FEED THE FUTURE
The U.S. Government's Global Hunger & Food Security Initiative

Fall Armyworm in Africa:

A GUIDE FOR INTEGRATED PEST MANAGEMENT

First Edition

USAID
FROM THE AMERICAN PEOPLE

CIMMYT
International Maize and Wheat Improvement Center

CGIAR RESEARCH PROGRAM ON Maize

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Editors

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in collaboration with international and national
research and development partners

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CHAPTER 05

Biological Control and Biorational Pesticides for Fall Armyworm Management

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1. Introduction

1.1. What are Biological and Biorational Pest Control Options?

In nature, the population of any organism is regulated. It is kept fluctuating within an upper and lower threshold, often below economically damaging levels, due to the actions of biotic regulations (availability of food, parasites, predators, and/or pathogens) and/or abiotic factors (climate and soil factors). Such population regulation is referred to as natural control. However, such natural control when disrupted due to biological, anthropogenic, or climatic factors results in the outbreak of organisms leading to economic damage. Invasiveness of a pest species into new geographies in the absence of biotic regulatory factors often results in the disruption of natural control, leading to devastating outbreaks (*e.g.*, fall armyworm (FAW), *Spodoptera frugiperda* [J.E. Smith]; tomato leaf miner, *Tuta absoluta* [Meyrick]). Anthropogenic changes in crop and pest management practices such as introduction of a susceptible crop/cultivar, monocropping, and irrational use of broad-spectrum pesticides, among others, also often result in disruption of natural control, leading to outbreaks of pest and diseases. Asynchrony in range expansion of pests and their natural enemies due to climate change could also disrupt the natural control.

The best approach to manage such outbreaks is to either revive or establish natural control as much as possible. Biological control primarily focuses on restoring the natural control. Biological control, as defined by Paul DeBach (1964), is the action of living organisms (parasites, predators, or pathogens) introduced by human intervention for regulating the population of another organism at densities less than those that would occur in their absence. Parasitoids are biological agents for which at least one of their life stages is intimately associated with specific life stages of the pest and with greater levels of specificity (*e.g.*, parasitoid species belonging to *Trichogramma* and *Telenomus* parasitizing eggs of insects including FAW). The larvae of parasitoids always kill their host as the outcome of their development. Predators, on the other hand, are never intimately associated with the insect pest, and the pest serves as prey for the predator often with less specificity (*e.g.*, insects such as ladybird beetles, earwigs, and sap-sucking insects such as *Orius* and *Podisus* prey on various life stages of FAW). Entomopathogens include bacteria, fungi, protozoans, nematodes, or viruses that infects and causes diseases in insects (*e.g.*, fungi such as *Metarhizium anisopliae* and *Beauveria bassiana;* viruses such as *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV); and bacteria such as *Bacillus thuringiensis* (*Bt*), and others that are known to infect FAW).

Based on how biological control is undertaken, it can be broadly classified as classical (inoculative) biological control, augmentation (inundative) biological control, and conservation biological control. Classical (inoculative) biocontrol is often undertaken to counter invasive pests; in this method, an exotic species of natural enemies from the region where the insect pest originated and with high level of host specificity is imported and released in the invaded regions. A successful classical biological control results in extensive, continuous, and widespread control of the invasive species (*e.g.*, release of *Cotesia flavipes* for the control of Asian stemborer *Chilo partellus* in Africa). Prior to invasion in Africa, FAW has been prevalent in the Nearctic and Neotropical regions of America for several centuries, associated with several natural enemies. Some of these natural enemies could be potential candidates for classical biological control initiatives in Africa.

An augmentation (inundative) biological control approach involves periodic releases of natural enemies or pathogens, which are either introduced or endemic, to foster biological control or to induce epizootics of pathogens against either invasive or endemic pests. In contrast to the first two forms, conservation biological control involves the manipulation of environment, cropping systems, and practices in a way that favors the natural enemies against the pest. During the process of invasion, invasive species are likely to encounter natural enemies of other species closely related to it. Some of these natural enemies could adapt to the invasive pest, often referred to as “new associations.” It is important to understand that prior to the invasion of FAW, Africa has been home to several lepidopteran pests belonging to the genus *Spodoptera*. African armyworm (*Spodoptera exempta* [Walker]), beet armyworm (*Spodoptera exigua* [Hübner]), and African cotton leafworm (*Spodoptera littoralis* Boisduval) are among the most widely prevalent species with effective natural enemies and entomopathogens enhancing the probability of new associations to establish against FAW. The use of a biocontrol method to control a pest species does not normally affect the performance of other biological agents important in regulating pest populations, although in some cases there is intraguild predation.

The concept of biorational pesticides encompasses pest control products that are efficacious against target pests but are safe to natural enemies and broadly to the environment. Biorational pesticides often refers to products that are derived from natural sources such as botanicals, biopesticides, and others. For this chapter, we will restrict our information on biorationals to botanical pesticides and biopesticides. Integrated use of management options such as biological control and biorational pesticides along with other cultural and host plant resistance is likely to significantly reduce dependence on pesticides for management of pests. In this regard, the focus of this chapter will be to take stock of the diverse biological and biorational pest control options that are available in the native region of FAW and show potential for its management in Africa.

2. Biocontrol-based IPM Strategies for FAW

FAW is native to the Americas and a newly introduced pest species in Africa. As is common with invasive species, most of the naturally occurring biocontrol agents for this pest are not present, or native species have not yet adapted to this new host or prey. Implementation of any IPM strategy in Africa for FAW control should seek to avoid disrupting biocontrol processes that are operational for other pests and those that are adapting to FAW.

Conservation of the diversity and density of natural enemies should be a key focus in such a strategy. A simple way to achieve this is to provide, near the maize area, conditions conducive to survival of natural control agents. Planting crops that provide shelter, alternative food sources, and conditions for multiplication of beneficial species may be key to regulating the FAW population. At the edges of maize cultivation areas, rows of crops such as Mexican sunflower or Crotalaria might be suitable components in landscape management with the goal of increasing the biodiversity of beneficial insects, even those that are not yet associated with FAW. A "Push-Pull" strategy can also be used, in which pest-repellent plant species are intercropped with the main crop to repel ("push") pests out of the field, which is also surrounded by a border of a pest-attractive species to "pull" both the pest and beneficial insects into it (http://www.push-pull.net/; see also Chapter 6).

The second step in the implementation of a biocontrol-based IPM strategy against FAW is to assess the economic injury levels (EIL); strengthen monitoring, scouting, and surveillance efforts (see Chapter 2); and undertake pest management efforts through inundative release of natural enemies or through application of biorational pesticides, such as botanicals, or biopesticides, especially when the pest density exceeds EIL.

2.1. Advantages of Using Biological Control of FAW in Africa

The smallholder-based maize-production systems in Africa are diverse especially in terms of size, mixed cropping, seasonality, and other characteristics, unlike the large-scale commercial monocropping systems of the Americas. Further, levels of pesticide sprays on maize at present are much lower in Africa than in the other parts of the world. These are ideal conditions for effective conservation of natural enemies and achieving the full benefits of biological control (Herren and Neuenschwander 1991; Macharia *et al.* 2005; Soul-kifouly *et al.* 2016). Biological control, especially classical and conservation biological control, is much cheaper and benefits smallholder production systems in Africa. Further there are no cases of resistance development among FAW to biological control agents. With effective capacity-building initiatives, Africa can take advantage of the available manpower, such as farmers' associations, to mass-produce and release biological control agents for FAW management in Africa, as with the biological control of millet head miner in Niger and Senegal.

Hence, based on the global experience of managing maize pests, biocontrol will serve as a necessary pillar of the IPM strategy for control of FAW in Africa. However, to harness this potential, it is important to assess the diversity and effectiveness of biocontrol species on the continent to identify new associations. Further, taking stock of the diversity of FAW biological control agents in America, selection of appropriate candidate agents for classical biological control of FAW in Africa based on ecological suitability assessments needs to be undertaken. Effective biorational pesticides that can aid in the management of FAW and conservation of natural enemies need to be identified and promoted. Preliminary assessments of biocontrol species on the continent suggest we should optimize the role of biocontrol in helping to manage FAW (IPM Innovation Lab 2017; https://ipmil.oired.vt.edu/wp-content/uploads/2017/07/Muni-FAW-PPT-1.pdf).

2.2. Inundative Release of a Biological Control Agent against FAW

As mentioned above, *Trichogramma* or *Telenomus* wasps are the best examples of species used in inundative release to control FAW eggs. Unlike pesticide treatments, which must cover the entire plant (whorl or maize ear) to reach the target pest, egg parasitoids may be released at some point in the target area. Once released, the wasps, with extreme search capacity, fly to the plants seeking the pest's eggs. Hence, the releases are made at strategic points ranging from 20 to 40 per hectare (Cruz et al. 2016).

Considering the very short (less than 3 days) longevity of the released female and the fact that a new parasitoid generation occurs 10 days after release, it is necessary to make three releases spaced at 3-day intervals to provide a continual presence of adults in the area. New releases may be necessary if there is a significant increase in the movement of moths into the production area, as indicated by monitoring traps. The inundative release of *Trichogramma/Telenomus* wasps in maize fields does not reduce the populations of other beneficial species. Interspecific competition studies should be done before the introduction of new natural enemies. FAW feeds primarily on leaves but can also use the grain as a food source. Inside the ear, the larvae are protected, making it difficult to use conventional control measures such as pesticide sprays. The presence of FAW in the maize ear results from migration of larvae during tasseling as they are pushed out of the whorl and into and on the ears. Release of *Trichogramma/Telenomus* early in the season helps to suppress FAW migration into the ears.

