

DRONE EVICTION IN HONEY BEES (*Apis mellifera* ssp.)

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

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August 2013

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ABSTRACT

Social insect colonies depend on individuals coordinating their efforts and adjusting their investment in various nest activities to maximize growth and reproduction. Honey bees (*Apis mellifera* ssp.) are constantly adjusting nest tasks depending upon availability of nectar and pollen. The production of drone honey bees is regulated by the queen and by the workers. While production of drones is important for reproduction, maintaining adult drones can place a strain on colony resources. At a certain point during the foraging season, the cost of maintaining drones outweighs the benefits, and adult drones are evicted from the colony. We investigated the causes of drone eviction and examined how quickly this process can occur. Using observation hives, colonies were either caged or uncaged and fed or unfed. By regulating access to resources in the field, we found that as little as 48 hours of poor foraging conditions is sufficient to produce a shift in drone location within the colony and significantly increase the rate of drone mortality ($P < 0.01$). These results highlight the rate at which the honey bee colony can respond to changing environmental conditions. The age of the workers involved in the drone eviction process and the impact of nectar availability vs. pollen availability remain to be determined.

BIOGRAPHICAL SKETCH

Rick was born in Endicott, New York on the 28th of January, 1978.

Always inquisitive, he showed a strong interest in many areas of study but chose to pursue Biology as an undergraduate at Hobart & William Smith Colleges. After completing a B.S. in 2000, Rick expanded his education through self learning and apprenticeship to become well versed in areas of local history, woodworking, outdoor leadership, engineering, salt water ecology, and viticulture. While he enjoyed being a Jack of all trades, he did want to be a master of some, and he found himself working at Cornell University. It was there that he decided to face one of his biggest fears; the stinging insects. He volunteered at Cornell's Dyce Laboratory for honey bee research and became the lab technician. With the discovery of the gentle honey bee came an insatiable desire to learn all that was known about their behavior. When he finally discovered questions that still needed to be answered, he chose one of the most intriguing questions and set out to answer it. His efforts culminated in this Masters thesis. Throughout this treacherous journey, his loving wife, Kimberly Griffiths, has remained by his side. Rick plans to continue with various intellectual endeavors and is happy to finally be able to say that he is a Jack of all trades and Master of Science.

To Cole, who has the ability to make any topic interesting due to his infectious enthusiasm and love of science.

To my parents, who have always supported my interests and taught me to pursue the things I love in life.

To Kimberly, the love of my life.

ACKNOWLEDGMENTS

I thank Nick Calderone for the many years of honey bee education and guidance throughout this project. I also thank the many undergraduate students who have assisted in this project including Emily Satinsky, Erin Loughlin, Shiliu Wang, and Eric Chang. Thanks to Tom Seeley for troubleshooting some of the details. I thank both Cole Gilbert and Linda Rayer, who have been like my entomology parents throughout this process. They have always had my back. A special thanks to Cole who encouraged me to pursue graduate studies in entomology. As a result, he spent countless lunch hours with me discussing every matter under the sun and probably wishes he had a better lock on his office door. But he is cool like that. I also want to thank my real parents who will drop everything if asked, and lend a hand on any given day. This includes the Father's day that was spent painting little colored dots on the back of several hundred drone honey bees. Finally, I thank my wife, Kimberly Griffiths. She has spent more time helping me with ALL of my endeavors than anyone will ever know, and for this, a special thank you goes to her.

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Drone eviction in honey bee colonies (*Apis mellifera* ssp.)

Richard P. Cicciarelli

INTRODUCTION

The success of a social insect colony depends on individuals working collectively to regulate the colony environment and provide for the needs of the group. Nest construction, food collection and storage, brood rearing and temperature regulation are all tasks that must be successfully accomplished for the colony to survive and reproduce. The collective activities of the nest inhabitants that maintain certain colony parameters within a particular range is called social homeostasis (Emerson, 1956). Members of social insect colonies exhibit a remarkable ability to coordinate their efforts and adjust their investment in various tasks required for colony growth and maintenance (B.-R. Johnson, 2002; Pratt, 2004; Schmickl & Crailsheim, 2002; T. D. Seeley & Mikheyev, 2003).

One key function of a colony is the ability to appropriately allocate available resources between growth and reproduction, and further, between male and female reproductives (Lee & Winston, 1987). Growth and reproduction are governed by both the queen and the workers. The ratio of drone honey bees to workers is in constant flux

depending on the time of the year and, more importantly, resource availability (K. Sasaki & Obara, 2001). There are several stages in the life cycle of a drone honey bee in which both the queen and the workers can play a role in the number of individuals that are ultimately produced.

In the northern United States, the production cycle of drones usually begins in late April to mid May and peaks about four weeks before swarming to coincide with the emergence of virgin queens (Allen, 1958; Lee & Winston, 1987; Page, 1981). As the season progresses, drone production remains fairly constant until late fall or early winter when production decreases, generally to zero (N. W. Calderone, 2005; Lee & Winston, 1987).

The colony produces drones at a rate that is controlled by both by the queen and the workers (Pratt, 1998a; Ken Sasaki, Satoh, & Obara, 1996; Wharton, Dyer, & Getty, 2008). The queen can affect sex allocation by varying the ratio of fertilized female and unfertilized male eggs that she lays (Wharton, Dyer, Huang, & Getty, 2007). Wharton et al. (2007) found that queens can modify their production of drone eggs depending on the number of drone eggs that have already been laid. Additionally, the proportion of male eggs to female eggs laid by queens is also influenced by the nutritional conditions of the colony (Sasaki & Obara, 2001). Queens laid a higher proportion of drone eggs to worker eggs in colonies that had been provided with larger amounts of a commercial pollen substitute.

While the queen can determine the ratio of fertilized to unfertilized eggs, the workers can also affect the sex ratio in several ways. Workers build the wax cells that make up the comb used by the colony to rear bees and store pollen and nectar. There are two sizes of cells making up the majority of the brood comb in a honey bee colony. About 83% of these cells are worker cells with an average cell internal width (wall-to-wall) of 5.21 mm and an average depth of 12.1 mm. The remaining cells, around 17% (T. D. Seeley & Morse, 1976), are drone cells with an average width of 6.42 mm and an average depth of 17.3 mm (Taber & Owens, 1970). The proportion of worker-to-drone cells is regulated by the workers building the comb (J. B. Free, 1967b; Pratt, 1998b, 2004). Once the comb is built, workers continue to control the use of these combs by actively cleaning the cells and making them available for egg deposition, or by choosing to use the cells for nectar or pollen storage. While workers clean both worker and drone cells during the summer (Ken Sasaki, et al., 1996), drone comb will mostly be used for brood during the early summer when peak drone production occurs, and it will mainly be used for nectar storage in the fall and winter months depending on its location within the hive (J. B. Free & Williams, 1975).

Workers are not only able to influence the percentage of worker and drone cells available to the queen for egg deposition, but they can also influence the sex ratio of developing larvae. Under certain environmental conditions, workers will consume some of the developing larvae (Woyke, 1977). Pollen availability appears to have a

significant effect on larval nursing frequency, particularly in young larvae. Workers will selectively feed older larvae when pollen becomes scarce and are able to recognize the sex of young larvae and will favor a certain sex depending on colony conditions (Haydak, 1958; Ken Sasaki, Kitamura, & Obara, 2004; Schmickl & Crailsheim, 2002). The amount of older male brood already in the colony affects the survival of younger drone brood, but has no effect on worker brood in the colony (Wharton, et al., 2008).

