



Influence of brood pheromone on honey bee colony establishment and queen replacement

David R. Tarpy , Eric Talley & Bradley N. Metz

To cite this article: David R. Tarpy , Eric Talley & Bradley N. Metz (2021): Influence of brood pheromone on honey bee colony establishment and queen replacement, Journal of Apicultural Research, DOI: [10.1080/00218839.2020.1867336](https://doi.org/10.1080/00218839.2020.1867336)

To link to this article: <https://doi.org/10.1080/00218839.2020.1867336>



© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 12 Jan 2021.



Submit your article to this journal [↗](#)



Article views: 32



View related articles [↗](#)



View Crossmark data [↗](#)

ORIGINAL RESEARCH ARTICLE

OPEN ACCESS

Influence of brood pheromone on honey bee colony establishment and queen replacement

David R. Tarpy^{a,b,*}, Eric Talley^c and Bradley N. Metz^a

^aDepartment of Entomology & Plant Pathology, North Carolina State University, Raleigh, NC, USA; ^bBiology graduate program—Ecology & Evolution, North Carolina State University, Raleigh, NC, USA; ^cNorth Carolina State Beekeepers Association, Hubert, NC, USA

(Received 14 October 2020; accepted 14 December 2020)

There is both anecdotal and empirical evidence to suggest that honey bee queen longevity has decreased in recent years, leading to premature supersedure and queen failure. This is particularly evident when beekeepers create new colonies from packages, where many queens are immediately rejected or replaced after only a few weeks. Relatively little is known about the mechanisms that trigger supersedure in honey bees, although previous studies have shown a strong link with open brood suggesting that brood ester pheromone (BEP) may be involved. We installed new packages into hive equipment with either no treatment (Control), exposure to BEP during package transport and for the first 10 days after installation (BEP), or one frame of open brood (Brood). We found that over the course of the 12-week experiment, Control colonies grew the least, Brood colonies started stronger but leveled off similar to Control colonies, and BEP colonies grew slowly initially but continued positive growth through the end of the experiment. Moreover, we found a highly significant effect of treatment on Outcome—whether the initial queens were immediately Rejected (within 5 weeks), Superseded (after 5 weeks), or Accepted, with Brood, BEP, and Control colonies having 86.7%, 53.3%, and 33.3% acceptance, respectively. Finally, we found that the open-brood:adult-bees ratio significantly diverged 3 weeks prior to queen replacement between accepting and replacing colonies. We suggest that while BEP alone is insufficient to deter premature supersedure, there are clear benefits to queen longevity and package-installation success when establishing new colonies with frames of young brood.

Keywords: *Apis mellifera*; queen replacement; colony establishment; collective decision making

Introduction

Given the central role that honey bee queens play in colony function and cohesiveness, it is not surprising that recent trends in their reduced longevity and other problems are of concern for beekeepers and the apiculture industry. Historically, the median lifespan of queens was 25.8 ± 0.498 months and thus beekeepers were recommended to replace their queens every 2 years (Szabo, 1993). However, in recent years a high percentage of queens fail to live more than 1 year (Tarpy et al., 2013), are prematurely superseded (Withrow et al., 2019), or lay unfertilized eggs at a very young age (Pettis et al., 2016). Indeed, these numerous “queen events” have been identified as one of the top management concerns for colony mortality in the US (vanEngelsdorp et al. 2013), with an average 22.4% of beekeepers reporting queen failure to explain why their colonies have died in annual surveys from 2008 to 2016 (Kulhanek et al., 2017 and references therein).

Of particular concern is the package industry in North America, where newly installed queens can often be replaced within a matter of weeks or months. Braun (1943) estimated that only 17.3% of package queens

were replaced in their first year including the 11.7% of them that were rejected during installation. More recently, however, Withrow et al. (2019) demonstrated that 27.2% of 195 package queens were rejected within 6–8 weeks after installation. At issue are numerous environmental factors, including extreme temperature exposure during transport (McAfee et al., 2020; Pettis et al., 2016), disease (Alaux et al., 2011; Chaimanee et al., 2014), and pesticide exposure (Chaimanee & Pettis, 2019; Chaimanee et al., 2016; Sandrock et al., 2014; Walsh et al., 2020; Williams et al., 2015). While no one single factor can explain all of the queen installation problems, the increased losses of newly mated queens during new colony formation is a troubling trend in the industry and of great concern to beekeepers.

While the regulation of swarming (reviewed by Grozinger et al., 2014; Winston, 1987) and emergency queen rearing (Fell & Morse, 1984; Tofilski & Czekonska, 2004) has been well studied, the mechanisms that govern supersedure (queen replacement in the presence of a laying queen) have been more difficult to ascertain in part because there is no easy means to

*Corresponding author. Email: drtarpy@ncsu.edu

induce it (Butler, 1957; Gary, 1959). Previous studies have used the construction of queen cells as a proxy for supersedure intent of the colony (Melathopoulos et al., 1996; Niño et al., 2012; Pettis et al., 1997). While there is a debate about whether or not queen mandibular pheromone (QMP) is an honest signal of queen quality (Kocher & Grozinger, 2011; Strauss et al., 2008), it appears that a lack of QMP alone is not responsible for queen rearing. Melathopoulos et al. (1996) showed that while the addition of synthetic QMP inhibited queen rearing when applied in the first 24 hours after queen loss, both QMP and worker larvae were required to fully deter queen rearing in later stages. Moreover, Pettis et al. (1997) showed that both QMP and open larvae suppressed queen rearing more effectively than QMP alone. As such, it appears that open brood is a secondary fecundity signal used in the collective decisions of workers to replace their queen.

