

Some time ago I completed a project on the effects of old comb versus new comb on honey bee colony growth, brood survivorship and adult mortality. This paper was originally published in the *Journal of Apicultural Research* 40(1): 3-8 (2001). (To read it in its entirety go to our website **www.ent.uga.edu/bees** and click on the research archives icon.) Here is a shortened version of that research.

This topic is still timely because of the more recent findings regarding chemical residues in wax and pollen in colony comb, and, because this is the time of the year it is easiest to remove that old, disease-ridden, chemical laden junk, and replace it.

Honey bees use structures like trees and man-made hives for shelter. but it is the beeswax that provides the basic building material for the interior nest substrate. Adult worker bees secrete oval shaped wax scales from glands located on their abdomen and then modify these scales with mandibular gland secretions in order to construct the comb. Wax secretion usually occurs during peak foraging times because large quantities of honey or nectar must be consumed by the worker bees in order to produce these wax scales (Gary, 1992). The comb, made up of an array of hexagonal cells placed back to back, is the site where immatures are reared and food is stored. The comb also plays an important role in communication by providing the substrate on which dances are performed and chemical messages

## Replace That Old Comb Here's why!

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transferred (Winston, 1987).

When comb is first constructed it is pliable and nearly white in color but changes over time due to constant use and incoming resources. Comb used for food storage takes on a yellowish hue due to the accumulation of pollen (Free & Williams, 1974). As comb used for brood rearing ages, it becomes darker, almost black, and more brittle (Hepburn, 1998) because of accumulated fecal material (Jay, 1963), propolis and pollen (Free & Williams, 1974). The darker color may also be a result of numerous contaminants that are collected and absorbed in the wax over time.

Wax comb consists primarily of hydrocarbons and ester components with a small percentage of free acids and alcohols. These minor components are believed to give wax its plasticity (Tulloch, 1980) and ability to absorb many types of materials. Some of these materials include fungal and bacterial spores, pesticides and heavy metals which may be detrimental to a colony's welfare. Here is a list of some biotic and abiotic contaminants found in wax.

Biotic: American and European foulbrood spores, Chalkbrood spores, Nosema

Abiotic: Amitraz, Arsenic, Azoxystrobin, Boscalid, Bromopropylate, Captan, Carbaryl (Sevin), Chlordimeform, Chlordane, 2-chloroethanol, Chlorpyrifos, Chlorothalonil, Chromated copper arsenate, Copper naphthenate, Coumaphos (CheckMite+ℝ), Diazinon, 4,4'-dibromobenzophenone, 1,4-dichlorobenzene, Dicofol, Endosulfan, Esfenvalerate, Ethion, Ethylene dibromide, Fenthion, Fluvalinate (Apistan<sup>™</sup>), Malathion, Menthol, Methomyl, Organochlorine (multi-residue), Organophosphorus (multi-residue), Methyl parathion (Penncap-M), P-dichlorobenzene, Pentachlorophenol, Phenkapton, Phenol, Phenothiazine, Polychlorinated biphenyls, 2,4,5-T,Tributyltin oxide (TBTO), Vinclozolin

As materials accumulate in wax comb the diameter of cells becomes smaller (Winston, 1987). Each time a larva pupates, it spins a silken cocoon, parts of which remain in the cell after the adult emerges (Jay, 1963). Over time, the mass ratio of silk to wax increases, and thereby wax comb goes from a single-phase material to a fiber-reinforced two-phase composite product (Hepburn & Kurstjens, 1988). The bees, along with the cell size in old comb, are smaller.

Pheromones also are absorbed and transferred in the wax comb and, depending on their volatility, may remain for a considerable time (Naumann et al. 1991). One pheromone group relevant in the current context is brood pheromones. These contact pheromones are emitted by brood and communicate to nurse bees the immatures' presence, age and nutritional needs (Free, 1987). Nurse bees, responsible for brood care, detect these pheromones more readily in older comb, and feed the brood more often. Therefore, larvae reared in comb with a previous history of brood rearing may receive somewhat better care with resultant higher survivorship (Free & Winder, 1983).