While workers are able to influence the colony sex ratio by the type and amount of comb they produce and by raising or eliminating young brood, they can also affect the adult sex ratio. During certain times of the year, or under certain environmental conditions, workers can be observed biting and pulling at adult drones (Ohtani, 1974). An early reference poetically refers to this behavior as “The massacre of the males...by an army of wrathful virgins” (Maeterlinck, 1936). However, from the few papers that reference this particular behavior, it is more accurately referred to as an “eviction” of the drones.

During periods of dearth, and in particular during the fall season, drones can be seen herded into a group on the bottom board of a colony and towards the bottoms of the frames nearest the entrance of the hive (Cicciarelli, personal observation). Eventually, many dead drones can be found around the entrance of the hive. It was originally thought that the workers actually slaughtered the drones during these times. Free (1957) described the behavior in some detail, observing that the workers pull at the

legs and wings of the drones but don't actually sting them. He suggested that this behavior was affected by the age of the drones and workers involved. He found that when worker aggression towards the drones was at its peak during the last three days of the experiment, drones with an average age of 6.9 days were still being fed by workers that averaged 9.8 days. However, during this same period, the average age of the drones being attacked was 23.0 days and the age of the workers attacking the drones averaged 21.2 days.

The mechanisms of eviction behavior are not well understood, though it has been suggested that it is closely associated with a dearth of incoming nectar (Ribbands, 1953). Morse et al. (1967) found that the rate of drone eviction is accelerated under starvation conditions, and drone mortality and eviction also increases at higher temperatures, although the process still takes many weeks to complete. Initially drones are denied access to stored honey (J. B. Free & Williams, 1975); and eventually, the workers force the drones outside of the colony (J B Free, 1957). As adult drones feed exclusively from honey in the comb rather than foraging, they eventually starve to death outside of the colony (Winston, 1987).

While both the Morse et al. (1967) and Free & Williams (1975) studies provide insight into the behavior of drone eviction, their experimental setups were not designed to examine how quickly this behavior takes place and what exactly causes the behavior.

Morse et al. (1967), used colonies of 40,000 to 50,000 bees fitted with dead bee traps at the entrance that allowed them to assess death rates of drones over time. Every few days, the numbers of drones found in the dead bee traps were tallied and the rate of drone mortality was assessed over the duration of the experiment (4 to 7 months). They found that workers will actively pursue and drive drones out of the hives in late summer and fall months over a period of many weeks.

Free & Williams (1975) used 10 colonies (size not given) established inside a cage and fitted with dead bee traps at the entrances. Five of the colonies were fed sugar syrup and five were not. Beginning four days later, daily counts were made of adult drones that were found in the dead bee traps or on the floor of the cages. Five days after the experiment began they counted the drones that were still alive inside the colonies that had not been fed. They found that few adult drones were dead in the colonies that had been fed sugar syrup and few adult drones remained alive in the unfed colonies.

In both studies, observations were made from outside of the colony after any eviction behavior took place. Neither study examined what was taking place inside the colony.

The purpose of this study is to examine what environmental factors cause drone eviction, define more clearly what behaviors are involved in drone eviction, and determine the time-frame over which the behavior can take place. If the eviction of drones is dependent upon foraging conditions, then there should be a significant

difference in the retention of drones in colonies that are allowed to freely forage or are fed compared to colonies that are not fed or are prevented from foraging.

METHODS

This study was conducted at the Dyce Laboratory for Honey Bee Studies, Cornell University, Ithaca, NY, USA (42° 26'N, 76° 30' W) from June to July, 2012. Eight observation hives (Figure 1), each consisting of four full-depth frames with drawn comb (total surface area of 2,200 cm²/frame) were set up in a shelter (11m x 3.5m x 3m) and populated with a queen and approximately 4,000 worker bees (Burgett & Burikam, 1985). The bees were obtained from previously established colonies from the same commercial supplier. The nest consisted of approximately 1 comb of brood, 1 comb of honey and 2 empty drawn combs. Shelter temperature was maintained between 32-36°C for optimal brood rearing conditions (Kovac, Stabentheiner, & Brodschneider, 2009). A grid consisting of 128 numbered squares (57mm X 57mm) was drawn on an acrylic sheet covering the glass on each side of the observation hives so drone locations could be recorded. In addition, an acrylic sheet with a grid consisting of 16 numbered squares (57mm X 57mm) was used to measure the bottom-board area of the hive, adjacent to the entrance. The bottom-board area consisted of 5.9% of the total nest surface area.

