

# PREPARATIONS OF VIRGINS AND DRONES FOR INSTRUMENTAL INSEMINATION

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## VIRGIN QUEENS REARING

These queens will endure stress, therefore every step in rearing and care procedures must be given extra attention. Optimal rearing conditions are essential. Give your cell builders an extra boost of young bees and graft fewer cells. This is especially important if forage resources are limited.

## EMERGING QUEEN CELLS

Queen cells should be caged and allowed to emerge in cages. Be sure to mark the queen mother and number all cages. After emergence, remove empty queen cells from cages (virgins will often crawl up into these and starve). Cull virgins of inadequate size or color.

## BANKING

Queen cells can be emerged and virgins banked in a queenless colony. Supply the bank frequently with open brood, young bees and feed. Never bank queens of various ages together, especially mated queens and virgins.

## AGE

Virgins should be inseminated between 6 and 10 days. After 10 days the Ph of the vagina changes and less sperm is stored. High mortality will be experienced when queens are inseminated 4 days old or younger.

## CO2 TREATMENTS

Two CO2 treatments are required to stimulate egg laying. One treatment is given during the insemination procedure and the second can be given either a day before or a day after insemination. If the queens are banked after insemination, the second CO2 treatment should be given when the queen is introduced. Give 2 to 3 minute CO2 treatments. Place caged queens in a plastic bag and fill with CO2. Queens will start laying 3 to 6 days after their second CO2 treatment. If a queen has not begun laying within 10 to 12 days a third CO2 treatment (2 to 3 minutes ) can be given.

## QUEEN INTRODUCTIONS

The most optimal condition is to introduce a caged newly emerged virgin queen into a nucleus colony. She can be released directly after insemination. If it is not possible to establish virgins in nucs, banked virgins can be introduced after insemination. Be sure these receive a second CO2 treatment, otherwise they will not lay for 4 to 6 weeks!

## Direct Release Of Queens

Be sure all nucs and queen cages are properly marked so they are returned to their original place. Virgins established in nucs and given a CO2 treatment 24 hrs. before insemination can be released directly after insemination. If released while they are still under the influence of CO2, the queens will be less defensive.

If awake when released, watch the response of the queen and bees for a few minutes to be sure the queen is accepted. A light spray of syrup over the bees and queen at time of release will facilitate distribution of queen pheromone and distract the bees during release.

### **Releasing Queens**

The optimal size colony for introductions is a 5 frame nucleus colony. A single deep with a division board works well and can be easily expanded as the population grows. Make these up with brood and young bees. Young bees will more readily accept a new queen. Banked queens should be given their second CO2 treatment and introduced in cages. These can be released 5 to 6 days after introduction. Upon release, lightly spray syrup over the bees and queen, watch the queen for several minutes noting any defensive behavior (rearing legs, running, etc) and the workers for aggressive behavior. If this occurs, cage the queen for an additional 2 or more days.

### **Exclude the entrance**

Exclude the entrances and patch all holes in boxes. Queens will sometimes attempt to take their own mating flights. After 5 to 10 days check for eggs. When eggs are seen, excluders may be removed. Zinc or plastic works nicely because these can be cut and stapled over the entrance.

### **Check For Queen Cells**

Be sure to check for natural queen cells during queen introductions and soon after the queen has started laying. This is critical during the first cycle of brood! Do not assume your I.I. queens will tear down natural queen cells. I.I. queens initially start egg production at a slower rate than naturally mated queens, though will catch up and perform as well. If the colony population size is too big initially, I.I. queens may be superseded as they cannot meet the production demands of the colony. For this reason it is important to introduce I.I. queens into small units. Allow the queen to build up her own population at her own pace and give her space as needed.

## **DRONES**

### **COLLECTING DRONES**

Select your best producing colonies as drone mothers. Drones can be marked at emergence for identification or colonies can be isolated to prevent drift. Drones must be 12 days or older. Usually if drones are flying, they are mature. Drones can be caught on return flights (preferably caught after taking cleansing flights). Place an excluder over the colony entrance during the afternoon drone flight time. During the peak season you should have handfuls of drones gathering quickly. Drones fly mid to late afternoon. They will fly later as the season progresses and later in the day as temperatures increase.