Brood pheromones, particularly brood ester pheromone (BEP) (Pankiw, 2004a), have been identified (Le Conte et al., 1990) and shown to have many different effects on the social physiology of colonies, including increasing colony pollen foraging (Metz et al., 2010; Pankiw et al., 1998), brood rearing (Sagili & Pankiw, 2009), initiation of foraging by individual workers (Le Conte et al., 2001), nursing behavior (Traynor et al., 2014, 2015), and capping of the cell (Le Conte et al., 1990). Through these effects, brood ester pheromone has been shown to increase queen egg laying rate, feeding, and ultimately colony brood area growth trajectory (Sagili & Pankiw, 2009). Brood ester pheromone has an equivocal history of use in managed apiaries with both positive (Sagili & Breece, 2012) and negative (Peso & Barron, 2014) impacts on colony growth. This variation in response is likely due to regional variance in population response, the bidirectional effect of relative dose, and colony level conditions (reviewed in Metz et al., 2010).

Given the central role that brood and brood ester pheromone may play in queen replacement during initial colony establishment, we investigated how this might help reduce supersedure of newly installed packages. Our hypothesis is that the prolonged absence of BEP by workers may help trigger queen replacement, so we either transported packages with BEP or installed them into hives with open brood compared to controls.

Materials and methods

Brood ester pheromone (BEP)

We formulated brood ester pheromone following Metz et al. (2010) using the proportions originally communicated by Yves LeConte (3.0 µg methyl palmitate, 3.0 µg ethyl palmitate, 17.0 µg methyl stearate, 7.0 µg ethyl stearate, 25.0 µg methyl oleate, 8.0 µg ethyl oleate, 2.0 µg methyl linoleate, 1.0 µg ethyl linoleate, 21.0 µg

methyl linolenate, and 13.0 µg ethyl linolenate). We dissolved 5,000 larval equivalents (LEq), each consisting of 560 ng total esters, in 1.0 mL 95% n-Hexane along with 0.05% w/w t-butyl hydroquinone (TBQ) to serve as an antioxidant to render the diluted pheromone shelf-stable. For the control treatment, we prepared a similar volume of 95% n-Hexane with 0.05% TBQ. This dose was predicted to approximate that promoting colony growth (Pankiw et al., 2004).

Packaged bees

We purchased 45 standard 3-pound packaged bees (~10,000 adult workers) with newly mated queens from Gardner's Apiaries (Baxley, GA). The day packages were created, we traveled to the commercial apiary to assist with the package installation. Just prior to when the bees were shaken into 15 packages, we pipetted 1.0 ml synthetic Brood Ester Pheromone (BEP; n = 15) onto individual 3 3/4 x 6-inch sections of tinfoil-wrapped cardboard (=foil plates), briefly allowed each to air dry (~20 min), and placed them into the bottom of the empty packages immediately prior to the bees being shaken into them. We did not add blank foil plates into the remaining packages. We transported the packages to Hubert, NC within 18 hours of their creation. The following day, we established the experimental colonies by shaking the packages into hive equipment.

Experimental colonies

The study was completed over a period of 12 weeks from the installation of packaged bees on 21 March 2020. We established three experimental groups of 15 colonies each. All hives were custom-made six-frame nucleus hive bodies each with one frame of drawn comb, three frames of undrawn wax foundation (depending on treatment group; see below), and one division-board frame feeder filled with 1:1 v:v sucrose solution (=syrup) that was provided *ad libitum* throughout the course of study. We organized the apiary with alternating placement of test groups, and we reduced drifting by painting the hive bodies in four different colors (blue, yellow, orange, or green). We spaced the hive bodies 5 feet apart and repeated the color pattern so that treatment and hive color were not confounded.

Treatment 1 colonies ("Control") were established from standard packages following the standard method of colony establishment from packaged bees (Laidlaw & Page, 1997). Each colony received a blank foil plate (formulated as above, but using 1.0 mL of 95% n-hexane as a solvent control) by pinning it to the top bar of an outside frame. Treatment 2 colonies ("BEP") were established from the 15 packages with BEP on the foil plates placed at the time the package was first created in Georgia. We similarly pinned each foil plate to a frame and replaced with fresh BEP daily between 8 am and 10 am for the first 10 days of the experiment. Treatment 3