Synchronization between the presence of FAW egg masses and release of parasitoids in maize is essential to the success of applied biocontrol. Monitoring the arrival of the moths in the target area using pheromone traps (see Chapter 2) is more effective than manually searching for egg masses. The first moth captures signal the arrival of the pest in the area and indicate that oviposition is close.

2.3. Importance of Other Beneficial Insects in the Natural Control of FAW

Considerable biodiversity of beneficial insects exists in maize fields in the Americas and the Caribbean (Molina-Ochoa *et al.* 2003; Cruz *et al.* 2009). The braconid wasp *Chelonus insularis* Cresson is one of the key natural biological control agents (Meagher *et al.* 2016). Like the egg parasitoids, *Chelonus* parasitizes the egg of FAW; however, the FAW eggs hatch into larvae and the parasitoid adult emerges from the FAW larva. Because *Chelonus* is a much larger insect than *Trichogramma/Telenomus* wasps, *Chelonus* is more competitive. The parasitized larvae gradually reduce their food intake, consuming less than 10% of the biomass consumed by a healthy larva (Rezende *et al.* 1994). Therefore, the presence of small larvae in the release area of *Trichogramma* does not necessarily mean a failure in biocontrol of FAW. Rezende *et al.* (1995a,b) provide further information on the role of *Chelonus* in IPM.

In addition to *C. insularis*, several other parasitoid species are also considered important in suppressing populations of FAW larvae (Figueiredo *et al.* 2009). For example, *Campoletis flavicincta* has been extensively used (Matrangolo *et al.* 2007; Matos Neto *et al.* 2004). So far in Africa, *Charops ater* Szépligeti (Ichneumonidae), *Chelonus curvimaculatus* Cameron, *C. maudae* Huddleston, *Coccygidium luteum* (Brullé) (Braconidae), and *Telenomus* spp. (Platygastridae) are egg and larval parasitoids found to be associated with *S. frugiperda* in East and West Africa (Mohamed *et al.* unpublished data; Goergen, unpublished data). Standardization of mass-rearing protocols of these parasitoids on *S. frugiperda* and assessment of their efficiency are ongoing. In addition to the benefits of parasitoids, the presence of insect predators of both eggs and larvae is important to keep the FAW population below the economic threshold level. For example, the predatory earwig *Doru luteipes* (Scudder) lays its eggs inside the maize whorl, the preferred location of FAW (Reis *et al.* 1988), and occurs throughout the maize crop cycle. Nymphs of *D. luteipes* consume 8–12 larvae daily, while in the adult stage they consume 10-21 larvae of *S. frugiperda* daily (Reis *et al.* 1988). Artificial diets for rearing of *D. luteipes* based on insect pupa flour and pollen were found to be equal to FAW eggs (Pasini *et al.* 2007). Several species of earwigs are also frequently observed in the whorl and ears of maize in Africa. Earwigs are frequently assessed as predators of stemborers and aphids in maize in Africa. Among them, *Diaperasticus erythrocephalus* (Olivier) is frequently observed. The predatory potential of these earwigs on FAW eggs and larvae needs to be assessed in detail. Laboratory and field studies with other identified beneficial insects associated with maize pests demonstrate the real possibility of having a sustainable management of maize pests based on biocontrol strategies.

In situations where the presence of biocontrol agents is not yet at the optimal level and where pesticide applications might be required, use of microorganisms such as Baculovirus or *Bacillus thuringiensis* should be considered (Valicente and Cruz 1991; Cruz 2000; Cruz *et al.* 2002; Figueiredo *et al.* 2009; see Section 3.2).

3. How to Recognize Natural Enemies of FAW

3.1. Insects

Table 1 describes the main group of natural enemies associated with FAW to help the farmer identify possible natural enemies of the pest in the different countries where the pest is already present.

Table 1. A summary of parasitoids and predators against FAW.

Scientific Name & Family	Description	Photograph
FAW Parasitoids		
Egg Parasitoids		
Trichogramma pretiosum (Riley) (Trichogrammatidae)[a]	• *Trichogramma* species are very small insects, with dimensions <1 mm. • *T. pretiosum* is used in the control of eggs of FAW and *Helicoverpa spp.*	 *Trichogramm*a parasitizing FAW eggs
Trichogrammatoidea armigera (Nagaraja) (Trichogrammatoidea)[b]	• Small insects (< 1 mm) with females bigger than males. • *T. armigera* is used in the control of eggs of *Helicoverpa armigera* and FAW. • *T. armigera* is being mass produced at ICRISAT-Niger Laboratory.	
Telenomus remus (Nixon) (Hymenoptera: Scelionidae)[c]	• Measures 0.5-0.6 mm in length and has a black, shiny body. • Presents high specificity for FAW. Each female parasitizes more than 250 eggs during its life span. • The total development period from egg placement to adult emergence is 10 days.	 *Telenomus remus* adult (left); females parasitizing FAW eggs (right).
Egg-Larval Parasitoids		
Chelonus insularis Cresson (Hymenoptera: Braconidae)[d]	• Measures about 20 mm in wingspan. • A very competitive parasitoid, usually predominant in maize fields. 91% of natural parasitism found in maize field samples was due to *C. insularis.* • Among the several biological control agents of FAW, it belongs to the most geographically dispersed, common in the USA and throughout South America. *C. insularis* has been found in South Africa and Egypt (CABI). • The parasitized FAW egg hatches, giving rise to a caterpillar, carrying within it the parasitoid. The larval period of the parasitoid has an average length of 20.4 days, close to that of a healthy caterpillar. However, the relationship of leaf consumption between healthy and parasitized caterpillars is 15:1, meaning less damage to the plant.	 Female of *Chelonus insularis* parasitizing FAW eggs (left); two FAW larvae of the same age. The smaller one has been parasitized by the wasp (right).

Scientific Name & Family	Description	Photograph
Larval Parasitoids		
Campoletis sonorensis (Cameron) (Hymenoptera: Ichneumonidae)[e]	• The insect wingspan is about 15 mm. Third-instar larvae are the most suitable stage for the parasitoid. The total cycle of the parasitoid is around 22.9 days. • The relation of consumption between a healthy caterpillar and a parasitized caterpillar is 14.4:1. Therefore, by parasitizing small-sized caterpillars, in addition to being efficient to cause death of the host insect, the parasitoid greatly reduces leaf consumption by caterpillars. • The skin of the dead FAW caterpillar lies next to the cocoon of the parasitoid, characteristic of this species.	 *Campoletis flavicincta* couple (upper left); female parasitizing FAW larva (upper right).  *C. flavicincta* pupa and remains of a parasitized FAW larva (bottom).
Cotesia icipe (Fernández-Triana & Fiaboe)[h]	• Known to parasitize several species of Spodoptera in Africa, including FAW. • Under laboratory conditions >50% parasitism has been observed on FAW.	 *Cotesia icipe*, seeking FAW larva (Source: Faris Samira Mohamed, ICIPE)
Habrobracon hebetor (Say) (Hymenoptera: Braconidae)[i]	• A small wasp, which has been used against pearl millet head miner, also attacks FAW larvae under laboratory conditions. • These parasitoids are reared in the laboratory at ICRISAT and INRA in Niger, and ISRA in Senegal on *Corcyra cephalonica* larvae, and released in pearl millet fields in Niger and Senegal. • In Africa, this parasitoid is found in Algeria, Burkina Faso, Egypt, Libya, Madagascar, Niger, Senegal, South Africa, Zimbabwe, and Mauritius (CABI).	 *Habrobracon hebetor* parasitizing on FAW larvae
Winthemia trinitatis (Thompson) (Diptera: Tachinidae)[i]	• The female places its eggs in the body of a fifth- and sixth-instar FAW near the head, making it impossible to be removed. The larvae of the parasitoid penetrate the body of the larva, delay pupation, and inflict up to 30% parasitism. • While acting on more developed instars that have already caused damage to the plant, these tachinids contribute to the reduction of future pest generations.	 Tachinid fly *Winthemia trinitatis* laying egg on FAW larva (left) and eggs on the host abdomen (right)

Scientific Name & Family	Description	Photograph
Larval-Pupal Parasitoids		
Archytas marmoratus (Townsend) (Diptera: Tachinidae)[k]	• A solitary larval-pupal parasitoid of several species of Noctuidae (Lepidoptera) including FAW. • Has a complex life cycle that allows it to parasitize a wide range of host larval instars. • The female does not lay the eggs directly on the host, but rather places several of them nearby. The eggs soon hatch and young larvae emerge. Parasitism occurs when these larvae meet a host and penetrate the body of the host. • Since the female of *A. marmoratus* lays several eggs at the same time at several places, the probability of superparasitism is very high. Often 75% of the parasitized larva are superparasitized. Survival of the parasitoid declines significantly if more than four parasitoid maggots are seen in a single host caterpillar. Hence, release rates of *A. marmoratus* need to be optimized to reduce superparasitism rates (Carpenter and Proshold 2000). • Mass-rearing protocols for *A. marmoratus* on corn earworm, Helicoverpa zea (Boddie) and Greater wax moth, Galleria melonella (L.) were standardized (Gross and Johnson 1985; Bratti 1993).	 *Archytas marmoratus*
Lespesia archippivora (Riley) (Diptera: Tachinidae)[l]	• A generalist parasitoid capable of parasitizing at least 25 species of Lepidoptera. • A female can oviposit between 15 and 204 eggs in her life span. • The female oviposits on the back end of the caterpillar. Three instars of *Lespesia archippivora* feed on the host caterpillar and upon maturity the parasitoid emerges out of the larva and pupates in the soil. Adult emerges from the pupa approximately 10–14 days from oviposition.	 *Lespesia archippivora* (Source: CBG Photography Group, Centre for Biodiversity Genomics)

