Effect of Brood Type on Varroa-Sensitive Hygiene by Worker Honey Bees (Hymenoptera: Apidae)

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ABSTRACT Honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae) have been selectively bred for varroa-sensitive hygiene (VSH), which is the removal of pupae that are infested by *Varroa destructor* Anderson & Trueman from capped brood cells. This hygienic behavior is a complex interaction of bees and brood in which brood cells are inspected, and then brood is either removed or recapped. Previous work has shown that VSH bees uncap and remove significantly more varroa-infested worker pupae than nonhygienic bees do, but nothing is known about the reactions of VSH bees to mite-infested drone brood. This study compared the reactions of VSH bees with mite-infested worker and drone brood in a laboratory test and a field test. VSH bees inspected brood cells containing mite-infested pupae of both types of brood, but they removed significantly fewer mite-infested drone pupae than mite-infested worker pupae after 1 wk. This result suggests that mite populations in VSH colonies could increase more rapidly when drone brood is available. Additionally, the percentages of uncapped pupae and uncapped mite-infested pupae were positively correlated to the natural infestation rate of brood after a 24-h exposure, but not after an exposure of 1 wk. This result suggests that the rate of uncapping brood by hygienic bees may depend on the infestation rate, which gradually decreases with longer exposures to bees that remove mite-infested pupae from capped brood.

KEY WORDS bees, varroa, hygiene, drone, brood

Varroa-sensitive hygiene (VSH) is the removal of varroa-infested pupae from capped brood by honey bees (*Apis mellifera* L.) (Hymenoptera: Apidae). It is an important mechanism of resistance to *Varroa destructor* Anderson & Trueman for the eastern hive bee (*Apis cerana* F.) (Peng 1988, Boecking and Drescher 1992), but it occurs naturally at much lower frequencies in the western hive bee (*A. mellifera* L.) (Boecking 1992a, Boecking and Drescher 1992, Boecking et al. 1993a, Boecking and Spivak 1999). Varroa-sensitive hygiene in *A. mellifera* has a genetic basis (Boecking et al. 2000), and it has been increased through selective breeding (Spivak 1996, Spivak et al. 2003, Harbo and Harris 2005). Breeding for the suppressed mite reproduction (SMR) trait (Harbo and Harris 2001) produced VSH bees that hygienically uncap and remove large numbers of mite-infested worker pupae (Harris 2007).

Hygienic removal of diseased or infested brood is a complex behavior involving more than one bee, which makes direct observations of the behavior difficult (Arathi et al. 2006). Hygienic removal of mite-infested pupae can be inferred by reduction of the infestation rate of capped worker brood after a naturally infested comb is exposed to honey bees (Harris 2007). Additionally, brood combs often contain uncapped pupae after exposure to hygienic bees. Many uncapped pupae are mite-infested, and some are chewed as hygienic bees remove them from combs (Corréa-Marques and De Jong 1998, Villegas and Villa 2006). Not all uncapped pupae are removed from their brood cells, and even mite-infested pupae are often recapped without injury to the host pupa or removal of the infesting mites (Boecking and Drescher 1994, Boecking and Spivak 1999, Aumeier et al. 2000, Boecking et al. 2000, Aumeier and Rosenkranz 2001, Arathi et al. 2006, Villegas and Villa 2006). Thus, hygienic manipulations of brood cells by bees can result in several measurable conditions (uncapped pupae, removed pupae, or recapped brood cells) in the absence of directly observing the behavior of adult bees.

