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Project title

Screening of field-collected bed bug isolates for resistance to non-chemical treatments: are they developing heat resistance?

Investigators

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I. Research Objectives: The two major objectives of this project were:

(1) To develop a diagnostic bioassay for screening bed bugs for heat resistance, and

(2) Determine the status of heat resistance in bed bug field populations/isolates.

II. Objective 1 (diagnostic bioassay standardization):

Bioassay description (Obj. 1): For each bed bug strain and time point, three replicates of 10 mixed sex adult bed bugs (5 M:5F) were placed into a 15-mL test tube with a strip of filter paper as harborage (Figs. 1A and 1B). The decision to use adult males for bioassays was made after collecting preliminary data that compared tolerance levels of adults and nymphs to heat exposure. This data indicated that adults were more tolerant to heat than nymphs (data not shown). Parafilm capped test tubes with bed bugs were then placed in a 12x6 test tube rack and secured with rubber bands to ensure they did not float (Figs. 1A and 1B). Then the rack was placed into a water bath (Isotemp 210, Fisher Scientific, Dubuque, IA) at 45°C (Fig. 1A). Preliminary experiments showed that 45°C or 113°F was an effective temperature, additionally this temperature was found to cause 100% bed bug mortality in other publications (Kells and Goblirsch 2011, Devries et al. 2013). The water level in the bath was marked and held constant for each exposure. Preliminary experiments indicated that if the water level was not consistent, mortality would vary. Additionally, variation in water temperature was monitored using an infrared thermometer. Multiple temperature measurements over time indicated that deviation from the mean temperature of 45°C was ~1% (or ± 0.5°C). Bed bugs were exposed to heat (45°C) for fifteen time points ranging between 10–27 minutes to generate a time mortality curve for each strain. Several exposure time intervals that provided 75–100% mortality was also included to increase the accuracy of LT_{99} estimations. Heated test tubes were removed from the water bath after their elapsed time and bed bugs were placed in a 35x10mm Petri dish (Fischer Scientific, Pittsburg, PA) with a Whatman No. 1 filter paper disc (Fig. 1C). Petri dishes were held in an environmental chamber with 25±1°C temperature, 50% relative humidity, and 12:12 h

light: dark conditions and mortality was scored 24 hours after heat exposure. Insects were scored as dead if they showed minimal body part movement and could not walk or right themselves after being prodded with a toothpick. To determine LT_{50} (lethal time) and LT_{99} PROBIT analysis was conducted using SAS 9.4 to estimate exposure periods (SAS Institute).

Differences and similarities between commercial heat treatment methods and the water bath based bioassay technique (Obj. 1): The diagnostic bioassay does not use a commercial-grade heater as is deployed for heat remediation in the field, but instead utilizes a tabletop temperature-controlled water bath (Fig. 1A). The benefit of using a water bath is that a uniform temperature can be rapidly achieved. With this type of assay, bed bugs are exposed to 45°C by placing them in sealed glass test tubes with paper harborage inside (Fig. 1B) for time intervals ranging from 10 to 27 minutes.

When a bed bug infestation is heat treated by a pest management professional (PMP), the temperatures inside the domicile are slowly increased for energy efficiency. This form of "ramp up" heat that bed bugs are exposed during a heat treatment is different from "heat-shock" (Kells and Gorblisch 2011), which is the exposure method used for this study. Nevertheless, heat shocking bed bugs represents an intense physiological challenge, which would occur under real-world conditions when a bed bug microhabitat is suddenly exposed to high heat.

When bed bugs or any other organisms are exposed to heat, they must respond in one of three ways: escape the heated zone, adapt to the temperature, or die. Our method of heat shocking bed bugs does not allow for the determination of how bed bugs would behaviorally respond to heat exposure, but instead tests their ability to tolerate heat at the thermal knockdown point of 45°C or 113°F.

