A Guide to Biological Control of Fall Armyworm in Africa Using Egg Parasitoids

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Foreword

The Integrated Pest Management Innovation Lab (IPM IL) has been addressing the Fall Armyworm (FAW) problem in Africa since May 2017, when it invaded Ethiopia. Even though FAW prefers maize, it is polyphagous and can infest over 300 species of plants. Several workshops supported by international organizations were held in different parts of Africa over the past three years; however, they mostly concentrated broadly on IPM approaches for FAW management on maize, with little or no emphasis given for management on other crops.

The IPM IL has consistently focused on biological control of FAW since its involvement, as this approach can tackle this pest not only on maize but also on other crops, and additionally, it can suppress the pest on a farm, village, province, country, or regional basis depending upon the effort and resources that are made available. Further, it is compatible with joint application with all other IPM tactics in the field, except that of chemical pesticides, which would most likely impede processes using biological control.

The IPM IL, in collaboration with *icipe* and ICRISAT-Niger, has identified egg parasitoids – *Trichogramma* spp. and *Telenomus remus* – and some larval parasitoids which attack FAW in Africa. It is known that these parasitoids are amenable for augmentative biological control and they are being used in augmentative biological control of FAW in the Caribbean and South America.

This publication provides information on rearing and release of these parasitoids under African conditions. The IPM IL, *icipe*, and ICRISAT-Niger have already jointly conducted two workshops for participating countries in Africa and Asia.

I am confident that this guide will be useful for the countries in Africa that would like to implement augmentative biological control of FAW.

John Bowman

USAID/Bureau for Food Security

AOR, IPM Innovation Lab

Preface

Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), a voracious agricultural pest native to North and South America, was first detected on the African continent in 2016 and has subsequently spread throughout the continent and across Asia. Fall armyworm (FAW) is known to feed on over 350 plant species and it has been predicted to cause up to $US 13 billion per annum in crop losses throughout sub-Saharan Africa, thereby threatening the livelihoods of millions of poor farmers.

Since the occurrence of FAW in Africa, synthetic chemical insecticides have been widely used as emergency responses to halt distribution of the pest and minimize damage in maize fields. Most smallholder farmers in Africa and Asia, however, cannot afford frequent insecticide applications. Furthermore, dependence on chemical insecticides results in the development of resistance to major classes of insecticides, effects on nontarget organisms, as well as other adverse effects to humans and the environment. This highlights the need for the development of integrated pest management (IPM) strategies that are suitable to African smallholder farmers.

Biological control using egg parasitoids particularly from the genus *Trichogramma* and *Telenomus remus* is part of the IPM approach presently underway to control FAW in North and South America. The approach involves mass rearing and release of these egg parasitoids to control FAW. These egg parasitoids are reared on factitious and natural hosts. Various species of both parasitoids are already present in Africa. After identifying the species/strain that best suit the local condition, the parasitoid wasps can be mass reared and used against FAW and other lepidopteran pests. Therefore, the purpose of this book is to provide guidelines on mass rearing systems for both the egg parasitoids and their hosts. The book describes the methods used to mass produce FAW (*S. frugiperda*), rice meal moth (*Corcyra cephalonica* (Stainton), Lepidoptera: Pyralidae), egg parasitoids - (*Trichogramma chilonis* Ishii, Hymenoptera: Trichogrammatidae) and (*Telenomus remus* Nixon, Hymenoptera: Platygastridae) in the facilities at *icipe*- Kenya and ICRISAT-Niger.

This guide is primarily intended for biological control practitioners at universities, research institutes and commercial laboratories particularly involved in managing FAW and other lepidopteran pests. The information in this document is also intended to assist those who are

relatively new at rearing FAW, rice meal moth, and the parasitoid wasps and to those who wish to improve existing rearing systems. The document covers virtually all aspects of information on the rearing techniques of each species such as colony establishment, stock culture maintenance, diet preparation, mass rearing, storage, quality control and field release. Each section is interrelated, contains step-by-step procedures, and is supported by colour pictures.

The guide produced jointly by the International Centre of Insect Physiology and Ecology (*icipe*), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Haramaya University, and Virginia Tech through support provided by the Feed the Future Innovation Lab for Integrated Pest Management, funded by the of the US Agency for International Development (USAID) under the Cooperative Agreement No. AID-OAA-L-15-00001.

International Centre of Insect Physiology and Ecology (*icipe*) is an international scientific research institute, headquartered in Nairobi, Kenya that works towards improving lives and livelihoods of people in Africa. The center's main objective is to research and develop alternative and environmentally friendly pest and vector management strategies that are effective, selective, non-polluting, non-resistance inducing, and which are affordable to resource-limited rural and urban communities. *icipe's* mandate extends to the conservation and use of the rich insect biodiversity found in Africa. Today, *icipe* is the only international center in sub-Saharan Africa working primarily on arthropods. *icipe* focuses on sustainable development using human health as the basis and the environment as the foundation for sustainability (http://www.icipe.org/).

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): is a non-profit, non-political organization that conducts agricultural research for development in the drylands of Asia and sub-Saharan Africa. Covering 6.5 million square kilometers of land in 55 countries, the semi-arid or dryland tropics has over 2 billion people, and 644 million of these are the poorest of the poor. ICRISAT and its partners help empower these poor people to overcome poverty, hunger and a degraded environment through better agriculture. ICRISAT is headquartered in Hyderabad, Telangana State, in India, with two regional hubs (Nairobi, Kenya and Bamako, Mali) and country offices in Niger, Nigeria, Zimbabwe, Malawi, Ethiopia and Mozambique. ICRISAT is a member of the CGIAR system Organization (https://www.icrisat.org/).

Virginia Polytechnic Institute and State University, commonly known as Virginia Tech, is a public, land-grant research university with its main campus in Blacksburg, Virginia (https://vt.edu/). The university houses the Feed the Future Innovation Lab for Integrated Pest Management, which aims to improve the livelihoods of smallholder farmers by implementing sustainable crop solutions in the developing world.

Haramaya University is a public academic and research university with its main campus in Haramaya, located at about 510 km East of Addis Ababa, Ethiopia. The university offers 264 academic programs of which 113 are undergraduate programs, 131 are second degree (M.Sc./M.Ed./MPH) and 20 are PhD level training programs. In addition, the university has been actively involved in research activities, primarily in the fields of agriculture. (http://www.haramaya.edu.et/).

Acknowledgement

Contents

1. Introduction

Maize (*Zea mays* L. Poaceae) is the most important staple food crop grown predominantly by smallholder farmers in sub-Saharan Africa (SSA) (Abate et al., 2017). In sub-Saharan Africa, maize occupies more than 36 million hectares of land each year. More than 208 million people in the region depend on maize for food security and economic well-being. Despite large production areas and the importance of maize, the average grain yield is <1.8 ton/ha (Abate et al., 2017). This may be due to several abiotic and biotic constraints. Among the biotic factors, the damage caused by cereal stem borers is major (Kfir et al., 2002). More recently, however, an invasive insect *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) also known as the fall armyworm (FAW), is becoming a major pest causing substantial yield losses on maize in the region (Goergen et al., 2016; Kumela et al., 2018). Estimates in September 2017 showed that in just six African countries, the pest has devastated almost 1.5 million hectares of maize crops (CIMMYT, 2017).

Since the occurrence of FAW in Africa, synthetic chemical insecticides have been widely used as emergency responses to halt distribution of the pest and minimize damage in maize fields (Prasanna et al., 2018). Synthetic insecticides play an important role in FAW management, but given confirmed reports of insecticide resistance development in FAW population (Yu, 1991), unavailability and high costs (Kumela et al., 2018), as well other adverse effects to humans and the environment (Lewis et al., 2016) sole dependence on insecticides is unsustainable in the long-run. Synthetic insecticides also disrupt integrated pest management (IPM) measures, such as biological control, targeted at other cereal pests (Day et al., 2017). Therefore, it is important to minimize the use of insecticides, especially the highly hazardous and broad-spectrum ones, and to develop, promote, and deploy proven and sustainable IPM technologies against *S. frugiperda* (Kenis et al., 2019).

Biological control, i.e., the use of natural enemies to control a pest, is central to the development of IPM systems. Biological control offers an economically and environmentally safer alternative to synthetic insecticides used for the management of this pest. A key part of an IPM approach is the identification and use of sustainable solutions for managing pests. In its native range, FAW has many natural enemies. An inventory of fall armyworm natural enemies in the Americas and Caribbean regions documented a total of 150 species of parasitoids (Molina-Ochoa et al., 2003;

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Meagher et al., 2016), indicating substantial natural enemy diversity and prospects for biological control. Therefore, based on the global experience of managing maize pests, biocontrol will serve as a necessary pillar of the IPM strategy for the management of FAW in Africa.

A recent survey conducted in three African countries *viz.,* Ethiopia, Kenya and Tanzania revealed the presence of four hymenopteran and one dipteran parasitoid (Sisay et al., 2018). These species are new associations with FAW and were never reported before from Africa, or North and South America. Among these parasitoids, *Cotesia icipe* Fernández-Triana & Fiaboe (Hymenoptera: Braconidae) parasitized 34 to 45% of FAW larvae in Ethiopia, two years after the confirmed arrival of FAW in Africa (Sisay et al., 2018). Surveys of FAW natural enemies conducted in 2017 and 2018 in maize and sorghum fields in Niger revealed the occurrence of three egg, one egg-larval, and four larval parasitoids. Among these parasitoids, parasitism by the egg parasitoid *Telenomus* sp. (Hymenoptera: Braconidae) ranged from 34 and 25% in 2017 and 2018, respectively (Amadou et al., 2018). Although FAW has rapidly invaded Africa, it is encouraging to see a reasonable level of biological control in its introduced place.

Likewise, surveys conducted in five African countries - Benin, Cote d'Ivoire, Kenya, Niger and South Africa indicated the presence of *Telenomus remus* Nixon (Hymenoptera: Platygastridae) (Kenis et al., 2019). A similar survey undertaken in Kenya found *Trichogramma chilonis* Ishi (Hymenoptera: Trichogrammatidae) parasitizing FAW eggs (Unpublished data). Among the natural enemies, *Telenomus remus* and *Trichogramma* spp. are the main egg parasitoids of FAW in North and South America, where they are already used in augmentative biological control (Cave and Acosta, 1999; Cave, 2000; Ferrer, 2001). *Telenomus remus* has been released in maize fields as part of IPM programs in Venezuela and resulted in 90% parasitism (Ferrer, 2001), demonstrating the high potential of biocontrol for several species of *Spodoptera.*

The egg parasitoids are considered excellent candidates among the biological control agents of FAW for augmentative release. The damaging stage of FAW is the larval stage and egg parasitoids prevent the FAW from causing damage to the host plant. The presence of these two parasitoid wasp species in Africa provides a great opportunity to develop augmentative biological control methods.

1.2. Fall armyworm

1.2.1. Spread of fall armyworm in Africa and Asia

The fall armyworm, *Spodoptera frugiperda*, is a lepidopteran pest that feeds in large numbers on the leaves, stems, and reproductive parts of more than 350 plant species, causing major damage to economically important cultivated grasses such as maize, rice, sorghum, sugarcane and wheat, as well as other vegetable crops and cotton (Montezano et al., 2018). It is native to the tropical region of the western hemisphere from the United States to Argentina (Sparks, 1986). It is a common pest of several crops in the tropics of North, South, and Central America including the Caribbean Islands, which suggests that the pest is tropical in origin (Vickery, 1929). Fall armyworm became important during the mid-19th century when it was reported attacking maize, sugarcane, rice and grasses in the southern USA (Hinds and Dew, 1915). The common name fall armyworm derives from its annual rapid range expansion northwards into North America where it lays eggs, and the larvae develop throughout the Fall (Autumn) (Du Plessis et al., 2018). Survival of FAW populations during winter months in the United States is frequently observed in southern Florida and southern Texas (Nagoshi et al., 2017). Fall armyworm has a remarkable dispersal capacity, a feature that is understood to have evolved as part of its life history strategy (Nagoshi and Meagher, 2008). During annual migrations, it is able to migrate northwards from its overwintering area in the warmer parts of central and South America more than 2,000 km across the USA and up into Canada; southwards, it reaches the northern parts of Argentina and Chile (Johnson, 1987).

Up until 2015, this pest had not been reported anywhere other than America. However, in early 2016 FAW was detected for the first time in West African countries such as Sao Tome, Nigeria, Benin and Togo (Goergen et al., 2016) and later in Ghana in 2017 (Cock et al., 2017). Within a short span of its introduction into Africa, FAW has spread to most of the sub-Saharan Africa, where it is causing extensive damage, especially to maize and to a less extent on sorghum and other crops (Prasanna et al., 2018; FAO, 2019). In July 2018, it was detected in Yemen and in India, the first reported infestation in Asia. In January 2019, it was reported in Bangladesh, Myanmar, Sri Lanka, Thailand and China (FAO, 2019) (Figure 1) and more recently, in Japan (IPPC, 2019). It is likely that it will spread further north to Europe and other countries in Asia. Recently published pest distribution and climatic suitability models have indicated that the environmental requirements for this pest to establish itself permanently are present through large

parts of Africa and Asia and some parts of Europe (du Plessis et al., 2018; Early et al., 2018). The ideal climatic conditions coupled with the abundance of suitable host plants in the regions suggests the pest can produce several generations in a single season.
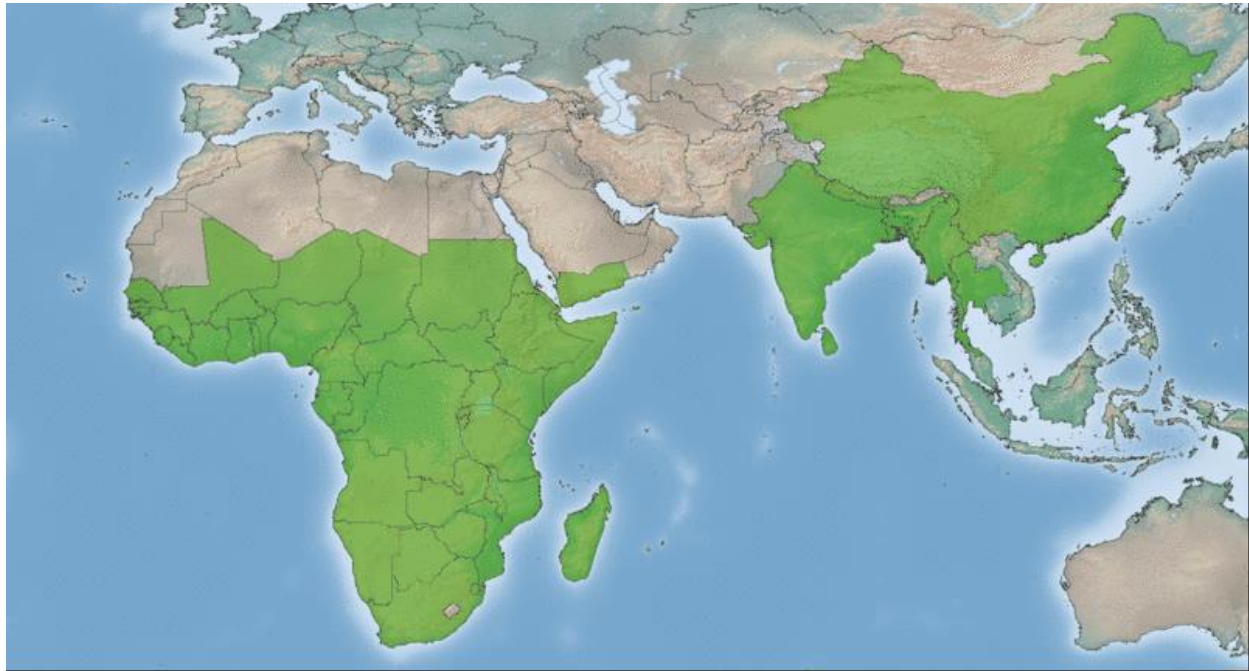
■ FAW detected and officially reported

Figure 1. Map of areas affected by FAW (until March 2019)

(Source: http://www.fao.org/3/BS183E/bs183e.pdf)

1.2.2. Description and lifecycle of fall armyworm

Temperature has a significant influence on the duration of FAW lifecycle. Sharanabasappa et al. (2018) reported that male and females complete their lifecycle in 32-43 and 34-46 days, respectively at 26±20C, 75 to 80% RH and L12: D12 photoperiod. Depending on the appearance of the migrating adults and climate, FAW can have up to eight generations per year in maize fields in tropical areas (Busato et al., 2005). According to Johnson (1987), FAW did not enter into diapause. Seasonal migration is a major factor in the life history of FAW and it is considered one of the most mobile noctuid crop pests (Johnson, 1987; Nagoshi and Meagher, 2008).

The egg is dome shaped; the base is flattened and the egg curves upward to a broadly rounded point at the apex. The egg measures about 0.4 mm in diameter and 0.3 mm in height. Eggs are laid at night mostly on the upper and sometimes on the lower surface of the leaves of the host plant (Figure 2. A and B) but occasionally they may lay on other parts of the host plants (Vickery, 1929; Johnson, 1987). Eggs are usually laid in masses of approximately 100-200 eggs, and total egg production per female averages about 1,500 with a maximum of over 2,000 (Johnson, 1987). The eggs of FAW are white in color and later turn into brown. The newly hatched eggs are covered with a protective, felt-like layer of white scales (setae) from the female abdomen and this gives a moldy appearance (Figure 3. A), but eggs laid by mature females are less or not covered (Figure 3. B.) and visible with naked eyes or through hand lens. Duration of the egg stage is only 2 to 3 days (20-30°C) (Capinera, 1999; Sharanabasappa et al., 2018).
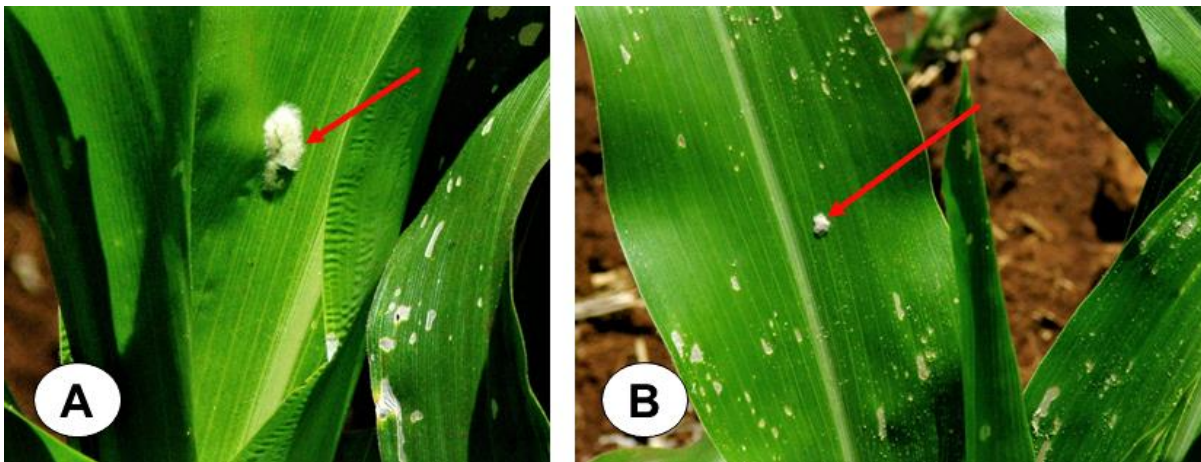
Figure 2. FAW eggs laid on the upper part of maize leaves (A and B) (M. Goftishu 2019).

