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Relating hygienic behaviour with the age of comb and colony strength of hived East African lowland honey bees, *Apis mellifera scutellata* Lepeletier, 1836 (Hymenoptera Apidae)

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ABSTRACT Hygienic behaviour in honey bees is known for associating with parasite and disease resistance but the mechanisms influencing this behaviour are not well known. We studied the hygienic relationship between the age of combs and colony strength of East African lowland honey bees *Apis mellifera scutellata* Lepeletier, 1836 (Hymenoptera Apidae) in Top-bar hives (n = 24) selected from an apiary located in an African tropical rainforest. The findings indicated that over 24 hours, there was no interaction between hygienic behaviour and colony strength (P=0.9707). Hygienic behaviour was also independent of the age of the honeycomb (P=0.9859). There was a non-significant difference in the number of cells cleaned, for both Young and Old combs; and between two levels of colony strength (Strong *Vs* Weak) during the day, with that number getting closer after 24 hours. There was an efficient rate of hygienic response during the night than during daytime when strong colonies cleaned 32.35% of debris while the weak colonies did 20.55%; but at night, the cleaning rate rose to 65.85% in strong and 73.9% among the weak colonies. Overnight, weak and new colonies cleaned >96% of the cells attributable to the presence of a big population of worker bees at night, and minimal colony activities notably foraging activity at that time. Whereas hygienic behaviour was not directed towards colony strength and age of the combs in 24 hours, there is a need for maintaining strong colonies. Since the rate of cell cleaning was higher at night than during the day, colony operations and hive disturbances should be done at night as opposed to daytime.

KEY WORDS *Apis mellifera scutellata;* Colony size; Dead brood; East African lowland honey bees; Hygienic behaviour.

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INTRODUCTION

Bees are economically important insects that belong to the order Hymenoptera with over 30,000 species known worldwide of which 30 are exploited by humans for honey production (Gupta et al., 2014; Prata & Martins da Costa, 2024). They also serve as plant pollinators among other roles (Thapa, 2006; FAO, 2019; Kugonza, 2022), and are part of the subcategory associated with biodiversity for food and agriculture. Uganda is endowed with a variety of honey bee species including the East

African lowland honey bee *Apis mellifera scutellata* Lepeletier, 1836, the most widespread and hence the target of this study; as well as *Apis mellifera adansonii* Latreille, 1804 (Kasangaki et al., 2017), *Apis mellifera monticola* F.G. Smith 1961 (Hepburn et al., 2000; Radloff & Hepburn, 2000; Kajobe, 2008) and a variety of stingless bee species of the genera *Trigona* Jurine, 1807 and *Meliponula* Cockerell, 1934 which exist in specific ecological zones (Kajobe, 2006; Kajobe, 2008).

Honey bees are attacked by a wide range of parasites (internal or external), predators and diseases. The diseases include bacterial diseases (e.g. American Foulbrood), fungal diseases (e.g. Chalkbrood), and viral diseases (e.g. Acute bee paralysis virus). The dangerous bee parasites, particularly the mite *Varroa destructor* (Anderson et Trueman, 2000), feed on body fluids of adult bees, pupae, and larvae, and also transmit viruses in the process (Nekoei et al., 2023). Parasites are a growing challenge to beekeeping in Uganda and neighbouring countries (Mwiza et al., 2013) although they have not reached economic threshold levels, showed by the absence of a correlation between mite infestation levels and colony productivity and strength (Chemurot et al., 2016). In some cases, they cause poverty to beekeepers after colonies are devastated, and also affect the ecological compatibility of the environment (Gyadan, 2012). The use of pesticides, insecticides and herbicides is an additional threat to beekeeping, but parasites, diseases and predators remain a significant growing concern to beekeepers in Uganda and a major driver in other parts of the world (Kugonza, 2022). Contamination and the development of resistance in pests are steadily challenging the continued use of chemicals by beekeepers in other parts of the world (Nekoei et al., 2023). This leaves physical means as the alternative to fight against these hazards by the honey bees. Fortunately, colonies of honey bees and other social insects (such as ants and termites) have a natural instinct to defend themselves against pests, parasites, and disease challenges through hygienic behaviour. Rothenbuhler (1964) introduced the term 'hygienic behaviour' to describe how worker honey bees detect, uncap, and remove diseased and dead broods from comb cells as a way to prevent infection.

Hygienic behaviour is an economically important source of natural immunity in the honey bee colony (Wilson-Rich et al., 2009). It has been studied as a level mechanism of resistance to the parasitic mite, *V. destructor* (Boecking et al., 2000) and these efforts have been ongoing for over eight decades (Spivak and Danka, 2020). However, it is not clear what mechanisms are responsible for the identification and removal of sick, damaged and dead brood from the capped cells (Gramacho, 2004; Downey, 1998). Hygienic behaviour is vital for the population dynamics of bees as it can delay or avoid the development of diseases in the colony (Gramacho and Goncalves, 2009). *Apis mellifera* colonies die from *V. destructor* infestation within a few years if the mite population growth is not regulated by the beekeeper and because chemical mite control has a lot of limitations and is hazardous (Milani, 1999; Walner, 1999); it is of both public and economic interest to breed bees with a high tolerance and resistance to this mite. In rural settings, proper identification and careful maintenance of strong and resistant honeybee colonies look to be the cheaper, harmless, affordable, and acceptable management practice. However, the factors governing this positive strength/hygiene remain unknown, hence this study.

MATERIAL AND METHODS

Study area

This study investigated the relationship between comb age and colony strength in relation to hygienic behaviour of East African lowland honey bees reared in Kenya Top-Bar (KTB) hives randomly selected from the Nyabyeya Forestry College apiary. Nyabyeya Forestry College (1° 02 15N, 33° 50 24E) is located on the fringes of the vast Budongo Forest, in the Lake Albert Crescent agro-ecological zone, 245 km from Kampala, the capital of Uganda and one of its nine cities. The college aims at contributing to the improved management and utilisation of Uganda's forest resources, and environmental conservation in Uganda. The study was based on Newton and Ostasiewski model (1986), where a sterile insect pin was used to kill 100 brood of worker larvae in the respective combs, taking care not to damage the free-hanging combs on the top bars, which were returned to their respective positions in their hives. The hygienic behaviour

Figures 1–4. Colony sizes and types of comb ages used in the study. Fig. 1: new white comb. Fig. 2: old dark combs. Fig. 3: weak colony (<50% hive full). Fig. 4: strong colony (more than ¾ hive full).

was determined as the removal of killed larvae from brood cells. The proportion of uncapped brood cells and dead brood removed by the colonies was recorded.

