Blood deprivation and heat stress increase mortality in bed bugs (Cimex lectularius) exposed to insect pathogenic fungi or desiccant dust

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Abstract. Bed bugs (Cimex lectularius L.) have returned as a nuisance pest in the last 20 years. Different bed bug control measures in combination have not been thoroughly studied, although induction of multiple stressors may improve extermination. The effects of heat stress only, heat stress followed by exposure to insect pathogenic fungi, and heat stress followed by exposure to desiccant dust on starved and blood-fed bed bugs were investigated. Five days at 22°C (control), 32°C, 34°C, or 36°C (heat stress) did not cause mortality in adults. However, their starved first instar nymphs produced after heat stress suffered mortalities of 33%, 56% and 100%, respectively. Exposure to insect pathogenic fungi after heat stress increased the mortality of adults and their progeny compared to exposure to fungi without heat stress. The beneficial effects of heat stress were not observed in blood-fed bed bugs. Desiccant dust killed all nymphs within 2 days and all adults within 3 days regardless of previous heat stress, but survival time was prolonged by access to blood. This study highlights the advantage of combining different methods in pest management, and points to heat stress combined with blood deprivation as possible management elements to increase the control success.

Key words. Blood deprivation, Cimex lectularius, combined effects, desiccant dust, insect pathogenic fungi, integrated pest management, sublethal heat.

Introduction

The common bed bug (Cimex lectularius) has re-emerged as a nuisance in private homes and in the accommodation industry during the last 20 years (Doggett et al., 2018). This hematophagous ectoparasite does not live permanently on the host, but takes obligate blood meals from humans to commence into subsequent instars and to maintain reproduction (Evison et al., 2018). Other blood feeding arthropods of medical and veterinary importance, such as sand flies, ticks, some fleas and red poultry mites, spend the majority of their time away from hosts and only temporarily visit them during feeding (Krasnov et al., 2002; Anderson & Magnarelli, 2008; Sparagano et al., 2014). Limited host exposure strongly subjects the ectoparasites to the surrounding environmental factors as determinants of the prevalence and intensity of ectoparasitism (Oorebeek & Kleindorfer, 2008; Tucci et al., 2008). This connection may consequently be utilized for improved management. Other control efforts can be shifted in favour of eradication success by manipulating the indoor habitat and the permanent surroundings.

To terminate pesticide-resistant bed bugs, Integrated Pest Management (IPM) combines several methods with a thorough monitoring regimen (Bennett et al., 2016; Kells, 2018). Since many possible methods may be used during the eradication of bed bugs, it is important to understand their combined effects. Although additive or even synergistic properties may influence mortality, few studies have examined these effects, even though it has been shown in ticks and stored product pest control (Arthur, 2000; Yoder et al., 2006).
Heat is frequently used to manipulate the indoor environment of bed bugs in apartments. To kill bed bugs within a few hours, a temperature of 55–65 °C in the air-space within a heat treated room may be required to ensure efficient mass transfer of heat (Kells & Goblirsch, 2011). Bed bugs die almost instantly at temperatures above 60 °C (Loudon, 2017). They can only survive for 1 h at 50 °C (Benoit, 2011), less than 2 days at 40 °C and as long as 9 days at 38.5 °C (Rukke et al., 2015). However, increased mortality may not be the only benefit from heat exposure. Lower, sublethal temperatures have been shown to reduce egg production, hatching success, offspring survival, moulting frequency and feeding (Rukke et al., 2015; Rukke et al., 2018). To counter these adverse conditions and improve survival, bed bugs may alter their metabolism to reduce water loss, adjust physiological processes, modify behaviour and even enter quiescence in the absence of a host (Benoit et al., 2009a; Benoit, 2011, 2018). Such strategies are likely to interact during a multi-method management approach, as exemplified by the variable effect of pesticides according to feeding status (Choe & Campbell, 2014; DeVries et al., 2015). These results highlight access to blood as a key factor for handling environmental stress. Additionally, access to blood is crucial for egg production (Matos et al., 2017), increasing the population size, and time to sexual maturation (Evison et al., 2018). Although slow-acting and weak stressors such as sublethal heat or starvation will eventually kill a bed bug (Benoit, 2011, 2018; Rukke et al., 2015, 2018), they have been considered of limited applicability in control situations because treatment time is of the essence. However, systematic manipulation of two or more stressors may increase the success of management by pushing the bed bugs towards extermination.