The FAW typically has six larval instars. The young larvae feed where eggs were laid; the first two instars feed gregariously on the young leaves, causing a characteristic skeletonizing or 'windowing' effect (Figure 4. A). Later instar feed near the funnel and upper leaves leaving sawdust-like frass which can be an easily spotted sign of larval feeding (Abrahams et al., 2017) (Figure 4. B). In a young crop, this feeding can kill the growing point, a symptom called 'dead heart' in maize, which prevents any cobs forming (Sparks, 1979; Johnson, 1987). Young larvae hide in the funnel during the day but emerge at night to feed on the leaves. In young plants, the stem may be cut, providing evidence of damage. Older larvae stay inside the funnel and so are

protected from insecticide spray applications and natural enemies. In older plants, the larger larvae can bore into the developing reproductive structures, such as maize cobs, reducing yield quantity and quality (Abrahams et al., 2017). Larger larvae are cannibalistic and have the ability to dominate interspecific competitors and reduce intraspecific rivals (Chapman et al., 1999). Due to this high level of cannibalism, which commences in the third instar, only between one and three fully grown larvae remain per plant, in spite of very high initial numbers of neonates per plant (Chapman et al. 1999). Larval development takes 14-21 days at 26^0C (Sharanabasappa et al., 2018). Larger larvae are nocturnal. The larva of FAW can be identified by four characteristic spots on the second to last segment, forming a square or rectangle. Moreover, the head of the mature larva has an inverted "Y" mark (Abrahams et al., 2017; Sharanabasappa et al., 2018) (Figure 5).
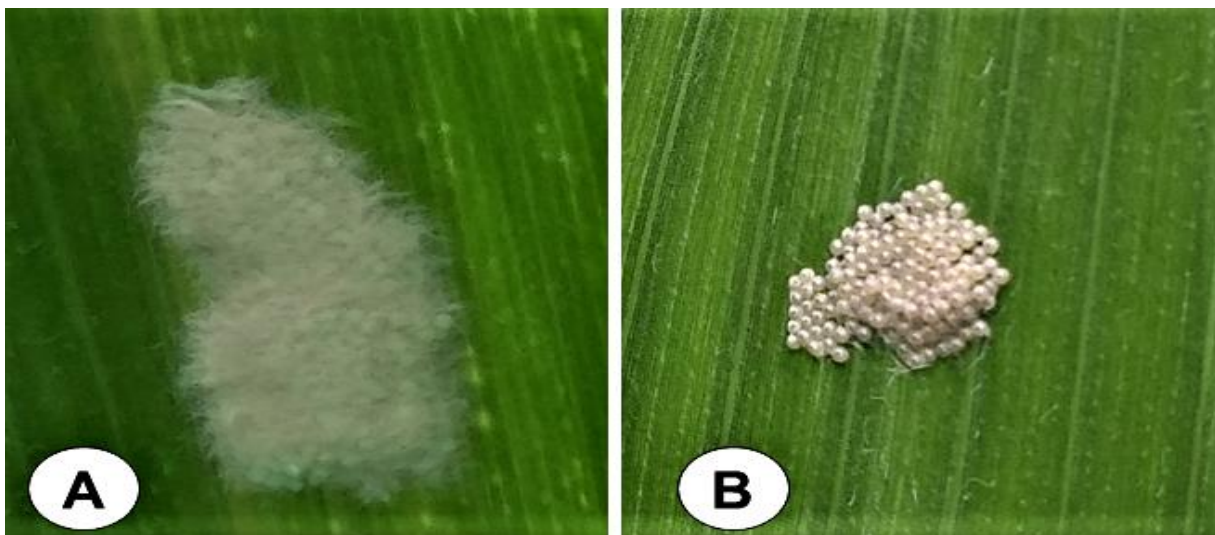
Figure 3. FAW eggs with hairs (A) and without (B) (P. Malusi 2019).
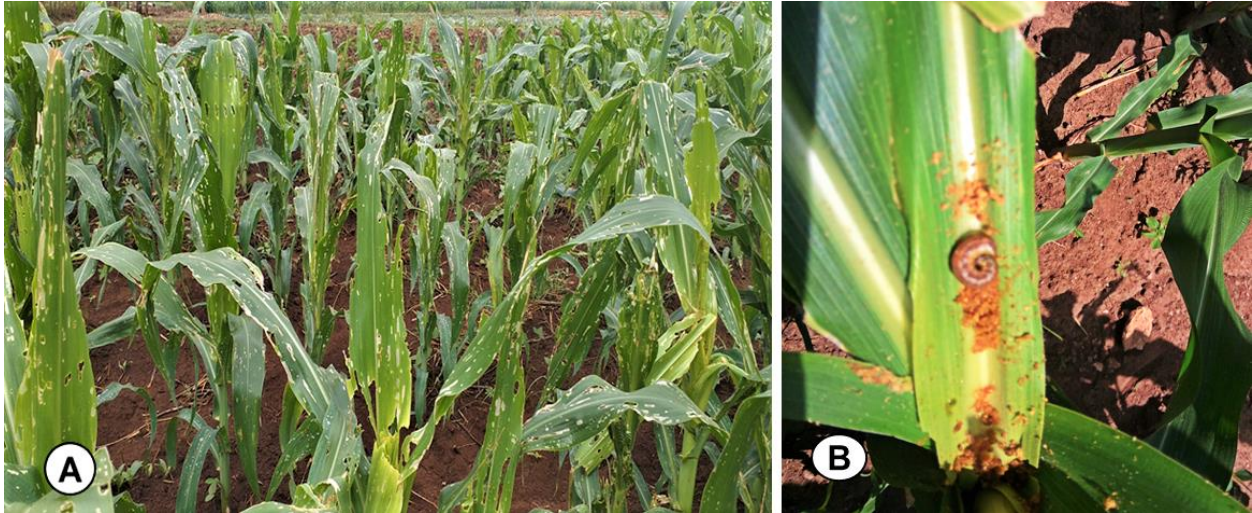
Figure 4. FAW damage skeletonizing or 'windowing' effect (A) and moist sawdust-like frass (B) (M. Goftishu 2019).

Figure 5. Distinguishing marks on medium to large-sized FAW larvae (Prasanna et al., 2018)

Pupation normally occurs 2-6 cm deep in the soil, but also occurs in mature leaf bases or maize ears (Sparks, 1979). If the soil is too hard, larvae may web together leaf debris and other material to form a cocoon on the soil surface. Duration of the pupal stage is about 9 to 13 days at 26^0C (Sharanabasappa et al., 2018).

Adults are nocturnal, and are most active during warm, humid evenings. Adults use their natural pre-oviposition period to fly for many kilometers before they settle to oviposit, sometimes migrating for long distances. After a preoviposition period of 3 to 4 days, the female moth normally

deposits most of her eggs during the first 4 to 5 days, although some females have been reported to oviposit for up to 3 weeks (Johnson, 1987; Sharanabasappa et al., 2018). Duration of adult life is estimated to average about 10 days, with a range of about 7 to 21 days (Figure 6) (Capinera, 1999; Sharanabasappa et al., 2018).
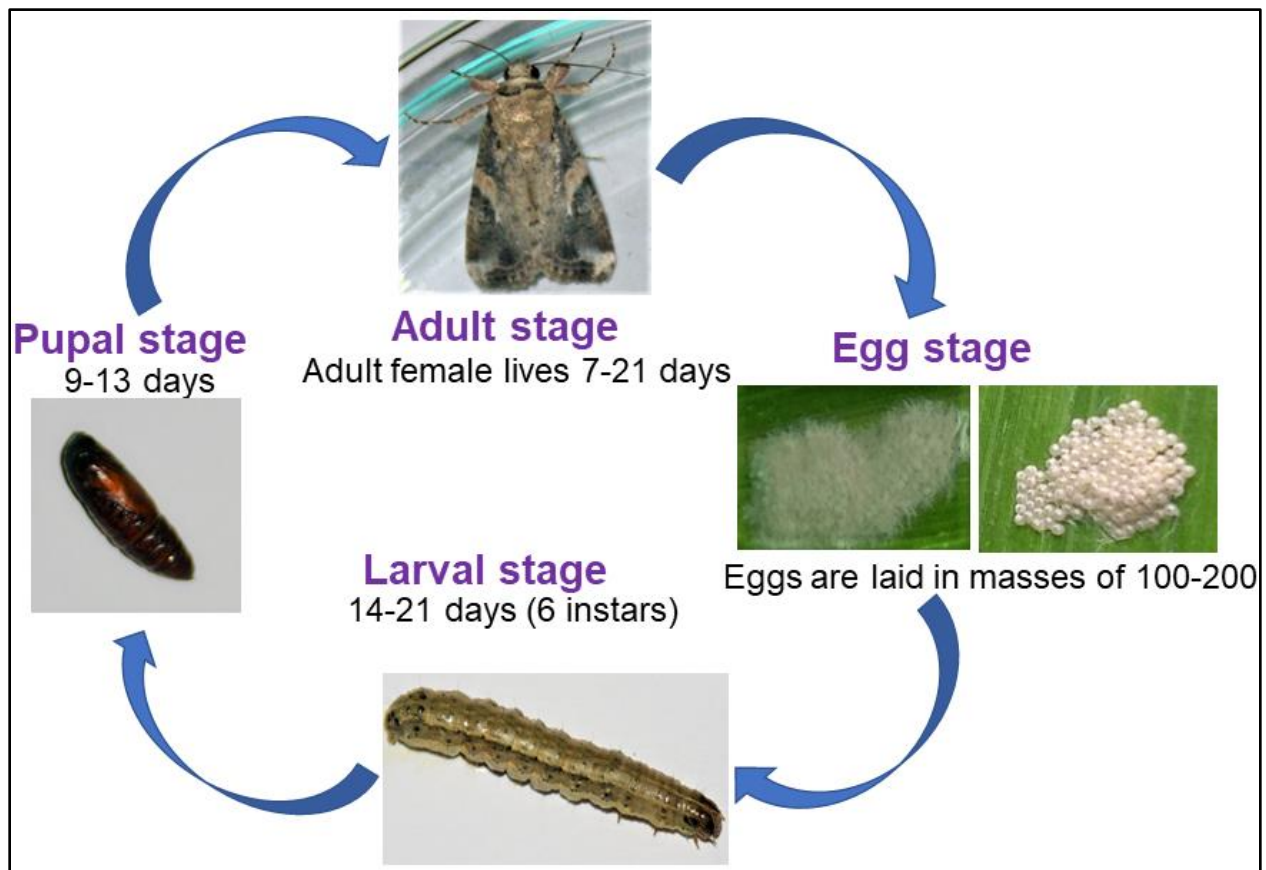
Figure 6. FAW life cycle of *S. frugiperda* (M. Goftishu 2019).

1.2.3. Damage and economic impact of fall armyworm in Africa

The invasion of *S. frugiperda* threatens the food security of more than 200 million people in Africa whose main staple crop is maize. Based on preliminary estimates in 12 African maize-producing countries, in the absence of proper control methods, *S. frugiperda* has the potential to cause maize yield losses of 8.3 to 20.6 million metric tons per year. This represents a range of 21-53% of the annual production of maize averaged over a three-year period in these countries. The value of these losses is estimated at between US $2.5 to 6.2 billion (Day et al., 2017).

Prior to the incursion of FAW, new agricultural pests were/are periodically introduced into Africa and cause some degree of damage on the agricultural environment. For example, the invasive *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae), a major insect pest of maize and sorghum in Africa was accidentally introduced from Asia (Kfir et al., 2002). The reason why FAW is particularly devastating to farmers across the region is that it is highly polyphagous and capable of feeding on both the vegetative and reproductive parts of more than 350 plant species (Montezano et al., 2018), although it has a preference for maize, the main staple crop in sub-Saharan Africa (SSA). Secondly, it has high dispersal ability. The adult moth can fly up to 100 km per night (Johnson, 1987; Cock et al., 2017; Nagoshi et al., 2017). When the wind pattern is right, moths can move much longer distances; for example, a flight of 1,600 km from Mississippi in southern United States to southern Canada in 30 hours has been recorded (Rose et al., 1975). Thirdly, FAW can have several generations per year particularly in the tropics (Busato et al., 2005), large reproductive capacity (Johnson 1987; Sharanabasappa et al., 2018) and absence of diapause (Johnson 1987). These characteristics make the FAW a significant risk to agricultural production, especially maize, the main staple crop in large parts of SSA (Otim et al., 2018).

2. Biological control of fall armyworm

2.1. Introduction to biological control

According to a recent definition by Eilenberg et al. (2001) biological control or biocontrol is "*The use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be*."

It should be stressed that the definition clearly states that 'living organisms' are used. Accordingly, natural enemies including predators, parasitoids, nematodes, fungi, bacteria, protozoa, and viruses, play a major role in the natural regulation of insect populations. 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. 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.

Entomopathogens include bacteria, fungi, protozoans, nematodes, or viruses that infect and causes diseases in insects (Eilenberg et al., 2001).

According to DeBach (1974), the use of biological control is essentially the antithesis of chemical pest control. Frequent application of chemical pesticides leads to the development of pesticide resistance, secondary pest outbreak, and causes biodiversity and environmental impacts as well as health risks to farmers and consumers (Yu, 1991; Abrahams, et al., 2017).

Based on how biological control is undertaken, it can be broadly classified as classical, augmentation, and conservation biological control. Classical biological control approach involves the intentional introduction of an exotic, usually coevolved biocontrol agent for stable establishment and long-term pest control. The objective of the approach is to introduce safe and effective biocontrol agents to suppress pest populations. The basic objective in this technique is to identify parasitoids that control a pest in its home location and introduce them to the pest's new location. This tactic is employed in cases of pests invading a new geographical location. A successful classical biological control results in extensive, continuous, and widespread control of the invasive species (e.g., release of *Cotesia flavipes* (Cameron) (Hymenoptera: Braconidae) for the control of spotted stem borer *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in Africa).

An augmentation (inundative/inoculative) biological control approach involves periodic releases of natural enemies, which are either introduced or endemic, to foster biological control against either invasive or endemic pests. 'Augmentation' falls into two categories: (a) Inoculative approach, in which natural enemies are collected, mass reared and released in relatively low numbers to establish a local population early in the season for long-or-short term suppression of a target pest or pest complex (eg., release of larval parasitoid *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) for control of pearl millet head miner, *Heliocheilus albipunctella* de Joannis (Lepidoptera: Noctuidae) early in the season in Niger), and (b) Inundative approach, in which large numbers of natural enemies are collected, mass reared, and released to obtain rapid pest suppression (i.e. use as a "biotic insecticide"). Pest control is mainly obtained from the released natural enemies and not from their offspring. E.g. egg parasitoids of the Hymenopterans

genus *Trichogramma* have been widely used worldwide for inundative releases against key pests (Li, 1994).

Conservation of biological control involves the modification of agroecosystem or existing practices to protect and enhance natural enemies or other organisms to reduce the effects of pests. It means the activities that preserve or protect the natural enemies and involves manipulation of agroecosystems to enhance the survival, fecundity, longevity, and behavior of parasitoids and predators to increase their effectiveness (Eilenberg et al., 2001). During the process of invasion, invasive species are likely to encounter natural enemies of other species closely related to them. Some of these natural enemies could adapt to the invasive pest, often referred to as "new associations" (Sisay et al., 2018).

2.2. Biological control agents of fall armyworm

It is important to understand that before the invasion of FAW, Africa has been home to several indigenous and exotic Lepidopteran insect pests. The African armyworm *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae), beet armyworm *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), African cotton leafworm *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), African stem borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), spotted stem borer, *C. partellus*; sugarcane borer, *Chilo sacchariphagus* (Bojer) (Lepidoptera: Crambidae), and African bollworm, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) are among the most dominant species associated with several natural enemies (Neuenschwander et al., 2003). Some of these natural enemies could be potential candidates for augmentation and conservation biological control initiatives in Africa. Based on this premise, many research groups in Africa are looking for natural enemies of FAW in several African countries.

Surveys conducted in three East African countries *viz.,* Ethiopia, Kenya and Tanzania revealed the presence of four hymenopteran (*Cotesia icipe* Fernandez-Triana & Fiobe (Braconidae), *Chelonus curvimaculatus* Cameron (Braconidae), *Coccygidium luteum* (Brulle) (Braconidae), *Charops ater* Szepligeti (Icheneumonidae)) and one dipteran (*Palexorista zonata* (Curran) (Tachinidae)) parasitoid (Sisay et al., 2018). With the exception of *C. curvimaculatus*, an egg-larval parasitoid,

the rest are larval parasitoids. All these species are new associations with FAW and were never reported before from Africa, or North and South America. Among these parasitoids, *C. icipe* was the dominant larval parasitoid in Ethiopia with parasitism ranging from 33.8 to 45.3%, while in Kenya, *P. zonata*, was the primary parasitoid with 12.5% parasitism, and *C. luteum* was the most common parasitoid in Tanzania with parasitism ranging from 4 to 8.3% (Sisay et al., 2018).

Likewise, surveys of FAW natural enemies undertaken in 2017 and 2018 in maize and sorghum fields in Niger revealed the occurrence of three egg parasitoids, *Trichogrammatoidea* sp. (Hymenoptera: Trichogrammatidae), *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) and *Telenomus* sp. (Hymenoptera: Platygastridae); one egg-larval parasitoid *Chelonus* sp. (Hymenoptera: Braconidae); four larval parasitoids, *Cotesia* sp. (Hymenoptera: Braconidae), *Charops* sp. (Hymenoptera: Ichneumonidae) and unidentified ichneumonid and tachinid fly (Amadou, et al., 2018).

Recent surveys conducted in five African countries including Benin, Cote d'Ivoire, Kenya, Niger, and South Africa indicated the presence of an egg parasitoid, *T. remus* (Kenis et al., 2019). A similar survey undertaken in Kenya found *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) parasitizing FAW eggs (Unpublished data). Entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana,* have also shown efficacy against eggs and second-instar larvae of FAW (Komivi et al., 2019). *B. bassiana* caused moderate mortality of 30% to second-instar larvae, while *M. anisopliae* caused egg mortalities of 79.5-87.0% under laboratory conditions. Cumulative mortality of eggs and neonates with *M. anisopliae* reached as high as 96% with some fungal isolates (Komivi et al., 2019).

Previous surveys undertaken in Africa recorded 18 *Trichogramma*, 8 *Trichogrammatoidea* and 11 *Telenomus* species from eggs of Lepidopteran pests (Polaszek and Kimani, 1999; Sithanantham et al., 2001). Among the natural enemies, parasitoid species belonging to *Trichogramma* and *Telenomus* are widely used to manage FAW damage in North and South America (Mihm, 1987: Cave, 2000; Figueiredo et al., 2002; Gutierrez-Martinez, et al., 2012). This is mainly because these wasps are easy to rear under laboratory conditions and effective in managing FAW populations (Cave and Acosta, 1999; Cave, 2000; Ferrer, 2001; Nagaraja, 2013). In Latin America, inundative

releases of *T. remus* in maize fields showed 90% parasitism, providing full control of FAW (Cave, 2000; Ferrer, 2001). Similarly, a preliminary field experiment undertaken at the *icipe* experimental field station in Kenya showed that augmentative releases of *T. remus* and *T. chilonis* effectively controlled the damage caused by FAW as compared to the control (Unpublished data). This shows the potential of egg parasitoids for FAW management. Therefore, for effective exploitation of egg parasitoids for FAW management in Africa, the experience gained elsewhere should be appropriately utilized and complementary research undertaken.

2.3. Advantages of using biological control of fall armyworm 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 developed world. Moreover, the amount 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 (Soul-kifouly et al., 2016). Biological control, particularly classical and conservation biological control, is much cheaper and benefits smallholder production systems in Africa. Further, there are no reports 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 against FAW in Africa.

Preliminary assessments of biocontrol species on the continent suggest we should optimize the role of biocontrol in helping to manage FAW.

Biological control of FAW has a multitude of benefits such as:

- **Location**: The parasitoids are naturally found in Africa, so introduction does not pose a threat of turning a non-native species into an invasive species on the continent.
- **Cost:** Mass-rearing the parasitoids on an alternative host such as Lepidopteran moths, as planned, instead of the fall armyworm will make it affordable. The cost benefit is crucial, as the FAW has the potential to cause billions of dollars of damage in multiple countries, many of them already plagued by poverty.

- **Natural enemies to many:** The biocontrol agents may act as natural enemies to most caterpillar pests that harm a wide array of crops, not just maize, further ensuring the goal of global food security.

2.4. Inundative release of biocontrol agent against fall armyworm

Previous and recent surveys conducted in Africa revealed that various species of *Trichogramma* and *Telenomus* wasps are already present on the continent (Polaszek and Kimani, 1999; Sithanantham et al., 2001; Amadou, et al., 2018; Kenis et al., 2019). After identifying the species/strains that best suit the local condition, the parasitoid wasps can be mass reared and released against FAW and other Lepidopteran pests. Once released, the wasps, with extreme search capacity, fly to the plants seeking the pest's eggs. Hence, the releases can be made at strategic points ranging from 20 to 40 points per hectare (Cruz et al., 2016).

Since the released female wasps have a short longevity (4-7 days on average) and a new parasitoid generation occurs 10 days after release, it is necessary to make four to five releases at weekly 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 FAW moths into the production area. The inundative release of *Trichogramma* and *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. These egg parasitoids kill the pest in the egg stage itself before the FAW could cause any damage to the maize crop. However, synchronization between the presence of FAW egg masses and release of parasitoids in maize is essential for successful management.

3. Insect rearing facility at *icipe* and ICRISAT-Niger

The fall armyworm and the egg parasitoids (*Trichogramma* spp. and *Telenomus remus*) rearing began at the International Center of Insect Physiology and Ecology (*icipe*) in 2019 with the support from the Feed the Future Innovation Lab for Integrated Pest Management, through the rice, maize, and chickpea IPM for East Africa project. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) facility in Niger, *Trichogramma* cultures were started in 2015, but both FAW and *Telenomus* cultures started in 2018. The main purpose of the mass rearing facility is to supply *Trichogramma* and *Telenomus* wasps for use in FAW management research activities at *icipe,* ICRISAT-Niger, and other sub-Saharan African countries. The facility is also used for training both students and staff members from African countries involved in biological control of FAW.

4. Mass production of fall armyworm

According to Mihm (1987), in order to efficiently mass rear an insect species, in addition to a thorough knowledge of the biology of that insect, the following components are required: 1) a rearing facility, 2) trained personnel, 3) natural, meridic or artificial diets, 4) rearing procedures, and 5) source of the insect species to initiate a colony.

4.1. FAW colony establishment

A large starter colony of at least 500 individuals collected from representative areas is used. The most important factor to be considered is that the culture of the pest must exhibit the vigor and vitality of the damaging pest population within the geographical and ecological areas that are affected. Even though it is not possible to include in the colony individuals from the total geographical distribution of the pest, emphasis should have to be given to include individuals from major maize producing areas within the country (Mihm, 1987; Tefera et al., 2010).

Before collection of FAW specimens', make sure the sampling field was not treated with insecticides. Materials required for field collection of FAW specimens include: scissors, lunchbox/plastic or glass jar, vial filled with artificial diet, cotton wool, notebook and pens, GPS, and icebox.

The FAW colony could be initiated from field collected specimens of any developmental stage. However, the easiest and least expensive method for colony establishment of insect pests like FAW is from its larvae. It is generally advisable to use late instar larvae. However, depending on the available developmental stage of FAW in the field, egg masses or larvae could be collected. If the target is to collect egg masses or neonate larvae, cut the maize leaves with egg batches (Figure 7. A) or neonate larvae using scissors and place in a lunchbox and provide fresh maize leaves for the larvae to feed on. A rectangular window in the middle of the lid of the lunchbox is cut and sealed with mesh for ventilation (Figure 7. B). Moreover, the inside of the box is lined with paper towel. The paper towel in the bottom of the box makes clean-up a little easier. Besides lunchbox, wide-mouthed (9 cm) 1-1.5 liter plastic jar could also be used (Figure 7. C) for field collection of egg mass or neonate larvae. Some of the eggs may also hatch immediately after collection, therefore,

provide some fresh maize leaves to the eggs as well. However, before supplying fresh maize leaves, wash the leaves with sodium hypochlorite (5ml/L), and rinse with distilled water to prevent any contamination. Replace the leaves every 2-3 days, depending on how long they remain green and fresh.

Figure 7. Field collection of FAW egg masses/neonate (A), container - lunchbox (B), plastic jar (C), and artificial diet (D) for holding the collected specimens (M. Goftishu 2019).

However, if the target is late instar larvae, prepare artificial diet as indicated in sub-section 4.4.1. As cannibalism is higher among late instar larvae (Chapman et al., 1999), place each aged larva individually in a separate vial with artificial diet (Figure 7. D) and cover the vial with cotton wool. Then, label all the necessary information such as date of collection, place and GPS reading of each location on the container. Place the collection boxes/jars/vials in icebox to protect the specimen from desiccation and/or physical damage during transportation.

