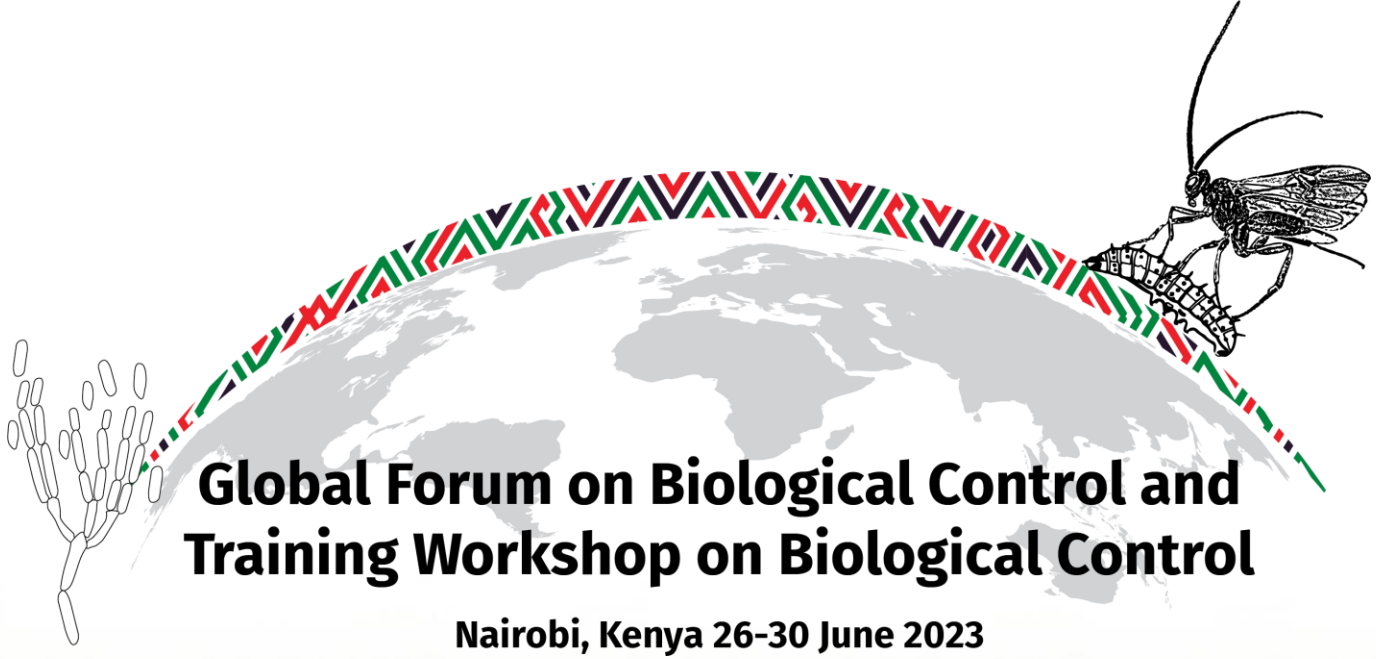

Global Forum on Biological Control and
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Mass rearing of fall armyworm larval parasitoids

Francis Obala and Samira Mohamed on behalf of Biological Control Group

Co-organized by

Food and Agriculture Organization of the United Nations

icipe

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Presentation Outline

- Overview of rearing larval parasitoids of fall armyworm (FAW)
- General overview of the biology of larval parasitoids: e.g., *Cotesia icipe*
- Mass rearing of larval parasitoids (with focus on *C. icipe* and *C. marginiventris)* in the laboratory
- Quality control for rearing of larval parasitoids

