

NOTES AND COMMENTS



## Individual feeding of honey bees: modification of the Rinderer technique

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Currently, inoculation with substances or infective microorganisms of individual honey bees involves many technical problems, such as long time periods of handling and a tedious manipulation of the bee to achieve the ingestion of the inoculum. Also, many operators are needed in order to simultaneously obtain large numbers of inoculated individuals.

Rinderer (1976) published a useful inoculation technique to achieve the feeding of large numbers of adult workers. By this method, bees are individually confined and the food is available on glass capillaries. This technique incorporates a wooden device which contains glass cylinders sealed by a cork, to form a compartment where the bee is confined. The inoculum, composed of sucrose solution and the test material, is placed at the internal end of the glass capillary, which passes through the cork. Confined bees are drawn to the glass capillary by the sugar solution and phototaxis and, finding the inoculum, consume it.

Our aim was to replace glass materials to achieve easier handling. Also, we propose different steps in the work sequence in order to speed the technique and reduce the number of operators required to produce large numbers of inoculated worker bees. A notorious reduction on the cost of materials was also achieved.

Initially, we proposed the replacement of the glass capillary with a plastic micropipette tip. The tip is truncated to increase the diameter of the feeding tube which allows the glossa introduction (Fig. 2b). Tips are loaded with a standard laboratory micropipette. The cylinders which delimit the capsule walls were made from rigid PVC pipe used in electrical installations (D = 16 mm). The support assembly was prepared with a polyethylene foam sheet, commercially available as insulator (0.5 cm). It was perforated with a punch of the cylinder diameter (Fig. 2a) and glued to a wooden board. The cork used in the technique of Rinderer was replaced by a polyethylene foam circle (the residual from the punched foam) and perforated in its centre to allow the insertion of the tip (Fig. 2b).

Workers used to test the technique were obtained from combs with sealed brood placed at incubator conditions ( $32^{\circ}\text{C} \pm 0.79$ ;  $60\% \pm 3.3$  HR) until the emergence. Imagoes were confined on wooden cages with plastic mesh and feed with sucrose syrup 60% (w/v) until

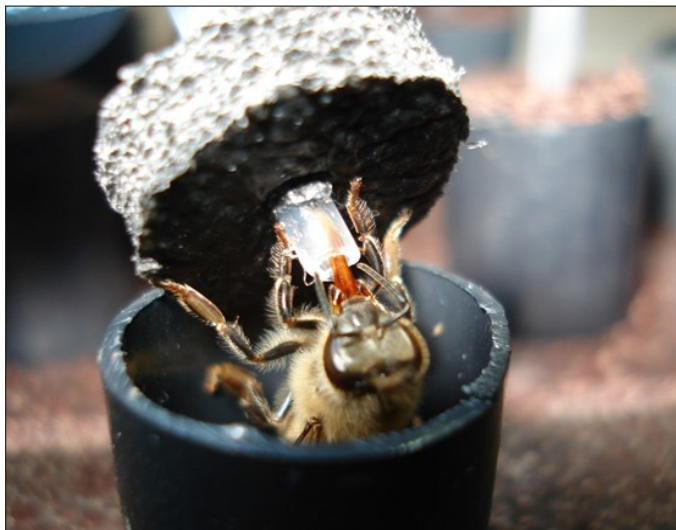


**Fig. 1.** Steps of the working sequence.

inoculation. Newly emerged bees up to 48 hours after emergence are recommended to perform the inoculation. Younger bees have shown a slower consumption of the inoculum.



**Fig. 2.** Device construction. 2a) Perforating the plastic base with a punch. 2b) Entire tip and truncated tip with foam plug. 2c) Capsule from inside.



**Fig. 3.** Bee consuming the inoculum before closing the capsule.

In general the following sequence was performed: 1) Plastic cylinders were inserted in a battery of foam plates (Fig. 1a); 2) the truncated micropipette tips were placed through the foam plugs (Figs 2b); 3) Bees were starved for at least 1.5 - 3 hours after inoculation; 4) If necessary, worker bees may be cooled at 4°C for 15 min. after inoculation (sometimes it helps for an easy handling); 5) Micropipette tips were loaded (2 - 10  $\mu$ l) with the inoculum diluted on sugar syrup 50% (w/v) (Fig. 1b); 6) Worker bees were placed into the capsules and then covered with cap + tip (Figs 1c and 3); 7) Plates with bees were held at 30°C for 30 min. to 1 h (Fig. 1d); 8) Caps were removed and the individuals which consumed the entire inoculum were used to perform the assays (Fig. 1e). Worker bees that did not consume the entire inoculum (generally, about 5% of confined bees) were discarded; 9) Materials were disassembled and were washed and disinfected using temperatures not exceeding 70°C for 2 days and soaking in sodium hypochlorite or detergents. This methodology has been employed on several occasions with the aid of two or three technicians. For example, up to 800 worker honey bees were infected with *Nosema ceranae* spores in a period of 3 to 4 hours. Time depends on the age of the worker bees, starving time and administered substance.

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## Reference

Rinderer, T E (1976) Honey bees: individual feeding of large numbers of adult workers. *Journal of Economic Entomology* 69(4): 489–491.