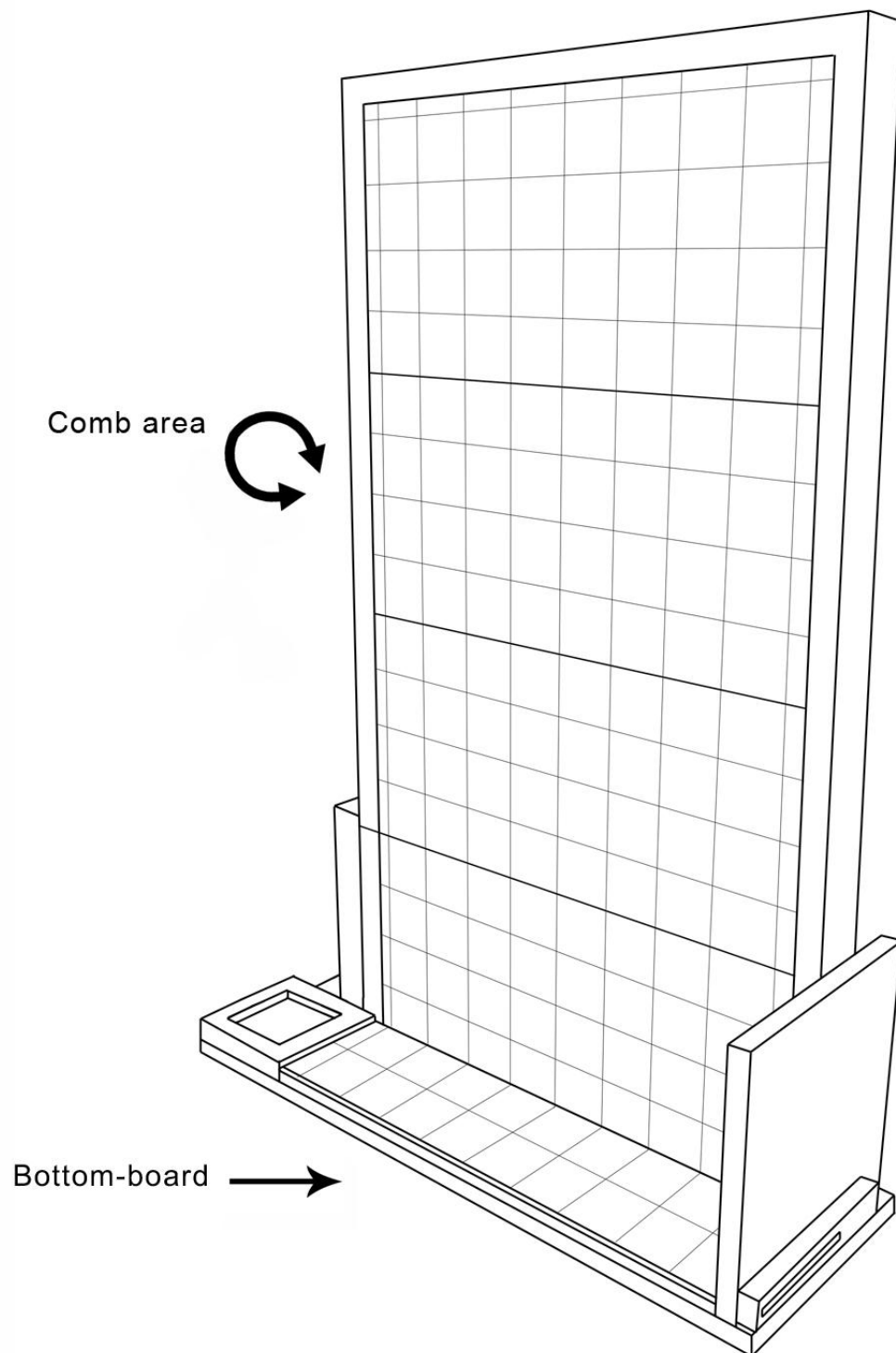


Figure 1. Diagram of observation hive setup. Total comb area consisted of 128 grid cells on each side. Bottom-board area consisted of a total of 16 grid cells.

Drone combs were placed in field colonies prior to the start of the experiment. As drones began to emerge, combs were removed and placed in a Percival Scientific incubator at 34.5 °C to allow adult drones to emerge. Newly emerged drones (<24 hours old) were marked on the thorax using Zinsser© white B-I-N shellac and placed in hoarding cages (Kulinčević, Rothenbuhler, & Stairs, 1973). Caged drones were misted with sucrose syrup (1:1 by weight) before being introduced to the observation hives through openings at the top of each hive. On July 5th, a total of 250 marked drones were introduced to each of the eight observation hives. On July 6th an additional 130 drones were introduced to each of the observation hives. Each of the observation hives was also supplied *ad libitum* with sucrose syrup (1:1 by weight) on this day. Any drones not accepted by the workers are immediately evicted from the colony (Cicciarelli, personal observations), so the first observations of drone locations were made more than 24 hours after the second introduction, on the evening of July 7th (Day 1). On Day 4 all colonies were switched over to 1:2 sucrose syrup (by weight) and provided with 30 grams of pollen pellets, which had been obtained from pollen traps during the Spring of 2011, and stored in a -74 °C freezer. On the morning of Day 5 all colonies were again provided with 30 grams of pollen.

Two treatments were employed to evaluate the effect of resource availability on the disposition of drones. For one treatment, four colonies were caged to prevent access to resources in the field; the other four were not caged. Colonies were caged or not

caged on the evening of Day 5. The four caged colonies were caged using nylon cages (1.83m X 1.83m X 3.66 m) erected on the outside of the observation shelter. For the second treatment, two caged colonies and two uncaged colonies were randomly selected to receive supplemental feed (*ad libitum* 1:2 sucrose syrup and 30 grams of pollen daily for the remainder of the experiment). The locations of drones (grid square) in the observation hives were recorded in the evenings between 9:00 PM and 11:00 PM for the duration of the experiment. Additionally, the locations of all drones were recorded between 9:00-11:00 AM and 3:00-5:00 PM on days 3-8. All colonies remained queenright throughout the experiment.

On Day 4 and Day 7, the contents of each colony were assessed to determine the quantities of brood, honey, pollen or empty space available to each of the colonies. Utilizing the same acrylic grid used in recording drone locations, a visual estimate of the contents of each of the 256 individual grid cells was recorded for each colony.

Two established colonies were set up on balance beam scales in the same apiary as the experimental colonies and weighed daily for the duration of the experiment to assess the foraging conditions in the apiary.

Statistical Analysis

Change in in-hive location

The change in the in-hive location of drones over time was analyzed using SAS/STAT® software, version [9.3](SAS Institute, 2008). The four combs in each hive were designated as one location (top) and the observation deck adjacent to the entrance as a second location (bottom). The number of drones present in both locations on each day and time period were converted to proportions of the total number of drones present with calculations for each day and time period made independently. The proportion of drones in the top location was used as the variable for analysis. Data were analyzed with a repeated measures model in SAS using PROC MIXED (SAS Institute, 2008) with random intercept, UN covariance matrix and SUB=colony. Feeding treatment (fed or unfed), cage treatment (caged or uncaged) and day with all interactions were modeled as fixed effects. Separate analyses were conducted for each time period. Significant interaction effects were resolved using multiple comparison tests.

The proportion of drones in the top location for each treatment was compared to the proportions for each of the other relevant treatments on the same day to determine whether feeding or caging had an effect on drone location within the hive. Comparisons among treatments were made for each day. Further, the proportion of drones for each individual treatment on Day 5 (final pre-treatment day) was compared to the proportion on each of the other days post-treatment to determine how quickly the location of drones within the hive was effected by the treatment. The pre-planned

multiple comparison tests listed above were performed with P -values adjusted using the Bonferroni correction method based on the specific number of tests involved.

Mortality over time

A second analysis was performed to evaluate treatment effects on mortality over time. For each colony, the largest number of drones present on any day was used as the starting population. The number of drones dying on each day (number of events) was calculated as the number of drones present on *day n* minus the number present on *day n+1*. Data were analyzed using a logistic regression with PROC GENMOD (SAS Institute 2008). A logistic regression for these data used a generalized linear model with the response equal to the binomial proportion r/n where r is the number of drones dying on a day (number of events) and n is the number of drones present the previous day (number of trials). Data were analyzed using a repeated measures model with feeding treatment, cage treatment, day and interactions modeled as fixed effects and colony as the repeated measure. Significant interaction effects were resolved using multiple comparison tests as described above.

Colony Contents

An analysis of the contents of each colony was performed to evaluate any differences that may have occurred between the two days prior to initiating treatments

and the two days after initiating treatments. The proportions of each grid cell two days prior to initiating the treatments that contained open brood, capped brood, open honey, capped honey, pollen or empty cells were summed to determine the total number of grid squares of each category. Similar totals were calculated for cell contents two days after initiating treatments, and the differences between the two estimates were calculated. The differences were analyzed in SAS using PROC MIXED (SAS Institute 2008). Feeding treatment (fed or unfed), cage treatment (caged or uncaged) and all interactions were modeled as fixed effects. Data were log transformed to equalize variances.

RESULTS

Change in locations of drones over time

The proportion of drones found on the four combs vs. the bottom board for each of the four treatments at three different times of day are shown in Figures 2, 3 and 4, respectively. Results of the statistical analysis are given in Table 1. Comparisons of means revealed no significant treatment effects on the location of drones during the three days prior to the initiation of the feeding and caging treatments ($P > 0.05$ all tests). No significant differences were found between the Fed-Uncaged (FU) and Unfed-Uncaged (UU) colonies during the experiment ($P > 0.05$). However, a comparison of Fed-Caged (FC) and Unfed-Caged (UC) colonies showed a significant difference two days after initiating treatment ($P < 0.05$) with an increasing proportion of drones being

located on the bottom-board in the unfed group (94.8%) compared to the fed group (36.8%).