### **BANKING DRONES**

Drones are extremely perishable. Unattended by workers and without food, drones will die within an hour or two. If you are collecting from an outyard- take a bank to put these in for travel. The best place to bank caged

drones is a cell builder or virgin bank which does not have many drones. Place the drone cages between a frame of open brood and a frame of pollen & honey. These can be banked for several days, though the less time the better. It is best to use the drones the same day or the following day. They are difficult to bank and build up a lot of feces - creating a major source of contamination during insemination.

Please note: A queenright colony will not take good care of old drones, especially if it already has a good drone population. If resource conditions are not plentiful- drones will die overnight in a normal colony.

### **DRONE HOLDING CAGES**

Drone cages can be made of queen excluder material to allow passage of attendant bees. Make cages so that 2 or 3 fit easily into one frame space in the bank and fit easily into a 1 ft. square flight box for semen collection. Drone cages 5" X 7" and 1"

wide will hold 100 to 150 drones. The opening can be a 3/4" cork. This size opening makes it easy to collect drones and allows them to exit slowly in the flight box.

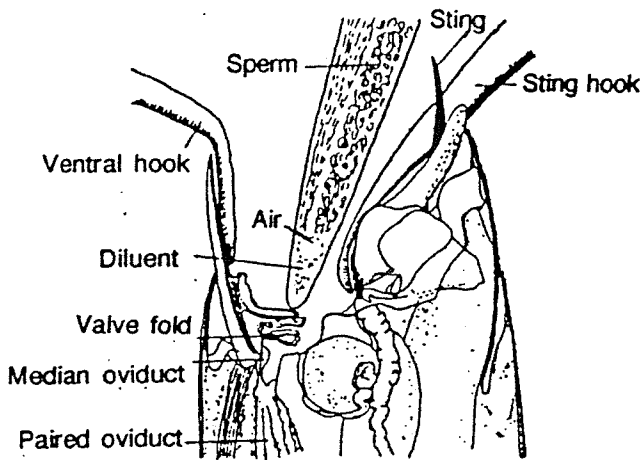
### **DRONE FLIGHT BOX**

The flight box should be large enough to hold two or three drones - a one ft. sq. box works well. The top should be screen hardware cloth (so light can be placed above for heat and stimulation). Three walls should be solid (so drones don't spray feces). The fourth side should be open and covered with a cloth with an elastic opening for easy access to drones. The open side needs a solid bottom lip to prevent drones from escaping.

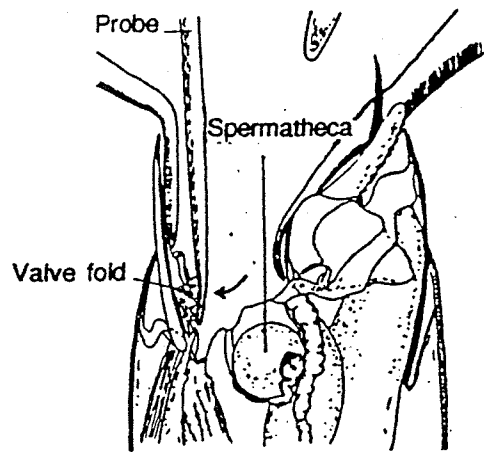
The drone brood should be moved to strong colonies to care for. Give each colony no more than the equivalent of one solid frame of drone comb (about 2000 drones). Keep in mind that it will take 60+ days from egg to mature drone.

Drone production and tolerance is a factor of population size. A small colony will not care for these. Colonies with older queens will have a higher drone tolerance. If things get difficult - dequeen or cage the queen in the colony. A queenless colony, colony with a virgin or caged queen will care for drones.

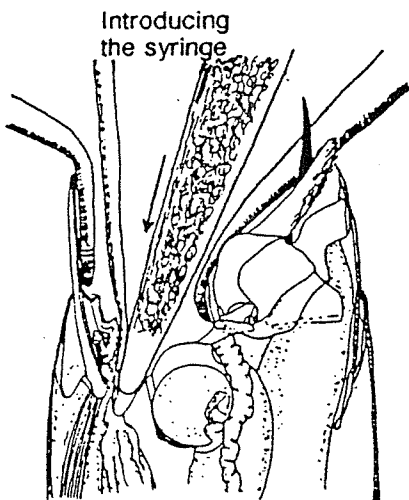
Providing a large number of mature drones late in the season is a difficult job. Drone production is easy during spring when the bees are naturally stimulated.