The field collected FAW specimens should pass through strict quarantine protocols before they are transferred to the insectary (Onyango and Ochieng'-Odero, 1994). In principle, field collected insects should be reared separately for 1-2 generations before mixed with lab population; this gives time to discard diseased and parasitized insects.

4.2. FAW colony maintenance

An important consideration when insects are mass-reared is to ensure that the laboratory colony exhibit the genetic diversity, aggressiveness, and vitality that the pest population exhibits in nature. For some insect species to maintain a healthy, vigorous colony, it is necessary to replace or genetically mix it with wild stock at least every year (about 10 generations). However, at the *icipe* and ICRISAT-Niger facilities, wild FAW specimens are collected every 6-12 months and infused with the laboratory colonies to ensure that colonies remain as genetically close as possible to their wild counterparts.

When rejuvenating the FAW colony at *icipe* insectary, about 20 to 30 FAW egg masses are collected from maize fields from different locations. In Niger, FAW eggs are collected from the maize and sorghum fields. The collected egg masses are surface sterilized (washed gently with sodium hypochlorite, 5ml/L solution) in the laboratory and reared in isolation for a generation in the quarantine room to avoid any contamination. During the process, any developmental stage that shows symptoms of disease is removed from the colony and destroyed by autoclaving. Strict sanitation is maintained to avoid infection and the spread of diseases. Slow-growing and stunted larvae, as well as malformed pupae and adults, are destroyed by autoclaving prior to disposal (*caution:* Never discard directly into the waste bin without autoclaving). When the second generation of the field collected populations reached pupal stage, the population is separated by sex and only the males are placed in cages with about the same number of laboratory reared female pupae for mating. This is to prevent introduction of diseases that might be transovarially transmitted by the wild females.

One additional safeguard against genetic deterioration is that the colonies are never reduced to only a few individuals. The average colony size during the maintenance production period is about 500 pupae per week.

18

Therefore, the maintenance of vibrant colonies of FAW is a matter of careful rearing, diligent monitoring of quality control parameters, and periodic strain restoration or replacement.

4.3. Rearing facilities

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

Rearing room

Fall armyworm is a very hardy insect; therefore, it can be successfully reared in any room with moderate temperature and relative humidity. However, efficient mass rearing is possible with the addition of more space, equipment, and controlled environment. These are 1) diet preparation, and infestation room, 2) larvae development room, 3) pupae harvesting room, and 4) adult emergence and oviposition room. Other facilities include dishwashing room, office and storage of supplies and equipment. The rearing laboratory is a new environment for the insects and should therefore have environmental conditions conducive to their development and effective field performance (temperature 25 ± 1°C; relative humidity of 75 ± 5%; and L12:D12 photoperiod). The entire building must be free from pesticide contamination and should be surrounded with a water trough at the ground level. The water trough prevents the entrance of crawling insects. The rooms should be kept free from disease, parasitoids, and predators.

Equipment

Installation of appropriate equipment in the rooms in relation to function saves time, increases efficiency, and enhances safety. Depending on the availability of resources and scale of operation, several pieces of equipment can be installed. The following items should be considered as essential or at least highly desirable equipment in any FAW insectary (Table 1). This list is not complete, but it is sufficient to run the existing rearing activities at icipe and ICRISAT-Niger. More specialized, industrial scale equipment may also be used for large-scale commercial production.

Table 1. List of equipment used for rearing FAW at *icipe* and ICRISAT-Niger insectaries.

No.	Equipment	Purpose
1.	Laminar hood	Clean, contaminant-free air during diet preparation, cooling and infesting the diet with larvae
2.	Microscope	Identification of microorganisms
3.	Electronic balance	Weighing diet ingredients
4.	Mixing pot	Diet container
5.	Spoon	Mixing diet ingredients
6.	Graduated cylinder	Measuring water
7.	Refrigerator	Storing larval diet, vitamin mix, and antibiotics, and chilling adults for transfer from emergence cages to oviposition cages
8.	Autoclave	Sterilizing autoclavable rearing materials
9.	Gas stove/microwave oven	Cooking diet
10.	Water distiller	Preparing distilled water for diet
11.	Room air conditioners	Temperature control in rearing rooms
12.	Room air humidifiers	Humidity control in rearing room
13.	Blender	Mixing diet components
14.	Thermometer	For measuring room temperature
15.	Heat fan	Maintain temperature
16.	Leaf grinder	To ground maize/sorghum leaf into powder
17.	Cages	Adult mating and oviposition
18.	Standby generator	To use in case of power failure

Laboratory consumables

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

Table 2. List of laboratory consumables and their function.

No.	Consumable	Purpose
1.	Detergent	Cleaning and disinfestations
2.	Cotton wool	Holding honey solution- food for moths, for covering vials
3.	Stationery	Recording diet, infestation date, collection date, etc
4.	Wax paper	Adult moth oviposition media
5.	Paper towels	Cleaning
6.	Glass wares	Diet preparation and rearing

Personnel

Insect rearing is a seven-day-a-week job. Like other animals, insects need daily care. To meet often tight scheduling, the rearing facility is independent from other units, which 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.

4.4. Diet

Laboratory colonies of herbivorous insects are commonly reared on artificial diets to reduce the labor, time, space, and associated costs of growing their host plants. These diets also simplify the synchronization of insect development with the availability of food and can be optimized to increase insect fitness above that of insects reared on natural foods (McMorran, 1965). Furthermore, the nutritional quality of these diets can be manipulated to facilitate research in areas including insect development, entomopathogens, insecticides, and plant resistance factors (George et al., 1960).

According to Singh (1983) an ideal diet for mass-rearing programs should have the following qualities:

a. Provide all nutrients needed to produce acceptable insects — nutritionally efficient and meet the insect's behavioral requirements.

b. Inexpensive and economical.

c. Safe to use and easily prepared from locally and readily available ingredients.

d. Long storage life.

e. Produce an average yield of adults of at least 75% from initial viable eggs. Size and rate of development should be similar to that in nature. The adults should mate, lay viable eggs and continuously reproduce without loss of vigor or fecundity. The behavior of the insects should be 'normal' and the quality 'acceptable.'

Rearing successive generations of Lepidoptera on nutritionally unbalanced diets can cause wing deformities, reductions in insect weight, fecundity, longevity, and increased mortality of all life stages (Hervet et al., 2016).

4.4.1. Diet ingredients and preparation

The insect diet is a mixture of nutritive substances including carbohydrates, proteins, fat, minerals, and vitamins, each with a specific function in the development of the insect and safe shelf life of the constituted diet (Table 3). Because of the polyphagous nature of FAW, it can be successfully reared on many diets that have been developed for cereal stem borers (Mihm,1983; Prasanna et al., 2018). Several synthetic diets for FAW have been optimized by various institutions, including *icipe*, the International Maize and Wheat Improvement Center (CIMMYT), International Institute of Tropical Agriculture (IITA), and the Agricultural Research Council (ARC)-South Africa, based on local availability of ingredients based on unpublished protocols. The descriptions below highlight how the synthetic diet for FAW insect mass rearing is prepared at *icipe*.

It is presented in three fractions (Fraction A, Fraction B and Fraction C).

Fraction A

Weigh all the powder ingredients using an electronic balance (Figure 8. A) following dietary checklist and tick/mark weighed components. Transfer all powdered ingredients in a mixing pot.

Mix all the powdered ingredients except Methyl paraben from fraction A using a spoon (Figure 8. B). Boil the distilled water, cool it to 60°C, and then mix with the pre-mixed ingredients using a blender for 1 minute (Figure 8. C). Add Methyl paraben (dissolved in 20 ml of absolute ethanol) to the mixture in the blender, and then mix for a further 2 minutes.

Figure 8. Weighing the diet ingredients (A), mixing all the powdered ingredients with spoon (B), and mixing pre-mixed ingredients using a blender (C) (M. Goftishu 2019).

Fraction B

Weigh agar powder in a separate container and then add to cold distilled water in a separate saucepan. Boil on a hotplate (Figure 9. A) while stirring periodically to achieve a mix with a spoon. Remove from the hotplate once it boils to raising point and stir while on the bench to avoid settling on the base of the saucepan, then cool to 60^0C. Add the ingredients of fraction B to fraction A and blend for 3 minutes (Figure 9. B).

Fraction C

After the diet has cooled to about 60^0C, add 40% formaldehyde, suprapen powder, and the heat-labile vitamin mixture to the ingredients of Fraction A and B and blend for 3 minutes continuously to be fairly distributed in the diet.
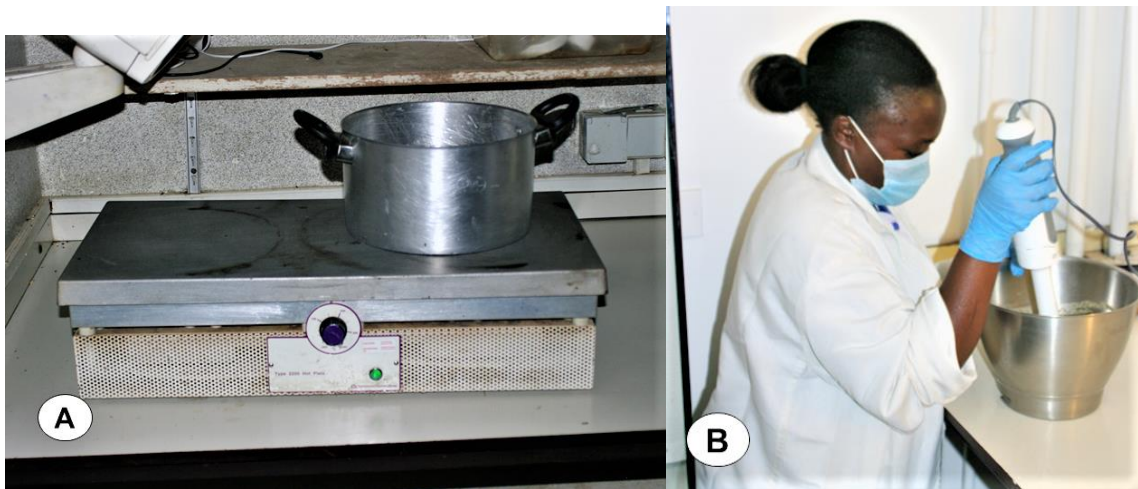
Figure 9. Boiling agar on a hotplate (A), and blending the ingredients of fraction B with fraction A (B) (M. Goftishu 2019).

Arrange rearing vials in the laminar hood for dispensing the diet. While still warm, using ketchup transfer pour (Figure 10. A), fill half of the volume of the vials (Figure 10. B) or one fourth of the volume of glass jars with the diet (Figure 10. C). Then, leave the diet uncovered in the laminar hood for at least 12 hours to allow the formaldehyde to volatize before inoculation of the larvae (Figure 10. B). If the diet is not to be infested within a day or two, the vials and jars are covered with cotton wool and metal lid (Figure 10. D), respectively, and refrigerated until needed.

Figure 10. Dispensing FAW artificial diet using ketchup transfer (A), to vials (B), glass jars (C) and capped vials (D) having diets ready for cold storage (M. Goftishu 2019).

Table 3. Diet ingredients for rearing FAW.

No.	Ingredient	Quantity (g or ml) per 1L of diet	Purpose
Fraction A			
1.	Bean powder	62.5g	Source of protein
2.	Wheat germ	50g	Source of roughage and mineral
3.	Maize leaf powder	25gm	Natural diet
4.	Milk powder	19g	Casein protein source
5.	Torula yeast	32g	Feeding attractant/induction and mineral source
6.	Ascorbic acid	3g	Vitamin C source
7.	Methyl paraben	2.5g	To prevent the growth of bacteria
8.	Sorbic acid	1.5g	To prevent the growth of fungi/mold
9.	Distilled water	500	Mixing the paste
Fraction B			
10.	Distilled water	350ml	For boiling agar
11.	Agar	11.5g	Gelling the diet
Fraction C			
12.	Vitamin mix	1ml	Vitamin B complex for healthy and uniform growth
13.	Formalin 40%	2ml	Long term preservative of food
14.	Suprapen powder (Tetracycline)	2.5g	Antimicrobial, treatment of insects if infected

FAW artificial diet ingredients given here are modified from stem borer rearing diet at *icipe* after having them tested with FAW for many generations and finally found suitable and recommended for FAW mass rearing.

4.5. Diet infestation

The surface of the diet in each vial and/or glass jar is first punctured in several places using a sterilized laboratory plastic rod to facilitate larval penetration. Before diet infestation, surface sterilize black eggs by immersing them in 10% formalin for 10 minutes or in 0.1% freshly prepared sodium hypochlorite to avoid microbial contamination. Then infest the diet using the sterilized blackhead eggs or neonate larvae from pre-sterilized eggs. Several FAW neonates can be introduced into one glass jar. However, starting from the third instar, the larvae need to be transferred to individual vials to avoid cannibalism. Transfer the latter instar larva from the glass jar with a sterile small fine-tipped camel hairbrush to glass vials containing the diet. Dip the brush in 0.5% of sodium hypochlorite, then in distilled water (Hathaway et al., 1971) each time a larva is transferred from the jar to the vial. After infestation, the mouth of the jar is covered with a paper towel and then covered with a screw cap that is ventilated with very fine wire mesh to prevent the larvae from escaping. The paper towel is meant to absorb excess moisture within the rearing jar during larval development. The vials are closed with tight fitting cotton-wool plugs and arranged in plastic trays (Figure 11.). The vials and jars containing the larvae are kept on shelves in larval rearing room at controlled environmental conditions (28 ± 2°C; 65 ± 5% RH; 12:12 light: dark photoperiod). The larvae are allowed to feed undisturbed until pupation.

Figure 11. Individual rearing of third instar FAW larvae in vials (M. Goftishu 2019).

4.6. Fall armyworm rearing procedures and colony handling

The science of insect rearing has advanced tremendously over the years as the need for laboratory-reared insects for entomological research such as bioassay testing, biological and toxicological studies. The success of any insect mass rearing program relies on continues supply of high-quality insects in adequate numbers, at specified times and at specific stages of development. In light of this statement, FAW is reared both at *icipe* and ICRISAT-Niger insectaries aiming to have enough insect population for biocontrol and related research studies.

Mass rearing procedures and colony handling of FAW are outlined below.

4.6.1. Management of larvae and pupae

i. Follow-up larval and pupal development daily to identify problems such as contamination with fungus or other arthropods such as ants or mites and discard any affected diet containers immediately. Moreover, closely examine the fourth and fifth instar larvae and select only healthy and vigorously growing ones.

ii. Monitor for pupal harvesting 14-20 days after diet infestation, and daily thereafter to avoid moth emergence within the rearing vials.

iii. Harvest pupae at once when at least 50% of the larvae have pupated. Keep larvae that have not pupated in the vial until they pupate. To harvest pupae, empty the diet from each glass jar onto a clean tray, sort and transfer the pupae into a plastic container lined with paper towel.

iv. Clean the pupae with a gentle spray of distilled water, and place on paper towel to drain excess moisture.

v. Transfer the pupae to clean Petri dishes (9 cm in diameter) lined with tissue paper. Each Petri dish can accommodate about 100 pupae.

vi. Place Petri dishes in the bottom of adult emergence cage, ventilated at the sides with fine wire mesh (Figure 12. A).

vii. Keep the emergence cages at room temperature ($25 \pm 1°C$); 12:12 light: dark photoperiod; and a relative humidity of $75 \pm 5\%$. The humidity can always be maintained by placing a plastic cup containing water-soaked cotton wool in the cage (Figure 12. A).

4.6.2. Management of adults

i. For adult moth oviposition, plan and plant maize seed- three to four maize seeds per pot. When the seedlings reach 3-4 weeks old, place them into oviposition cage for moth to lay eggs (Figure 12. B) or detach fresh maize leaf from the field planted maize and place in oviposition cage/jar as oviposition substrate. To keep the leaf green, soak cotton wool in water and wrap the bottom tip of the leaf inside a water-soaked cotton wool (Figure 12. E). Replace the leaves every 2-3 days, depending on how long they remain green and fresh. Wax paper could also be used as a substrate for oviposition. Line the lateral sides of the oviposition cage with a sheet of wax paper (Figure 12. C).

ii. Place 50% honey solution soaked in cotton wool inside the cage for the moths to feed on (Figure 12. D). Keep about 100 moths (50 pairs) in each oviposition cage.

iii. Daily, check each oviposition cage and:
   a. Collect eggs that have been oviposited on maize leaves or wax papers.

   b. Remove dead moths from each cage.

   c. Pick and transfer the live moths to a freshly prepared cage containing fresh maize leaves/fresh wax paper and fresh honey solution-soaked cotton wool.

   d. Clean and disinfest the cage for reuse.

FAW prefers maize seedlings for oviposition and lays more eggs on the natural substrate than on wax paper. Most likely, the natural host/maize supports the adult moth during resting (Figure 10. F).
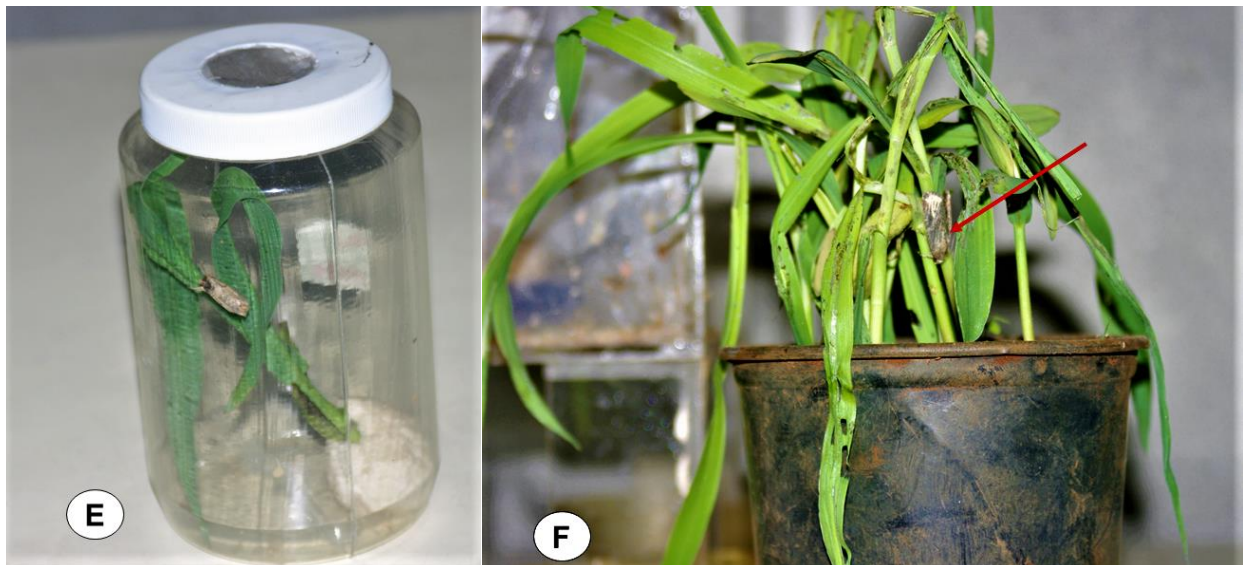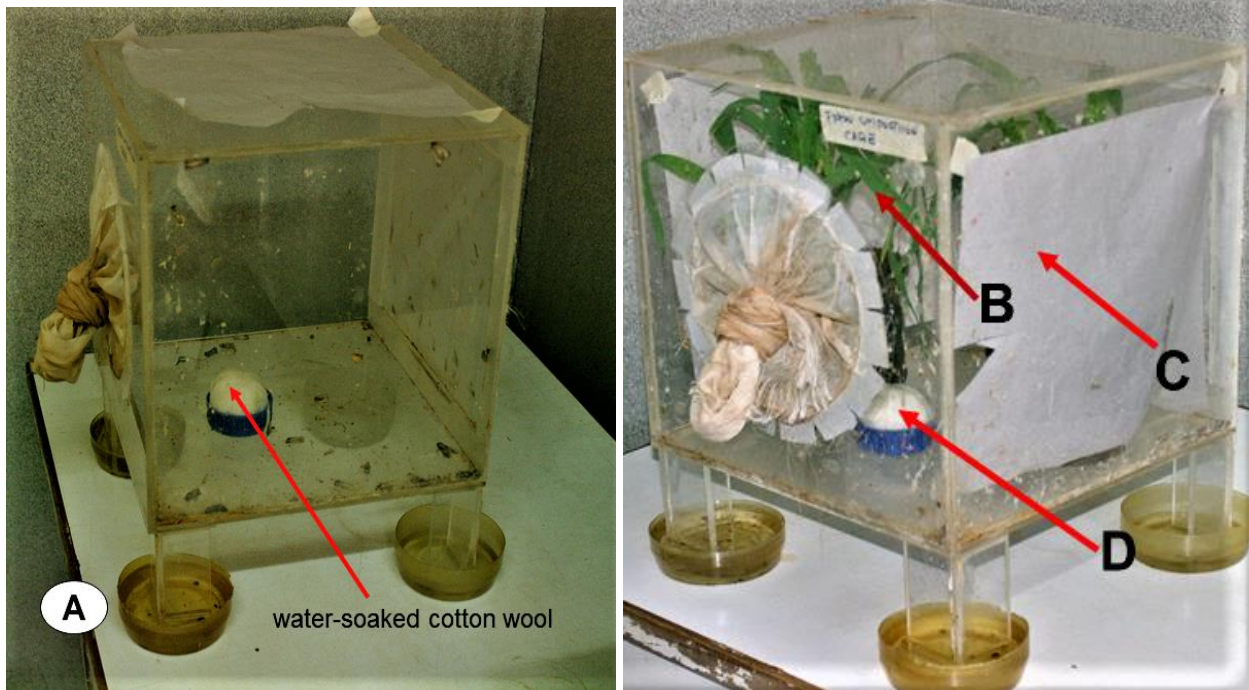
Figure 12. Adult emergence cage (A), Oviposition cage- maize seedling (B), wax paper (C), honey solution soaked in cotton wool (D), maize leaves on water-soaked cotton wool (E), adult moth at rest (F) (M. Goftishu 2019).