Results (Obj. 1): Using the abovementioned protocol we have screened multiple bed bug strains from different geographical regions and with different insecticide resistance profiles, and heat exposure histories (Fig. 1 D) for thermotolerance. Initially, exposure time mortality curves were generated for

strains with no prior heat exposure history (Table 1). Based on the statistically determined LT estimates that kill 99 to 100% of test insects (Table 1) and consultation with raw time mortality data (Fig. 2), exposure of bed bugs at 45°C for 27 minutes was found to kill 100% of bed bug strains with no prior heat exposure history. We considered both statistically determined probit LT data and raw mortality data for determining the diagnostic exposure time because if the mortality response exhibited by strains is heterogeneous, it can lead to over or underestimation of LT_{99} values (see footnote for Table 1). Raw mortality data graphs (Fig. 2) indicated that exposure to 45°C for 22 to 27 minutes was sufficient to kill all bed bugs of strains with no prior heat exposure history (Table 1 and Fig. 2). If there are bed bug strains that would survive exposure to 45°C for 27 minutes, it would indicate the presence of heat resistance. Strains with greater heat resistance (if identified) may have a higher chance of surviving heat exposure in the field in comparison to other field strains.

III. Objective 2 (screening of bed bug strains for heat resistance):

Establishment of strains from heat-treated accounts (Obj 2): Initial requests for bed bug collections received a significant level of response from several pest management companies. However, collections that we received (from >15 different locations) typically included only a few bed bugs (1 to 5 or 6) that were not sufficient to develop laboratory colonies. It should be noted, however, that under field conditions bed bug infestations can start from even a single gravid female. Additionally, adapting field-collected bed bugs to laboratory conditions was constrained by: (i) the collection of only male bed bugs from some accounts and (ii) challenges associated with acclimating bed bugs to feed on rabbit blood.

Five bed bug populations that had survived heat exposure in the field did successfully develop into lab strains. However, three of the total five strains from heat-treated accounts are still not breeding prolifically on rabbit blood source and we haven't been able to test these strains yet. Since, we could not

reach our intended goal of developing 10 or more heat exposed bed bug strains, full exposure time-mortality curves and LT_{99} values for some of these strains were determined using similar procedures described under objective 1. Generating complete time-mortality curves for many different bed bug populations allows for a more robust statistical analysis and comparison.

Results (Obj. 2): Results of the exposure time-mortality curve at 45°C or 113°F indicate that the time required to kill ~99-100% of insects from certain heat-treated accounts (Lafayette, IN and Raleigh, NC) is not significantly different (based on overlap of 95% fiducial limits) than that for other field strains from non-heat treated accounts (Table 1 and Figs. 2 and 3). At present, our initial results indicate the absence of physiological heat resistance in field strains previously exposed to heat. Additional bed bug strains collected from heat-treated accounts will be tested using funds from other grants. Interestingly, the Richmond strain, which has >5000-fold deltamethrin resistance (Adelman et al. 2011) and the Knoxville strain that shows reduced susceptibility to chlorfenapyr and bifenthrin (Ashbrook et al. 2017), displayed slightly increased, but statistically non-significant, thermo-tolerance in comparison to other previously heat-exposed and non-exposed bed bug populations (Table 1 and Fig. 4). The Richmond strain possesses high-level deltamethrin resistance due to increased expression of detoxification enzymes, point mutations in the pyrethroid target site, and decreased insecticide penetration through the cuticle. Perhaps the up-regulation of detoxifying enzymes and/or the presence of thicker cuticle is responsible for thermo-tolerance and cellular recovery from heat stress in the Richmond strain. The possibility of insecticide resistant strains showing higher heat tolerance needs to be further explored.

IV. Conclusions, impacts, and future directions:

1. Through this project we have developed a simple diagnostic bioassay test (exposure at 45°C or 113°F for 27 minutes) that can be used to screen bed bugs that are surviving heat treatments for heat resistance or their ability to physiologically tolerate heat exposure.

2. Thus far we have not identified the presence of high heat resistance in bed bug strains that have been collected from previously heat-treated accounts.

3. By the fall of 2017 we plan to submit a manuscript to the Journal of Economic Entomology that describes the diagnostic bioassay and data collected for previously heat-exposed and insecticide resistant strains. Additionally, PMP-oriented publications, which describe the diagnostic bioassay method can be made available in trade magazines at an earlier date.

4. The diagnostic bioassay method may be implemented in-house by pest control companies with the proper equipment. In addition, the Purdue Urban Center can be a permanent resource for companies to send live bed bug samples (at least 30 insects) for heat resistance screening.

5. To overcome the problems associated with adapting a few field-collected bed bugs into laboratory colonies, larger collections (~30 to 35 or more insects) can be made from accounts where bed bug re-infestations have occurred after heat treatments. These insects could then be directly tested in the diagnostic bioassay.

6. The detection of slightly higher, but statistically non-significant thermo-tolerance within the insecticide resistant Richmond and Knoxville strain is an important finding that requires further investigation.