Although the use of insect pathogenic fungi is not a traditional bed bug management method, Beauveria bassiana kills bed bugs both through direct conidia contact and through horizontal transfer in harboursages (Barbarin et al., 2017; Aak et al., 2018). When using insect pathogenic fungi in an IPM solution, the defence against the pathogen may affect bed bug resilience towards the total management protocol. This is also the basis for the manipulation of bed bugs’ water balance using lipid-absorbing desiccant dusts. Desiccant dusts may yield effects directly or through horizontal transfer to conspecifics in harboursages (Benoit et al., 2009b; Akhtar & Isman, 2013; Aak et al., 2016), but more importantly, they should increase water loss and mortality when combined with thermal stress.

In the present study, we investigated how multiple stressors applied in sequence may improve bed bug management. We tested the ability of sublethal heat to increase the effects of insect pathogenic fungi and desiccant dust in blood-fed and unfed individuals. In order to understand the effects at the population level of this parasite, both adults and their progeny were investigated.

Materials and methods

Stock cultures and experimental units

The stock cultures at the Norwegian Institute of Public Health originated from bed bugs collected at two locations in Norway in 2009 and are fed heated human blood through a Parafilm membrane (Aak & Rukke, 2014). Stock cultures were maintained under a photoperiod of 16:8 (light:dark) hours at 22 °C and 60% relative humidity in climate-controlled chambers (Sanyo MLR-351H; Medinor ASA, Oslo, Norway). The experiments were conducted inside four identical climate chambers set at 22 °C (control), 32 °C, 34 °C, or 36 °C. The latter three temperatures represent three levels of sublethal heat stress. Experimental bed bugs were kept inside 140 mL polyethylene boxes (VWR straight sample container, VWR, Oslo, Norway) throughout the study. Each experimental box had a lid with a 40 mm diameter circular opening secured by a metal mesh screen (0.25 mm openings; Burmeister AS, Oslo, Norway) that allowed aeration and blood feeding without handling of the bed bugs. A folded filter paper (Whatman No. 1, 47 mm) was used in each box to provide a substrate for the bed bugs and to allow deposition of eggs.

Insecticides

The insect pathogenic fungi were prepared from the product BotaniGard 22WP (B. bassiana strain GHA, 2 × 1013 cfu/kg, Laverlam International, USA). Conidia were suspended in water according to the manufacturer’s instructions to create a 0.02% conidia suspension (approximately 6.9 × 10⁸ conidia/cm²). Bed bugs were exposed to conidia through circular patches of cotton cloth (woven bed sheets, 100% cotton, Jysk – Oslo, Norway) with a diameter of 47 mm, which were dipped into the fungal solution. The patches were dried on petri dishes for 3–4 days at room temperature before being given to the bed bugs. Desiccant dust Syloid 244 FP (GRACE GmbH & CO, Germany) was used as the second killing agent. Syloid is synthetic amorphous silica powder (99.6% SiO₂) with a particle size of 5.5 μm and is a close approximation of commercially available silica gel products. Fresh filter papers (Whatman No. 1, 47 mm) were coated with dust, and the weight before and after coating was used to calculate the dose. An average (±SE) of 0.62 ± 0.04 mg of Syloid per filter paper produced an effective dose of 0.18 g/m².

