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Effects of a Synthetic Queen Mandibular Pheromone on Emergency Queen Rearing in the Honey Bee (*Apis mellifera*)

Abstract

This study aimed to investigate the effectiveness of an artificial queen pheromone (TempQueen) in honey bee (*Apis mellifera*) colonies that lack a queen to ensure that beekeepers are not using a product that is an unnecessary expense. Honey bee queens signal their presence in a colony by a specialized pheromone called Queen Mandibular Pheromone (QMP). In the absence of this signal, honey bee workers will initiate emergency queen rearing, in which they raise many queen cells to increase the chance that one new queen bee will be viable. This process can take a month before a new queen begins laying eggs, which can inhibit pollination services and honey production. Beekeepers who financially rely on their colonies may use an artificial QMP, a product called "TempQueen," as a false signal of queen presence so that workers will continue to be productive. However, there is no data supporting the claims TempQueen advertises. This study looks at if TempQueen stops a colony from raising queen cells in the claimed product exposure time of three weeks due to a lack of other queen-presence signals. Queenless colonies provided with TempQueen not only raised queen cells, but did not raise a significantly different number of queen cells compared to colonies that did not receive TempQueen. This is consistent with other studies and supports the hypothesis that queens have an additional signal that indicates her presence to worker bees.

Introduction

Honey bees (*Apis mellifera*) colonies are eusocial, complex superorganisms that are able to function due to thousands of individual smaller organisms, or bees (1). Individual honey bees can be thought of as cells that carry out necessary biological processes that allow the organism as a whole to pass on its genetic lineage (2). In honey bees, the social structure is a single reproductive female, the queen, supported by a colony of nonreproductive female worker bees (1). Worker bees carry out multiple essential tasks every day that keep the colony functional (1). Worker bees perform tasks that include caring for the developing offspring and the queen, keeping the hive clean, thermoregulation of the colony, foraging for food and water, processing honey, and defending the colony (1). Honey bee colonies are perennial so a healthy queen is essential to ensure the health and survival of its workforce (1). A queen bee must be able to lay fertile worker bee eggs to grow the colony population and replace the worker bees that die. A honey bee colony must have a large enough population and ample food stored to survive winter.

This level of preparation and functionality is achieved in part through pheromone communication (3). Pheromones are chemical signals produced by animals that can trigger behavioral or physiological changes (3). Honey bees utilize multiple different pheromones to communicate an array of information to each other (3): alarm pheromones are used to notify other bees of potential threats to the colony, Nasonov pheromone marks the location of a honey bee's home colony, and brood pheromone signals that there is healthy offspring in the colony (3). While some pheromones are produced by all castes of honey bees (workers, queen, and drones), the queen bee is able to emit a specialized pheromone known as the Queen Mandibular Pheromone (QMP) (3). The presence of QMP in the colony enables worker bees to perform important functions because there is an active signal of a viable queen (1). This pheromone is produced in a queen’s mandibular glands and is maintained at a constant concentration on the outside of her body (3). This is due to QMP being spread throughout the colony through physical contact between worker bees (4). When a worker bee comes in contact with the queen (either through feeding, grooming, or antenning), some amount of QMP is transferred to the worker bee’s body. As that worker bee moves throughout the hive, she continues to transfer QMP to other workers through bodily contact (4). This effectively transmits the message that an active queen bee is present in the hive to her workers. When worker bees come in contact with QMP regularly, they receive a signal that a queen is present and there is no need to raise a new queen. If there is no QMP circulating in the colony, this indicates to worker honey bees that their queen is unexpectedly absent (3). This can occur when a queen is killed by an external threat, such as a beekeeping error, predator, or chemical application. When worker bees are unable to detect QMP within their colony, they initiate an emergency queen rearing process in which they attempt to raise multiple queens to increase the chance that one of them will be viable (5). Colonies typically start raising queen cells within 12-24 hours into being queenless (3). Honey bee queens take 16 days to develop before emerging from their cells as an adult and roughly two weeks to mate and start laying eggs (6). Due to this, emergency queen rearing can take over a month before a new, mated queen is able to start laying eggs, causing a significant break in brood production and therefore a reduced workforce that is able to carry out foraging and pollination tasks (5). Requeening is risky for a colony. Beekeepers can see significant honey and survival losses from a requeening colony. Beekeepers may use this product to halt the colony from producing a new queen themselves, allowing the beekeeper to introduce a queen from a specific genetic line. However, if a colony is raising their own queens as the beekeeper introduces a new queen, they may reject and kill the desired queen. This would be at the financial loss of the beekeeper because mated queens can be expensive. Due to this, the beekeeper would want their colonies to not attempt to raise, or build, any queen cells on their own.

