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NOTES AND COMMENTS

A new design for honey bee hoarding cages for laboratory experiments

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Honey bees are the subject of research around the world due to their great economic importance and current population declines (vanEngelsdorp and Meixner, 2010). Many studies cannot be conducted at the colony level. Controlled cage experiments provide insight into behavioural interactions (Elzen *et al.*, 2001), diseases (Martín-Hernández *et al.*, 2009), nutritional requirements (Altaye *et al.*, 2010) and effects of insecticides and genetically engineered plants on these important pollinators (Malone *et al.,* 1999; Medrzycki *et al.*, 2003).

Honey bees have been kept in hoarding cages of various materials and sizes (reviewed in Williams *et al.*, 2013). Wood is widely used, with mesh for ventilation, and glass fronts for observations (Altaye *et al.*, 2010; Malone *et al.*, 1999). Others have used Plexiglas® / Perspex® (polymethyl methacrylate) cages (Grozinger *et al.*, 2003; Medrzycki *et al.*, 2003), wire mesh cylinders and Petri dishes (Melathopoulus *et al.*, 2000). Disposable alternatives include plastic cups (Evans *et al.*, 2009; Iwasa *et al.,* 2004) and cardboard boxes (Moncharmont *et al.*, 2003; Picard-Nizou *et al.*, 1997). Research papers often lack detail on cage design, making it difficult to replicate the experimental conditions. Here we propose a durable cage design that has been successfully tested in our laboratory.

We recommend constructing cages from polycarbonate (PC), which is very break-resistant. Transparent PC is ideal for observations and video recordings, an obvious advantage over wood. PC is resistant to various foodstuffs, such as sugars and oils. It can withstand temperatures of 120°C (40°C higher than polymethyl methacrylate) and can therefore be autoclaved between trials, which is particularly important for studies on bee pathogens. Ethanol can also be used for sterilisation. Resistance to sodium hypochlorite is limited, although bleach commonly contains < 8% sodium hypochlorite, which PC can endure. It is important to note that PC is not resistant to acetone, a solvent often used in toxicological studies. Its otherwise good resistance and durability makes PC a suitable material for hoarding cages, although potential negative effects of PC on honey bees remain to be investigated.

The bottom of the hoarding cage consists of wire-mesh for ventilation; this should be made from a rust-resistant material (e.g. stainless steel) to withstand high humidity. Cages should be assembled using screws: glue may contain toxic ingredients, and may gradually dissolve when exposed to high temperature and humidity.

An outside measurement of 11.5 × 10 × 14 cm for the cage is ideal to sustain groups of 100 workers, but leaves space for > 300 if needed. Many experiments have used only 20–60 workers per cage (Evans *et al.*, 2009; Martín-Hernández *et al.*, 2009). However, larger groups are preferred because queens mate with up to 60 drones (Adams *et al.*, 1977), so not all patrilines may be represented in a cage with too few bees, resulting in genetic differences between cages from the same colony. Furthermore, the fewer bees there are in a group, the less they act as a social unit. Darchen (1957), for instance, showed that comb construction only starts in groups of 75–100 workers.

The top and two sides of the cage consist of 10 mm thick PC for stability (Fig. 1). Thin PC slides (3 mm) in grooves serve to close the front and back of the cage and can be lifted for access to the inside. The wire-mesh bottom slides into grooves. It is convenient to have a second pair of grooves cut above those for the wire-mesh, so that the back slide can be lifted for insertion of a second base before removing the first for quick removal of dead workers.

An additional small PC piece with openings sits underneath the front slide. Feeders made from plastic test tubes with screw-on lids are inserted horizontally. Feeder pieces can be exchanged to accommodate different numbers and sizes of feeders. The hole for feeding cut into the plastic tube should be no bigger than 1 × 0.3 cm for liquids, in order to minimize evaporation and prevent drowning of the bees. Larger feeding holes can be used for solid diets so that the bees can walk into the feeder to collect the food.

Groups of 100 *Apis mellifera scutellata* workers provided with honey comb (5 × 5 cm) survive better than those given a wax foundation of the same size (Fig. 2). There are two reasons why the presence of a

Fig. 1. The honey bee hoarding cage made from polycarbonate.
Photograph taken by A.K. Switala

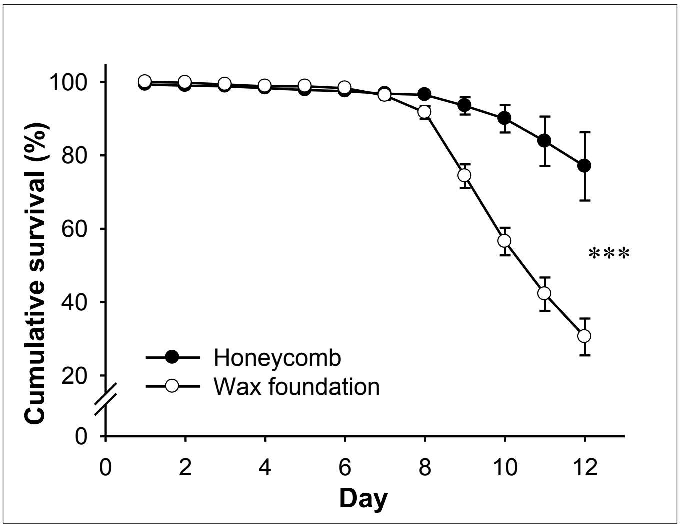

Fig. 2. Survival of caged *A. m. scutellata* workers, incubated at 34˚C and on a 0.63 M sucrose diet, when provided with either a piece of honeycomb or wax foundation (N = 12 cages from six colonies; mean ± SE). Survival was lower in workers with wax foundation (Gehan's Wilcoxon; $Z = 16.03$, ***$P < 0.001$).

comb may improve survival. Firstly, young bees keep warm by crawling inside the cells, as endothermic heat production only develops in workers older than two days (Stabentheiner *et al.*, 2010). Secondly, honey bees store sugar solution in the comb, which is later consumed. Honey bees do not defecate inside the cage and may benefit from ingesting a more concentrated diet after storage. The stored solution may further assist with humidity regulation.

Different laboratories have developed various cage designs for controlled honey bee experiments. We feel that standardization of maintenance cages would increase comparability between studies. We have proposed a robust, re-usable cage design that can sustain honey bees over several weeks, with easy feeder access and removal of dead individuals. Its transparency allows for behavioural observations. Given the cost of polycarbonate, the proposed hoarding cage is perfect for longer-term experiments, while inexpensive disposable cages may be favoured for short experiments. The advantage of the polycarbonate cage is that it is long-lasting due to the resistant material and can be autoclaved and disinfected to prevent cross-contamination.

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