MgCl2 concentration is 1.5 mM MgCl2.

The *C. sesamiae*-specific primer is CsV1394F 5'AACGAACACTTTCGATGAA3' and the *C. flavipes*-specific primer is CfV1634F 5'GAGTATTTTCCGAAAATGG3'.

Chelex® (BioRad) protocol

- insert one specimen Cotesia into a 0.5 ml Eppendorf tube and crush the insect;
- add 250µl of Chelex at 10% preheated at 60 °C, and then mix;
- add 10µl of proteinase K and let it to react with it for 1h at 50 °C;
- insert the tube into a PCR machine, preheated at 95°C for 30 min;
- centrifugate at 12 000 rpm for 5 min;
- keep the sample at -20 °C.

Advantages of using biological control of stemborers in Africa

The following highlights are among the advantages of biological control of stemborers in Africa:

- ✓ This is a very specific strategy since whichever parasitoid is introduced, it will only control the population of the pest that they are meant to target. For example, *C. sesamiae* specifically controls *B. fusca* on maize, whereas *C. flavipes* specifically controls *C. partellus* and not *B. fusca*.
- ✓ The natural enemies introduced to the environment are capable of sustaining themselves, often by reducing whatever pest population they are supposed to manage. This means that after the initial introduction, very little effort is required by the farmers to keep the system running fluidly. It also means that biological control can be kept in place for a much longer time than other methods of pest control, such as chemical controls. This is true in Africa since many resource-poor farmers cannot afford to use insecticides, thus rendering the amount of pesticide sprayed on maize much lower in Africa than in the other parts of the world.
- ✓ Biological control can be cost effective and benefits smallholder production systems in Africa in the long run. Although it may cost at the beginning to introduce a new species to an environment, it is a tactic that only needs to be applied once due to its self-perpetuating nature.
- ✓ Most important of all, it is effective. Midingoyi et al. (2016) show that the biological control intervention, using *C. flavipes*, has contributed to an aggregate monetary surplus of USD 1.4 billion to the economies of Kenya, Mozambique and Zambia, with 84% coming from maize production and the remaining 16% from sorghum production.
- ✓ Biological control has no environmental side effects since the natural enemies used are environmental friendly.

Nevertheless, some disadvantages are known:

- × The introduced parasitoids can disappear or simply may not establish in their new environment. This was the case with the introduction of *C. sesamiae* in the Taita Hills in Kenya, where it has not been found in the years following its release (Midingoyi et al., 2016). Furthermore, while they are supposed to manage a specific pest, there is always the possibility or risk that the parasitoids will switch to a different non-target species.
- × It is a slow process, providing no immediate results as pesticides do. It can take a long time for the parasitoids to establish in any environment where they have been released. Nevertheless, the opposite to this is the long-term effect that biological control provides.
- × If the farmers are looking to completely wipe out a pest, biological control may not be the right choice, as the natural enemies can only survive if their host pest is still present; hence, eliminating the pest population would risk their own survival. Therefore, they can only reduce the number of harmful pests.
- × While biological control is cheap in the long run, the process of actually setting up a biological control system is a costly endeavour; much planning and money goes into developing a successful system (e.g. the biological control programme of icipe against *C. partellus* using *C. flavipes*).

Inundative release of biocontrol agent against stemborers

Within the continent, there has been an interest in redistributing the populations of *C. sesamiae* as a biological control of stemborers (Schulthess et al., 1997, Rousse & Gupta, 2013). For example, the introduction of a Kenyan population of *C. sesamiae* into West Africa is thought to have improved the biological control of stemborer in the region (Schulthess et al., 1997; Mochiah et al., 2002a). More recently, *C. sesamiae* has successfully been introduced in Cameroon and its full impact assessed (Ndemah et al., 2012; Kaiser et al., 2017).

Although *C. sesamiae* is the most abundant larval endoparasite in Africa (Mohyuddin & Greathead, 1970; Polaszek & Walker, 1991), it has not managed to efficiently control the *C. partellus* population in Kenya (Overholt et al., 1994b).

The exotic larval parasitoid *C. flavipes* was imported from Asia in 1991 by icipe (Nairobi, Kenya) and released from 1993 onwards in East and Southern Africa. The first releases were done in the coastal region of Kenya (Midingoyi et al., 2016). Within four years after its release, the parasitoid had successfully established itself in the coastal region, and later spread to other areas of the country. The parasitoid was established between 1995 and 2004, while the average annual parasitism due to the parasitoid has increased linearly since the time of its introduction (Zhou et al., 2001a; Songa et al., 2002; Omwega et al., 2006). At the coast, *C. flavipes* reduced the *C. partellus* population densities by 57%, while at the same time maize yields increased by 10–15% (Zhou et al., 2001b). Following its success in Kenya and western Tanzania (Omwega et al., 1995; 1997), eleven other countries in Eastern and Southern Africa adopted this control method and the parasitoids were released and became established in 10 of these countries (Omwega et al., 2006). Since then, evidence of the establishment and spread following the release of *C. flavipes* has been highlighted in several studies and surveys. The parasitism-effect and suppression-effect of the released *C. flavipes* has demonstrated and confirmed its effectiveness in reducing *C. partellus* densities (Midingoyi et al., 2016).

Mass production of stemborers

Colony establishment

A colony of stemborers can be obtained from established laboratories or directly collected from the field. Although a start collection of 500 individuals and above is generally advisable, 200 individuals might be enough where it is not possible to collect a genetically diverse sample of 500, on the condition that the 200 individuals are collected over a wide geographical area (i.e. both from different maize fields and from different regions).

Before assembling a collection of stemborer specimens, make sure that the sampling field has not been treated with insecticides, as insects collected from such fields may result in mass mortality, or in some cases the total failure, of the collection.

Materials required for field collection of stemborer specimens include: long knives (for dissection of maize stems), big empty plastic jars for collecting insect specimens (eggs, larvae, pupae) in the field, and small glass vials, filled halfway with an artificial diet, to individually hold larvae. Alternatively, small empty glass vials can be used to harvest pupae, for example, using cotton wool to cover each small vial. In addition, have notebooks and pens, GPS equipment to record the GPS position of each location where the insects have been collected, and a box (not necessarily icebox) in which to store and transport the field-collected insects (Figure 17).

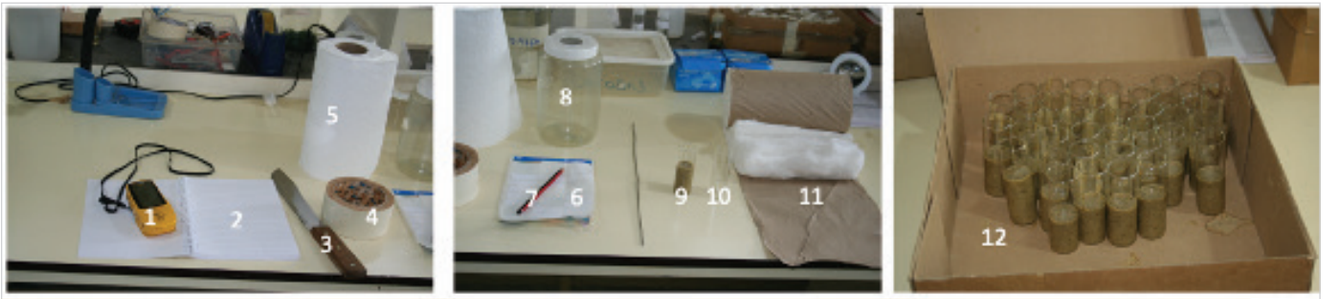


Figure 17: Materials necessary for field insect's collection (1: GPS, 2: notebook, 3: knife to dissect the maize stem to collect stemborer larvae, 4: masking tape used to label tubes, 5: Paper towel (Velvex) for cleaning, 6: labels, 7: pencil, 8: empty plastic jar to collect insects while in the field, 9: small glass vials filled halfway with artificial diet to start rearing the collected larvae, 10: empty glass vials to store pupae or cocoon masses found, 11: cotton wool used to enclose the small glass vials, 12: boxes to store and transport collected material).

The stemborer colony can be initiated from field-collected specimens of any developmental stage, targeting infested maize plants that show symptoms of infestation (See section 2.1). However, the frequently used for colony establishment is to collect from larval stages. It is generally advisable to use late instar larvae. Nevertheless, depending on the developmental stage of stemborers available in the field, egg masses or pupae can also be collected. In all cases, it is important to verify the species identity (see the identification criteria given in section 2.3).

Any field-collected material should be kept under quarantine, in isolation, for at least one or two generations to ensure a disease-free or parasitism-free parental stock and to prevent contamination of the laboratory culture. It is recommended to preserve some field specimens, both dry and wet, in 70% ethanol as references.