Experimental protocol

About 1200 bed bugs in 202 boxes were used in the experiments, and male: female and fed: unfed ratios were balanced between all treatments (Table 1). Each box in the adult experiments contained three males and three females, while the boxes in the offspring experiments contained six first instar nymphs. Prior to the experiments, fifth instar nymphs were collected from stock cultures and fed for 15 min. Fully engorged nymphs were transferred to new boxes and kept at room temperature for 2 weeks. Freshly moulted adults were sorted into experimental boxes for different treatments (see below and Table 1) and fed one day prior to the start of the experiments. From day 1 to day 5 in the adult experiments, the bed bugs were exposed to temperatures of 22 °C (control), 32 °C, 34 °C, or 36 °C. From day 6 to day 27, they were all kept at 22 °C. On day 6, a new blood meal...
was offered to the adults in half of the boxes in each temperature treatment, while the rest remained unfed. On day 7, the bed bugs were split into three groups: those receiving no further treatment, those being exposed to fungi, or those being exposed to Syloid. For fungal exposure, the bed bugs were allowed to crawl over the conidia-loaded substrate for 5 min before being put back into new boxes with clean filter papers. For Syloid exposure, the bed bugs were released onto Syloid-coated filter papers and allowed to crawl on them until the end of the experiment. Mortality was recorded daily from day 8 to day 17, and then every 2 days until day 27 of the experiments. When bed bugs appeared dead, air was blown into the boxes. If the bugs still did not move, a soft hairbrush tip was used to poke the animals gently to provoke movement. Dead bed bugs were then removed. The adult experiments were terminated on day 27. At this stage, the bed bugs kept at the control temperature or having received heat stress only had produced first instar nymphs in their respective boxes. These nymphs were collected and transferred to new boxes on day 28 for the offspring experiments. Half of the nymphs were fed on day 29. All the nymphs were kept at room temperature (22 °C) and had, therefore, received heat stress or control temperature (22 °C) only indirectly through their parents. From day 30 to day 50, the nymphs were either kept on clean filter paper or exposed to one of the two killing agents in the same way as the adults. They were checked for mortality daily from day 31 to day 40, and then every 2 days until day 50 of the experiments.

The methods used to kill bed bugs in the experiments were combined in sequence. We did not use stressors or killing agents in parallel because the high temperature and dry conditions would likely negatively affect the fungi and consequently mask any potential effects. Too few nymphs were produced by the heat-stressed adults to allow for a fully balanced number of replicates in the offspring experiments (Table 1). Therefore, based on the results of the adult experiments, we chose to allocate fewer nymphs to the unfed Syloid treatment, as rapid and complete mortality was expected.

### Statistical methods

All data were analysed using SigmaPlot 13.0 (Systat Software Inc. Son Jose, CA, USA) and JMP pro 13.0.0 (SAS Institute, Cary, NC, USA) software. To investigate the progression of the population mortality during the experiments, we conducted survival analyses using the Kaplan-Meier product limit method with the log-rank test. The level of significance was set to 0.05 for all analyses.

### Results

**Effect of heat stress and feeding on survival of adults and their offspring**

All the adults and their offspring survived the control treatment (22 °C) (Fig. 1A–D). All the adults also survived the heat stress (Fig. 1A,B), but an effect was seen on their offspring. When deprived of blood, nymph survival was significantly reduced at all temperatures (Kaplan-Meier (only least significant test shown); 32 °C vs. control: $\chi^2 = 6.99, df = 1, P = 0.008$, Fig. 1C), and the mortality increased as the temperature the parents were exposed to increased. Fed nymphs were only affected when their parents had experienced the highest heat stress temperature (Kaplan-Meier; 36 °C vs. all other temperatures: $\chi^2 = 4.37, df = 1, P = 0.037$, Fig. 1D).

**Effect of heat stress, feeding and fungi on survival of adults and their offspring**

When exposed to fungi after heat stress and with access to blood, neither adults nor nymphs showed additional mortality (Fig. 2B,D). When kept unfed, however, the adults showed significantly reduced survival after exposure to 36 °C (Kaplan-Meier; 36 °C vs. 22 °C (Control): $\chi^2 = 6.54, df = 1, P = 0.011$, Fig. 2A), and previous exposure to 34 °C resulted