Larval parasitoids of FAW in East Africa

Indigenous larval parasitoids

Charops ater

Cocygidium luteum

Cotesia icipe

Tachinid fly

Introduced parasitoid

Cotesia marginiventris

Overview of rearing of larval parasitoids

Points to note

- **Rearing of larval parasitoids requires synchronization of:**

1. Mass rearing of host species/stage
2. Host plant or artificial diet
3. Adoption of right protocol for the parasitoid

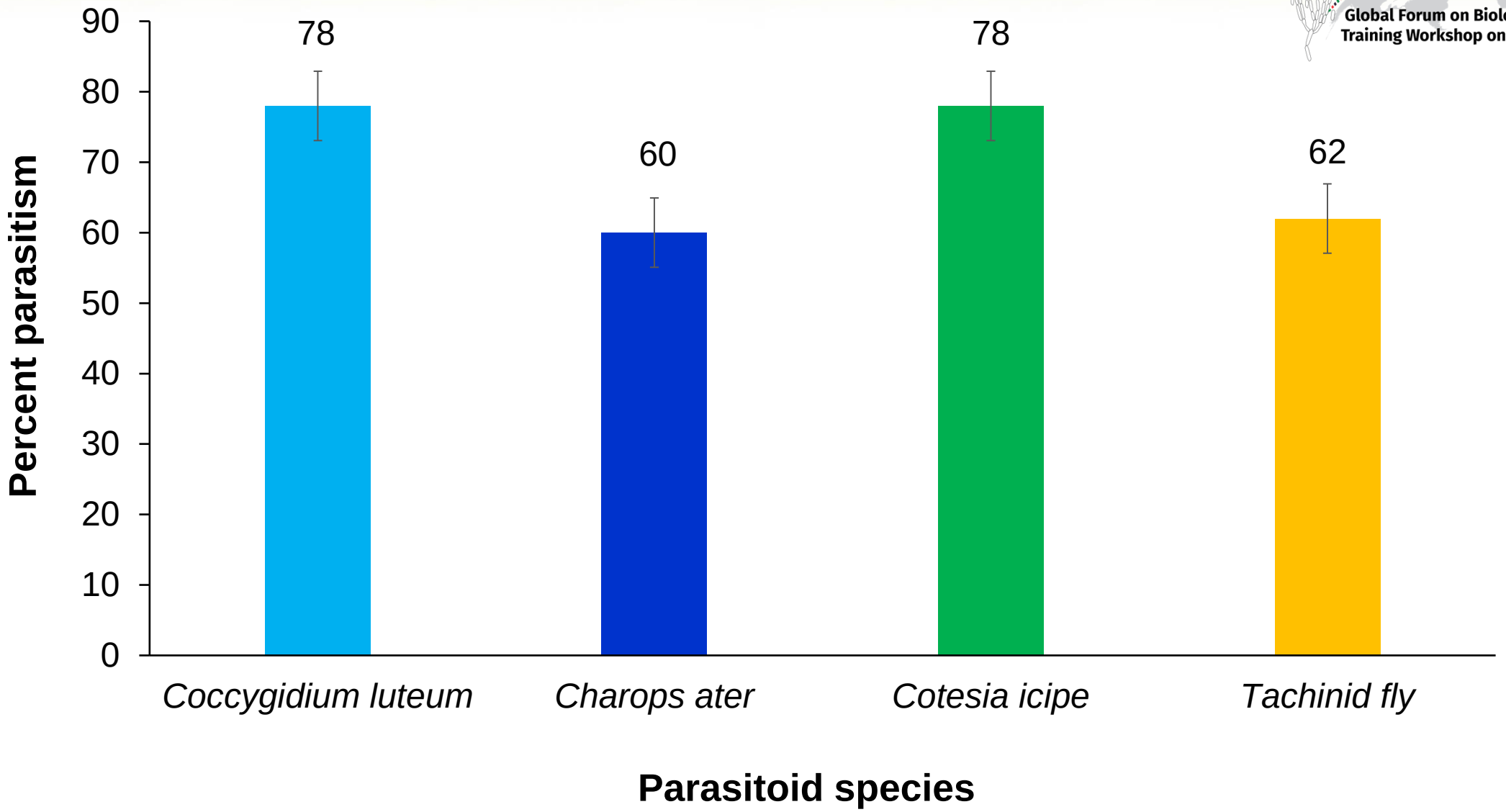
- **In order to achieve the above, mass rearing of parasitoids should be done using:**

1. Suitable host insect and host stage
2. Cost-effective diet (either host plant or artificial diet)
3. A combination of the right host insect and feed that gives high quality parasitoids

Other aspects to consider:

- Biology of the parasitoids
- Performance of the larval parasitoids under lab conditions

Performance of FAW larval parasitoids in the laboratory



General information about the biology of larval parasitoids

- Some larval parasitoids are solitary while other are gregarious.

- Parasitoids are endoparasitoids while others are ectoparasitoids

Cotesia icipe

Tachinid fly

Biology of *Cotesia icipe*

Adult *Cotesia icipe*

C. Icipe parasitizing FAW larvae

Parasitized FAW larvae

C. Icipe pre-pupae exiting parasitized FAW

Cocoon

Biology of *Cotesia icipe*

Parasitized FAW and unparasitized FAW (same age)

- Parasitized FAW larvae becomes inactive, slow growth and eventually dies upon exit of the pre-pupae
- Spins cocoon and eventually a wasp emerges from a cocoon.

