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COLONY STRENGTH SHAPES THE REMOVAL OF GREATER WAX MOTH (*GALLERIA MELLONELLA*) EGGS AND LARVAE BY HONEY BEE WORKERS AND THE SPREAD OF COMB DAMAGE IN *APIS MELLIFERA* COLONIES

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ABSTRACT

The greater wax moth, *Galleria mellonella*, is a common pest of honey bee combs, and beekeepers usually meet its worst damage in weak colonies. Yet the path from colony strength to worker removal of the pest, and then to comb damage, has rarely been measured in one trial. We infested 24 *Apis mellifera* colonies (eight each with 10, 6 or 3 frames of bees) with wax moth eggs placed on open comb or in crevices, and with young (1st-2nd instar) and old (4th-5th instar) larvae. Eggs and larvae were counted from 2 to 72 h, and comb damage was followed for 21 days. Strong colonies removed 92.5% of young larvae within 24 h, against 74.8% in medium and 41.2% in weak colonies. Old larvae were removed less often at every strength level (strength $F_{2,21}$ = 344.6; larval age $F_{1,21}$ = 87.7; both P < 0.001). Removal followed first-order kinetics, and the rate constant fell from 0.240 to 0.089 h^{-1} as colonies weakened. Eggs hidden in crevices were poorly removed (36.5% versus 94.3% on open comb), and 52.8% of them hatched. By day 21, weak colonies carried 142.6 cm^2 of damaged comb against 4.0 cm^2 in strong ones, and their damage doubled every 5.5 days. Damage rose as a power function (exponent 1.56) of the share of young larvae left unremoved. Dark, brood-used combs gave 2.7 times higher larval survival than new comb. Keeping colonies strong, removing surplus unguarded combs and closing crevices should limit wax moth losses.

Keywords: *Apis mellifera; Galleria mellonella; colony strength; hygienic removal; comb damage; social immunity; beekeeping management.*

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INTRODUCTION

Honey bees pollinate many of the crops people eat, and this service is worth far more than their honey and wax [1]. Colony losses are reported from many regions and seldom have one cause; parasites, pesticides, poor forage and weather extremes act together and weaken the immune defences of the bees [2-4]. Pests of the comb itself get less attention, although beekeepers in Sub-Saharan Africa rank wax moths among the main causes of colony loss and absconding [5].

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), is the most damaging of these pests. Its larvae bore through the comb to the midrib, feeding on wax, pollen and brood remains. Their silk lets honey leak and traps emerging bees, which then starve, a condition known as galleriasis [6]. Hot regions suit the moth well [6], and in northern India its numbers peaked in months with mean temperatures of 32-34 °C [7]. In central and southern Iraq, infestation ranged from 10.7% to 73.3% between provinces, and the province with the most strong hives had the least infestation [8].

Workers defend their combs by finding and removing unhealthy or foreign material, a part of colony social immunity [9]. Larger groups of workers do this better, at least at small scales [10], and field surveys find fewer wax moths in strong colonies than in weak ones [11,12]. This evidence is mostly correlational, though. We know little about how fast colonies of different strength remove eggs and larvae of different ages, how hiding places change this, and how much later damage is decided in the first hours.

We therefore ran a field trial with 24 colonies in three strength classes. We measured the removal of exposed and hidden eggs and of young and old larvae over 72 h, modelled removal kinetics, followed comb damage,

tunnels, galleriasis and absconding for 21 days, and compared larval survival on dark and light combs.

2. MATERIALS AND METHODS

2.1. Apiary and colonies

The experiment was run in an apiary at Babil Governorate, Iraq (32.62° N, 44.42° E) from September 2025 to March 2026. We used 24 colonies of *Apis mellifera* meda Skorikov kept in standard Langstroth hives, each headed by a queen of similar age (spring-mated, < 1 year). Colonies were assigned to three strength classes of eight colonies each according to the number of frames covered with adult bees [13]: strong (10 frames), medium (6 frames) and weak (3 frames). Colony strength was checked one day before infestation and then weekly. No acaricide or wax moth treatment was given during the trial.

2.2. Wax moth material

Eggs and larvae came from a laboratory culture of *G. mellonella* reared on a modified Haydak artificial diet (beeswax 36%, glycerol 23%, honey 15%, dry yeast 10%, wheat flour 8%, milk powder 5% and wheat bran 3%) at 30 ± 1 °C and 65 ± 5% relative humidity in darkness. Eggs were used within 24 h of laying. Larval stages were identified from head capsule width and body size with the pictorial key of Pietrykowska-Tudruj et al. [14]. In this paper, first- and second-instar larvae are called young larvae, and fourth- and fifth-instar larvae are called old larvae.

2.3. Experimental treatments

Every colony received the same set of treatments on separate frames (Figure 1). Fifty eggs were placed on the open surface of a brood comb and fifty eggs were placed inside a crevice between the top bar and the comb. Ten young larvae and ten old larvae were released on the comb surface of separate brood frames. One frame in each colony received no wax moth stages and was kept as a control.