Data collection and analysis

The new/white combs (Fig. 1) and old/dark combs (Fig. 2) used were obtained from six weak honeybee colonies (Fig. 3) and six strong colonies (Fig. 4) in randomly selected Kenya Top-Bar (KTB) hives. The colonies with combs >75% brood and high populations of adult bees were chosen as strong colonies. Those KTB hives with combs having 50–75% brood were selected as weak colonies. Six strong and six weak colonies were evaluated over 12 hours overnight, and another set of six strong and six weak colonies were evaluated over a 24-hour period. The number of cells cleaned by honey bees at different time intervals was counted and recorded.

ABBREVIATIONS. FAO: Food and Agriculture Organisation of the United Nations. KTB: Kenyan Top Bar hive MAAIF: Ministry of Agriculture Animal Industry and Fisheries

RESULTS

Detection of dead brood in the combs and expression of uncapping behaviour after four hours from perforation

Worker bees from both strong and weak East African lowland honey bee colonies detected and uncapped the injured/dead brood in the different

comb types within the first four hours of observation. The number of cells detected, uncapped, and cleaned was higher in strong colonies than in weak colonies. The average proportion of cells uncapped in strong colonies with new combs was 9.4%, and for strong colonies in old combs was 6.7% (Table 1). Strong colonies registered an average proportion of 8.05% uncapped cells (for both fully and partially cleaned). Weak colonies were uncapped and cleaned within the first four hours, with an average of 4.4% of the cells that had been perforated. Regardless of colony strength, the number of cells uncapped and cleaned in new combs was 6.9% while in old combs it was 5.25%.

The process of damaged brood removal at various times across the day

During the first four hours, the number of cells cleaned by strong colonies with new combs was 9.4% while for strong colonies with old combs, it was 6.7%. These values are higher than those recorded for weak colonies (4.4% and 3.9% respectively). During the subsequent four hours from 1100 hr to 1500 hr, the cleaning effort of the colonies improved by 21.7% in both comb types for strong colonies. This was much higher than the rate in weak colonies only registering an increase of 13.9% in new combs and 11.7% in old combs for the same duration (Table 1).

From 1500 hr to 1900 hr, weak colonies registered a higher percentage of cells cleaned than strong colonies with a difference of 1.1% between weak old and strong old combs and 0.6% between weak new and strong new combs. Similarly, weaker colonies cleaned a greater number of cells than strong colonies overnight, from 1900 hr to 0700 hr. Overall, the number of cells cleaned was 73.9% in both new and old combs with weak colonies; while elsewhere it was 65% and 66.7% brood removal from new and old combs with strong colonies respectively (Table 1).

The effect of change in day conditions on the cell cleaning rate

The accumulated cells cleaned up to 1500 hr (8 hours from perforation) were 31.1% in new combs and 28.4% in old combs of strong colonies. On the other hand, the weak colonies registered a lower accumulated value at the same time range of 18.3% in new combs and 15.6% in old combs. The additional percentage of cells cleaned between 1500 hr and 1900 hr was much lower than the additional percentage from 1100 hr to 1500 hr in combs of different colony strength. The lowest additional value between 1100–1500 hr was 11.7% but the maximum additional from 1500 hr to 1900 hr was 4.4% (Figs. 5, 6).

The effect of night versus day on the cell cleaning rate

The number of cells cleaned during the night (12 hours) was higher than the number of cells cleaned during the day (12 hours) (Figs. 7–12). During the day, the cleaning rate was 33.0% in new and 31.7% in old combs of strong colonies and was a low 21.1% in new and 20% in old combs of weak colonies. At night, the cell cleaning rate was higher in weak colonies than in strong colonies. Both new and old combs of weak colonies had the same num-

Time Range	Colony strength and comb age (%)			
	Strong new	Strong old	Weak new	Weak old
0700–1100 hr	9.4	6.7	4.4	3.9
1100–1500 hr	21.7	21.7	13.9	11.7
1500–1900 hr	2.2	3.3	2.8	4.4
1900–0700 hr	65.0	66.7	73.9	73.9

Table 1. Mean percentage of cells cleaned in new and old combs from different colony sizes.

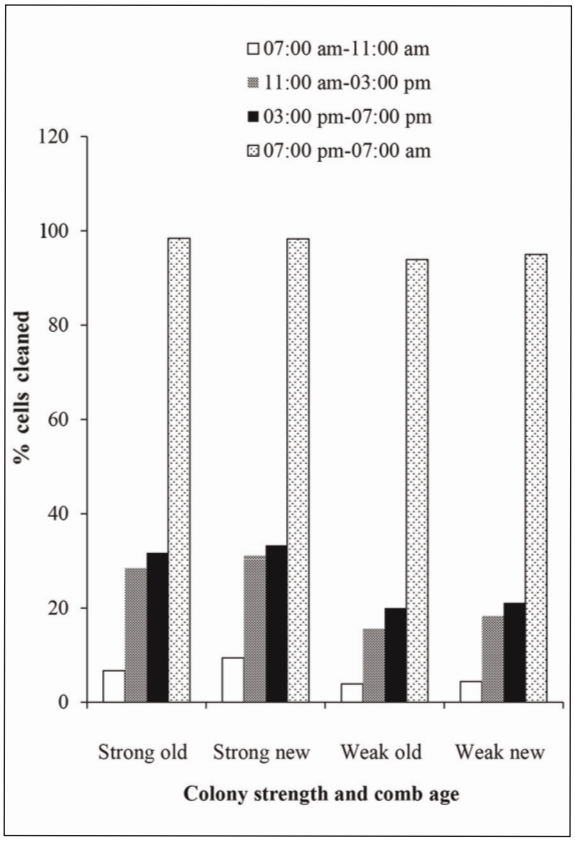


Figure 5. The effect of change in day conditions on cell cleaning, considering time factor.

ber of cells cleaned during the night of 73.9%. The strong colonies removed only 65% of new combs and 66.7% of old combs. The number of old-comb cells cleaned in strong colonies (66.7%) was higher than the number of new-comb cells cleaned during the night (65%). The number of new cells cleaned during the day was higher (33.3%) than the number cleaned in old cells (31.7%). Weak colonies had the same number of cells cleaned in old and new combs at night (73.9%) with a higher number cleaned in new cells (21.1%) than old cells (20%) during the day.