8. This study used a “heat-shock” or “step-up” method of heat exposure as opposed to slow “ramp-up” and “ramp-down”, which is widely used in the field. In future research, it will be important to test both of these techniques simultaneously to determine differences in bed bug mortality response.

9. Overall, this study did not find any evidence for physiological heat tolerance/resistance in bed bugs, but it is likely that some of the strains reported here may display different behavioral (escape) responses at the population level. These studies are currently underway at the Purdue University urban center.

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References:

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Table 1. Lethal time estimates required for achieving 99 to 100% mortality (LT_{99}) in bed bug strains with different heat exposure and insecticide resistance histories

Strain category	Strain name (collection state)	n (# of adult bed bugs tested)	Slope (±SE)	Chi-square (degrees of freedom)	LT_{99} value in minutes (and 95% fiducial limits
No history of previous heat exposure	Harlan (NJ)	480	10.99 (0.96)	12.31 (13)	23.16 (21.41–25.35)
	Hackensack (NJ)*	480	11.99 (2.17)	80.18 (13)	26.50 (23.32–35.19)
	Poultry House (TN)	480	8.63 (0.71)	15.72 (13)	26.97 (24.51–30.03)
	KVS (FL)*	290	12.04 (1.86)	26.31 (13)	28.35 (25.26–35.31)
	Bradenton (FL)	190	10.93 (2.43)	7.32 (7)	26.13 (19.56–36.44)
Strains with heat exposure history	Raleigh (NC)*	480	17.26 (3.23)	56.33 (13)	22.35 (20.22–28.32)
	Lafayette (IN)*	480	11.46 (1.50)	41.49 (13)	26.37 (23.58–35.44)
Insecticide resistant strains	Richmond (VA)*	480	7.26 (0.87)	26.98 (13)	30.00 (26.05–37.41)
	Knoxville (TN)*	420	8.77 (1.11)	38.10 (13)	29.46 (26.05–37.07)

(*) asterisk indicates bed bug strains that showed heterogeneous mortality responses in bioassays.

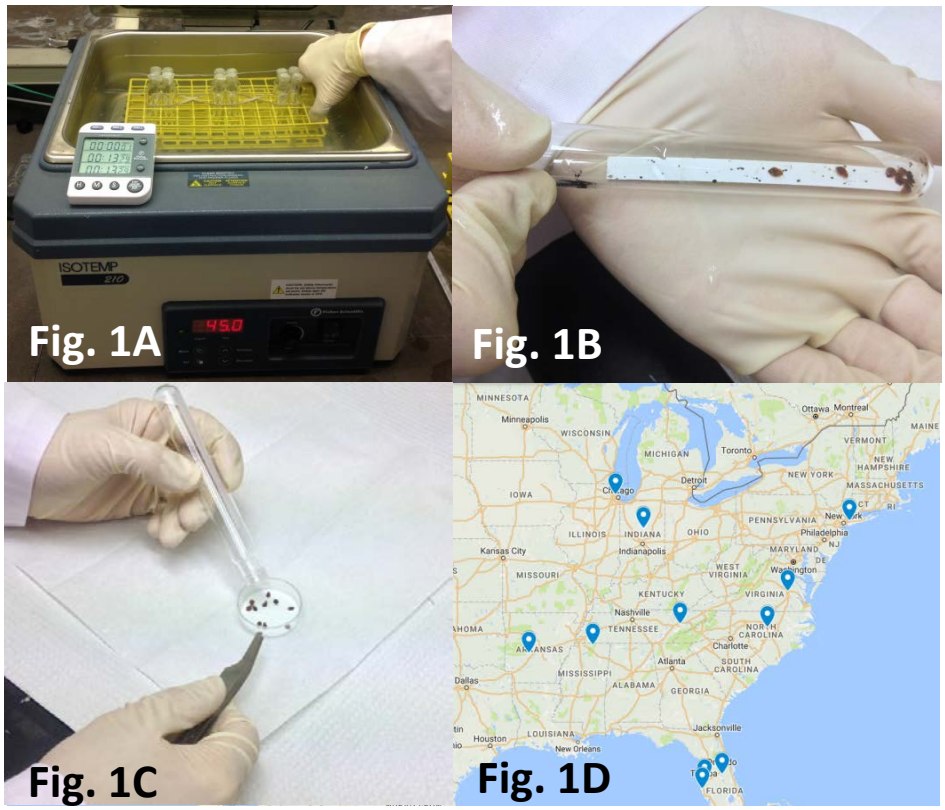

Figure 1. Experimental set up and a map showing geographical origins of bed bug populations used in this study. (1A) Shows the water bath setup used for heat exposure experiments. (1B) Shows bed bugs confined in a 15-mL glass test tube with filter paper harborage just prior to testing. (1C) After heat exposure all bed bugs (alive and dead) were transferred to a filter paper lined Petri dish for 24 h, i.e., until mortality observations were recorded. (1D) Shows geographical origins of bed bugs strains used in this study (note that multiple populations were collected from some locations).