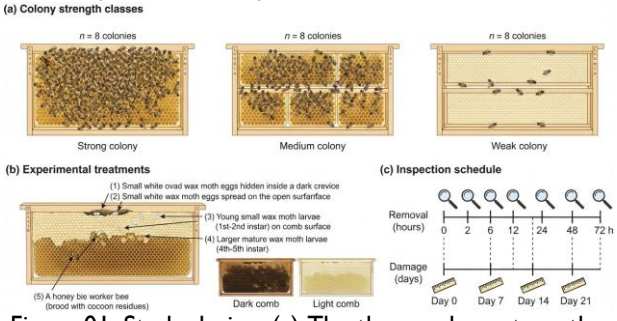

Figure 01: Study design. (a) The three colony strength classes, eight colonies each, set by the number of frames covered with bees. (b) Treatments placed in every colony: eggs hidden in a crevice (1), eggs on open comb (2), young larvae (3) and old larvae (4); a worker removing a larva is shown at (5). Dark and light combs were used for the comb-type test. (c) Inspection schedule for removal (hours) and for damage (days).

2.4. Removal of eggs and larvae

Frames were inspected briefly with minimal smoke at 2, 6, 12, 24, 48 and 72 h after introduction, and the eggs and larvae still present were counted. Removal (R, %) at time t was calculated as:

$$R_t = (N_0 - N_t) / N_0 \times 100 \quad (1)$$

where N_0 is the number introduced and N_t the number still present. Removal time (h) was recorded for each colony as the arithmetic mean of the last time the larva was observed and the first time it was found absent. Eggs that escaped removal were followed until day 7, and the percentage of introduced eggs that hatched inside the colony was noted.

2.5. Comb damage, tunnels, galleriasis and absconding

Damaged comb area (cm^2) was measured on days 7, 14 and 21 with a transparent acetate grid of 1 cm^2 squares laid over the frame. Silk tunnels were counted on each infested frame on day 21. Galleriasis was scored as the percentage of capped brood cells showing bald brood or bees trapped in silk within a fixed area of 100 capped-brood cells on each infested frame. A colony was recorded as absconded when the bees left the hive before day 21.

2.6. Dark and light combs

The effect of comb type was tested with twelve dark combs that had held brood for at least two brood-rearing seasons and twelve light, newly drawn combs. Each comb received ten young larvae in six medium-strength colonies (one dark and one light comb per colony). Larval survival was recorded after 72 h and damaged area on day 14.

2.7. Calculations

The cumulative removal of young larvae in each strength class was fitted with a first-order model:

$$R(t) = R_{max} [1 - \exp(-kt)] \quad (2)$$

where R_{max} is the plateau (%) and k the removal rate constant (h^{-1}). The time needed to reach half of the plateau is $t_{1/2} = \ln 2/k$ (Eq. 3). A Weibull form was fitted as a check:

$$R(t) = R_{max} \{1 - \exp[-(t/\lambda)^{\beta}]\} \quad (4)$$

where λ is a scale parameter (h) and β a shape parameter; $\beta < 1$ means that removal slows down with time. The time to remove half of the introduced larvae (t_{50}) was read from the observed curve by linear interpolation. A removal efficiency index (REI, %) was taken as the area under the observed curve from 0 to 72 h divided by the largest possible area (100% × 72 h). The spread of comb damage was summarized by the relative growth rate $r = \ln(D_{21}/D_7)/14$, in d^{-1} (Eq. 5), and the doubling time $T_d = \ln 2/r$ (Eq. 6), where D_7 and D_{21} are the damaged areas on days 7 and 21. The area under the damage curve (cm^2 d) was found by the trapezoid rule. Finally, the link between damage and the escape of young larvae was described by a power model:

$$D_{21} = a\, E^b \quad (7)$$

where $E = 100 - R_{24}$ is the percentage of young larvae not removed within 24 h. The constants a and b were estimated by linear regression of log D_{21} on log E. Equations 2 and 4 were fitted by nonlinear least squares.

2.8. Statistical analysis

The colony was the experimental unit (n = 8 per class). Percentages were arcsine square-root transformed, and damaged area, removal time and tunnel counts were $\log_{10}$ transformed before testing. Class means were compared by one-way ANOVA followed by Tukey's HSD test. Levene's test showed unequal variances for young-larva removal (P = 0.037), so Welch's ANOVA and the Kruskal-Wallis test were run as checks. Larval age and egg position were tested inside colonies with mixed (split-plot) ANOVAs, with strength as the between-colony factor. Damage on days 7, 14 and 21 was analysed in the same way, with the Greenhouse-Geisser correction because sphericity was violated (Mauchly's W = 0.27, P < 0.001). Absconding was compared with Fisher's exact test. Pearson and Spearman correlations were computed on all 24 colonies. We also computed pooled within-class correlations, after centring each variable on its class mean (df = 20), to see whether the relationships held inside the strength classes and not only between them. A principal component analysis (PCA) was run on eight standardized variables. All analyses were done in Python 3 with the SciPy, statsmodels and pingouin libraries, and P < 0.05 was taken as significant.