Notes on Egg and Egg-Larval Parasitoids

- The egg parasitoids are considered the most important among the agents of biological control. These species prevent the pest from causing any damage to the host plant. In addition, these parasitoids have been easily reared on a large scale and are therefore commercially available from biofactories in several countries.
- The *Trichogramma* female oviposits inside the egg of its host. Within a few hours its larva emerges and feeds on the contents of the host's egg. The whole cycle of the parasitoid occurs inside the pest egg. Soon after emergence, the adult wasp immediately begins the process of searching for a new egg mass, which continues the multiplication of the species. The total life cycle of the parasitoid is about 14 days.
- 100,000 adult parasitoids per hectare, released at about 40 points, is the recommendation for maize. Field efficiency, rearing ability on a commercial scale, and competitive prices are the main reasons for using *Trichogramma* as the main biological control agent in an inundative release method.
- The presence of scales/hairs over the egg masses acts as a barrier against parasitism by *Trichogramma* spp. This difficulty can be overcome by using a more aggressive parasitoid that is capable of breaking the physical barrier. Therefore, it is essential to know the species/strains present in the agroecosystem when choosing the Trichogramma species to be used for applied biological control of FAW.
- There are 12 species of Telenomus and 27 species of *Trichogramma/Trichogrammatoidea* found in Africa, indicating the adaptability of these genera in the continent.
- A species of *Trichogramma/Trichogrammatoidea* has been collected from the eggs of FAW in Niger. Twenty-four government and private laboratories in Egypt are producing *Trichogramma* spp. and other natural enemies. The Economic Entomology Laboratory at Cairo University is producing *T. achaeae, T. euproctidis, Chrysoperla* sp., *Orius* sp., and coccinellids in large scale for research and distribution. The laboratory is capable of providing training on mass culture of these natural enemies. In 2016, the laboratory trained technicians from Mali and Niger on mass production of Trichogramma spp.
- The use of *Telenomus* in the control of FAW follows the same dynamics as Trichogramma but is used at a quantity of 60,000 insects per hectare. A species of *Telenomus* has been found parasitizing 60% of FAW eggs in Niger (ICRISAT).

Notes on Larval Parasitoids

- *Cotesia marginiventris* has been found in Central African Republic and Egypt (CABI). Incidentally, *C. marginiventris* has been introduced into Cape Verde prior to 1981 where it has established on *Helicoverpa armigera, Trichoplusia ni,* and *Chrysodeixis chalcites* (Lima and van Harten 1985; van Harten 1991).
- Several other species of *Ichneumonidae* larval parasitoids are common in samples taken from FAW larvae in different maize-producing regions in Brazil, indicating their potential for use in biological control programs. For example, *Eiphosoma laphygmae, Ophion flavidus,* and *Colpotrochia* mexicana cause a significant reduction in the food ingestion by the pest, thereby reducing the potential to cause economic losses. In addition to *O. flavidus, Aleiodes laphygmae* (Braconidae), *Meteorus* spp. (Braconidae), and *Euplectrus platyhypenae* (Eulophidae) also associate with FAW larvae (e.g., Meagher et al. 2016).

Notes on Some Pupal Parasitoids

Five species of *Ichneumonidae, Diapetimorpha introit, Cryptus albitarsis, Ichneumon promissorius, Ichneumon ambulatorius,* and *Vulgicheneumon brevicinctor;* two species of *Chalcididae, Brachymeria ovata* and B. robusta; and one eulophid species, *Trichospilus pupivora*, have also been reported on FAW pupae from the USA, Argentina, and Barbados.

Scientific Name & Family	Description	Photograph
FAW Predator Insects		
Coleomegilla maculata (De Geer) (Coleoptera: Coccinellidae) **Ladybird beetle[m]**	• Adults are 6 mm in length and generally red with six black spots on each elytra. • Females lay clusters of 10 to 20 yellow eggs on the plants. • Both adults and larvae feed on aphids, mites, eggs, and larvae of various insects such as FAW. • Pollen and fungal spores are also important components of this species' diet.	 *Coleomegilla maculata* (adults, eggs, larva and pupa)
Hippodamia convergens (Guérin-Méneville) (Coleoptera: Coccinellidae) **Ladybird beetle[n]**	• Adults are ~6 mm in length and have orange-colored elytra, typically with 6 small black spots on each. • The body section behind the head is black with white margins and two white lines converging. • The females lay clusters of 10-20 yellow-colored eggs on the plants. • The larva grows through four stages.	 *Hippodamia convergens*
Olla v-nigrum (Mulsant) (Coleoptera: Coccinellidae) **Ladybird beetle[o]**	• Adults are initially light in color, and over time, become darker. • Adults come in two different color patterns. The black-colored adult acquires a brilliant black color, while the spots of their elytra become orange. The yellow-straw colored adult shows a slight increase in its tonality and the spots located along its elytra become black. • An efficient predator, both in the larval and adult stages. • The average eggs per oviposition is around 21. The total egg to adult cycle lasts about 20 days.	 *Olla v-nigrum*
Cycloneda sanguinea (L.) (Coleoptera: Coccinellidae) **Ladybird beetle[p]**	• A red insect with no spots on the elytra of adults but two black spots on the clear area of the head. • The female lays her eggs in the host plant, in groups, each containing about 20 yellowish eggs. • The insect passes through four nymphal stages. The larval period lasts for ~8 days. The larvae to adult cycle is ~15 days. • Both the larva and the adult are predators.	 *Cycloneda sanguinea*

Scientific Name & Family	Description	Photograph
Doru luteipes Scudder (Dermaptera: Forficulidae) **Earwigs**[q]	• One of the most important natural enemies of FAW. Bioecological studies with the predator, feeding on FAW larvae, showed that the number of eggs per oviposition is 25-30, with an incubation period of around 1 week. • The nymph stage comprises four instars, ranging from 37 to 50 days. • Adults with sieves at the extremity of the abdomen can live up to 1 year.	 *Doru luteipes*
Euborellia annulipes (Lucas) (Dermaptera: Carcinophoridae) **Earwigs**[r]	• In summer, the incubation period is 7 days. Time from egg until adult emergence is ~60 days. • The newly deposited eggs are oval, of yellowish cream color, 0.95 mm in length and 0.75 mm in diameter. The newly hatching nymphs have white coloration, black eyes, and black or brown abdomen. When they become adults, the initial coloration is white, and then turn dark color. • The insects do not have wings.	 *Euborellia annulipes*
Zelus longipes (L.) *Zelus leucogrammus* (Perty) *Zelus armillatus* (Lepeletier & Serville) (Hemiptera: Reduviidae) **Assassin bug**[s]	• The genus *Zelus* is one of the most common killer bugs in maize. • Average adult length is 1.3-1.9 cm. • Brown or blackish in color, and commonly found in maize fields. • Usually have a long, narrow head with a distinct neck behind the eyes, which are often reddish. • The females lay the eggs in groups on the leaves of the plants or even on the ground. The nymphs resemble an adult but without a wing.	 *Zelus* spp.: adults and egg mass (right, above)
Geocoris punctipes (Say) Hemiptera: Lygaeidae **Big-eyed bugs**[t] *Orius insidiosus Say* (Hemiptera: Anthocoridae) **Flower bug**[u] *Nabis rugosus* (L.) (Hemiptera: Nabidae) **Pirate bug**[v]	• Small insects (approximately 4 mm in length) occurring in many parts of the world. • Generally considered beneficial because they attack various pests including insects and mites in ornamental and agricultural crops. • Very common predator of *Lepidoptera* species. • Predator of small arthropods, such as thrips, mites, whiteflies, aphids, and lepidopteran eggs. • Highly abundant species with the high potential for use in biological control programs. • Predators of aphids, moth eggs and small Lepidoptera larvae	 Big-eyed bug *Geocoris punctipes* (Lygaeidae) (upper left); small pirate bug *Orius insidiosus* (Anthocoridae) (lower left); *Nabis* sp. (Nabidae) (right)

Scientific Name & Family	Description	Photograph
Podisus maculiventris (Say) (Hemiptera: Pentatomidae) **Spined soldier bug**[w]	• *Podisus* spp. are found in different ecosystems, with nymphs and adults feeding mainly on *lepidopteran* larvae. • Pricks its prey and injects a toxin that paralyzes it in relatively short time. The prey is killed as its internal fluids are sucked out by the predator.	 *Podisus* spp. (egg mass, nymphs, and adult feeding on FAW larva)
Calosoma granulatum (Perty) (Coleoptera: Carabidae) **Ground beetles**[x]	• A greenish, iridescent beetle (25-30 mm in length). • Mated female lays the eggs on the surface of the soil or slightly below. The immature stage passes through three instars, before pupation in the ground. • Eggs are light yellow. The larval stage is ~12 days. Adult longevity is ~83 days.	 Eggs and pupa (left); adult and larva of *Calosoma* sp. feeding on FAW larva (right)

Note: References provided here are representative for each species.
[a]Buttler and Lopez (1980); [b]Manjunath (1972); [c]Pomari *et al.* (2013); [d]Rezende *et al.* (1995a), Figueiredo *et al.* (2006a); [e]Isenhour (1985); [f]Figueiredo *et al.* (2006b); [g]Jalali *et al.* (1990); [h]Fiaboe et al. (2017); [i]Landge *et al.* (2009); [j]Silva *et al.* (2010); [k]Gross and Johnson (1985); [l]Etchegaray and Nishida (1975); [m]Lundgren *et al.* (2004); [n]Cardoso and Lázzari (2003); [o]Chazeau *et al.* (1991); [p]Cardoso and Lázzari (2003); [q]Choate (2001); [r]Bharadwaj (1966); [s]Cogni *et al.* (2000); [t]Champlain and Sholdt (1967); [u]Coll and Ridgeway (1995); [v]Tamaki and Weeks (1972); [w]Mukerji and LeRoux (1965); [x]Pasini (1995)

3.1.1. Host Suitability Studies

Suitability is defined as the ability of a host to successfully support parasitoid development from egg to adult.