Most studies of varroa-sensitive hygiene have followed the fate of artificially inoculated brood cells, and there are few studies of hygiene involving naturally infested brood (Boecking and Spivak 1999). Additionally, varroa-sensitive hygiene toward worker brood has been well studied, but little is known about the hygienic behavior of honey bees to varroa-infested drone brood. Occasionally, workers of *A. cerana* remove mite-infested pupae from capped drone brood (Rath and Drescher 1990), but more often they plug the pore of the drone cap (Boecking et al. 1999) as a defense against varroa mites and other diseases of drone brood (Boecking and Ritter 1994, Boecking,

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1999). Drone caps of *A. mellifera* do not have central pores and are softer than those of *A. cerana*, which makes them more vulnerable to hygienic inspections by worker bees. Boecking (1992b) (also see Boecking et al. 1993a, 1993b) reported hygienic removal of varroa-infested drone pupae from artificially inoculated cells placed into six colonies of *A. mellifera* for 10 d. There has been no report of hygiene toward naturally infested drone brood. Because drone brood was not allowed during selection of VSH bees (Harbo and Harris 1999), there is also no information on their reaction to mite-infested drone brood.

Hygienic removal of mite-infested drone brood may be important in *A. mellifera* because varroa mites have a much higher preference for drone brood than worker brood (Fuchs 1990, Boot et al. 1995), and the mites produce more adult daughters during reproduction on drone pupae than on worker pupae (Fuchs 1992). So, although the availability of drone brood is more limited than worker brood, mite populations can grow more rapidly when drone brood is present, especially when populations are initially low. However, the lower availability of drone brood becomes limiting as mite populations grow larger, and competition for food leads to diminished reproduction by mites in drone brood cells (Martin and Medina 2004).

The primary purpose of this study was to compare the hygienic responses of VSH bees to varroa-infested drone and worker brood. Hygienic responses of VSH bees and commercial Italian bees to drone and worker brood were compared by exposing naturally infested brood to caged bees within an incubator for 24 h. A subsequent field experiment compared the hygienic responses of VSH bees to both types of mite-infested brood by placing infested combs into free-flying colonies for 1 wk.

Materials and Methods

Sources of Bees. Honey bees with high levels of VSH were bred at the USDA–ARS Honey Bee Breeding, Genetics, and Physiology Laboratory in Baton Rouge, LA (Harris 2007). All VSH queens used in this study were derived from instrumental insemination among queens from various VSH lines. Most control queens were produced by artificially inseminating queens from a commercially available Italian stock with drones from unrelated queens of the same stock. Some control queens were naturally mated queens of unknown origin, but each was known to produce colonies that grew populations of mites with high percentages of reproductive mites.

Incubator Test with Both Types of Brood. This test was repeated twice in May 2005. One comb of worker brood with the oldest larvae in the third instar was removed from each of five source colonies, and a comb of drone brood with the oldest larvae in the third or fourth instar was removed from each of another five source colonies. Drone combs were made from drone foundation and contained no worker brood cells. Similarly, combs of worker brood contained no drone cells. Combs were chosen only if all larvae were about the same age (1–2 d apart). All 10 combs (standard deep Langstroth) of uncapped brood were labeled and randomly placed into a single mite source colony for 5 d until all cells were capped.

A second trial was done with a second group of five worker and five drone combs that were removed from l0 different colonies. The larvae in these combs were in the same stages of development as in the previous group. Combs were placed in the same mite source colony that was used in the first trial, and they were removed after cells were capped 5 d later. For both trials, the infestation rate was determined for each comb by recording the number of infested cells in 200 capped brood cells (100 cells per comb side) on worker combs and 10% (15–40 cells) of all available capped cells in drone brood.

Each comb of infested brood was cut into equal halves (outside frame dimensions, 23.6 by 23.3 by 2.7 cm [length by depth by width]; includes 1.6-cm "ear" on the length for hanging frame) by using a radial arm saw. Each half was then attached to a similar comb half that contained only honey and pollen. The two pieces of comb were combined using thin strips of hardware cloth and staples so that the combination would hang as a single frame in the test cage. Both sides of each comb were photographed with a digital camera before the combs were caged with bees. The total number of capped brood cells was determined for each comb by counting cells from the digital photographs. VSH bees were given drone combs with 172 ± 65 capped pupae and worker combs with 958 ± 474 capped pupae (mean $\pm$ SD). Control bees were given drone combs with 179 ± 115 capped pupae and worker combs with 898 ± 417 capped pupae. One combination comb from each infested comb was housed with $\approx$2,000 worker bees from a VSH colony in a single frame hive (5 by 25 by 48.5 cm i.d.), and the other combination comb was housed with 2,000 control bees in a similar hive.