4.6.3. Management of eggs

i.   Each day cut the maize leaves or waxed paper sheets with attached eggs using scissors (Figure 13. A).

ii. Use some part of the eggs for continuation of the laboratory colonies and the rest of the eggs for rearing the parasitoid *T. remus* (Figure 35)

iii. Surface-sterilize the eggs found on the maize leaves or on the wax paper intended for maintaining the culture of FAW by dipping them in 10% formaldehyde or 0.1% freshly prepared sodium hypochlorite for 15 minutes, rinse them carefully using distilled water, and then dry them on paper towel.

iv. Transfer the surface-sterilized egg masses in lunchbox on moist paper towel, cover the box with a well-ventilated top, and place the boxes in the oviposition room (Figure 13. B).

v.   Maintain a relative humidity of 80-90% in the lunchbox by placing a Petri dish with water-soaked cotton wool at the bottom of the box, below the paper towel.

vi. The development of the eggs can be arrested by placing them in a refrigerator at 10^0C for up to three days without loss in hatchability.

vii. In about 4-6 days, the eggs develop into a blackhead stage, and then hatch into neonate larvae. Both the black-head stage eggs and the neonate larvae can be used to infest the artificial diet or can be used for any other operation.
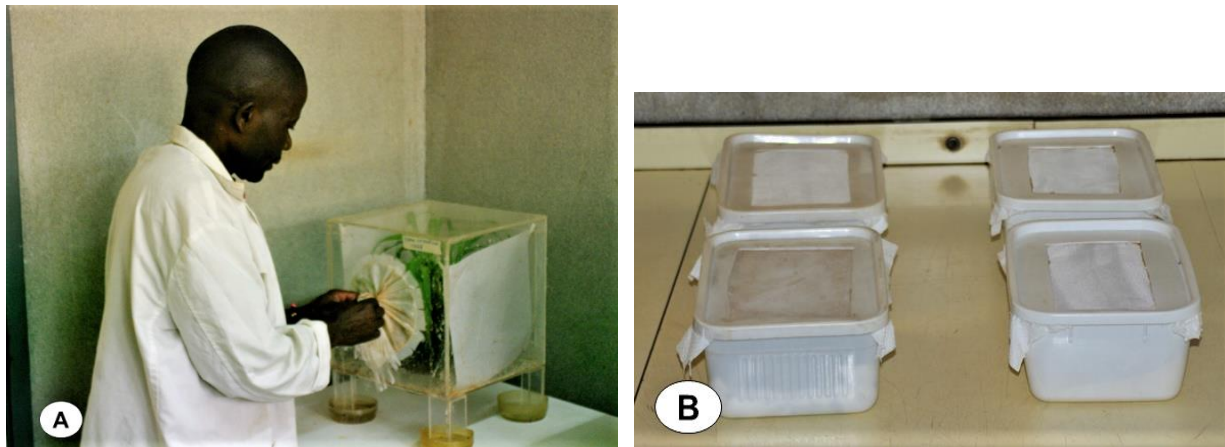
Figure 13. Collecting FAW eggs from maize leaves and waxed paper (A), and surface-sterilized egg masses in lunchbox (B) (M. Goftishu 2019).

4.7. Quality control parameters

Quality control in insect mass production involves the production of individuals with fitness and vigor comparable to wild population. Quality control is a 'fitness for use' philosophy (Rogers and Winks, 1993) and related to 'biological vigour' (Moore, 1985). According to FAO/IAEA/USDA (2003) quality control is a systematic process whereby management critically evaluates the elements of production, establishes standards and tolerances, obtains, analyses, and interprets data on production and product performance, and provides feedback to predict and regulate product quality and quantity. A quality management system should therefore be implemented if long-term use of insects is planned.

As an integral part of the production system, quality control provides a means of optimizing insect mass-rearing by identifying and gradually correcting deficient production processes, thereby preserving the quality of the strain (Leppla and Ashley, 1989).

Over the generation period of laboratory rearing, insects could be exposed to natural inbreeding, genetic drift, and inadvertent selection and adaptation to rearing conditions, which can unknowingly affect insect behavior and performance in the field and produce insects of reduced quality (Calkins and Parker, 2005; Parker, 2005; Rull et al., 2005).

In this regard, the *icipe* and ICRISAT-Niger insectaries attempt to produce insect populations that are as competitive as the wild population of the same species. The wild populations are therefore used for relativity studies against the performance of the laboratory-reared insects. The parameters used in determining quality of laboratory-reared insects include survival rate, developmental period (egg to adult), deformities, reproductive capacity (sex ratio, adult longevity, fecundity, hatchability), growth index (the ratio of percent pupation over mean larval development period), and adaptability under field conditions. The quality of the laboratory-reared insect is monitored periodically against the aforementioned quality parameters.

Generally, tests used to monitor the quality of laboratory-reared insects must be reproducible, economical and simple (Huettel, 1976). The concept of quality control in insect mass rearing became critical when the field behavior and performance of released insects was the most important element (Webb et al., 1981).

Generally, quality control is divided into three categories: production, process, and product quality control (Leppla and Ashley, 1989; Leppla and Fisher, 1989; Calkins and Parker, 2005; Dyck, 2010). Production quality control involves the inputs into rearing (e.g. diet ingredients and environmental conditions), process quality control involves the actual procedures involved (diet preparation, insect collection and holding) and product quality control focuses solely on the produced adult insects and their ability to complete the purpose for which they were reared (Dyck, 2010).

Leppla and Ashley (1989) categorized quality control of mass reared insects into three main interrelated categories:

1. Production quality control addresses the availability, standard/quality and timeliness of the inputs to rearing such as diet ingredients, materials and equipment. At the *icipe* facility, production quality control ensures the availability and efficient storage of diet ingredients and rearing equipment.

2. Process quality control assures the performance of the production process so that unacceptable deviations do not occur in product quality. Here, logistical consideration

particularly strict compliance to rearing procedures and maintenance schedules, are crucial. Moreover, environmental conditions such as temperature, RH, and photoperiod are checked regularly to adjust the conditions of the rearing operations.

3. Product quality control regulates the conformity of the product to acceptable standards of quality and predicts the effectiveness of the product in performing its intended function. In this regard, biological parameters are evaluated to identify possible deficiencies and to predict insect quality. Production can immediately be improved through testing of key and sensitive parameters and feedback mechanisms. Tests should be practical, uncomplicated, efficient, and reproducible. A minimum number of parameters using the smallest sample size are recommended.

Tests used to monitor the quality of laboratory-reared insects must be reproducible, economical, and simple (Huettel, 1976). The important parameters used in determining quality of laboratory-reared insects are described above. For FAW for example, if the laboratory insect population quality declines below a threshold of at least 300 well-formed, disease-free eggs per female, discard the population (Abrahams et al., 2017), collect a fresh founder colony of wild insects, and repeat establishment of a fresh colony (see section 4.1.).

5. Mass rearing of the rice meal moth (*Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae)): factitious host for *T. chilonis*

5.1. Factitious prey

The successful production of any parasitoid/predator is dependent upon the effective mass production of its host- natural or factitious. Cost of production may be reduced when predatory insects are produced on unnatural or factitious prey that are easier and less expensive to rear than on the natural prey. Factitious prey is comprised of organisms that are not normally attacked by the parasitoid/predator, mostly because they do not occur in its natural habitat, but do sustain its development in a laboratory environment (De Clercq, 2008). Factitious prey may be offered fresh, but in many cases, they are irradiated or frozen for improved storage or use in parasitoid/predator cultures (Riddick, 2009).

Trichogramma wasps can be mass reared, usually not on the target host but instead on a factitious host that allows for easy production and minimizes cost, which maximizes efficiency and output. The most common lepidopterous insect hosts used for mass rearing of *Trichogramma* are the angoumois grain moth (*Sitotroga cereallela* (Olivier) (Lepidoptera: Gelechiidae), rice meal moth (*C. cephalonica*) and Mediterranean flour moth (*Ephestia kuehniella* Zeller) (Lepidoptera: Gelechiidae). All these species of moths can be easily and inexpensively raised on a diet of good quality grains and are commonly used to rear *Trichogramma*. (Wang et al., 2014).

5.2. Description and life cycle of *C. cephalonica*

Adults mate shortly after emergence. There is a pre-oviposition period of about 2 days. The female moth lays 250-300 small white eggs. Egg-laying mainly occurs during the night. The greatest numbers are laid on the second and third days after emergence, although oviposition may continue throughout life. Eggs take about 4-5 days to hatch. There is a variation in the number of larval instars; however, males generally have seven and females have eight instars. At 28^0C and 70% relative humidity, larval period takes 28-34 days after which pupation takes place. The last-instar larva spins a closely woven, tough, double-layered cocoon in which it develops into a pupa.

The last-instar larvae pupate within the food. Pupa is pale brown in colour and pupal period is nine days. Total duration of the life cycle at 28^0C and 70% relative humidity takes 40-50 days. The adults emerge through the anterior end of the cocoon, where there is a line of weakness. The sex ratio is 1:1 (Mbata, 1989).

5.3. Colony establishment

To begin the mass production with nucleus culture, collect a large starter colony of at least 500 adult *C. cephalonica* individuals from granaries or warehouses located at distant locations to minimize inbreeding. Collect adults with sweeping nets or by gently aspirating them to prevent injury. Place 20 to 25 randomly selected adults in each oviposition jar (Figure 14. A) and place them in the rearing room. The lid of the jar has a circular window sealed with wire mesh for ventilation (Figure 14. B). Keep records of the source of the insect strain and the date that the colony was initiated.
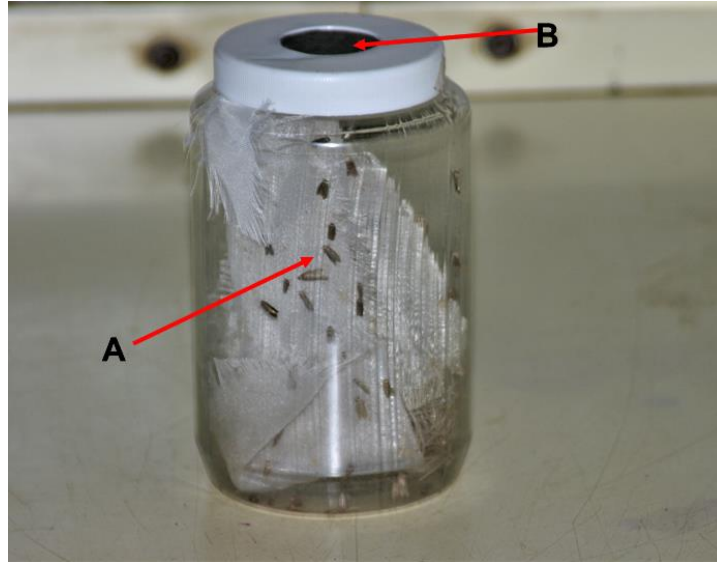

Figure 14. Adult *C. cephalonica* in oviposition jar (A), and window for ventilation (B) (M. Goftishu 2019).

Once adults are acquired, they should remain in isolation until it is reasonably certain that they are free of diseases and parasitoids. Generally, these problems can be detected by regular visual

inspections of larval and adult populations in the new colony. The presence of pathogens and parasites should become evident in new cultures within a few weeks. Moreover, closely observe the occurrence of other contaminants such as mites.

If colonies are contaminated with mites and psocids, they may be difficult to disinfest. The adults from the colony can be transferred frequently and destroying the old medium immediately. Lowering the RH makes the colony environment less desirable for mites and psocids. If this procedure does not work new breeding stock may be necessary. Appearance of mites and psocids may be an indicator of high moisture in colony because of overcrowding or poor moisture control.

5.4. Colony maintenance

An important consideration when insects are mass-reared is to ensure that the laboratory colony exhibit the genetic diversity and aggressiveness that the pest population exhibits in nature. Accordingly, colony maintenance involves periodic gene infusion done by mating the laboratory colony with the wild population. It is used to avoid genetic decay, maintain heterozygosity among insect populations and to preserve the original wild behaviour of the insects. At the *icipe* insectary, adult *C. cephalonica* are periodically collected from diverse area. The collected wild populations are reared in isolation for one generation to avoid any contamination. Parasitized, diseased, and deformed insects are discarded. The second generation can be cross-breed with the laboratory colony.

5.5. Rearing facilities

The rearing room

Ideally, a tightly closed room or other enclosed space should be designated for rearing *C. cephalonica*. The entire building must be free from pesticide contamination and should be surrounded with a water trough at the ground level. The water trough prevents the entrance of crawling insects. The room should be protected from fluctuations in temperature and relative humidity (RH). All cultures should be maintained at a constant temperature of 27°±1°C, 65 ± 5% relative humidity and 12:12 light: dark cycle. Equipment to maintain these conditions includes

humidifiers, air conditioners, and heaters with control mechanisms such as humidistats and thermostats.

Rearing containers

The most simple and convenient container for rearing *C. cephalonica* is a transparent plastic bucket/ rearing cage. The rearing cage is fabricated from a 5-10-liter capacity plastic container with lid (Figure 15. A). A window of 15 to 20 cm diameter is cut from the centre of the lid and covered with mesh by heat sealing for ventilation (Figure 15. B). In addition to the rearing cage, a transparent plastic jar with 1 to 1.5 litters capacity for adult oviposition is used (Figure 14). The lid of the oviposition jar has a circular window sealed with wire mesh for ventilation.

Each rearing cage should be labeled on the outside, indicating the species contained and the date on which the colony was set up. This information can be written directly on the outside surface of the jar using a felt-tip marker with water-soluble ink. This method of labelling is especially convenient because the labels are easily removed by washing, particularly if the jars are to be reused. However, the simplest way is to use printable labels and to fix it on the outside of the cages.
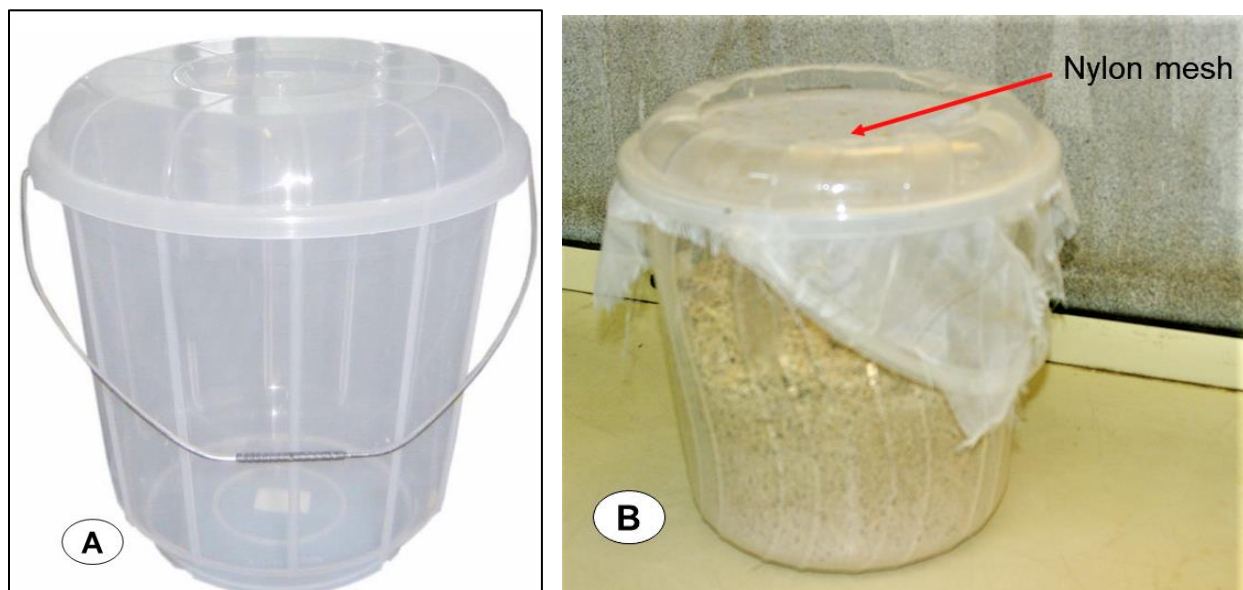

Figure 15. Transparent plastic bucket/ *C. cephalonica* rearing cage (A), and rearing jar covered with nylon mesh for ventilation (M. Goftishu 2019).

A list of equipment used in rearing *C. cephalonica* at the *icipe* insectary is given below (Table 4). This list by no means exhaustive; however, it is enough to rear the factitious host *C. cephalonica* for mass production of *Trichogramma*.

Table 4. List of equipment used for rearing *C. cephalonica*.

No.	Equipment	Function
1.	Cylindrical rearing cage	Rearing *C. cephalonica*
2.	Rectangular cage	Collecting adults from rearing cage
3.	Plastic jar	Adult mating and oviposition
4.	Glass vials	Transferring adults to oviposition jar
5.	Nylon mesh	Oviposition media
6.	Sweeping net	Collecting adults from warehouses
7.	Aspirator	Collecting adults from warehouses and rearing cages
8.	Face mask	To protect inhalation of moth scales
9.	Electronic balance	Weighing diet ingredients
10.	Graduated cylinder	Measuring eggs volumetrically
11.	Grain moisture tester	Determine grain moisture content
12.	Petri dish	Collecting *C. cephalonica* eggs for sterilization
13.	Tweezers	Holding insects
14.	Oven	Sterilizing autoclavable rearing materials
15.	Fumigation barrel	Disinfestations of grain
16.	Refrigerator	Disinfestations of grain, temporary storage for *C. cephalonica* adults and eggs
17.	Laminar flow	Sterilizing *C. cephalonica* eggs
18.	Home milling machine	Grinding grains
19.	Room air conditioners	Temperature control in rearing rooms
20.	Room air humidifiers	Humidity control in rearing room
21.	Thermometer	Temperature recording
22.	Heat fan	Heating the room

Rearing media

C. cephalonica is reared on coarsely milled or broken maize/sorghum/rice/pearl millet/wheat or wheat bran. When the grain is first received, it must be cleaned and dried before storage or usage. Cleaning could be done by sieving to remove any excess dirt, dust, fine materials, or moldy, broken or shriveled kernels. Once the grain is clean and dry, it should be held in a deep freezer at -20°C for two weeks or fumigated with phostoxin tablets in a plastic drum or barrel for seven days, then transferred to cold storage (4°C) until it is needed for cultures. This prevents unwanted infestation of the grain.

For a grain to be used for an insect colony, it should be checked for proper moisture content. To be in approximate equilibrium with the relative humidity of the rearing room (at 27°C, 65% RH), whole grains should have the following moisture content levels: wheat 12 to 13%; millet 12 to 13.5%; sorghum 12 to 13.5%; maize 13 to 14%. If the moisture content of the whole grain intended for the colony media is not suitable, it must be adjusted; if too high, the grain can be dried by spreading on a clean floor and blowing air over it. Once the desired moisture level is reached, the grain is returned to the freezer to destroy any possible infestation before it is ready for insect rearing. If the moisture content of the grain is too low, then water must be added. This is done by placing grain in a container or any mixing device, then gently and evenly adding distilled water over the surface. The formula below gives a guide on the amount of water to add:

where Q = weight of water to be added, A = initial weight of grain, a = initial grain moisture content (wet weight basis) and b = desired final grain moisture content of grain (wet weight basis) (Boxall, 1986). Seal the container and rotate or mix it for 30 minutes. If the moisture is determined to be appropriate, put the grain in storage for future use. If not, then repeat the tempering process or dry as needed.

During mass rearing of the factitious host, knowledge of grain varietal characteristics is also important. Varieties susceptible to stored-grain insects should be used in cultures. Furthermore, make sure the grain used for mass rearing of meal moths is not treated with pesticide. Purchasing grain for mass production from a commercial dealer is unwise, because untreated grain may have been mixed with insecticide treated grain. It is important to know the storage history of grains if they are not purchased directly from the field.

If there is any concern about chemical contamination in a batch of grain, a bioassay should be conducted. To do this, add 100 adult insects of the same species to each of several 250 g samples of the grain. In addition, prepare control samples with grain that is known to be uncontaminated. All the jars should be set up on the same day and checked one week later to determine mortality. If the suspect grain is contaminated, the numbers of insects surviving in those samples should be significantly lower than those in the control samples.

5.6. Mass production of *C. cephalonica*

The reproductive capacity of parasitoids depends on the suitability of the selected host for its development (Vinson and Iwantsch, 1980). Accordingly, parasitoids are able to discriminate between different qualities of host. Different criteria can be considered by the parasitoid after contacting a potential host, like host size, age, nutritional suitability and previous parasitism (Schmidt, 1994). Among *Trichogramma*, there is generally a preference for younger eggs over more developed ones (Tuncbilek and Ayvaz, 2003; Saour, 2004; Makee, 2005). For this reason, mass rearing of *Trichogramma* requires large amounts of high-quality host eggs. Moreover, those eggs must be available at the precise time and amount when farmers/growers need *Trichogramma*. In order to keep large amounts of young eggs, host eggs are sterilized early and so stop their development. Sterilization also prevents the subsequent cannibalism by hatched larvae on unhatched parasitized eggs (Mansour, 2010).

A large number of *Trichogramma* is needed for inundative releases and in most cases, mass rearing them on the target pests is too difficult and costly. Thus, mass rearing of quality factitious host is imperative in inundative and augmentative releases of *Trichogramma*. Among the lepidopterous insect hosts, eggs of *C. cephalonica*, also known by the common name "rice meal moth," is extensively used for mass rearing of *Trichogramma*.

Corcyra cephalonica is a stored-product pest of grains and can be raised on a diet of good-quality maize/sorghum/rice/pearl millet/wheat or wheat bran. It is a well-known storage pest with a global distribution.

In this section, we aim to provide general guidance on the necessary steps, procedures and tools used at *icipe* and ICRISAT-Niger insectaries for mass production of *C. cephalonica* eggs.

Preparation of rearing containers and equipment
- The rearing cages, oviposition jars, and any equipment (Petri dishes, vials, nylon mesh) used for *C. cephalonica* mass rearing should be properly cleaned with detergents, rinsed in tap water and shade dried. Whenever the rearing cages are emptied after a cycle of rearing, they must be cleaned preferably with 2% formaldehyde and returned to store until further use. The cleaning steps are repeated during reuse.