3. RESULTS AND DISCUSSION

3.1. Removal of young and old larvae

Colony strength had a large effect on how fast workers cleared introduced larvae (Table 1, Figure 2). Within 24 h, strong colonies removed 92.5 ± 3.9% of young larvae, medium colonies 74.8 ± 3.5% and weak colonies only 41.2 ± 9.6%. The same order held for old larvae (81.3, 58.7 and 23.5%). All three classes differed from each other for both larval ages (Tukey, P < 0.05), and weak colonies also needed about five times longer to remove larvae (21.8 against 4.2 h). Weak colonies were the most variable group; one of them removed 58.2% of young larvae, while four removed only about a third. Because of this spread, we repeated the young-larva test with Welch's ANOVA ($F_{2,12.2}$ = 91.6) and the Kruskal-Wallis test (H = 20.5), and both gave P < 0.001.

Table 01: Removal of wax moth larvae and eggs by workers of strong, medium and weak colonies (mean ± SD, n = 8 colonies per class).

Variable	Strong	Medium	Weak	$F_{2,21}$	P	η^2
Young larvae removed within 24 h (%)	92.5 ± 3.9^{a}	74.8 ± 3.5^{b}	41.2 ± 9.6^{c}	131.4	< 0.001	0.93
Old larvae removed within 24 h (%)	81.3 ± 4.2^{a}	58.7 ± 6.3^{b}	23.5 ± 4.3^{c}	243.4	< 0.001	0.96
Removal time (h)	4.2 ± 0.8^{c}	9.6 ± 2.1^{b}	21.8 ± 3.4^{a}	151.4	< 0.001	0.94
Exposed eggs removed at 72 h (%)	97.4 ± 1.4^{a}	94.5 ± 2.5^{ab}	90.9 ± 4.9^{b}	8.2	0.002	0.44
Hidden eggs removed at 72 h (%)	55.0 ± 5.5^{a}	35.0 ± 6.1^{b}	19.5 ± 5.0^{c}	79.0	< 0.001	0.88

Means in a row followed by different letters differ significantly (Tukey HSD, P < 0.05); a marks the highest mean. Percentages were arcsine square-root transformed and removal time $\log_{10}$ transformed before analysis. η^2, eta squared.

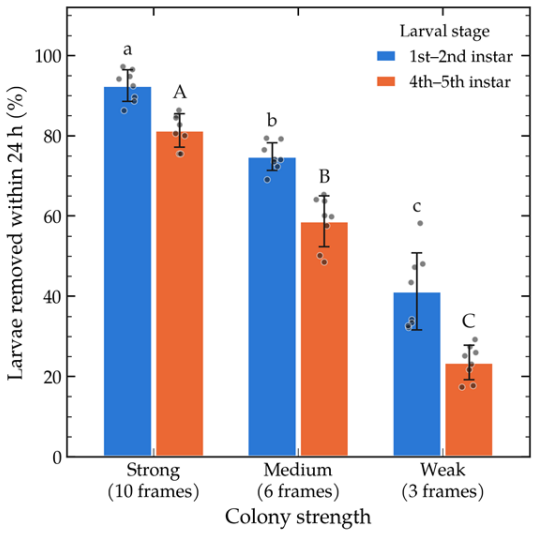

Figure 02: Removal of young (1st-2nd instar) and old (4th-5th instar) wax moth larvae within 24 h in strong, medium and weak colonies. Bars are means ± SD (n = 8); dots are single colonies. Different lowercase (young larvae) or uppercase (old larvae) letters mark significant differences between classes (Tukey HSD, P < 0.05).

The split-plot ANOVA separated the two factors cleanly (Table 2). Strength explained most of the variation ($F_{2,21}$ = 344.6, P < 0.001, partial η^2 = 0.97), larval age had a smaller but clear effect ($F_{1,21}$ = 87.7, P < 0.001), and the two did not interact ($F_{2,21}$ = 0.09, P = 0.91). On average, old larvae were removed 15.0 percentage points less often than young ones; the gap was 11.2 points in strong, 16.1 in medium and 17.7 in weak colonies, and it was significant in every class (paired t, all P < 0.01). In other words, older larvae were harder to remove everywhere, but colony weakness added its own, much larger, penalty on top of that.

Table 02: Mixed-design (split-plot) ANOVA for larval removal, egg removal and comb damage (colony as subject, n = 24).