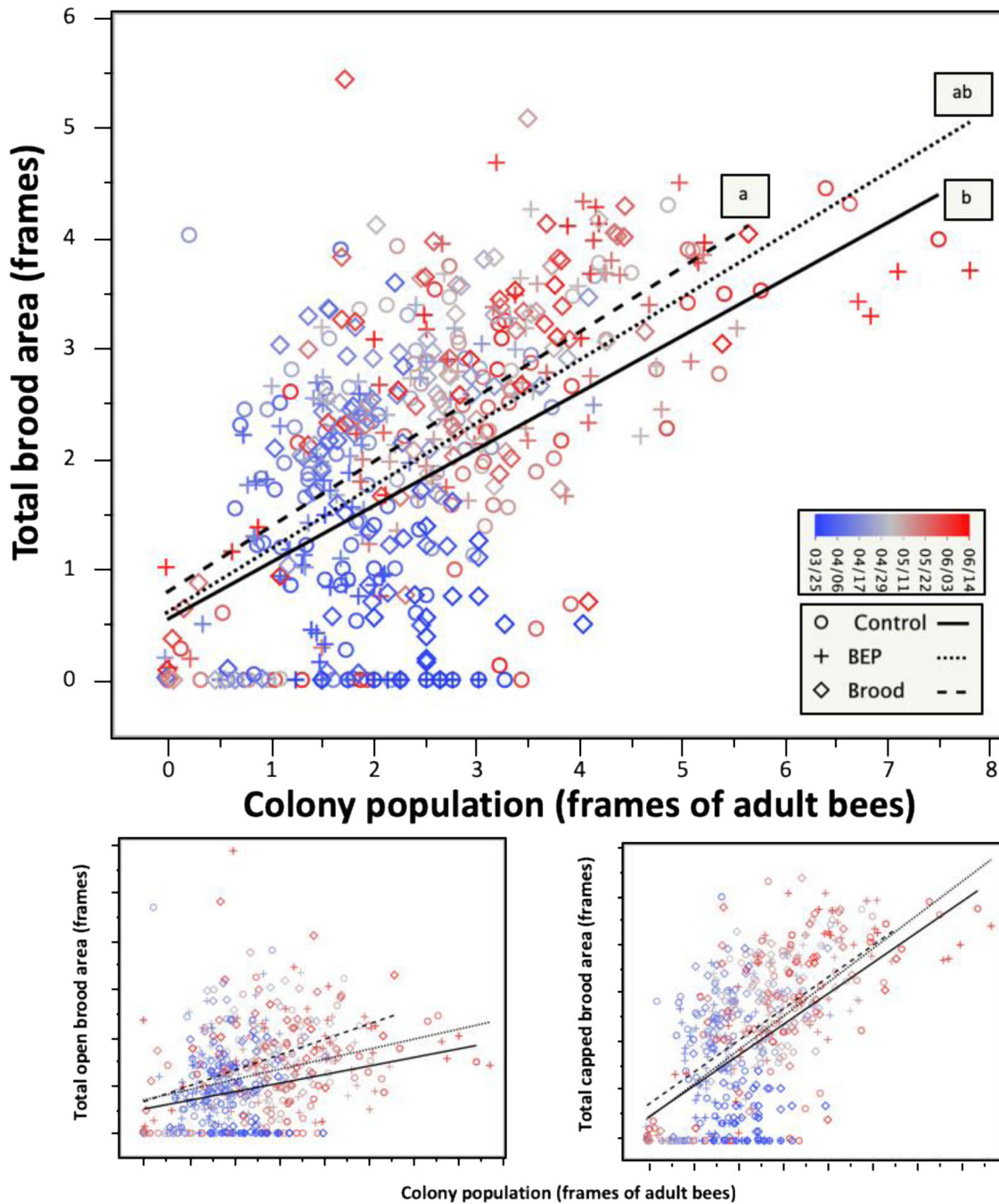


Figure 1. Top: The association between colony population (number of frames of adult bees) and total frames of brood (sum of capped and open brood). Different colors (blue to gray to red) signify the dates recorded. The different symbols distinguish the three different experimental treatments: Control (normal package = open circle), BEP (brood-ester pheromone added to the package and installed colony = cross), and Brood (package installed onto open brood = diamond). Trend lines are designated by dashed lines, which are statistically different from each other as indicated by different letters. Bottom left: The same association between colony population and capped brood only. Bottom right: The same association between colony population and open brood only.

colonies (“Brood”) were established from standard packages with blank foil plates, but in place of the frame of drawn comb we placed one frame of open brood from an unrelated source and without adult bees adhering to them.

We inspected each colony weekly for 12 weeks following Delaplane et al. (2013). All evaluations were performed by a single observer (ET) and recorded on a field data sheet by a volunteer scribe. We took

photographs of frames containing the queen, queen cells, or both. For each frame in each hive, we recorded the number of frames of adult bees (to the nearest 10% of frame coverage), queen status (present, virgin, cells, laying workers, or drone-layer), number of new queen cells, estimated number of frames with capped brood, estimated number of frames of open brood (larvae), relative brood pattern (0 to 5; very poor to excellent), and estimated number of frames of eggs.

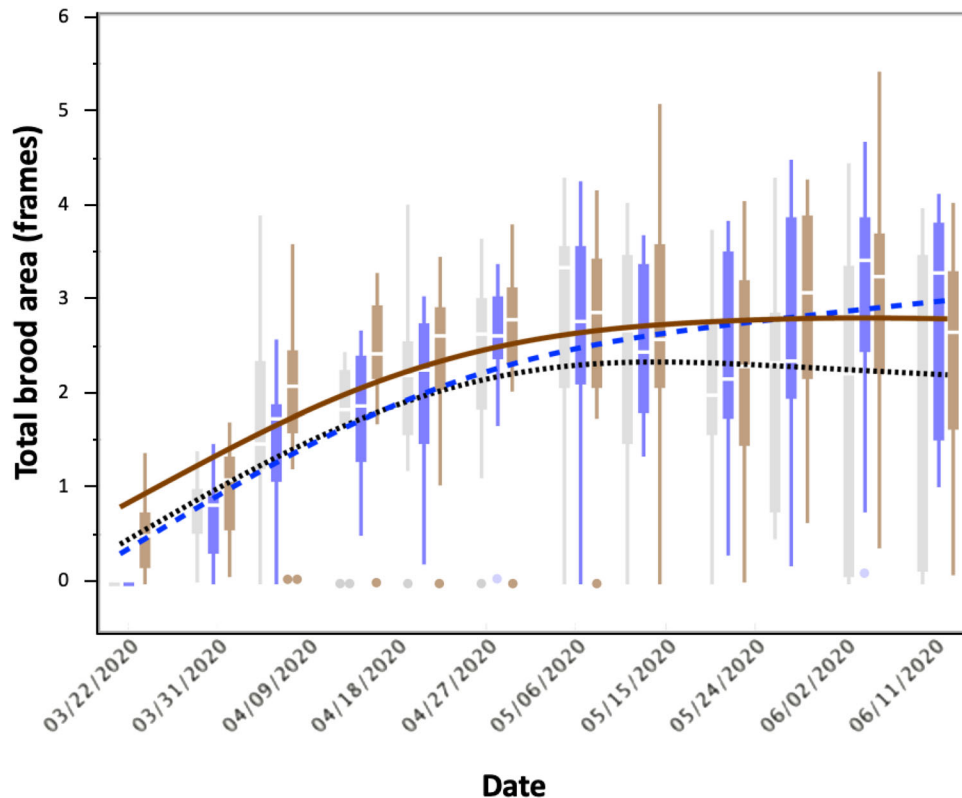


Figure 2. The total frames of brood over the course of the experiment. Standard box plots are given for each inspection period and different lines signify the average trendlines for the three experimental treatments (grey dotted: Control; blue dashed: BEP; brown solid: Brood). These results demonstrate that the established colonies grew normally and as expected, with some subtle but statistically significant differences among the experimental treatments.