3.1.1.1. Egg parasitoid host suitability

i. Expose 100 eggs to a naïve mated parasitoid female in a glass vial.

ii. After 6 hours of exposure, remove the female parasitoid from the vial and incubate the eggs at 25±1°C and 70±5% RH.

iii. Record data on the following parameters: percentage parasitism, percentage emergence, F1 sex ratio (percentage of females), and developmental time.

3.1.1.2. Host acceptability and suitability for larval parasitoid

i. Select L2-L5 instars for these experiments. Conduct the experiments in the laboratory at 25±1°C, 50–70 % RH, and 12h:12h (L: D) photoperiod.

ii. Use the hand stinging technique, *i.e.*, offer larvae held in a soft forceps to the female parasitoid in a sleeve cage. A host is defined as having been accepted when the parasitoid inserts its ovipositor. Verify oviposition (*i.e.*, egg deposition) later via dissection of a subsample of larvae.

iii. Put the probed larvae individually into glass vials containing artificial diet.

iv. Monitor the fate of both host larvae and parasitoids daily. Only larvae that produce cocoons are considered as suitable. Record the number of hosts that die, produce cocoons that pupate, or form adult moths. In addition, compute parasitoid emergence, total progeny per host larvae, sex ratio (proportion of female progeny), and parasitoid mortality. When neither eggs nor dead parasitoids are found, the larvae are regarded as unparasitized.

3.1.1.3. Host suitability study of pupal parasitoids

i. Expose one- to five-day-old pupae of FAW to a naïve female pupal parasitoid by releasing a mated female into the vial. Stopper the vial with cotton wool to prevent the parasitoid from escaping.
ii. Remove the parasitoid and the pupae after 6 hours. Place all FAW pupae that were exposed to female parasitoids into individual vials to assess host suitability. Stopper the vials with cotton wool and maintain at 25±1°C, 50-70% RH, and 12h:12h (L: D) photoperiod.
iii. Check pupae daily for moth emergence, parasitoid emergence, or pupal mortality. Record the developmental time and sex of adult parasitoids.

3.1.1.4. Host suitability study of FAW predators

i. Place potential predators individually into a petri dish containing moist cotton wool and starve them for 24 hours prior to the experiment.
ii. Offer potential predators, enclosed in a petri dish, either one batch of FAW eggs, 20 first-instar larvae, 10 late-instar larvae (10- to 14-day-old larvae) or five pupae under laboratory conditions (25±1°C and 70±5% RH).
iii. Record prey acceptance and consumption capacity after 24 hours.

3.2. Entomopathogens

3.2.1. Viruses

Among the microbial control agents, virus-based insecticides, which are mostly in the Baculovirus group, have been identified as having the highest potential for development as bioinsecticides due to specificity, high host virulence, and the highest safety to vertebrates (Moscardi 1999; Barrera *et al.* 2011). Two types of Baculovirus have been studied for the control of *S. frugiperda*, namely granulovirus (SfGV) (Betabaculovirus) and multiple nucleopolyhedrovirus (SfMNPV) (Alphabaculovirus). However, SfMNPV has greater potential for use in the management of FAW (Behle and Popham 2012; Gómez *et al.* 2013; Haase *et al.* 2015). SfMNPV is specific to only FAW larvae. Under natural conditions, the pest is infected orally by ingesting the contaminated food (maize leaf). Once ingested, the polyhedral inclusion bodies (PIB) dissolve in the alkaline midgut, releasing the infective virions. These virions infect the midgut epithelium cells and multiply in the nucleus. Further, the virus spreads to the body cavity and infects other tissues such adipose tissue, epidermal, tracheal matrix and even salivary glands, Malpighian tube, and blood cells, causing its death from 6 to 8 days after ingestion. A caterpillar infected with the nucleopolyhedrovirus eats only 7% of the food normally eaten by a healthy caterpillar (Valicente 1988). The symptoms of Baculovirus infection include appearance of blemishes, yellowing of the skin, and decline in feeding. An infected larva moves to the higher parts of the plant and upon death hangs head down, with some prolegs still attached to the plant. The dead larvae are soft, dark in color, and disintegrate easily to release the body fluids rich in polyhedrons which aids in further spread of the virus (Figure 1).

Age of FAW larva at infection, amount of virus ingested, virulence of the virus, and prevailing climatic conditions, especially temperature, humidity, and solar radiation, are key factors that influence the efficacy of the virus and speed of kill. Therefore, these factors have marked effects on the virus action when it is applied in the field. In addition, other factors such as type of spray equipment, formulation used, and time of spray also influence the efficacy of the virus (Hamm and Shapiro 1992; Cisneros *et al.* 2002).

Better efficiency of Baculovirus for the control of FAW is obtained when applied on maize plants at the 6- to 8-leaf stage or 8- to 10-leaf stage with a costal-manual sprayer, using a wettable powder formulation containing the recommended dose of the product (2.5×10^{11} PIB / ha) on newly hatched larvae, applied at one time or at intervals of one week. An evaluation carried out

seven days after virus application indicated a minimum larval mortality from 79.2 to 97.2%. In a second evaluation, carried out three days after the second virus application, mortality varied from 86.6 to 100%. Viral efficiency did not vary between the two stages of plant growth. A commercial formulation for FAW NPV, SPOBIOL (prepared by CORPOICA, the Colombian public-private ag research partnership) is available and has been licensed from Certis LLC, a U.S. company (see Section 7 for a method of small-scale production).

It should be considered also that, as the caterpillar develops, it becomes more resistant to virus. Therefore, the newer the larvae, the higher the efficiency of the virus. Hence, it is recommended to apply Baculovirus to larvae of a maximum of 1.5-cm long. Spraying is performed with the same equipment used for the application of a conventional chemical. Particularly for FAW, it is recommended to use a fan nozzle (8004 or 6504). The more uniform the planting, the more efficient the application with backpack or motorized sprayers. Appropriate nozzles to facilitate uniform application with the type or sprayer used need to be considered. Improved formulations of SfMNPV with maize flour and 1% boric acid (Cisneros *et al.* 2002) and microencapsulation (Gómez *et al.* 2013) are effective for the control of FAW.

Despite various developments in terms of *in vitro* multiplication of baculoviruses, large-scale production of baculoviruses as a commercial biopesticide has been based on *in vivo* multiplication in the host insects due to the significantly low cost involved and less technology-intensive nature of production. Factors such as the ability to maintain a diseased colony of the host insect, age of the caterpillar when exposed to the pathogen, temperature at which the infected colony is maintained, concentration of virus inoculum used, nutritional profile of the larval diet, and mechanization/availability of labor are some of the critical factors that govern the efficiency of Baculovirus production (Moscardi 1999; Subramanian *et al.* 2006; Moscardi *et al.* 2011; Paiva 2013). The cannibalistic nature of FAW further adds to the complexity of SfMNPV production. Inoculation of 8-day-old larva with 1×10^{7} PIB/ml and maintained at 25°C has been reported to be optimal to maximize the yield of SfMNPV. The cost of the biopesticide product produced is largely dependent on the cost of maintaining a disease-free colony. Use of natural diets such as castor leaves for rearing SfMNPV can greatly reduce the cost of production; however, such a system is largely prone to contamination due to extraneous virus/microsporidians. *In situ* field-level production using infection of field-collected larva has been developed for *Spodoptera exempta* nucleopolyhedrovirus (*Spex*NPV) in Tanzania, Africa. Early outbreaks of the African armyworm are sprayed with potent *Spex*NPV. Diseased insects are harvested, formulated using a kaolin formulation, and used for treatment of subsequent outbreaks (Mushobozi *et al.* 2006).

3.2.2. Entomopathogenic Fungi

Entomopathogenic fungi (EPF) have a broad spectrum of action with the ability to infect several species of insects and different stages, causing epizootics under natural conditions (Alves *et al.* 2008). The fungus spores infect through the integument, multiply in various tissues within the insect body, and kill the insect due to destruction of tissues and by production of toxins. Induction of epizootics depends on climatic factors such as wind, rain, or frequency of contact among the insects. Diseased insects stop feeding, become discolored (cream, green, reddish, or brown), and ultimately die as a hard-calcareous cadaver from which the fungus sporulates. Moisture is essential to the success of fungi as a biological control agent. *Beauveria bassiana, Metarhizium anisopliae*, and *Nomuraea rileyi* are the common fungi with potential uses against insect pests.

Beauveria bassiana has been used in the control of *Spodoptera* (*e.g.*, Fargues and Maniania 1992). Compared to other lepidopteran pests, FAW larvae seem to be least susceptible to *Beauveria bassiana* (Wraight *et al.* 2010). Several fungal isolates belonging to three different genera (*Metarhizium, Beauveria,* and *Isaria*) have been screened for efficacy against second-instar larvae of S. frugiperda at ICIPE, but only one isolate of *B. bassiana* was able to cause moderate mortality of 30% (Akutse et al. unpublished data). Current efforts are underway to screen EPF isolates for efficacy against other life stages of FAW such as adults and eggs.

