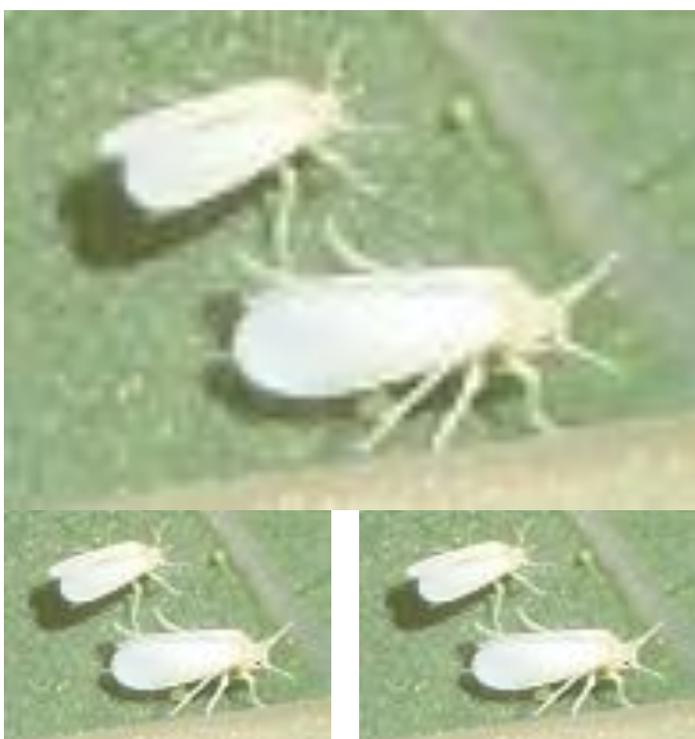


PROTOCOL

RAISING PURE WHITEFLY COLONY



Raising and maintaining pure colonies of Bemisia tabaci



National Agricultural Research Organisation
National Crops Resources Research Institute

Citation

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Contents

| | |
|---|---|
| Citation | 2 |
| Photo credits: | 2 |
| Introduction | 4 |
| Life cycle of <i>Bemisia tabaci</i> | 5 |
| Production of pure colonies of <i>B. tabaci</i> | 6 |
| Raising pure colony | 6 |
| Raising of plants for rearing whiteflies..... | 6 |
| Whitefly colony establishment | 6 |
| Anesthetizing whiteflies | 8 |
| Sexing whiteflies | 8 |
| Maintenance of pure colonies | 8 |
| Termination of the colony | 9 |
| Lock and Lock pots, and cube cages | 9 |

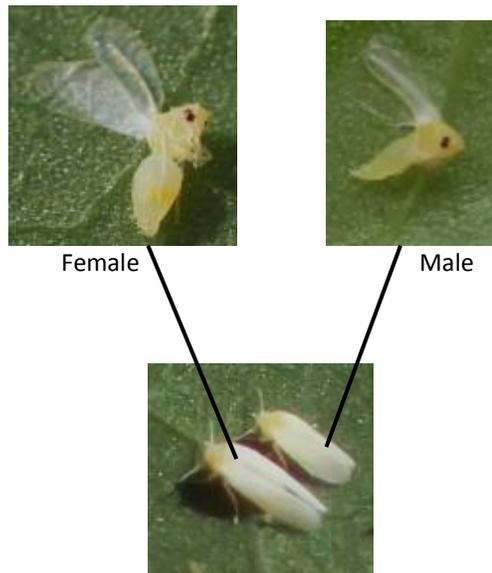
Introduction

Bemisia tabaci is a plant sap-sucking insect in family Aleyrodidae. *B. tabaci* is a species complex that cannot be distinguished morphologically. Therefore, to produce a pure colony it is important to raise the initial population from a single fertilized female.

The adult female usually lays eggs underneath the tender leaf of the host plant as it feeds. Eggs are initially whitish and change to brown towards hatching. Eggs hatch into first nymphal instar (usually semi-transparent) known as crawler, which move short distances for about 1 – 3 hours looking for a suitable place to feed and develop. After the first moult into the second instar, the nymph becomes immobile, and is oval and flattened. The process of moulting continues leading to third and fourth instar.