We performed all statistical analyses in JMP Pro 14 with $\alpha = 0.05$, and all means are reported with \pm SEM unless otherwise noted.

Results

Colony development

We compared the colony population (as measured by the number of frames with adult bees) with the amount of brood within the colonies (Figure 1). We applied a full-factorial ANOVA model including experimental treatment, date, and frames of bees as independent variables. We found a highly significant, positive relationship between colony population (frames of bees) and the total frames of brood (capped plus open brood frames) ($F_{1,480} = 60.9$, $p < 0.0001$). Date was also highly significant ($F_{1,480} = 77.1$, $p < 0.0001$), which is not surprising since colonies would be expected to grow in size over the course of the experiment (see below). Importantly, experimental treatment was also highly significant ($F_{1,480} = 14.2$, $p < 0.0005$), suggesting that the relationship between population size and brood rearing was measurably different depending on how the new colonies were established. Colonies established onto open brood had a slightly but significantly higher efficiency of rearing brood for a given population, those established from normal packages had slightly lower efficiency, and

those established from packages onto BEP were statistically intermediate. These same relationships were the same when separately comparing capped brood only and open brood only (Figure 1).

We also measured colony growth over time, using the total frames of brood as a proxy for colony size and strength (Figure 2). As would be expected, upon establishment colonies had little to no brood but soon grew in strength in the following weeks, on average plateauing after 5–6 weeks. Using a full-factorial ANOVA mixed model, we analyzed the total frames of brood using experimental treatment, date, and adult population as independent variables. As above, there were highly significant effects of worker population ($F_{1,480} = 68.2$, $p < 0.0001$) and date ($F_{1,480} = 86.3$, $p < 0.0001$) with no significant interaction terms. Interestingly, there was also a significant effect of treatment ($F_{1,480} = 7.98$, $p < 0.0005$), with Control colonies growing the least, Brood colonies starting stronger (since they were shook into hives with one frame of open brood) but leveling off similar to Control colonies, and BEP packages starting slowly initially but continued positive growth through the end of the experiment.

Propensity of queen rearing

Of the 45 colonies in this experiment, nearly half (48.9%) raised queen cells within the first 12 weeks of

colony establishment. The likelihood for a colony to raise queens was significantly dependent on the experimental treatment ($\chi^2 = 6.84$, $df = 2$, $p < 0.05$), with Control colonies having a significantly higher likelihood (73.3%) of rearing queens, Brood colonies having a lower likelihood (26.7%), and BEP colonies being intermediate (46.7%). Interestingly, there was wide variation in when queen-cell construction was initiated among the colonies, with some rearing new queens almost immediately (weeks 1–2) and others only raising queens much later after colony establishment (weeks 11–12). We therefore make a distinction between those that raised new queens and replaced the original package queen based on whether or not the colony population had turned over from the original packaged bees; prior to 5 weeks, the colony consisted of predominantly unrelated package bees, whereas after 5 weeks the vast majority of the population were the daughters of the package queen. As such, we had three separate “Outcomes” for the initially installed queens in these packages: those that rarely if ever raised queen cells and accepted the original queen (“Accepted”), those that reared queens and replaced the original package queen early in the establishment phase (“Rejected”), and those that successfully superseded the package queen after population turnover (“Superseded”).

There were significant differences in the Outcome of queens depending on package treatment ($\chi^2 = 13.7$, $df = 4$, $p < 0.01$; Figure 3). Brood colonies had queens that were significantly more likely to be accepted than either Control ($\chi^2 = 12.7$, $df = 2$, $p < 0.005$) or BEP colonies ($\chi^2 = 8.13$, $df = 2$, $p < 0.05$), and there was no significant difference between Control and BEP acceptance rates ($\chi^2 = 1.47$, $df = 2$, $p = 0.48$). Installing the package into a hive with one frame of open brood (Brood treatment) resulted in 86.7% acceptance of the original queen, whereas the BEP and Control treatments resulted in 53.3% and 33.3% acceptance, respectively.

Numbers of queen cells

Of the 22 colonies that raised queen cells at some point during the 12-week experimental period, there was high variation among them as to the total number of new queen cells recorded. Colonies that built queen cells constructed on average 5.07 ± 1.09 total cells, with one colony building 25 distinct queen cells (and all in the last week of the experiment). The distribution is not normal, however, and thus the data were natural-log transformed for subsequent analyses. We should note that cells were not double-counted in this total because only new queen cells were recorded each week. Many were torn down before virgin queens emerged (see above), but each individual cell was not tracked further so we do not know the specific fate of each. There were three colonies that built 1–2 queen cells but were torn down by the following hive inspection and thus