3.2.3. Bacteria

Among the various biopesticides used for insect control, *Bacillus thuringiensis* (*Bt*) Berliner biopesticides are the most widely used. These are ubiquitous, soil-dwelling, gram-positive bacteria that produce crystal proteins named delta-endotoxins, which are insecticidal. These endotoxins have relative levels of specificity to specific groups of insects. Although there are several commercial *Bt* products available in the market for management of lepidopteran pests, only a few are effective in controlling FAW. Among the various strains of *Bt*, FAW is more susceptible to *Bt aizawai* and *Bt thuringiensis* (Polanczyk *et al.* 2000), and not to *Bt kurstaki*, which is effective against many other lepidopteran pests (Silva *et al.* 2004). Further aspects such as the susceptibility of the endotoxin to UV, inability to reach the pest and induce consumption of the toxins, and high cost of production limit their wide adoption and use. Efforts to screen for effective *Bt* strains against FAW has been ongoing by several research groups. Variations among populations of FAW in their susceptibility to different Cry toxins have also been observed (Monnerat *et al.* 2006), which needs to be considered during the choice of *Bt*-based biopesticides for FAW management in different regions. With the objective of development of Bt-based biopesticides from Africa, 19 Bt strains have been screened against second-instar larvae of FAW at ICIPE. Seven *Bt* strains were recorded highly effective, causing 100% mortality 7 days post-treatment, with lethal time mortality (LT_{50}) values ranging between 2.33±0.33 and 6.50±0.76 days (Akutse *et al.*, unpublished data). Further biological and molecular characterization of these isolates are currently ongoing. Mass production of *Bt*-based biopesticides has been undertaken using fermentation technology, either as liquid or semi-solid or solid-state fermentation (Fontana Capalbo *et al.* 2001). Apart from the Cry toxins, FAW is also susceptible to some of the vegetative insecticidal proteins found in the *Bt* culture supernatants (Barreto *et al.* 1999). Commercial *Bt* biopesticides based on strain *Bt aizawai* are registered and available to a limited extent in Africa. Efficacy of these biopesticides against FAW in Africa needs to be assessed.

3.2.4. Entomopathogenic Nematodes

One of the less explored but promising strategies in biological control is the use of entomopathogenic nematodes (EPNs), especially *Heterorhabditis bacteriophora*, *Heterorhabditis indica*, and *Steinernema carpocapsae.* These have proved to be human- and eco-friendly alternatives to chemical pesticides in controlling many soil-dwelling insect pests including armyworms. It is reported that FAW is very susceptible to these beneficial nematodes at the rate of 23,000 nematodes per sq. ft., to target both young and mature larvae. Beneficial nematodes need to be applied early in the morning or late at night when armyworm larvae are very active and can be easily found by the nematodes. Another advantage of applying nematodes during these timings is the low exposure of the nematodes to UV as they can die instantly if exposed to UV light (Shapiro-Ilan *et al.* 2006).

Similarly, Garcia *et al.* (2008) reported that 280 infective juveniles of *Steinernema* sp. were required to kill 100% of third-instar FAW in petri dishes, as compared to 400 infective juveniles of the *H. indica* nematode to obtain 75% FAW control. It is possible to spray EPNs without significant loss in their concentration and viability, with equipment that produces electrical charges to the spraying mix, and with those using hydraulic and rotary nozzle tips. The concentrations of infective juveniles of *H. indica* and *Steinernema* sp. nematodes were reduced by 28% and 53%, respectively, when hydraulic spraying nozzles that require 100-mesh filtrating elements were used. Furthermore, Molina-Ochoa *et al.* (1999) reported earlier that *Steinernema carpocapsae* and *S. riobravis* are very effective in controlling FAW prepupae. The authors demonstrated that the combination of EPNs and resistant maize silks could enhance the mortality of FAW prepupae and could be used for integrated management of this pest. Negrisoli *et al.* (2010a) reported that several commercial insecticides were compatible with the three species of EPNs including *Heterorhabditis indica*, *Steinernema carpocapsae*, and *Steinernema glaseri* under laboratory conditions. It was also reported that the efficacy of *H. indica* was enhanced against FAW when mixed with an insecticide, Lufenuron (Negrisoli *et al.* 2010b). However, it is critical to study and evaluate the compatibility of insecticides, including biopesticides and EPNs, before recommending their use in an IPM program for FAW.

According to Kaya *et al.* (2006) the African continent provides great potential for occurrence and exploration of EPNs, but only a few countries have been surveyed so far. Extensive work on nematodes has been done on survival, infectivity, and virulence in Egypt and these studies have shown promising results for development and incorporation of EPNs into IPM programs in some cropping systems in Egypt. While some studies reported successful pest management using EPNs, limited field success was achieved against the lepidopteran sugarcane stalk borer, *Eldana saccharina*, in South Africa. The failure was attributed to the cryptic nature of the larvae and frass/sap in the infested sites of the infested stems. Although commercial applications have not yet been reported from Africa, there is a need to delve into active research on EPNs and explore the potential and fitness of EPNs for biological control plans and IPM programs.

3.3. Botanical Pesticides

Plant-derived pesticides are commonly referred as botanical pesticides. A large diversity of plants are known to have insecticidal properties and some of them have been used for the management of FAW in America (Table 2). The botanical pesticides are biodegradable, environmentally safe, less harmful to farmers and consumers, and often safe to natural enemies and hence amenable for use in biocontrol-based IPM strategies. Further, based on the availability of the pesticidal plants in the ecosystem, botanical pesticides could be easily prepared by smallholder farmers.

Table 2. Potential botanical pesticides against FAW, based on studies in America.

Species	Family	Extract	Mode of action	Reference
Neem: Azadirachta indica	Meliaceae	0.25% Neem oil	Larvicidal with up to 80% mortality in the lab	Tavares *et al.* (2010)
Aglaia cordata Hiern	Meliaceae	Hexane and ethanol extracts of seeds	Larvicidal with up to 100% mortality in the lab	Mikolajczak *et al.* (1989)
Annona mucosa Jacquin	Annonaceae	Ethanolic extract from seeds	Larval growth inhibition	Ansante *et al.* (2015)
Vernonia holosenicea, Lychnophora ramosissima, and Chromolaena chaseae	Asteraceae	Ethanol extracts from leaves	Ovicidal	Tavares *et al.* (2009)
Cedrela salvadorensis and Cedrela dugessi	Meliaceae	Dichloromethane extracts of wood	Insect growth regulating (IGR) and larvicidal with up to 95% mortality	Céspedes *et al.* (2000)
Myrtillocactus geometrizans	Cactaceae	Methanol extracts of roots and other aerial parts	Insect growth regulating (IGR), larvicidal, delayed pupation	Céspedes *et al.* (2005)
Long pepper, *Piper hispidinervum*	Piperaceae	Essential oil from seeds	Affects spermatogenesis and hence egg laying	Alves *et al.* (2014)
Melia azedarach	Meliaceae	Ethanolic extracts of leaves	Antifeedent to larva; synergistic with pesticide	Bullangpoti *et al.* (2012)
Jatropha gossypifolia	Euphorbiaceae	Ethanolic extracts of leaves	Antifeedent to larva; synergistic with pesticide	Bullangpoti *et al.* (2012)
Ricinus communis	Euphorbiaceae	Castor oil and Ricinine (seed extracts)	Growth inhibition and larvicidal	Ramos-López *et al.* (2010)

The use of botanicals in pest management is a cultural practice of African smallholder farmers, which could be an arsenal in FAW management. Several plant extracts have insecticidal properties against stemborers infesting cereals in Africa. These include Neem (*Azadirachta indica*), Persian lilac (*Melia azedarach*), Pyrethrum (*Tanacetum cinerariifolium*), Acacia (*Acacia* sp.), Fish-poison bean (*Tephrosia vogelii*), Wild marigold (*Tagetes minuta*), Wild sage (*Lantana camara*), West African pepper (*Piper guineense*), Jatropha (*Jatropha curcas*), Chillies (*Capsicum* sp.), Onion (*Allium sativum, Allium cepa*), Lemon grass (*Cymbopogon citratus*), Tobacco (*Nicotiana* sp.), Chrysanthemum (*Chrysanthemum* sp.), and Wild sunflower (*Tithonia diversifolia*) (Ogendo *et al.* 2013; Mugisha-Kamatenesi *et al.* 2008; Stevenson *et al.* 2017). The efficacy of these botanicals against FAW needs to be quickly assessed

and effective botanicals disseminated among maize growers in Africa. Preliminary evidence indicates the insecticidal property of seeds and leaf extracts of Neem, Melia, and Pyrethrum in Africa to FAW, which needs to be further explored.

4. Protocol for Monitoring Biological Control Agents of FAW

Considering that biological control is an important strategy in the management of FAW, and that FAW has likely been in at least some African countries for some time, it is likely that endemic biocontrol species, primarily parasitoids, have already started using FAW as a host. Accordingly, a protocol for the identification of biological control agents of FAW in maize follows below.

4.1 Parasitoids

- This protocol should be carried out preferably for at least three consecutive years, covering municipalities of different regions in each country.
- In each municipality, randomly select three rural properties.
- At each location, identify a maize area of at least one hectare. Choose five sampling points at random and at each point, mark 100 plants and count the number of FAW-damaged plants.
- For each plant, collect all FAW larvae and egg masses, noting the date and place of collection.
- To find the larvae, it is often necessary to open the still-rolled leaves of the plant because that is the insect's preferred feeding location. When the plant is in the reproductive stage, the larva will be found feeding inside the ear.
- In the laboratory, place each egg mass individually in a closed container to prevent the escape of larvae after hatching.
- Use maize leaves washed and dried (in the shade) as a food source for rearing the FAW larvae.
- Change the food in the case of the wilted leaves or when totally consumed.
- If possible, use an artificial FAW diet to rear larvae.
- Daily, observe the presence of newly hatched larvae, considering the incubation period of three to four days; at the end of this period, if there is no egg hatch and eggs are blackened, isolate the remainder of the eggs, and wait for the possible emergence of egg parasitoids.
- Keep neonate larvae in the laboratory for a minimum of 10 days to observe if egg-larval parasitoids are present.
- Keep collected larvae isolated individually to prevent cannibalism.
- Data should include date of collection, location, an estimation of the FAW larval instar at the date of collection. With these data, it is possible to determine the approximate date that parasitism began.
- Monitor the development of the larvae in the laboratory until FAW pupation or until the emergence of parasitoids.
- If the parasitoid species cannot be identified, send it to a specialist for identification (*e.g.*, ICIPE in east and southern Africa; IITA in west and central Africa).