Bees for each hive were obtained by mixing worker bees from two brood combs that were taken from the center of the broodnest of a source colony, completely filling a jar (650-ml volume) with bees, and dumping the bees into a cage containing a comb before quickly closing the lid. In total, eight VSH and eight control colonies were used as sources of bees. Most sources provided bees to more than one cage, but the bees within each cage came from a single source. All 20 cages from each of the trials were housed in an incubator held at 34.5°C and 65% RH for 24 h.

All bees were brushed from each comb at the end of the test, and both sides of the comb were digitally photographed. Comparison of the pre- and posttest photographs allowed determination of the total number of host pupae that were completely removed from the comb. The infestation rate for removed pupae was not determined because of the difficulty in deciding whether all evidence of varroa infestation had been cleaned from some cells during the 24-h period. The number of cells that were uncapped but still contained either an entire or partially eaten host pupa was counted. Each uncapped pupa was inspected for evidence of infestation by varroa mites (foundress mites,

mite offspring, and feces). Most of the infested and uncapped pupae were uneaten, and these often retained the foundress mite and one to two offspring. Other brood cells contained only the terminal abdominal segments from eaten pupae. These cells often retained only a fecal patch as evidence of infestation by a varroa mite.

Statistical Analyses. The variables measured were 1) the percentage of pupae that were removed from capped brood cells, 2) the percentage of pupae that were uncapped, and 3) the percentage of uncapped pupae that were mite-infested. An analysis of variance (ANOVA) for a 2 by 2 factorial treatment structure over two blocks was used to test for differences in these responses that were related to the type of bees, type of brood, and the interaction of these factors. Variation related to the two experimental blocks was included as a random effect in the mixed model (SAS Institute 2000). The model included random factors to account for source of bees and source of brood used to make each experimental unit. Because response variables were percentages, they were arcsine transformed before analysis. Given that the final infestation should correlate with the initial infestation even if there were no hygiene, and the natural infestation rates for combs were variable, an analysis of covariance (ANCOVA) was conducted by incorporating the initial infestation rate (also arcsine transformed) as a covariate. The model included interactions between the covariate and the main factors. Nonsignificant interaction terms were removed sequentially from the model beginning with the highest order interaction, and the data were reanalyzed until only significant interactions remained.

Field Test with Both Types of Brood. The experiment was conducted during May–June 2007. Hygiene toward naturally infested worker and drone brood were measured in 14 VSH colonies in one apiary. All colonies had VSH queens that had been laying eggs for >2 mo prior; thus, most if not all of the worker bees in a colony were daughters of the resident queen. Colonies were manipulated so that they were of similar size, having six to seven frames of adult bees within a single deep Langstroth hive body that had 10 combs. All colonies had three to four combs of capped brood before the experiment. The queen in each colony remained free running during the test.

Combs of worker and drone brood were chosen so that the predominant stage of host bee in the capped brood was a white-eyed pupa (4 d postcapping for workers and 5 d postcapping for drones). Although the postcapping development for drones is longer than workers, the duration of the test was limited to 1 wk for both types of brood. After this time, the majority of worker pupae had brown eyes, tanned bodies and white wing pads. The drone pupae also had tanned bodies, but the eye color varied from dark purple to light brown. Twenty-five different colonies were used as sources of naturally infested worker and drone brood, and some colonies provided both types of infested brood.