Colony maintenance

It is important to ensure that the colony of each insect's species is genetically different, with a good vitality and aggressiveness, and free of disease generally involving microsporidia. Such disease can appear if the larvae do not develop correctly (giving malformed pupae and adults), or develop slowly, or if the females give sterile eggs. To avoid infection and the spread of diseases, it is important to maintain strict sanitation. Never discard infected material or any material coming from the insects' mass rearing (including the artificial diet) directly into the waste bin, without previous autoclaving or a 5-day-stay in a freezer.

It is advisable, once a year, to replace or infuse each colony with wild insects directly from the field, using the same procedure and quarantine precaution given in section 4.1. This replacement or infusion will allow for the maintenance of genetic diversity and limit the risk of disease caused by microsporidia.

An important safeguard to avoid genetic deterioration is to ensure that each colony is never reduced to only a few individuals. The average colony size during the maintenance production period is about 100 pupae, minimum, per week.

Rearing facilities

The main components of the stemborer mass rearing facility include laboratory space, equipment, diet, consumables, and personnel.

Insectary design

The insectary should be divided, if possible, into 3 sections depending on the nature of the work to be performed:

- one area comprising rooms for diet storage, media preparation, surface sterilization of eggs and diet inoculation;
- one area for larval and adult holding rooms;
- one area for the washing room, pupal harvest room, and quarantine room.

All stemborers can be successfully reared continuously under the following conditions: temperature 25 ± 2 °C; relative humidity of 60–70%; and L12:D12 photoperiod.

Each area must be free from pesticide contamination and each support (table or shelf) where the larvae and adults are reared should be isolated from the floor by petroleum jelly or water to prevent the entrance of crawling insects (such as ants). All areas should be kept free from disease, parasitoids, and predators.

Equipment

The following items should be considered as essential, or at least highly desirable, equipment in any stemborer insectary (Table 1). This list is not complete, but it is sufficient to run the existing rearing activities at icipe. More specialized, industrial-scale equipment may also be used for large-scale commercial production.

Table 1: List of equipment used for rearing stemborers at icipe insectaries.

Equipment	Purpose
Laminar hood	Clean, contaminant-free air during diet preparation, cooling and infesting the diet with larvae
Binocular loup	Sexing the pupae
Balances	Weighing diet ingredients
Small glass vials (e.g. 75 x 22 mm) and big plastic jars	Diet containers
Spoons	Mixing diet ingredients
Graduated cylinders	Measuring liquid (water, ethanol and formaldehyde)
Diet dispensers (such as ketchup dispenser)	Dispensing the diet into small glass vials or big plastic jar
Metallic pots at different sizes	Warming the ingredients and agar into water
Refrigerator	Storing larval diet, vitamin mix and antibiotics, and chilling adults for transfer from emergence cages to oviposition cages
Incubators/oven	Sterilizing the glass vials and plastic jars before use, and drying leaf and bean powder
Electric stove/microwave oven	Cooking diet
Water distiller	Preparing distilled water for diet
Blender	Mixing diet components
Thermo-hygrometers	For measuring room temperature and relative humidity
Heat fan	Maintain temperature at 25-27°C during winter periods
Leaf grinder	To ground maize/sorghum leaves into powder
Cages	Adult mating and oviposition

Laboratory consumables

Consumables are products that are recurrently consumed by the insectary (Table 2).

Table 2: List of laboratory consumables and their function.

Consumable	Purpose
Liquid detergent (Teepol) and disinfectant (Jik, Dettol, 70% ethanol solution)	Cleaning and disinfecting
Cotton wool	Holding water in cages for adults; for covering small glass vials
Masking tape	Recording diet, infestation date, collection date
Butter paper	Oviposition substrate
Paper towel (Velvex)	Cleaning
Camel hair brush No.1	First instar larvae infestation
Scissors or scalpel blade	To cut the oviposition substrates
Soft forceps	To hold/collect and soak into a formaldehyde solution the pieces of oviposition substrates with egg batches or detached eggs, and to hold the host larvae for parasitism
1 litre plastic jars (10 × 20 cm)	Larval rearing, pupal formation and egg eclosion
Small glass vials (e.g. 75 x 22 mm)	Larval rearing

Personnel

Insect rearing is a seven-days-a-week job. Like other animals, insects need daily care. To meet often tight scheduling, the rearing facility is independent from other units that operate only 5 days per week. The minimum personnel requirements are an insectary manager, laboratory technical assistant, and laboratory cleaner. This number can vary, depending on the scale of operation and the number of insect species reared.

Rearing procedures and colony handling

The different steps to rear the stemborers range from egg hatching, to rearing the larvae on an artificial diet for about 5 weeks, and then to holding the adults in cages for mating and oviposition (Figure 18).

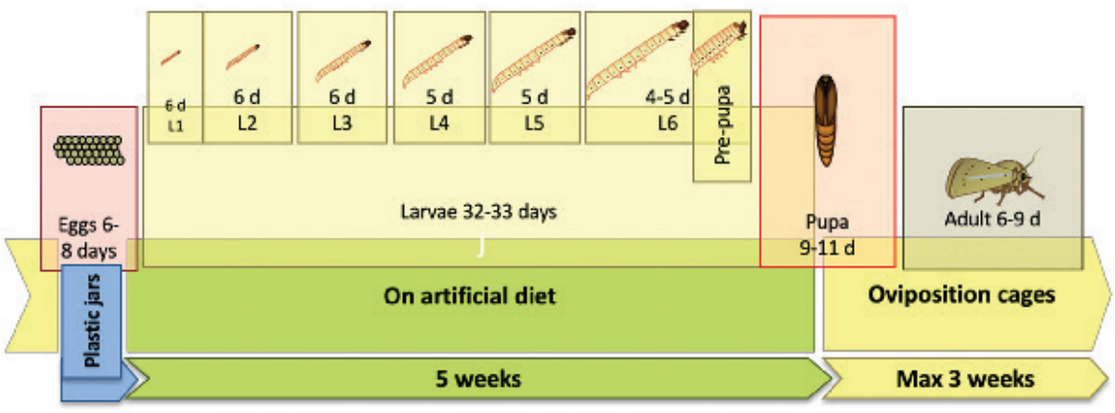


Figure 18: Different steps and estimated durations to mass rear stemborers at 25 ± 2°C; relative humidity of 60-70%; and L12:D12 photoperiod.

Artificial diet for larval development

Ingredients

The artificial diet valid for all stemborers (even for *C. partellus*) is composed by the following ingredients according to Onyango & Ochieng'-Odero (1994) (each quantity is given per 2 litres of diet):

Fraction A	Quantity per 2 litres	Purpose
Distilled water	800 ml	Mixing the ingredients
Brewer's yeast	45.0 g	Feeding attractant/induction and mineral source
Sorbic acid	1.3 g	To prevent the growth of fungi/mould
Methyl p-hydroxybenzoate	2.0 g	To prevent the growth of dissolved in 6 ml ethanol bacteria
Ascorbic acid	5.0 g	Vitamin C source
Vitamin E (300 i.u.)	4.2 g	Vitamin E source
Maize leaf powder[1]	50 g	Natural diet
Seeds of bean	175 g	Source of protein (Phaseolus vulgaris) powder[2]
Sucrose	70.0 g	Source of energy and feeding attractant/induction
Fraction B		
Agar (tech. No 3)	25 g	Gelling the diet
Distilled water	800 ml	For boiling agar
Fractions C		
Formaldehyde 40%	2 ml	Long-term preservative of food

[1] 6-week old leaves, washed and dried at 60°C for 12 hours and then ground to a fine powder.
[2] Seeds of bean washed with water dried at 60°C for 24 hours and then ground to a fine powder.

Making 40% formaldehyde solution

Formaldehyde (ml)	Distilled water (ml)	Total volume (ml)
40	60	100
200	300	500
400	600	1 000

Diet preparation procedure

For both diet compositions, each fraction is prepared as follows:

Fraction A: All powdered ingredients are weighed, pre-mixed in a clean container and mixed together with ingredients of Fraction A in a blender for 1 minute (Figure 19A). Formaldehyde 40% and dissolved methyl p-hydroxybenzoate are added into the blender at this stage and mixed together with the other ingredients in the blender for 1 minute.

Fractions B: Agar powder is weighed in a separate container, added to cold distilled water in a separate saucepan, boiled while stirring periodically, cooled to 60 °C and mixed together with ingredients of Fractions A and C in the blender for 1 minute.

Figure 19: Laboratory staff weighing and mixing the ingredients (A); mass rearing of stemborers in small glass vials for *B. fusca* (B) or big plastic jar for *C. partellus* (C).

It is preferable to avoid any risk of unsuccessful feeding of the small larvae of *B. fusca* (particularly during dry seasons). Accoringly, it is preferable to rear this species in small glass vials, 75 x 22 mm (Figure 19B). *Chilo partellus* can be reared in 1-litre plastic jars, 10 × 20 cm (Figure 19C).

