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FEEDING VARIATION AND ENVIRONMENTAL EFFECTS ON SUSCEPTIBILITY OF *CIMEX LECTULARIUS* (HEMIPTERA: CIMICIDAE) TO CHLORFENAPYR INSECTICIDE

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Abstract Bed bugs were evaluated in the laboratory to determine the effects of varying feeding regimens and environmental conditions on their susceptibility to two commercial products containing chlorfenapyr, Phantom® II Aerosol and Phantom® SC Insecticide. In the first study, bed bugs were reared under four different feeding regimens (daily, random, five-day period, and seven-day period) and exposed to both products over a three-week period. In the second study, bed bugs were exposed only to Phantom SC Insecticide and held under differing environmental conditions (temperature, humidity, and light) to determine their susceptibility to chlorfenapyr over 21 days. The temperature regimens maintained in the second study were either 20ºC or 26ºC and the humidity levels were 20-60%, 40%, or 75%. The light to dark cycle was 10 hours light:14 hours dark, 14 hours light:10 hours dark, or variable at 8-12 hours light:8-12 hours dark. Results show higher mortality with Phantom II Aerosol under the seven-day feeding regimen and daily feeding with Phantom SC Insecticide. Higher temperatures and increased humidity regimens increased the susceptibility of bed bugs to Phantom SC Insecticide while the duration of light or dark photoperiod did not have any effect.

Key words Bed bug, Phantom II Aerosol, Phantom SC Insecticide, temperature, humidity, light

INTRODUCTION

Chlorfenapyr, the active ingredient in the Phantom line of products from BASF, is a pyrrole in the Insecticide Resistance Action Committee (IRAC) Class 13. The mode of action is uncoupler of oxidative phosphorylation via disruption of the proton gradient. PT Phantom II Aerosol and Phantom SC Insecticide are registered in a number of countries for bed bugs. A number of researchers have tested the efficacy of chlorfenapyr on bed bugs with mixed results. (Doggett et al., 2012; Moore and Miller, 2006; Moore and Miller, 2008; Potter et al., 2008; Romero et al., 2010; Tawatsin et al., 2011). Due to the mode of action of chlorfenapyr, mortality takes longer to occur because the insecticide is converted to the active form in the insect which then affects the mitochondria in the cells resulting in energy depletion and ultimately death of the insect. Any factors that speed up the insect metabolism will result in more rapid conversion of chlorfenapyr to the active form leading to faster mortality of the insect. Some of the factors that could potentially influence insect metabolism include feeding frequency, temperature, humidity, and light:dark cycle. The objective of these studies was to test these factors to determine if there was any effect on the susceptibility of bed bugs to chlorfenapyr.

MATERIALS AND METHODS

Harlan susceptible strain bed bugs were used for these studies. The membrane feeding method developed by Chin-Heady et al. (2013) was used to feed defibrinated rabbit blood to the insects. A reptile heating pad was used to keep the blood dispensed in a Petri dish warmed to 35–37^{0}C. A fine nylon mesh at the bottom of the rearing jar allowed bed bugs to consume a blood meal. Rearing was conducted at 26^{0}C, 40% RH and 12:12 h light: dark cycle.

Varying Feeding Regimens Study. Bed bugs were reared on 4 different feeding regimens for 1–2 generations before using them in insecticide bioassays. To initiate the feeding regimens, unfed first instar nymphs were used. Feeding regimens continued during the 3-week insecticide susceptibility bioassays.
The treatments were as follows:

1. Daily Feeding: Bed bugs were offered a blood meal daily for 30 minutes.
2. Random Feeding: Bed bugs were fed randomly every 1 to 7 days. Generally, they were fed 1–3 times per week.
3. 5-Day Feeding: Bed bugs were provided a blood meal every 5 days.
4. 7-Day Feeding: A blood meal was provided once every 7 days.