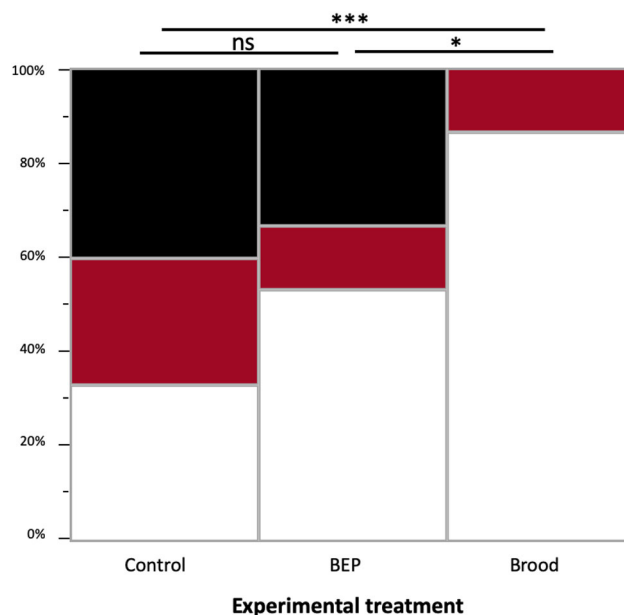


Figure 3. The proportion of colonies in each experimental treatment that did not rear any queens and accepted the package queen (“Accepted” = white), those that reared new queens and replaced the package queen before the colony population turned over (“Rejected” = red), and those that successfully superseded the package queen after the colony consisted of her offspring (“Superseded” = black). There were statistically significant differences among the three experimental treatments with respect to the outcome of queen replacement, with the Brood treatment having a significantly higher acceptance rate than the BEP or Control groups. * $p < 0.05$; *** $p < 0.005$.

were not considered supersedure attempts. As would be expected, there was a significant difference in queen cell number among Outcomes ($F_{2,42} = 124.8$, $p < 0.0001$), although there was not a significant difference between Rejected (9.38 ± 2.39) and Superseded (13.4 ± 2.08) queens (Tukey post-hoc test, $p > 0.05$). As such, it does not appear that the number of constructed queen cells is a measure of how ardent a colony is to replace its queen.

There was a weak but statistically significant effect of colony treatment on the total number of new queen cells constructed ($F_{2,42} = 3.54$, $p < 0.05$), such that Brood colonies had significantly fewer constructed queen cells (1.87 ± 1.10) compared to Control colonies (8.13 ± 2.16) with BEP colonies being intermediate (5.2 ± 1.97). A full-factorial ANOVA with both Treatment and Outcome was highly significant ($F_{7,37} = 34.4$, $p < 0.0001$) with Outcome being the only significant factor ($F_{2,37} = 99.5$, $p < 0.0002$) because colonies that Accepted their package queen built few if any queen cells compared to those that Rejected or Superseded them (Figure 4).

Comparison of last 4 weeks

In an attempt to compare the colony status among those that retained or replaced their queen, we

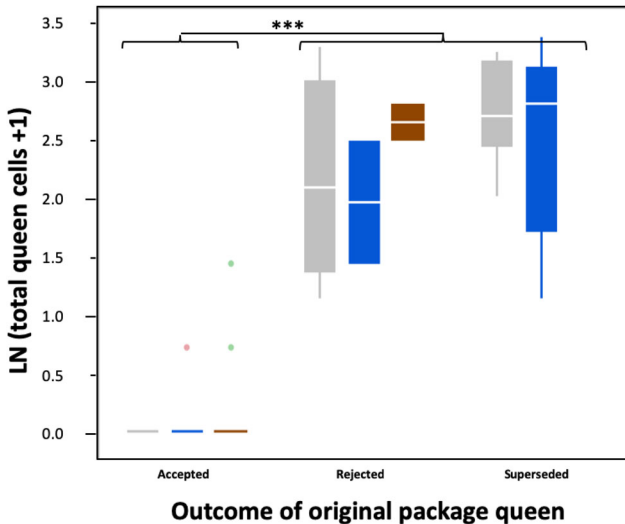


Figure 4. The total number of queen cells (ln-transformed) constructed by colonies that either Accepted, Rejected (within 5 weeks), or Superseded their queens (after 5 weeks). Colonies that successfully replaced their queen during the 12-week experimental period built significantly more queen cells (***) but were not significantly different among experimental treatment (grey = Control; blue = BEP; brown = Brood).

adjusted the timeline for each colony to include only the last 4 weeks in which the original package queen was present. For the “Accepted” queens, those were the last 4 weeks of the experiment (Weeks 9–12), but for the “Rejected” or “Superseded” queens they were whichever 4 weeks preceded the original queen no longer being seen within a given colony and with documented queen-rearing activity. Some of the Rejected colonies replaced their queens within the first month of establishment, and as such those were only included for less than 4 weeks.

Because of the high variation in the number of queen cells built by the various colonies, we attempted to see if any of the colony variables were associated with the initiation of queen rearing. We therefore used a full-factorial mixed ANOVA model with new weekly queen cells as the dependent variable and week and Outcome as independent variables with either frames of adult bees, capped brood, open brood, and brood viability as covariates (including all four colony measurements into a unified model was not possible because of a limited degrees of freedom). While Outcome ($F_{2,158} = 21.8$, $p < 0.0001$) and week ($F_{3,158} = 11.4$, $p < 0.0001$) were highly significant, none of the phenotypic covariates were significantly associated (adult population: $F_{1,158} = 0.21$, $p = 0.64$; capped brood: $F_{1,158} = 0.79$, $p = 0.38$; open brood: $F_{1,125} = 1.51$, $p = 0.47$; brood viability: $F_{1,158} = 0.27$, $p = 0.60$).

The most associated variable with Outcome over the last 4 weeks for each queen was the ratio of open brood to adult bee population (Figure 5), which was normalized by $\ln + 1$ transformation. A two-way ANOVA with week, Outcome, and their interaction

