USE OF SOLID CARBON DIOXIDE FOR CONTROLLING BED BUGS
CIMEX HEMIPTERUS (FABRICIUS) UNDER LABORATORY CONDITIONS

Sutira Nanoudon, Yaowaluk Chanbang

Department of Entomology and Plant Pathology, Faculty of Agriculture,
Chiang Mai University, Chiang Mai 50200, Thailand

ABSTRACT

This study investigated the effect of using solid carbon dioxide (dry ice), which sublimates to gaseous carbon dioxide, to eliminate the tropical bed bug Cimex hemipterus (Fabricius) (Hemiptera: Cimicidae). All work was conducted in a laboratory with the temperature between 25 and 30 °C, and 75% relative humidity. In experiment 1, the tolerance of egg, nymph and adult bed bugs to dry-ice treatment was studied. 10 individuals from each group were exposed to 2.5 g of dry ice pellets for 9 hours in a 2,000 ml flask, which provided an average of 40.87% carbon dioxide (CO2) by volume. Complete mortality was observed in the nymphal stage, while the mortality rates in the egg and adult stages were 22.50% and 90.00%, respectively. The results indicated that bed-bug eggs are the most tolerant life stage to CO2 gas. In experiment 2, groups of bed-bug eggs were exposed to 4 different amounts of solid CO2 - 5, 10, 15, and 20 g, which provided averages of 86.02, 97.88, 98.51 and 99.07% CO2 by volume. Each amount of CO2 was used for 5 different durations, i.e., 3, 6, 9, 12, and 15 hours, creating 20 experimental groups in all. The experiments were housed in 2,000 ml flasks, and hatchability was examined 10 days post-treatment. In an untreated control (no dry ice), the mean bed-bug hatching rate was 86.30%. Bed-bug mortality was first observed after 3 hours in the 10g CO2 group (97.88% CO2). Mortality increased with exposure time and amount of CO2 from this initial point. Total mortality first occurred using 96.2% CO2 (15 g of dry ice in 2,000 ml flask) for 6 hrs.

Keywords: Cimex hemipterus, solid carbon dioxide, dry ice, bed bug, tolerance

INTRODUCTION

The tropical bed bug, Cimex hemipterus, is one of the most numerous insect pests in urban areas worldwide. They are obligatory hematophagous (bloodsucking) insects of the Order Hemiptera (Mullen and Durden, 2002). Many kinds of accommodation, especially hotels, serviced apartments, and other temporary lodgings often face problems with bed-bug infestation. During the 1950s, dichlorodiphenyltrichloroethane (DDT) insecticide was very effective against bed bugs, but many kinds of insects have since developed resistance to DDT due to repeated exposure. Organophosphates, such as chlorpyrifos, malathion, and pirimiphos-methyl (IRAC, 2008); pyrethroids including alpha-cypermethrin, beta-cyfluthrin, and bifenthrin; and carbamates, namely bendiocarb, fenobucarb, and propoxur (Tawatsin et al., 2011) have all been used to replace DDT in the control of bed bugs in different areas around the world. Residual sprays and crevice treatments are used to control bed bugs in households, especially by pest-control services. Some populations of bed bugs have developed very high levels of resistance to most common insecticides used for pest control, including the pyrethroid group (bifenthrin 25% WP, alpha-cypermethrin 5% SC and lambda-cyhalothrin 10% CS, etc.) (Tawatsin et al, 2011), especially deltamethrin and lambda-cyhalothrin (Romero et al, 2007).
Some new insecticides recommended to control bed bugs include chlorfenapyr, a pyrrole insecticide; and imidaclorprid and dinotefuran, neonicotinoid insecticides (How and Lee, 2010). Some other inorganic insecticides, such as diatomaceous earth, boric acid, and limestone, provide advantages in the control of bed bugs due to their greater residual effectiveness in dry environments (Potter, 2009). However, combining insecticide use with alternative control methods would provide more effective control. Some previously reported methods include insect removal using vacuum machines, thorough cleaning, steam heating of bedding, and reducing cracks and crevices (Wang and Cooper, 2011). Of more concern are bed-bug eggs, since the eggs often stick tightly to surfaces or are hidden, and are usually resistant to insecticides. For mattress or bed-frame treatment, few insecticides are available due to the unacceptable danger many varieties cause to humans. Some synthetic pyrethroids, including permethrin, resmethrin, and deltamethrin (EPA, 2014), and inorganic insecticides, such as diatomaceous earth, are suitable for use on mattresses. However, bed bugs are also highly resistant to most of these pyrethroids, especially bifenthrin and alpha-cypermethrin (Suwannayod et al, 2010).

Fumigation is a method of using a lethal gas to exterminate pests within an enclosed space. The fumigant, sulfuryl fluoride (Vikane®), has completely eliminated bed bugs in some multi-unit housing situations (Miller and Fisher, 2008). The use of fumigation, or atmosphere modification, can control an infestation while minimizing dangerous side effects. Modified atmospheres, with high concentrations of carbon dioxide (CO₂), can be used to control many insects, such as those in stored products (Annis, 1997). CO₂ is of very low risk to people and the environment, and is a natural component of the atmosphere. Wang et al (2012) reported that CO₂ from dry ice can kill Cimex lectularius, the common bed bug. To be proven, the potential benefits of using CO₂ to control tropical bed bugs requires evidence of effective application, and data on required doses and efficacy. The objective of this study was to examine the efficacy of CO₂ gas originating from solid dry ice on Cimex hemipterus, the tropical bed bug.