Adult males and females (1:1 ratio) were used for all bioassays. Ten adults (males: females in 1:1 ratio) were used per replicate and four replicates were performed for each treatment (n = 40 per treatment). Feeding regimens of insects were continued during the 3-week bioassay period or until all insects were dead.

For the PT Phantom II Aerosol bioassays, bed bugs were held in 10 cm diameter plastic Petri dishes with a sanded surface. The sanded surface allowed the bed bugs to easily walk in the Petri dish and prevented aggregation or balling which is commonly seen on smooth surfaces. Each replicate was treated with two 1-second passes of Phantom II Aerosol. The Phantom SC Insecticide (24% chlorfenapyr SC) was diluted to 0.5% in tap water. A 3.3-ounce (100 ml) spray bottle was used for treating bed bugs. Bed bugs held in sanded 10 cm Petri dishes were treated with 0.26 ml of insecticide solution which is equivalent to the 1 gallon per 1000 sq. ft. application rate. For the water-treated control treatment, a 3.3-ounce (100 ml) spray bottle was used for treating bed bugs with tap water. Bed bugs held in sanded 10 cm Petri dishes were treated with 0.26 ml of water which is equivalent to the 1 gallon per 1000 sq. ft. application rate. Treated bed bugs were left in sanded Petri dishes for 3 h after treatment and then transferred to meshed jars with folded index card harborages that were used for feeding.

Bed bugs that were unable to walk or display significant body movement other than occasional leg twitching were scored as dead. Mortality was recorded at 1 h, 6 h, 24 h, 3 d, 7 d, 14 d and 21 d intervals.

Data Analysis Data from each treatment were subjected to probit analysis in SAS 9.4 to determine lethal time (LT) required to achieve 50 or 90% mortality. Mortality observed in water-treated control bed bugs was corrected/ accounted for while performing probit analysis. Additionally, to depict mortality trends in line graphs, data were converted to percentage and averaged across the four replicates for each treatment.

Environmental Effects Study. Adult males and females (1:1 ratio) were used for testing. Ten adults (males: females in 1:1 ratio) were used per replicate and three replicates were performed for each treatment (n = 30 per treatment). Bed bugs were fed 3 days prior to the initiation of the study.
The treatments were as follows:

1. 68°F 75%RH and 10:14 light:dark cycle
2. 68°F 75%RH and 14:10 light:dark cycle
3. 78°F 75%RH and 10:14 light:dark cycle
4. 78°F 75%RH and 14:10 light:dark cycle
5. 68°F 40%RH and 10:14 light:dark cycle
6. 68°F 40%RH and 14:10 light:dark cycle
7. 78°F 40%RH and 10:14 light:dark cycle
8. 78°F 40%RH and 14:10 light:dark cycle
9. 68°F with variable RH (20-60%) and light:dark cycle (8–12 hours light and 8–12 hours dark)
10. 78°F with variable RH (20-60%) & light: dark cycle (8–12 hours light and 8–12 hours dark)

Phantom SC Insecticide was diluted in tap water to achieve 0.5% concentration. Water or Phantom SC 0.5% was sprayed onto the 10 cm diameter Petri dish at 1 gallon per 1000 sq. ft. At this rate, the volume of application for the Petri dish corresponded to 0.26 ml of Phantom SC Insecticide or water. Insecticide or water was applied to the Petri dish using a 3.3 oz (100 ml) handheld sprayer. Treated plates were allowed to dry overnight. After overnight (16 h) drying of Phantom SC Insecticide or water-treated Petri dishes, ten adult bed bugs (males: females in 1:1 ratio) were released into each dish. Exposure of insects to different environmental conditions began immediately after they were released in the treated or control Petri dish. After 24 h exposure in the treated or control Petri dishes, bed bugs were transferred to a 3.5 cm diameter Petri dish with filter paper substrate. Mortality characterized by lack of body movement or ability to walk were recorded at 1, 3, 7, 10, 14 and 21 d.

Data Analysis. Day 21 mortality data was transformed to percentage and averaged across the three replicates conducted for each treatment. No statistical analysis test was performed with day 21 mortality data reported here.

