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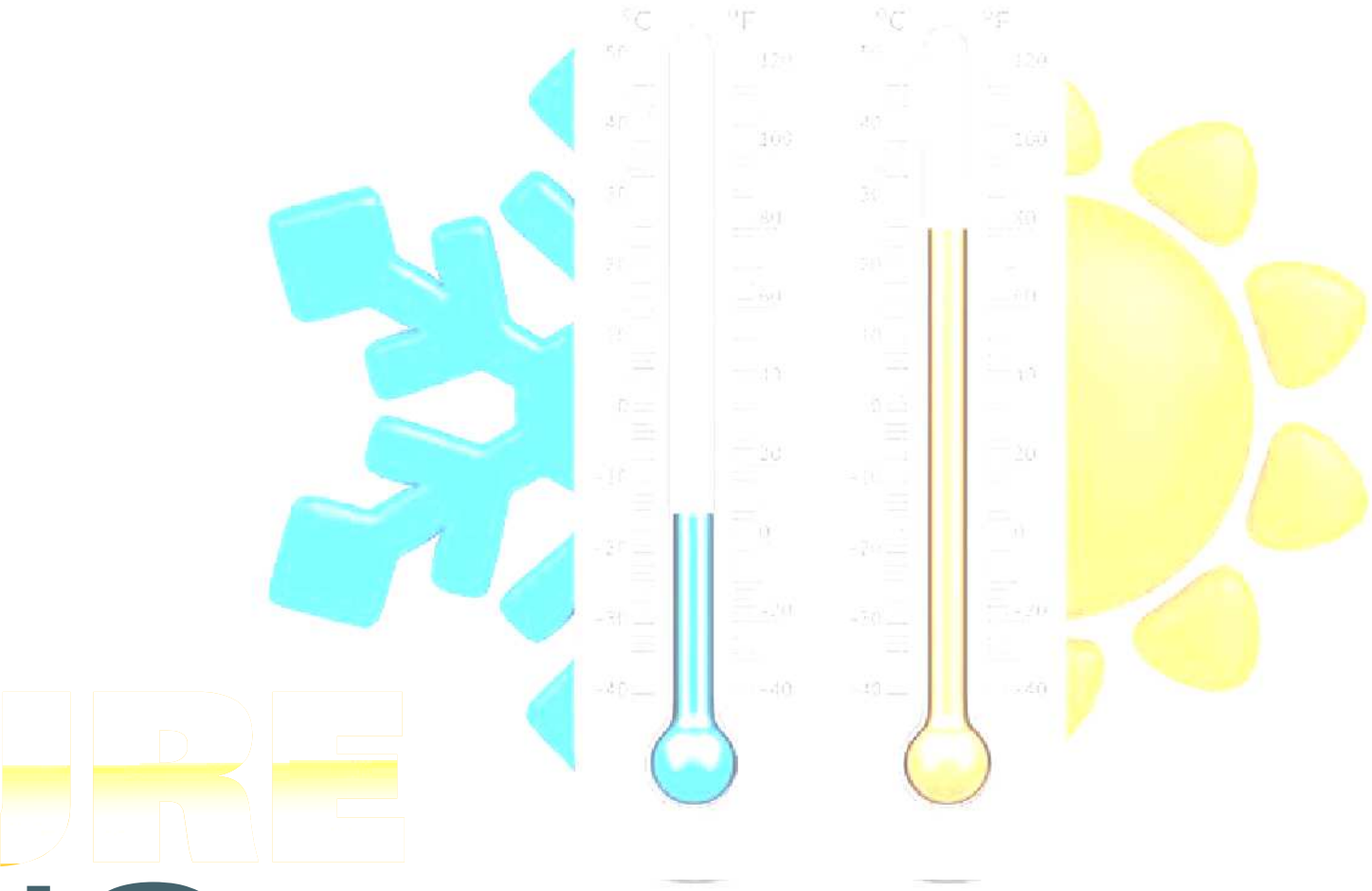
VARIABLE TEMPERATU CONDITION

Executive Summary

Recently laid bed bug eggs were divided into two groups, eighty-eight and sixty-four, and subjected to different rearing temperature conditions using two laboratory incubators. The first group of eggs was reared at a constant temperature 78 °F (25.5 °C) for 24-hrs/day and the second group was reared at a variable temperature that oscillated between 58 °F (14.4 °C) for 12 hours and 68 °F (20 °C) for 12 hours. Each incubator was equipped with automated temperature recording device and software. All eggs were checked daily for hatch. The study continued until eggs for both incubators had hatched or were determined to be non-viable based on weekly inspections with a stereo dissection microscope. Days to egg hatch were significantly different for the two rearing temperatures; the mean days to hatch for the variable temperature incubator was 2-fold greater (15.7 versus 6.1 days) than those eggs reared at constant temperature. Egg survivorship was similar for both constant and variable temperature rearing groups, 98% and 97%, respectively. The current pest management professionals (PMP) practice is to wait 7-10 days after treatment before making claims of treatment outcome. However, our results support modification to this strategy. Alternatively, post-treatment evaluation periods for egg hatch could be based on a day-degree model that more accurately reflect development



