NOTES AND COMMENTS

Bee cups:
single-use cages for honey bee experiments

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Honey bees face challenges ranging from poor nutrition to exposure to parasites, pathogens, and environmental chemicals. These challenges drain colony resources and have been tied to both subtle and extreme colony declines, including the enigmatic “Colony Collapse Disorder” (CCD). Understanding how various challenges affect bees, and especially how these challenges interact with each other, requires numerous controlled experiments. In practice, these experiments are often carried out on a small number of bee larvae or adults under laboratory conditions. Insights from such experiments can pave the way to field experiments and results from actual management.

Numerous methods for ‘caging’ and observing adult bees have emerged over decades of study. One cage commonly used in research is a 12 cm x 12 cm x 12 cm chamber constructed of wood, mesh, and glass that provides an environment for up to 100 bees (Kulincevic and Rothenbuhler, 1975). Bees survive well in these cages and investigators are able to introduce chemicals and / or pathogens to the caged bees via liquid feeders or surface exposure. The main drawback with these cages is their expense in time and materials, which makes them poor choices for single-use experiments with infectious agents or chemicals. Other experiments have been carried out with wire mesh canisters (Herbert, 1975) or disposable cardboard ‘ice cream cups’ (David Knox, pers. comm.) which are less expensive but can preclude behavioural studies. Others have used small plastic cups (Iwasa et al., 2004) and even ‘Benton’ wooden queen cages for experiments with a small number of workers (S. Pernal, pers. comm.). Cages have also been divided by a permeable screen in order to contrast the impact of physical contact with that of volatiles (Lindberg et al., 2000). While these studies provide clear instructions for caging bees, most other experiments, including important pre-market tests of pesticides to determine their possible impacts on bees, provide poor documentation of cage design and protocols. Here we describe our attempts to design and test cages for exposing adult honey bees to pathogens and other factors that impact their health.

We present two designs that have been used successfully, the latter of which is suitable for experiments with captive Varroa destructor mites.

We had five criteria in designing our bee cups. They had to be: 1. fully disposable, so as to avoid contamination across runs; 2. transparent to allow easy counting and observation of bees; 3. inexpensive and made of commonly available supplies to allow hundreds of trials and shared results across groups; 4. easily opened for the addition or removal of bees, food, water, or treatments; and 5. capable of sustaining bees.

Our most successful bee rearing cups consisted of 14 oz. clear plastic tumblers (Party City; Laurel, MD, USA; Fig. 1a.) with an opening diameter of 84 mm. These cups were paired with standard plastic laboratory Petri dishes and glass or plastic 20 ml scintillation vials. The smaller (bottom) Petri dish in each pair has a diameter of 87 mm, just clearing the outside diameter of the cup. For our first design, approximately 80 holes were burned into the sides of the plastic cups with a heated piece of 5 mm hardware cloth (Fig. 1b.). A circular hole was burned in the bottom of the cup by heating the mouth of a glass scintillation vial and pressing it to the bottom of the cup with a slight swirling motion (Fig. 1b.). This produced an opening big enough to allow the lidded end to pass through the hole, yet small enough to have the shoulders rest on the opening. Two holes were drilled into the lids of the scintillation vials with a 1 mm drill bit and these vials were filled with a 1:1 sugar: water mix and inverted over the top of the cup to provision caged bees (Fig. 1c.). A circular hole was burned in the bottom of the cup by heating the mouth of a glass scintillation vial and pressing it to the bottom of the cup with a slight swirling motion (Fig. 1b.). This produced an opening big enough to allow the lidded end to pass through the hole, yet small enough to have the shoulders rest on the opening. Two holes were drilled into the lids of the scintillation vials with a 1 mm drill bit and these vials were filled with a 1:1 sugar: water mix and inverted over the top of the cup to provision caged bees (Fig. 1c.). Finally, a piece of disposable incontinence underpad was cut to be slightly larger than the Petri dish (roughly 10 cm square) so that occasional drips from the scintillation vials were absorbed. As a second design, ventilation was provided by cutting a circular hole in the side of the cup and gluing a small square of fine nylon mesh over this hole (Fig. 1d.). These cups were then...
Bee cups for experimentation

Fig. 1. Steps in building 'top-feeder' plastic rearing cups: A. heated steel mesh for making cup ventilation; B. melting of feeder vial hole; C. finished ventilated cup; D. finished 'mite-proof' cup.

suitable for studies with live V. destructor mites. We maintained bees in a 32-34°C incubator with a pan of water in the bottom for humidity. We loaded bees into each cup after plugging the top hole with tissue paper or a number 5 stopper, and only provided the sucrose source once bees were settled in. Depending on the numbers of caged bees (20 - 60), the sugar water had to be replaced about every two weeks. With these cups, we have carried out several hundred trials with adult bees on sugar water alone, with a median survival of 36 days, and maximum of 60+ days.

An alternate, "cup-in-cup" design analogous to that used by Iwasa et al., (2004) but using 14 oz plastic cups also proved successful. A 4 mm hole was drilled in one cup, and a wick rolled from 2 cm x 4 cm sterile tissue paper was pressed through this hole, with even lengths on each side. Once bees were placed into this cup, the opening was covered with nylon mesh held in place by a rubber band. The cup was then nested in a second cup containing 10 ml of 1:1 sugar water, and sugar water was replenished weekly.

Advantages of the top-feeder design include ease of sampling live or dead bees, as cups had only to be tipped slightly and bees removed from the bottom edge by forceps. In addition, these cups did not show the incidental microbial growth found with feeders on the floor of the cup. Challenges were occasional leaks from the scintillation vials, and (more rarely but more seriously) blockage of the small holes due to crystallization of the sugar water source. Maintaining high incubator humidity appeared to reduce the latter.

We have successfully used the described top-feeder cups to assess the impacts on bee longevity of exposure to bacteria, viruses, and Nosema, and to collect material for determining the immune responses of adult bees to this exposure (Evans, 2006). We also anticipate experiments testing the impacts of pesticides or other environmental chemicals on bee health and behaviour. We are also using these cups for survival experiments involving the genetically important, but more fragile, drones. Experiments with drones offer a potentially more efficient route for exploiting genetic disease resistance and we anticipate a system like this could be used to screen adult drones (with worker helpers) for resistance prior to harvesting their sperm for instrumental insemination. Finally, while we saw no evidence of such effects, plastics of all types have the potential to release volatiles that could affect bee survival or behaviour. As a minimum, any given controlled experiment should use cups from the same source.

We realized belatedly how much effort has been put into bee cage designs over the years, and we are eager for feedback from other researchers who have developed methods for exposing bees to environmental insults of all kinds. Repeatable, high-throughput methods for determining how adult bees respond to various chemicals and organisms should help improve both management and breeding issues in honey bees. Ideally, the research community will soon develop and advertise standard methods along those lines. As one way to enable this discussion, and provide a place to cite additional references beyond what could be cited here, we have established a resource site within the BRL "Research Protocols" folder at: http://www.ars.usda.gov/Services/Services.htm?modecode=12-75-05-00.

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References