Response and source	df	F	P	Partial η^2
Larval removal within 24 h				
Colony strength	2, 21	344.6	< 0.001	0.97
Larval age	1, 21	87.7	< 0.001	0.81
Strength × age	2, 21	0.09	0.914	0.01
Egg removal at 72 h				
Colony strength	2, 21	55.5	< 0.001	0.84
Egg position	1, 21	1551.5	< 0.001	0.99
Strength × position	2, 21	14.4	< 0.001	0.58
Damaged comb area, days 7-21				
Colony strength	2, 21	265.9	< 0.001	0.96
Day[a]	1.15, 24.2	758.0	< 0.001	0.97
Strength × day[a]	2.31, 24.2	57.4	< 0.001	0.85

Percentages were arcsine square-root transformed and damaged area $\log_{10}$ transformed. [a] Greenhouse-Geisser corrected degrees of freedom (ε = 0.577).

The age effect makes biological sense. Young larvae are tiny, move on the comb surface and have spun little silk. Older larvae soon push into the comb toward the midrib and cover themselves with silk galleries [6], where workers find them less easily. The strength effect is less simple than it looks. Snyder et al. [10] found that larger groups of workers removed dead brood better in small-scale tests, but at full colony scale the size of the colony did not change hygienic scores. Our classes differed in another way too, since a weak colony with three frames of bees leaves much of its comb without bees. Larvae placed on such comb are simply not met by workers for hours. We therefore read the strength effect mainly as a matter of bee cover and patrolling, not as proof that each worker in a strong colony is more hygienic. The speed of our strong colonies is close to what is reported for hygienic stock. Hygienic colonies in India took about 20.6 h to clear pin-killed brood, against 44.6 h for non-hygienic ones [15], and native Yemeni bees in Saudi Arabia had cleaned 87% of such cells after 8 h [16]. Our strong colonies had removed 88.6% of young larvae by 12 h, while weak colonies had not reached half by 24 h.

3.2. Kinetics of larval removal

Cumulative removal of young larvae rose quickly and then levelled off in all classes (Figure 3). The first-order model described the curves well (R^2 = 0.979-0.992; Table 3). The rate constant fell from 0.240 h^{-1} in strong colonies to 0.133 h^{-1} in medium and 0.089 h^{-1} in weak ones, and the plateau dropped from 95.0% to 82.2% and 51.7%. So weak colonies were not only slower; they also stopped short, leaving nearly half of the larvae in place whatever the waiting time. Half of the introduced larvae were gone after 3.4 h in strong and 7.3 h in medium colonies, but only after 46.7 h in weak colonies. The removal efficiency index told the same story (89.0, 72.5 and 42.6%). For a beekeeper the most useful number is probably t_{50}. A strong colony halves a fresh larval load within a morning, whereas a weak colony needs about two days, which may be long enough for young larvae to reach the inside of the comb.

Table 03: Kinetic parameters for the removal of young larvae by colonies of different strength.

Parameter	Strong	Medium	Weak
First-order model (Eq. 2)			
R_{max} (%)	95.0 ± 1.2	82.2 ± 1.6	51.7 ± 2.0
k (h^{-1})	0.240 ± 0.014	0.133 ± 0.010	0.089 ± 0.011
$t_{1/2}$ (h)	2.9	5.2	7.8
R^2	0.992	0.992	0.979
RMSE (%)	1.82	2.11	2.31
Weibull model (Eq. 4)			
λ (h)	4.25	7.82	12.68
β	0.88	0.90	0.84
R^2	0.997	0.994	0.984
Observed curve			
t_{50} (h)	3.4	7.3	46.7
Removal efficiency index (%)	89.0	72.5	42.6

± values are standard errors of the estimates. $t_{1/2}$ = ln 2/k; t_{50}, time to remove 50% of the introduced larvae; RMSE, root mean square error.

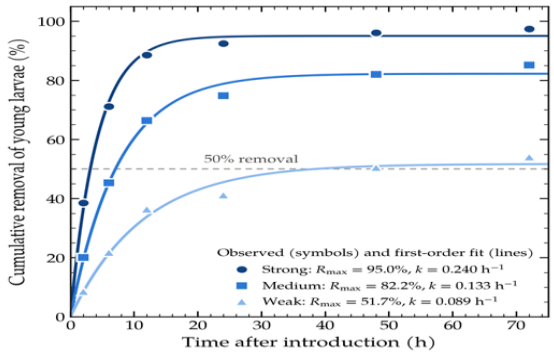

Figure 03: Cumulative removal of young larvae over 72 h. Symbols are observed class means; lines are first-order fits (Eq. 2). The dashed line marks 50% removal.

The Weibull fits were slightly better (R^2 = 0.984-0.997) and gave shape values below one in every class (β = 0.84-0.90). A shape value below one means that the chance of a larva being removed falls with time. The easy larvae go first, and the rest become steadily harder to reach, probably because they have moved into cracks, under cappings or into the comb. This fits the plateau values. It also suggests that the first few hours after hatching are the main window in which a colony can stop an infestation; once a larva survives this window, its chance of being removed later is small.