RESULTS AND DISCUSSION

Varying Feeding Regimens Study

In the PT Phantom II Aerosol treatments, LT_{50} and LT_{90} were highest for the daily fed group (1.38 and 4.66) and lowest for those fed every 7 days (0.08 -0.29) (Table 1). Time to 100% mortality was quicker in the 7-day fed group while control mortality was higher in the daily fed groups (Figure 1). The LT_{50} and LT_{90} of Phantom SC Insecticide were highest (9.79 and 33.60, 14.65 and 30.24, respectively) and mortality the lowest in the 5- and 7-day feeding regimens (Figure 2). These results clearly show that chlorfenapyr kills bed bugs with Phantom II Aerosol providing quicker mortality than Phantom SC Insecticide. This may be due to the presence of crystals of the active ingredient on surfaces when applied as an aerosol which are adsorbed quicker or in greater amounts than the SC formulation. The uptake of the active ingredient by bed bugs from a Phantom II Aerosol application appears not to be influenced by the frequency of feeding. Dose acquisition and metabolism of chlorfenapyr by bed bugs when applied as an SC formulation appears to be influenced by the frequency of feeding. The higher mortality and lower LT_{50} and LT_{90} values observed with Phantom SC Insecticide in daily and random-feeding regimens may be due to the increased metabolism of the bed bug which converts the active ingredient to the active form more rapidly. The current practice of most researchers is to feed bed bugs on a weekly basis which may not be representative of wild bed bug populations which feed on a more frequent basis. A daily or random feeding regimen better mimics a real-world situation which is the recommendation for feeding bed bugs when testing Phantom SC Insecticide.

Table 1. LT_{50} and LT_{90} of bed bugs exposed to PT Phantom II Aerosol and Phantom SC Insecticide under various feeding regimes.

Treatment	Slope (±SE)	LT_{50} in days (95% fiducial limits)	LT_{90} in days (95% fiducial limits)	Heterogeneity Factor*
PT Phantom II Pressurized Insecticide				
Daily Feeding	2.26 (0.68)	1.28 (0.11–3.26)	4.66 (1.93–104.95)	4.35
Random Feeding	1.46 (0.23)	0.22 (0.11–0.35)	1.62 (1.01–3.15)	-.--
5 Day Feeding	1.26 (0.17)	0.17 (0.09–0.28)	1.8 (1.10–3.54)	-.--
7 Day Feeding	2.35 (0.43)	0.08 (0.05–0.12)	0.29 (0.19–0.56)	-.--
Phantom SC Insecticide/Termiticide				
Daily Feeding	3.53 (1.82)	7.07 (Not Determinable)**	16.32 (Not Determinable)**	9.03
Random Feeding	3.25 (1.19)	6.63 (0.046–15.92)	16.43 (8.40–388302)	5.86
5 Day Feeding	2.39 (0.78)	9.79 (2.81–23.25)	33.60 (16.90–12367)	3.11
7 Day Feeding	4.07 (2.32)	14.65 (Not Determinable)**	30.24 (Not Determinable)**	5.25

Environmental Effects Study

Mortality in the water-treated controls was mostly in the 5 to 10% range. The duration of light or dark photoperiod did not affect mortality levels (Figure 3) since there did not appear to be any differences between the two L:D cycles. Higher temperature and humidity increased the susceptibility of bed bugs to Phantom SC Insecticide with temperature clearly having a greater effect. A temperature of 78°F and a relative humidity of 75% exhibited 100% bed bug mortality followed by the treatments of 78°F and relative humidity of 40% which exhibited 80% mortality at 21 days. This study shows that combinations of higher temperatures and humidity levels likely lead to a synergistic increase in bed bug susceptibility to Phantom SC. This is likely due to the bed bug being more active which results in increased metabolism of the chlorfenapyr molecule into the active form. For future efficacy testing on bed bugs, feeding regimens and environmental factors should be taken into consideration when designing the studies.