One theorized solution is the usage of synthetic QMP artificially applied to the colony by the beekeeper (7, 8). In this way, the synthetic QMP would mimic the presence of a viable queen and inhibit the emergency queen rearing process. However, previous research using the chemical formula of QMP reported that it does not completely suppress emergency queen cell rearing (7, 8). A product known as TempQueen is being widely sold (9) as a "temporary queen replacement" (10) due to it containing synthetic QMP—but without research to support its efficacy. TempQueen's manufacturer reported that there was no quantitative data collection on the synthetic pheromone's colony effects before being marketed (11). The product claims to be able to keep a queenless colony "queenright" (meaning believing the false signal of the presence of a queen) for up to three weeks (10). However, no public study has demonstrated that artificial queen pheromone is able to prevent honey bees from raising queen cells for longer than four days (7, 8). In addition, this may not be a feasible solution for beekeepers as leaving a colony queenless for three weeks would significantly weaken the colony itself due to a decreased and older population of worker bees by the time a queen is introduced to the colony by the beekeeper. Older bees are less accepting of a new queen, which may lead to rejection of the introduced queen.

It is important to investigate the advertised product claims made to beekeepers to prevent unnecessary financial or colony loss. This paper demonstrates the efficacy of TempQueen exposure to queenless colonies in its ability to inhibit emergency queen rearing. This study examines if TempQueen stops a colony from raising queen cells in the product's labeled exposure time of three weeks due to a lack of a complex queen pheromonal composition. The findings of this paper will help create usage guidelines for beekeepers who buy this product to mitigate financial loss and standardize expectations.

Methods

Colony Setup

This study was conducted at the University of Minnesota Bee Lab Teaching Apiary. Twenty colonies were established in single 10 frame deep Langstroth hive boxes in early May using adult bees and brood from overwintered colonies. Twenty sister queens were raised using the methods in Successful Queen Rearing (12). Five additional sister queens were raised as backups and kept in 5-frame nucleus colonies (further referred to as nucs). Queen cells were introduced to the experiment colonies roughly one day prior to emergence. Experimental colonies and backup nucs were then left alone for two weeks to allow for natural queen mating. Queen cell introduction to experimental colonies and backup nucs had a 92% acceptance rate with 23 out of 25 queens being accepted, meaning that the queens mated and were laying eggs. Queens were marked on their thorax with a blue paint pen for identification and tracking. Weekly management checks were conducted every week for the following five weeks to ensure the colonies had the original active laying queen, were managed to prevent swarming, and weren't experiencing any issues from disease or lack of food. Four colonies were pulled from the study: one swarmed and therefore did not have the original queen, two experienced brood breaks due to queen issues, and one was at risk of swarming and had queen cells before the addition of TempQueen. Two colonies were replaced by backup nucs that had been transitioned to single 10 frame deep Langstroth hive boxes. Since the other two colonies were pulled the day of queen removal, they were not replaced. This resulted in 18 total colonies left in the experiment. Each colony started the study with 9 full frames of adult bees in a deep box and two honey super boxes.