In order to assess the impact of the cages on the treatments, comparisons between the Fed-Uncaged (FU) and Fed-Caged (FC) treatments and Unfed-Uncaged (UU) and Unfed-Caged (UC) were performed. No significant differences were found between Fed-Uncaged (FU) and Fed-Caged (FC) treatments ($P > 0.05$) during the experiment, however a comparison of the Unfed-Uncaged (UU) and Unfed-Caged (UC) treatments revealed significant differences on Day 7 and Day 8 during the 9:00-11:00 AM time period ($P < 0.01$ both, Figure 2), and for Day 7 during the 3:00-5:00 PM time period ($P < 0.01$, Figure 3) with an increasing proportion of drones being located on the bottom-board in the caged group.

Within treatment comparisons were made between Day 5 and each of the following days (post-treatment) separately for each of the four treatments. A significant shift in the location of drones from the upper combs to the bottom-board is first seen on Day 7 in the Unfed-Caged (UC) treatment ($P < 0.01$). By Day 8, a significant shift in the location of drones from the upper combs to the bottom-board is also seen in the Unfed-Uncaged (UU) treatment ($P < 0.05$). No significant shift in the location of drones occurred in either the Fed-Caged or Fed-Uncaged treatment groups ($P > 0.05$)

Drone Mortality

Results of the statistical analysis are given in Table 2. The probability of a drone dying on each day for each of the four treatments is shown in Figure 5. To evaluate the effects of the feeding treatment in both the caged and uncaged environments on mortality, comparisons were made between the Fed-Uncaged (FU) and Unfed-Uncaged (UU) treatments (uncaged environment) and the Fed-Caged (FC) and Unfed-Caged (UC) treatments (caged environment). During the pre-treatment days, there were significant differences between the Fed-Uncaged (FU) and Unfed-Uncaged (UU) treatments (FU=0.021 vs. UU=0.121) on Day 5 and between the Fed-Caged (FC) and Unfed-Caged (UC) treatments on Day 2 (FC=0.008 vs. UC=0.083), however these differences were relatively small compared to changes in drone mortality that occurred post-treatment on Day 7 and Day 8. There were significantly higher rates of mortality both on Day 7, in the Unfed-Caged (UC) treatment when compared to the Fed-Caged (FC) treatment ($P < 0.01$; UC=0.709 vs. FC=0.059), and Day 8 ($P < 0.01$; UC=0.642 vs. FC=0.117). A significantly higher rate of drone mortality also occurred in the UU treatment when compared to the FU treatment both on Day 7 ($P < 0.01$; UU=0.322 vs. FU=0.020) and on Day 8 ($P < 0.01$; UU=0.319 vs. FU=0.025).

In a comparison between Fed-Uncaged (FU) and Fed-Caged (FC), and Unfed-Uncaged (UU) and Unfed-Caged (UC) treatments, a significant difference in the rates of drone mortality was found during the pre-treatment days (Day 3, FU=0.067 vs.

FC=0.126; Day 5, FU=0.021 vs. FC=0.107), these differences, however, are also relatively small when compared to differences in drone mortality that occurred post-treatment on Day 7 and Day 8. There were significantly higher rates of mortality in the Unfed-Caged (UC) treatments than in the Unfed-Uncaged (UU) treatments on Day 7 ($P < 0.01$, UC=0.709 vs. UU=0.322) and Day 8 ($P < 0.01$, UC=0.642 vs. UU=0.319) and significantly higher rates of mortality in the Fed-Caged (FC) treatment when compared to the Fed-Uncaged (FU) treatment on Day 8 ($P < 0.01$, FC=0.117 vs. FU=0.025).

Contents of Combs Pre- and Post-treatment

Results of the statistical analysis are shown in Table 3. The estimates for each of the various content groups for each treatment for both periods and the difference between the values for the two periods are shown in Figure 6. Significant treatment effects were found for both changes in pollen and capped honey. There were significant differences found in the change in pollen content between the Fed-Uncaged (FU) treatment and all other treatments ($P < 0.05$). Significant differences were also found between the Unfed-Uncaged (UU) and Unfed-Caged (UC) treatments ($P < 0.05$) and between the Fed-Caged (FC) and Unfed-Caged (UC) treatments ($P < 0.05$). The Fed-Uncaged (FU) treatment had the largest increase in the amount of stored pollen while the Unfed-Caged (UC) treatment was the only treatment that had a decrease in pollen stores.

A comparison of capped honey revealed a significant difference in the change in the amount of capped honey between the Fed-Uncaged (FU) and Unfed-Uncaged (UU) treatments with the Unfed-Uncaged (UU) treatment having a larger increase in the amount of capped honey ($P < 0.05$).

Scale colony weights

Figure 7 shows the weights and change in hive weights for the two scale colonies over the course of the experiment. Both colonies gained very little weight during the experiment; and during most days, actually lost weight.

Table 1. Table of fixed effects for the location of drones over three time periods.

9:00-11:00 A.M. for Days 3-8				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	9.16	0.0389
Cage	1	4	8.79	0.0414
Day	5	20	12	<.0001
Fed*Cage	1	4	4.86	0.0922
Fed*Day	5	20	6.43	0.001
Cage*Day	5	20	6.1	0.0014
Fed*Cage*Day	5	20	3.26	0.0258

3:00-5:00 P.M. for Days 3-8				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	7.07	0.0565
Cage	1	4	3.01	0.158
Day	5	20	21.34	<.0001
Fed*Cage	1	4	2.65	0.179
Fed*Day	5	20	4.26	0.0085
Cage*Day	5	20	2.57	0.0594
Fed*Cage*Day	5	20	2.33	0.0807

9:00-11:00 P.M. for Days 1-9				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	10.8	0.0303
Cage	1	4	0.91	0.3942
Day	8	32	12.24	<.0001
Fed*Cage	1	4	0.59	0.4844
Fed*Day	8	32	4.44	0.0011
Cage*Day	8	32	1.05	0.4199
Fed*Cage*Day	8	32	0.31	0.9574