URE NS » DELAY EGG HATCH FOR THE BED BUG, CIMEX LECTULARIUS

times for all bed bug life stages based on actual temperatures encountered under field conditions.

Introduction and Justification

The greatest challenge in the detection and control of bed bugs involves their eggs. Bed bug eggs are small-1 mm, have a sticky coating, and females tend to lay them in concealed and hard-to-see locations (Usinger 1966). Among the developmental forms of bed bugs it's virtually impossible to visually detect all eggs and difficult to kill them (Cooper 2011, Pinto et al. 2007).

Determining the time to bed bug egg hatch is also challenging. Egg hatch rates have been reported going back 70 years, and based on experimental laboratory studies, were highly variable (4-21 days) based on the rearing temperature (18-33 °C) (Usinger 1966). Reviewing the literature today, there are no published studies that verify these earlier results. The current industry practice for follow-up inspections and assurances of the success of control includes searching for hatched eggs 7-10 days post-treatment (Snetsinger 1997, Cooper and Harlan 2004, Pinto et al 2007, Cooper 2011). However, there are increasing complaints of bites 4-5 wks post-treatment (Cooper 2006) and the source of these bites, either new introductions or hatching of eggs after previous control attempts, still remains unclear. Additionally, egg hatch rate is impacted

when people employ energy conservation tactics by lowering overall room temperature or only using heat when room occupants are home and awake. For our investigations, we compared the hatch rate of eggs maintained in incubators at a constant 78 °F (25.5 °C) temperature for 24-hrs to those maintained in a variable temperature incubator that oscillated between 58 °F (14.4 °C) for 12 hours and 68 °F (20 °C) for 12 hours. Our hypothesis was that eggs reared at the variable temperature would take significantly longer to hatch compared to eggs reared at constant temperature. The relevance of this research has greatest importance for those locations in the United States that undergo winters, especially those that experience significant snowfall and where heating of rooms and structures is most extreme, but could also include those structures in locations that have adopted energy conservation tactics. The objectives and methods for our investigation are listed below.

FIGURE 1.

Laboratory setup for determining bed bug egg hatch rates for constant and variable temperatures. 1A) 12.7 cm diameter round plastic Petri dishes for maintaining female bed bugs and eggs. The small writing on labeling tape and filter paper pads denotes the number of eggs and date laid. Each small black circle contains a small cluster of bed bug eggs. 1B) Exterior view of variable temperature incubator (Echotherm, Torrey Pines Scientific, Carlsbad, CA). 1C) Interior view of same variable temperature incubator and large plastic containers containing bed bugs eggs at UC Richmond Field Station, Richmond, CA.

Objectives

1. To compare the hatch rate for eggs reared in incubators at constant and variable temperature.
2. To document any differences in mortality for eggs reared in incubators at constant and variable temperature.

Materials and Methods

Bed bug source. Live and healthy female bed bugs were purchased from Sierra Research Laboratories in Modesto, CA. This laboratory has a federal permit to rear bed bugs on laboratory maintained mammals. The particular strain we used for testing was called "Earl", and was field collected from Modesto, CA in 2007. This field strain has been in colony for five years and based on Sierra Research Laboratory tests is susceptible to pyrethroids, imidacloprid, and propoxur (Sierra Research Laboratory, unpublished data). We used females that were recently fed, within a few days from the supplier; however to maintain females for egg laying we occasionally fed them on an artificial feeding system following the methods of Lewis et al. 2012.

Inserting female bed bugs into incubators. Fifty recently fed females were shipped overnight to our lab from Sierra Research Lab in Modesto, CA. The group of females were equally divided into five groups of five and placed inside a 12.7-cm dia clear plastic Petri dish and fitted with a friction fitting top (Fig. 1A). A single Petri dish containing five females was inserted into a constant 78 °F (25.5 °C) temperature incubator for 24-hrs (Heratherm IMC 18, Theromo Scientific, Langenselbold, Germany) and a second container and group of five females was inserted into a variable temperature incubator that oscillated between 58 °F (14.4 °C) for 12 hours and 68 °F (20 °C) for 12 hours (Echotherm incubator, Torrey Pines Scientific, Carlsbad, CA) (Fig. 1B, C). Both incubators were equipped with automated temperature recording devices and software (Hobe temperature data logger U10; and Hobo temperature logger kit, U10-001; Onset Computer Corporation, Cape Cod, MA). Females were allowed to lay eggs on provided filter paper in each Petri dish.