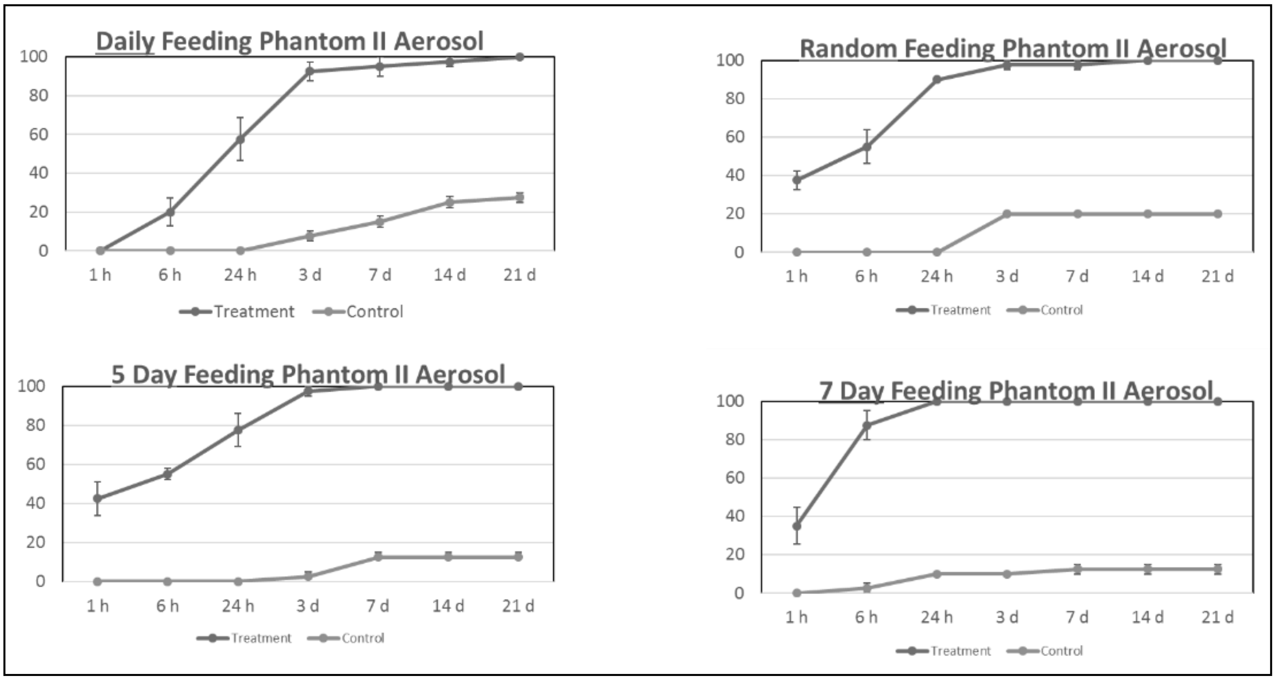

Figure 1. Percent bed bug mortality over time when exposed to PT Phantom II Aerosol under various feeding regimes.