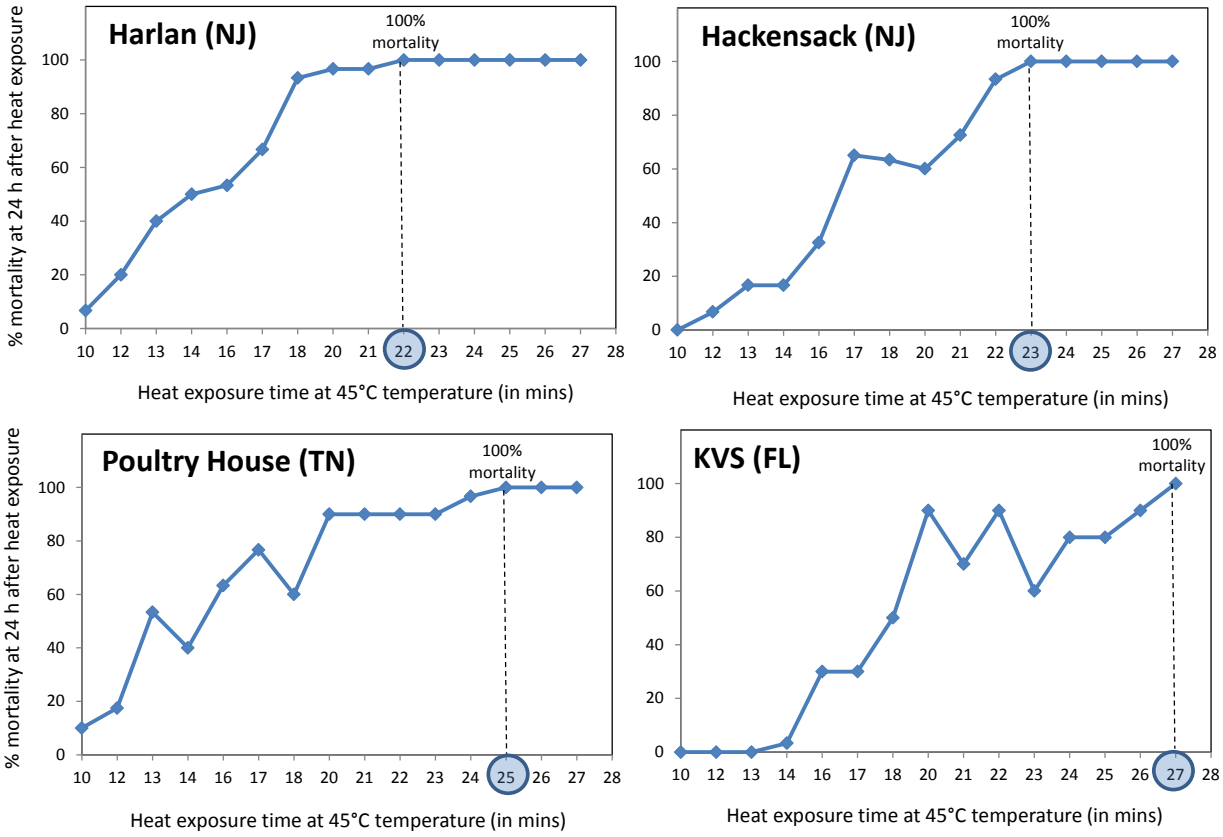

Figure 2. Exposure time-mortality curves at 45°C for bed bug strains with no prior heat exposure history. For each strain data is based on 400 to 480 adult bed bugs (1:1 male: female ratio). The vertical dotted line shows the exposure time at which 100% mortality was first observed. Note that in strains from more northern regions, (Harlan and Hackensack) 100% mortality was achieved after 22 to 23 minutes of exposure, but in southern strains (Poultry House and KVS) 100% mortality was achieved after 25 to 27 minutes of exposure. However, the higher exposure times required for complete mortality in southern strains was not statistically different from northern strains.