Mass production of *Cotesia icipe* in the laboratory

Requirements for rearing of parasitoids on natural diet

Nursery of potted maize plants in a screen/net house; soil mixed with organic manure in ratio of 1:5 parts

Open field for growing maize; using organic manure only

Transparent plastic containers for rearing parasitized FAW larvae

Perspex cages for maintaining adult parasitoids

Requirements for the rearing

Plastic/glass jar for holding eggs and neonates

Perspex cages for incubation of larvae

Plastic boxes with netted lids for incubation

Scissors, counter, brush, aspirator and forceps

- Trained personnel
- Laboratory facility with an AC, proper lighting and working benches
- Natural diet (maize grown in open field/greenhouse
- Artificial diet
- Stable host colony

Adult and immature parasitoids diet

Honey

Artificial diet and preparation unit

Adult wasps

- 100% honey solution, fine drops put underside of the top surface of the rearing cage
- Water on a moistened cotton wool

Immature stages

- Natural diet (corn leaves/castor oil leaves)
- Artificial diet (Fall Armyworm Diet)

Mass production of larval parasitoids using:

1. Natural diet
2. Artificial diet

Mass production of *Cotesia icipe* using Natural diet

Collection of host eggs for rearing larval parasitoids

Maize plant in a cage holding FAW adults

Harvested egg mass in a Petri Dish

- Potted maize plants (3 wks old) are put in a clean Perspex cage
- Mature, mated FAW adults introduced into the cage
- Female FAW allowed to lay eggs on the leaves overnight

Collection of host eggs

Egg masses

plastic jar with neonates

- Fresh egg masses are collected from the vibrant *S. frugiperda* colony (maize leaves).
- 3 egg masses/batches are placed in a clean plastic jar (2litres) and allowed to hatch.
- Depending on laboratory conditions, eggs hatch after 3 days (at 25±2°C) and maintained on maize leaves.

Maintenance of FAW larvae before parasitization

Early larval instars

- Neonates/early larval instars are maintained on leaves (up to L2)
- Only fresh clean maize leaves are used to feed the host larvae
- To prevent dumping or moisture in the plastic jars, use a few leaf cuttings and place pieces of paper towel at the bottom of the jars
- Old maize leaves; no feeding and eventually larvae dies
- For oviposition, choose either L1 or L2; expose to mature mated parasitoids

Exposure of FAW to *C. icipe*

Counting and transferring larvae into jars

Oviposition cage

- Using clean soft camel hairbrush, and a counter, transfer 250 L1/L2 into a clean plastic jar holding pieces of maize leaves
- Introduce the larvae into a Perspex cage with mature mated wasps (25:1 Host: Para) and allow parasitization for 24 hours
- Working with an approx. of 20 cages of at least 100 ♀; 20,000 wasps can be produced per day with only 80 jars (with 250 L2)

Exposure of FAW to *C. icipe*

Oviposition cage

- Exposure time = 24 hours
- Parasitoid: Host = 1♀: 20 L1/L2
- 2-Litre jar can hold up to 250 L1/L2
- 4 jars can fit in a 40 x 40 x 30cm Perspex cage.

Removal of parasitized larvae and incubation in plastic boxes

- Transfer the parasitized larvae into plastic boxes (2L) lined with paper towel
- Maintain the larvae on maize leaves (introduce fresh leaves every two days) until cocoon formation.
- To avoid cannibalism by the unparasitized larvae, remove these larvae everyday (as from the 3rd day)
- Place ≤250 parasitized larvae in a plastic box (2/4L)

Maintaining larvae in plastic boxes and harvesting cocoons

Plastic boxes

- For plastic boxes, maintain the parasitized larvae on fresh maize leaves
- Upon cocoon formation (7-8 days), use soft camel hairbrush to remove the cocoons
- Count the cocoons, place on clean Petri dish and introduce in a clean Perspex cage (30×30×30cm) for rearing or parasitoid field release cages

Incubation of parasitized larvae in Cages

Perspex cages for incubation of larvae

- For rearing of parasitized larvae in Perspex cages, maintain the larvae on fresh maize leaves until cocoon formation.
- Emerging wasps are aspirated and transferred into clean Perspex cage (30×30×30cm) or parasitoid release cages