The rate of cell cleaning in strong Versus weak colonies

The interaction between the number of cells cleaned in strong and weak colonies was not significant (P=0.9707). The P values at different observation intervals were P=0.5097 at 8 hours and P=0.7612 at 12 hours. The mean proportion of cells cleaned was 8.1% in strong and 4.2% in weak colonies after four hours of cell perforation. The accumulated percentage after 8 hours was 29.8% for strong and 17.0% for weak colonies. There was a slight rise from 8 hours to 12 hours in both types of colonies (2.2% in strong and 3.6% in weak

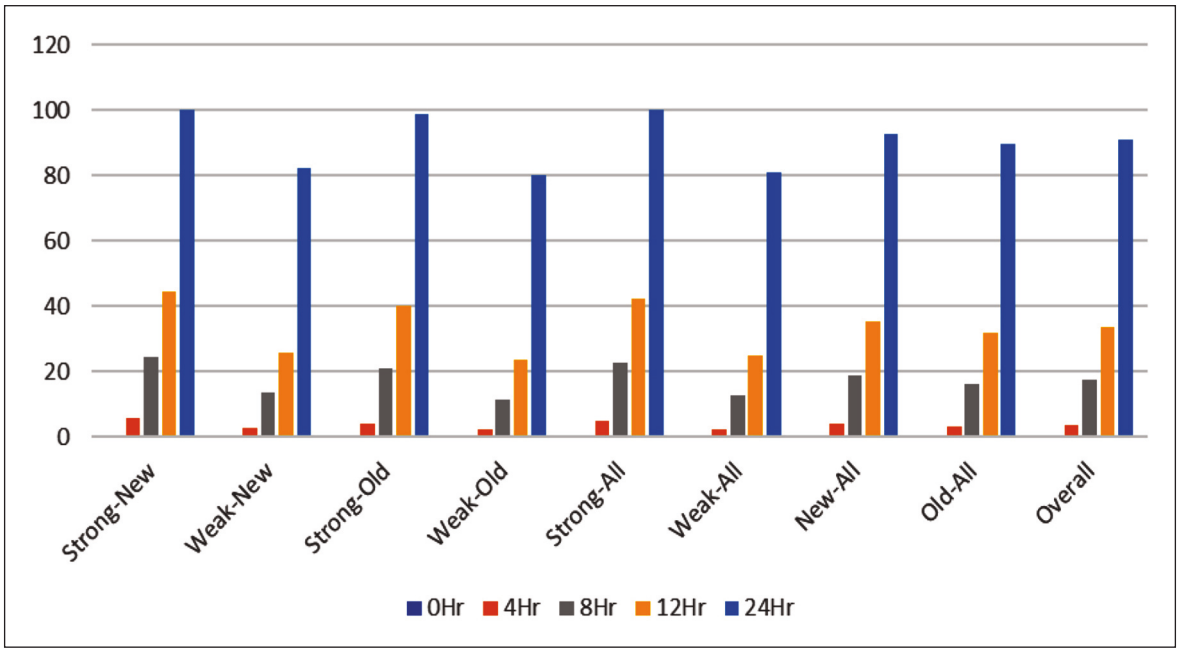


Figure 6. The effect of change in day conditions on cell cleaning, considering colony strength.

colonies) making a slight increase in accumulated values to 32.5% in strong and 20.6% in weak colonies. Strong colonies cleaned more cells in the first 12-hour phase from perforation (32.5%) than weak colonies which cleaned 20.6% in the same duration. Weak colonies cleaned a higher number of cells during the last 12-hour phase (73.9%) than strong colonies did (65.9%) in the same duration. The cell cleaning rate showed the same trend in both colonies during the day and night but with varying values (Figs. 7–13).

The comparative rate of cell cleaning in old and new combs

The interaction between hygienic behaviour (number of cells cleaned) between old and new combs was not significant after 24 hours (P=0.9893) (Table 2). The similarity between the number of cells cleaned was very strong at all checks but this was low in the period soon after cell perforation, rising subsequently (P=0.8798 at 8 hours, P=0.9467 at 12 hours and P=0.9893 after 24 hours). However, there was a difference of only 1.6% between the old and new comb cells cleaned after four hours from perforation. The worker bees cleaned 6.9% of new combs and 5.3% of old combs. The accumulated percentages after eight hours were 22.0% in old combs and 24.7% in new combs with a difference of 2.7% from the fourth to the eighth hour. There was an accumulated difference of 5.6% in 12 hours during the day between the number of cells cleaned in old and new combs. The new combs had a relatively high number of cleaned cells with a value of 27.2% during the day. The worker bees cleaned 25.9% of old combs in the same time range. There was a slight difference of less than 4% for all the accumulated values of cells cleaned.

The cell cleaning process for cell perforation overnight

All colonies removed over 95% of the dead brood in only 12 hours from perforation. There was a uniform mean percentage for the number removed in both comb types of the strong colonies with a value of 97.2%. The weak colonies also registered a uniform number of 96.6% in both comb types.