Preparation of rearing medium for C. cephalonica
- Kindly refer to section 5.5 on methods of preparation and disinfestation of culture media (grain) for *C. cephalonica* rearing. Fumigation or deep-freezing of grains to prevent unwanted infestation may take one to two weeks. To shorten the time elapsed for grain disinfestation and to hasten the rearing process, heat sterilize the coarsely broken grains at 100^0C for 30 minutes. This clears the residual population of stored product insect pests, namely, *Rhyzopertha dominica* (Fabricius) (Coleoptera: Bostrichidae)*, Sitophilus zeamais* (Linnaeus) (Coleopteran: Curculionidae)*, S. cerealella, Tribolium castaneum* (Linnaeus) (Coleopteran: Curculionidae)*,* mites and other fungal contaminants.

- After sterilization, cool the grains under fan in a clean area and spray with 0.1% formalin to prevent the growth of moulds as well as to increase the grain moisture content to the optimum (see section 5.5. about rearing media preparation), which is lost due to heat sterilization. Then transfer the grain to each rearing cage. To provide adequate space for the emerging adult moths, pour the grain to the level of one-third to half of the volume of the rearing cage (Figure 16).
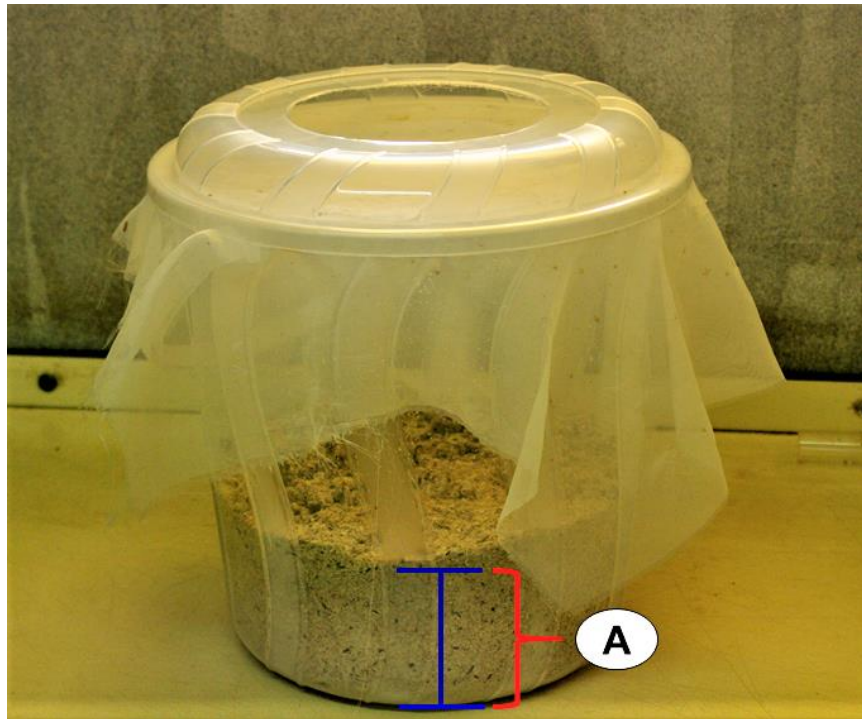
Figure 16. Volume (amount) of grain filled in the rearing container for *C. cephalonica* rearing (A) (M. Goftishu 2019).

- The number of buckets (rearing cage) required for egg infestation is decided based on the anticipated needs and the amount of *C. cephalonica* eggs prepared for charging the grain.

Preparation of C. cephalonica eggs

- Collect the freshly laid *C. cephalonica* eggs. Clean the eggs from contaminants like the moth scales and broken appendages and do not expose to UV light.

- Measure the volume of overnight laid eggs to determine the number of buckets that can be inoculated with eggs. The quantity of the egg is assessed in a graduated cylinder volumetrically. One cc of *C. cephalonica* eggs estimated to contain approximately 16,000 eggs (Jalali and Singh, 1989).

Infestation of medium with eggs

- To begin the mass production, infest the medium with *C. cephalonica* eggs in desired quantities. Carefully spread the eggs evenly on top of the grain and close the rearing cage. For each 5 kg of grain, apply one cc of *C. cephalonica* eggs and secure the lid.

- Record infestation data: date of infestation, kg of medium used, and g of eggs used. This data will be needed later to calculate the average output of the rearing facility.

Handling of larvae

- After 3-4 days, the larvae hatch and begin to feed on the grain. At this stage, light webbings are noticed on the surface. As the larvae grow up, they move down. During this period, the larvae can grow undisturbed in the buckets (Figure 17).

Figure 17. Larval rearing inside plastic cages (M. Goftishu 2019).

Handling of adults

- The adults begin to emerge in 40-50 days after infestation of the eggs (at 27^0C and 70% RH). The adults can be seen hanging on the inner side of the lid on the nylon mesh.

- Collect the moths from the rearing cage at least once a day; it is best to do this in the morning, due to low mobility of insects. At times of peak moth emergence, collect a second time in the afternoon. This is to avoid moths starting to lay eggs in the rearing cage. The collection extends over a period of 15 to 20 days.

- To collect the moths, place the rearing jar inside a big rectangular cage, then open the lid of the rearing jar and empty the adult moths. The rectangular cage prevents escape of the moths. A screened window (15 cm in diameter) on each side of the cage provides ventilation. The front of the cage has an opening (15 cm in diameter) with a cloth sleeve attached to provide access into the cage (Figure 18. A).

- Collect the newly emerging moths from the cage using a glass vial. When the mouth of the glass vial is brought near them, the moths tend to jump back and thus get into the vial. Once the moth gets into the vial, the mouth is closed with fingers (Figure 18. B). Use the same tube until enough moths (up to the capacity of the vial) are collected and then transfer them to the oviposition jar (Figure 18. C) by tapping the vial with finger. In each oviposition jar, place 30 to 50 moths. Workers involved in the collection of moths should wear mask to avoid inhalation of scales.

- As much as possible, keep moths emerging on different days in separate jars.

Figure 18. Cloth sleeve that provides access into the cage (A), glass vial for adult moth collection (B), and oviposition jar for adult *C. cephalonica* moths (C) (M. Goftishu 2019).

Handling of eggs

- Every 24 hours, collect *C. cephalonica* eggs from the oviposition jar. The moths lay eggs in large numbers loosely. To collect the eggs, place the oviposition cage inside a bigger cage and empty the adult moths as indicated above to prevent escape of moths. Adult *C. cephalonica* lay their eggs on nylon mesh lined inside the oviposition jar (Figure 18. C). A strip of nylon mesh suspended inside the oviposition jar where the top portion of it fitted with the lid of the jar provides area for oviposition, walking and resting for adult moths.

- Thoroughly shake the nylon mesh to drop the eggs at the bottom of the jar and collect the eggs onto printing paper. Transfer those moths released in the rectangular cage for egg harvesting into the oviposition cage for further egg laying. Repeat this operation for 3 to 4 days and discard the moths then after.

- Clean the freshly laid eggs from contaminants like moth scales and broken insect appendages by gently rolling the eggs on printing paper and pour the cleaned eggs in a small Petri dish (Figure 19. A).

- Weigh the eggs with sensitive balance (Figure 19. B) and quantify the number of eggs.

- Use some part of the eggs for maintaining the culture of mill moth and the rest of the eggs for rearing the parasitoid, *Trichogramma*.
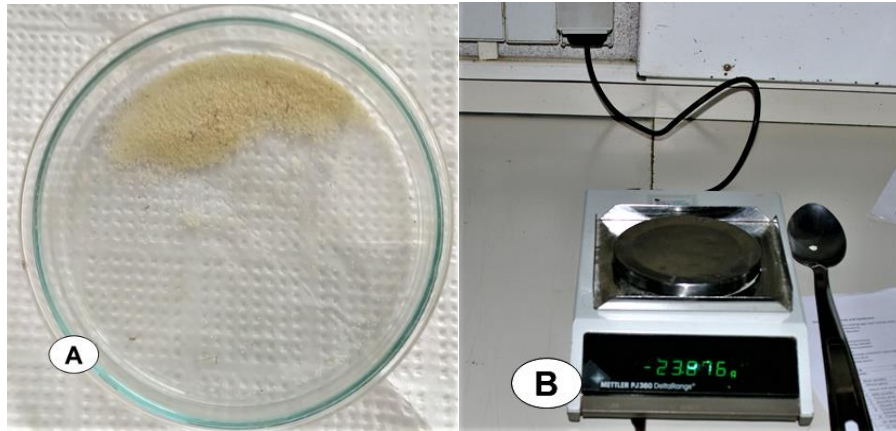
Figure 19. Cleaned *C. cephalonica* eggs (A), sensitive balance (B), and laminar flow for egg sterilization (M. Goftishu 2019).

As with nearly all lepidopterans, adult mill moths have scales on their wings that might affect the lungs of workers in the insectary. A small number of people also show a certain level of allergic reaction to the scales. Therefore, it is important to keep the operation clean and to consider wearing masks in the rooms where adult moths are kept.

Egg sterilization
- Expose those eggs used for parasitoid rearing to ultraviolet rays of 15-watt UV tube for 45 minutes or 30-watt UV tube for 10 minutes to prevent hatching of mill moth eggs. During UV exposure, maintain 12-15 cm between the eggs and the tube for optimum egg sterilization. The precise time will depend on the exact distance to the light bulb and may need to be slightly adapted according to experience. Be careful to increase just the time, because placing the eggs too close to the UV light will lead to desiccation, a major obstacle in *Trichogramma* production. Laminar flow or UV chamber could be used for sterilization (Figure 19. C).

- Using this treatment, the embryo is killed, whereas the egg quality is not affected. This prevents the destruction of parasitized eggs by rice meal moth larvae if they are not treated with UV rays. The eggs could also be made inviable by exposing them to very low temperatures $0\text{-}2^0$C in the freezer for 3-4 hours. By freezing, the quality of the host eggs is found to shrink and the eggs form lumps due to moisture.

- Keep the sterilized egg for tricho card preparation (see section 6.3.).

Storage of C. cephalonica eggs

A carefully planned system is needed to optimize synchronization of host and *Trichogramma* production; storage of eggs is one component of this system. However, storing host eggs at low temperatures decreases their fitness. Research results indicated that the storage of host eggs within

the host ovariol is a more suitable strategy than cold storage of the eggs for mass rearing of egg parasitoids (Ayvaz and Karaborklu, 2008).

In case of storing eggs used for *C. cephalonica* rearing, the eggs should be kept at about 10°C for not more than 10 days because longer storage time increases the mortality of larvae and adults, and also because moths produced from such low-quality eggs will have decreased egg production themselves. *Corcyra cephalonica* eggs sterilized by UV at 4°C should not exceed 15 days for mass-rearing of *Trichogramma*. The survival rates of *Trichogramma* embryos, larvae and pupae decreased significantly when *C. cephalonica* eggs were cold-stored for more than 15 days (Wu et al., 2018). *Corcyra cephalonica* eggs stored for longer can also be used for *Trichogramma* production, but will be less attractive to *Trichogramma* adults, resulting in a reduced parasitism and emergence rates (Wu et al., 2018).

Always make sure that the correct-sized containers are used in order to avoid egg desiccation. Use well-sealed containers with not much air compared with the volume filled by the eggs or simply place the eggs in glass or plastic tubes/vials and cover with cotton wool wrapped with paper towel.

Treatment of used medium
The used medium cannot simply be discarded away, as *C. cephalonica* is a storage pest. Even after a few months, there will still be a few moths emerging from the used grain, which could possibly infest storage facilities in the nearby surroundings.

- If no risk exists, i.e. there is no storage of grains nearby, then one of these recommendations should be followed:
    - ✓ Use the medium as a feed for pigs and chickens if it is dry.
    - ✓ Use it for composting or for producing organic fertilizer.

- If all the remaining *C. cephalonica* need to be killed, either heat or cold treatments can be used. Place the used medium in an oven for about 2 h at 100°C, or in a deep freezer (-20^{0}C) for a few days.

5.7. Quality control

- The most important quality criteria for *C. cephalonica* is the hatch rate (proportion of healthy host larvae hatching from the eggs).

- Another relevant parameter is egg size, since *C. cephalonica* eggs that are too small will not support the development of a *Trichogramma* adult.

- Mark paper card to the size of 10 mm x 20 mm with ruler and pencil and cut the marked area with scissors. Apply a thin layer of glue on the paper card, then sprinkle about 100 freshly laid (unparasitized) eggs of *C. cephalonica* and distribute evenly over the glue-covered area. These pieces of paper card are placed in a labelled glass vial, sealed with a lid and stored at about 25°C and 70–80% RH. After 5 days, check how many larvae have hatched. Between five and ten sample cards should be checked.

- Cut 10 mm × 20 mm pieces of cardboard paper with scissors. Apply a thin layer of glue and then sprinkle about 100 freshly laid (unparasitized) eggs. Place the cardboard in a labelled glass vial, sealed with cotton wool a lid and stored at about 27°±1°C and 65 ± 5%RH. For the assessment, 5-10 samples should be checked. After six days, check how many larvae have hatched.

- The normal hatch rate is over 95%, but slightly lower rates up to 75% is acceptable. If hatch rate becomes less than 70%, something must be wrong, and action needs to be taken. The most likely causes for low hatch rate are high temperature or low humidity, but it could also be due to bad-quality grains being used.

6. Mass Rearing of *Trichogramma* spp.

Interest in using insect parasitoids and predators to manage pest populations has increased as the undesirable consequences of excessive pesticide use has become more evident.

Parasitoid wasps of genus *Trichogramma* (Hymenoptera: Trichogrammatidae) includes several species that are frequently used as biological control agents worldwide (Consoli et al., 2010). *Trichogramma* has a cosmopolitan distribution and parasitize more than 200 species of insect pests, the majority of them primary egg parasitoids of Lepidoptera (Tanwar et al., 2006). *Trichogramma* has the distinction of being the maximally produced and released natural enemy in the world (Manjunath et al., 1988). This is mainly because of the following attributes: short life cycle, most in tropical climate have 8-10 days from egg to egg-laying period; high breeding potential (a female can produce in its lifetime of 6-7 days of active egg-laying period between 25 and 80 females); high percentage of female progenies (60–90 %); kills the pest in the egg stage before the pest could cause any damage to the crop; amenable for mass production in the laboratories; most adapt to laboratory factitious hosts of lepidopteran insects (Nagaraja, 2013). Generally, the mass production technique of all *Trichogramma* spp. is very similar.

Naturally occurring parasitoids and predators are often not present in sufficient numbers at the right time to keep pest species within an economically sustainable limit. *Trichogramma* release programs can be used to overcome these limitations. There are two ways to use *Trichogramma* release in pest control. "Inoculative" releases create the conditions for an early start, to maintain and augment an existing population, or "inundative" releases introduce large numbers of insectary-reared *Trichogramma* to coincide with maximum host presence. Both approaches aim to increase *Trichogramma* parasitism of the pest to reduce crop losses.

6.1. *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) description and life cycle

The parasitoid, *T. chilonis* are free living, tiny wasps found in most crops. The development of all *Trichogramma* spp. is very similar. Being an egg parasitoid, the female *Trichogramma* is able to locate the pest egg on the crop and parasitize it by inserting her egg in it (Figure 20). Instead of pest larvae emerging from the eggs, more adult *Trichogramma* appear, which can then search and parasitize additional hosts in the crop. The number of eggs laid per host may vary from 1 to 20 or more depending upon the size of the host egg. *Trichogramma* wasps are the most commonly used biocontrol agents in open field crops worldwide.

Generally, the development of all *Trichogramma* spp. is very similar. *Trichogramma* spp. undergo complete metamorphosis. There are three larval instars, all sacciform. These are followed by a prepupa, when the adult characters form, and a pupa. At the beginning of the third larval instar, the host egg turns black due to the deposition of black granules at the inner surface of the chorion, an invaluable diagnostic character for parasitized eggs. The egg, larval and pupal stages of *Trichogramma* at 28 ± 2^oC are completed in 1 day, 3 to 4 days, and 4 to 5 days, respectively (Figure 20). Thus, *Trichgramma* wasp completes the development within the host egg and emerges out after 8 to 10 days. Mating and oviposition occur immediately after emergence. Adults are very small, ranging in size from 0.2-1.5 mm (Nagaraja, 1987). They have a yellow black compact body, red eyes and short antennae. *Trichogramma* adults live for about 4-7 days under laboratory condition. Fecundity varies from 20 to 200 eggs per female according to the species, the host and the longevity of the adult. The sex ratio is generally 1:1. One female *Trichogramma* is capable of parasitizing up to 120 eggs of the host in her life. The parasitized eggs turn uniformly black after 3 to 4 days (Figure 20), an invaluable diagnostic character for distinguishing them from unparasitized eggs. A female parasitoid can distinguish already parasitized eggs, thereby avoiding super-parasitism or multiple parasitism under natural conditions.
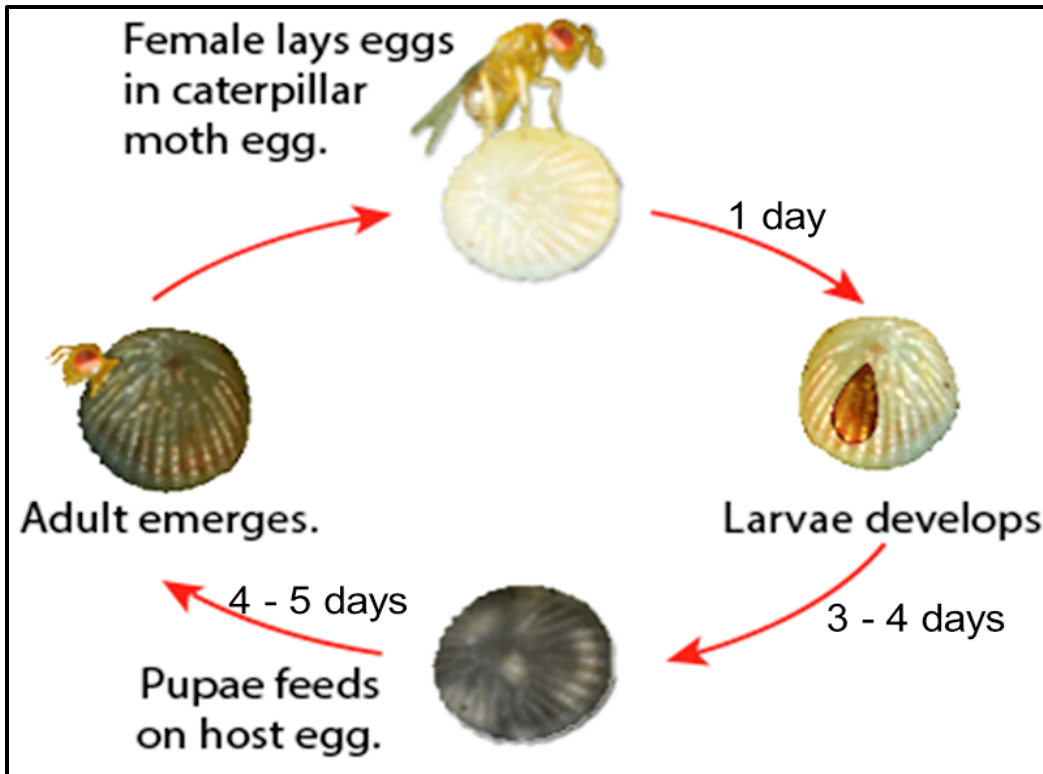
Figure 20. Life cycle of *Trichogramma* spps.
(https://gardeningzone.com/products/trichogramma-sp-pretiosum-for-caterpillar-control-short-plants-low-flyer)

6.2. Colony establishment: field sampling, collection, handling and transportation

Materials required:

- Vials
- Lunchbox
- Ice box
- Hand lens
- Scissors
- GPS
- Cotton wool
- Paper towel
- Labels and pencils
- Masking tape

The first step in establishing *Trichogramma* culture is to obtain adults of the desired species. Keep records of the source of the *Trichogramma* strain and the date that the culture is initiated. The two most common sources of the predator are field populations and cultures maintained at other laboratories from which adults may be obtained upon request. However, regardless of the non-specificity of *Trichogramma*, the strain/species should preferably be collected on the target pest and in a region with climatic conditions similar to those of the target area, expecting an optimal adaptation to pest and environment in local populations. Field collected material has a closer resemblance of their behavioral and genetic traits to the wild population than those older laboratory colonies collected from elsewhere.

Another important factor to be considered is that the culture of the natural enemy must have a wide genetic base of as many individuals as possible from a selected locality. When the colony is raised from a very small number of individuals, the laboratory culture may become highly inbred and evolve into 'laboratory ecotypes' (Mackauer, 1972); such individuals when released against target pests may not produce satisfactory results. The collection of founder parasitoid population therefore should preferably be from the center of the distribution of the species. This population possibly has low potential of evolving into laboratory ecotypes (Remington, 1968) and could be expected to produce satisfactory results.

It is important that the stocks of the same morphospecies of *Trichogramma* from different areas, both contiguous as well as isolated regions, should never be mixed up for raising the culture. Otherwise, it is possible that some such cultures may in fact have undetected contamination, as in samples from different localities and regions. They might also contain ecotypes or sibling species, where the morphological characters are the same, but have discreet traits as observed in different spatially isolated populations of *Trichogrammatoidea cojuangcoi* Nagaraja in the Philippines and elsewhere (Nagaraja, 1987). Therefore, the identity of a species, its diverging populations – strains/sibling species – should be confirmed before selecting for mass production in the laboratory (Nagaraja, 2013).

To initiate the *Trichogramma* colony, parasitized FAW egg masses collected over a wide area are used. To collect parasitized FAW eggs from field, target four to six weeks old maize plants. Fall armyworm eggs and parasitoid wasps are easily washed from maize leaves with rain. Therefore, plan the parasitoid wasp collection during the dry season from irrigated fields.