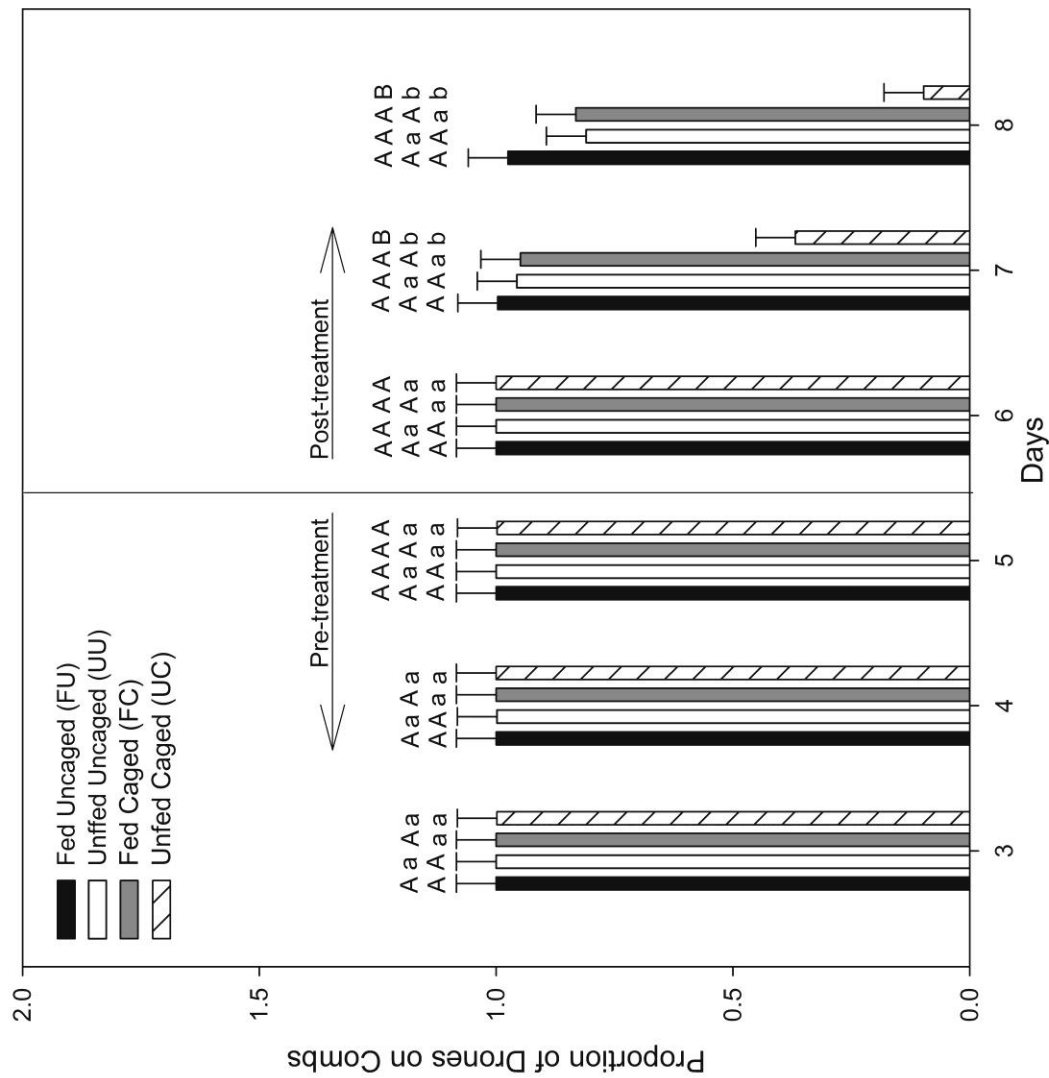


Figure 2. Proportion of drones on combs vs. bottom-board between 9:00-11:00 AM. The bottom row of letters denotes comparisons between FU vs. UU (Caps) and FC vs. UC (Lowercase) on each day. The middle row denotes comparisons between FU vs. FC and UU vs. UC on each day. The top row denotes comparisons of each individual treatment on Day 5 with the same treatment on each of the post-treatment days.

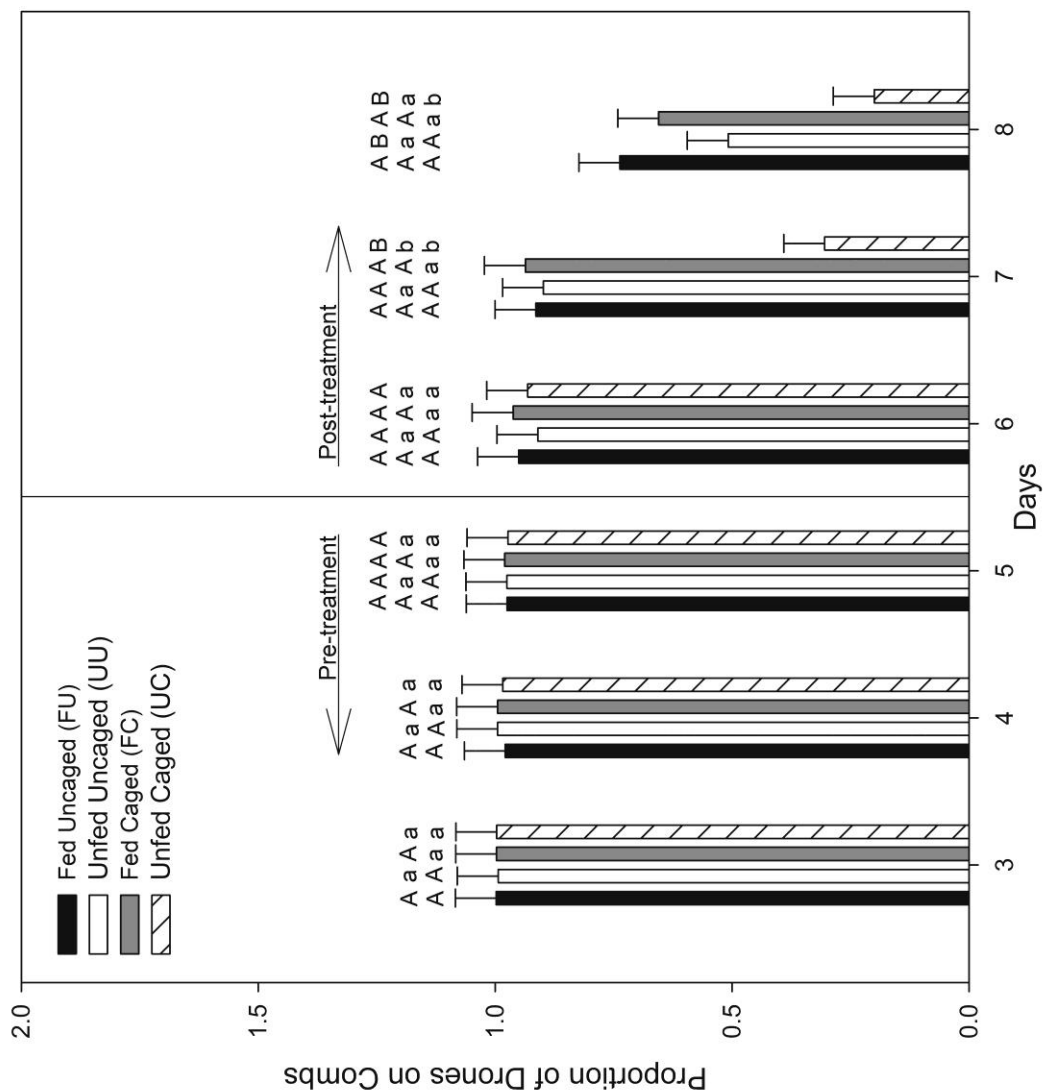


Figure 3. Proportion of drones on combs vs. bottom-board between 3:00-5:00 PM. The bottom row of letters denotes comparisons between FU vs. UU (Caps) and FC vs. UC (Lowercase) on each day. The middle row denotes comparisons between FU vs. FC and UU vs. UC on each day. The top row denotes comparisons of each individual treatment on Day 5 with the same treatment on each of the post-treatment days.