DISCUSSION

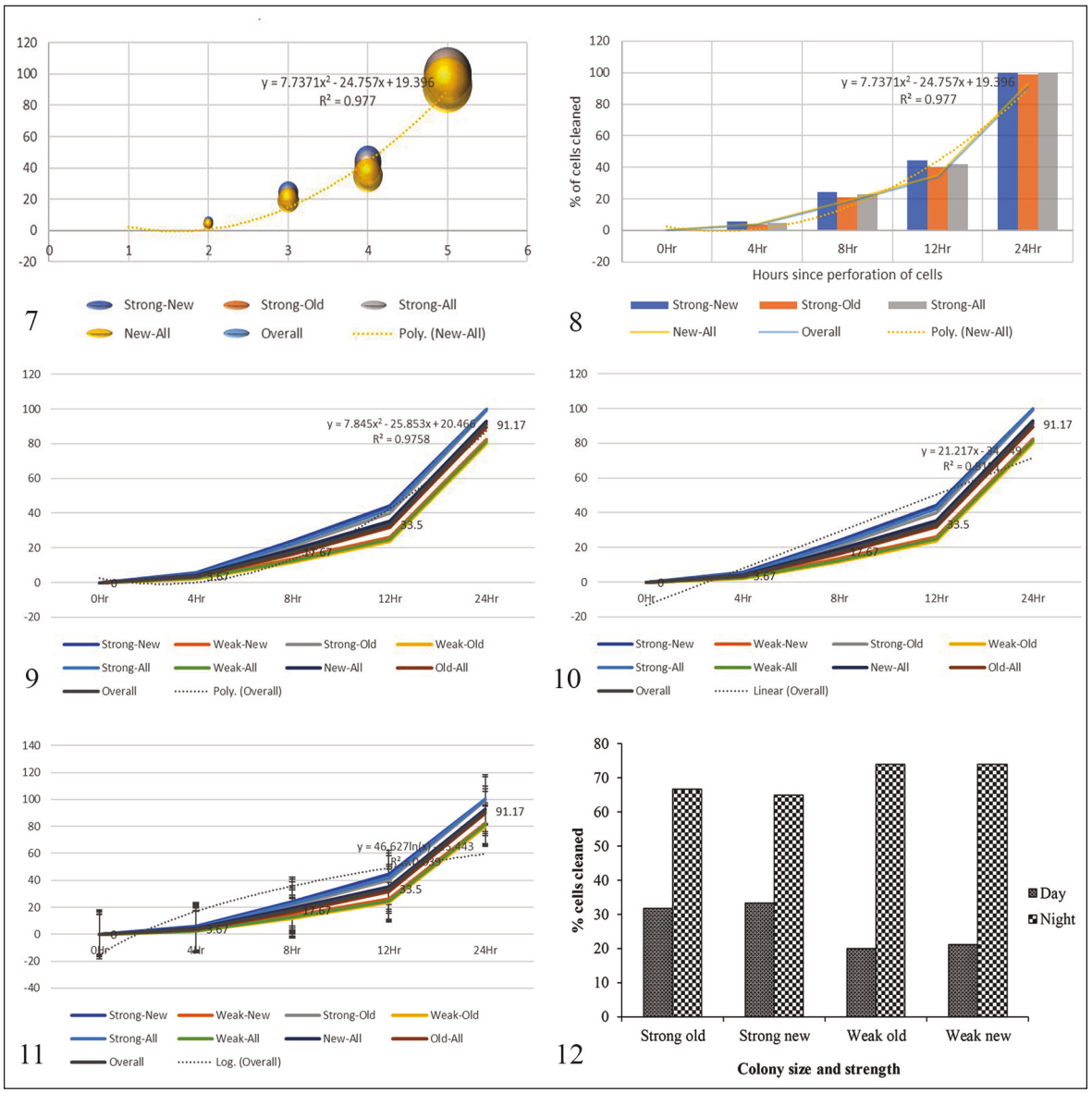
The East African lowland honey bees demonstrated the ability to detect and uncap injured broods in both new and old combs. The fluids released from injured brood cells after perforation likely served as a stimulus for detection. The liberation of hemolymph and other volatile substances that come out after the insect pin damage initiates uncapping and dead brood removal (Spivak & Downey, 1998). Studies elsewhere have documented that bees that are hygienic respond to odour signals from dead bees as the odours stimulate them to detect, uncap and remove dead brood, that is just diseased or parasitized (Rosenkranz et al., 1993; Palacio et al., 1996; Masterman et al., 1998; Palacio et al., 2010). The numbers of uncapped cells in strong colonies were higher than the cells uncapped in weak colonies after four hours of observation (Figs. 1–4), though this not attributed to the hemolymph exuding from combs of strong colonies vis-à-vis the weak colonies. This is because the same insect pin was used to perforate a similar number of cells. Indeed, previous research

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.36125	1	0.36125	0.0003858	0.98496588	5.987377607
Within Groups	5618.0975	6	936.349583			
Total	5618.4588	7				

Table 2. ANOVA table for the effect of age of the comb on brood cell cleaning response (hygienic behaviour) in 24 hours. Ho: $\alpha 1=\alpha 2$, Ha: $\alpha 1 \neq \alpha 2$. Where α is the mean of the respective column.

suggests that the release of body fluids is not the main factor influencing brood removal (Gramacho et al., 1999). The rate of uncapping in strong colonies must have been higher than in weak colonies mainly because of the differences in alertness and population of worker bees in the hive that do the cleaning of the hive. There was a difference in the number of cells uncapped from new and old combs with a higher number registered in new combs. The softness of new combs with fresh and fragile beeswax provides easiness in uncapping thus enhancing the uncapping process.

The rate of dead brood removal during the daytime did not take a uniform trend (Figs. 1–4), increasing slowly in the first four hours, later tripling in all the colonies in the next four hours, though the rate slowed thereafter in the next four hours of the evening (Table 1). The requirement of the participants to detect and start the cleaning process rendered the initial process a slow-paced one. The



Figures 7–12. The rate of cell cleaning during the day Vs the rate during the night. Polynomial plot (Fig. 7), Exponential plot (Fig. 8), Linear plot (Fig. 9), Logarithmic plot (Fig. 10), fitted with Standard errors (Fig. 11), and showing proportion of cleaned cells (Fig. 12).

rapid foraging activity that is a normal routine in honey bees during the morning hours must have been the main reason for this sluggish cleaning process.

As previously reported, for most African tropical honey bees, pollen foraging is at its peak at low temperatures, especially in the early morning hours (Kajobe, 2008). This is because at low temperatures, there is high moisture that makes pollen sticky and firm for easy transportation by foragers. As the temperature increases, pollen on flowers becomes lighter as it dries thus reliable to loss by wind. Also, with hotter day temperatures, bees tend to forage less concentrated nectars, therefore they would forage the concentrated nectars in the cooler times of the day (Kajobe, 2007). The rate of dead brood removal was thus low in the morning hours because this was the time when pollen collection was at its peak. During this time, many foragers exit the hive for foraging activities leaving a reduced labour force for hive cleaning duties.