Collecting eggs. Both Petri dishes were checked daily between 3-5 PM for newly laid eggs. When eggs were seen on the filter paper, the entire assembly was removed and placed into a large stainless steel pan. To collect eggs, adult females were subjected to a brief exposure (several seconds) of CO_2 to safety sedate them. A small paintbrush was used to slide female bed bugs off of the filter paper, exposing the laid eggs. Since female bed bugs can secrete an adhesive and affix eggs to surfaces, small clusters of eggs were often encountered. A small piece of filter paper and attached eggs was later cut and transferred to a new Petri dish, and labeled with the date eggs were laid, number of eggs in the cluster, and incubator identification number. Since each bed bug female laid several eggs a day over several weeks, the actual number and replicate for eggs for each treatment/incubator group was variable. However, we targeted at least 50 individual eggs for each treatment group.

Monitoring eggs for hatch. We visually searched all containers daily between 3-5 PM for newly hatched eggs. In addition to our visual searching, once a week we also looked at all eggs under a 20X magnification stereo-viewing microscope for the condition of developing embryos (specifically, dark eye spots on head). We continued to check unhatched eggs for 60 days or until the egg no longer looked viable. At the termination of the study, any remaining unhatched eggs were visually checked using the same microscope and a final determination (dead or unknown) was given.

Statistical analyses. The variable of interest compared days to egg hatch with rearing temperatures between incubators. For our investigation, a single egg was considered a replicate. Histogram plots were created showing the frequency distributions in days to hatch between the two incubator rearing conditions. The mean days to hatch between the two incubators were compared for significant differences using a Welch Two sample *t*-test (SAS 1994). Temperature line traces for both incubators during the entire study were also plotted using graphic methods provided by R-Development Core Team 2004.

Results and Discussion

The mean days to egg hatch were dramatically different between the two temperature conditions (Fig. 2). The mean days to hatch for the constant temperate group was 6.05 ± 0.45 days; however for eggs reared in the variable temperature group, there was a 2-fold higher number of days needed for hatch, 15.7 ± 1.7. This difference was significant (*t*-test = 42. 55; DF = 67/147; $P < 0.002$). The line traces from both incubators also collaborates the constant temperature conditions inside the control incubator and cycle temperature conditions for the variable incubator (Fig. 3). Survivorship to hatch between constant and variable temperature groups were almost identical; 98% and 97%, respectively.