Queen Removal and TempQueen Application

On the day of queen removal and TempQueen addition, each queen was located and removed. Additionally, an initial check through for queen cells occurred to ensure there were no queen cells prior to the removal of the queen. Colonies had been split prior to the study (one colony split into two colonies), and colonies from the same parent colony were labeled “a” and “b.” “A” colonies were control colonies while “b” colonies were experimental or “TempQueen” colonies. TempQueen was added to nine experimental colonies on the middle (5th) frame on the “front” of the frame (the side that was facing to the right when facing the front of the colony). As seen in Figure 1, the TempQueen lure was secured to the top bar of the frame using a ziptie and a thumbtack. Nitrile gloves were worn to prevent contamination of artificial QMP on control colonies. Control colonies did not have anything added to them.

Figure 1. TempQueen secured to the frame of an experimental colony.

Data Collection

For the next three weeks, queen cell checks were conducted every four days. The location and number of queen cells were tracked using specialized data sheets (Figure 2) so that the same queen cell could be followed over each check. After 21 days, TempQueen was removed from the colonies.

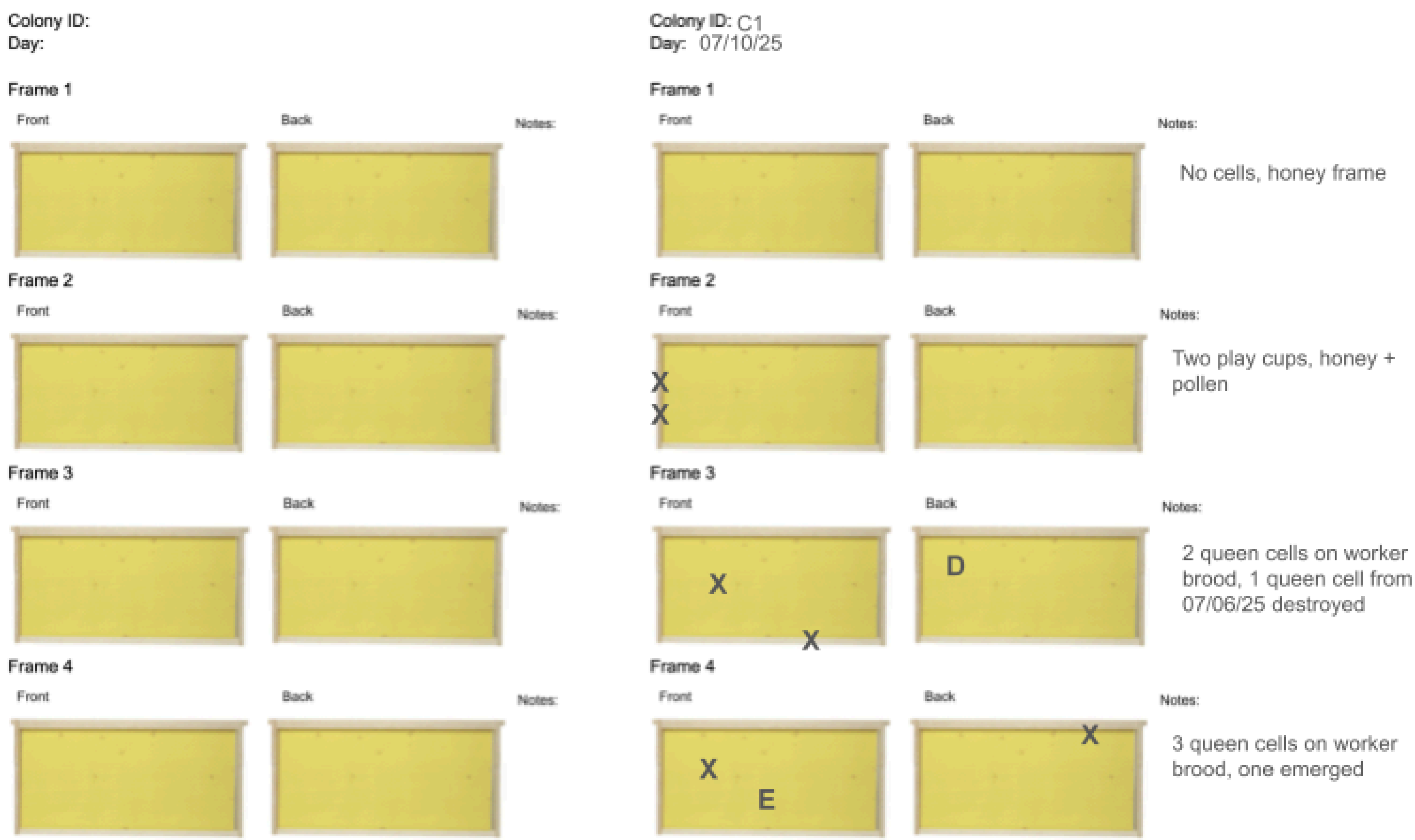

Figure 2. An example of the specialized data sheet used to track queen cells and what it would look like filled out.

Data Analysis

Analysis of data was done using JMP 18 Pro (13). Because cells were tracked over multiple weeks, we need to avoid accidentally double counting a cell that appeared in multiple checks. To do this, data sheets of each colony were compared after every investigation to determine which ones were newly built and not seen in the previous checks. The total number of queen cells each colony built was calculated by adding the new cells found during each check for each colony. A T-test was conducted to compare the total queen cells built by the colony. A Chi-Square Analysis was conducted to compare the percentage of queenright and queenless colonies at the end of the study.

Results

Average Queen Cells Built

A t-test was performed on the actual number of queen cells built by the colonies in each treatment. There was no difference in the number of queen cells built by each colony between treatments (t-Ratio=-1.148, df=15.704, p=0.1341).