Diet dispensing procedure

The *B. fusca* and *S. calamistis* diet is dispensed while warm in sterilized glass vials, 75 x 22 mm (previously heated at 100 °C for at least 1 hour), using a ketchup dispenser, 10 ml per vial (Figure 20A). The vials are then covered with a clean white cotton fabric or paper towel and left to stand overnight in the laboratory before use (Figure 20C).

The *C. partellus* diet is dispensed while warm in scrupulously clean and dry 1-litre plastic jars (10 × 20 cm), each jar containing about 200 ml of diet (Figure 20B). The jars are covered with clean paper towels or white cloth and left on the bench in the laboratory to release any condensate (Figure 20C).

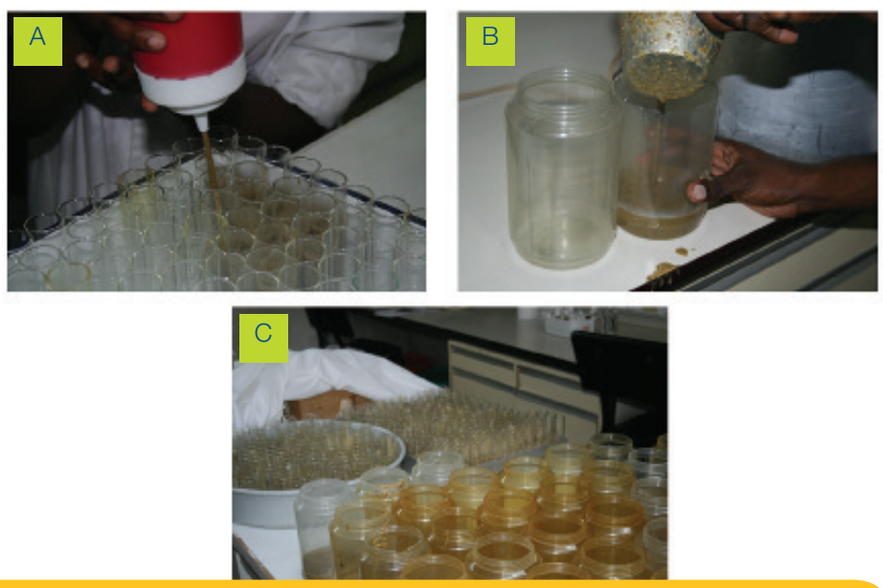


Figure 20: The diet is dispensed into each glass vial using a ketchup dispenser (A) or directly into each plastic jar (B). All diet containers are covered with a clean white cotton fabric or paper towel and left to stand overnight in the laboratory before use, or if urgently needed, left to cool for 6-7 hours before inoculation.

The glass vials are sterilized by heat treatment (100 °C for at least 1 hour, daily), with the plastic jars being soaked overnight in sodium hypochlorite solution (200 ml in 60 litres of water). Both glass vials and plastic jars are rinsed with water and dried at 40 °C before diet dispensation procedure.

Diet infestation

For *B. fusca* and *S. calamistis* diet, each glass vial is infested by 2 first-instar larvae, using a camel hair brush No.1 (dipped in 70% ethanol and rinsed in distilled water). Each vial is then closed immediately with a tight-fitting sterilized cotton wool plug to prevent larval escape. Then, the vials are placed in groups of 50 or 100, and each group labelled with date of inoculation, lab generation, and diet batch number.

For *C. partellus* diet, one sterilized egg mass (approximately 50 eggs at the black head stage) is held, using sterilized fine-nose forceps, and then dropped into each plastic jar. A paper tissue is placed on each jar, across the cover of the , and the jar is closed tightly with a screw cap, ventilated with fine wire mesh to prevent larval escape. Each jar is then labelled with date of inoculation, lab generation, diet batch numbers, and number of jars infested.

Making 70% ethanol solution

Ethanol (ml)	Distilled water (ml)	Total volume (ml)
70	30	100
350	150	500
700	300	1 000

Management of larvae and pupae

All stemborer larvae are allowed to feed ad libitum in the vials or jars, respectively until they pupate. The vials or jars are observed weekly and any vial or jar showing contamination is discarded, away from the rearing rooms. The pupae are collected weekly from vials or jars and the number of pupae collected is recorded. All pre-pupae are placed on a soft paper towelling medium in a jar to complete pupation. All pupae are then placed in adult cages for emergence.

In some case, it is necessary to sex the pupae, for example to estimate the number of males and females that will be placed into the adult cages. This is done in anticipation for the enhancement of adult mating and egg viability.

Management of adults and eggs

The adult cages (for mating and oviposition) are made of aluminium frame (45 cm L x 60 cm H x 45 cm W) with wire mesh sides, a vertically sliding full door, and aluminium plates at the bottom and top of the cage (Figure 21). The top plate has horizontal slits.

It is important to wash the cages with soapy water and mild Jik solution weekly and sun-dry before next use. The dead moths have to be removed from the cage daily and faecal droppings, scales and detached appendages must be cleaned away before introducing oviposition substrate. Up to 200 pairs of pupae per cage can be introduced at a time.

Figure 21: Adult cages used for *Busseola fusca* and *Sesamia calamistis* with butter paper, wound spirally (A) and for *Chilo partellus*, with butter paper sheet (B) used as oviposition substrates

The process to distinguish male and female pupae is shown in Figure 22.

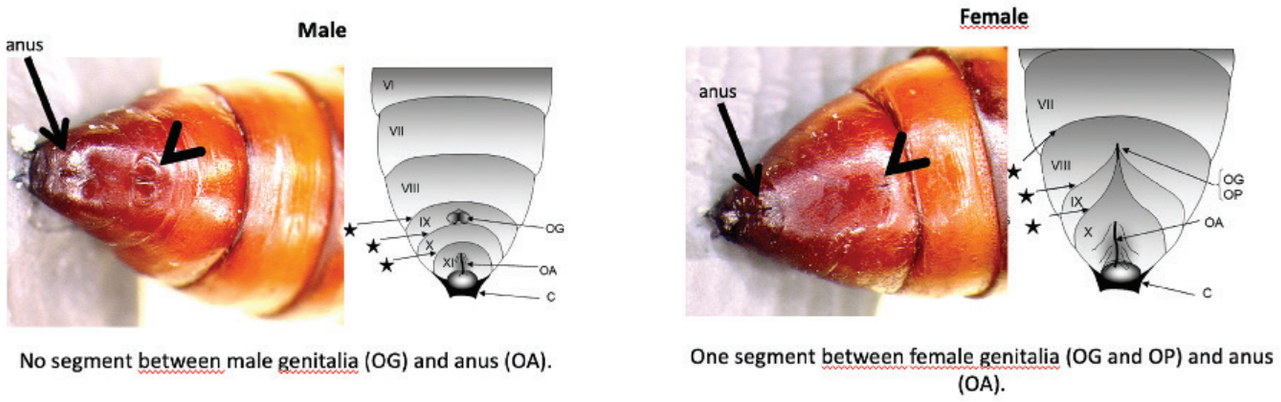


Figure 22: Sexual dimorphism between male and female pupae of stemborers

The process to distinguish male and female adults is shown in Figure 23.

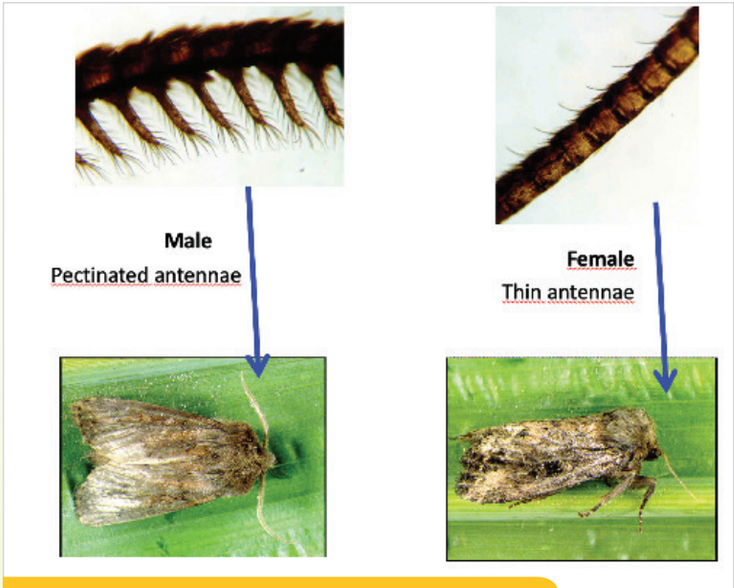


Figure 23: Sexual dimorphism between male and female adults of stemborers

For *B. fusca*, cut pieces of butter paper are used, 6 cm W x 75 cm L, wound spirally with each end twisted to hold the spirals firm (Figure 24). The spiral papers are placed into the cages slanting vertically in the cage with the right side up to simulate cereal stems with leaf sheaths (*B. fusca* females hide their eggs between the leaf sheath and stem of the plant) (Figure 21A). Eight spiral papers per cage are sufficient for oviposition of 50 pairs of adults.