3.3. Removal of exposed and hidden eggs

Where an egg lay mattered as much as the colony it was in. Eggs on open comb were removed quickly (71.4% at 24 h and 94.3% at 72 h), while eggs placed in crevices were mostly left alone (18.6% at 24 h and 36.5% at 72 h; Figure 4a). Across the 24 colonies, the difference at 72 h averaged 57.8 percentage points (paired t_{23} = 21.1, P < 0.001). The consequence was plain in the hatching data: 52.8% of hidden eggs hatched inside the colonies, compared with 3.1% of exposed eggs, a 17-fold difference (Figure 4b). The ratio between the two positions narrowed from 3.8 at 24 h to 2.6 at 72 h, so workers did reach some hidden eggs with time. They reached them too slowly, and more than half of the hidden eggs hatched before they were found.

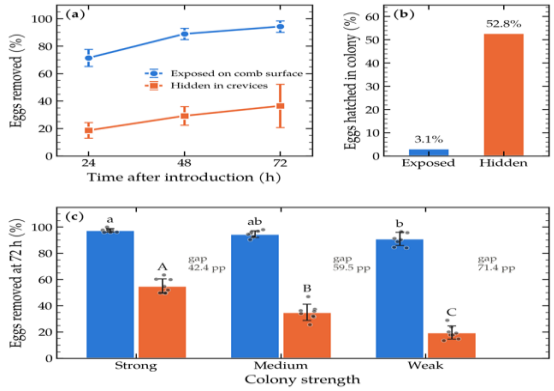

Figure 04: Removal of wax moth eggs. (a) Time course for eggs on the comb surface and eggs hidden in crevices (means ± SD across colonies) . (b) Percentage of introduced eggs that hatched inside the colonies. (c) Egg removal at 72 h by colony strength (means ± SD; dots are single colonies); letters as in Figure 2, and the gap between exposed and hidden eggs is given in percentage points (pp).

Strength and egg position interacted ($F_{2,21}$ = 14.4, P < 0.001; Table 02). Removal of exposed eggs changed little between classes (97.4, 94.5 and 90.9%), but removal of hidden eggs fell steeply from 55.0% in strong to 35.0% in medium and 19.5% in weak colonies (Figure 4c). As a result the gap between the two positions widened from 42.4 to 59.5 and 71.4 points. A crevice therefore protects eggs in any colony, and it protects them even better where few bees are present. Female moths are known to choose their egg sites carefully. In choice tests, gravid females used smell to find suitable sites and contact cues on their tarsi to avoid sites with bees, and far more moths emerged from hives without bees (37.2) than from hives with bees (9.8) [17]. Our results add the other side of the story: when eggs do end up in narrow gaps that workers cannot enter, most of them survive. Tight hive joints, repaired cracks and well-fitted top bars should reduce these refuges.

3.4. Comb damage, tunnels, galleriasis and absconding

Damage grew in all classes, but at very different speeds (Table 04, Figure 05). On day 21, damaged area was 4.0 ± 1.5 cm^2 in strong colonies, 31.2 ± 9.9 cm^2 in medium colonies and 142.6 ± 17.5 cm^2 in weak colonies, a 36-fold range. The mixed ANOVA gave strong effects of strength and day and a clear interaction (Greenhouse-Geisser corrected $F_{2.31,24.2}$ = 57.4, P < 0.001), which means the classes drifted further apart with time. Summed over the three weeks, the area under the damage curve differed 24-fold between strong and weak colonies (45.0 against 1060.8 cm^2 d). Damage doubled every 5.5 days in weak colonies, every 7.7 days in medium colonies and only every 16.1 days in strong colonies. In strong colonies the increase between days 14 and 21 was not significant (paired test on log values, Bonferroni P = 0.22), so the damage had more or less stopped. In medium and weak colonies it was still rising (P = 0.002 and P < 0.001).

Table 04: Comb damage, tunnels, galleriasis and absconding in colonies of different strength (mean ± SD, n = 8 colonies per class).

Variable	Strong	Medium	Weak	$F_{2,21}$	P
Damaged area, day 7 (cm^2)	2.1 ± 0.4[c]	8.7 ± 2.6[b]	24.9 ± 5.9[a]	160.9	< 0.001
Damaged area, day 14 (cm^2)	3.4 ± 0.9[c]	19.5 ± 3.3[b]	67.8 ± 9.1[a]	425.8	< 0.001
Damaged area, day 21 (cm^2)	4.0 ± 1.5[c]	31.2 ± 9.9[b]	142.6 ± 17.5[a]	232.6	< 0.001
Area under damage curve (cm^2 d)	45 ± 13[c]	276 ± 66[b]	1061 ± 143[a]	331.3	< 0.001
Relative growth rate (d^{-1})	0.043 ± 0.016[c]	0.091 ± 0.007[b]	0.126 ± 0.010[a]	104.7	< 0.001
Doubling time (d)	16.1	7.7	5.5	-	-
Tunnels per frame	1.4 ± 0.5[c]	4.8 ± 0.7[b]	13.8 ± 4.2[a]	139.9	< 0.001
Galleriasis (% of capped brood)	0.8 ± 0.2[c]	4.6 ± 1.7[b]	17.3 ± 3.9[a]	153.7	< 0.001
Absconded colonies	0/8	0/8	2/8	-	0.10[a]