As the temperatures increase in the external environment during the afternoon hours, most foragers switch back to hive duties. This leads to a significant increase in the number of worker bees inside the colony, thereby shifting focus to hive duties. This is supported by Kajobe & Echazarreta (2005) who found out that, for tropical honey bees, nectar foraging increases with temperatures up to an optimum beyond which the bees' foraging changes to resin collection, mud, and removal of debris from the hive. The return of worker bees in the colony increased the internal labour force. This could have been the reason why the rate of dead brood removal increased rapidly from 1100 hr to 1500 hr (Figs. 1–4). Between 1500 hr to 1900 hr, the accumulated maximum additional cleaning percentage was 4.4%, and other colonies cleaned was even lower (Table 1). It can be suspected that the rate must have started to decrease earlier in the hot hours of the day (from 1400 hr) but there is no data to confirm this since the checks were done at four hours intervals.

The return of most worker bees to the colony during the hot conditions results in overcrowding. This causes a rise in the internal temperature due to the high amount of heat generated by the high bee population. This makes it a necessity for the worker bees to regulate the internal hive temperature. Worker bees tend to widely space themselves inside the hive during the hot and warm conditions as a strategy to regulate the internally generated hive heat. This condition may also prevent work bees from concentrating on the perforated area to perform the uncapping duties. The heat regulation activity in the colony during the warm conditions of the day (social breathing) attracts the worker bee's attention and other activities in the hive may be given little attention. This explains the very low percentage rise in cleaning observed during that time. As the external temperatures go calm during the early evening, the rate of fanning is cut down because the hive temperatures also reduce. However, the rate of cell cleaning remained low during cool conditions towards seven o'clock in the evening (Figs. 1–4). During this time, fanning is expected to cease being a priority within the hive due to the low ambient temperatures present therein. However, the population of worker bees inside the hive tremendously reduced. The workers go foraging and the cell cleaning participants remain few. This is in line with Gilbert (1973) who concluded that there is always a smaller peak in flight activity during the sunset for pollen collection. The slow rate between 1500 hr to 1900 hr across the combs of both colony types was thus influenced by the continuous fanning activity in the hot hours after midday and the immediate commencement of foraging activity in the cool hours of the late evening.

The first four hours of observation show that the mean value of cells cleaned was higher in strong colonies (8.1%) compared to 4.2% observed in weak colonies. The activities of the colonies like foraging, hive guarding, and debris cleaning from the hive are always effective when the worker bee population is sufficient enough to accommodate most of them simultaneously. When the population is not sufficient to run the activities at ago, some tasks may be slowed down or delayed in favour of the most demanding duties happening at that time. The quick detection and uncapping rate in strong colonies than weak colonies must therefore have been due to the high population of the worker bees that provided the required cleaning labour force. The variations in the accumulated number of cells cleaned from 0700 hr to 1500 hr between the two types of colonies (29.8% in strong and 17.0% in weak) can be explained by the similar phenomenon. However, there were a higher additional percentage of cells cleaned in weak colonies (3.6%) than strong colonies (2.2%) between 1500 hr–1900 hr.

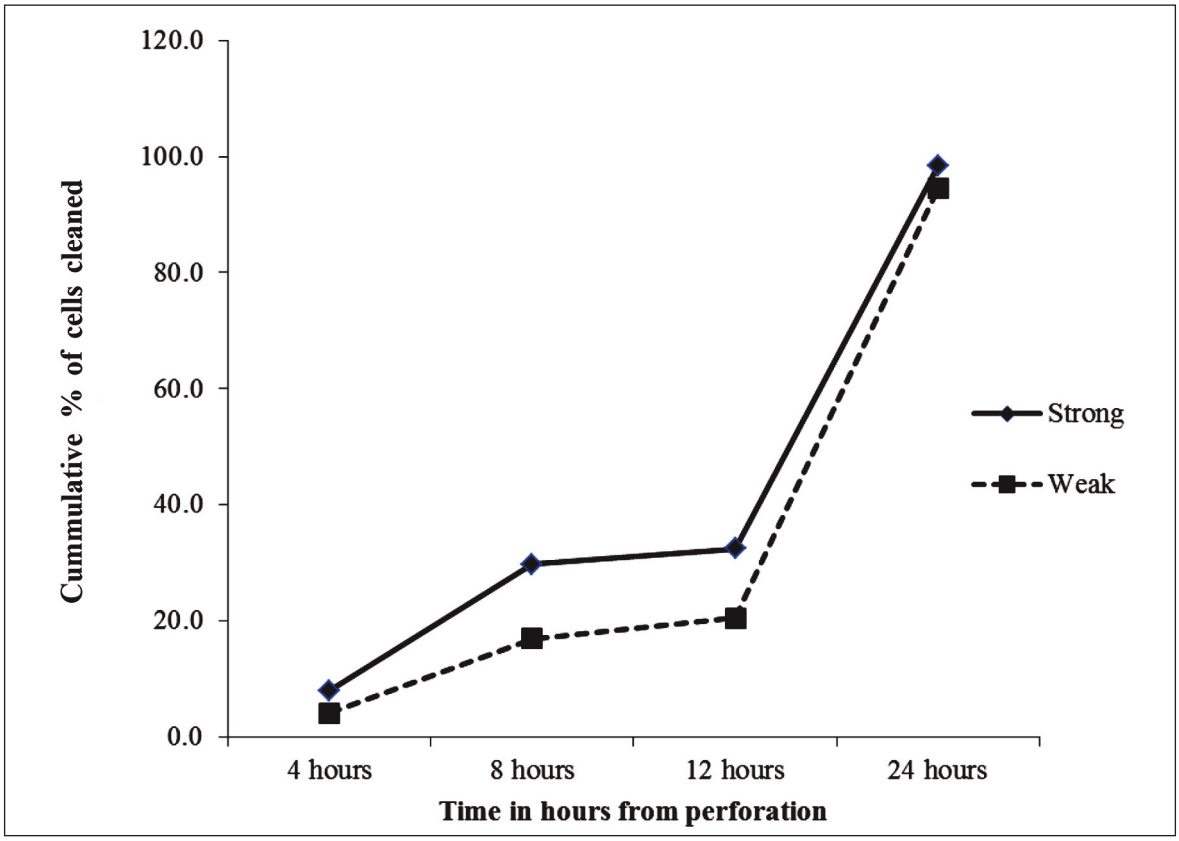


Figure 13. The rate of cell cleaning in strong colonies Vs weak colonies.

The high bee population in strong colonies results in overcrowding after the return of worker bees during the hot hours of the day. This may lead to higher internal hive temperatures in strong colonies than in weak colonies. The need for temperature regulation may be thus more demanding in strong colonies than weak ones. The number of cells cleaned therefore may have been higher in weak colonies than strong colonies within that time due to the differences in hive temperatures and fanning activity. This however, did not affect the accumulated percentage trend. Strong colonies maintained a higher accumulated percentage in the respective time (32.5 %) than weak colonies (20.6 %).