Varroa-sensitive hygiene was quantified by comparing the infestation rate of capped brood before and after exposure to honey bees. Initially, a comb of naturally infested worker brood was placed in the center of the broodnest of each VSH colony for 1 wk. The initial infestation (sum of multiply and singly infested cells) was found by sampling 200 capped worker brood cells in straight line transects on each side of the comb (100 cells per side). In addition to the presence of mites, all cell caps were carefully examined to determine whether the cap was lined with an entire layer of silk or if the cap had been opened and recapped by previous hygienic activity (Boecking and Drescher 1994, Boecking et al. 2000). Combs were rejected if >10% of the cells had been recapped. The 14 combs of worker brood averaged 3,468 ± 469 capped worker cells, and ≈2 ± 1% (range, 0–8%) of these were recapped at the start of the test (mean ± SE).

Within 1–2 wk of this initial test, a comb of naturally infested drone brood was placed into the center of the broodnest of each colony for another week. The infestation rate of each comb was determined by sampling 50–200 capped cells. Few pupae were sampled for the initial infestation because the patches of drone brood were smaller (704 ± 451 cells) than the patches of worker brood. ≈1 ± 0.4% (range, 0–6%) of the drone cells were recapped at the start of the test.

The final infestation rate was estimated for each comb at the end of the test by sampling ≈200 capped brood cells for worker brood and 146–300 capped cells for drone brood. This sample was evenly divided between both sides of combs for worker brood, but much of the drone brood only occupied one side of a comb.

Three different types of pupae were examined after combs were exposed to VSH bees. Uncapped pupae were those in which the cell cap was completely or partially removed by bees, and the face of the pupa was exposed and visible. All uncapped pupae were examined for evidence of infestation by mites (adult mites, mite offspring, or mite feces). Normally capped and recapped pupae were found in the sample that was used to determine the final infestation rate of capped brood. As with uncapped pupae, the infestation rates for normally capped and recapped pupae were determined.

Statistical Analyses. The final percentage of mite-infested pupae from capped brood (normally capped + recapped cells) was compared between the two types of brood using ANCOVA with type of brood as a fixed effect and the initial percentage of mite-infested cells included as a covariate (with all interaction terms). A similar ANCOVA was used to analyze each of the following variables: the percentage of pupae found uncapped at the end, the percentage of uncapped pupae that were mite-infested, the percentage of mite-infested pupae in recapped cells, and the percentage of mite-infested pupae in normally capped cells. The proportion of the two types of capped brood cells (normally capped and recapped) in the final sample was compared between the two types of brood by analyzing the log (base 10) of the ratio of recapped

Table 1. Hygienic response measured after combs with either capped worker or drone cells were cut into halves, and each half was caged with ≈2,000 VSH or control bees in an incubator for 24 h

Variable (mean ± SD)	VSH bees		Control bees	
	Drone brood (n = 10)	Worker brood (n = 10)	Drone brood (n = 10)	Worker brood (n = 10)
% brood cells that were removed	5 ± 0.9a	4 ± 0.9a	1 ± 0.9b	0.8 ± 0.9b
% pupae that were uncapped at end	4 ± 1a	4 ± 1a	2 ± 1b	1 ± 1b
% uncapped pupae infested with mites	27 ± 10b	71 ± 11a	20 ± 11b	46 ± 11a,b

Data are least square means adjusted for a significant effect of the initial infestation rate of combs as a covariate in analysis of covariance. Means ± SE with the same letter within a row are not significantly different (α = 0.05).

cells to normally capped cells in a similar ANCOVA. All percentage data were arcsine transformed. Each ANCOVA also included random factors for the sources of bees and sources of brood used to establish experimental units.

Results

Incubator Test with Both Types of Brood. Although combs in each trial were simultaneously infested by mites within a single colony, the infestation rates varied greatly among combs. The average initial infestation rate was 26 ± 5% for worker brood and 37 ± 5% for drone brood (mean ± SE; n = 10 for each type). Drone brood was expected to have much higher infestation rates than worker brood because varroa mites prefer drone brood (Fuchs 1990, Boot et al. 1995), and as to why this was not the case here is unclear.