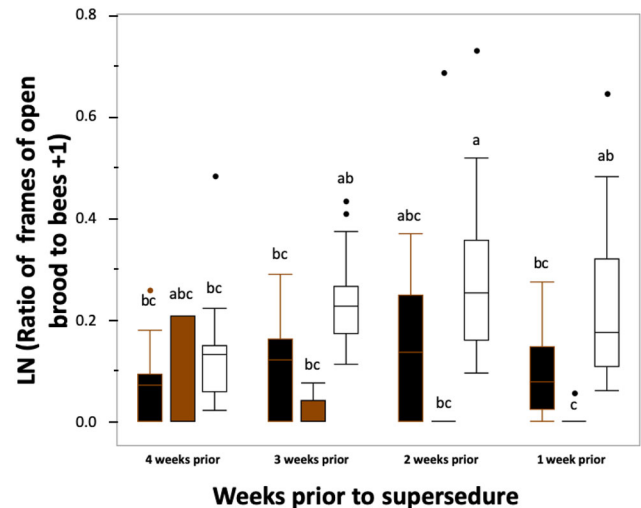


Figure 5. The ratio of frames of open brood to frames of adult bees (ln-transformed) for colonies that Superseded (black), Rejected (red), or Accepted (white) their original package queen for the last 4 weeks leading up to the queen’s final fate. Box plots with different letters are significantly different at $\alpha = 0.05$ using Tukey post-hoc tests. The open-brood:adult-bees ratio was equivalent among all three Outcome groups 4 weeks prior to queen fate, but such ratios became divergent starting 3 weeks prior to the final inspection in which the original queen was seen in the colony.

showed no effect of week ($F_{3,162} = 1.50$, $p = 0.22$) or interaction ($F_{6,162} = 1.04$, $p = 0.40$) but a highly significant effect of Outcome ($F_{2,162} = 20.3$, $p < 0.0001$). Four weeks prior to the original queens’ fate, there were no significant differences in the open-brood:adult-bees ratio among colonies, but starting 3 weeks prior to final queen fate the ratios significantly diverged between colonies that accepted its queen and those that replaced them (Figure 5).

Discussion

While it is well-known anecdotally that newly established packages frequently attempt supersedure, it has neither been well quantified nor demonstrated to be so highly variable depending on the initial starting conditions or treatment of the packages. Our results clearly demonstrate a positive effect of installing packaged bees into hives with some open brood, both in terms of colony growth but especially for reduced supersedure attempts. This was not seen with the BEP treatment so it appears that the effect is mediated by more than just brood ester pheromone.

The effect of brood ester pheromone in this experiment was generally intermediate to that of added brood. This implies that at least some brood signal is responsible for the perception of the queen as viable, and that the extended loss of brood signal during package shipment, queen installation, and the rearing of the first generation of workers appears to trigger supersedure. Brood ester pheromone does not appear to be the complete signal, but may constitute part of an honest—

if not always accurate—signal of queen fertility. One possible component to consider is volatile brood pheromone (vBP, or E- β -Ocimene). vBP is preferentially released by young larvae (Carroll & Duehl, 2012), while BEP increases in quantity dramatically in older larvae (Maisonasse et al., 2010). Interestingly, vBP also affects colony-level pollen consumption and may therefore be expected to elicit similar effects on colony growth parameters. Thus, signals of both young and old larvae may be necessary to suppress the supersedure response in newly installed packages.

There is an equivocally positive effect of hiving packages onto open brood, providing additional fecundity signaling to the package workers as well as decreasing the time to first replacements for the colony. Our results suggest that this affects the initial strength of the colony without necessarily affecting the rate of growth (Figure 1). Shaking packages onto brood rather than foundation appears to be an easy, if potentially more laborious, method for promoting colony growth. Colony growth over time differed among the three treatments, suggesting that the fecundity signal alone, if a sufficiently effective signal is elucidated and applied, is enough to promote colony growth from package installation. Previous research supports this assertion, with simulated packages provided brood pheromone rearing significantly more brood over a 4 week period (Pankiw, 2004b).

The installation of packages onto brood obviously should provide colonies with a head-start in growth, productivity, and longevity. What is surprising in our findings is that this seems to translate to the longevity of the queen as well. The presence of brood *sensu stricto* should elicit no effect on intrinsic drivers of mortality, and brood was provided independent of any knowledge of the queen's reproductive quality. Therefore, the decrease in queen supersedure must be due to a change in worker response to the queen as a result of the presence of brood. Previous research with BEP suggests this to be the case; simulated packages treated with brood pheromone had queens that laid more eggs, were fed for longer, and workers that spent more time preparing cells for larval rearing (Sagili & Pankiw, 2009). This suggests an effect of brood signals on queen behaviors either directly or through changes in worker tending efforts ultimately promoting the very behaviors hypothesized to generate the signals that prevent her supersedure.

The number of queen cells raised by a colony does not seem to be a function of its strength, which has been shown before (Butler, 1957; Gary, 1959). As such, we do not have a good understanding of what governs the number of queen cells that colonies build or around which larvae, suggesting that factors other than major internal colony variables are responsible for cell number (e.g., age demographics, the paternity of available brood, nutrition). The number of queen cells may therefore

not be as good a proxy for “relative intent” of supersedure since cell number does not translate to outcome, but until a better alternative is demonstrated it remains the most logical empirical measure.

To conclude, establishing new packages into hives with at least one frame of open brood clearly helps to hedge against colonies wanting to reject the queen immediately after installation. Brood ester pheromone (BEP) alone does not fully mimic brood in this context, but it shows promise that more-complete proxy brood signals may serve in the place of brood to stimulate package growth and promote queen survival in this context.

Acknowledgements

This project was conducted for partial fulfillment of the Master Craftsman level in the North Carolina Master Beekeeper Program by ET. The authors would like to thank Garner Apiaries for allowing us to work with their packages. We greatly appreciate Rick Welton and Marie Bowman for their assistance conducting the experiment, as well as the NC Master Beekeeper Program committee for their many comments on the experimental design. Additional helpful suggestions were provided by the Tarpay lab think tank.

Disclosure statement

No financial interest or benefit that has arisen from the direct applications of this research.

Funding

Funding was provided in part by USDA-NIFA grant 2016-07962 and U.S. Army Research Laboratory grant W911NF1920306.