Summary of rearing of *C. icipe* on natural diet

Counting of FAW larvae and placing in a jar with maize leaf

C. Icipe searching for host on maize leaf

C. Icipe ovipositing on FAW larvae

Parasitized larvae placed in plastic container with fresh maize leaf

Parasitized and unparasitized FAW larvae

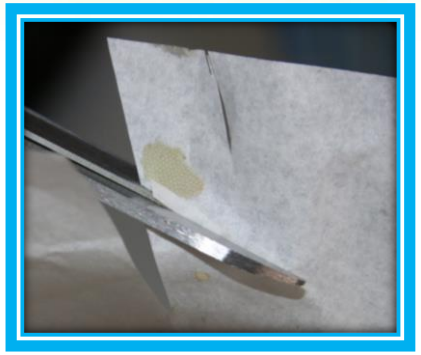
Parasitoid larva exiting the parasitized host

Parasitoid cocoon

Newly emerged wasp

Rearing of *C. icipe* on artificial diet

Host eggs collection on wax paper and inoculation on artificial diet



- Wax paper are carefully lined in the host rearing cages for 24 hours
- Sections of the paper with egg masses are then cut from the paper, egg masses counted and placed in clean Petri Dishes
- In each plastic/glass jars (2 liters capacity), place 3 egg masses and allow them to hatch.

Maintenance of host larvae (L1/L2) for *C. icipe* rearing

L1 larvae feeding on artificial diet

- Transfer 300 L1 into a clean 1L plastic jar (with 10gms artificial diet) using clean soft camel hairbrush
- Maintain the larvae at ambient temperature and humidity until L2

Exposing Larvae to *C. icipe*

Larvae in jars ready for exposure to the parasitoids

- Introduce L2 into a Perspex cage (10:1 Host: Para) and allow parasitization for 4 hours
- The number of jars placed in the adult parasitoid holding cages varies with the size of cages
- The best size is 40 x 40 x 20cm Perspex cages
- In the above cages, place 5-6 jars.

Removal of the exposure jars and maintenance of Larvae

Plastic boxes jars with artificial diet

- The jars are removed from the cages after 4 hours, covered with netted lids and maintained for 2 days in the jars.
- On the third day, larvae are transferred into plastic boxes with diet (30mgs of artificial diet carefully spread on the inner surfaces)
- Pieces of clean paper towel are placed into the plastic boxes

Mantainance of the parasitized larvae on artificial diet

Parasitized and unparasitized
(bigger in size and is removed)

- Remove the parasitized larvae and transfer into a clean 4L plastic boxes with artificial diet
- Maintain the larvae in the diet until cocoon formation.
- As from 6th day (after parasitization), check for cocoons.
- Remove cocoons every 24 hours, put in a clean Petri dish and transfer into a clean Perspex cage for eclosion.

Rearing methodology of *Cotesia icipe*

Counting of FAW larvae and placing in a jar with artificial diet

Parasitized larvae placed in plastic container with fresh maize leaf

Parasitized and unparasitized (removed) FAW larvae

Parasitoid larva exiting the parasitized host

Parasitoid adult emerging from a cocoon

Newly emerged wasp

Quality control in rearing of larval parasitoids

Rearing procedures and maintenance of equipment

- Physical layout, organization of equipment and supplies, design of apparatus (e.g., diet preparation unit) and access to the facility
- Spread of contamination should be minimized by a restricted access to the rearing facility
- Areas of high contamination potential such as artificial diet preparation unit should remain hygienic
- Flow of materials into the rearing facility should be unidirectional (prevent contamination duet to backflow)
- There should be periodic evaluation of microbial load such as fungi within the facility

Maintaining high quality parasitized larvae

Fungal infection on parasitized larva

- Poorly maintained larvae are weak and show stunted growth
- **Cannibalism** is common among poorly fed larvae.

  Regularly check for unparasitized larva (based on size, color and activity) and separate to prevent cannibalism

- **Mites and fungal** growth and possible contaminants

  To prevent fungal growth and presence of mites, use clean natural host or artificial diets; disinfect the rearing containers and destroy the infected culture

Mantainance of cocoons

- Less then 60% parasitism in the lab is an indication of poor exposure strategies for the host larvae to parasitoids in the cage
- Small cocoons indicates poor larval feeding.
- To ensure high quality cocoons, feed the larvae and remove the formed cocoons every 24 hours (to avoid cannibalism of the spinning cocoons)

Strategies to maintain high quality of the wasps

- To get high sex ratio, avoid injury to the female wasps, and to obtain high quality generations, expose L1/L2 for 24 hours in a plastic jar.
- Exposing appropriate hots stages to parasitoids to avoid the problems of physical defense by the host larvae/killing effect
- Group the adults according to filial generations.
- Avoid overcrowding of the wasps in the holding cages
- Provide proper lighting (12H light: 12H dark regimes).
- Proper feeding of the wasps; 100% natural honey; water on moistened cotton wool.