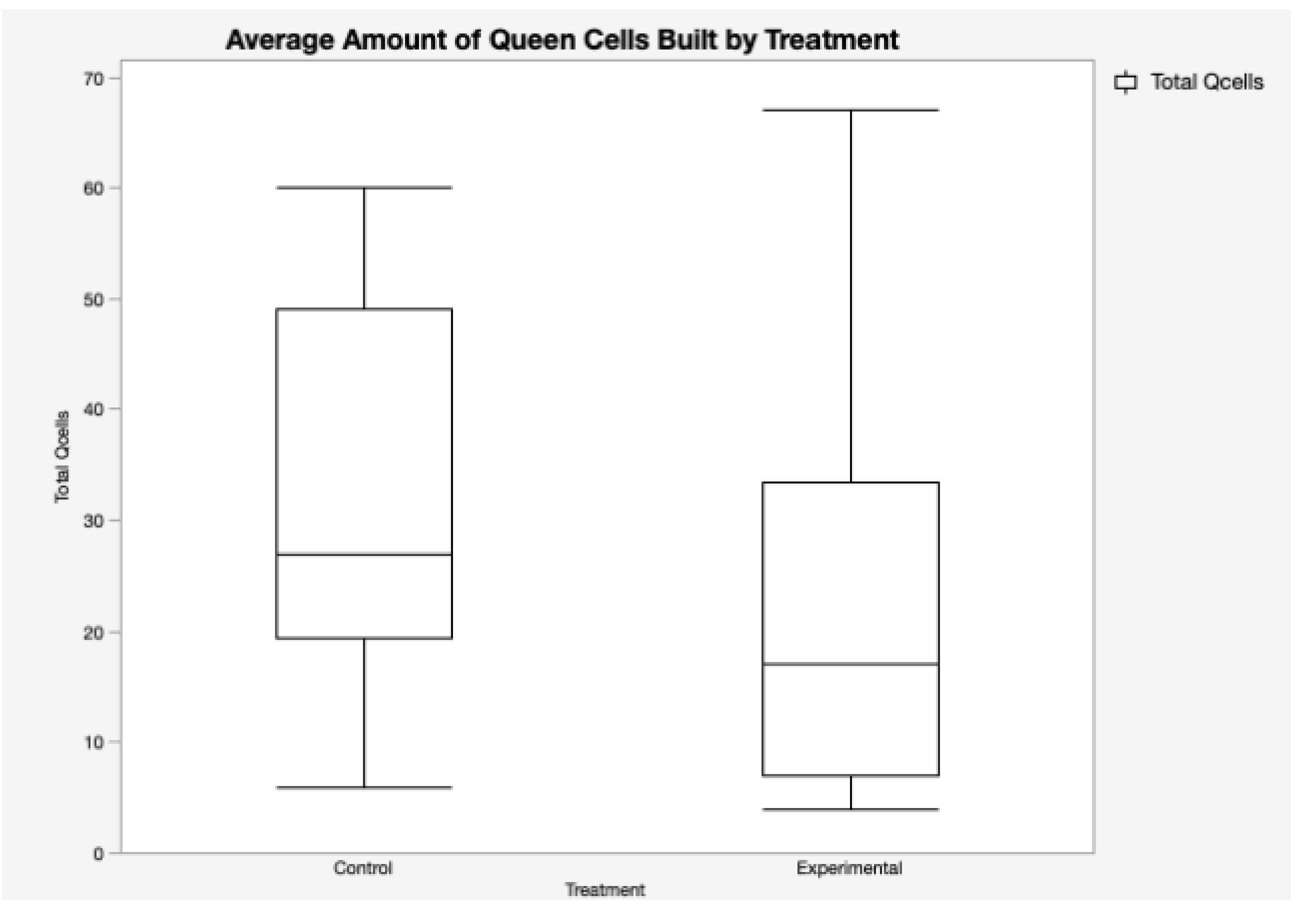

Figure 3. The average number of queen cells built by each colony. Colonies in the control treatment (n=9) built an average of 27±8.905 queen cells. Colonies in the TempQueen treatment (n=9) built an average of 17±8.905 queen cells.

Percent Queenright and Queenless Colonies

After the three week experimental period, the queen status of the colonies were determined. A queenright (QR) colony had an actively laying queen, while queenless (QL) had no queen at the end of the experiment. A Chi-Square test was conducted to compare the number of QR to QL colonies in each treatment. It was found that colonies in the two treatments did not differ significantly in the number that were QR or QL (χ^2=0.407, df=1, p=0.5237).