The percentage of pupae that were completely removed from combs was positively correlated to the initial infestation rate (F = 5.53; df = 1, 35; P = 0.025). There were no significant interactions between the initial infestation rate (covariate) and either the type of bees or the type of brood. VSH bees removed a significantly higher percentage of pupae than did control bees (F = 30.0; df = l, 35; $P < 0.001$) (Table 1). There was no significant difference in percentage of removed pupae between worker and drone brood (F = 0.99; df = l, 35; P = 0.33), and the interaction between type of bees and type of brood was not significant (F = 0.26; df = 1, 35; P = 0.62). Although some of the emptied cells retained a fecal patch, most cells did not show evidence of mites. Thus, although the removal of pupae was positively correlated to infestation rate, it could not be confirmed that the majority of pupae removed by the bees were mite-infested.

The percentage of pupae that were uncapped at the end was positively correlated to the initial infestation rate of brood (F = 10.5; df = l, 35; P = 0.003). There were no significant interactions between the initial infestation rate (covariate) and type of bees or type of brood. VSH bees uncapped significantly more pupae than did control bees (F = 4.2; df = 1, 35; P = 0.047) during the 24-h period. VSH bees uncapped 4% of the available pupae, whereas control bees uncapped 1–2% (Table 1). There was no significant difference in percentage of uncapped pupae between the two types of brood (F = 0.43; df = 1, 35; P = 0.52), and the interaction between type of bee and type of brood was not significant (F = 0.0; df = 1, 35; P = 0.96).

The percentage of uncapped pupae that were mite-infested was positively correlated to the initial infestation rate (F = 5.6; df = 1, 26; P = 0.026), but there was no significant difference between the two types of bees (F = 1.5; df = 1, 26; P = 0.23) (Table 1). For all combs (drone and worker), 32% of the uncapped pupae from combs with control bees were mite-infested, whereas 47% of the uncapped pupae from combs with VSH bees were mite-infested. There were significantly more uncapped worker pupae that were mite-infested than were uncapped drone pupae that were mite-infested (F = 9.1; df = 1, 26; P = 0.006) (Table 1). ≈57% of all uncapped worker pupae were mite-infested, whereas only 25% of the uncapped drone pupae were mite-infested (combined for both types of bees). The interaction between type of bee and type of brood was not significant (F = 0.35; df = l, 26; P = 0.56).

Field Test with Both Types of Brood. The final infestation rate for all capped cells was positively correlated to the initial infestation rate (F = 32.8; df = l, 24; $P < 0.001$), which should be the case, even if no mite-infested pupae were removed by VSH bees. If no mite-infested pupae were removed by hygienic bees (or if pupae were removed at random without regard to the presence of mites), the estimates of the initial and final infestations should be the same, and the slope of the line relating the final and initial infestations should equal 1. If mite-infested pupae were preferentially removed, there may be a correlation between the final and initial measurements, but the slope of the line should be <1.

There was a significant interaction between the initial infestation rate (covariate) and the type of brood (F = 20.67; df = 1, 24; P = 0.0001) in the ANCOVA of the final infestation rate. This significant second order interaction suggested nonparallel lines for the responses of VSH bees toward the two types of brood. Consequently, slope parameter estimates were determined for the lines for each type of brood, and a 95% confidence interval around the difference in slope estimates was calculated. The difference was considered significant if zero was not contained within the confidence interval (CI). When all 28 combs were included in the analysis (Fig. 1A), the difference in slope estimates $\beta_{drone} - \beta_{worker} = 1.10 \pm 0.28$ (±95%