Figure 24: Cut pieces of butter paper (6 cm W x 75 cm L) are wound spirally, with each end twisted to hold the spirals firm.

For *C. partellus*, pleated butter paper sheets, folded in an accordion form (Figure 25), are placed horizontally in slits in the cage top for the moths to oviposit upon (*C. partellus* females lay eggs on plant leaf surfaces) (Figure 21B). Two sheets, each approximately 40 cm W x 50 cm L, are sufficient. The butter papers are replaced daily following egg collection.

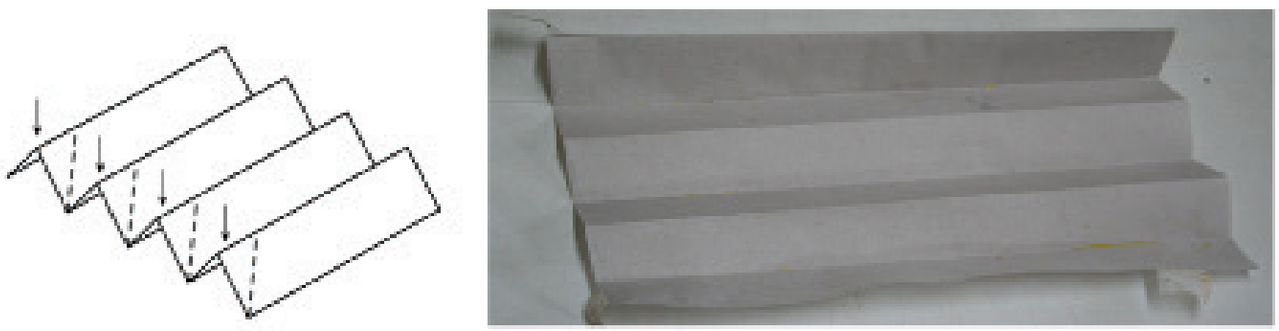


Figure 25: Cut pieces of butter paper (40 cm W x 50 cm L) are folded in accordion form

Management of eggs

For both *B. fusca* and *C. partellus*, the oviposition substrates are removed from the cage (opened up for the spiral papers) and each batch of eggs is cut around by using a pair of scissors or scalpel blade. The portions of paper cut with eggs batches (Figure 26A) or eggs detached from the paper are sterilized by dipping them in 10% formaldehyde solution for 20 minutes, then rinsed thoroughly with running distilled water and dried between filter papers. They are then placed in a plastic jar lined with moist tissue paper and closed tightly to incubate (Figure 26B). Each jar is labelled properly. Thereafter, to allow the eggs to incubate in the jar, a few drops of distilled water are added when the paper tissue is dry to provide moisture. The first instar larvae are expected to emerge after 6 days (Figure 26C).

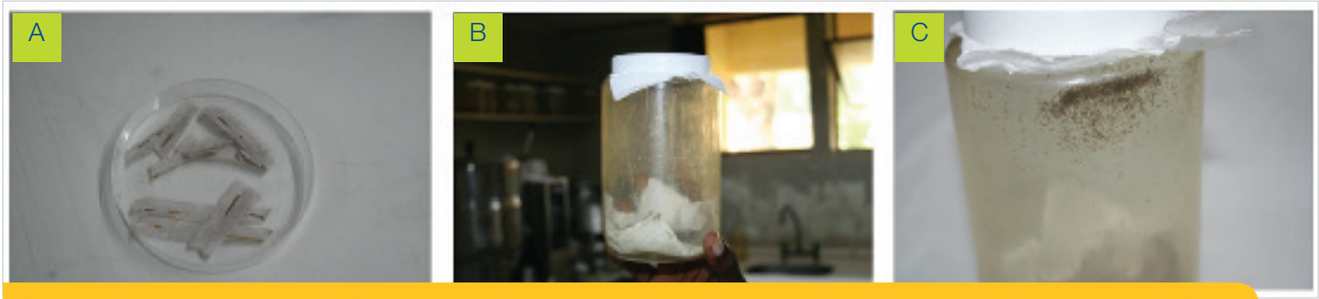


Figure 26: Portions of paper cut with *C. partellus* eggs batches ready to be soaked in 10% formaldehyde solution for 20 minutes (A). The first instar larvae are expected after 6 days from a plastic jar lined with moist tissue paper for egg incubation (B & C).

Making 10% formaldehyde solution

Formaldehyde (ml)	Distilled water (ml)	Total volume (ml)
10	90	100
20	180	200
50	450	500

Quality control

As described by Tefera et al. (2019), the quality control procedure is divided into three categories: production, process, and product quality.

- The production quality control ensures the availability and efficient storage of diet ingredients and rearing equipment.
- The process quality control ensures strict compliance with rearing procedures and maintenance schedules.
- The product quality control ensures the insect quality produced and in our case, good insect quality for parasitism.

Quality controls are also linked to the avoidance of microbial contamination in the rearing facility. In order to avoid microbial contamination, it is important to maintain a sanitary environment by mopping the floor with Jik mixed with Teepol regularly and by wiping bench tops with 70% alcohol before and after use. It is also important to sterilize the rearing equipment (see section 6.4). The insectary should be restricted to rearing personnel only and quarantine regulations should be enforced for field introductions. All escaped larvae and adults, as well as insects escaping from contaminated containers, should be discarded in a separate room. Antimicrobial compounds in artificial diets should be used. The insectary staff should observe strict personal hygiene.

Mass production, rearing procedures and colony handling of *Cotesia sesamiae* and *Cotesia flavipes*

Colony establishment

The colonies of *C. sesamiae* and *C. flavipes* can be obtained from the established colonies in the laboratory, or can be directly collected from the field. There is less need to start with a large number of individuals, as is the case with stemborers, since they are gregarious and do not suffer from loss of genetic diversity. Furthermore, even isolines (i.e. from one cocoon mass or one female) can be envisaged to initiate a colony. However, for a likely establishment after release in a biological control programme, it is advisable to initiate a parasitoid colony from several cocoon masses that come from a wide range of geographical areas (i.e. both different maize fields and different regions). Generally, a start colony with a minimum of 10–15 cocoon masses from the field is sufficient.

Using the same protocol to collect stemborers in the field (see section 6.1), the stemborer larvae or cocoon masses found in maize stems are collected. If the collected stemborer larvae have been parasitized, the cocoon masses might appear from the larvae a day after collection or several days, about 5–7 days after collection date. If the cocoon masses are collected from *B. fusca* larvae, the parasitoid is likely to be *C. sesamiae* and if they are from *C. partellus* larvae, the parasitoid is likely to be *C. flavipes* (see section 2.3 for the species identification criteria of stemborer larvae). From *S. calamistis* larvae may produce both *C. sesamiae* and *C. flavipes*.

After emergence of adult parasitoids, the species identity can be confirmed by using the species identification criteria of parasitoids given in section 3.2.4.

Colony maintenance

Because of the gregarious characteristic of both *C. sesamiae* and *C. flavipes*, it is not necessary to regularly infuse or replace each colony with wild materials. Nevertheless, the state of their hosts is important, and the colony should be genetically different, with good vitality and aggressiveness, and free of disease, generally involving microsporidia. It is then recommended to regularly infuse the laboratory colonies with wild individuals (i.e. wild cocoon masses obtained from stemborer larvae collected from the field).

Rearing facilities

Since continuous rearing of the hosts is necessary, the same rearing facilities as for stemborers are then needed for parasitoid rearing. However, additional equipment and consumables specific to the parasitoids are necessary, such as Perspex cages (30 × 30 × 30 cm) (mass-parasitize the host larvae), an incandescent lamp to heat/stimulate the parasitoids, and honey (to prepare a 20% honey–water solution to feed parasitoid adults).

The parasitoids must be maintained in different rooms to those used to rear the stemborer hosts. Similarly to stemborers, they have to be placed in areas free from pesticide contamination and each support (table or shelf) where the parasitoids are reared should be isolated from the floor by petroleum jelly or water to prevent the entrance of crawling insects (such as ants). All areas should be kept free from disease, other parasitoid species, hyperparasitoids and predators.

Both *C. sesamiae* and *C. flavipes* can be successfully reared continuously under the following conditions: temperature 25 ± 2 °C; relative humidity of 60–70%; and L12:D12 photoperiod.

Rearing procedures and colony handling

The various steps to rear the parasitoids involve the use of medium-to large-size host larvae (3-6th instar) for parasitism. Hosts reared on artificial diet should be removed from the artificial diet and allowed to feed on a natural diet (cut pieces of maize or sorghum stems) for 24 hours prior to exposure to the parasitoids. The parasitized larvae are then put on an artificial diet for incubation for a duration of about 12 days, after which cocoons form. The cocoon masses are then collected for adult obtention (Figure 27). It can take about 3 weeks to obtain adult parasitoids.