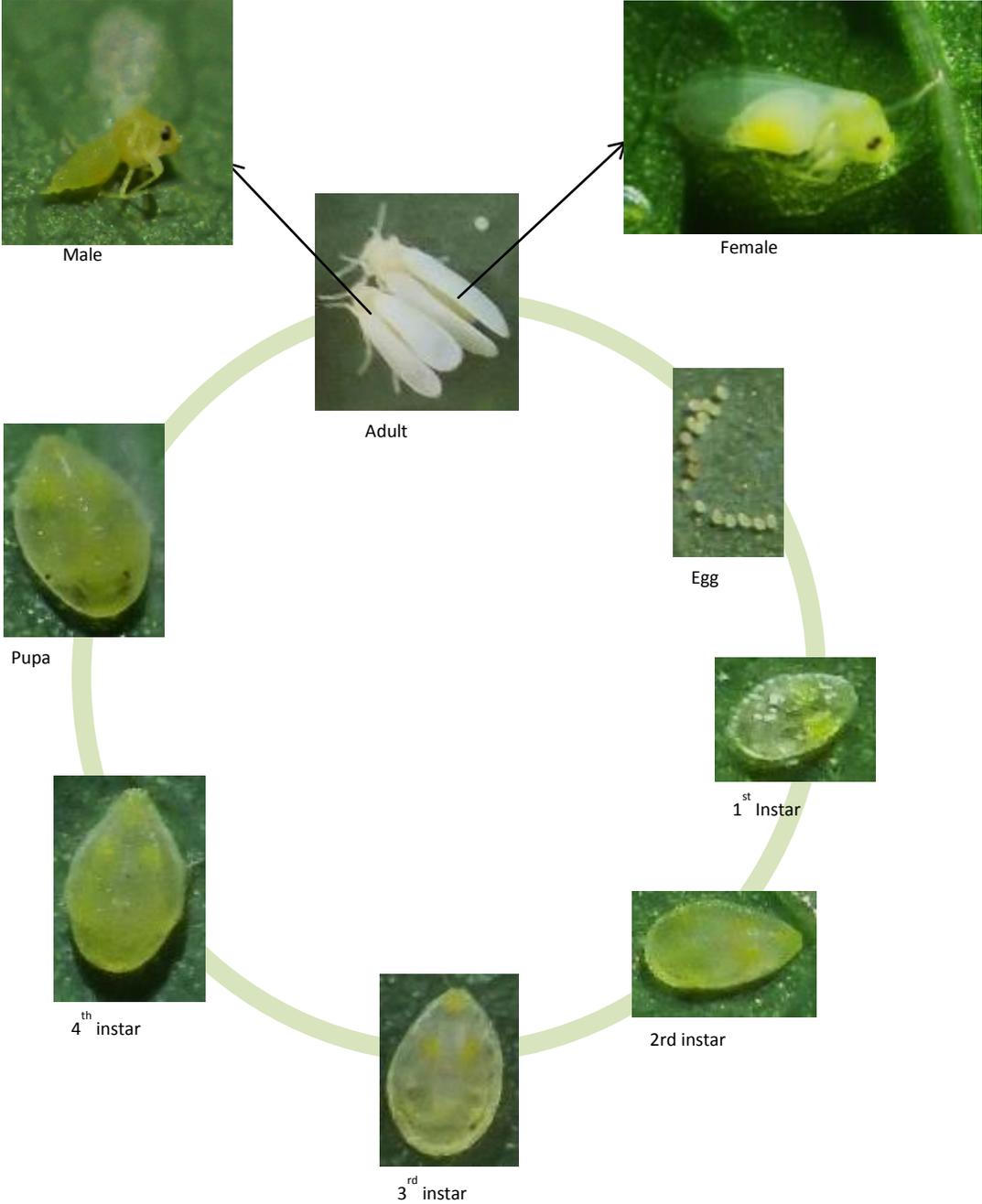
The second, third and fourth instars secrete a waxy material at the margins of their body that helps adhere them to the leaf surface. The different instars subsequently increase in size and become more yellow, and the fourth instar has noticeable red eyes when viewed under a stereo microscope. The fourth instar does not pupate but emerges from the last molt as adult leaving an exoskeleton called exuvium with a characteristic T-shaped exit hole.

Freshly emerged males and females are sexually immature and have clear wings that gradually get coated with wax. The emerged adults sexually mature during the next 24 hours. Virgin females are able to lay eggs but offspring will be only males. Mated females will produce both male and female offspring.



Life cycle of Bemisia tabaci

Bemisia tabaci has incomplete metamorphosis with 4 nymphal stages.