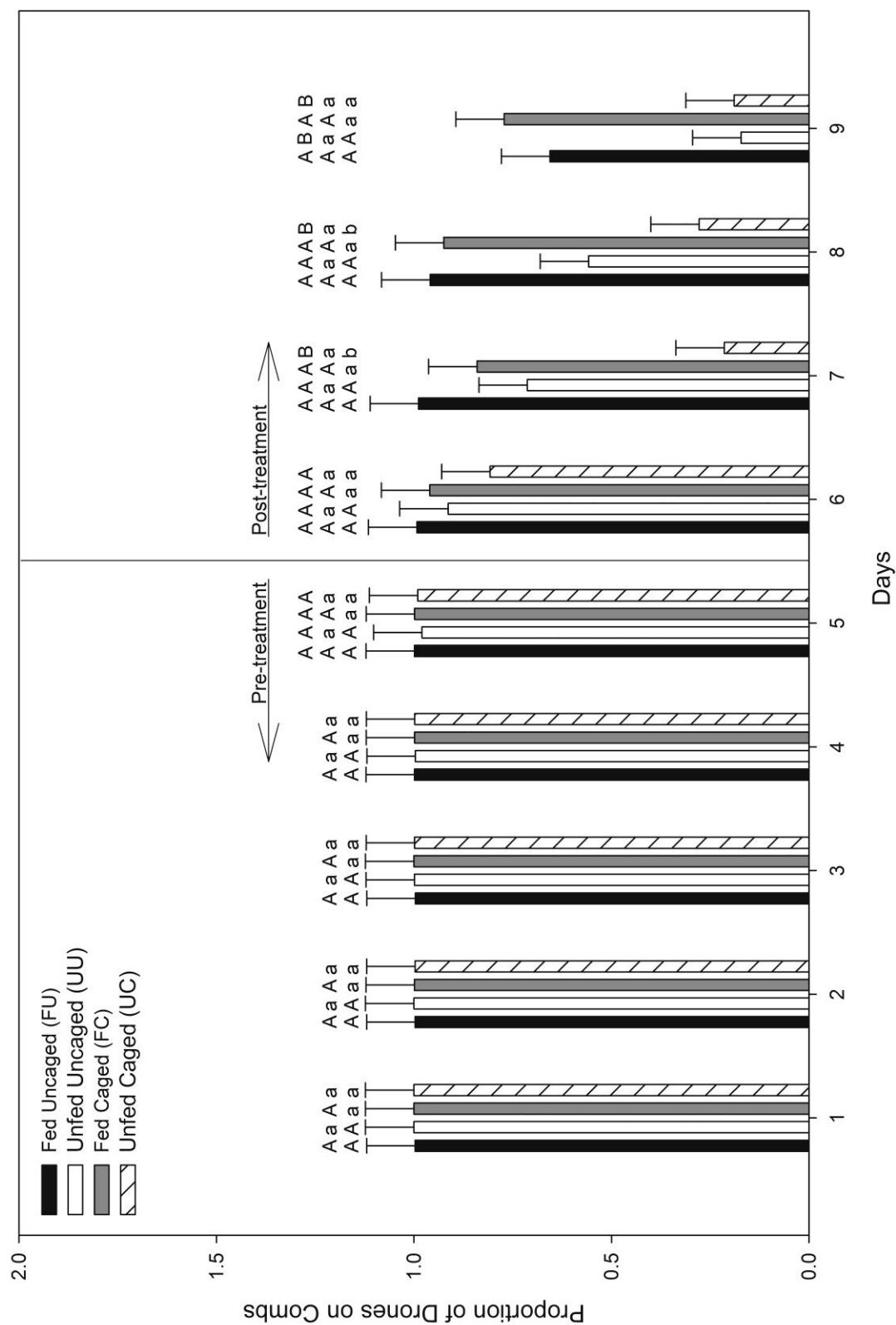


Figure 4. Proportion of drones on combs vs. bottom-board between 9:00 - 11:00 PM. The bottom row of letters denotes comparisons between FU vs. UU (Caps) and FC vs. UC (Lowercase) on each day. The middle row denotes comparisons between FU vs. FC and UU vs. UC on each day. The top row denotes comparisons of each individual treatment on Day 5 with the same treatment on each of the post-treatment days.

Table 2. Table of likelihood ratios for drone mortality.

Source	DF	Chi-Square	Pr > ChiSq
Fed	1	294.21	<.0001
Cage	1	64.55	<.0001
Day	7	446.24	<.0001
Fed*Cage	1	0.15	0.701
Fed*Day	7	432.92	<.0001
Cage*Day	7	108.88	<.0001
Fed*Cage*Day	7	42.41	<.0001