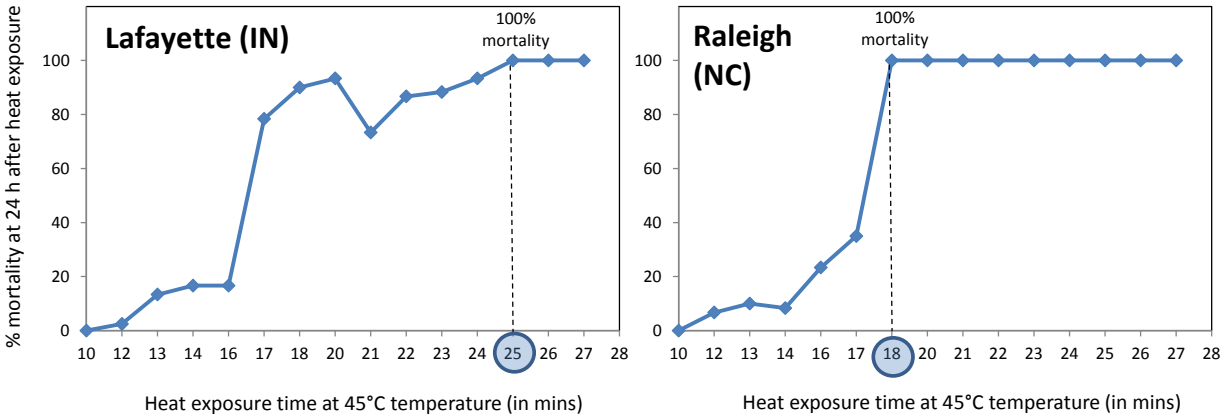

Figure 3. Exposure time-mortality curves at 45°C for bed bug strains with prior heat exposure history. For each strain, data is based on 480 adult bed bugs (1:1 male: female ratio). The vertical dotted line shows the exposure time at which 100% mortality was first observed. The time required to achieve 100% mortality was not significantly different from the time required to achieve complete mortality in strains with no prior heat exposure history (see Fig. 2).

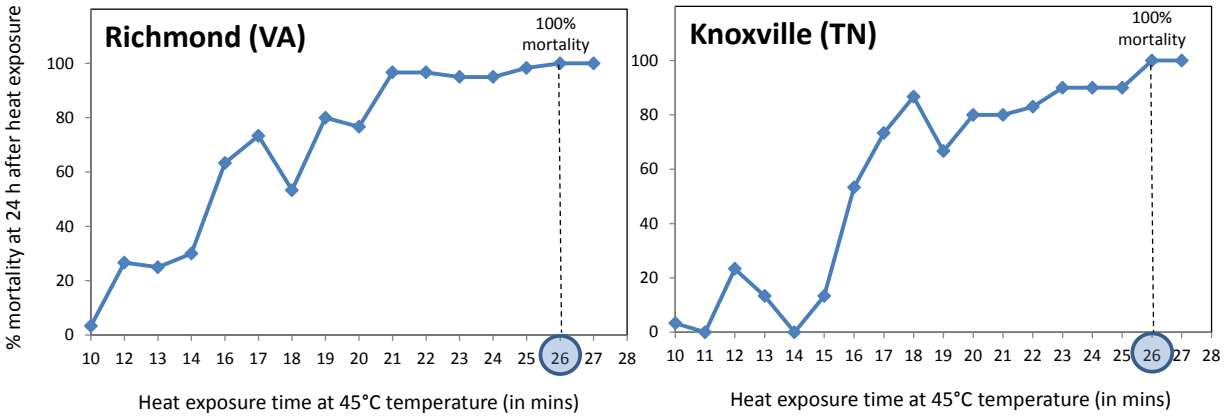

Figure 4. Exposure time-mortality curves at 45°C for insecticide resistant bed bug strains. For each strain, data is based on 480 adult bed bugs (1:1 male: female ratio). The vertical dotted line shows the exposure time at which 100% mortality was first observed. Based on these mortality graphs, time required to achieve 100% mortality in insecticide resistant strains was similar to other strains shown in Figs. 2 and 3. However, probit analysis estimated that slightly higher exposure time (~ 30 mins) is required to achieve complete mortality in these insecticide resistant bed bug strains (see Table 1).