Letters as in Table 1. Damage, area under the curve and tunnel counts were $\log_{10}$ transformed and galleriasis arcsine square-root transformed before analysis. Doubling time = ln 2 divided by the class mean relative growth rate. [a] Fisher's exact test, weak colonies against medium and strong colonies combined.

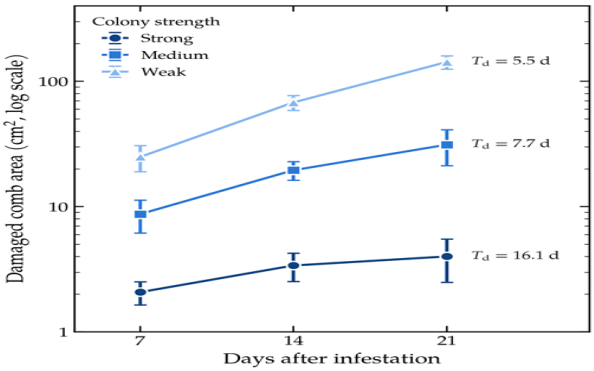

Figure 05: Damaged comb area on days 7, 14 and 21 in strong, medium and weak colonies (means ± SD, log scale). T_d is the doubling time of the damaged area (Eq. 6).

Tunnels and galleriasis followed the same pattern. Weak colonies had 13.8 tunnels per frame and 17.3% of capped brood with galleriasis, against 1.4 tunnels and 0.8% in strong colonies. Two of the eight weak colonies absconded before day 21, and none of the medium or strong colonies did. These were the two colonies with the largest damage (173.5 and 154.8 cm^2), the most tunnels (21 and 19 per frame) and the slowest removal (26.8 and 25.5 h). With only two events the difference was not significant (Fisher's exact test, P = 0.10), and we would not build much on it. The direction, however, agrees with field reports. In Nigeria, 68.4% of weak colonies carried both small hive beetle and wax moth against 26.9% of strong colonies, and colonies that had absconded left badly damaged combs [12]. In India, adult wax moth numbers fell as colony strength and brood area rose (r = −0.499 and −0.607) [11], and hives with small bee populations and poor hive hygiene were attacked most, to the point that bees left the worst of them [18]. The Iraqi survey mentioned earlier gives a similar picture at province level [8]. Table 05 compares these studies with ours. The rise in galleriasis is the part that hurts the colony directly, because trapped bees die and the brood nest shrinks [6]. Weak colonies usually carry other burdens too; in declining commercial colonies, weak colonies held 3.6 times more virus species than strong ones [19]. Wax moth damage is therefore more likely to deepen an existing weakness than to start it.

Table 05: The present results compared with recent studies on colony strength, comb condition, worker removal behaviour and wax moth infestation.

Study (country)	Design	Main finding
Al-ettaby and Khlaf [8] (Iraq)	Survey of apiaries in six provinces, 2023-2024	Infestation ranged from 10.7% (Maysan) to 73.3% (Basra); strong hives made up 81.7% of colonies in Maysan and 30.3% in Basra
Naveen et al. [11] (India)	Seasonal monitoring of *A. mellifera* colonies	Adult wax moths were negatively correlated with colony strength (r = −0.499) and brood area (r = −0.607)
Akinwande and Murele [12] (Nigeria)	45 managed colonies and 12 nucleus colonies	Wax moth and small hive beetle occurred together in 68.4% of weak and 26.9% of strong colonies; absconded colonies left badly damaged combs
Parepely et al. [17] (India)	Oviposition choice tests	37.2 moths emerged from hives without bees against 9.8 from hives with bees
Kumar et al. [20] (India)	Larval rearing on combs of different age	Three-year-old comb was the best larval diet; processed wax gave slow development and high mortality
Mohindru and Chhuneja [15] (India)	Pin-killed brood assay	Hygienic colonies responded after 20.6 h and non-hygienic colonies after 44.6 h
Al-Kahtani and Taha [16] (Saudi Arabia)	Pin-killed brood, infrared video recording	Yemeni bees had cleaned 87% of cells after 8 h, Carniolan bees 67%
Present study (Babil, Iraq)	24 colonies in three strength classes; introduced eggs and larvae	Young-larva removal in 24 h was 92.5% in strong and 41.2% in weak colonies; damage on day 21 was 4.0 and 142.6 cm^2