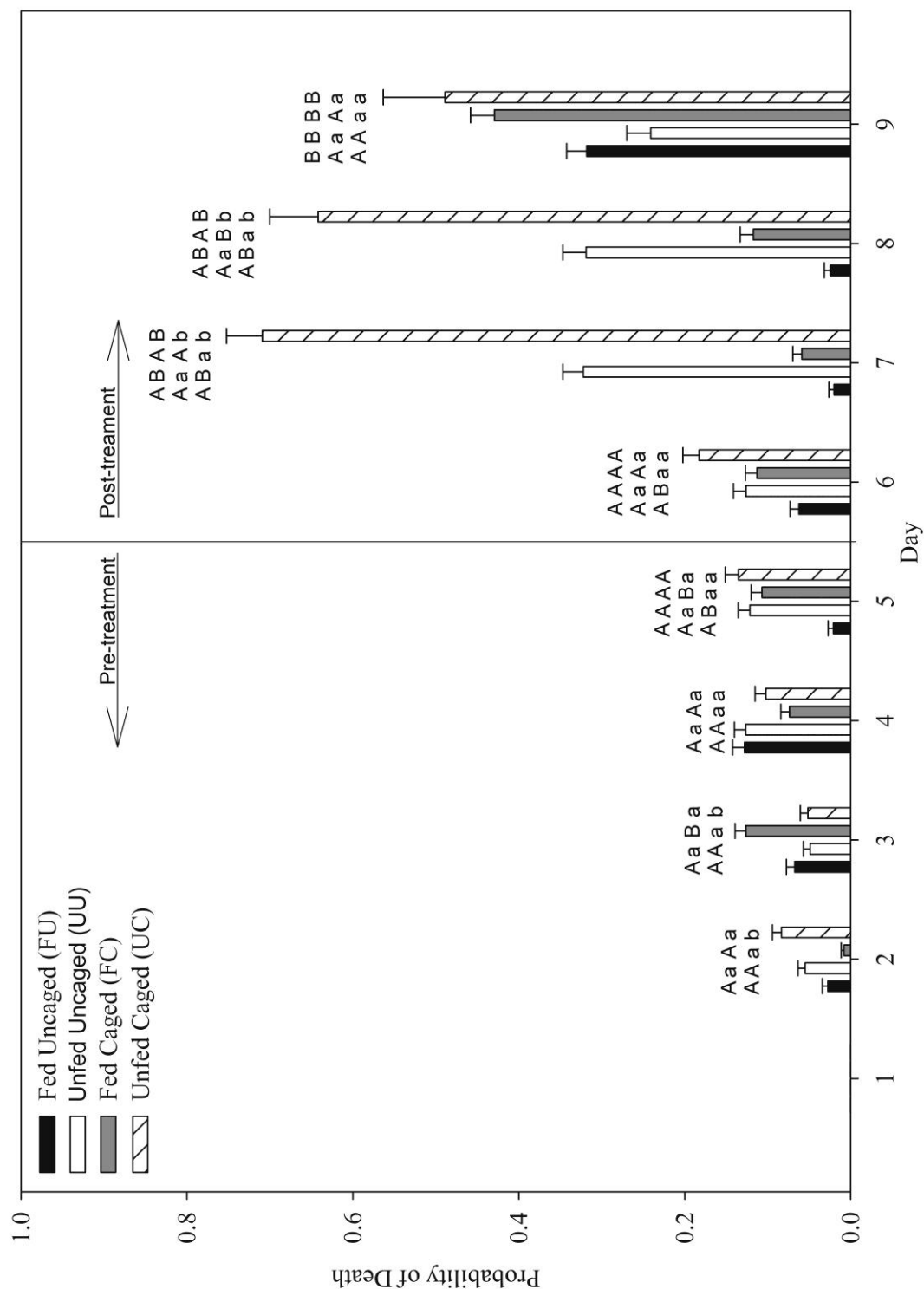


Figure 5. Probability of drones dying on each day. The bottom row of letters denotes comparisons between FU vs. UU (Caps) and FC vs. UC (Lowercase) on each day. The middle row denotes comparisons between FU vs. FC and UU vs. UC on each day. The top row denotes comparisons of each individual treatment on Day 5 with the same treatment on each of the post-treatment days.

Table 3. Table of fixed effects for colony contents pre- and post-treatment.

Empty Cells				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	0.68	0.4564
Cage	1	4	0.06	0.8165
Fed*Cage	1	4	2.74	0.1732

Pollen Cells				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	105.48	0.0005
Cage	1	4	26.4	0.0068
Fed*Cage	1	4	11.72	0.0267

Capped Honey Cells				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	1.07	0.3585
Cage	1	4	0.16	0.7059
Fed*Cage	1	4	24.53	0.0077

Open Honey Cells				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	4.28	0.1073
Cage	1	4	0.34	0.5888
Fed*Cage	1	4	2.48	0.1903

Capped Brood Cells				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	6.74	0.0603
Cage	1	4	0.21	0.6703
Fed*Cage	1	4	0.52	0.5125

Open Brood Cells				
Effect	Num DF	Den DF	F Value	Pr > F
Fed	1	4	1.86	0.2444
Cage	1	4	0.23	0.6587
Fed*Cage	1	4	1.34	0.3115

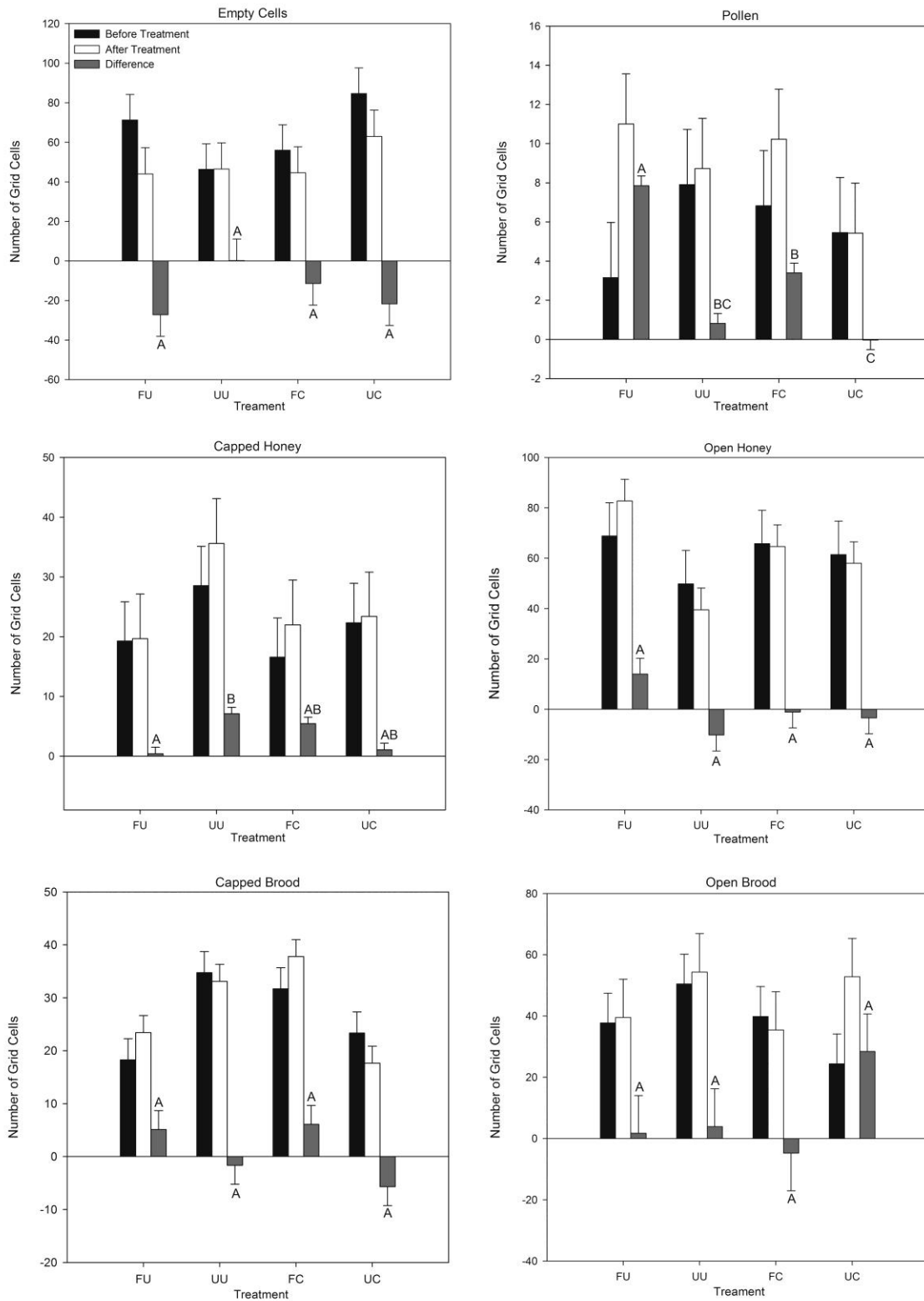


Figure 6. Comparison of colony contents pre- and post-treatment. The letters represent significant differences between treatments ($P < 0.05$)