4.2 Predators

Predator insects are generalists and in maize areas they can feed on FAW eggs and or larvae as well as other pest species. Therefore, it will also be important to sample for the presence of predators in the same points of the parasitoids.

Predator sampling can be performed in three ways: (a) Bag the maize whorl with a plastic or mesh bag and immediately remove the leaves for further insect counts and identification; (b) perform sampling with a sweep net; (c) use direct visual observation.

4.3. Entomopathogens

In general, when a FAW larva is infected by a pathogen the larva will change color, increasing in paleness and decreasing movement, especially when touched. However, the best way to identify a diseased larva is when it is already dead. Particularly for FAW larvae infected with Baculovirus (see Section 3.2.1), the dead larvae will generally be observed in the upper parts of the maize plant and will hang upside down. Dead larvae covered with a powdery white or greenish mass suggest fungal infection. Regardless of the symptoms, any larva displaying abnormal behavior should be taken to the laboratory and kept at a low temperature (refrigerator) until the cause of the symptoms is determined.

5. Procedures for Rearing Natural Enemies of FAW in the Laboratory

5.1. Production and Use of Egg Parasitoid *Trichogramma*

Embrapa has an efficient rearing technique for *Trichogramma* that is being passed on to farmers (Cruz *et al.* 2013; Almeida and Cruz 2013; Almeida *et al.* 2013). Artificial rearing of *Trichogramma* has progressed over the last 20 years through the discovery of alternative hosts that support parasitoid development in a manner like that of the preferred host. The use of these alternative hosts is advantageous due to the low cost of rearing, ease of procedures, and high reproduction capacity. Among the insects most used as alternative hosts are stored grain pests or stored flour pests such as *Corcyra cephalonica* (Stainton), *Sitotroga cerealella* Oliver, and *Anagasta kuehniella* (Zeller). This latter species has been the most frequently employed in the production of eggs as alternative hosts for *Trichogramma*, although *Corcyra* is deployed in Niger and Senegal. Known as the flour moth, *A. kuehniella* is a small moth, dark gray in color, with a life cycle lasting around 40 days. One gram of insect eggs is equivalent to 36,000 eggs.

The larval period varies according to temperature, being, on average, 29 days at 27.9°C and 73% RH. The number of larvae per growing container can also affect the duration of development of the flour moth. An increase in the number of larvae leads to a decrease in adult size, increase in cycle length, and mortality.

Pupae exhibit a development period of 8 to 16 days at summer temperatures, which can be lengthy if conditions are adverse. At 30°C and 73% RH, the pupal period is 8 days. Adults have a relatively short life cycle. At 30°C and 73% RH, copulating couples have a much shorter cycle (6 days for females and 7 days for males) than those that do not mate (11 and 10 days, respectively, for females and males).

The laying capacity reaches an average of up to 350 eggs, with 80-90% of eggs produced between the 3rd and 4th days of laying. Usually, eggs are placed shortly after mating and oviposition usually completes two to five days after emergence.

A temperature of 27°C is considered the best for fertility. Females can initiate oviposition 24 to 48 hours after emergence. A 24-hour photoperiod can cause reduction of fecundity, and the viability of eggs from couples where the males were kept under these conditions is less than that of couples where the males were reared in 24-hour darkness. The development from egg to adult, at 28-30°C and 73% RH, lasts about 41 days.

5.1.1. Rearing Procedure for *Anagasta*

Flour-moth larvae are grown on a diet of maize or wheat bran, alone or in equal mixtures, enriched with beer yeast (3%), distributed in 5-L plastic trays, following the procedures below.

5.1.1.1. Container preparation

i. Use plastic trays (10 cm high × 20 cm wide × 30 cm long) with Snap-On caps.

ii. To provide ventilation inside the tray, make a cut (9 cm wide × 19 cm length) on the top of the cover.

iii. To prevent penetration of natural enemies, replace the removed part with fine-woven fabric (organza), fixed with adhesive tape both inside and outside.

5.1.1.2 Diet preparation and set-up of larval rearing vessel

Neither maize nor wheat used in this procedure can be treated with any type of pesticide; therefore, it is essential to observe the provenance of the cereal acquired.

i. Finely grind the grain.

ii. Depending on the milling grit size, sift the material using a 1.5-mm mesh sieve.

iii. After sieving, store the flour of each cereal in an airtight environment to avoid infestations by insects; freezer storage is preferential. Mix the flour with the brewer's yeast in advance, if desired, or immediately prior to use.

iv. Place the food (500 g of maize bran, 500 g of wheat bran, 30 g of beer yeast) evenly inside the plastic tray with a slight compaction to level the diet.

v. On the surface of the diet, spread about 0.20 g of *Anagasta* eggs (about 7,200 eggs), then place the lid and seal it with adhesive tape to prevent the entry of parasitoids.

vi. Keep the trays on shelves in an air-conditioned room (25°C) to allow for good ventilation inside.

5.1.1.3. Construction of oviposition cage

i. Construct the cage using PVC pipe 300 mm in diameter and 25 cm high.

ii. To seal the ends of the cage, use PVC rings (2 cm high) and 0.5-mm nylon mesh.

iii. Glue the mesh to the rings with "araldite" glue.

iv. Use a plastic dish (of the type used under potted plants) as an egg collector.

5.1.1.4. Collection of Anagasta adults

i. After observing the emergence of the first adults of the *Anagasta* (about 40 days), collect them daily by means of a vacuum cleaner. The collection period extends over a period of 15 to 20 days. Adult moth collecting is usually performed in the morning, due to the lower mobility of insects.

ii. Collect insects from about ten trays and transfer them to a plastic bag (20-L capacity). After removing insects from 40 trays, transfer them to the oviposition cage.

5.1.1.5. Obtaining flour-moth eggs

i. After obtaining desired number of adults (about 10,000-12,000 insects), attach the rings to the PVC pipe with crepe tape.

ii. Place the base of the cage within the plastic dish in which the eggs will be collected.

iii. Do not provide any type of food. Maintain adult moths at a temperature of about 25°C and humidity of at least 70%. Adults remain in the cage for 5 days, on average.

5.1.1.6. Egg collection

i. Usually, begin egg collection the day after assembly of the oviposition cage. A lot of eggs will fall directly into the dish. Others will stick to the screen. Therefore, pass a brush over the outside of the screen covering both the top and bottom ring and then knock on the cage to complete removal of the eggs.

ii. Pass the eggs through a 0.50-mm sieve to remove residues such as flour remnants or insect scales. Clean the eggs again with the aid of a thin brush and a cotton pad passed lightly on the eggs.

iii. Measure daily productivity by weighing the eggs, considering an average of 36,000 eggs per gram.

iv. Use the vast majority of eggs to produce the parasitoid and the rest for flour-moth maintenance. Place the eggs inside a plastic tube, without moisture, to prevent them from sticking to each other.

5.1.1.7. Quality control of eggs produced

i. Before assembling the trays for multiplication of the flour moth, evaluate the viability of the eggs. To do this, individualize the eggs, with the aid of a brush, into the holes of a plastic plate (*e.g.*, a 96-well ELISA plate) and then seal the plate with plastic tape.

ii. After six days, on average, count the number of *Anagasta* larvae and determine the viability of the eggs, considering as normal a viability above 75%.

5.1.2. *Trichogramma* Production

There are several systems for the production of *Trichogramma* with *Anagasta* eggs, but they usually follow a basic technique. Initially, moth eggs are placed on rectangular paperboard cards, maintaining an egg-free edge of 1.5 to 2.0 cm along its shorter length. The cards are then placed in plastic or glass containers. For parasitism, a ratio of parasitized to non-parasitized eggs of about 1:15, with a 48-hour exposure period, may be used.

5.1.2.1. Card preparation

i. Cut white-colored cardboard to size 10 × 15 cm.

ii. With the exception of a 2-cm space at one end, cover the entire area with gum arabic glue. First dilute the glue in water (20% glue and 80% water), then spread it evenly over the card with the aid of a sponge.

iii. Immediately distribute eggs uniformly on the glue, avoiding the formation of layers because it impairs parasitism. To facilitate distribution, place the eggs inside a small tube covered by a mesh fabric fine enough to pass only one egg at a time. In addition, place the card at a 45° angle. Record the date of egg distribution.

iv. For better preservation, store the cards in a refrigerator (up to a week) and, if possible, inside Styrofoam boxes. Approximately 25,000 eggs are distributed on each card.

5.1.2.2. Rearing of parasitoids

i. Once the cards with the flour-moth eggs have dried, introduce three to five cards into a 1.6-L plastic or glass container. Inside these containers should already be a card that is totally parasitized and shows the emergence of the first adults.

ii. As food for the *Trichogramma*, use drops of honey free of pesticide residues (eight small droplets, as large droplets can trap miniature wasps) scattered on one wall of the container.

iii. Two days after the first distribution, additional egg cards could be added to the containers.

iv. Seal the containers with PVC film and keep them on shelves. About three to four days after card placement, the parasitized eggs become dark, providing a qualitative evaluation of the rate of parasitism. At that time, remove the cards from the containers and place them, by date of distribution, in other identical containers without adult parasitoids. Usually the rate of parasitism is above 90%. If for some reason the parasitism rate is smaller, eliminate the hatched larvae.