Scout the field for egg parasitoids by examining egg masses for parasitism using hand lens (compare both eggs with parasitoids and without for better understanding of the differences). Parasitized FAW eggs turn deep black compared (Figure 21) to unparasitized FAW eggs, where sometimes adults may also be found on the egg mass. Accordingly, cut maize leaves showing visible parasitized eggs or egg masses that you suspect are parasitized using scissors and place in a vial. Keep each egg mass separately in a plastic/glass vial. Close the vial with cotton wool wrapped with paper towel. *N.B*. If the vial is covered only with cotton wool, the parasitoid may hide themselves inside the cotton fibre upon emergence. In each vial, label the genus/species name of the parasitoid/the suspected parasitoid, date of collection, place, and GPS reading of the location. Place the vials having the sample together in a lunch box and tightly pack the lunch box with masking tape to avoid physical damage during transport. Before/during specimen collection, make sure the sampling field was not treated with insecticides.
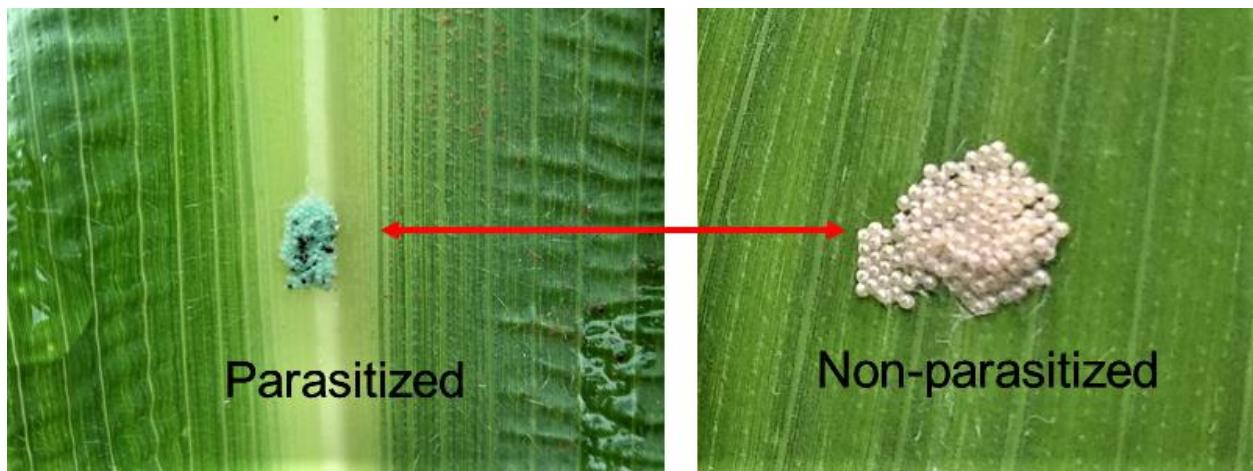

Figure 21. Parasitized and non-parasitized FAW eggs (P. Malusi 2019)

In the laboratory, keep the collected specimens at room temperature and check the samples at least twice a day for emergence of the parasitoid. The parasitoid can take 8 to 10 days for emergence

(Figure 20). As soon as the emergence of the first *Trichogramma* wasp/s, place a piece of printing paper coated with a thin layer of 50% honey solution in the vial so that the parasitoid wasps have food after emergence. *Caution:* The honey solution painted on the paper must be thin; otherwise, the wasps will get trapped in the honey and die.

When opening the vial with active *Trichogramma* inside, always make sure that a light source is available and directed to the side of the vial, opposite to the opening, to attract the wasps and ensure that they do not escape from the vial (Figure 22). Close the vial with cotton wool wrapped with paper towel. *Trichogramma* adults are positively phototropic and negatively geotropic.
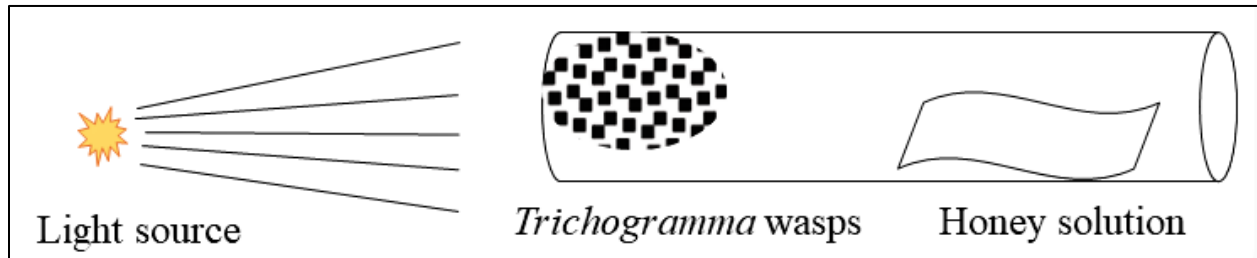
Figure 22. Scheme showing *Trichogramma* stock culture management (M. Goftishu 2019).

6.3. Colony maintenance

Colony maintenance/stock culture is very critical for sustaining the rearing cycle and ensuring the fecundity and efficiency of the *Trichogramma* wasps produced. Colony maintenance rearing of *Trichogramma* takes place in small containers such as glass vials. To keep healthy and vigorous colony, introduce measures such as familiarising the *Trichogramma* wasps to field conditions, alternating diverse host eggs, and/or introducing wild parasitoids from the field.

Strain deterioration can occur when insects are reared continuously in the laboratory for longer periods and over many generations. This can lead to parasitoids becoming less efficient or less healthy because they are adapted to the special conditions of the laboratory. When released, they might have difficulties with host finding, etc. In order to prevent this, certain practices can be adopted to keep the culture healthy. These are:

- **Periodical introduction of wild parasitoids.** This is the most efficient method. To do so, collect naturally parasitized FAW eggs of the target hosts from maize fields and rear the *Trichogramma* wasps in isolation for a generation to avoid any contamination. Then the second generation of *T. chilonis* is allowed to crossbreed with the laboratory colony. At *icipe* and ICRISAT-Niger insectaries, *Trichogramma* colonies are rejuvenated every eight to ten generations by crossing laboratory-reared populations with wild populations collected from similar area and host.

- **Acclimatization of *Trichogramma* to outdoor conditions.** In the laboratory, allow the *Trichogramma* wasps to parasitize the eggs at 20-25°C for 2 days, then take the vials with the newly parasitized eggs outside and allow the *Trichogramma* to continue their development under field conditions. After 5-6 days, take the vials back to the laboratory for the parasitoids to emerge. Follow this procedure over several generations.

- **Use natural host eggs.** Another way to avoid degradation of the stock culture is to rear the parasitoid on the natural host (FAW eggs or *S. littoralis*) instead of *C. cephalonica*. When natural host eggs are substituted for at least two to three generations, the colony deterioration of the *Trichogramma* culture will be reduced.

6.4. Rearing facilities

Rearing room

The fundamental and most essential requirement for sustainable *Trichogramma* mass-production is availability of well-secured building. As indicated above for other insectaries, the *Trichogramma* rearing room should be free from pesticide contamination and should be surrounded with a water trough at the ground level. The water trough prevents the entrance of crawling insects. Laboratory windows should be equipped with glass with the upper portion made up of fine wire mesh to ensure the provision of adequate natural light as well as to provide good ventilation. Proper lighting regime is vital for *Trichogramma* rearing and additional artificial light (fluorescent lights) controlled by a timer should be installed to provide a uniform daylight source in the insectary. For most of the *Trichogramma* species photoperiod of 14:10 light: dark cycle has

been found to be adequate. Heaters and/or cooling systems should also be installed to ensure that temperature is kept at the optimum. At the *icipe* rearing facility, temperature is maintained at 26-28°C and relative humidity is kept at the range of 75-80%.

Rearing containers

The most simple and convenient container for rearing *Trichogramma* is a transparent plastic jar (rearing/parasitization/exposure jar). The jar is fabricated from 1.5-liter capacity cylindrical plastic container with lid (Figure 23. A). The lid of the oviposition jar has a circular window sealed with wire mesh for ventilation (Figure 23. B). In addition to the rearing jar, there is also a glass/plastic vial of about 10 cm length and 3 cm diameter for colony initiation and stock culture rearing activities (Figure 23. C).

Each rearing jar and/or vial should be labeled on the outside, indicating the species contained and the date of exposure (parasitization). This information can be written directly on the outside surface of the jar using a felt-tip marker with water-soluble ink. This method of labelling is especially convenient because the labels can easily be removed by washing, particularly if the jars are to be reused. However, the simplest way is to use printable label and to fix it on the outside of the cages (Figure 23. D).
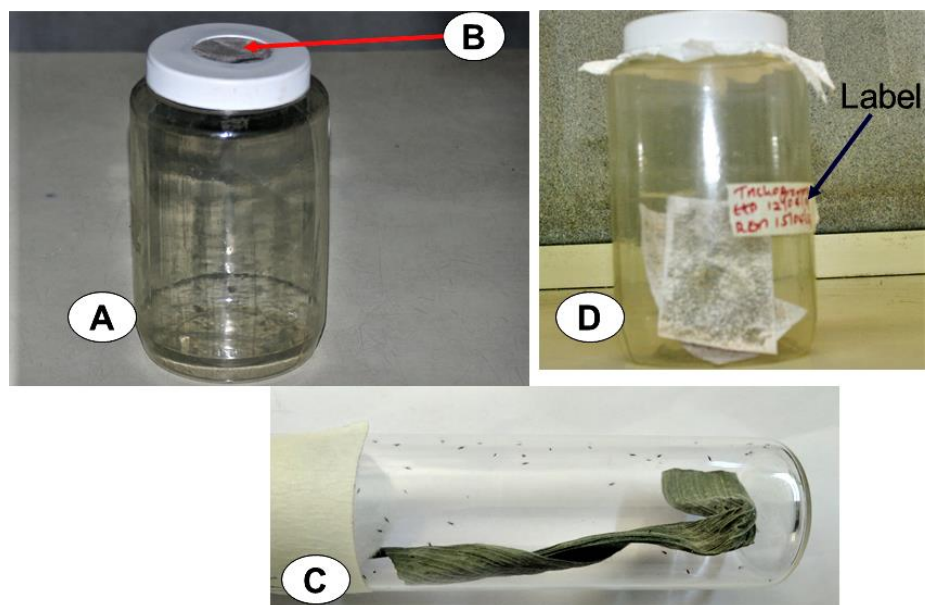

Figure 23. *Trichogramma* wasp rearing containers (M. Goftishu 2019)

Depending on the availability of resources and scale of operation, several pieces of equipment can be installed. A list of equipment used in rearing *Trichogramma* at the *icipe* insectary is given below (Table 4). Moreover, a list of consumables that are regularly consumed for rearing *Trichogramma* is indicated below (Table 5).

Table 4. List of equipment used for rearing *Trichogramma*.

No.	Equipment	Purpose
1.	Laminar hood	*C. cephalonica* egg sterilization
2.	Microscope	Identification of parasitoids and their sex
3.	Graduated cylinder	Measuring *C. cephalonica* eggs volumetrically
4.	Refrigerator	Storing adult and pupal parasitoids
5.	Autoclave	Sterilizing autoclavable rearing materials
6.	Room air conditioners	Temperature control in rearing rooms
7.	Room air humidifiers	Humidity control in rearing room
8.	Thermometer	For measuring room temperature
9.	Heat fan	Maintain temperature
10.	Cages	Adult mating and oviposition
11.	Glass vials	Stock culture maintenance
12.	Plastic jar	Parasitization
13.	Lunchbox	Collecting parasitized egg masses
14.	Hand lens	Examining egg masses for parasitism
15.	Icebox	Maintain the temperature during parasitoid transport
16.	Standby generator	To use in case of power failure

Table 5. List of laboratory consumables and their function.

No.	Consumable	Purpose
1.	Tricho cards	Pasting the sterilized eggs
2.	White glue (a non-toxic gum)	Sticking the sterilized eggs to the card
3.	*Trichogramma* adult wasps	Initiating the rearing colony
4.	Sterilized mill moth eggs	Host for *Trichogramma*
5.	Camel hair brush/sponge	Applying the glue uniformly over the card
6.	Honey solution	Food for adult wasps
7.	Scissors	Cutting the tricho card
8.	Cotton wool	Covering the vial
9.	Ruler	Measuring the length and width of the card
10.	Pencil	Marking and labelling information on the card
11.	A4 printing paper	Placing the card during tricho card preparation
12.	Paper towel	Covering the rearing jar
13.	Ethanol 75%	Cleaning the operation desk

6.5. Mass production of *Trichogramma*

Some of the most important lepidopteran pests of field crops are attacked by *Trichogramma* wasps. However, in most crop production systems, the number of eggs destroyed by native populations of *Trichogramma* is not sufficient to prevent the pest from reaching damaging levels. Therefore, rearing a large number of *Trichogramma* is needed for inundative releases. From the stock culture, the colony is enlarged repeatedly until it is big enough for mass production.

Different mass rearing procedures and field release techniques of *Trichogramma* have been optimized by several researchers and institutions including *icipe*, the Food and Agricultural Organization (FAO), Indian Council of Agricultural Research (ICAR), and the Indian Ministry of Agriculture, based on the efficacy of species/strains of *Trichogramma* for the specific crop-pest situation and local availability of the factitious host. The descriptions below provide the procedure followed in mass rearing of *T. chilonis* at *icipe* facility. Even though the production of

Trichogramma biocontrol agents is not particularly difficult, a practical training on the rearing methods is recommended before starting production.

Materials required:

Refer to table 5 and table 6 about the kind of materials and their function required for mass rearing of *Trichogramma.*

Procedure:

Tricho card preparation

i.   Clean the operation desk with 75% ethanol.

ii. Prepare "tricho card" also known as egg card. Mark white coloured art paper card (Ivoary board) to the size of 15 X 10 cm with ruler and pencil and cut the marked area with scissors. On the backside of the paper, write date of exposure/parasitism, card number, estimated number of *C. cephalonica* eggs exposed and expected date of parasitoid emergence.

iii. Place the tricho card on A4 paper sheet on laboratory bench (Figure 24. A). Apply white glue (non-toxic gum) (Figure 24. B) on the card starting from middle to edge in a spiral way or vice-versa. Quickly spread/paint the glue uniformly over the card with the aid of brush or sponge.

iv. Measure one cc of sterilized *C. cephalonica* eggs using sensitive balance. One gram of *C. cephalonica* eggs is estimated to contain approximately 16,000 eggs (Jalali and Singh, 1989). Approximately 12,000 eggs cover one card of 15 x 10 cm size.

v.   Immediately distribute the sterilized eggs uniformly on the glue (Figure 24. A), avoiding the formation of layers as it impairs parasitism. To facilitate uniform distribution, place the eggs inside a small vial covered by a mesh fabric fine enough to pass only one egg at a time. The extra loose eggs found on the card can be collected in A4 paper sheet by tiling and tapping the tricho card with a finger. These eggs can be used for further egg cards.

vi.   Allow the card for shade drying for 5 to 10 minutes (Figure 24. C). Make sure the card is well dried. Otherwise, the *Trichogramma* wasps will likely get stuck in it.
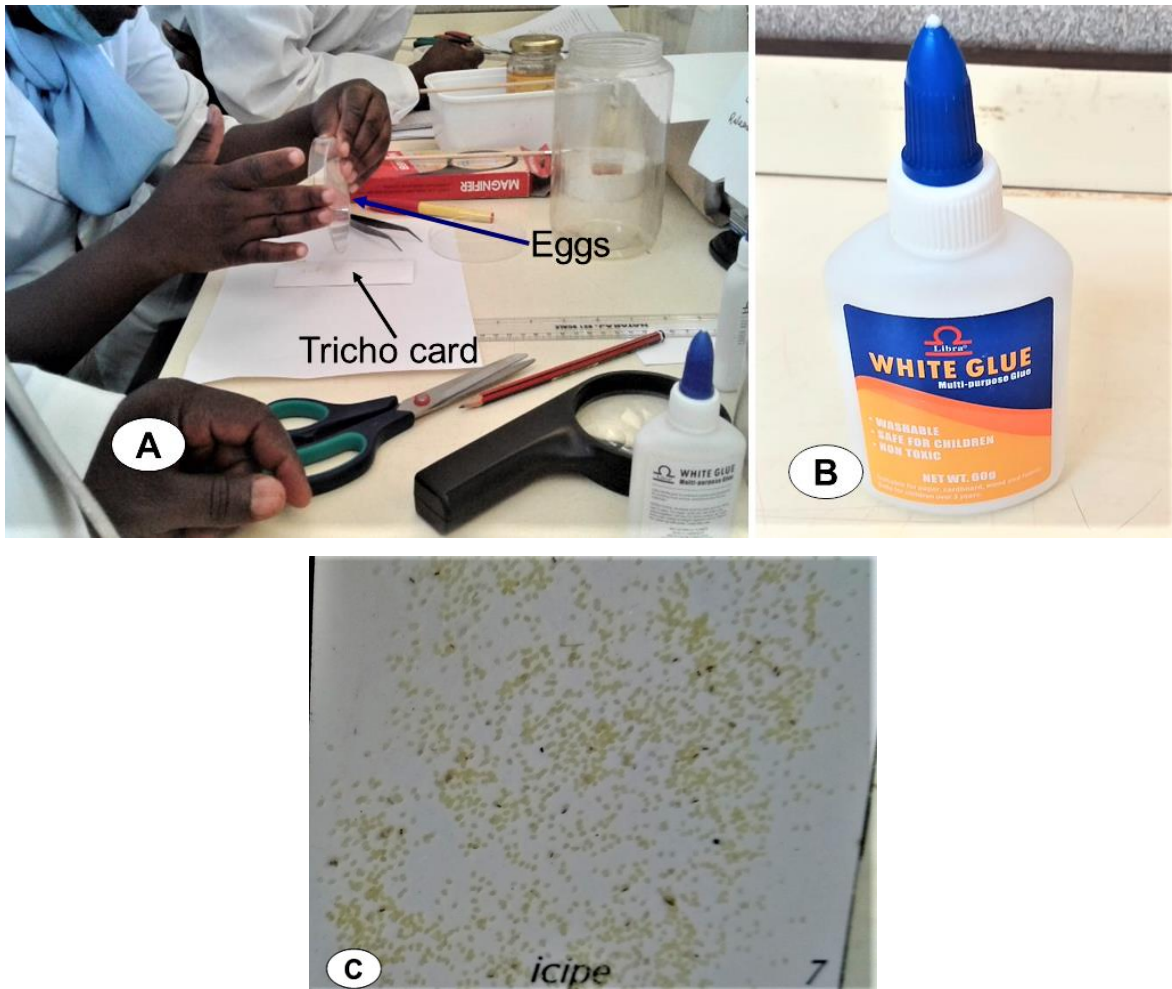
Figure 24. Tricho card preparation and sterilized egg distribution (A), white glue (B), and tricho card (C) (M. Goftishu 2019).

Egg infestation/parasititzation

i.   Paint a thin layer of 50% honey solution with brush on a clean paper sheet (~5 cm X 15 cm) to feed *Trichogramma* adults (Figure 25. A). While staining the honey, leave a small area around 2 cm at one end to fix the paper sheet on the inner wall of the jar with masking tape (Figure 25. B). Provide honey solution regularly as food source until the death of the *Trichogramma* adults.

ii. Once the tricho cards with the *C. cephalonica* eggs have dried, introduce one to three cards (Figure 26. A) into a 1.5-liter plastic or glass jar. Then transfer adult *Trichogramma* wasps from the vials to rearing jar (Figure 26. B). To transfer the *Trichogramma*, shake the vials tightly with fingers to drown the wasps to the bottom of the vial. Place the mouth of the rearing jar upside down on the vial, slowly remove the cover of the vial inside/under the rearing jar, and release the wasps to the rearing jar. After transferring adult *Trichogramma* wasps, cover the opening of the rearing jar carefully. The eggs on tricho cards are exposed to adult female *Trichogramma* in the ratio of 30:1 for 24 hours. *N.B. If there are not enough parasitoids available at the beginning, considering the host:parasitoid ratio, the number of host eggs or size of tricho card prepared for infestation will be modified according to the available number of adult parasitoids.*

iii. Once sufficient parasitoid culture is established, the easiest way to parasitize the tricho cards is to introduce a card with eggs that have previously been parasitized by *Trichogramma* wasps (could be referred to as "mother card") from the existing stock (Figure 27). As soon as the first set of wasps have emerged from the mother card, place tricho cards containing unparasitized eggs ("daughter cards") just opposite to that of the mother card in the same rearing jar. When opening the jar with active *Trichogramma* inside, always make sure that a light source is available and directed to the side of the jar, opposite to the opening, to attract the wasps and ensure that they do not escape from the jar (Figure 22). Finally, cover the lid carefully. The parasitoid-host population may be adjusted in the ratio of 1:6, i.e., to the parasitoid emerging from one card, six fresh egg cards of similar size may be offered for parasitization.

iv. Leave the rearing jar in the laboratory while the *Trichogramma* parasitize the host eggs. If a large number of the *Trichogramma* have emerged from the mother card on the same day in which fresh eggs/daughter cards were introduced, it is recommended that eggs/ daughter cards are left to be parasitized for just 24 hours; if emergence is less well synchronized then keep them in for 48 hours.

v. After 24/48 hours, remove the parasitized tricho cards and place them in a separate new labeled jar.

vi. After 3-4 days, the eggs turn black (dark) in colour, indicating parasititzation of eggs (Figure 28). Normally 80-90% successful parasitization is observed to occur.

vii. *Trichogramma* survives 4-7 days after emergence. Expose fresh new batch of egg cards to *Trichogramma* adults every 24/48 hours until it dies.

viii. The parasitized egg cards can be released immediately or stored in refrigerator at 10^0C up to 21 days. Prolonged storage beyond 21 days would impair emergence as well as longevity and fecundity of the resulting progeny.

A summary of the production of *Trichogramma* wasps is presented in the form of flow chart (Figure 29).
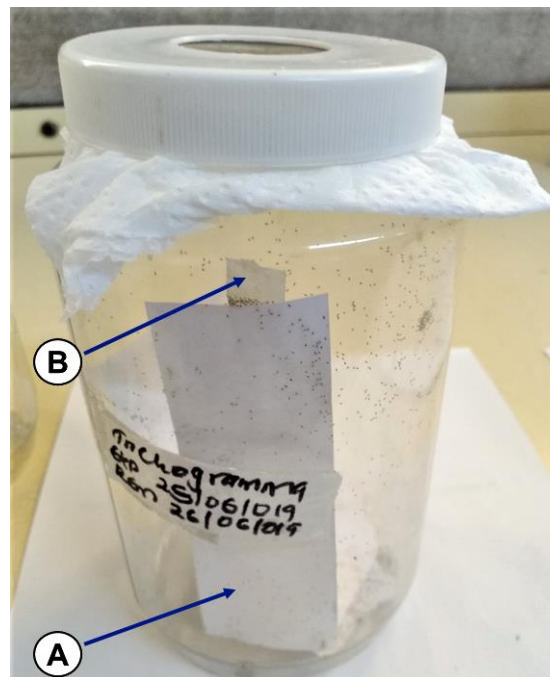
Figure 25. Honey solution stained paper (A) fixed with masking tape (B) (P. Malusi 2019).