References

- Alaux, C., Folschweiller, M., McDonnell, C., Beslay, D., Cousin, M., Dussaubat, C., Brunet, J. L., & Le Conte, Y. (2011). Pathological effects of the microsporidium *Nosema ceranae* on honey bee queen physiology (*Apis mellifera*). *Journal of Invertebrate Pathology*, 106(3), 380–385. <https://doi.org/10.1016/j.jip.2010.12.005>
- Braun, E. (1943). Supersedure of queens in package bees. *Scientific Agriculture*, 23, 424–438.
- Butler, C. G. (1957). The process of queen supersedure in colonies of honeybees (*Apis mellifera* Linn.). *Insectes Sociaux*, 4(3), 211–221. <https://doi.org/10.1007/BF02222154>
- Carroll, M. J., & Duehl, A. J. (2012). Collection of volatiles from honeybee larvae and adults enclosed on brood frames. *Apidologie*, 43(6), 715–730. <https://doi.org/10.1007/s13592-012-0153-x>
- Chaimanee, V., Chantawannakul, P., Chen, Y., Evans, J. D., & Pettis, J. S. (2014). Effects of host age on susceptibility to infection and immune gene expression in honey bee queens (*Apis mellifera*) inoculated with *Nosema ceranae*. *Apidologie*, 45(4), 451–463. <https://doi.org/10.1007/s13592-013-0258-x>
- Chaimanee, V., Evans, J. D., Chen, Y., Jackson, C., & Pettis, J. S. (2016). Sperm viability and gene expression in honey bee queens (*Apis mellifera*) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate

- acaricide coumaphos. *Journal of Insect Physiology*, 89, 1–8. <https://doi.org/10.1016/j.jinsphys.2016.03.004>
- Chaimanee, V., & Pettis, J. S. (2019). Gene expression, sperm viability, and queen (*Apis mellifera*) loss following pesticide exposure under laboratory and field conditions. *Apidologie*, 50(3), 304–316. <https://doi.org/10.1007/s13592-019-00645-4>
- Delaplane, K. S., Dag, A., Danka, R. G., Freitas, B. M., Garibaldi, L. A., Goodwin, R. M., & Hormaza, J. I. (2013). Standard methods for estimating strength parameters of *Apis mellifera* colonies. *Journal of Apicultural Research*, 52(4), 1–12. <https://doi.org/10.3896/IBRA.1.52.4.12>
- Fell, R. D., & Morse, R. A. (1984). Emergency queen cell production in the honey bee colony. *Insectes Sociaux*, 31(3), 221–237. <https://doi.org/10.1007/BF02223608>
- Gary, N. E. (1959). *A study of natural and induced supersedure of the queen honey bee (Apis mellifera L.)* Cornell University. Dissertation Abstracts.
- Grozinger, C. M., Richards, J., & Mattila, H. R. (2014). From molecules to societies: Mechanisms regulating swarming behavior in honey bees (*Apis* spp.). *Apidologie*, 45(3), 327–346. <https://doi.org/10.1007/s13592-013-0253-2>
- Kocher, S. D., & Grozinger, C. M. (2011). Cooperation, conflict, and the evolution of queen pheromones. *Journal of Chemical Ecology*, 37(11), 1263–1275. <https://doi.org/10.1007/s10886-011-0036-z>
- Kulhanek, K., Steinhauer, N., Rennich, K., Caron, D. M., Sagili, R. R., Pettis, J. S., Ellis, J. D., Wilson, M. E., Wilkes, J. T., Tarpy, D. R., Rose, R., Lee, K., Rangel, J., & vanEngelsdorp, D. (2017). A national survey of managed honey bee 2015–2016 annual colony losses in the USA. *Journal of Apicultural Research*, 56(4), 328–340. <https://doi.org/10.1080/00218839.2017.1344496>
- Laidlaw, H. H., Jr., & Page, R. E. Jr. (1997). *Queen rearing and bee breeding*. Wicwas.
- Le Conte, Y., Arnold, G., Trouiller, J., Masson, C., & Chappe, B. (1990). Identification of a brood pheromone in honeybees. *Naturwissenschaften*, 77(7), 334–336. <https://doi.org/10.1007/BF01138390>
- Le Conte, Y., Mohammedi, A., & Robinson, G. E. (2001). Primer effects of a brood pheromone on honeybee behavioural development. *Proceedings of the Royal Society of London Series. Biological Sciences*, 268(1463), 163–168. <https://doi.org/10.1098/rspb.2000.1345>
- Maisonnasse, A., Lenoir, J.-C., Beslay, D., Crauser, D., & Le Conte, Y. (2010). E-beta-ocimene, a volatile brood pheromone involved in social regulation in the honey bee colony (*Apis mellifera*). *PLoS One*, 5(10), e13531. <https://doi.org/10.1371/journal.pone.0013531>
- McAfee, A., Chapman, A., Higo, H., Underwood, R., Milone, J., Foster, L. J., Guarna, M. M., Tarpy, D. R., & Pettis, J. S. (2020). Vulnerability of honey bee queens to heat-induced loss of fertility. *Nature Sustainability*, 3(5), 367–376. <https://doi.org/10.1038/s41893-020-0493-x>
- Melathopoulos, A. P., Winston, M. L., Pettis, J. S., & Pankiw, T. (1996). Effect of queen mandibular pheromone on initiation and maintenance of queen cells in the honey bee (*Apis mellifera* L.). *The Canadian Entomologist*, 128(2), 263–272. <https://doi.org/10.4039/Ent128263-2>
- Metz, B. N., Pankiw, T., Tichy, S. E., Aronstein, K. A., & Crewe, R. M. (2010). Variation in and responses to brood pheromone of the honey bee (*Apis mellifera* L.). *Journal of Chemical Ecology*, 36(4), 432–440. <https://doi.org/10.1007/s10886-010-9775-5>
- Niño, E. L., Malka, O., Hefetz, A., Teal, P., Hayes, J., & Grozinger, C. M. (2012). Effects of honey bee (*Apis mellifera* L.) queen insemination volume on worker behavior and physiology. *Journal of Insect Physiology*, 58(8), 1082–1089. <https://doi.org/10.1016/j.jinsphys.2012.04.015>
- Pankiw, T. (2004a). Cued in: Honey bee pheromones as information flow and collective decision-making. *Apidologie*, 35(2), 217–226. <https://doi.org/10.1051/apido:2004009>
- Pankiw, T. (2004b). Worker honey bee pheromone regulation of foraging ontogeny. *Die Naturwissenschaften*, 91(4), 178–181. <https://doi.org/10.1007/s00114-004-0506-z>
- Pankiw, T., Page, R. E. J., & Fondrk, M. K. (1998). Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). *Behavioral Ecology and Sociobiology*, 44(3), 193–198. <https://doi.org/10.1007/s002650050531>
- Pankiw, T., Roman, R., Sagili, R. R., & Zhu-Salzman, K. (2004). Pheromone-modulated behavioral suites influence colony growth in the honey bee (*Apis mellifera*). *Die Naturwissenschaften*, 91(12), 575–578. <https://doi.org/10.1007/s00114-004-0568-y>
- Peso, M., & Barron, A. B. (2014). The effects of brood ester pheromone on foraging behaviour and colony growth in apicultural settings. *Apidologie*, 45(5), 529–536. <https://doi.org/10.1007/s13592-014-0270-9>
- Pettis, J. S., Higo, H. A., Pankiw, T., & Winston, M. L. (1997). Queen rearing suppression in the honey bee: Evidence for a fecundity signal. *Insectes Sociaux*, 44(4), 311–322. <https://doi.org/10.1007/s000400050053>
- Pettis, J. S., Rice, N., Joselow, K., vanEngelsdorp, D., & Chaimanee, V. (2016). Colony failure linked to low sperm viability in honey bee (*Apis mellifera*) queens and an exploration of potential causative factors. *PLoS One*, 11(2), e0147220. <https://doi.org/10.1371/journal.pone.0147220>
- Sagili, R. R., & Breece, C. R. (2012). Effects of brood pheromone (SuperBoost) on consumption of protein supplement and growth of honey bee (Hymenoptera: Apidae) colonies during Fall in a northern temperate climate. *Journal of Economic Entomology*, 105(4), 1134–1138. <https://doi.org/10.1603/ec11437>
- Sagili, R. R., & Pankiw, T. (2009). Effects of brood pheromone modulated brood rearing behaviors on honey bee (*Apis mellifera* L.) colony growth. *Journal of Insect Behavior*, 22(5), 339–349. <https://doi.org/10.1007/s10905-009-9176-1>
- Sandrock, C., Tanadini, M., Tanadini, L. G., Fauser-Misslin, A., Potts, S. G., & Neumann, P. (2014). Impact of chronic neonicotinoid exposure on honeybee colony performance and queen supersedure. *PLoS One*, 9(8), e103592. <https://doi.org/10.1371/journal.pone.0103592>
- Strauss, K., Scharpenberg, H., Crewe, R. M., Glahn, F., Foth, H., & Moritz, R. F. A. (2008). The role of the queen mandibular gland pheromone in honeybees (*Apis mellifera*): Honest signal or suppressive agent? *Behavioral Ecology and Sociobiology*, 62(9), 1523–1531. <https://doi.org/10.1007/s00265-008-0581-9>
- Szabo, T. I. (1993). Length of life of queens in honey-bee colonies. *American Bee Journal*, 133, 723–724.
- Tarpy, D. R., vanEngelsdorp, D., & Pettis, J. S. (2013). Genetic diversity affects colony survivorship in commercial honey bee colonies. *Die Naturwissenschaften*, 100(8), 723–728. <https://doi.org/10.1007/s00114-013-1065-y>
- Tofilski, A., & Czekonska, K. (2004). Emergency queen rearing in honeybee colonies with brood of known age. *Apidologie*, 35(3), 275–282. <https://doi.org/10.1051/apido:2004014>
- Traynor, K. S., Le Conte, Y., & Page, R. E. Jr. (2014). Queen and young larval pheromones impact nursing and reproductive physiology of honey bee (*Apis mellifera*) workers. *Behavioral Ecology and Sociobiology*, 68(12), 2059–2073. <https://doi.org/10.1007/s00265-014-1811-y>
- Traynor, K. S., Le Conte, Y., & Page, R. E. Jr. (2015). Age matters: pheromone profiles of larvae differentially influence foraging behaviour in the honeybee, *Apis mellifera*. *Animal Behaviour*, 99, 1–8. <https://doi.org/10.1016/j.anbehav.2014.10.009>