Prior to the presence of *Varroa destructor* (Anderson and Trueman) in the United States, wild, temperate honey bee colonies were known to survive for about six years (Seeley, 1978). Once the colony died, wax moths, mice and other nest scavengers usually removed the wax comb, leaving an empty cavity for the next colony to inhabit (Gilliam & Taber,



1991). Modern beekeeping practices disrupt this natural recycling process by housing bees on semi-artificial comb that may be years or even decades old. Advances in beekeeping equipment, like the Langstroth hive and wire-reinforced foundation, have added years to the longevity of wax comb.

In the United Kingdom, beekeepers are encouraged to replace old combs as part of good husbandry practices (Brown, 1999). In the United States, Bonney (1990) recommends replacing two of the oldest combs each year to ensure that the hive body will not contain comb over five years old. Nowadays this may even be too old. Even so, many beekeepers believe that it is not economically feasible to regularly remove and replace old comb. Not only is the new foundation expensive and time consuming to replace, there is an energetic cost for the bees who must draw out the foundation into a functional comb using metabolicallyderived beeswax. The typical nest contains around 100,000 cells (Seeley & Morse, 1976) which takes about 1,200 g of wax to construct. The amount of sugar required to secrete the wax is energetically equivalent to 7.5kg (16.5 lbs.) of honey, about onethird of the honey stores consumed by a colony over winter (Seeley, 1985). Therefore, beekeepers believe they lose money, time and honey yields by replacing old comb.

However, it is possible that the economic savings of using long-lasting comb may be offset by deleterious effects of old comb acting as a biological sink for toxins and pathogens or as a physical constraint on larval development. This question led me to investigate the effects of comb age on honey bee colony growth, brood survivorship and adult mortality.

In a three-year field study, we compared the quantity of brood

produced, brood survivorship, average body weight of adult bees and population of adult bees in colonies housed on brood combs comprised of either old beeswax or newly drawn, first-year beeswax.

Outcome for this particular study resulted with colonies maintained on new comb having a significantly higher area of total brood, area of sealed brood and higher young bee weight. Comb age produced no statistically significant treatment effects in ending adult bee population or change in adult bee population; however, the trend was for higher ending bee populations in new comb and, correspondingly, a greater loss of bees in old comb. Brood survivorship was either unaffected by treatment or higher in the old comb class.

The increased brood production measured in the new comb may have been the result of several different events taking place inside the colony. It may have been due to the survivorship of the brood, quality of brood care delivered by nurse bees, or the queen's egg production. Let's review the latter. Queens are able to distinguish between worker cells and drone cells by appraising the width of the cell with their forelegs (Koeniger, 1970). The cell diameter in old comb become smaller over time (Abdellatif, 1965); thus, an average reduction of cell diameter in old comb may have a negative effect on a queen's egg-laying productivity.

Older comb is also known to harbor numerous pesticide residues and diseases which may be detrimental to the brood's health. They're spread from colony to colony by tainted wax and materials brought into the hive. The queen may be sensitive to these contaminants and not lay eggs in particular cells. Also, the old comb may harbor brood pheromones (Free & Winder, 1983) that act as egglaying inhibitors to the queen because she perceives the cell to be already occupied.

Another phenomenon relevant to this study is the observation that bees prefer to store honey and pollen in cells that have been previously used for brood rearing. In the wild, as a colony grows and continues to add new comb, brood rearing gradually shifts into this new comb and the honey is stored in the old brood comb (Free & Williams, 1974). In unmanaged colonies this behavior may serve to avoid the negative effects of old comb on brood production. However, modern beekeeping practices inhibit this natural process by forcing bees to reuse old brood comb for brood rearing and to store honey in comb usually only used for honey storage.