### Table 1. Number of Cimex lectularius adults or nymphs and boxes (in brackets) used in the different experiments.

| Adults | # individuals (boxes) | # individuals (fed: unfed) | 1st instar nymphs | # individuals (boxes) | # individuals (fed: unfed) |
|--------|------------------------|-----------------------------|-------------------|------------------------|-----------------------------|
| Heat stress only | 22 °C (Control) | 36 (6) | 18:18 | 36 (6) | 18:18 |
| | 32 °C | 36 (6) | 18:18 | 36 (6) | 18:18 |
| | 34 °C | 36 (6) | 18:18 | 36 (6) | 18:18 |
| | 36 °C | 36 (6) | 18:18 | 36 (6) | 18:18 |
| Heat stress followed by syloid | 22 °C (Control) | 60 (10) | 30:30 | 60 (10) | 30:30 |
| | 32 °C | 60 (10) | 30:30 | 50 (9) | 30:20 |
| | 34 °C | 60 (10) | 30:30 | 45 (8) | 30:15 |
| | 36 °C | 60 (10) | 30:30 | 37 (7) | 30:7 |
| Heat stress followed by fungi | 22 °C (Control) | 60 (10) | 30:30 | 60 (10) | 30:30 |
| | 32 °C | 60 (10) | 30:30 | 60 (10) | 30:30 |
| | 34 °C | 60 (10) | 30:30 | 60 (10) | 30:30 |
| | 36 °C | 60 (10) | 30:30 | 60 (10) | 30:30 |

Heat stress was applied prior to the desiccant dust (Syloid 244 FP) or fungus (Beauveria bassiana) exposure. The nymphs were offspring only produced by control adults or heat stressed adults, and they have only experienced heat stress through their parents.

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Fig 1. Survival of adult *Cimex lectularius* exposed to 22 °C (control), 32 °C, 34 °C or 36 °C for 5 days and then (A) unfed or (B) fed. The nymphs were offspring produced by the heat-stressed adults and had only experienced heat stress through their parents. The nymphs were either (C) unfed or (D) fed. Different letters (a, b or c) denote significant differences between treatments (Kaplan-Meier log-rank tests: \( P \leq 0.05 \)). Benefit of different heat stress temperatures compared to the control temperature (Temperature benefit) is shown as the percentage of increased mortality at the end of the experiment (+%).

in minor additional mortality (Kaplan-Meier; 34 °C vs. 22 °C (Control): \( \chi^2 = 2.92, \text{df} = 1, P = 0.087 \) and 32 °C vs. 22 °C (Control): \( \chi^2 = 0.19, \text{df} = 1, P = 0.662 \), Fig. 2A). All the unfed nymphs showed increased mortality when their parents had been heat stressed, and none survived exposure to fungi when their parents had been exposed to 36 °C (Kaplan-Meier; 32 °C vs. 22 °C (Control): \( \chi^2 = 4.68, \text{df} = 1, P = 0.031 \), 34 °C vs. 22 °C (Control): \( \chi^2 = 3.72, \text{df} = 1, P = 0.054 \), 36 °C vs. 22 °C (Control): \( \chi^2 = 13.78, \text{df} = 1, P < 0.001 \), Fig. 2C).

**Effect of heat stress, feeding and desiccant dust on survival of adults and their offspring**

Chronic exposure to desiccant dust killed adults and nymphs considerably faster than brief exposure to fungi (Fig. 3 vs. Fig. 2). All adults died within 3 days and all nymphs within 2 days regardless of previous heat stress or blood access. The rapid mortality did not allow relevant temperature discrepancies to be investigated in detail. However, when all individuals were pooled across temperatures, adults with access to blood lived significantly longer (Kaplan-Meier; fed vs. unfed adults, \( \chi^2 = 21.49, \text{df} = 1, P < 0.001 \), Fig. 3A). On average (±SE), 80 ± 5% of the fed individuals were alive on day 1 and 8 ± 3% on day 2, compared to 52 ± 7% and 0%, respectively, among the starved individuals. For nymphs that died even faster than the adults, only a minor and close to significant effect was observed (Kaplan-Meier; fed vs. unfed nymphs, \( \chi^2 = 3.66, \text{df} = 1, P = 0.056 \), Fig. 3B).

**Discussion**

Bed bug management is challenging, and studies often report control failures (Bennett et al., 2016). The present study emphasises some important aspects that favour higher control success. *First*, we showed that even a moderate environmental manipulation of ambient temperature to 32 °C reduced bed bug survival or population development and elevated the effect of the insect pathogenic fungi, *Beauveria bassiana* combined well with prior heat stress to increase mortality, while chronic exposure to desiccant dust was a highly efficient option regardless of temperature stress. *Second*, we illustrate that denial of blood feeding is a major factor in successful bed bug management, as access to blood may diminish the beneficial effects seen from environmental stressors.