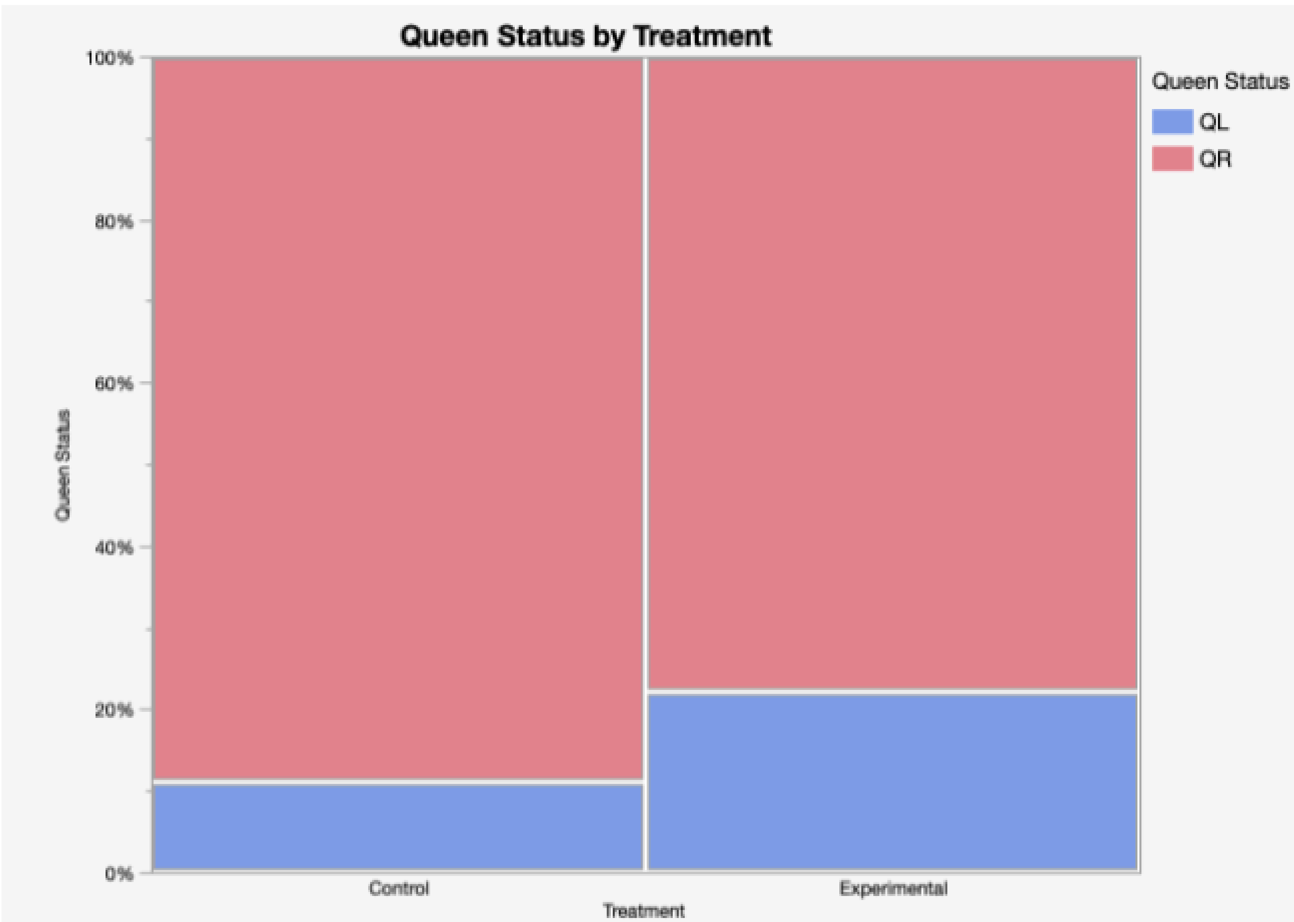

Figure 4. The percent of control and treatment colonies that were queenright or queenless at the end of the experimental period. In the control colonies (n=9), 11.11% (or 1) of colonies were queenless and 88.89% (or 8) of colonies were queenright. In the TempQueen colonies (n=9), 22.22% (or 2) of colonies were queenless with 77.78% (or 7) of colonies were queenright.

Discussion and Conclusion

The purpose of this experiment was to determine the efficacy of an artificial queen mandibular pheromone that is currently being marketed and sold to beekeepers worldwide without quantitative data supporting its claims. The product claims (10) that TempQueen is able to keep queenless colonies "queenright" for up to three weeks before the product gets chewed away by the worker bees. This implies that the colony would not attempt to raise any new queens because TempQueen is emitting an artificially synthesized QMP. However, the average number of queen cells raised by colonies with TempQueen was not significantly fewer than colonies without TempQueen, let alone none at all. All but one experimental colony had built queen cells by day four of being queenless, with all colonies having queen cells by day eight. This finding is consistent with other studies that looked at the use of artificial QMP in queenless colonies that found all colonies had queen cells by day four of being queenless (7, 8).

Additionally, by the end of the experimental period of this study, eight out of nine control colonies were queenright and seven out of nine experimental colonies were queenright. This difference was not significant, demonstrating that TempQueen would not be able to inhibit emergency queen rearing in queenless colonies. One issue encountered in this experiment was having no recorded emerged cells for colonies that ended the study with a queen. While we know that there must have been at least one emerged cell, it is unusual for a colony to only have one successful emergence of a queen. Therefore, we are unable to estimate the true number of queen cells. It is possible that emerged cells were incorrectly recorded, as sometimes workers can start destroying them after emergence and before the beekeeper sees it. In Figure 5 below, the leftmost cell is an emerged cell, which we can tell from the circular hole cut out at the bottom (resulting from the queen chewing her way out from the inside). The middle cell on the left is a sealed cell (no openings), but the three cells on the right have been chewed down by worker bees (multiple noncircular holes in the cell) and it's unclear whether a virgin queen emerged from them before they were destroyed (Fig. 5). In this way, it's possible that in colonies where no emerged cells were recorded, they were too destroyed to recognize them as emerged cells.