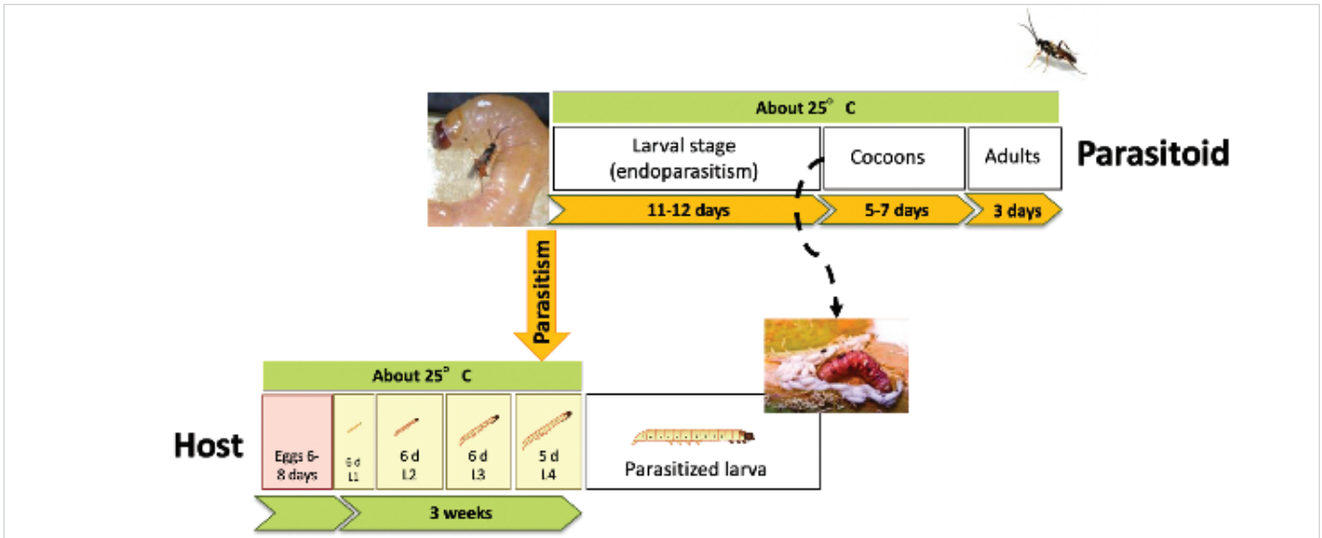


Figure 27: Different steps and estimated durations to mass rear *Cotesia sesamiae* and *Cotesia flavipes* at 25 ± 2°C; relative humidity of 60–70%; and L12:D12 photoperiod.

Parasitism, management of parasitized larvae and cocoon collection

Mass parasitisms (i.e. using several host larvae) are done in Perspex cages (30 × 30 × 30 cm) (Figure 28). The surfaces of two sides (at the least) of the cage should have several small droplets of 20% honey–water solution applied to them, or the same solution on cotton wool provided in a petri-dish at the bottom of the cage, to maintain the adults in the cage.

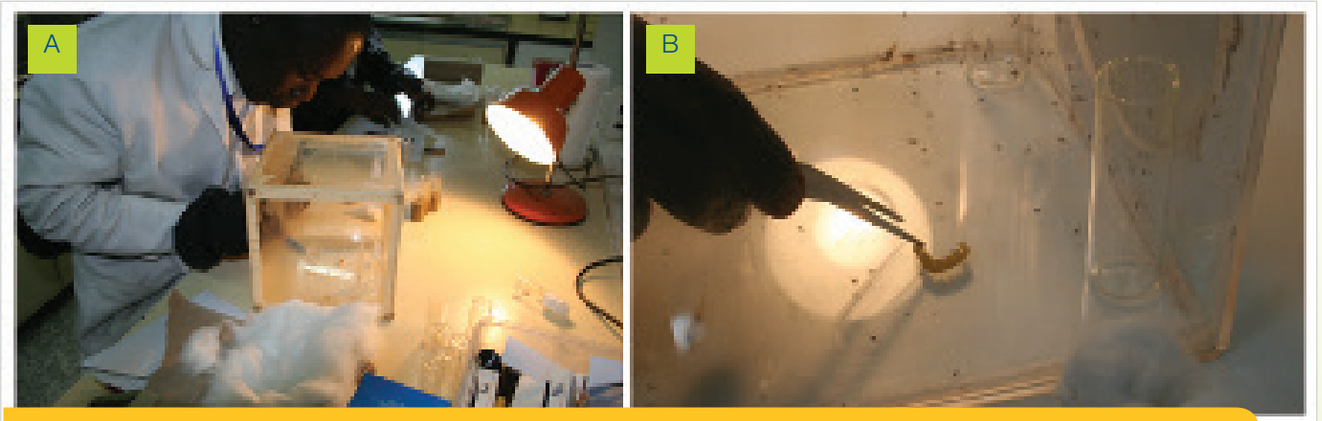


Figure 28: Mass parasitism in a Perspex cage (A). The adult parasitoids are released into the cages having on the surface of two sides several small droplets of 20% honey–water solution. The larvae are parasitized by hand-stinging method (B).

Inside the cage, the hand-stinging method described by Overholt et al. (1994c) is employed to parasitize several hosts in a short time (Figure 29).

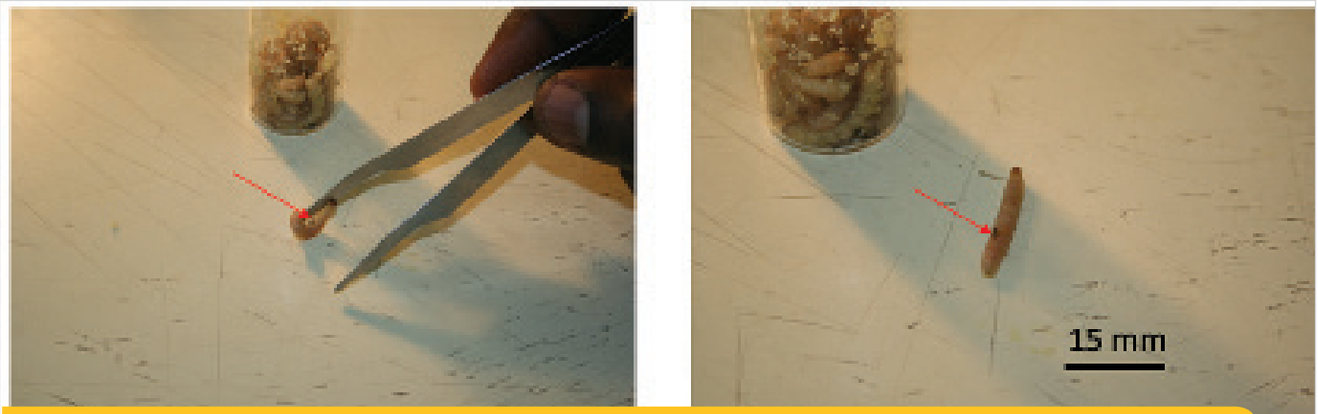


Figure 29: Hand-stinging method includes holding a host larva via soft forceps towards a parasitoid female to ease the parasitism process (each arrow indicates the parasitoid parasitizing the larva).

After parasitism, all larvae are placed individually in glass vials with diet (Figure 30B). After about 12 days, the cocoon masses are removed from the diet (Figure 30C), and transferred into another empty glass vial for adult emergence and mating (each tube with black cocoons contains on its side a cotton wool infused with 20% honey–water solution)(Figure 30D). The adults emerge after 5 days. One day after adult emergence, during which mating mostly mating occurs, the parasitism process in the cage can be carried out.

The adults are released into Perspex cages with droplets of 20% honey–water solution for parasitism. The cage is then put under light to stimulate female activity for oviposition. All larvae used for parasitism are previously fed for 24 h on pieces of maize stem in 10 × 20 cm jars to produce frass that facilitates host acceptance by the parasitoid wasps (Figure 30A).

Figure 30: Before parasitism, all stemborer larvae are fed on pieces of maize stem (A). After parasitism, the parasitized larvae are inserted individually into glass vials filled with diet until cocoon masses form (B). After cocoon mass formation, each cocoon mass is removed from the diet (C). Then, several cocoon masses are placed in empty glass vials for adult emergence and mating (D).

Quality control

As described by Tefera et al. (2019), "quality control in mass production involves the production of parasitoid individuals with fitness and vigor comparable to wild populations.

Besides the adaptation to the target pest and keeping the correct parasitoid species, the quality of the final parasitoid product depends also on the following parameters:

- appearance of the cocoon mass (the cocoons are well formed);
- number of emerged adults per cocoon mass;
- sex ratio (for example, if only males emerge from a cocoon mass this indicates that the female which parasitized the larva was not mated); parasitoids should therefore be given ample time to properly mate before being exposed to the host for steady continuation of the colony.
- the activity or movement of the adults and the aggressiveness of the females for parasitism is an important aspect to observe during exposures.