Strategies to maintain high quality of the wasps

Fitness parameters for parasitoids (hind tibia length, forewing length; and forewing width)

- After every three generations, consider assessing the fitness of the parasitoids (measuring the wing length, wing width and hind tibia lengths)
- Smaller female wasp sizes (hind tibia <0.7mm; Wing width < 2.4mm and wing length <0.8mm) are indications of poor-quality parasitoids

Strategies to maintain high quality of the wasps

- Longevity of the wasps at ambient conditions in the lab is used as a measure of the quality of the reared wasps.

- Poor quality wasps that are not well fed, overcrowded and subjected to higher temperatures (>35°C) have a shorter lifespan (less than 7 days in case of *C. icipe*).

- To ensure longest survival of the wasps, feed the wasps with natural honey every 48 hours and moistened cotton wool changed every 72 hours.

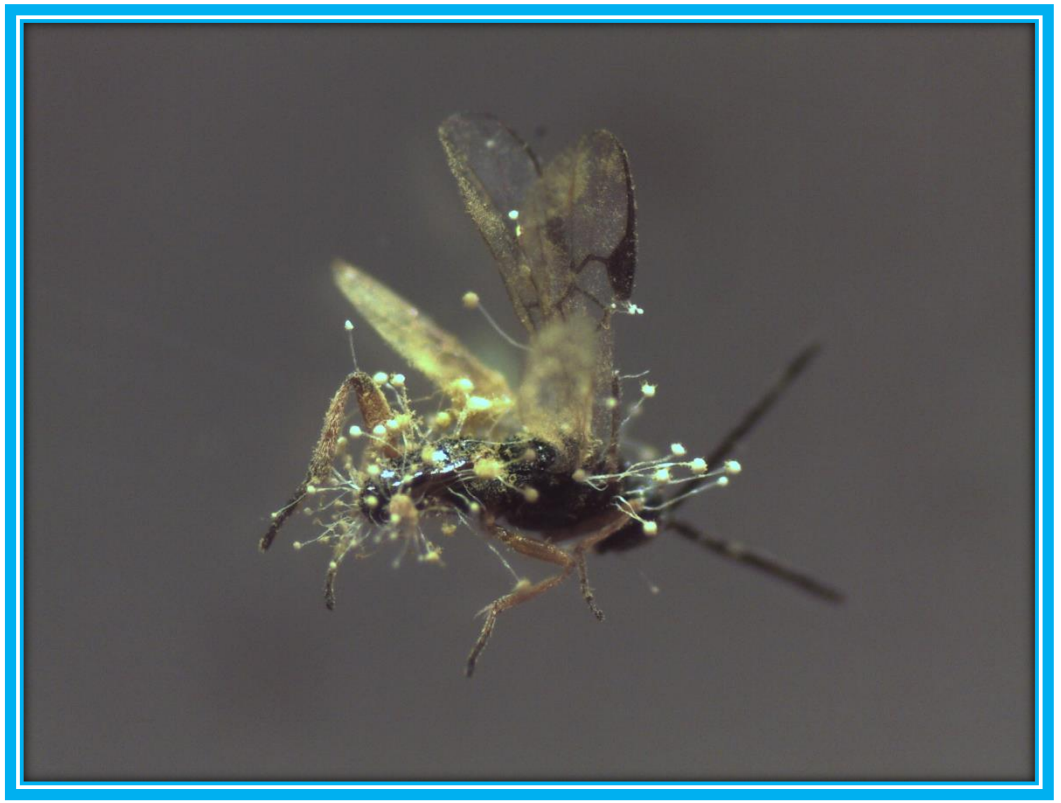
Rejuvenation of the Lab reared *Cotesia icipe*

Cotesia icipe cocoon on maize leaf

- Maintenance of lab colony should be done under conditions as close as possible to wild populations or from the laboratory where insects were obtained.
- Rejuvenation of existing laboratory culture should be done by:
  - ✓ Sourcing for parasitoids from the local wild populations
  - ✓ Importing parasitoids from other areas or rearing facilities.

Strategies to maintain high quality of the wasps

❖ Dead wasps should be removed from the cages to prevent fungal growth



Fungal growth on dead *C. icipe* wasp

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