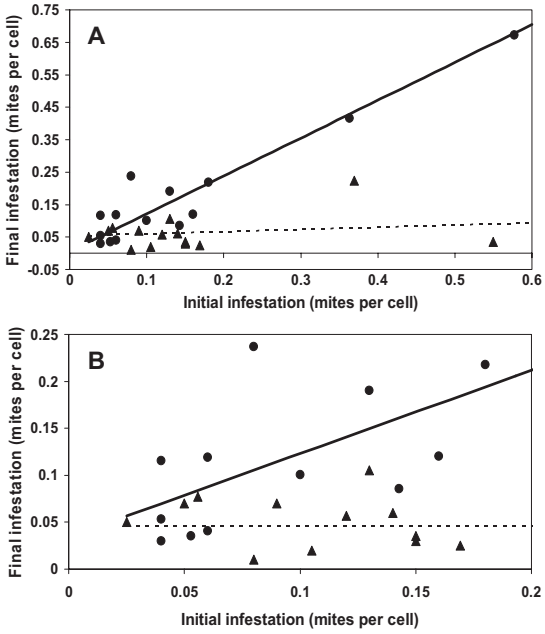

Fig. 1. Comparison of initial and final infestation rates for mite-infested worker (dashed line, triangles) and drone brood (solid line, circles) that had been exposed to VSH bees for 1 wk. The reduction in infestation for capped worker brood was significantly different than the response to capped drone brood when all the data were analyzed (A) or when data were limited to infestations <0.2 mites per cell (B).

CI) for the two types of brood was significantly greater than zero ($\alpha = 0.05$). The predicted line (solid line, Fig. 1A) for drone brood had a slope close to one, which suggested that pupae were either removed randomly (without regard to the presence of mites), or no significant numbers of mite-infested pupae were removed. The slope of the predicted line (dashed line, Fig. 1A) for worker brood was much less than one, which suggests that mite-infested pupae were preferentially removed from capped brood.

However, the lines for both types of brood were potentially biased by a few combs with initial infestations >0.3 mites per cell, whereas the majority of combs had initial infestations <0.2 mites per cell (Fig. 1A). Therefore, the data were reanalyzed with exclusion of the four combs having initial infestations >0.3 mites per cell. The ANCOVA produced results similar to the previous analysis. The interaction between the initial infestation and type of brood was significant ($F = 5.1$; df = l, 20; $P = 0.035$), and the difference in slope estimates $\beta_{drone} - \beta_{worker} = 0\,0.88 \pm 0.68$ (±95% CI) for responses to the two types of brood was significantly greater than zero ($\alpha = 0.05$). The final infestation rates for capped drone brood (solid line, Fig. 1B) were similar to the initial infestation rates, whereas the final infestation rates for capped worker brood (dashed line, Fig. 1B) tended to be lower than the initial infestation rates.

The capped brood cells that were sampled to determine the final infestation rate were subdivided into cells with normal caps and those that had been recapped after being previously opened by bees. The ratio of recapped to normally capped cells was not significantly different between the two types of brood (Table 2), which suggests that similar rates of recapping had occurred in the two types of brood. Approximately 20–30% of the brood cells had been recapped during the experiment. The ratio of recapped to normally capped brood cells and the percentage of recapped cells that were mite-infested were not correlated to the initial infestation rate of capped brood, and there were no significant differences between the two types of brood for both variables. Slightly >20% of all recapped cells were mite-infested, regardless of type of brood (Table 2).

The percentage of normally capped brood cells with varroa mites was significantly correlated with the initial infestation rate ($F = 22.4$; df = l, 24; $P < 0.0001$). As with the final infestation rate for all capped brood, there was also a significant interaction between the initial infestation rate and type of brood ($F = 59.7$; df = l, 24; $P < 0.0001$) for the percentage of normally capped cells that were mite-infested. The infestation rate for normally capped drone brood was more similar to the initial infestation rate (solid line, Fig. 2), whereas the infestation rate for normally capped worker cells was significantly lower than the initial infestation (dashed line, Fig. 2). The difference in slope estimates $\beta_{drone} - \beta_{worker} = 1.18 \pm 0.19$ (±95% CI) was significantly greater than zero ($\alpha = 0.05$).