Storage, packing and field release of the larval parasitoids

Storage

Before releases, the parasitoids reared under laboratory conditions can be stored in the refrigerator/incubator only at cocoon stage at between 15–20 °C, for only a few days in order to meet the number of parasitoids required for the release dates. However, the emergence rates, sex ratio and fecundity can be affected by such storage process and duration. It would therefore be better to avoid this process or not to exceed 7 days at 15 °C.

Field release

Pre-release site assessment

It is preferable to release the parasitoids in field sites where the parasitoids will get a chance to establish themselves. The target fields should have maize cultivated on them every year (which enhances the chance of presence of the host stemborers every year) and should not be treated with insecticides (particularly necessary after releases), or should be at a distance away from fields that are sprayed. Moreover, the target sites should be easy to access in order to visit them periodically for parasitoid releases and recoveries.

Number and frequency of releases

Of course, higher numbers and frequencies of parasitoids released increase the chance for the parasitoids to become establish in the released sites. For example, for *C. sesamiae*, 5,000 to 14,000 cocoon masses have been released in Cameron over a total of 8 to 18 times per year to recover the released parasitoids in the field (Ndemah et al., submitted). For *C. flavipes*, 10,000 to 2,300,000 parasitoids have been released over a total of 100 to 500 times per year in Uganda (Omwega et al., 2006).

Timing and field releasing techniques for parasitoids

It is always important to verify the presence of the host stemborers when the parasitoids are released. It is preferable to release the parasitoids during maize cropping seasons and close to maize plants that show damages by stemborers (see section 2.1), to allow the released parasitoids to reach their hosts faster and easily.

Three release methods are generally employed (Omwega et al., 2006):

- the most common method is to release adults (although synchronizing the timing between the mass release process and transportation to the release sites is not always possible);
- the second method is to release the parasitoids in cocoon form (cocoon masses are taken to the field and placed in the release sites to emerge and disperse);
- mated parasitoids in a cage are taken to the field and allowed to singly sting medium-size larvae dissected from stems in an infested field, and stung larvae are then reintroduced into the stems of infested plants in the field (this process can be done before going to the field in the laboratory and then carrying and releasing parasitized larvae to the released sites).

Monitoring and assessment of parasitoid establishment

Using the same protocol to collect stemborers in the field, the stemborer larvae or cocoon masses found in maize stems are collected from release sites. After emergence of adult parasitoids, the species identity should be confirmed by using the species identification criteria for parasitoids given in section 3.2.4.

Quality control of released larval parasitoids

Three aspects have to be considered in the quality of the releases: i) the genetic variation required to allow the parasitoids to adapt in the field; ii) their Wolbachia presence and strain characteristics, an endosymbiotic bacterium involved in cytoplasmic incompatibilities (i.e. cross incompatibilities between parasitoid populations), and iii) their dispersal capacity to spread as much as possible and then to gain greater chance of becoming established.

To release a greater genetic variation of the parasitoid population, it is advisable to release at least 1 000 mated females (Omwega & Overholt, 1996). This estimate was given for *C. flavipes*. The percentage of females per cocoon mass generally obtained from field populations in Kenya is 45% (mean) with 24 (mean) progenies per cocoon mass (n=84 cocoon masses) for *C. sesamiae* and 69% (mean) with 28 (mean) progenies per cocoon mass (n=50 cocoon masses) for *C. flavipes* (Obonyo Julius, Pers. Observ.). We can therefore estimate to release 1,000 females needed to obtain 91 cocoon masses of *C. sesamiae* (11 females per cocoon mass) and 53 cocoon masses of *C. flavipes* (19 females per cocoon mass).

The *C. flavipes*, parasitoid species has no Wolbachia and since the population spread in East and Southern Africa has come from icipe mass rearing, there is no restriction against re-introducing individuals of *C. flavipes* in both Uganda and Ethiopia from icipe. In fact, *C. flavipes* was first released by icipe in Uganda in 1998 (Matama-Kauma et al., 2001) and thereafter in 10 other countries in East and Southern Africa (Omwega et al., 2006). The establishment of this parasitoid species has been recorded in all of these countries, including Ethiopia where releases were never made, with the population settling in Ethiopia probably coming from Somalia where the species had been released in 1997 (Getu et al., 2003).

For *C. sesamiae* the situation is more complex. *Cotesia sesamiae* is known to harbour Wolbachia, an endosymbiotic bacterium widespread in arthropods that is known for inducing unidirectional (when infected males mate with non-infected females) or bidirectional (when crosses are effected between insects harbouring different strains of the bacterium) cytoplasmic incompatibilities. Such incompatibilities may reduce the performance of the

parasitoid if crosses between infected males and uninfected females occur, particularly in hybrid zones, where some bidirectional incompatibility may be expressed. Therefore, introducing and releasing incompatible populations will not reinforce the existing *C. sesamiae* population already involved in the biological control of *B. fusca*.

Moreover, it has been recently shown that each population of *C. sesamiae* (coming from Kenya and introduced into Cameroon) remained strongly associated with its original Wolbachia component, even after releases (Ndemah et al., submitted). In western Kenya, the parasitoid that parasitizes *B. fusca* is infected by the Wolbachia WCsesB1 strain (the population reared massively at icipe on *B. fusca*), while in the eastern coastal region, *S. calamistis*, it is infected with two strains of Wolbachia WCsesA and WCsesB2 (Branca et al., 2019). There are then likely to be successful crosses with the native *C. sesamiae*, if the Kenyan population parasitizing *B. fusca* (infected by Wolbachia WCsesB1 strain) is introduced, and released in Ethiopia and Uganda and thus giving more chance for successful establishments.

The last criteria of quality of release is the capacity of the released parasitoid species to disperse. The dispersal rates of parasitoids have been estimated for *C. flavipes* and Cotesia glomerata (L.) (Hymenoptera: Braconidae) (Table 3).

Table 3: Average of dispersion rate of *Cotesia flavipes* and Cotesia glomerata (Taiajana Fortuna, pers. comm.)

Cotesia species	Dispersion rate (m/day)	Reference	Body size (mm)*	Comparison with *C. sesamiae* body size**
C. flavipes	34	Botelho et al. (1980)	1.8	< *C. sesamiae*
C. flavipes	164	Omwega et al. (1997)	1.8	< *C. sesamiae*
C. flavipes	4	Sallam et al. (2001)	1.8	< *C. sesamiae*
C. flavipes	34	Omwega et al. (2006)	1.8	< *C. sesamiae*
C. flavipes	6.67	Dinardo-Miranda et al. (2014)	1.8	< *C. sesamiae*
C. glomerata	72	Bezemer et al. (2010)	2.6	> *C. sesamiae*
Average	45.83	without Omwega et al. (1997)		

* *C. flavipes* body length of the females in Potting et al. (1997); C. glomerata body length in Le Masurier (1991);
** Body size of *C. sesamiae* female is, on average, 2.1 mm.

According to the Table 3, as adult wasps of both *C. flavipes* and *C. sesamiae* live 3–4 days, we expect a dispersion rate of between 60–138 m in 3 days after releases, and (as estimated by Omwega et al. (2006) in Kenya for *C. flavipes*) a diffusion rate of 11.23 km per year when they become established.

References

Abate, T., Van Huis, A., Ampofo, J.K.O. (2000). Pest management strategies in traditional agriculture: An African perspective. Annual Review of Entomology 45, 631-659.

Altieri, M., Nichols, C.I., Fritz, M.A. (2005). Manage insects on your farm: A guide to ecological strategies. Sustainable agriculture network handbook series book 7. (Available online at: http://www.sare.org/Learning-Center/Books/Manage-Insects-on-Your-Farm) (verified 19 May 2020).

Bezemer, T.M., Harvey, J.A., Kamp, A.F.D., Wagenaar, R., Gols, R., Kostenko, O., Fortuna, T., Engelkes, T., Vet, L.E.M., Van Der Putte, W.H., Soler, R. (2010). Behaviour of male and female parasitoids in the field: influence of patch size, host density, and habitat complexity. Ecological Entomology 35, 341-351.

Bonhof, M., Overholt, W., Van Huis, A., Polaszek, A. (1997). Natural Enemies of Cereal Stemborers in East Africa: A Review. Insect Science and Its Application 17, 19-35.

Bosque-Pérez, N.A., Mareck, J.H. (1990). Distribution and species composition of lepidopterous maize borers in southern Nigeria. Bulletin of Entomological Research 80, 363-368.

Botelho, P.S.M., Macedo, N., Mendes, A.C., Silveira Neto, S. (1980). Aspects of the population dynamics of Apanteles flavipes (Cameron) and support capacity of its host Diatraea saccharalis (Fabr.) In: Congress of International Society of Sugarcan technologists, Manila.

Branca, A. (2009). Ecological diversification of the African parasitoid *Cotesia sesamiae*: role of symbiotic partners (In French). PhD Thesis, Pierre and Marie Curie University, Paris, France, 236p.