Figure 6 shows a sharp rise in the rate of cell cleaning from 1900 hr to 0700 hr in both colony types. The numbers of cells cleaned in weak colonies were the same in both comb types (73.9%). The numbers of cells cleaned in new combs of strong colonies were less than those cleaned in old combs of the same colony type. This does not necessarily reflect that the rate of cell cleaning during the night period was higher for weak colonies than strong colonies. Due to the fact that a larger number of brood was removed during the day in strong colonies, meant that logically there was a smaller number of cells left to be cleaned during the night for strong colonies to complete the cleaning process (cell cleaning was finished in less than 24 hours for all colonies). Analysis of the interaction between hygienic behaviour and the period of the day found that there are differences between periods of the day within the hygienic lines (Pereira et al., 2013).

The individual workers from hygienic strains spent less time uncapping the cells during the day than they did at night but the total time spent was considerably higher at night. Strong colonies have a higher population mass of worker bees compared to weak colonies. Almost all activities notably foraging, temperature regulation, debris removal and hive repair are always performed better in strong colonies than weak colonies. This is partly because of the large number of participants involved in performing those duties in strong colonies. Though

strong colonies offer a greater number of foragers to the field in the day than weak colonies, the number that stays in the hive to perform inside hive duties remains higher than that in weak colonies. The consistently higher number of active workers in strong colonies explained why the rate of removal remained much faster than in weak colonies. The fact that the process of cleaning was completed in no more than 24 hours in weak colonies just like in strong colonies explains the hygienic independence of the East African lowland honey bees from colony strength.

The observable differences in the rate of dead brood removal in both types of combs were very close to each other. It was, however, noted that there has not been a time when the rate of removal was higher in old combs than in new combs. The fact that the rate in new combs was always higher or at worst uniform but never less than that of old combs makes it a matter of concern. However, it should be noted that slight differences were noted in the daytime. After 24 hours, the rate was similar in all types of combs and all colonies.

Physically, old combs are thicker compared with new combs. In the process of harvesting honey from KTB hives, it is always slightly harder to cut a comb of sealed honey from the top bar if the comb is old than when new. When using the drip method of honey processing, in removing cell cappings from new combs is always easier in new combs than old combs. This means that new combs consist of a higher percentage of soft beeswax than old combs. When the same quantity of empty honeycombs is processed to produce beeswax, pure white combs and cappings produce a larger portion of beeswax than old cappings and old combs. The colour of the product also differs greatly being pure yellow for new cappings and new combs and partly grey/dull yellow for old combs. This explains that old combs are associated with other compounds more than new combs which renders it difficult for the bees to remove the cappings. This may therefore delay the rate of dead brood removal due to uncapping challenges.

In an experiment to study hygienic behaviour in response to non-hygienic and hygienic colonies, bees from hygienic colonies spent more time uncapping cells from old combs than cells from new combs (Morais, 2009). They explained that this influence was probably due to the existence of substances deposited on the wax of old combs such as propolis and other water-soluble substances. These substances contribute to the increased thickness and cell wall resistance of the old combs. The composition of the new combs varies significantly on the other hand from new combs (Kugonza, 2009). By composition, new combs are purely beeswax with thin and more fragile cell walls which facilitate efficient uncapping besides the time of the day, condition and the population of the performers. Colonies with a great proportion of white (new) combs thus may successfully have high and fostered cleaning rates in relation to similar colonies but with old combs.

The number of dead brood removed from both strong and weak colonies was higher at night (no foraging activity) than in the daytime (Figs. 1–6) when there is foraging. It is plausible that night conditions are not necessarily favourable for removal more than day conditions, but rather, the foraging activity intensity may explain this behaviour. During the day, workers are busy foraging, leaving a small workforce cleaning the hive, and performing dehydration and feeding duties. During the night, all the foragers are back to the hive and they concentrate on hive cleaning hence the rapid and efficient rate of removal. Individual bees are known to spend more time uncapping cells in hygienic colonies during day time than at night (Pereira et al., 2013). However, the total time is much greater during the night than during the day because of the many bees involved.

Our study was conducted in January which is the peak of the dry season when most trees in Budongo forest and the nearby places are in deep bloom. With reference to the beekeeping calendar of Budongo Sub County, the study was conducted in a honey flow period that runs from December-February and repeats in July for a short time (only one month). This is a period when there is sufficient forage (pollen and nectar) and the foragers show a lot of response to this stimulus. According to Kajobe (2008), most African honey bees normally go foraging on a variety of forage plants including *Acacia* spp, *Eucalyptus* spp, *Prunus africana* (Hook. f.) Kalkman, *Senna spectablis* (DC.) H.S.Irwin & Barneby, *Tectona grandis* L.f. and others for they mostly bloom heavily in the period of February to May in Budongo. The very low rate of dead brood removal in the day time was thus not a

confirmatory indicator that the colonies were non-hygienic. Since the colonies were able to express more than 90% of removal in 24 hours, this proves their hygienic status right.

This study was conducted in nectar and pollen abundances (honey flow period), hence these conditions must have delayed the cleaning rate in the daytime. Forage availability and the desire by the foragers to bring it home explain the low cleaning rate in the foraging period. This is in accordance with Gramacho et al. (1998) and Gramacho (1999) who said that the expression of hygienic behaviour is highly influenced by environmental factors.

CONCLUSIONS

The impact of colony strength on East African lowland honey bees is higher than the influence of the age of combs on hygienic response during the day, but the superiority diminishes approaching 24 hrs. There is a more efficient rate of hygienic response in both strong colonies and new combs compared to weak colonies and old combs during the early hours after perforation but the rates also converge before 24 hours. East African lowland honey bees show more intense engagement in hygienic activities in the hive during times of limited foraging. Generally, the cell cleaning rate was influenced mostly by the condition of the day rather than colony strength and comb age.

During the process of hive inspection and preparing colonies for honey flow and space creation in the hive, emphasis should not be put on old comb removal but instead, half broken, damaged or very small combs should be of concern. Opening of colonized hives for inspection or any other hive service must be particularly done close to sunset/night when the hive cleaning process is at maximum peak. Though colony strength has no significant impact on the hygienic response of East African lowland honey bees, weak colonies should be strengthened through colony unification. This helps minimize maintenance costs, labour, and the need for frequent apiary inputs.