Neither the percentage of uncapped pupae nor the percentage of uncapped mite-infested pupae was cor-

Table 2. Comparison of worker and drone brood combs that were exposed to VSH bees for 1 wk in the field study

Variable (mean ± SD)[a]	Worker brood ($n = 14$)	Drone brood ($n = 14$)	F	df	P
% normally capped cells	66 ± 7	78 ± 7	1.37	1, 26	0.25
% cells that were recapped	34 ± 7	22 ± 7			
% recapped cells with mites	21 ± 9	23 ± 8	0.65	1, 24	0.43
% pupae that were uncapped	1 ± 1	2 ± 1	1.50	1, 26	0.75
% uncapped pupae with mites	62 ± 10	50 ± 14	0.54	1, 17	0.55

Each colony was initially given a comb of naturally-infested worker brood. A naturally infested drone brood was tested in each colony within 1–2 wk of the first trial.

[a] Because the first two variables are different components of a pool of capped cells, the log ratio between the two was analyzed in an analysis of covariance. All of the remaining percentage data were arcsine transformed before analysis, but the least squares means (mean ± SE) for the raw data are reported here.

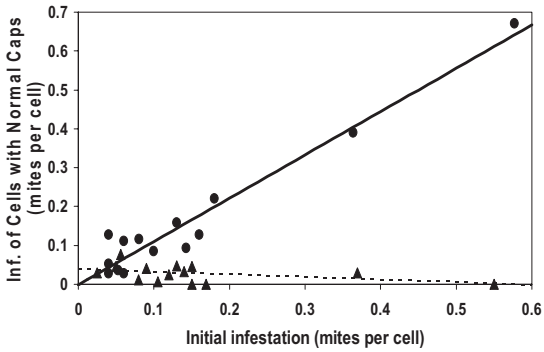

Fig. 2. Comparison of initial and final infestation rates for only the normally capped brood cells in worker (dashed line, triangles) and drone brood (solid line, circles) after combs were exposed to VSH bees for 1 wk. Infestation rates declined in worker brood and remained relatively unchanged in drone brood, which suggested preferential hygiene of mite-infested pupae for worker brood.

related to the initial infestation rate, so the covariate and its interaction term were dropped from the ANCOVA. There was no significant difference in percentage of pupae that were uncapped between the two types of brood (Table 2). Approximately l-2% of all pupae were uncapped after 1 wk, regardless of the type of brood. In addition, there was no significant difference in the mite infestation rate of uncapped pupae between the two types of brood (Table 2). Approximately 50–60% of all uncapped pupae were mite-infested.

Discussion

Mixed results were obtained in the comparison of hygienic activity of honey bees to mite-infested worker and drone brood. The removal of pupae from brood combs was similar between worker and drone brood in a short incubator test (Table 1). VSH bees removed significantly more pupae than control bees, which was similar to previous studies (Harbo and Harris 2005, Ibrahim and Spivak 2006, Harris 2007). The infestation rate for cells that were emptied by removal of pupae was not determined; therefore a preference for removing mite-infested pupae could not be compared between the two types of brood. Results were different for worker and drone brood combs that were exposed to VSH bees for 1 wk. The final infestation rate for capped brood was much lower than the initial infestation for worker brood, whereas the final and initial infestation rates for drone brood compared closely (Fig. 1). This suggested a preferential removal of mite-infested worker pupae and either no removal or random removal of mite-infested drone pupae.

Preferential removal of mite-infested worker pupae was supported by examining the final infestation rate of only normally capped brood cells after 1 wk of exposure to VSH bees. Normally capped cells were those with a complete layer of silk lining the interior surface of the cell cap. They were cells not manipulated by adult bees after the larvae inside had spun their cocoons. The final infestation rate measured from these cells should be the same as the initial infestation rate if hygienic activity (uncapping, removal, and recapping) occurred randomly. The final infestation rate for normally capped worker pupae was much lower than the initial infestation, which suggested preferential hygiene toward mite-infested pupae (Fig. 2). The final infestation of normally capped drone cells was similar to the initial infestation and suggested no hygiene or hygiene not biased to mite-infested pupae.