- vanEngelsdorp, D., Tarpy, D. R., Lengerich, E. J., & Pettis, J. S. (2013). Idiopathic Brood Disease Syndrome and queen events as precursors of colony mortality in migratory bee-keeping operations in the Eastern United States. *Preventive Veterinary Medicine*, 108(2–3), 225–233. <https://doi.org/10.1016/j.prevetmed.2012.08.004>
- Walsh, E. M., Sweet, S., Knap, A., Ing, N., & Rangel, J. (2020). Queen honey bee (*Apis mellifera*) pheromone and reproductive behavior are affected by pesticide exposure during development. *Behavioral Ecology and Sociobiology*, 74(3), 33. <https://doi.org/10.1007/s00265-020-2810-9>
- Williams, G. R., Troxler, A., Retschnig, G., Roth, K., Yanez, O., Shutler, D., Neumann, P., & Gauthier, L. (2015). Neonicotinoid pesticides severely affect honey bee queens. *Scientific Reports*, 5, 14621. <https://doi.org/10.1038/srep14621>
- Winston, M. L. (1987). *The biology of the honey bee*. Harvard University Press.
- Withrow, J. M., Pettis, J. S., & Tarpy, D. R. (2019). Effects of temperature during package transportation on queen establishment and survival in honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology*, 112(3), 1043–1049. <https://doi.org/10.1093/jee/toz003>