Branca, A., Le Ru, B., Calatayud, P.-A., Obonyo, J., Musyoka, B., Capdevielle-Dulac, C., Kaiser-Arnauld, L., Silvain, J.-F., Gauthier, J., Paillusson, C., Gayral, P., Herniou, E.A., Dupas, S. (2019). Relative influence of host, Wolbachia, geography and climate on the genetic structure of the Sub-saharan parasitic wasp *Cotesia sesamiae*. Frontiers in Ecology and Evolution 7, 309. doi: 10.3389/fevo.2019.00309

Branca, A., Vavre, F., Silvain, J.-F., Dupas, S. (2009). Maintenance of adaptive differentiation by Wolbachia induced bidirectional cytoplasmic incompatibility: the importance of sib-mating and genetic systems. BMC Evolutionary Biology 9, 185. doi: 10.1186/1471-2148-9-185

Cairns, J. E., Hellin, J., Sonder, K., Araus, J. L., MacRobert, J. F., Thierfelder, C., Prasanna, B. M. (2013). Adapting maize production to climate change in sub-Saharan Africa. Food Security 5, 345-360.

Calatayud, P.-A., Clarke, C. W., Sforza, R. F. H., Ndemah, R. N., Nyamukondiwa, C., eds. (2020). Parasitoids' Ecology and Evolution. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-480-4

Calatayud, P-A., Le Ru, B., Van den Berg, J., Schulthess, F. (2014). Ecology of the African Maize Stalk Borer, *Busseola fusca* (Lepidoptera: Noctuidae) with Special Reference to Insect-Plant Interactions. Insects 5, 539-563.

CIBC (1968-72). Annual Reports of the Commonwealth Institute of Biological Control, Farnham Royal, UK.

De Groote, H. (2002). Maize yield loss from stem borerstemborers in Kenya. Insect Science and its Application 22, 89-96.

Demissie, G., Admassu, S., Getu, E., Azerefegn, F. (2011). Review of the past decade (2001-2011) research on pre-harvest insect pests of maize in Ethiopia. In M. Worku, et al (Eds.), Meeting the Challenges of Global Climate Change and Food Security through Innovative Maize Research. Proceedings of the Third National Maize Workshop of Ethiopia.Mexico, DF: CIMMYT.

Dinardo-Miranda, L.L.; Fracasso, J.V., Pereira da Costa, V., Lopes, D.O.T. (2014). Dispersal of *Cotesia flavipes* in sugarcane field and implications for parasitoid releases. Bragantia, Campinas 73, 163-170.

Dupas, S., Gitau, C., Le Ru, B., Silvain, J.-F. (2006). Single-step PCR differentiation of *Cotesia sesamiae* (Cameron) and *Cotesia flavipes* (Cameron)(Hymenoptera: Braconidae) using polydnavirus markers. Annales de la Société Entomologique de France 42, 319-323.

Food and Agriculture Organization (FAO) (2020). FAOSTAT Crop Production Compare Data. Rome. Accessed on 1st May, 2020. http://www.fao.org/faostat/fr/#compare

Getu, E., Overholt, W.A., Kairu, E., Omwega C.O. (2003). Evidence of the establishment of *Cotesia flavipes* (Hymenoptera:

Braconidae), a parasitoid of cereal stemborers, and its host range expansion in Ethiopia. Bulletin of Entomological Research 93, 125-129.

Gifford, J.R., Mann, G.A. (1967). Biology, rearing and trial release of Apanteles flavipes in the Florida everglades to control sugarcane borer. Journal of Economic Entomology 60, 44-47.

Godfray, H.C.J. (1994). Parasitoids: behavioral and evolutionary ecology. Krebs J.R. & Clutton-Brock, T. (Eds.). (PP.473). Princeton University Press, Princeton, USA.

Goftishu, M., Assefa, Y., Niba, A., Fininsa, C. (2017). Cereal stem borerstemborer management practices in subsistence farms of eastern Ethiopia. International Journal of Pest Management 63, 289-298.

Hajek, A. (2004). Natural Enemies: An Introduction to Biological Control. Cambridge University Press, Cambridge. http://34.250.91.188:8080/xmlui/handle/123456789/1001

Ingram, W.R. (1958). The lepidopteran stalkborer associated with Gramminae in Uganda. Bulletin of Entomological Research 49, 367-383.

Kaiser L., Dupas S., Branca A., Herniou E.A., Clarke C.W., Capdevielle-Dulac C., Obonyo J., Benoist R., Gauthier J., Calatayud P.-A., Silvain J.-F., Le Ru B.P. (2017). The *Cotesia sesamiae* story: insight into host-range evolution in a Hymenoptera parasitoid and implication for its use in biological control programs. Genetica 145, 455-468.

Kaiser, L., Le Ru, B. P., Kaoula, F., Paillusson, C., Capdevielle-Dulac, C., Obonyo, J. O., Herniou, E. A., Jancek, S., Branca, A., Calatayud, P.-A., Silvain, J.-F., Dupas, S. (2015). Ongoing ecological speciation in *Cotesia sesamiae*, a biological control agent of cereal stem borerstemborers. Evolutionary Application 8, 807-820.

Kalule, T., Khan, Z.R., Bigirwa, G., Alupo, J., Okanya, S., Pickett, J.A., Wadham, L.J. (2006). Farmer's perception of importance, control practices and alternative hosts of maize stemborer. International Journal of Tropical Insect Science 26, 71-77.

Kalule, T., Ogenga-Latigo, M.W., Okoth, V.A.O.C. (1998). Efficacy of different insecticides for the management of stemborer in Uganda. African Crop Science Journal 6, 103-108.

Kfir, R., Overholt, W.A., Khan, Z.R., Polaszek, A. (2002). Biology and management of economically important lepidopteran cereal stem borerstemborers in Africa. Annual Review of Entomology 47, 701-731.

Khadioli, N., Tonnang, Z. E. H., Ong'amo, G., Achia, T., Kipchirchir, I., Kroschel, J., Le Ru, B. (2014). Effect of temperature on the life history parameters of noctuid lepidopteran stem borerstemborers, *Busseola fusca* and *Sesamia calamistis*. Annals of Applied Biology 165, 373–386.

Kipkoech, A. K., Schulthess, F., Yabann, W. K., Maritim, H. K., Mithöfer, D. (2006). Biological control of cereal stem borerstemborers in Kenya: A cost benefit approach. Annales de la Société entomologique de France 42, 519-528.

Le Masurier, A.D. (1991). Effect of host size on clutch size in Cotesia glomerata. Journal of Animal Ecology 60, 107-118.

Le Ru, B. P., Ong'amo, G. O., Moyal, P., Muchugu, E., Ngala, L., Musyoka, B., Abdullah, Z., Matama-Kauma, T., Lada, V. Y., Pallangyo, B., Omwega, C. O., Schulthess, F., Calatayud, P.-A., Silvain, J.-F. (2006b). Geographic distribution and host plant ranges of East African noctuid stem borerstemborers. Annales de la Société Entomologique de France 42, 353-361.

Le Ru, B. P., Ong'amo, G. O., Moyal, P., Ngala, L., Musyoka, B., Abdullah, Z., Cugala, D., Defabachew, B., Haile, T. A., Matama-Kauma, T., Lada, V. Y., Negassi, B., Pallangyo, B., Ravololonandrianina, J., Sidumo, A., Omwega, C. O., Schulthess, F., Calatayud, P.-A., Silvain, J.-F. (2006a). Diversity of lepidopteran stemborers on monocotyledonous plants in eastern Africa and island of Madagascar and Zanzibar revisited. Bulletin of Entomological Research 96, 1-9.

Macauley, H., Ramadjita, T. (2015). Cereal Crops: Rice, Maize, Millet, Sorghum, Wheat. Bacground paper. Feeding Africa: An Action Plan for African Agricultural Transformation, Abdou Diouf International Conference Center, Dakar, Senegal.

Mahr, D.L., Whitaker, P., Ridgway, N.M. (2008). Biological control of insects and mites: An introduction to beneficial natural enemies and their use in pest management. University of Wisconsin Cooperative Extension, No. A3842.

Matama-Kauma, T., Kyamanywa, S., Ogwang J.A., Omwega, C.O., Wilson, H.R. (2001). Cereal stem borerstemborer species complex and establishment of *Cotesia flavipes* Cameron in eastern Uganda. Insect Science and Its Application 21, 317-325.

Matama-Kauma, T., Schulthess, F., Ogwang, J. A., Mueke, J. M., Omwega, C. O. (2007).Distribution and relative importance of lepidopteran cereal stemborers and their parasitoids in Uganda. Phytoparasitica 35, 1, 27-36.
Matama-Kauma, T., Schulthess, F., Mueke, J. M., Omwega, C. O., Ogwang, J. A. (2006). Effect of wild grasses planted as border

rows on stem borerstemborer infestations in maize in Uganda. Annales de la Societe Entomologique de France 42, 3-4, 455-460.