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REFERENCES

Arathi H.S., Burns I. & Spivak M., 2000. Ethology of hygenic behaviour in honey bee - *Apis mellifera* L. (Hymenoptera: Apidae): Behavioural report on hygenic bees. Ethology, 106: 365–379.

Bienefeld K., Haberl M. & Radtke J., 1998. Does the genotype of honey brood influence the attractiveness for *Varroa Jacobsoni* and the reproduction of the parasite? Hereditas, 129: 125–129.

Biesmeijer J.C., Born M., Lukacs S. & Someijer M.J., 1999. The response of the stingless bee *Melipona beecheii* to experimental pollen stress, worker loss and different levels of information input. Journal of Apiculture Resources, 38: 33–41.

Boecking O., 1999. Sealing up and non removal of diseased and *Varroa jacobsoni* infested brood cells in part of the hygenic behaviour in *Apis cerana.* Journal of Apiculture Resources, 38: 159–168.

Boecking O., Bienefeld K. & Drescher W., 2000. Heritability of the Varroa specific hygienic behaviour in honey bees (Hymnoptera: Apidae). Journal of Animal Breeding and Genetics, 117: 417–424.

Boecking O. & Genersh E., 2008. Varrosis - the ongoing crisis in bees. Journal for Verbrauchutz Lebensmittelsicherheit, 3: 221–228.

Bradbear N., 2009. Bees and their role in forest livelihoods. Non-wood Forest Products Journal, 19: 69–79.

Breed M.D., Garry M.F., Pearce A.N., Hibbard B.E., Bjostad L.B. & Page R.E. Jr., 1995. The role of wax comb in honey bee nest mate recognition. Animal Behaviour, 50: 489–496.

Chemurot M., Akol A.M., Masembe C., de Smet L., Descamps T. & de Graaf D.C., 2016. Factors influencing the prevalence and infestation levels of *Varroa destructor* in honeybee colonies in two highland agro-ecological zones of Uganda. Experimental and Applied Acarology, 68: 497–508. https://doi.org/10.1007/s10493-016-0013-x

Downs S.G. & Ratnieks F.L., 2000. Adaptive shifts in honey bee (*Apis mellifera* L.) guarding behaviour

predictions of the acceptance threshold model. Behavioural Ecology, 11: 326–333.

FAO (Food and Agriculture Organisation of the United Nations), 2019. The State of the World's Biodiversity for Food and Agriculture. Bélanger J. & Pilling D. (Eds.). FAO Commission on Genetic Resources for Food and Agriculture Assessments. Rome. doi:10.4060/CA3129EN

Gramacho K. & Spivak M., 2003. Differences in olfactory sensitivity and behaviour responses among honey bees bred for hygienic behaviour. Behavioral Ecology, 54: 472–479.

Gramacho K.P. & Goncalves L.S., 2009. Comparative study of the hygenic behaviour of Carniolan and Africanised honey bees directed towards grouped versus isolated dead brood cells. Genetics and Molecular Research, 8: 744–750.

Gramacho K.P. & Goncalves L.S., 1996. A comparative Study of the hygenic behaviour in several honey bee races. In: Proceedings of 20th International Congress of Entomology, Firenze, 445 pp.

Gramacho K.P., Goncalves L.S., Rosenkranz P. & De Jong D., 1999. Influence of body fluid from pin-killed honey bee pupae on hygienic behavior. Apidologie, 30: 367–374.

Gramacho K.P., Goncalves L.S. & Rosenkranz P., 1997. Influence of the body fluid of *A. m. cornica* originated from the brood pin killed method on hygienic behaviour of worker bees, In: XXXV International Apiculture congress-Apimondia, Antuerpia-Belgica, Apimondia Publishing House.

Gramacho K.P., Goncalves L.S. & Rosenkranz P., 1998. Study of the temperature of brood killed by the pin killing method in worker bees of *Apis mellifera carnica*. Apicarta, 33: 33–41.

Gramacho K.P., 2004. Sensorial perception of hygenic honey bee. In: Harterfelder K.H., De Jong D., Pereira R.A. & Dos Santos C.A. et al. (Eds.). Proceedings of the 8th IBRA International Conference on Tropical Honey Bees on 6th Oct, 2004.

Hepburn H.R., Radloff S.E. & Oghiakhe S., 2000. Mountain honey bees of Africa. Apidologie, 31: 205-221.

Jamal Z.A., Abou-Shaara H.F., Qamer S., Alotaibi M.A., Khan K.A., Khan M.F., Bashir M.A., Hannan A., Al-Kahtani, S.N., Taha El-K.A., Anjum S.I., Attaullah M., Raza G. & Ansari M.J., 2021. Future expansion of small hive beetles, *Aethina tumida*, towards North Africa and South Europe based on temperature factors using the maximum entropy algorithm. Journal of King Saud University. Science, 33: 101242.

Kajobe R., 2006. Pollen foraging by *Apis mellifera* and stingless bees *Melliponula bucandei* and *Melliponula nebulata* in Bwindi Impenetrable National Park, Uganda. African Journal of Ecology, 45: 256–274.

Kajobe R., 2007. Botanical sources and sugar concentrations of the nectar collected by two stingless bee species in a tropical African rain forest. Apidologie, 38: 110–121.

Kajobe R., 2008. Foraging behaviour of Equatorial Afrotropical stringless bees: Habitat selection and competition for resources. PhD Thesis, University of Utrecht, The Netherlands.

Kajobe R., 2008. Foraging behaviour of equatorial Afrotropical stingless bees: habitat selection and competition for resources, Sephine Enterprises (U).

Kajobe R. & Echazaretta M.C., 2005. Temporal resources partitioning and climatology influences on colony flight and foraging of stingless bees in Uganda tropical forests. African Journal of Ecology, 43: 267–275.

Kugonza D.R., 2009. Beekeeping: Theory and Practice, Fountain Publishers, Kampala. 289 pp.

Kugonza D.R., 2020. Africa under attack: a continent-wide mapping of pathogens, parasites and predators afflicting the hived honey bee *Apis mellifera* L. (Hymenoptera: Apidae). African Journal of Rural Development, 5: 1–27. http://dx.doi.org/10.6084/m9.figshare.21587514

MAAIF (Ministry of Agriculture, Animal Industry and Fisheries), 2005. The National Monitoring Plan for Residues in Honey from Uganda, The Republic of Uganda.