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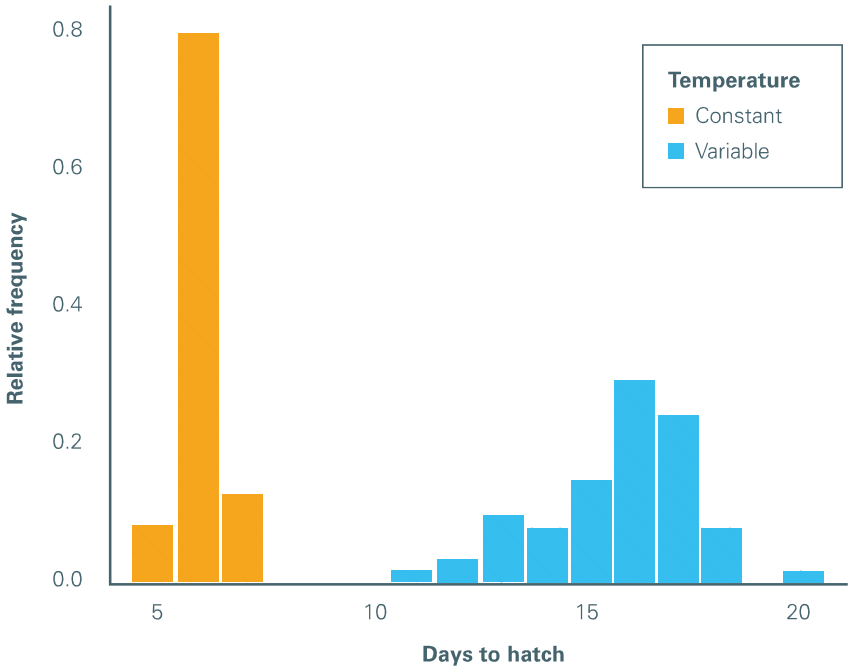
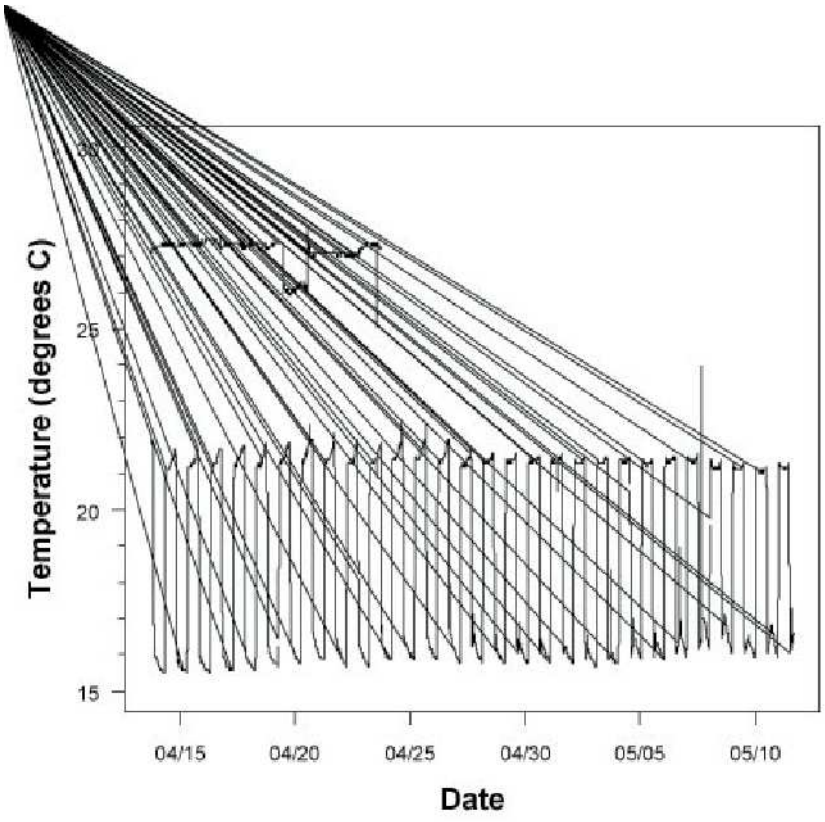
FIGURE 2.



Histogram plots of days to hatch for bed bug eggs reared at constant temperature (Heratherm IMC 18, Theromo Scientific, Langenselbold, Germany) and variable temperature (Echotherm, Torrey Pines Scientific, Carlsbad, CA) incubators at UC Richmond Field Station, Richmond, CA.

Time to bed bug egg hatch is variable and depending on temperature can range from 4 days at 33 ^{0}C to 21 days at 18 ^{0}C (Usinger 1966). Our results lie in-between these previous investigations and provide further documentation on the flexibility of this public health pest and its ability to exploit and expand into new habitats. The current practice of waiting 7-10 days after treatment for egg hatch has its roots going back to the 1990s when bed bugs had virtually disappeared from urban habitats in North American due to the successful post-World War II control campaigns using synthetic insecticides and increasing standards of habitat cleanliness (Ebeling 1978, Moore and Miller 2006). However, today with increased international travel and pesticide resistance, a resurgence of bed bugs is occurring (Cooper 2011) and points to the need to re-evaluate post-treatment practices based on egg hatch. Our results support this revised strategy. As an alternative strategy, post-treatment evaluation periods for egg hatch can be based on a day-degree model that more accurately reflect development times for bed bug life stages based on actual temperatures encountered under field conditions. These day-degree models are common for agricultural pest systems (Roltsch et al. 1999); however more recent models have been developed for cockroaches (Stejshal et al. 2003). Perhaps future day-degree models can be developed for bed bugs that include egg hatch. The results from our investigation were presented at Pest World 2012 in Boston, MA. «

FIGURE 3.



Daily temperature traces for temperatures recorded from constant (Heratherm IMC 18, Theromo Scientific, Langenselbold, Germany) and variable temperature incubators (Echotherm, Torrey Pines Scientific, Carlsbad, CA). The upper line trace is from the constant temperature incubator. The two dips in the curve represent the removal of eggs to check on development and the later date represents the termination of the study. The lower trace shows the oscillation between 58 oF (14.4 oC) for 12-hours and 68 oF (20 oC) for 12-hours pattern for the variable temperature incubator. Both incubators contained automated temperature recording devices and software (Hobe temperature data logger U10; and Hobo temperature logger kit, U10-001; Onset Computer Corporation, Cape Cod, MA) maintained at UC Richmond Field Station, Richmond, CA.

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