Midingoyi, S. G., Affognon, H. D., Macharia, I., Ong'amo, G., Abonyo, E., Ogola, G., De Groote, H., Le Ru, B. (2016). Assessing the long-term welfare effects of the biological control of cereal stemborer pests in East and Southern Africa: Evidence from Kenya, Mozambique and Zambia. Agriculture, Ecosystems and Environment 230, 10-23.

Mochiah, M.B., Ngi-Song A.J., Overholt W.A., Richard S. (2002a). Wolbachia infection in *Cotesia sesamiae*(Hymenoptera: Braconidae) causes cytoplasmic incompatibility: implications for biological control. Biological Control 25, 74-80.

Mochiah, M.B., Ngi-Song, A.J., Overholt, W.A., Stouthamer, R. (2002b). Variation in encapsulation sensitivity of *Cotesia sesamiae* biotypes to *Busseola fusca*. Entomologia Experimentalis et Applicata 105, 111-118.

Mohyuddin, A.I. (1971). Comparative biology and ecology of Apanteles flavipes and A. sesamiae Cameron as parasites of gramineous borers. Bulletin of Entomological Research 61, 33-39.

Mohyuddin, A.I. (1990). Biological control of Chilo spp. in maize, sorghum and millet. Insect Science and Its Application 11, 721-732.

Mohyuddin, A.I., Greathead, D.J. (1970). Annotated list of parasites of gramineous stemborers in East Africa with a discussion of their potential in biological control. Entomophaga 15, 241-247.

Ndemah, R., Le Ru, B.P., Capdevielle-Dulac, C., Schulthess, F., Kaiser, L., Hanna, R., Depoilly, A., Obonyo, J., Calatayud, P.-A., Abang, A., Dupas, S. (Submitted). Managing and monitoring genetic isolation and local adaptation of endemic and introduced *Cotesia sesamiae* for the biological control of the cereal stemborer *Busseola fusca* in Cameroon. Biological Control.

Ndemah, R., Schulthess, F., Abang, A., Ghogomu, R. T., Ntonifor, N., Dupas, S., Le Ru, B. (2012), Suitability of cereal stemborers in Cameroon to Kenyan populations of the braconid larval parasitoid *Cotesia sesamiae*. Journal of Applied Entomology 136, 60-69.

Ngi-Song, A. J., Overholt, W. A., Ayertey, J. N. (1995). Suitability of African gramineous stemborers for development of *Cotesia flavipes* and *C. sesamiae* (Hymenoptera: Braconidae). Environmental Entomology 24, 978-984.

Ngi-Song, A.J., Overholt, W.A., Stouthamert, R. (1998). Suitability of *Busseola fusca* and *Sesamia calamistis* (Lepidoptera: Noctuidae) for the development of two populations of *Cotesia sesamiae* (Hymenoptera: Braconidae) in Kenya. Biological Control 12, 208-214.

Nye, I. W. B. (1960). The insect pests of graminaceous crops in East Africa. Report of a survey carried out between March 1956 and April 1958. Colonial Research Studies No 31.

Odendo, M., Ouma, J., Wachira, S., Wanyama, J. (2003). Economic assessment of maize yield loss due to stem borerstemborer in major maize agro-ecological zones of Kenya. African Crop Science Conference Proceedings 6, 683-687.

Omwega, C.O., Kimani, S.W., Overholt, W.A., Ogolv, C.K.P.O. (1995). Evidence of the establishment of *Cotesia flavipes*Cameron (Hymenoptera: Braconidae) in continental Africa. Bulletin of Entomological Research 85, 525-530.

Omwega, C.O., Muchugu, E., Overholt, W.A., Schulthess, F. (2006). Release and establishment of *Cotesia flavipes*Cameron (Hymenoptera: Braconidae) an exotic parasitoid *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) in East and Southern Africa. Annales de la Société Entomologique de France 42, 511-517.

Omwega, C.O., Overholt, W.A. (1996). Genetic changes occurring during laboratory rearing *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), an important parasitoid for the control of gramineous stem borerstemborers in Africa. African Entomology 4, 231-237.

Omwega, C.O., Overholt, W.A., Mbapila, J.C., Kimani-Njogu, S.W. (1997). Establishment and dispersal of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae), an exotic endoparasitoid of *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in northern Tanzania. African Entomology 5, 71-75.

Onyango, F. O., Ochieng'-Odero, J. P. R. (1994). Continuous rearing of the maize stem borerstemborer *Busseola fusca* on an artificial diet. Entomologia Experimentalis et Applicata 73, 139-144.

Overholt, W. A., Ogedah, K., Lammers, P. M. (1994a). Distribution and sampling of *Chilo partellus* (Lepidoptera: Pyralidae) in maize and sorghum on the Kenya coast. Bulletin of Entomological Research 84, 367-378.
Overholt, W.A., Maes, K.V.N., Goebel, F.R. (2001). Field guide to the stemborer larvae of maize, sorghum and sugarcane in Eastern and Southern Africa. ICIPE Science Press, Nairobi, Kenya.

Overholt, W.A., Ngi-Song, A.J., Kimani, S.K., Mbapila, J., Lammers, P., Kioko, E. (1994b). Ecological considerations of the introduction of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) for biological control of *Chilo partellus*(Lepidoptera: Pyralidae), in Africa. Biocontrol News and Information 15, 19N-24N.

Overholt, W.A., Ngi-Song, A.J., Omwega, C.O., Kimani-Njogu, S.W., Mbapila, J., Sallam, M.N., Ofomata, V. (1997). A review of the introduction and establishment of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) in East Africa for biological control of cereal stemborers. Insect Science and Its Application 17, 79-88.

Overholt, W.A., Ochieng, J.O., Lammers, P., Ogedah, K. (1994c). Rearing and field release methods for *Cotesia flavipes*Cameron (Hymenoptera: Braconidae), a parasitoid of tropical gramineous stem borerstemborers. Insect Science and its Applications 15, 253-259.

Overholt, W.A., Songa, J.M., Ofomate V., Jeske R. (2000). The spread and ecological consequences of the invasion of *Chilo partellus* (Swinhoe) Lepidoptera: Crambidae in Africa. http://www.icipe.org/invasive/talks/stemborer.cfm

Pedigo, L.P., Rice M.E. (2009). Entomology and Pest Management. 6th Edition. Prentice-Hall, Upper Saddle River, NJ, 784 p.

Polaszek, A. & Walker, A.K. (1991). The Cotesia species complex: Parasitoids of cereal stemborers in the tropics. Redia 74, 335-341.

Potting, R.P.J., Snellen, H.M., Vet, L.E.M. (1997). Fitness consequences of superparasitism and mechanism of host discrimination in the stemborer parasitoid *Cotesia flavipes*. Entomologia Experimentalis et Applicata 82, 341-348.

Rousse, P., Gupta A. (2013). Microgastrinae (Hymenoptera: Braconidae) of Reunion Island: a catalogue of the local species, including 18 new taxa and a key to species. Zootaxa 3616 (6), 501-547.

Sallam, M.N., Overholt, W.A., Kairu, E. (2001). Dispersal of the exotic parasitoid *Cotesia flavipes* in a new ecosystem. Entomologia Experimentalis et Applicata 98, 211-217.

Schulthess, F., Bosque-Pérez, N.A, Chabi-Olaye, A., Gounou, S., Ndemah, R., Goergen, G. (1997). Exchange of natural enemies of lepidopteran cereal stem borerstemborers species between African regions. Insect Science and Its Application 17, 97-108.

Songa, J.M., Overholt, W.A., Mueke, J.M., Okello, R.O. (2002). Colonization of *Cotesia flavipes* (Hymenoptera: Braconidae) in stemborers in semi-arid eastern province of Kenya. Journal Economic Entomology 21, 289-295.

Tams, W.H.T., Bowden, J. (1953). A revision of the African species of Sesamia Guenée and related genera (Agrotidae, Lepidoptera). Bulletin of Entomological Research 43, 645–678.

Tefera, T., Goftishu, M., Ba, M., Muniappan, R. (2019). A Guide to Biological Control of Fall Armyworm in Africa Using Egg Parasitoids. First Edition, Nairobi, Kenya.

Wale, M., Schulthess, F., Kairu, E.W., Omwega, C.O. (2006). Cereal yield losses caused by lepidopterous stem borerstemborers at different nitrogen fertilizers rates in Ethiopia. Journal of Applied Entomology 130, 220-229.

Zhou, G., Baumgärtner, J., Overholt, W. A. (2001a). Impact assessment of an exotic parasitoid on stemborer (Lepidoptera) population dynamics in Kenya. Ecological Applications 11, 1554-1562.

Zhou G., Overholt W.A., Mochiah M. (2001b). Changes in the distribution of lepidopterans maize stemborers in Kenya from the 1950s to 1990s. International Journal of Tropical Insect Science 21, 395-402.

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