5.1.3. Parasitism Quality Control

For control of parasitism quality, take three 100-egg samples from a card and record the number of parasitized eggs, the wasp emergence percentage, and the sex ratio (number of females divided by the total number of insects emerged). This is important both for the continuity of breeding and for release in the field. Because there is the possibility of having more than one wasp in each parasitized egg, count the number of exit holes in the *Anagasta* eggs to determine viability.

5.1.4. Care in Rearing

To avoid interruption in the insect flow of both the host and the parasitoid, maintain strict control of the asepsis conditions at the breeding sites. After the adult moths have been collected from the flour, place the trays to be discarded in a freezer to avoid contamination in the breeding environment.

During *Anagasta* breeding, care must be taken to avoid the presence of a larval parasitoid (*Habrobracon*), which generally reaches high populations when the trays are not well protected. If these parasitoids occur, discard contaminated trays immediately by placing the material in a freezer for at least 24 hours to kill the contaminating parasitoid.

When hygiene conditions are not adequate and the moth collection exceeds 20 days, a predatory mite can be found preying on *Anagasta* eggs, and, consequently, compromising the parasitoid production. The same procedure used to control *Habrobracon* can be followed for the mite.

5.1.5. Field Release

5.1.5.1. Factors affecting efficiency

The factors that affect the efficiency of the artificially released parasitoid in the field include number of insects released, pest density, *Trichogramma* species, season and number of releases, distribution method, crop phenology, number of other natural enemies in the target area, and climatic conditions.

Number of insects per hectare: The number of insects to be released per unit area varies in relation to the population density of the pest. On average, around 100,000 individuals are released per hectare, which is roughly equivalent to the number of insects on five cards.

Number of releases: Depending on the inflow of the pest in the area, especially in places where the biological imbalance is evident, new releases will sometimes be necessary.

Method of release: To release the parasitoid, there are several methods, but the most recommended is through the release of the adult insect.

i. To release adult insects, use 1.6- to 2-L plastic or glass containers containing three to five 150-cm^2 cards with parasitized eggs. Wrap containers with a black cloth, secured by a rubber band.

ii. A few hours after adult emergence, take the containers to the field.

iii. Intermittently open and close the containers as the site of release is crossed, calibrating the pace of the workers to evenly cover the field.

iv. The next day, bring the containers back to the same location, for distribution of the remaining material that emerged, carefully depositing the cards on the plants at the end. Perform this second release from the opposite direction of that used the first day (e.g., first day – north-south; second day – south-north).

v. When using the technique of carrying the container open all the time, keep the container in a horizontal position, with the mouth facing in the opposite direction from the direction of walking, allowing the insect to jump onto the plants.

Another method of distribution is by placing the card itself before the emergence of adults. When the emergence of the first adults is observed, take the material to the field, distributing it inside the plant whorl.

Release points: The more uniform the release of insects, the better the control efficiency. If parasitoids that are still as pupae inside eggs of *Anagasta* i.e., near emergence, are used then release points should range from 25 to 30 per hectare. In this case, subdivide the cards according to the number of release points and then distribute them at the established points.

Time of release: The distribution of *Trichogramma* in the field should be synchronized with the appearance of the first eggs and/or adults of the pest. Repeat the releases at less than weekly interval, depending on the degree of infestation of the pest eggs. The correct timing of initiation of releases, frequency, and amount used are fundamental factors in ensuring the efficacy of biological control with *Trichogramma*. It is very important to make evaluations before and after the releases, to quantify the behavior of the parasitoid and measure its regulatory action. In this way, one can also make the necessary adjustments. If possible, perform egg distribution at strategic points to determine the rate of parasitism. Otherwise, make this determination by collecting eggs from natural populations of the pest. The efficiency can also be assessed through the damage to maize leaves or ears, using a visual scale of injury.

5.1.6. Precautions during Release

- *Trichogramma* species are phototrophic positive, *i.e.*, they exhibit oviposition activity during the day; therefore, they may be very prone to the toxic effects of nonselective insecticides.
- The efficiency of *Trichogramma* in the field is also affected by climatic conditions. It has been verified, for some species, that relative humidity has no effect on survival and dispersion capacity of the parasitoid in the range of 33 to 92%. Also, the action of the wind, at speeds less than 3.6 m/sec, had no influence on the dispersion of the females. The dispersion rate (cm/min) of the parasitoid, in both sexes, increases at higher temperatures. Males appear to be more sensitive to high temperatures than females, although temperatures below 20°C have reduced dispersal capacity.
- When making the releases, it is essential to consider the direction of the wind, the amount of solar radiation (heat), and the presence of rainfall.

- For greater efficiency of the parasitoid, the reduction or elimination of the use of chemical insecticides is necessary. If pesticide application is required, select less-toxic products and continue releasing the parasitoids two or three days later, increasing dose and frequency, to restore biological balance.
- The integration of releases with other cultural, microbiological, physical, and mechanical measures may increase the overall efficiency of control.

5.2. Production of Egg Parasitoid *Telenomus remus*

For small-scale production, *T. remus* are reared in eggs of FAW, as described in the method below. It is also possible to rear this parasitoid on *Corcyra cephalonica.* Initially, host egg masses are pasted onto rectangular cards, which are placed in plastic or glass containers to allow parasitism to occur. For parasitoid multiplication, a proportion of parasitized to non-parasitized eggs of about 1:5, with a 48-hour exposure period, may be used.

5.2.1. Card Preparation

i. Cut white or black boards to size 10 × 15 cm.

ii. With the exception of a 2-cm space at one end, coat the entire area with "gum arabic" glue, which should be initially diluted with water (20% glue, 80% water) and spread evenly on the paperboard with the aid of a sponge.

iii. Immediately, distribute egg masses of the host evenly over the glue, with the aid of surgical tweezers. Distribute about 60 egg masses (approximately 18,000 eggs) onto each card.

iv. Record the date of egg distribution, to better calibrate the expected adult emergence date.

v. Store the cards in a refrigerator (up to a week) and, if possible, inside Styrofoam boxes. The age of the host can influence the performance of the parasitoid. Experiments conducted with different egg ages when the parasitoid has a choice, show that it prefers oviposition in egg masses of up to 36 hours of embryonic development, although it may, to a lesser extent, parasitize eggs up to 60 hours of age, in a non-choice trial.

5.2.2. Egg Infestation

1. Once cards with FAW eggs are dried (room temperature), introduce six cards (about 100,000 eggs) into a 1.6-L plastic or glass container already containing a card that is totally parasitized and a day or less from adult emergence.
2. As food for the parasitoid adults, scatter drops of honey (eight small drops, as large droplets can trap the tiny wasps) on a wall of the container.
3. Seal the containers with plastic film and keep them on shelves. About three to four days after card placement, the parasitized egg becomes dark, providing a qualitative way to evaluate the rate of parasitism. When that occurs, remove the cards from the containers and place them, by date of distribution, into other identical containers without adult parasitoids.

Usually the rate of parasitism is above 90%. If, for some reason, the parasitism rate is lower and larvae hatch from the host, remove them by means of a brush or transfer the parasitized card to another container.

Both temperature and relative humidity can also influence the performance of the parasitoid, especially when it is less than 70%.

5.3. Production of *Chelonus* spp.

The establishment of a small colony of *Chelonus* can be initiated with parasitized eggs or larvae of FAW or from parasitoid adults collected in the field.

i. In the laboratory, keep the collected insects in rooms with little temperature oscillation (optimum is 25±2°C). Soon after emergence, place adults in oviposition cages. Maintain parasitized FAW larvae individually on artificial or natural diet until adult emergence. If using artificial diet, do not use anti-contaminants.

ii. Place five couples of *C. insularis* in an oviposition cage (a transparent container such as glass or plastic pot, for example, with a 1.6-L capacity) containing a food source composed of 10% sugar solution, enriched with 0.1% of ascorbic acid. This solution can be previously prepared (kept in the refrigerator) and offered by means of a cotton dental roll introduced into plastic cups (50 ml) and fitted into a hole made in the middle of a polystyrene lid or another lid type.

iii. Cover the oviposition cage with a fine mesh fabric for ventilation. Keep insects in a lab room with an average temperature of 25±2°C, relative humidity of 70±10%, and a 12-hour photoperiod for one day to allow mating.

iv. After the mating period, replace the food three times per week. Also, offer daily, for a week, about three batches of FAW with less than 24 hours of embryonic development. In case of death of the female parasitoid, add another to the container if available. After each period of parasitism, remove the parasitized FAW egg masses and individualize them in plastic cups containing artificial diet, noting the date of parasitism.

v. Place the cups in Styrofoam stands and keep them on shelves under the same environmental conditions as the *C. insularis* adults. Seven days after hatching, individualize the parasitized larvae to avoid cannibalism, keeping them within the rearing container until the appearance of parasitoid adults, usually 30 days after parasitism. The parasitoid pupation occurs inside the diet. At the time of adult emergence, record the sex of each individual and initiate a new generation. Sex separation of *C. insularis* can be performed through the antenna, which is markedly longer in males.

Apparently, there is no pre-oviposition period for *C. insularis*; the mean incubation period is about 1.8 days. The larval period varies from 17 to 23 days, with an average of 20.4 days; the mean pupal period is 6.2 days. The average duration of the total cycle is 28.6 days. The average longevity of mated females is, on average, 11.6 days, with a maximum of 18 and a minimum of 5 days. The number of parasitized eggs and the longevity varied greatly from female to female, and the parasite capacity is reduced considerably near death. The highest rate of parasitism occurs when females are three days old, with a maximum of 92.2 and a minimum of 48.1 eggs parasitized on that day. In the interval between the 3rd and 6th day, the females had a 72% to 80% parasitism rate.

Although the food consumption of a parasitized larva is much lower than that of a normal larva, it is not possible to reduce the amount of food because the diet will dry out, causing high mortality.