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Starvation and heat stress in bed bugs

Fig 2. Survival of adult *Cimex lectularius* exposed to 22 °C (control), 32 °C, 34 °C, or 36 °C for 5 days and then (A) unfed or (B) fed before being exposed to fungi (*Beauveria bassiana*). The nymphs were offspring produced by the heat-stressed adults and had only experienced heat stress through their parents. They were either (C) unfed or (D) fed and then exposed to fungi. Different letters (a, b or c) denote significant differences between treatments (Kaplan-Meier log-rank tests: \( P \leq 0.05 \)). Benefit of different heat stress temperatures compared to the control (Temperature benefit) is shown as the percentage of increased mortality at the end of the experiment (+%).

Fig 3. Survival of fed or unfed *Cimex lectularius* (A) adults and (B) nymphs exposed to 22 °C (control), 32 °C, 34 °C, or 36 °C for 5 days (pooled survival across temperatures) before being exposed to desiccant dust (Syloid 244FP). The nymphs were offspring produced by the heat-stressed adults and had only experienced heat stress through their parents. Different letters (a or b) denote significant differences between treatments (Kaplan-Meier log-rank tests: \( P \leq 0.05 \)).
We selected two killing agents with a high potential to kill pesticide-resistant bed bugs (Benoit et al., 2009b; Aak et al., 2016, 2018; Barbarin et al., 2017) and combined these treatments with the easily obtainable environmental stressor of sublethal heat. The purpose of this study was to examine the overall benefits of multiple treatments in succession to potentially offer more efficient control. Interactions among different control measures may significantly affect insects survival (Arthur, 2000; Singh et al., 2016; Meyling et al., 2018). Environmental stressors like ambient temperature are subtle, and their effects may be masked by more dramatic control methods such as exposure to pesticides, fungi, or desiccant dusts. They may nevertheless support the control measures and are likely to impact the total outcome (Arthur, 2000; Yoder et al., 2006). In this respect, it is of interest that adults appeared unaffected by the heat until we checked for offspring effects, or until they were exposed to the fungi stressor. Offspring effects due to sublethal heat have previously been documented (Rukke et al., 2015, 2018), but not at temperatures as low as 32 °C. When combined with the fungi, the underpinning temperature stress was clearly visible in both adults and nymphs. We failed to identify similar effects regarding the desiccant dust treatments, most likely because of chronic exposure and because the dose used was still too high to reveal any benefits from the thermal stress. Future studies should avoid chronic exposure to the dust or reduce the dose further. However, regardless of the failure to observe thermal stress effects, the use of silica gel appears effective at extremely low doses. These results are in agreement with a previous dose-response study where chronic exposure of adults without thermal stress yielded 100% mortality later, but still quickly within 4 or 5 days, at doses just above and below the dose used in this study (Aak et al., 2016). This may be considered equivalent to effects observed in the fungi experiment where a five-minute brief exposure killed as efficient as with chronic exposure to the same dose (Aak et al., 2018) only when bed bugs were heat stressed.

The mechanisms underlying the impact of sublethal temperatures in bed bugs have not yet been described in detail, even though the outcome may be prominent (Rukke et al., 2015, 2018). The effect of thermal stress on survival could be a result of multiple factors, including the denaturation of proteins, desiccation, nutrient deprivation (Chown & Nicholson, 2004), production costs of heat-shock proteins (Silbermann & Tatar, 2000), symbiont imbalance (Wernegreen, 2012), fungi-desiccation interactions (Yoder et al., 2006) or dehydration-starvation effects (Rosendale et al., 2017). A more comprehensive understanding of the driving force behind the increased mortality may focus indirect decimation more precisely. The effects on the bed bug nymphs are possibly more intricate but could also be explained by a depletion of resources. For example, heat-stressed adults may produce lower quality eggs that carry fewer nutrients to supply the offspring or fewer symbionts to ensure later vital vitamin B supply (Moriyama et al., 2015). The more subtle effect of heat stress in the adults and the clear temperature-dependent effects in the nymphs also highlights the importance of investigating more than one stage or generation when examining the effect of control efforts. We did not expose the juveniles to heat directly, but if a natural, mixed population is exposed to sublethal heat, it is likely that the reduced survival will be even more evident.