3.5. Effect of comb type

Comb type had a strong effect as well (Figure 6). After 72 h, 38.4% of the larvae placed on dark, previously brood-reared combs were still alive, against 14.2% on light, new combs, a 2.7-fold difference (t_{22} = 6.9, P < 0.001). By day 14 the dark combs had 41.7 cm^2 of damage and the light combs 9.8 cm^2, 4.3 times less. Old combs hold cocoons, cast larval skins and pollen residues, which give the larvae protein that pure wax lacks; wax moth larvae are known to feed on all of these [7]. In a feeding study, larvae grew best and died least on three-year-old comb, and processed wax was the poorest diet [20]. The attraction of old comb is strong enough that dark comb has been used as a lure in wax moth traps [21]. For beekeepers the message is practical: replacing old brood combs in rotation removes much of the food that makes an infestation succeed.

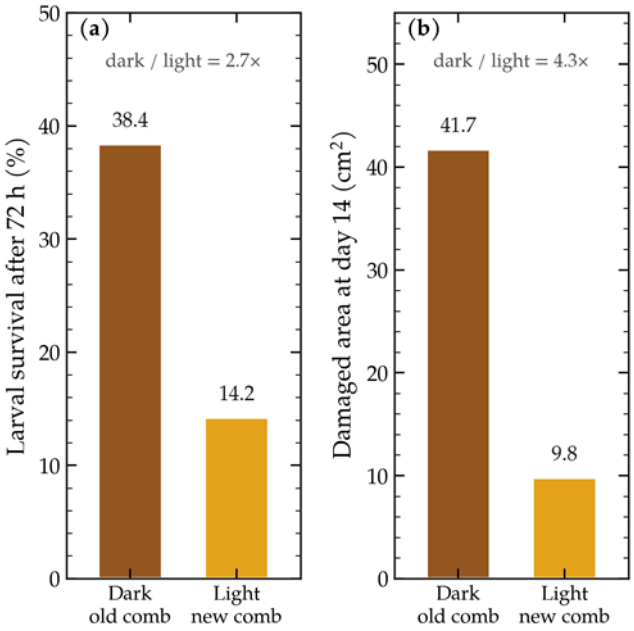

Figure 6. Effect of comb type on (a) survival of young larvae after 72 h and (b) damaged area on day 14. Values are means ± SD (n = 12 per comb type).

3.6. From early removal to later damage

Across colonies, removal of young larvae within 24 h was strongly and negatively related to damage on day 21 (r = −0.977, P < 0.001; Table 6). The same was true for old larvae (r = −0.941), and removal time was closely tied to the number of tunnels (r = 0.976). Such high values are expected when three well separated classes are pooled, so we looked inside the classes as well. There the picture changed in an informative way. Young-larva removal still predicted damage within classes (pooled within-class r = −0.856, P < 0.001), but old-larva removal did not (r = 0.068, P = 0.76), and neither did the removal of hidden eggs (r = 0.010, P = 0.96). Removal time kept its link with tunnels (r = 0.867) and tunnels with galleriasis (r = 0.908).

Table 06: Correlations between removal and damage variables across the 24 colonies, and pooled within-class correlations.

Variable pair	Pearson *r*	Spearman *ρ*	Within-class *r*	*P* (within)
Young-larva removal × damage, day 21	−0.977	−1.000	−0.856	< 0.001
Old-larva removal × damage, day 21	−0.941	−0.896	0.068	0.762
Hidden-egg removal × damage, day 21	−0.849	−0.900	0.010	0.965
Removal time × tunnels per frame	0.976	0.992	0.867	< 0.001
Removal time × damage, day 21	0.984	1.000	0.930	< 0.001
Tunnels per frame × galleriasis	0.983	0.992	0.908	< 0.001
Damage, day 21 × galleriasis	0.989	1.000	0.976	< 0.001

All pooled correlations had $P < 0.001$. Within-class r was computed after centring each variable on its class mean (df = 20).

The power model put a number on this link (Figure 7). Damage on day 21 rose with the percentage of young larvae left unremoved as $D_{21} = 0.213\ E^{1.56}$ ($R^2 = 0.958$, $P < 0.001$; 95% CI of the exponent 1.42-1.70). Because the exponent is above one, every doubling of the larvae that escape removal multiplies the damage about three times ($2^{1.56} = 2.95$). Put simply, damage was decided early. The first day of contact between workers and newly hatched larvae set the scale of the losses seen three weeks later, while the fate of larvae that were already large added little once colony class was taken into account.