Figure 26. Tricho card (A) and vial with adult *Trichogramma* wasps (B) (P. Malusi and M. Goftishu 2019).
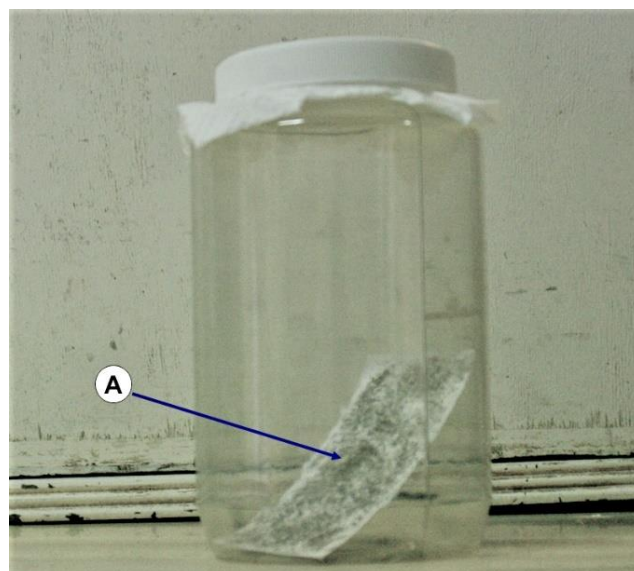

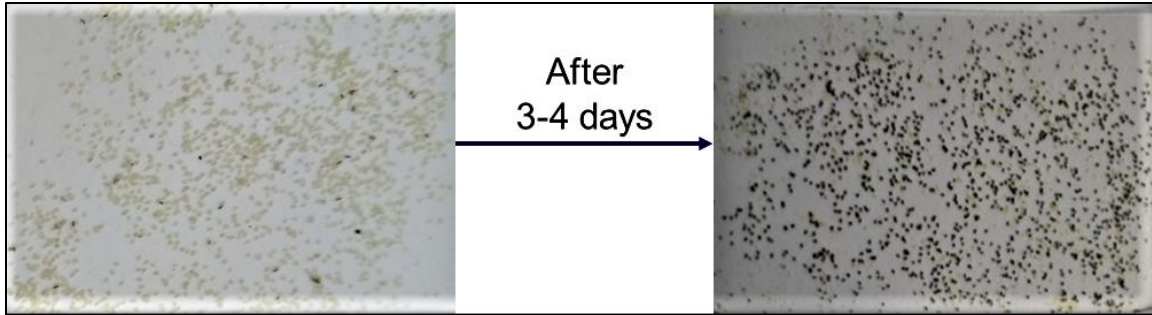**Figure 27.** Mother card (A) (M. Goftishu 2019).

Figure 28. Unparasitized (left), and parasitized eggs (right) (M. Goftishu 2019).
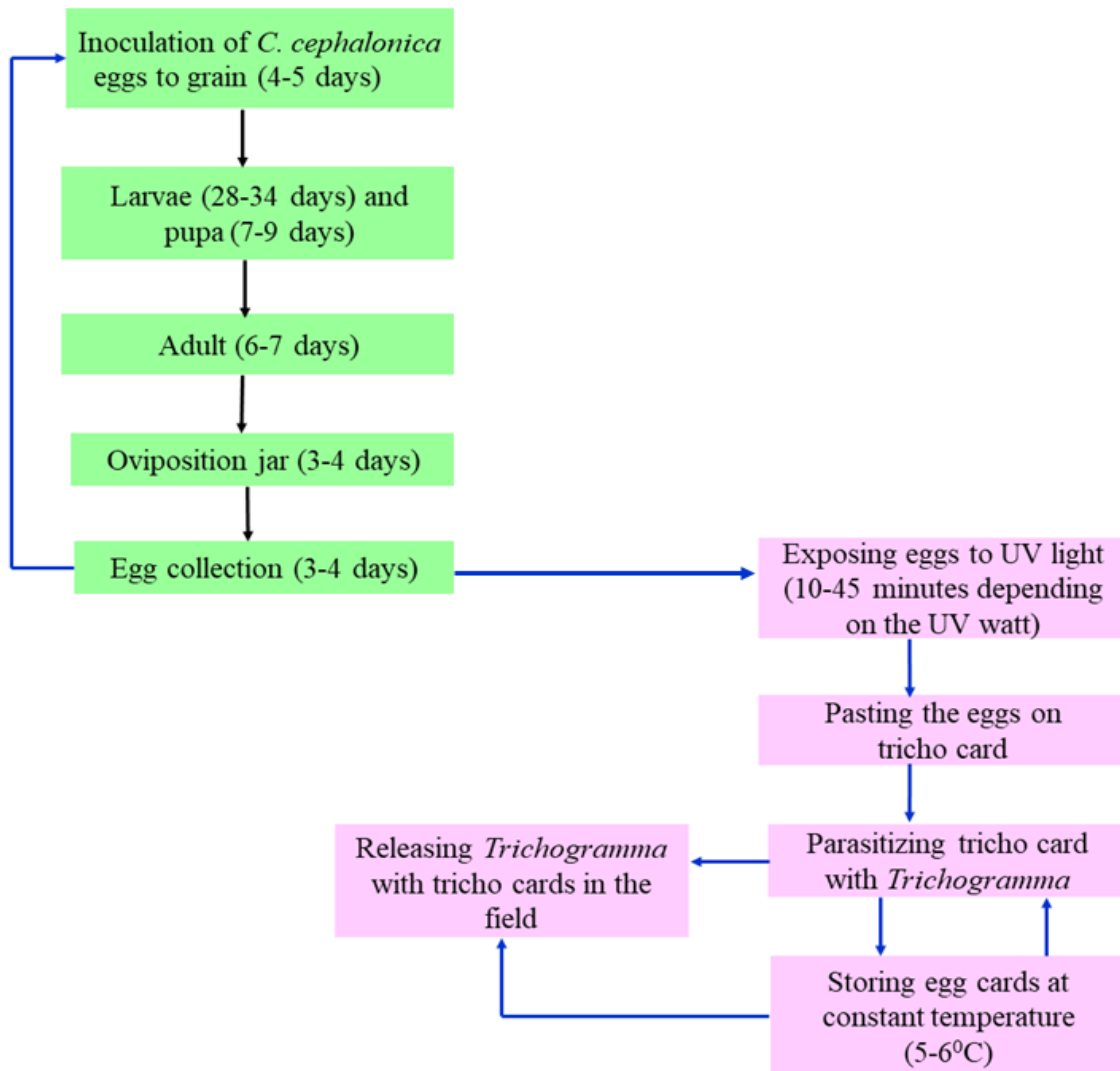

Figure 29. Flow chart for production of *C. cephalonica* and for mass rearing of *Trichogramma* (M. Goftishu 2019).

7. Mass rearing of *Telenomus remus*

The egg parasitoid *Telenomus remus* Nixon (Hymenoptera: Platygastridae) is an effective biological control agent for various pest species of the genus *Spodoptera* Guenée (Lepidoptera: Noctuidae) (Pomari et al., 2012). *T. remus* is a native of Asia and has been introduced against *Spodoptera* spp. to various parts of the world, including India, Israel, Pakistan, Australia, New Zealand, the Caribbean, Colombia, and Venezuela (reviewed in Cave, 2000). The parasitoid is now found in most of the distribution range of *S. frugiperda* in the Americas (Cock et al., 1985). *T. remus* was also recorded in five countries of East, South and West Africa (Kenis et al., 2019). In the Americas, *T. remus* can be produced under laboratory conditions on *S. frugiperda* or other hosts and released in the field (Cave, 2000). Inundative release of *T. remus* resulted in 90% parasitism in *S. frugiperda* eggs, releasing 5,000 to 8,000 parasitoids per hectare (Cave and Acosta, 1999; Cave, 2000). Similarly, the parasitism of *T. remus* on *S. frugiperda* under laboratory conditions varied from 80 to 100% (Cave and Acosta, 1999; Cave, 2000). This parasitoid has been released in maize fields as part of IPM programs in Venezuela and resulted in 90% parasitism (Ferrer, 2001), demonstrating the high potential of biocontrol for several species of *Spodoptera*.

7.1. *Telenomus remus* **Nixon (Hymenoptera: Platygastridae) description and life cycle**

The adult of *T. remus* measures 0.5-0.6 mm in length. The body is shiny black. *T. remus* males have a filiform antenna and short abdomen, whereas females have four-segmented clubbed antennae and longer and wider abdomen (Figure 30) with the ovipositor clearly visible under 10X hand lens.

Telenomus remus is primarily produced on the eggs of FAW, *S. frugiperda* and tobacco caterpillar, *S. litura,* which are the preferred hosts. It could also parasitize *S. exigua*, *Spodoptera littoralis* (Bosiduval) (Lepidoptera: Noctuidae), *H. armigera*, *Plusia signata* Fabricus (Lepidoptera: Noctuidae), *Agrotis segetum* Denis and Schiffermuller (Lepidoptera: Noctuidae), *Agrotis biconical* Kollar, *Agrotis ipsilon* (Hufngel) (Lepidoptera: Noctuidae), and *C. cephalonica*, etc. However, in factitious hosts, poor initial parasitism was recorded with *T. remus*, but after a few generations, 100% parasitism was obtained without any significant difference in the developmental period and sex ratio of the parasitoid (Kumar et al., 1986).

The cycle of this parasitoid, inside the host egg, can be divided into egg, larva, and pupa, and the mean development period ranges from 11–13 days in *C. cephalonica* and in 10 days in other hosts. Optimum conditions for rearing are 27 ± 2 °C and $75 \pm 5\%$ RH (Goulart et al., 2011). Each female of *T. remus* produces about 270 eggs during its reproductive lifespan (Morales et al., 2000).
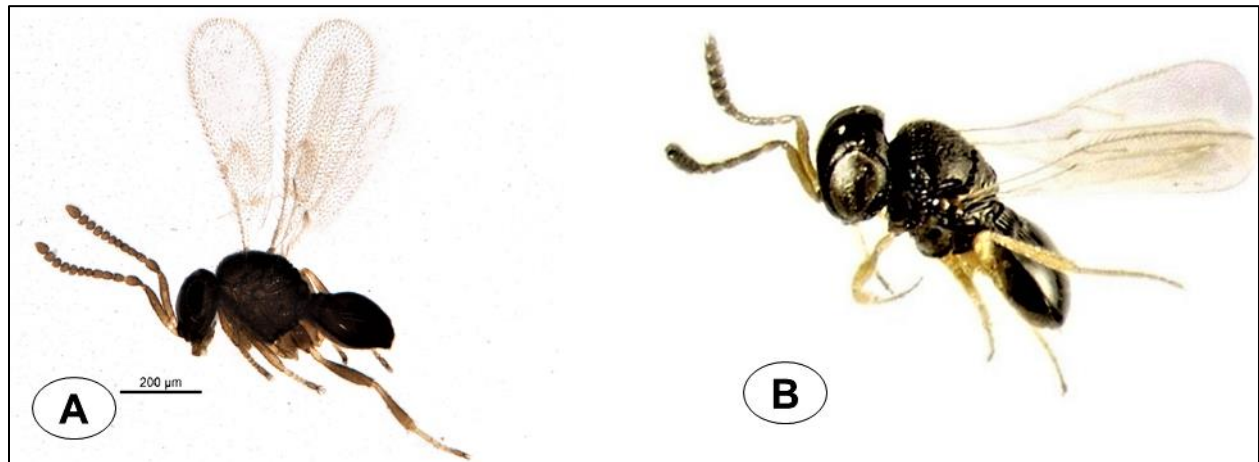
Figure 30. Male (A), and female (B) *Telenomus* parasitoid
(https://www.cabi.org/news-and-media/2019/scientists-confirm-first-report-of-egg-parasitoid-in-africa-to-fight-devastating-fall-armyworm/)

7.2. Colony establishment: field sampling, collection, handling and transportation

Refer to section 6.2. Both *Telenomus* and *Trichogramma* wasps have relatively similar life cycles, habitat, feeding habit, and ecological requirement. Thus, colony establishment, culture maintenance, and rearing facility including rearing containers and equipment described for *Trichogramma* wasps apply for *T. remus* as well.

7.3. Colony maintenance

In the colony maintenance the rearing is focused not on high output, but on the quality of the parasitoids reared, targeting maintenance of a healthy and efficient colony for subsequent mass production. Maintenance of reared *Telenomus* wasps in such a way that laboratory colonies are genetically similar with wild populations is a criterion for ensuring quality of the reared

parasitoids. Accordingly, *T. remus* is periodically collected from the field, reared in isolation for F1 to avoid any contamination. Then the second generation of *T. remus* is allowed to crossbreed with the laboratory colony. At the facilities of *icipe* and ICRISAT-Niger, *Telenomus* colonies are rejuvenated every ten generations by crossing laboratory-reared populations with wild populations collected from similar area and host. However, Queiroz et al. (2017) found no difference in body size, flight activity, and parasitism capacity of *T. remus* which had been reared for 45 generations on *C. cephalonica* eggs in laboratory.

7.4. Rearing facilities

Refer to section 6.4.

7.5. Mass production of *T. remus*

Telenomus remus is currently being mass reared and released in several Central and South American countries for the control of *Spodoptera* pest species, FAW in particular (Cave, 2000; Gutierrez-Martinez, et al., 2012). This small parasitic wasp is notable for its aggressive and efficient ability to parasitize the large egg masses associated with FAW, whose multiple, superimposed layers are covered with scales (Figure 31) that limit attack from other parasitoids (Cave and Acosta, 1999). *T. remus* can penetrate all layers of the egg mass, routinely producing 80–100% parasitization in laboratory studies (Pomari et al., 2012), characteristics that favor its use as a biological control agent.

Figure 31. *T. remus* parasitizing FAW eggs covered with scales

(https://entomologytoday.org/2017/11/01/pennies-for-parasitoids-savings-add-up-when-rearing-wasps-on-alternate-hosts/)

The descriptions below provide the procedure followed in mass rearing of *T. remus* at *icipe* and ICRISAT-Niger insectaries.

Materials required:

Refer to table 5 and table 6.

Production procedures:

Card preparation

i. Clean the operation desk with 75% ethanol.

ii. Mark white coloured art paper card to the size of 15 X 10 cm with ruler and pencil and cut the marked area with scissors (Figure 32. A). On the backside of the paper, write date of exposure/parasitism, card number, estimated number of FAW eggs exposed, and expected date of parasitoid emergence.

iii. Paint the entire area of the rectangular cardboards with a thin layer of non-toxic glue (Figure 32. B) with the aid of sponge or brush.

iv. Immediately, transfer the freshly harvested (0-24 hours old) FAW egg masses evenly over the glue, with the aid of surgical tweezers (Figure 30. C). Transfer about 40 egg masses (approximately 12,000 eggs) onto each card.

v. Allow cards with FAW eggs to dry for 5 to 10 minutes at room temperature. Make sure the card is well dried. Otherwise, the parasitoid wasps will be likely to get stuck in it.

vi. The eggs cards can be stored in the refrigerator at 4°C up to a week for accumulation of eggs prior to infestation by *T. remus*.
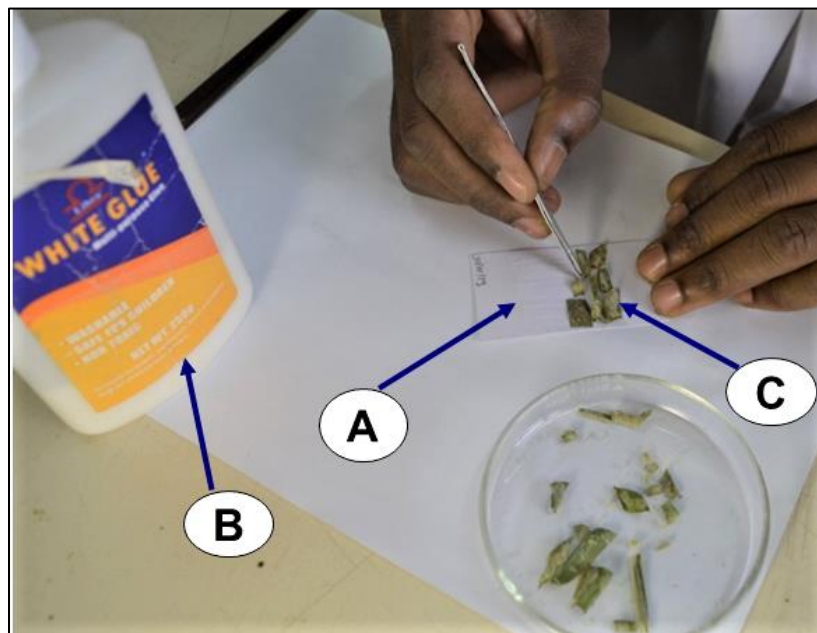
Figure 32. *T. remus* egg card preparation and egg pasting on the card (M. Ba 2019).

Egg infestation/parasititzation

i. Paint a thin layer of 50% honey solution with brush on a piece of printing paper to feed *T. remus* adults (Figure 33. A). While staining the honey, leave a small area around 2 cm at one end to fix the paper on the inner wall of the jar with masking tape. Provide honey solution regularly as food source until the death of the *Telenomus* adults.

ii. Once the cards with the FAW eggs have dried, introduce one to three cards into a 1.5-liter plastic or glass jar (exposure jar). Then transfer adult *Telenomus* wasps from the vials to

rearing jar (Figure 33. B). To transfer *Telenomus,* shake the vials tightly with fingers to drown the wasps to the bottom of the vial. Place the mouth of the rearing jar upside down on the vial, slowly remove the cover of the vial inside/under the rearing jar and release the wasps to the rearing jar. After transferring adult *Telenomus* wasps from the vials, cover the lid of the jar lined with paper towel (Figure 33. C) and opening of the rearing jar carefully. The egg cards are exposed to adult female *Telenomus* in the ratio of eggs: wasp, 20:1 for 24 hours. *N.B. If there are not enough parasitoids available at the beginning, considering the host-parasitoid ratio, the number of host eggs or size of tricho card prepared for infestation will be modified according to the available number of adult parasitoids.*

iii. Once enough parasitoid cultures are established, the easiest way to parasitize the egg cards is to introduce wax paper/maize leaves with FAW eggs that have previously been parasitized by *Telenomus* wasps (could be referred as "mother cards") from the existing stock. Two to three days after the emergence of most wasps from the wax paper/maize leaves, place cards containing unparasitized FAW eggs ("daughter cards") in the same rearing jar (Figure 34. A). Finally, cover the lid of the jar carefully. The parasitoid-host population may be adjusted in the ratio of 1:6 i.e., to the parasitoid emerging from one card six fresh egg cards of similar size may be offered for parasitization.

iv. Label the species name, date of exposure of FAW eggs, and expected date of parasitoid emergence on the exposure jar (Figure 33. D).

v. After 48 hours of exposure/infestation, remove the daughter cards from the exposure/ parasitization jar and transfer them into another identical jar without adult.

vi. About three to four days after parasitoid wasp exposure, the parasitized egg becomes dark, providing a qualitative way to evaluate the rate of parasitism (Figure 34. B).

vii. *Telenomus remus* survives 5-10 days after emergence. Expose fresh FAW egg masses to adult parasitoids every 2 days until it dies.

viii. The parasitized egg cards can be released immediately or can be safely stored in refrigerator at 10 ± 1 °C for 7 days without affecting the efficacy of the parasitoid. Storage in refrigerator beyond 7 days may affect the quality of the parasitoids.