5.4. Production of *Campoletis flavicincta*

The same rearing procedures for *Chelonus* are used to rear *C. flavicincta* but with this species the artificial diet is complete; that is, it is prepared with anti-contaminants.

i. After separation by sex, which is facilitated due to the exposed female ovipositor, place five couples of *C. flavicincta* in an oviposition cage in acclimatized rooms at 25±2°C, relative humidity of 70±10% and photophase of 12 hours, for a period of five days for mating.

ii. After the mating period, replace the adult food source three times per week. Each day for one week, offer about 150 three-day-old FAW larva to the parasitoid. After each 24-hour parasitism period, individualize the parasitized larvae in plastic cups containing diet. Keep insects on shelves under the same environmental conditions as the *C. flavicincta* couples.

iii. The first pupae of the parasitoid usually appear eight days after the individualization of the larvae. Three days after the appearance of these first pupae, eliminate non-parasitized larvae and compute the number of insects parasitized. Seven days after this period, the emergence of adults begins. Unlike *Chelonus*, whose pupa occurs within the diet, the *Campoletis* pupa usually occurs in the higher parts of the breeding recipient. The total cycle of the parasitoid is, on average, 22.9 days: 14.5 days from egg to pupal period and from 7.3 days for completion of pupal period. Parasitized larvae live about a week less than healthy caterpillars.

iv. As the food consumption of a parasitized larva is only 16.9% of the consumption of a normal larva, it is possible to use only one-third the amount of food used by a larva without parasitism.

6. Actions Complementary with Biological Control

6.1. Classical Biological Control implemented by Government Intervention

FAW has been researched for many years in the Americas. Notably in Brazil, there are many agents of natural biological control of the pest of both eggs and larvae. In addition to their demonstrated efficiency under field conditions, technologies for laboratory rearing already exist. Considering the relative similarity in terms of soil and climatic conditions, careful introduction of these biological control agents is highly promising to keep the pest at acceptable population levels and, especially, avoiding the application of chemicals, particularly under smallholder farming. Moreover, given the technical and institutional capacity of many African countries, government incentive is very important in pest mitigation now and in the future. In addition to being a sustainable solution to the pest, it certainly provides an opportunity for exchanges of experience and continued knowledge among countries with so many partnerships already existing in other areas of common interest.

Although there is much knowledge about FAW, especially in the Americas, it is important that researchers make local surveys of natural enemies and then generate necessary evidence on efficacy, selection, mass production, and release of the most effective natural enemies. When gaps exist, emphasis should be given on classical biocontrol for species with proven efficiency against FAW, including *Telenomus remus, Trichogramma pretiosum, Chelonus insularis,* and *Cotesia marginiventris*, following appropriate guidelines, including proper environmental assessment of such introductions.

To achieve effective impact, governments should be willing to invest in mass rearing and large-scale releases, and farmers should be involved in all processes. In fact, the involvement of farmers is critical. As an initial incentive, governments could provide for free effective natural enemies of FAW to the farmers to do their own release and appreciate the effect of the technology on the pest.

6.2. Using Traps with Female Pheromone for Monitoring and Making Decisions

The effectiveness of the biological control of FAW is directly related to the synchronization of the presence of the pest with the presence of the beneficial insect.

When traps are placed at the time of planting (see Chapter 2), the moth catch indicates that the pest has reached the farmer's area and soon the female will begin oviposition. That is, the trap will indicate to the farmer that the pest is present, but it is still not causing damage because there are no larvae yet.
The presence of eggs is the indicator to use, for example, the parasitoid *Trichogramma* or *Telenomus*. The continued capture of moths in the trap suggests that the farmer should continue to observe the plants for the presence of larvae. Larvae up to 12 mm (usually 10 days after the first catch of moths in the traps) can be efficiently controlled either by beneficial insects or through biopesticides such as *Metarhizium, Beauveria, Baculovirus, Bacillus thuringiensis,* fungi, or plant extracts such as Neem products, after local evaluation against the pest. That is, the farmer should use only biopesticides that are compatible with natural biological control. However, success only will be achieved when spraying the products directly into the maize whorls.

6.3. Awareness of Farmers Regarding the Benefits of Biological Control

A great difficulty in establishing a culture of biological control in rural areas is the lack of knowledge on how to recognize and separate insect pests from beneficial insects. There is a need for empowerment of these farmers by showing them that beneficial insects are those that feed on the pests that attack the crops and those that are essential in agricultural production as pollinators, such as bees.

Brochures (with good photographs), videos, and training courses (with sufficient time for field visits) will be useful to help raise the awareness of the farmer and his/her family about the importance of biodiversity of beneficial insects.

Using the photos provided in this FAW IPM Guide and gradually updating them with new photos of biological control agents found locally in Africa against FAW will provide an important resource for continuous training of the farmers. This should be coupled with an open-access database of natural enemies of FAW identified across the African continent.

6.4. Suggestions for Continued Training of Rural Extension Agents and Farmers

- Train farmers and extension people to identify/collect eggs masses that have turned dark (egg masses become dark three days after parasitism) and ship them to Ministry personnel for identification, remembering that local parasitoids could be better than foreign species.
- Provide materials for farmers to collect parasitized eggs.
- Remind farmers and extension people that FAW lays eggs in masses, never isolated, and that each egg mass may contain up to 300 eggs, usually covered with scales.
- Farmers should be aware that by avoiding the use of chemicals on their property, they will contribute to maintain natural biological control agents. But it is important to use strategies that favor the increase of these beneficial insects not only on the individual farmer's property but also throughout the entire community.
- Encourage farmers to manage habitats and use conservation agriculture to augment naturally occurring parasitoids and predators (see Chapter 6).

7. Establishing Small-Scale Biofactories for Regional Use of FAW Biological Control Agents

7.1. Small-Scale Production of Baculoviruses Infecting FAW

Since 1984, Brazil has been researching entomopathogens for the control of FAW, especially with Baculovirus (see Section 3.2.1). Here, a simple method is provided to produce Baculovirus in a small or medium-size biofactory that can be applied in African countries where the pest is already established, as described in several Embrapa publications (Valicente and Tuelher 2009; Valicente *et al.* 2010).

7.1.1. Obtaining Baculovirus-Infected Larvae

Baculovirus-infected larvae can be obtained in the field (Figure 1) from maize plants or purchased from other sources, such as in laboratories where Baculovirus is already grown.

Figure 1. FAW larva killed by Baculovirus on the maize plant (left); dead FAW larvae showing integument rupture (right).

7.1.2 Formulation of Baculovirus Wettable Powder

The formulation of the Baculovirus in wettable powder is carried out in three steps: selection and collection of larvae, maceration, and drying.

7.1.2.1 Selection and collection of larvae

i. Use tweezers to collect larvae killed by Baculovirus infection, selected by color and external appearance, and store them in clean containers.

ii. If there is time, dead larvae may be processed and formulated immediately. Usually a larva killed by Baculovirus has a ruptured integument, making it difficult to collect the insect (Figure 1). For this reason, dead larvae may be placed in the freezer before being collected.

7.1.2.2. Maceration of larvae and incorporation of kaolin clay

i. Macerate the dead larvae using a standard or industrial blender, with a small amount of water, just enough to spin the blender blades. The larvae should be ground in the blender for approximately 10 minutes without interruption.

ii. During blending, incorporate an inert agent (kaolin clay), which acts as a filler and aids in drying the product in the wettable powder formulation. Kaolin clay is inert (does not react with other elements) at widely varying pH and temperature and it often exists in nature as a free element.

The farmer may utilize the Baculovirus macerate provided that the material from the blender is suitably filtered to remove any impurity that may cause nozzle clogging when applied in the field.

7.1.2.3. Drying of Baculovirus formulated in wettable powder

1. Place the larvae/inert mixture in trays (Figure 2) that have been washed and cleaned with 70% alcohol.
2. Dry the material in the laboratory with a forced-air jet. After three to four days, all material will be dry (Figure 2).
3. Crush the dry material using a grinder (Figure 3).
4. Package the material in transparent plastic bags (Figure 3) or bags of aluminum-laminated paper (coffee packaging).

Figure 2. Baculovirus wettable powder preparation. Distribution in tray (left); Mixture of Baculovirus and kaolin clay, completely dry (right).

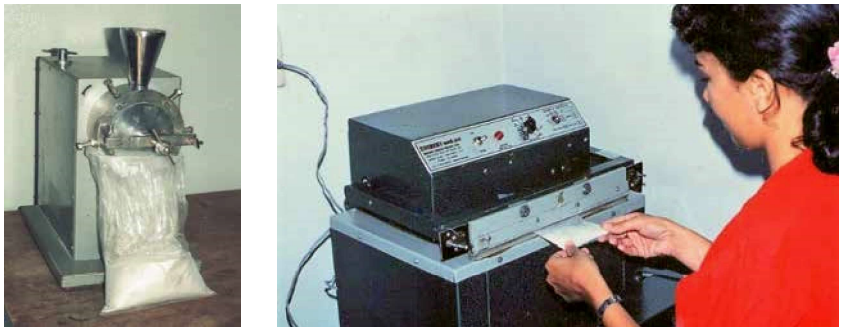

Figure 3. Milling and packaging. Milling of the Baculovirus wettable formulation (left); Baculovirus wettable packaging for application on one hectare of maize crop (right).

7.1.2.4. Stability of FAW Baculovirus formulated in wettable powder

Storage conditions may affect Baculovirus infectivity. Thus, the shelf life of a biological product must be determined so that it can be used safely, obtaining the desired control efficiency. The efficiency of the Baculovirus was verified with the use of two different inert materials: kaolin and zeolite. After one year of storage, there was no decrease in the control efficiency of FAW larvae and no significant difference between the evaluation times or inert materials used in the formulation.