Another interesting result in the present study is the removal of the heat stress effects upon blood feeding. Starved individuals appeared unable to assemble resilience towards the fungal infection, and the bed bugs benefited from a blood meal in all our experiments. Based on this, water-balance and energetic cost are the likely contributing elements because the increased mortality was systematically linked to the level of thermal stress, and it was absent when water and nutrition were supplied through blood. This may be a pivotal element in field situations where bed bugs often experience limited and more sporadic contact with the killing agents than in our laboratory setting. Partial exposure in combination with feeding may, therefore, allow survival until eggs are deposited or until a physiological reset is obtained through moulting. This may contribute to control failure or a prolonged duration of the infestation. Denial of feeding therefore appears to be important for eradication success with fungi, dust and heat (this study) as well as with pesticides (Choe & Campbell, 2014; DeVries et al., 2015; Singh et al., 2016), and it is surely beneficial for controlling egg development (Matos et al., 2017) and life cycle progression through moulting (Reinhart & Siva-Jothy, 2007).

The simplicity of rising temperatures to sublethal levels allows this tool to be easily included in management routines. The laboratory findings are therefore noteworthy because the environmental stress starts at temperatures that may be reached in infested rooms without any specialized equipment. The study also holds relevance for other indoor ectoparasites, because Immature cat fleas and red poultry mites are severely affected by 35 °C (Silverman et al., 1981; Tucci et al., 2008). Previous bed bug studies show shortened life spans among unfed individuals, i.e. a significant cost of elevated temperatures above 34 °C (Rukke et al., 2015; Rukke et al., 2018). Increasing temperatures 10–15 °C above normal room levels is easy and, when simulating such conditions, we found a nymph mortality of up to 100% from heat alone. Additionally, the importance of dual stressors was illustrated by fungal exposure in heat-stressed adults that showed no effect from temperature alone but yielded 30% increased mortality when combined. This indicates some synergy between the heat stress and fungi comparable to benefits observed with pesticides and fungi (Yoder et al., 2006; Farenhorst et al., 2010; Meyling et al., 2018). The importance of an additional stressor is further highlighted by the indirectly affected nymphs and would likely positively affect control efficiency.

Many infestations of are not handled immediately, and the residents often vacate their room or apartment when infestations are discovered. This will leave the bed bugs starving, and if advised to turn on the heat pending actual control, residents or hotel managers may contribute towards a significant increase in control efficiency without any further cost than the electricity used. It is often difficult to vacate premises for a long enough time to truly starve the bed bugs, but elevated temperatures will also contribute towards the eradication process. At 30 °C, eggs will develop rapidly and hatch after 5 days (Omori, 1941), making first instar nymphs emerge faster and consequently become more susceptible to control. Existing nymphs and adults will also use up their energy resources quicker. This may
accelerate the entire population to a point of energy or nutrition shortage, which may further enhance the effectiveness of the control measures as seen in this study. The present results are in line with other laboratory observations that point at reduced survival at higher temperatures (Omori, 1941; Rukke et al., 2015, 2018). However, the transition from the laboratory to field applications is uncertain and should be interpreted with caution. No field studies have evaluated these effects systematically.

This laboratory study suggests that adding several minor contributions to more conventional measures may enhance the efficiency of a dynamic IPM strategy. This demands a combination of both theoretical and applied knowledge among the pest control technicians. Instead of enforcing one general strategy against all bed bug infestations, the technicians must interpret individual situations and select the appropriate means of control to deliver a more precise management. If it is possible to vacate the room and turn on the heat, it is likely to bring forth an increase in the eradication success of other more standard methods. There is, however, a great need for further laboratory and field studies that focus on such combination approaches to more clearly measure and detect optimal strategies. This should be done by evaluating both control efficiency and treatment cost. In this respect, the results presented here highlight some of the smaller, low-cost methods that may tip the balance in favour of bed bug control.

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The authors declare no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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