The percentage of uncapped pupae was not significantly different between worker and drone brood after a 24-h exposure of combs to honey bees (Table 1). Similarly, the percentage of uncapped pupae was not significantly different between the two types of brood after a 1-wk exposure to VSH bees (Table 2). However, the percentage of uncapped pupae that were mite-infested was significantly higher in worker than in drone brood in the 24 h test. In particular, 70% of the uncapped worker pupae were mite-infested after exposure to VSH bees, whereas 27% of the uncapped drone pupae were mite-infested. The infestation rate for uncapped worker pupae also exceeded the natural infestation, which implies that mite-infested pupae were targeted and the uncapping of pupae was not random (Corréa-Marques and De Jong 1998, Villegas and Villa 2006). The infestation rate for uncapped drone pupae was slightly lower than the natural infestation rate (37%) in the 24-h incubator test, which suggests that specificity toward mite-infested drone pupae was lacking. Control bees also showed greater specificity for uncapping mite-infested worker pupae than for mite-infested drone pupae (Table 1).

Differences in specificity for uncapping mite-infested pupae between worker and drone brood were not apparent after a 1-wk exposure to VSH bees (Table 2). About half (50–62%) of the uncapped pupae were mite-infested, regardless of type of brood. However, the percentages of uncapped pupae (1–2%) after 1 wk were lower than those (4–5%) after 24 h. It may be that uncapping rates and specificity for uncapping mite-infested pupae are highest during the first hours of exposure when odors or other stimuli that trigger hygiene are most concentrated within the infested comb. This notion is supported by correlations between the percentages of uncapped pupae and uncapped mite-infested pupae in this study. Both percentages were positively correlated to the natural infestation rate of brood after a 24-h exposure, but not after a 1-wk exposure to VSH bees. The lack of a positive correlation between initial infestation of brood and the incidence of uncapped pupae after a 1-wk exposure may result from confounding events such as removal and recapping of mite-infested pupae that do not occur during the shorter exposures to the hygienic bees.

Although the results were mixed, it seemed that VSH bees were less hygienic to mite-infested drone

brood than to mite-infested worker brood. The higher specificity for uncapping mite-infested worker pupae after 24 h and the similarity between final and initial infestations of drone brood after a 1 wk of exposure support this conclusion. Reduced hygiene toward mite-infested drone brood allows the possibility of greatly increased growth of varroa mite populations in colonies of VSH bees (Fries et al. 1994). How seriously the lack of hygiene toward mite-infested drone brood compromises the varroa resistance of VSH bees remains unknown. In the worst case, the growth of mite populations would be highest when drone production is high, which is March–May and September–October in Louisiana. However, it is also very likely that mite populations of well established VSH and their hybrid colonies would be low in the early spring before drone production begins (Harbo and Harris 2001). A recent 3-yr study showed that the varroa resistance of VSH bees remained high when used in commercial beekeeping operations in which drone brood production was not restricted (Ward et al. 2008). Although the amount of drone brood was not monitored in that study, it is very likely that significant amounts of drones were produced by colonies during all years of the experiment.

One potentially positive consequence of reduced hygiene to mite-infested drone brood is that drone brood could serve as a refuge for mites that retain genes which make them susceptible to varroa-sensitive hygiene. As with chemical treatments that are used to kill mites, there is a possibility that selection pressure caused by intense behavioral resistance of honey bees could produce populations of mites that are genetically resistant to the bees. Thus, highly intense varroa sensitive hygiene directed to mites in worker brood could result in the development of resistant mites that evade the hygienic behavior of the bees.

For drone brood to be an effective refuge, there needs to be a mechanism by which genes in susceptible mites can dilute the frequency of genes conveying resistance to the behavior. In singly infested brood cells the usual mating scheme for varroa is between brother and sister (Donzé et al. 1996), which does not introduce new genes to the next generation. The best chance for genetic exchange among mites occurs in multiply infested cells where unrelated males can encounter unrelated females (Reich et al. 1998). Because the frequencies of multiply infested cells are usually higher in drone brood (Fuchs and Langenbach 1989), the chances for genetic dilution of resistance genes through nonsibling matings should be higher in drone brood (Reich et al. 1998).

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