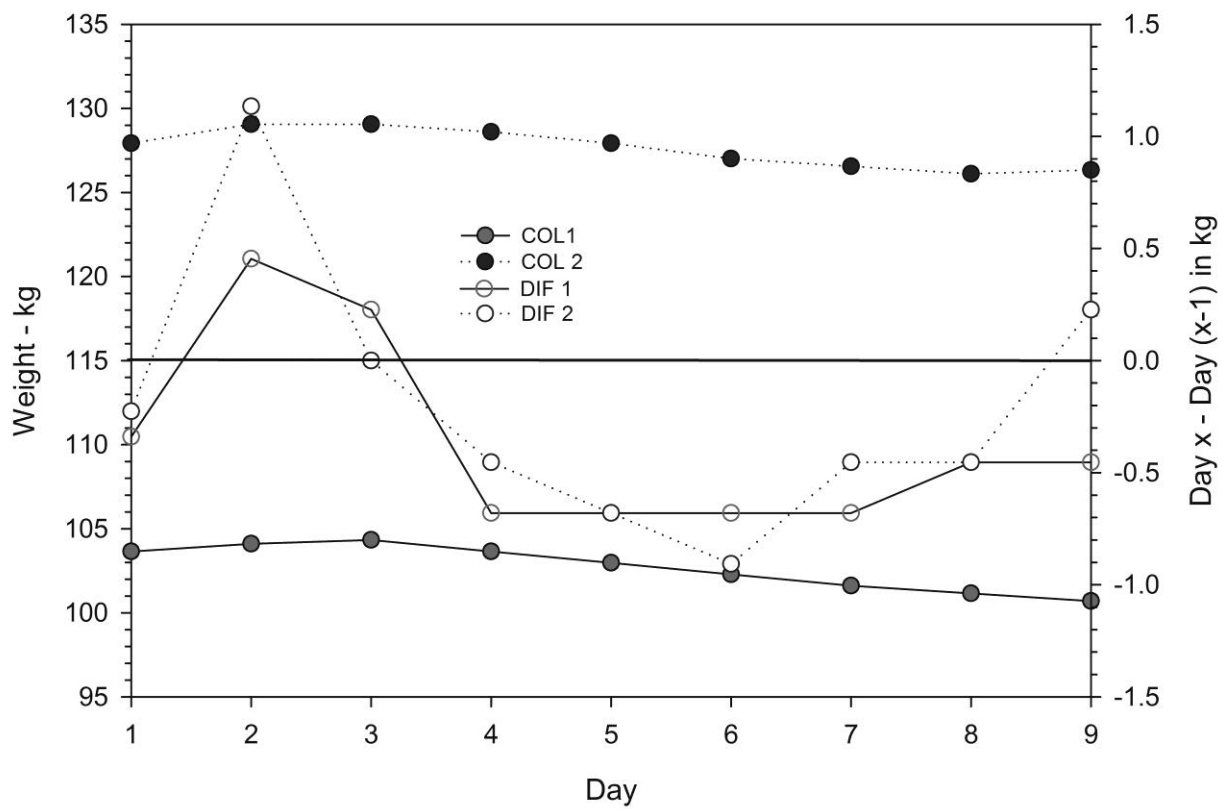


Figure 7. Patterns of changing hive weight between two scale colonies.

DISCUSSION

This study provides strong support for the hypothesis that drone eviction is affected by changes in foraging conditions. The increased rate of drone eviction observed in the Unfed-Caged colonies compared with Unfed-Uncaged colonies suggests that although foraging conditions around the study site were poor, foragers were able to find some amount of forage when compared to the caged colonies in which foraging was completely cut off. However, the change in drone location and drone mortality was significantly higher in both the Unfed treatment colonies compared to the Fed treatment colonies. These findings are similar to those of Free & Williams (1975) in which the significant factor controlling drone eviction was the amount of forage being collected. The present study is the first to show how quickly the eviction process can take place. Both Morse et al. (1967) and Free & Williams (1975) suggested that the drone eviction process takes place over several weeks. Our results demonstrate that as little as 48 hours of poor foraging conditions is sufficient to produce a shift in the location of drones from the combs to the bottom-board nearest the entrance and significantly increase the rate of drone mortality.

The rapid eviction and high mortality of drones are surprising considering the amount of resources and energy that are invested in the production of drones. The cost of producing drone comb, drone rearing, drone maintenance, and fueling adult drones for mating flights is significant (T.-D. Seeley, 2002). This investment in producing

drones takes place over the course of the reproductive season (several months). While these results show that workers can quickly adjust the care they provide the drones depending on foraging conditions, it does not answer the question of how the workers are able to assess the conditions of the hive in such a short time-frame.

In order for insect societies to be successful, individuals must coordinate their behavior with other individuals, often dispersed over a very large cavity or volume in which the nest resides. Many studies have examined the question of timing of comb construction, brood rearing, foraging, and other task allocations (Boes, 2010; B. R. Johnson, 2002; Pratt, 1998a; Ken Sasaki, et al., 2004; Schmickl & Crailsheim, 2002; T. D. Seeley & Mikheyev, 2003; Vaughan & Calderone, 2002). The main question behind most of these studies is whether the individuals are sharing information about colony needs between each other, or whether individuals are making decisions by directly assessing the needs of the colony. Support for the direct assessment hypothesis has been found for the regulation of pollen stores. Calderone & Johnson (2002) found that pollen foragers inspect individual cells and make decisions based on the contents of the cells they inspect. Pollen foragers entered approximately 10% of the 288 grid cells used in that set-up. Using a similar observation hive set-up, Johnson (2008) reported that patrolling workers (workers over 14 days old that walk around the comb and inspect the cells) inspect cells while moving widely throughout the nest, contacting over 80% of the grid cells within 30 minutes. Considering these findings, it is certainly possible that

within 48 hours of an onset of poor foraging conditions, workers are able to assess the changing conditions of the colony and begin to make decisions about what to do with the drones.

Adult drones are costly for the colony to maintain during conditions of dearth as they do not go on foraging flights and thus, do not contribute to the overall food stores of the colony. Adult drones are fed by workers only during the first few days after emergence, after which they feed themselves on the honey from the comb (J B Free, 1957). Sexually mature drones make multiple mating flights a day, returning to the colony several times to refuel from the honey comb (Ruttner, 1966). This behavior can quickly put a strain on colony reserves that are required to provide the colony with nutrients for the duration of non-foraging conditions.

Significant questions remain regarding which workers regulate drone eviction, which cues they use in this process and how they acquire the relevant information. It has been suggested that the unemployed foragers evict the drones during times of dearth (J B Free, 1957). Furthermore, are the workers responding to changes in nectar foraging or pollen foraging? Colonies that were fed in this experiment received both nectar and pollen. Consequently, it is not clear if the results are due to a change in access to both resources or to any one of them. A slight modification of the present study might provide some insight into this question. Changes in the estimates of stored pollen, suggests that pollen availability may be a significant factor in initiating drone

eviction. It has been shown that the presence of stored honey does not appear to have any effect on the amount of nectar foraging taking place, while changing conditions in the colony can have significant effects on pollen foraging (Dreller, Page, & Fondrk, 1999; Fewell & Winston, 1996; J. B. Free, 1967a). Is it a change in stored pollen that initiates the eviction of drones or is simply the lack of forage itself? While statistically significant changes in drone position was not observed in this study until approximately 48 hours after treatment began, the data do suggest that the impact of dearth conditions may actually be more influential than changes in the amount of stored pollen in the colony. Further investigations will need to be conducted to provide a better understanding of the complex interplay between foraging conditions and drone eviction.

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