Figure 5. A frame from a control colony (LA09a) showing both emerged and destroyed cells and a virgin queen bee.

The data exemplified in this paper indicates that there is likely some other signal(s) in addition to QMP that worker bees look for to determine if their queen is absent from the colony. This experiment, along with historical research (3, 5, 6), demonstrates that the presence of artificial QMP is not effective in "tricking" worker bees and preventing them from raising queen cells. It is likely that the absence of brood (or developing bee offspring), and thereby brood pheromone, also plays a role in triggering queen cell raising. While in this study we did not continually add new brood frames to the colonies, a study by Pettis et al. found that queenless colonies that were exposed to both artificial QMP and brood pheromone had significantly fewer queen cells than colonies solely exposed to artificial QMP (7). It is possible that beekeepers who use TempQueen and also give their queenless colony new brood frames could see a reduction in queen cells. Additionally, Pettis et al. found that colonies with QMP and new brood frames still built cells, indicating that there is potentially a third unknown signal that cues worker bees to the presence of their queen (7).

This additional signal could be attributed to the queen signal/control hypotheses (14). This pair of hypotheses theorize whether queen bees produce an honest signal of their quality through their pheromone composition. The queen signal hypothesis predicts that the pheromonal composition of queens truthfully reflects their reproductive capacity (15). The queen control hypothesis theorizes the opposite, in which the pheromonal composition does not truthfully reflect the reproductive quality of the queen and therefore worker preference is not based on reproductive capacity (15). This experiment would lend support to the queen signal hypothesis due to TempQueen having a poor pheromonal composition and therefore workers may have detected that TempQueen had no reproductive potential, if they did recognize it as a “queen.” In this way, having only artificial QMP and brood pheromone in a colony may not be enough to suppress queen rearing because the worker bees sense a lack of a complex queen pheromone composition which indicates to them that this “queen” does not have a good (or any) reproductive capacity. One possible fecundity signal could be esters secreted by Dufour’s gland, which seems to indicate ovary development in laying workers (16, 17). While the secretion from Dufour’s gland was originally thought to act solely as a signal for a fertilized or unfertilized egg, it has been postulated that it could also be an essential indicator of reproductive quality in a queen’s pheromone make-up (16, 17).

Potential future studies could look at adding an artificial queen pheromonal composition including QMP, Dufour's gland secretion, and brood pheromone in queenless honey bee colonies to see if it would inhibit queen rearing by worker honey bees. If successful, this would indicate that ester compounds in Dufour's gland potentially acts as a queen signal in conjunction with QMP and brood pheromone. This study could also include colonies that are only exposed to Dufour's gland secretion and not QMP and brood pheromone. This would test the queen signal hypothesis by seeing if make-up of multiple pheromones is necessary for worker bees to determine the reproductive quality of a queen honey bee.

TempQueen is unable to inhibit emergency queen rearing in queenless honey bee colonies. It does seem to be effective as a lure for calming flying honey bees and attracting them to one spot (18), which might be useful for sampling or cleaning. However, these uses are advertised secondarily to emergency queen rearing inhibition (10). It is important that beekeepers use this product with the knowledge that it will not stop honey bee colonies from raising queen cells and that they will need to go into the colonies to remove queen cells if they are trying to avoid requeening done by the colony. It is also important to note that using this product for the claimed time of three weeks is potentially damaging to the health of honey bee colonies. Honey bee colonies should be requeened as soon as possible to support the strength and productivity of the colony. Beekeeper suppliers selling this product should include warnings and considerations for the use of this product as a way to prevent a queenless colony from raising cells to any consumers. It is important to ensure that beekeepers are effectively using products that promote the health of their colonies and are aware of the potential pitfalls. The beekeeping industry is fragile in terms of its stability, especially with the 2024 colony loss experienced by US beekeepers (19). Research into advertised products is essential to mitigate financial losses for commercial, sideliner, and hobby beekeepers alike.

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