Masterman R., Ross R., Mesce K. & Spivak M., 2001. Olfactory and behavioural response thresholds to odours of diseased brood differ between hygenic and non hygenic honey bees (*Apis mellifera* L.). Journal on Comparative Physiology, 187: 441–452.

Masterman R., Smith B.H. & Spivak M., 2000. Brood order discrimination abilities in hygienic honey bees (*Apis mellifera* L.) using proboscis extension reflex conditioning. Journal of Insect Behaviour, 13: 87–101.

Masterman R., Mesce K.A., Smith B.H. & Spivak M., 1998. Odor discrimination by hygienic honeybees using proboscis-extension conditioning in *Apis mellifera*. American Bee Journal, 138: 297–298.

Matheson A., 1996. World bee health update. Bee World, 77: 45–51.

Milani N., 1999. The resistance of *Varroa jacobsoni* to acaricides. Apidologie, 30: 229–234.

Milne C.P. Jr., 1983. Honey bee (Hymenoptera: Apidae) hygienic behaviour and resistance to chalkbrood. Annals of the Entomological Society of America, 76: 384-387. https://doi.org/10.1093/aesa/76.3.384

Morais M.F., 2009. Africanised honey bees are efficient at detecting, uncapping and removing dead brood. Genetics and Molecular Resources, 8: 718–724.

Mwiza W.K., Kanyandekwe C., Nshimiyimana A.M., Mukamurenzi E. & Kugonza D.R., 2013. Evaluation of three varroacides in honeybee *Apis mellifera* (Hy-

menoptera: Apidae) colonies in Rwanda. In: Gaidashova S.V., Kagabo D.M. & Ebong C. (Eds.), Book of Abstracts of First RAB Biennial Conference on Agricultural Research and Extension, 21–23 August 2013, Kigali, Rwanda, p. 52 (Abstr.).

Nekoei S., Rezvan M., Khamesipour F., Mayack C., Molento M.B. & Revainera P.D., 2023. A systematic review of honey bee (*Apis mellifera*, Linnaeus, 1758) infections and available treatment options. Veterinary Medicine and Science, 9: 1848–1860. https://doi.org/10.1002/vms3.1194.

Newton D.C. & Ostasiewiski J., 1986. A simplified bioassay for behavioral resistance to American Foulbrood in honeybee strains (*Apis mellifera* L). Bee Journal, 126: 278–281.

Palacio M.A., Figini E., Rodriguez E.M., Rufinengon S., Del Hoyo M.L. & Bedascarrasbure E., 1996. Selección para comportamiento higiénico en una población de *Apis mellifera* (Evaluation of different methods used to estimate hygienic behavior in honeybee – Abstract). In: Anales del V Congreso Ibero latino americano de Apicultur, Mercedes, Uruguay, 5th Ibero-Latin American Congress of Beekeeping, May 30–June 2, 1996, Mercedes, Uruguay, pp. 148–150.

Palacio M.A., Rodriguez E., Goncalves L., Bedascarrasbure E. & Spivak M., 2010. Hygienic behaviours of honey bees in response to brood experimentally pinkilled or infected with *Ascosphaera apis*. Apidologie, 41: 602–612. https://doi.org/10.1051/apido/2010022

Prata J.C. & da Costa P.M., 2024. Honeybees and the One Health Approach. Environments, 11: 161. https://doi.org/10.3390/environments11080161

Radloff S. & Hepburn R., 2000. Population structure and morphometric variance of the *Apis mellifera scutellata* group of honey bees in Africa. Genetics and Molecular Biology, 23: 305–316.

Rodrigues I., Beetsma J., Boot W.J. & Calis J., 1996. Testing hygienic behaviour in four different honey bee strains (*Apis mellifera* L.). Application Entomology, 7: 83–88.

Rosenkranz P., Tewarson N.C., Singh A. & Engels W., 1993. Differential hygienic behaviour towards *Varroa jacobsoni* in capped worker brood of *Apis cerana* depends on alien scent adhering to the mites. Journal of Apicultural Research, 32: 89–93. https://doi.org/10.1080/00218839.1993.11101292

Rothernbuchler W.C., 1964a. Behavioural genetics of nest cleaning in honey bees. IV. Responses of FI and backcross generations to disease-killed brood *Apis mellifera*. Zoology, 4: 11–23.

Rothernbuchler W.C., 1964b. Behavioural genetics of nest cleaning in honey bees. I. Responses of four inbred lines to disease-killed brood. Animal Behaviour, 12: 578–583.

Spivak M., 1996. Honey bee hygienic behaviour and defence against *Varroa jacobsoni*. Apidologie, 27: 245–260.

Spivak M. & Gillian M., 1998a. Hygenic behaviour on honey bees and its application for control of brood diseases and varroa mites. Part I: Hygenic behaviour and resistance to American foul brood. Bee World, 79: 165–182.

Spivak M. & Gilliam M., 1998b. Hygienic behaviour on honey bees and its application for control of brood diseases and varroa mites. Part II: Studies on hygienic behaviour since the Rothenbuhler era. Bee World, 79: 165–178.

Spivak M. & Danka R.G., 2020. Perspectives on hygienic behaviour in *Apis mellifera* and other social insects. Apidologie, 1–16.

Spivak M. & Downey D.L., 1998. Field assays for hygenic behaviour in honey bees (Hymenoptera: Apidae). Journal of Economic Entomology, 91: 64–70.

Tabler S., 1982. Bee behaviour - Determining resistance to brood diseases, *Apis mellifera*. Bee Journal, 122: 422–423.

Thapa R., 2006. Honeybees and other Insect Pollinators of Cultivated Plants: A Review. Journal of the Institute of Agriculture and Animal Science, 27: 1–23. https://doi.org/10.3126/jiaas.v27i0.691

TUNADO (The Uganda National Apiculture Development Organisation), 2006. Promoting bee keeping for sustainable development Newsletter, October, 2006.

Wallner K., 1999. Varroacides and their residues in bee products. Apidologie, 30: 235–248.

Wilson-Rich N., Spivak M., Fefferman N.H. & Stalks P.T., 2009. Genetics of individual and group facilitation of disease resistance in insect societies. Annual Reviews of Entomology, 54: 405–423.