Higher weight of emerging young bees in new comb is best explained by differences between the average diameter of cells in the two comb age classes. As mentioned before, while brood comb ages, the diameter of the cells decreases due to accumulated cocoons and fecal material that are deposited by the larval and pupal instars developing within the cell (Jay, 1963). The body weight of a worker bee is mediated by genetics (Ruttner & Mackensen, 1952) as well as by environmental effects such as the amount of food fed to larvae (Daly & Morse, 1991; Fyg, 1959) and the size of the natal cell (Jay, 1963; Abdellatif, 1965). Buchner (1955) determined that the mean weight of newly emerged bees from old comb in which



Would you raise your young in this environment? (Jaycox photo)

68 generations had emerged was about 19% smaller (96.1 mg) than the controls (118.3 mg). Morphological characteristics of European worker bees reared in smaller Africanized comb were smaller than those of European bees reared in the larger European comb (Rinderer et al., 1986). Daly & Morse (1991) found that larger worker bees could be reared from the large cells of drone comb. Glushkov (1958) discovered that bees reared in enlarged cells were heavier and larger resulting in more honey being produced by the colony and larger cells constructed. Worker larvae reared in enlarged cells received more food (21% more protein and 39.7% more glucose) than worker larvae reared in normal worker cells (Volosevich & Kulzhinskaya, 1953). The bulk of the evidence suggests that the weight of newly emerged bees is proportional to the volume of the cells in which they are reared (Nowakowski, 1969) and the amount of food fed to them by the nurse bees.

In this study bees reared in new comb weighed about 8.3% more than those reared in old comb, which is similar to Abdellitif's (1965) finding that worker bees reared in old comb in which 70 generations had been reared have an 8% reduction in body weight.

Lower bee populations in the old comb may result from an accumulation of foreign contaminants sequestered in the older comb causing higher mortality. Also, contaminants in the wax comb may mask hive signature and nestmate recognition cues, making it difficult for foraging bees to return to their own colony. Some nestmate recognition cues are obtained from the wax comb (Breed & Stiller, 1992), and Breed et al. (1988a) discovered that colony odor acquired from wax comb can mask the genetic differences between bees. Colony odor is transferred to the adult bees by exposure to the comb substrate and can alter the recognition phenotype in as little as five minutes (Breed et al., 1988b).

Brood communicate to the worker bees their presence in the cell, caste, age and hunger levels through mechanical and chemical signals (Free, 1987). The chemical signals are the brood pheromones that may be the causative agent responsible for the increased survivorship found in old comb in this study. Wax comb acts as a reservoir for absorbing and transmitting pheromones which may explain why honey bee swarms are more attracted to older comb (Naumann et al., 1991). The presence of brood pheromones stimulates pollen foraging (Pankiw et al., 1998), enhances brood recognition (Le Conteet al., 1994) and stimulates nurse bees to feed larvae (Le Conte et al., 1995), all of which are important factors in brood survivorship. Free & Winder (1983) determined that brood survival was greater in cells which had been used previously for brood rearing than in comb cells never used before. Taken together these studies demonstrate that pheromones incorporated in wax comb may improve brood survivorship. The differences in brood survivorship noted in this study may be partly explained by more optimal concentrations of brood pheromones in older comb. In this study we found the seemingly paradoxical results of higher brood production in new comb but higher brood survivorship in old comb. We believe that this is best reconciled, internally and with the literature, by positing that the egg-laying rate of queens is highest in new comb, but once placed in a cell the chances of a larva's survival are best in old comb. Nevertheless, overall brood production is highest in new comb. Apparently the benefits of maximized egg production exceed the benefits of maximized brood survival.

Over three years of field study, honey bee colonies housed on new comb had higher area of total brood, area of sealed brood, and weight of newly emerged bees. Brood survivorship was the only variable significantly higher in old comb. And finally, mortality of adult bee as affected by the age of comb in which they were reared or maintained was lower in new comb but not significantly. The



Naturally drawn new comb is white, and generally quite fragile

bulk of the evidence suggests that new combs optimize overall honey bee colony health and reproduction. These findings suggest that beekeepers should eliminate very old brood combs from their operations.

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*References for this article will appear on Bee Culture's web page.*