A summary of the production of *T. remus* is presented in the form of flow chart (Figure 35).
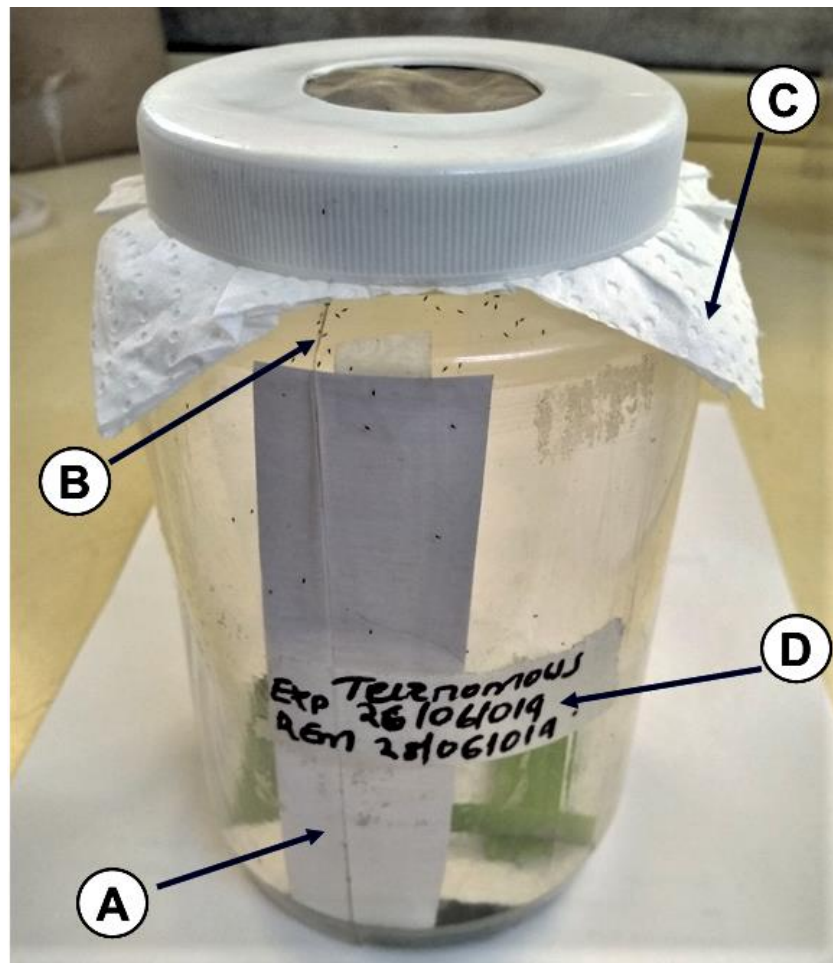

Figure 33. Honey solution-stained paper (A), adult *Telenomus* wasps (B), and paper towel cover (C) (P. Malusi 2019).
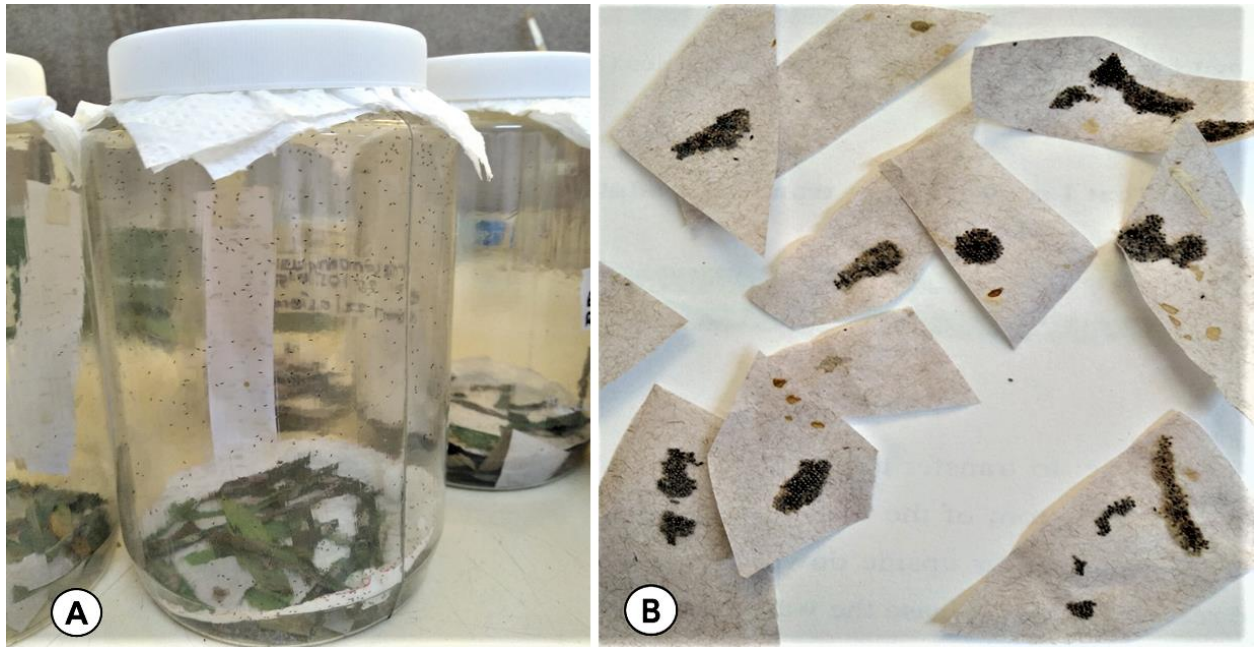
Figure 34. Confinement of FAW eggs with *T. remus* females (A), and FAW egg masses parasitized by *T. remus* (B) (P. Malusi 2019).
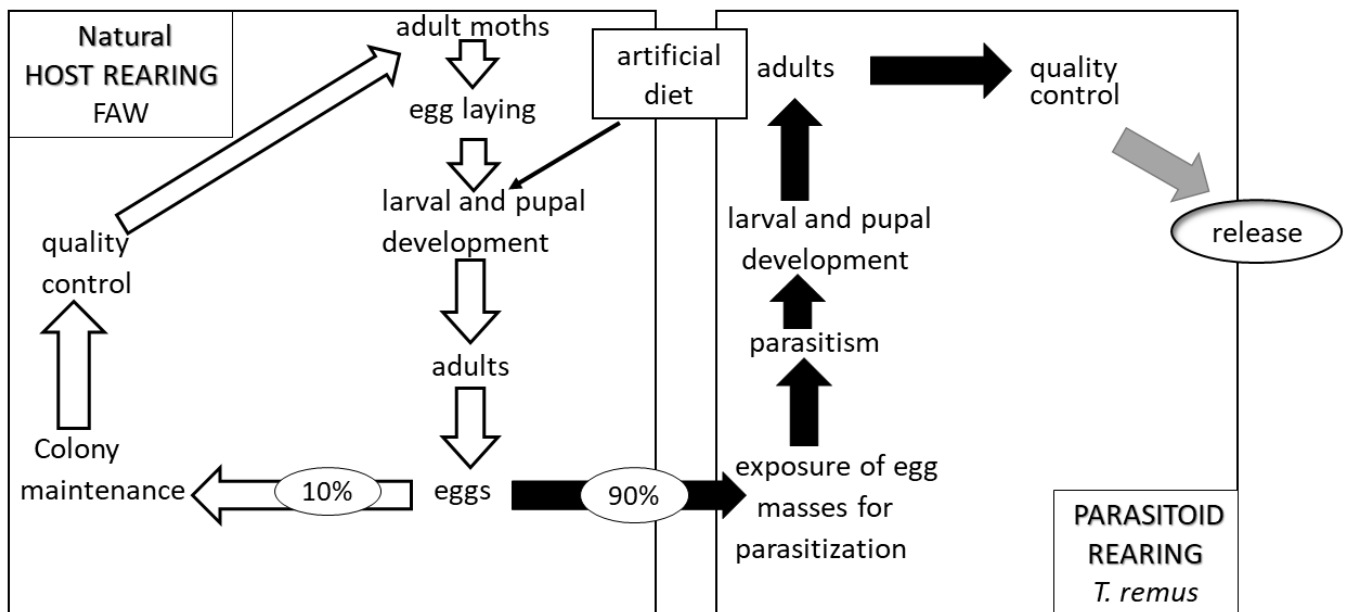

Figure 35. Flow chart for the production of *T. remus* (M. Goftishu 2019).

8. Storage, packing and field release of the egg parasitoids

8.1. Storage

Identifying the optimum storage temperature of parasitoids and host eggs would enable insectaries to store the cultures and to utilize them at the required time. Thus, it is necessary for rearing facilities to have reliable systems for storing parasitoid eggs and pupae, or adults.

Irrespective of species/strains, adult *Trichogramma* can be stored in a refrigerator at 10ºC for 30 days. Parasitized egg cards showing blackening of eggs can be stored at 10^0C for 15-21 days (Wang et al., 2014). After returning to the room temperature, they complete their development process. In order to meet the mass rearing of *Trichogramma* with the release dates, the factitious eggs are usually stored in a cold room. However, emergence rates from the parasitized eggs, sex ratio, and fecundity are affected by storage duration. To keep good quality levels in *Trichogramma* rearing, the storage period for the factitious host eggs at 4°C should not exceed 15 days (Wu et al., 2018).

Parasitized eggs of *T. remus* can be stored at 10°C for 7-9 days. There will be a sudden decline in the fecundity of the parasitoid after the ninth day of cold storage (Gautam, 1987). Storing adult parasitoids for later release in the field is another option. Gautum (1986) found that adult females of *T. remus* stored at 10ºC survived up to seven days without negative effects on their biological attributes; males did not survive for more than three days. Storage of adult females for more than seven days significantly reduced fecundity.

8.2. Field release

8.2.1. Pre-release site assessment

Although improved rearing methods have allowed for the production and release of greater parasitoid numbers, each parasitoid is still costly to produce. Therefore, parasitoids should be released at sites where they have the highest probability of establishment. The other most important factor in field selection is the grower's assurance that it will not be treated with insecticides once the parasitoids releases begin. To reduce the effects of insecticide drift, the selected fields should

be distant from other fields that will be sprayed. In addition to the parasitoids release site, secure control plots for comparing the effect/control efficiency of parasitoids. The selected site should also be relatively easy to access because personnel will need to visit the site periodically for parasitoid release and recovery activities.

8.2.2. Number and frequency of releases

In theory, a higher number and frequency of parasitoids released increases the probability of establishing stable parasitoid populations. However, the number and frequency of parasitoid releases are often limited by the available resources for parasitoid production.

Trichogramma chilonis, at the rate of 100,000-150,000 pupae per hectare, released at about 40 points, is the recommendation for the control of FAW on maize; however, depending on the level of infestation, the number could be increased or decreased. Each egg card has approximately 12,000 parasitoids. For even distribution of the parasitoids on the field, each egg card should be cut into three to five parts with 2,500-3,750 eggs per card. Likewise, the use of *T. remus* in the control of FAW follows the same dynamics as *Trichogramma* but is used at a quantity of 120,000 pupae per hectare. Releases of the egg parasitoids will start 3 weeks after the emergence of maize crop or with the appearance of FAW. For both parasitoids, a total of one to two releases at weekly intervals will be made, until maize flowering. Before release of the egg parasitoids, FAW egg sampling should be done randomly in maize field, both from treatment and control plots.

8.2.3. Packing and transporting parasitoids

Before packing the egg cards, make sure that date of emergence is written on the cards for the guidance of the user. The cards may be packed immediately after the eggs are parasitized or after the eggs have turned black; the former allows a couple of more days for transport. The consignment has to reach the destination within 4 to 5 days before adult parasites emerge. Therefore, it is always better to avail the fastest mode of transport.

Parasitoid egg cards can be easily transported in the pupal stage (3-4 days after parasititzation). These cards can be kept in exposure jars or aerated polythene bags and packed in cardboard boxes

for transport. Care should be taken to keep the cardboard out of direct sunlight or other potentially hot (e.g., a sealed vehicle) environments. An icebox can be used to provide an insulation layer. Place an ice pack or some other cold material inside to maintain a cool temperature, ideally in the range of 10–12°C. To ensure that the ice packs do not come into direct contact with egg cards they should be covered with materials such as lined newspaper or cotton. This method should allow the eggs to be kept cool for several hours. However, make sure that the parasitized eggs will reach their destination in the shortest time possible to avoid any damage to the egg cards during storage. Place a thermometer inside the transport box. If the temperature inside the box reaches over 35°C, the parasitoids will die without any changes being visible in the eggs.

8.2.4. Timing and field releasing techniques of parasitoids

Timing of field release

The timing of release of parasitoids should be considered for the successful biological control strategy. The emergence, mating, and oviposition of egg parasitoids take place in the morning hours. Therefore, it is better to release the parasitoids in the morning so that they can start their activity immediately (Manjunath, 1988). Generally, early morning and early evening or night application of parasitoids is recommended to avoid solar radiation and high temperatures (Firake and Khan, 2014). Moreover, the release of egg parasitoids should coincide with the egg-laying period of the pest. Female *Trichogramma* and *Telenomus* usually oviposit in all ages of FAW eggs. However, eggs in the early stages of embryonic development appear to have greater acceptance and suitability.

Field releasing techniques

Generally, the use of egg parasitoids tends to be inoculative and inundative. The objective is to start the population early in the season before the pest builds up and outnumber the pest before it causes serious damage to the crop. There are two methods of field release of parasitoids: release on cards (as pupae) and release as adults.

Both *Telenomus* and *Trichogramma* parasitoids are typically released as pupae inside the host egg just prior to the emergence of adult wasps. Tricho/Tele card with parasitized host eggs glued to

the cards is the most practical method (Manjunath, 1988; Jalali et al., 2005). A more convenient method of release is to cut the tricho/tele cards bearing the parasitized eggs into small pieces of convenient size and distribute them in the field. The cards may be placed on the plant, stapled or tied to the underside of the leaves. It is advisable to place such cards, before emergence of the parasitoids. Field release of egg parasitoids with tricho/tele card is a simple technique but stapling of the card might not be fully understood by farmers (Jalali et al., 2005).

Adult wasps can be released by holding the adult parasitoids with a variety of containers (preferably transparent glass or plastic jar) with a tight-fitting lid. Any size of jar can be used depending on convenience and size of release. Adults can be released by holding the container open and slowly moving in all directions in the field (Manjunath, 1988; Sarwar and Salman, 2015). Put shredded paper, confetti, or strips of cloth in the container for the adult wasps to easily fly out from the container. The benefit of using a transparent jar is to see the activities of the wasps and the proportion that remains to be released (Sarwar and Salman, 2015).

According to Chowdhury et al. (2016) adult parasitoids released directly in the field parasitized higher number of host eggs than those on tricho cards. This might be due to absence or lower risk of predation in adult release method. However, this method requires a considerable amount of time and makes large-scale releases impossible (Jalali et al., 2005). Both *Telenomus* and *Trichogramma* parasitoids oviposit soon after emergence and are short lived. Therefore, the parasitoids will have to be released upon emergence or within the next 24 hours (Manjunath, 1988; Sarwar and Salman, 2015). Long distance shipment of such parasitoids requires cold storage facility.

Materials required for field release:
- Cards with parasitoid eggs
- Envelopes for hanging egg cards
- Transporting buckets/jar
- GPS
- Meter and rope
- Marking sticks
- Notebook

- Pen

- Marker pen

- Stapler and pins

- Stickum or grease to apply at the base of the plants to prevent ants and others predating on the eggs in the tricho/tele cards.

Procedure for field release

i. After a site is chosen for parasitoid release, measure the maize farm area attacked by FAW, mark the center of the release point generally in the middle of the farm with stick/peg.

ii. Move the equipment and materials to the marked point.

iii. Transfer egg cards/tricho cards on the envelopes or tricho envelopes (Figure 36. A) for hanging on the plants at the marked points.

iv. Release envelops with the egg card after every 10-meter covering north, south, east and west directions.

v. If there is high infestation, release additional cards covering northeast, southeast, northwest and southwest directions to increase the wasp coverage area.

vi. Mark the date of release and quantity released at each point of release.

Precautions during parasitoid field release

i. Parasitized egg cards should not be stored in deep freezer.

ii. To minimize the risk of damage through solar radiation, the envelopes should be placed at the middle or lower levels of maize leaf or under shade (Figure 36. B).

iii. Inside the envelope, tricho/tele card should be placed keeping the surface with the parasitized eggs facing the outer side (Figure 36. C). This facilitates flying and host searching ability of parasitoid upon emergence.

iv. Avoid application of insecticides in the field where egg parasitoids are released. If the need arises use selective/safer insecticides 15 days after or before the release of parasitoids.

v. It is best to distribute the egg parasitoids just before wasp emergence. However, if the egg cards are placed in the crop several days before the emergence of wasps, there is a higher risk of the eggs being attacked by predators or damaged by climatic conditions (e.g. heat, rain).

vi. Egg cards should not be placed while it is raining.

vii. Release should be made over the entire area, every season, and coinciding with the egg-laying period of the pest. This results in more uniform and effective control.

viii. Release should be made over the entire area, every season, and simultaneous to the egg laying period of the pest. This results in more uniform and effective control.

8.3. Monitoring and assessment of parasitoids establishment

In order to assess the establishment of the field released egg parasitoids (*T. chilonis* and/or *T. remus*), sample egg mass of FAW from the parasitoid release site and control site weekly. However, in order to establish a basis for comparison with data from subsequent assessments, the sampling should have to begin before the release of the egg parasitoids. During sampling, care should be taken that no more than one-half of the egg mass present is collected at a time in order to ensure further establishment of the parasitoids. Otherwise, the parasitoids would be adversely impacted. Sampling for the initial as well as subsequent assessments should be done on randomly collected egg masses in the field and brought to the laboratory for evaluation and comparison. In the laboratory, rear each sample/egg masses individually in a separate container for recovery of emerging parasitoids and/or FAW larvae. Determine percentage of parasitism by different parasitoids. Then, to determine the parasitoid establishment, identify the recovered species of parasitoids, estimate their percentages, and compare the release and control site.
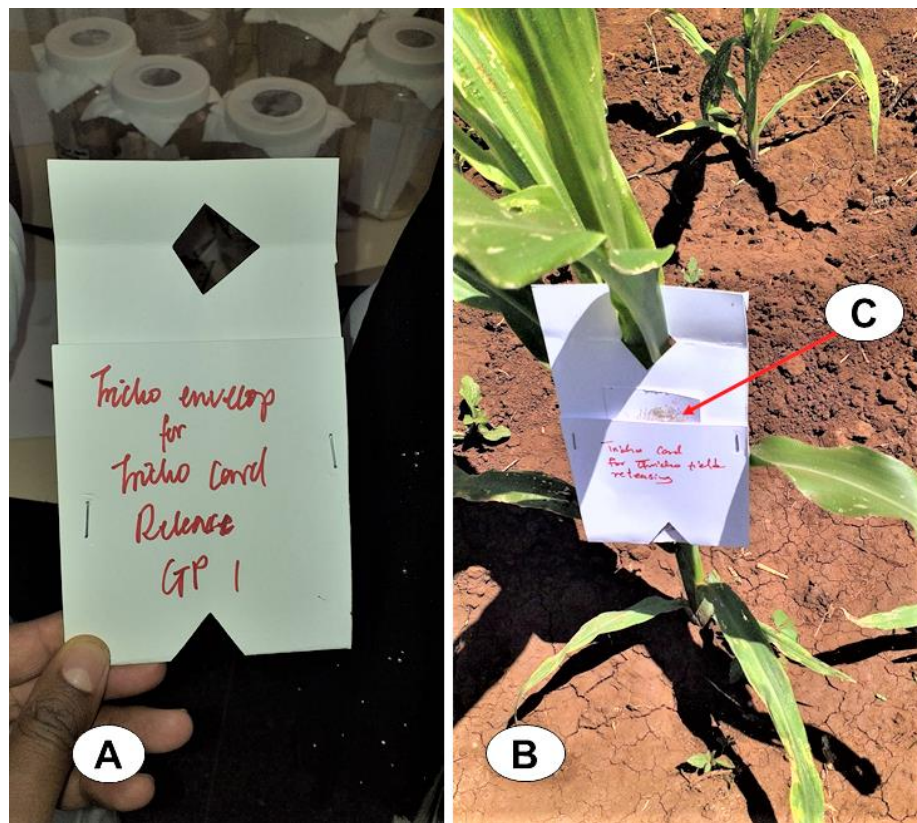

Figure 36. Tricho envelope (A), tricho envelope placed at the middle of maize leaf (B), and tricho card with the parasitized eggs facing the outer side (C) (M. Goftishu 2019).

8.4. Quality control of mass reared egg parasitoids

Quality control in mass production involves the production of the parasitoid individuals with fitness and vigor comparable to wild population.

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

- the number of eggs per cards;
- parasitism rate;
- emergence rate: number of emerged parasitoids/total number of parasitized eggs;
- sex ratio of the emerging parasitoids;
- viability of the emerging parasitoids (measured by wing deformity).

Determining these parameters is important both for the continuity of mass rearing and field release. In addition to the above parameters, activity of adult wasps (walking speed), longevity, and lifetime fecundity are also good indicators of the quality of egg parasitoids but it is considered too time consuming to assess these parameters on a regular basis.

Technical procedures for quality control of egg parasitoid:
Materials required:

- Tricho/Tele cards
- White glue (a non-toxic gum)
- *Trichogramma* parasitized eggs
- *Telenomus* parasitized eggs
- Camel hair brush/sponge
- Forceps
- Scissors
- Vials
- Pins
- Cotton wool
- Ruler

- Pencil
- Counter (ideally with two categories), Paper towel
- Microscope
- Hand lens
- Stickum or grease

Procedure:

i. Mark paper card to the size of 10 mm x 20 mm with ruler and pencil and cut the marked area with scissors. Apply a thin layer of glue on the paper card, then sprinkle about 100-200 eggs of each *Trichogramma* and *Telenomus* parasitized eggs on a separate paper card over the glue-covered area (be sure to handle the eggs gently). This number ensures that enough eggs will adhere to the paper to provide an adequate sample size, while at the same time minimizing the work. Between five and ten cards should be prepared for each parasitoid species and control check.

ii. The 10 mm × 20 mm paper with eggs glued to it can then be placed into a labelled glass vial, which is closed with a lid and stored at about 25°C and 75% R.H. One day after the eggs turn black, the parasitism rate should be determined. It is also possible to do this at a later point. On each vial, label the species name of the parasitoids and the check.

iii. After all the parasitoids have emerged, the quality control check can be conducted for all the other parameters. Each species of the adult parasitoid wasps that have emerged can be killed by placing the vials in a freezer for several minutes.

iv. To assess the parasitism rate, place the egg card under a microscope and count all the black (parasitized) eggs as well as all the others. The parasitism rate should be higher than 70%.

v. To assess the emergence rate, place egg cards under the microscope and look for emergence holes of the parasitoid wasps. Use a pin to remove the eggs that have been counted to avoid recounting them. For eggs without evident holes, use the pin to rotate or open them to look for signs of parasitism. In some cases, remainders of the parasitoid or even fully-grown

adults that were not able to emerge will be found. If any liquid material erupts from the eggs, this means that the parasitoid adults have not successfully emerged.

vi. Parasitized eggs of good quality show a high emergence rate (percentage of black eggs with holes) of between 90 and 100%. If the emergence rate is lower than 85%, check whether the eggs are over-parasitized (too many parasitoids have developed inside one host egg). Check the abundance of the parasitoids in the parasitization cage and reduce the number if necessary. The parasitized eggs have been stored under the wrong conditions (temperature lower than 10°C or higher than 35°C), the eggs have been stored for too long in the refrigerator at 10°C (more than 10 days), or the eggs were stored close to pesticides and other chemicals.

vii. The sex ratio can be assessed by placing all the emerged adult parasitoids under the microscope. Determine the sex ratio by looking at one species and individual at a time. In case of *Trichogramma* adults', males have more hairs on their antennae than females. In the case of *T. remus*, males have a filiform antenna and short abdomen whereas females have four-segmented clubbed antennae and longer and wider abdomen (Figure 30) with visible ovipositor. Use a wet paint brush to remove the counted parasitoids from the collection to avoid recounting. The sex ratio of the emerged parasitoids should be more than 50% females. A high ratio in favor of males may indicate overcrowding in the parasitization jar.

viii. Wing deformity can be assessed at the same time of checking the sex ratio, again looking at one individual at a time. Determine if the wings of each adult are deformed or not. The number of individuals with undeveloped wings should be very low (less than 5%).

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