Life cycle of *Bemisia tabaci*

Production of pure colonies of *B. tabaci*

Introduction

A pure colony is a population of whiteflies originating from a single fertilized female. It is imperative that precise whitefly studies in laboratory and screenhouse are conducted using known whitefly species, hence production and molecular characterization of a pure colony. The other value of pure colony is live preservation of a known species for future comparative research undertaking.

Raising pure colony

Materials

The following materials are necessary when one intends to produce a pure whitefly culture: petri dishes, pooter, plants, sterile soil, lock and lock pots (LLPs), cages, hand lens, camel hair brush and ice (see annex 1).

Raising of plants for rearing whiteflies

Plants propagated from seeds or stakes are raised in sterile soil to avoid soil pathogens and pests. Potting cups with propagules (seeds or stakes) are placed on a tray and provided with adequate water for soil absorption, and covered with transparent polythene until one open leaf is seen.

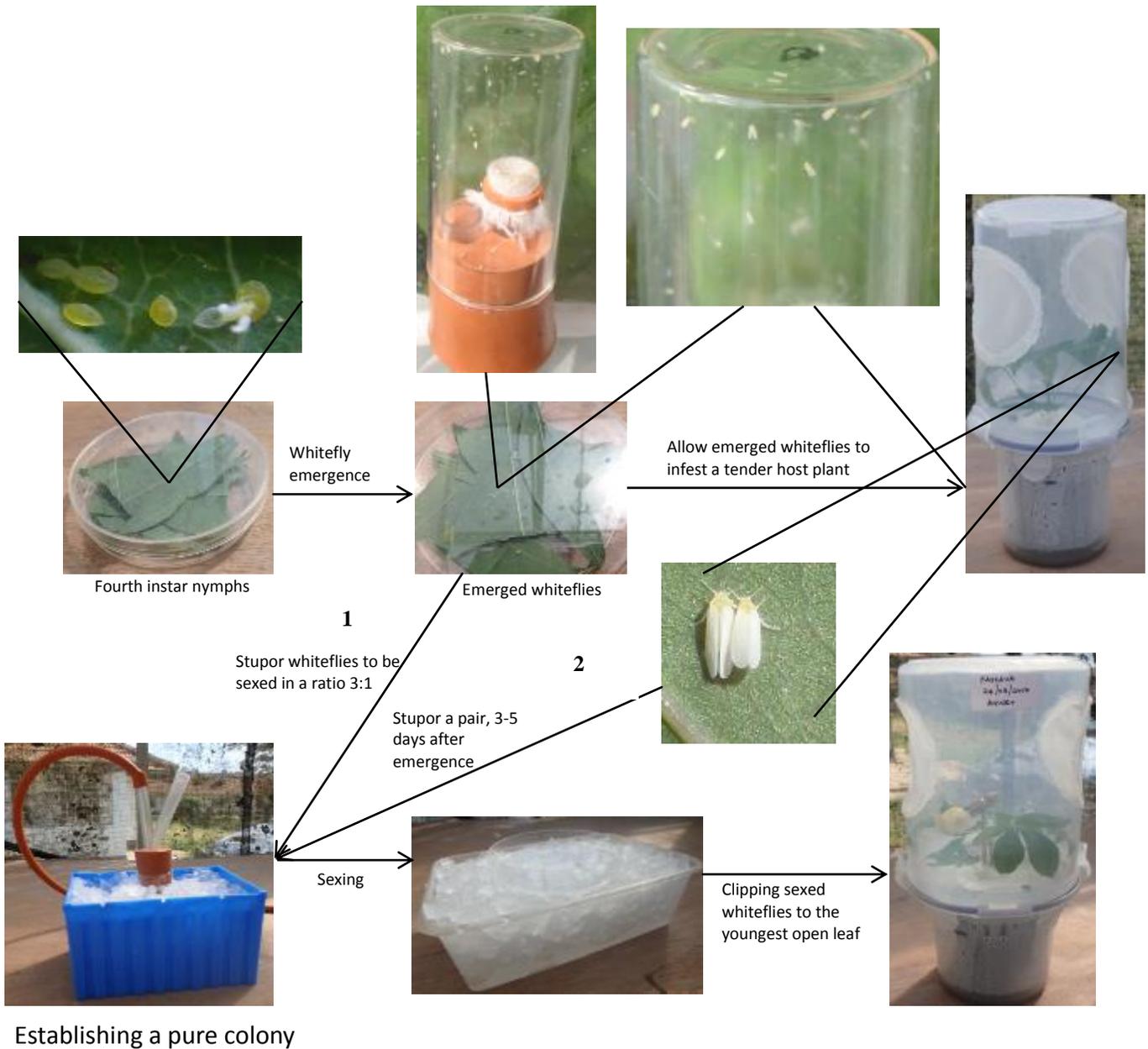
Remember to remove excess water that drains off into the tray to avoid reabsorption into the potting cups, which may lead to rotting of the propagules. The plants are raised in a clean room that is regularly fumigated to kill foreign pests and pathogens. The plants are ready for use when they have 3-5 leaves