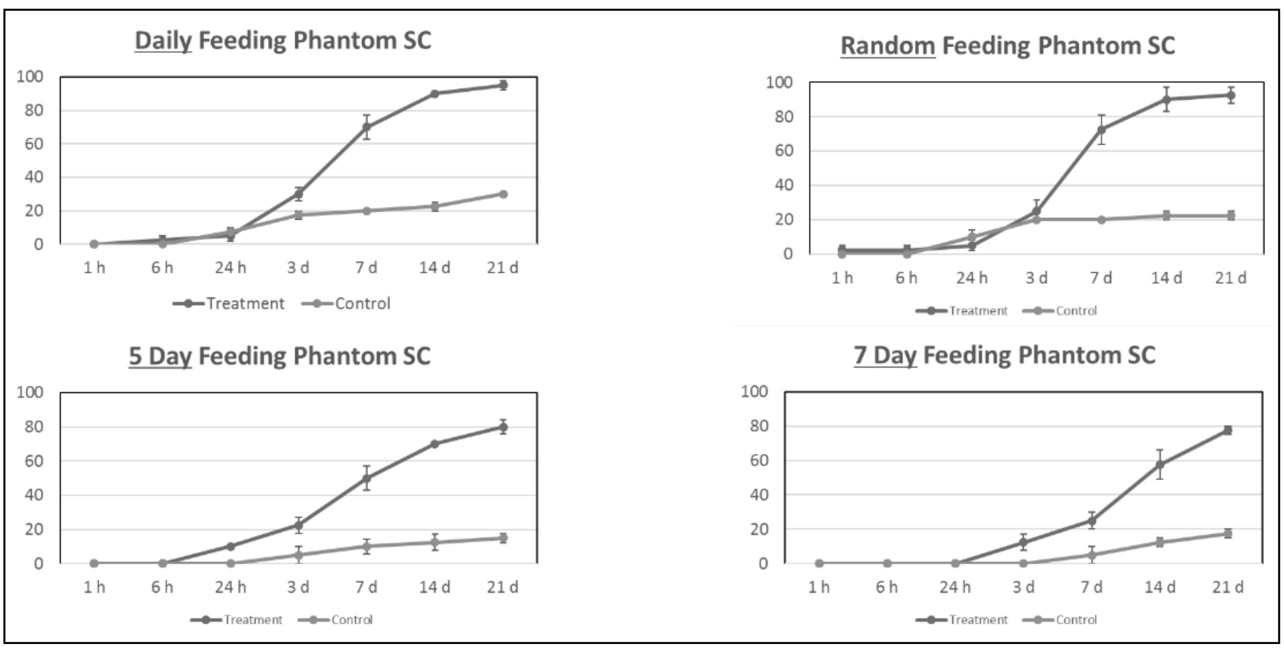

Figure 2. Percent bed bug mortality over time when exposed to Phantom SC Insecticide under various feeding regimes.

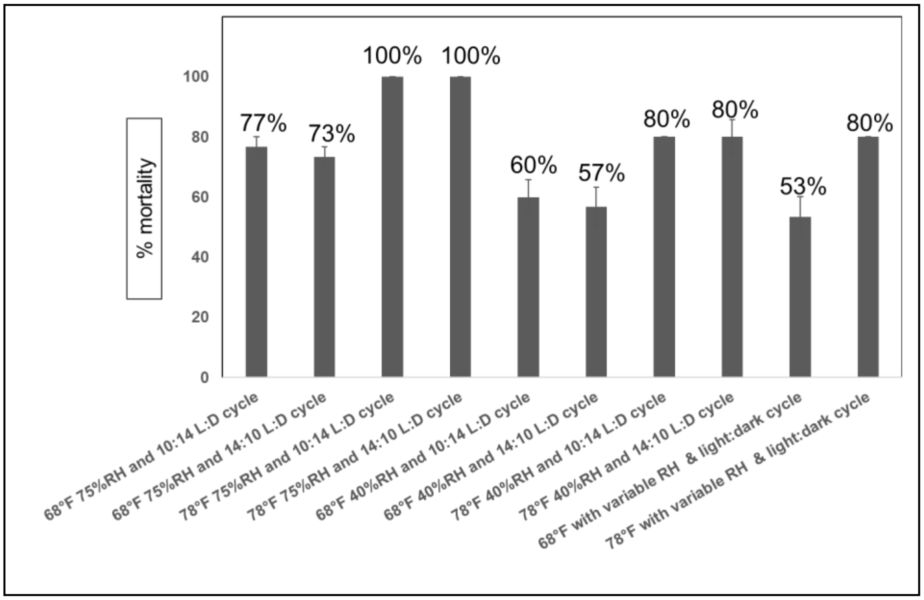

Figure 3. Percent bed bug mortality at 21 days after exposure to Phantom SC Insecticide under different temperatures, humidities, and light:dark cycles.

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