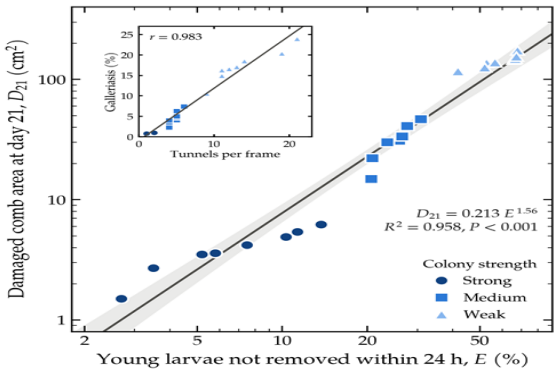

Figure 07: Damaged comb area on day 21 against the percentage of young larvae not removed within 24 h (log-log axes). The line is the power model (Eq. 7) with its 95% confidence band. Inset: galleriasis against the number of tunnels per frame.

The PCA summarized all of this in one axis. The first component explained 87.3% of the variance and loaded the removal variables negatively (−0.65 to −0.99) and damage, tunnels, galleriasis and removal time positively (0.96-0.99) (Figure 8). Colonies separated along this axis by class without overlap. The second component (8.2%) was driven mainly by removal of exposed eggs, which varied little between colonies.

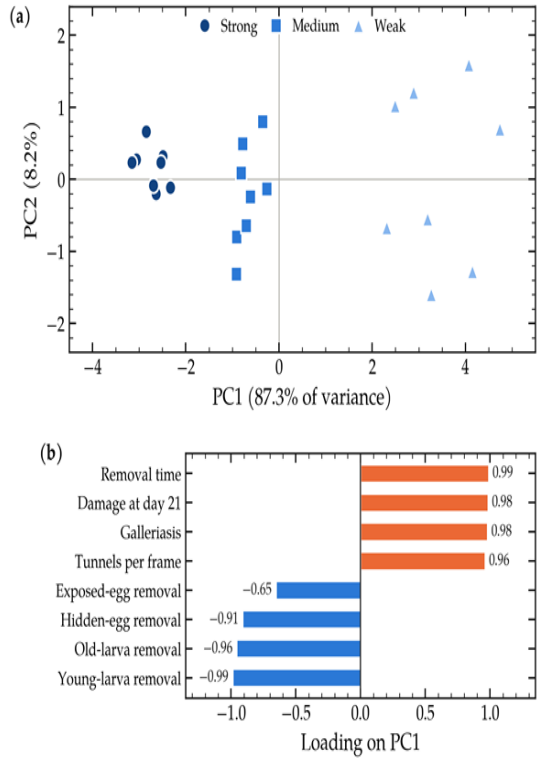

Figure 08: Principal component analysis of eight colony variables. (a) Scores of the 24 colonies on the first two components. (b) Loadings of the variables on the first component.

3.7. Practical meaning and limits of the study

These results point to management steps that most beekeepers can apply. Weak colonies should be given only the combs their bees can cover, or be united with other colonies, because comb without bees is where young larvae escape. Surplus combs should be stored

out of the hive and protected. Freezing works well for this: holding larvae at −16 °C for 60 min killed all of them [22], and Iraqi beekeepers already report good results from freezing frames for two days [8]. Clove and orange oils also cut the number of larval tunnels and the silk area in stored combs, and colonies accepted the treated combs without bee deaths [23]. Crevices should be closed and old dark combs replaced in rotation. Where extra help is needed, *Bacillus thuringiensis* products and traps that keep bees away from the treated lure are promising [21], and local plant extracts are being tested in Iraq; a 10% *Ajuga iva* leaf extract killed about half of third-instar larvae and reduced pupation [24]. In the longer term, queens from hygienic lines may help, since breeding for hygienic behaviour brought lower mite and virus loads without any clear cost to the workers [25]. These steps matter most in the hot months, when wax moth numbers peak [7], and in areas where many hives are weak, such as Basra, where infestation was highest and strong hives were fewest [8].

The study has limits. It covered one apiary and one season, with eight colonies per class. The egg time course and the comb-type test were summarized as class means, so their variation between colonies could not be tested fully. Larvae and eggs were introduced by hand, and natural infestations may start differently. Absconding was too rare for firm conclusions. Repeating the trial over several seasons, with continuous recording of worker activity on the infested frames, would help to separate the effect of bee cover from the hygienic tendency of individual workers.

4. CONCLUSION

Colony strength controlled almost every stage of the contest between honey bee workers and the greater wax moth in this trial. Strong colonies removed most young larvae within hours, stopped short of the damage seen in weaker colonies and kept comb losses near 4 cm^2 after three weeks. Weak colonies removed larvae slowly, left about half of them in place and saw their damage double every 5.5 days, with more tunnels, more galleriasis and the only cases of absconding. Old larvae and eggs hidden in crevices escaped removal in all classes, and dark, brood-used combs helped larvae survive. The strongest predictor of later damage was the share of young larvae removed in the first 24 h, which suggests that the fight is largely won or lost in the first day. In practice, beekeepers should keep colonies strong, give weak colonies only the combs their bees can cover, store surplus combs frozen or treated, close hive crevices and renew old brood combs. Studies over more seasons and sites are needed to confirm these patterns and to measure the benefit of each step.

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