Whitefly colony establishment

Colonies of host-plant specific species can be raised from adults or nymphs. However, it is better to raise from nymphs to avoid picking visiting whiteflies. Leaves of host-plant of interest are examined and the ones with higher numbers of fourth instar are collected and allowed to emerge in a petri dish.

The emerged adults can be i) sexed and in a ratio of 3:1 (male to female) and clipped on the youngest fully open leaf of the host plant to initiate a pure colony, ii) allowed to infest a tender host plant in a lock and lock pot (LLP) and then after 5-7 days a pair is picked, sexed and used to initiate a pure colony. In circumstances where nymphs are hard to find on the host plant, adults may be picked and allowed to infest the host plant in a LLP. A pair from the resulting F1 is picked, sexed and then used to start a pure colony.

Once the pure colony has been established, the species identity should be determined by molecular characterisation. Furthermore, it is imperative that regular sequencing of samples from the colony is done to check for possible contamination.



Anesthetizing whiteflies

Anesthetising is done to temporarily immobilize the whiteflies to allow ample time for sexing. This can be achieved by using carbon dioxide or ice. Carbon dioxide (CO₂) works very well, keeping whiteflies immobile for long periods of time with no side effects. However, CO₂ is expensive and a CO₂ source and delivery system are necessary, increasing the costs.

Anesthetizing whiteflies by cooling is the simplest, requiring only a freezer or ice and petri dishes or a simple container. In order to stupor the whiteflies, place the pooter glass in the freezer or on ice for about 1-2 minutes until they are not moving. Remove the pooter and empty the insects onto a petri dish placed on ice and sex. Place the sexed whiteflies in a clip cage using a camel brush and quickly clip to the tender leaf. Whiteflies will “wake up” quickly once off the ice.

Sexing whiteflies

It is quite easy to tell males from females with a little practice. The male is usually smaller than the female and the tip of abdomen is pointed while that of the female is round with a cleft.

Maintenance of pure colonies

Pure colonies of different whitefly species can be raised and maintained in a single room with regulated temperature and humidity. The optimum rearing condition is 25°C and 60% humidity. However, with a temperature of 30°C, the generation time is shorter.

It is important to regularly check your colony and once the rearing plant starts aging, put a younger one in the cage so that whiteflies can move to it. This process should be continuous depending how long the colony is to be maintained. The rearing plants are normally placed on a tray inside the cage. Note that the tray should be positioned closer to the cage sleeves to aid watering through the sleeve surface. Water the plants regularly maintaining a small pool of water in the tray to ensure sufficient moisture to the plant. Stop watering the aging plant once you see majority of the whiteflies on the new younger plant.

To avoid contamination, the following practice should be adhered to: i) ensure restricted access to the whitefly rearing room to minimize a possible introduction of foreign pests and pathogens, ii) strategically place yellow sticky traps in the rearing room to capture any foreign whiteflies flying around in the working area, iii) always put on a lab coat and hair net before entering the rearing room, iv) open one cage per day and/or per room when accessing different whitefly species, v) regularly sequence whitefly samples from the colonies to validate purity, vi) routinely remove the algae from the tray and sooty mould from the surface of the cage.

Termination of the colony

To terminate the colony, watering of colony plants must be stopped to kill plants and whiteflies. Dead plants should be removed and put in a polythene bag, thereafter frozen for 2days. Cube cages should be washed in detergent. LLPs should be frozen for 2days and then washed with detergent.

Lock and Lock pots, and cube cages

Lock and lock pots (LLPs) are very useful when initiating whitefly colonies and for ease of culturing. Cube cages are used mainly for maintenance of large populations of whiteflies whereas LLPs are useful for maintaining smaller populations and are preferred for constructing pure colonies.

Materials for making LLPs can be purchased in any supermarket and covers screwed together. Cages can be purchased from the BugDorm Store (<https://shop.bugdorm.com>).

National Crops Resources Research Institute, 2017