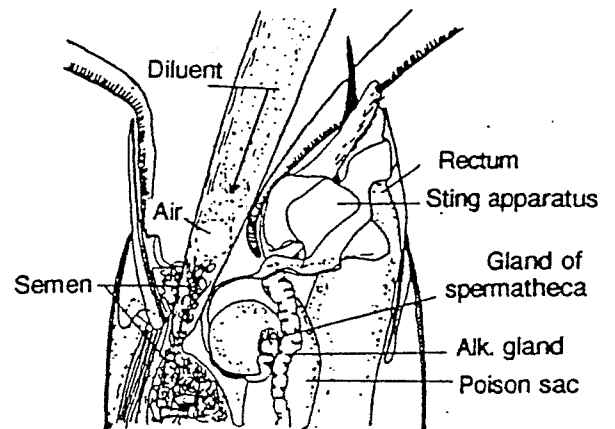
1. Stinging chamber opened with hooks. Syringe is adjusted to the vaginal orifice.



2. Syringe is pulled back. Valve fold is pressed to the side with a vaginal probe.



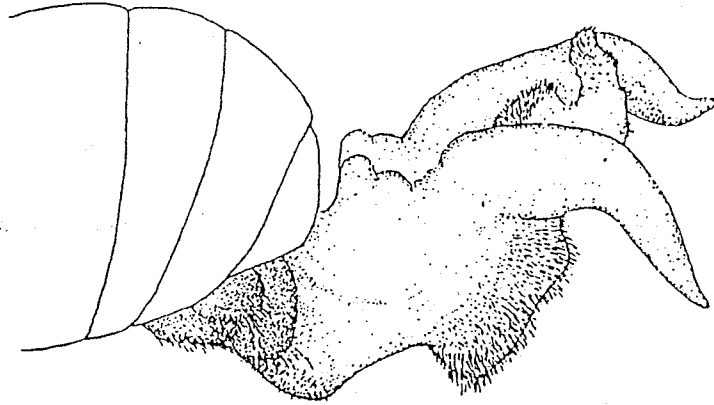
3. Introducing the syringe into the median oviduct (genital orifice).



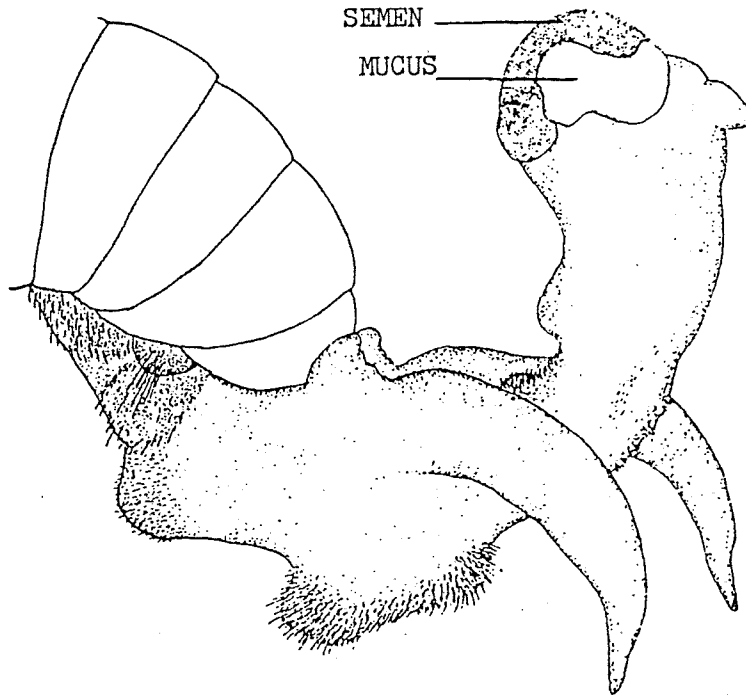
4. Injecting semen into the paired oviduct.

Fig. 13. Insemination process using the conventional dorsal hook without hole and with the aid of a probe (Illustrations: HLA - Field Office for Apiculture/Kirchhain)

ABDOMEN OF THE DRONE SHOWING STAGES OF  
EVERSION FOR SEMEN COLLECTION



PARTIAL EVERSION



COMPLETE EVERSION EXPOSING SEMEN AND MUCUS  
(adapted from Mackensen and Roberts, 1948)