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Scientific note

Scientific note on small hive beetle infestation of stingless bee (*Tetragonula carbonaria*) colony following a heat wave

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Abstract – We present the first study of infestation by the small hive beetle, *Aethina tumida*, in a queenright colony of the stingless bee *Tetragonula carbonaria*, a species used for pollination services. Nine managed hives were deployed outside the bee's endemic range. After 2 months, including a heat wave of four consecutive days above 40 °C, one colony was visibly weakened with fewer foragers. This hive was removed from the site for the colony to recover. After a second heat wave, another colony was found weakened and infested with small hive beetle (SHB). Inside the hive, 14 adults and 133 larvae of SHB were discovered. Extreme daily maximum temperatures and low humidity appear to have weakened this colony. Our study highlights the need for careful placement of managed hives, as the SHB has potential to be a significant pest of stingless bees under adverse conditions.

Aethina tumida **/ Hive pest /** ***Tetragonula carbonaria***

The small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera: Nitidulidae), is a global pest that infests honey bee colonies (Neumann et al. 2016) as well as other bee hosts including bumblebees (Hoffmann et al. 2008) and stingless bees (Mutsaers 2006; Halcroft et al. 2011; Lóriga Peña et al. 2014; Bobadoye et al. 2018). Infestations occur in physically damaged or recently split stingless bee hives, but healthy colonies are usually able to defend themselves, e.g., by covering adult beetles in propolis (Greco et al. 2010; Halcroft et al. 2011). Knowledge regarding factors leading to SHB susceptibility of hosts other than honey bees is still limited, and only anecdotal observations have been reported of infestation in healthy, managed Australian stingless bee (e.g., *Tetragonula carbonaria* Smith) colonies (Heard 2016).

In November 2018, nine queenright *T. carbonaria* colonies with strong foraging activity (hive weights ranging from 11.0 to 13.4 kg) were moved from a florally diverse site in Richmond, NSW (− 33.610068, 150.746820) to a commercial farm at a florally limited site in Cowra, NSW (− 33.779647, 148.617618). Colonies are housed in hardwood Original Australian Trigona Hive (OATH) boxes (Fig. S1a) and placed under trees which provided shade. Temperature loggers (iButtons, Temperature Technology, Ashford, South Australia) were attached to the top rear external surface of each hive to record ambient conditions. Two heat waves during which ambient temperature reached > 40 °C on at least two consecutive days occurred from 15 to 18 January and from 25 to 26 January 2019.

On 5 February, a liquid was observed leaking from the front corner of one hive (#8), indicating a disruption of internal hive conditions and subsequently attracting scavenging ants and wasps (Fig. S1b, c). On 26 February, the forager activity of this colony had ceased, and it was returned to Richmond on 1 March. Upon return, the hive was immediately opened and inspected during the

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afternoon, when it was discovered to contain SHB. Using forceps and a magnifying lamp, we combed through the hive box and contents, recording 14 SHB adults and 133 larvae (57 fourth instar, 47 third instar, 22 second instar, and 7 first instar and no eggs) (Fig. S1d). Development from egg to adult can take as little as 18 days when temperature and humidity are optimal (Neumann et al. 2016). Because we observed mostly late instar SHB larvae, this suggests the infestation likely occurred after the first heat wave event. Most larvae are found in the bottom box containing the bee brood (Fig. S1a), and the bee brood clump is greatly reduced in size, containing only ~ 100 healthy capped cells consisting of all life stages. Because bee eggs were observed, and the egg stage lasts only ~ 6 days (Heard 2016), this indicates the recent presence of a laying queen (although not directly observed) and eliminates queenlessness as a possible cause of infestation (Fig. S1e). Few adult workers (< 50) remained inside the hive, and "slimy" internal conditions caused by SHB larvae (Heard 2016) were apparent. The hives of remaining colonies, which exhibited normal foraging activity, were not opened to inspect for SHB, and no SHB infestations were subsequently recorded for other colonies returned from Cowra or those that had remained at the Richmond site. Both Cowra and Richmond are known locations for SHB in honeybee colonies (Spooner-Hart et al. 2017); thus, all colonies were potentially exposed to SHB threat.

To test whether the microclimate differed between individual colonies and thus may have led to the SHB infestation in one colony, we compared daily thermal maxima recorded at the external surface of each hive box using generalized linear models with a Poisson distribution in the *lme4* package (Bates et al. 2015) and then used a pairwise least squared means contrast with false discovery rate (FDR) corrected P values (at $\alpha = 0.05$) (Verhoeven et al. 2005) in *emmeans* package (Lenth 2018). All analyses were conducted in R (v.3.5.1, R Core Team 2018). We found that the external average thermal maxima were significantly higher for the SHB-infested colony (#8) and colony #27 (Fig. S2a), likely due to their greater exposure to direct sunlight in uneven shading conditions. On 23 January, colony #27 also had low forager activity and was returned early to Richmond, where it recovered without being infested by SHB. Colony #17 also received relatively high temperatures; however, it did not reach the thermal maxima (~ 48C^{o}) of #8 and #27 (Fig. 2a).

We then used a Student t test on data sourced from the Bureau of Meteorology (2019) to compare daily maximum temperature and % relative humidity between the endemic site in Richmond and the relocation site in Cowra over the study period (21 Nov 2018–1 Mar 2019). On average, the daily maximum temperatures in Cowra were higher by 3 °C than in Richmond ($t = 3.86$, df = 173.72, $p < 0.001$), and relative humidity was lower by 23% than in Richmond ($t = -11.583$, df = 340.75, $p < 0.001$; Fig. S2b, c).

We conclude that the heat wave events coupled with low relative humidity at the Cowra field site may have damaged brood within the infested colony due to its elevated exposure to heat. If larvae or pupae reached their critical thermal limits and mortality occurred, this would interrupt the overlapping generations of workers in the colony. With few or no new workers eclosing after the heat wave, the subsequent drop in worker number could have facilitated pest invasion, and olfactory cues regarding host status could have attracted SHB (Bobadoye et al. 2018). A previous study of the Brazilian stingless bee *Scaptotrigona depilis* Moure indicated that 38 °C inside the hive was lethal to bee brood (Vollet-Neto et al. 2015), but thermal limits of *T. carbonaria* are currently unknown.

We provide the first report of SHB infestation in a stingless bee colony that was not caused by physical damage or hive splitting but was probably linked to weather extremes. Further research is needed to determine the lethal thermal limits of the commonly kept stingless bee species in Australia. Our observation of the likely effects of weather conditions on stingless bee colonies contributes to an emerging body of evidence that stingless bees have a wider range of parasites and diseases than previously reported (Porrini et al. 2017; Shanks et al. 2017). Many stingless beekeepers place additional coverings over managed hives to reduce heat and redirect rainwater away from hives (Klumpp 2007). Adding these when relocating colonies may further aid in protecting them from direct sunlight during hot summer months, thereby reducing the chance of colony loss from heat extremes and SHB invasion.

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AUTHORS' CONTRIBUTIONS

SN, MH, JC, and MR conceived the research. SN and MH collected data. MD and RSH participated in data interpretation. SN and MH wrote the paper. MD, JC, MR, and RSH participated in revisions.

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Une note scientifique sur l'infestation par le petit coléoptère des ruches d'une colonie d'abeilles (*Tetragonula carbonaria*) après une vague de chaleur.

Aethina tumida **/ parasite des ruches.**

Eine wissenschaftliche Notiz über den Befall eines Volks der Stachellosen Biene *Tetragonula carbonaria* durch den Kleinen Beutekäfer nach einer Hitzewelle.

Aethina tumida **/ Nestschädling.**

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