MATERIALS AND METHODS

Bed bug mass rearing

Individuals adult C. hemipterus were reared by being fed rabbit blood* from feeding cups. These were made by covering clear plastic drinking cups with satin cloth. Bed bugs were allowed to feed on the blood through the cloth for 15 minutes per meal. Bed bugs fed 2 times per week. Bed bug colonies were kept in closed plastic containers with sodium chloride solution to maintain 75% relative humidity. All bed bugs were reared in the laboratory in temperatures of 28-32 °C.

*Remarks: the vertebrates (rabbits) used in this experiment conformed to the international and national guidelines for ethical conduct and care of animals. Faculty of Agriculture, Chiang Mai University.

Bed bug preparation for carbon dioxide testing

Four to five corrugated-paper pieces were put into each plastic drinking cup, which maintained about 50 pairs of bed bugs. After the bed bugs laid eggs on the corrugated paper, the adults were separated from the eggs. Insects were kept in the moist box, maintained at 75% relative humidity with a room temperature of between 25 to 30 °C. The 0-8 day-old eggs, 3-5 instar nymphs, and 3-9 day-old adults, were used for exposure to CO₂ in the experiment.

Tolerant stages of bed bugs to carbon dioxide

Ten bed bugs at each developmental stage were put into net bags of 5x7 cm to prevent the insects from escaping. The net bag, which was
placed in a 2,000 ml flask, was used as the test arena for the carbon dioxide chamber. Solid carbon dioxide (dry ice pellets) weighing 2.5 g were put into the sealed 2,000 ml flask as the CO$_2$ source for 9 hours. The amount of CO$_2$ gas produced in the flask was quantified, with gas concentrations being measured at the start of the experiment and every 3 hours thereafter. The bed bugs were exposed to CO$_2$ for 9 hours. All experiments were replicated 4 times and compared with the untreated control (untreated control with 0.03% CO$_2$). After exposure to CO$_2$, bed bug mortality was recorded after 14 days for the egg stage, and 24 hrs for the nymphal and adult stages.

**Efficacy of carbon dioxide on tolerant bed bug stages**

The objective of this experiment was to examine the amount of CO$_2$ required to control the most tolerant life stage of tropical bed bugs in the laboratory. The most tolerant stage, determined from the previous experiment to be the eggs, was used to test for complete mortality. Ten bed-bug eggs were placed into net bags and exposed to 5, 10, 15, and 20 g of solid carbon dioxide in a 2,000 ml flask for 3, 6, 9, 12, and 15 hours. Gas concentrations were measured once the solid blocks of carbon dioxide had completely sublimated, and the insects were introduced to the flask after this point. Each exposure time was prepared individually. Each combination treatment underwent 4 replications. Bed-bug mortality was examined after 10 days of CO$_2$ exposure. The experimental design was split plot, with the concentration of CO$_2$ as the main plot, and exposure times as the sub-plot (4 concentrations x 5 exposure times = 20 treatments). CO$_2$ gas was measured using a Bridge Headspace Gas Analyzer (Bridge Analyzers, Inc., CA) at the beginning and end of the exposure-time-per-treatment.

**RESULTS**

**Tolerant life stages of bed bugs to carbon dioxide**

Carbon dioxide at 40.87% (Table 1) on average caused complete mortality (100%) of tropical bed bug nymphs and 90.00 ± 8.16% for adults, while egg mortality (22.50 ± 20.61%) was significantly lower (Table 2). The results indicated that bed-bug eggs were the most tolerant life stage to carbon dioxide.

**Efficacy of carbon dioxide to tolerant stage of bed bug**

The carbon-dioxide concentration in each tested flask fluctuated. For example, 82.25% of CO$_2$ at 3 hours and 85.25% at 15 hours were detected when using 5 g of solid dry ice (Table 3). The concentration of CO$_2$ decreased slightly after insect exposure. The concentration of gas in the flask increased with the weight of solid dry ice being used. When 15 to 20 g of dry ice was used, the CO$_2$ concentration increased to 90-100% for the duration of the test. In the

<table>
<thead>
<tr>
<th>Exposure time (hours)</th>
<th>CO$_2$ % (± SD)$^{1/}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>45.70 ± 5.13</td>
</tr>
<tr>
<td>6</td>
<td>41.87 ± 4.66</td>
</tr>
<tr>
<td>9</td>
<td>40.87 ± 4.00</td>
</tr>
</tbody>
</table>

$^{1/}$ Gas concentration measured using the Bridge Headspace Gas apparatus (Bridge Analyzers, Inc., CA).
untreated control, insect mortality ranged from 2.5 to 25% each exposure time. The egg life stage was the most durable to carbon dioxide gas produced from solid carbon dioxide in laboratory conditions at temperatures of 25-30 °C. In the untreated control, on average, 86.3% of bed bug eggs could hatch and survive. The results showed that the effect of solid dry ice concentration/CO2 gas produced (main plot) and exposure time, were significantly different (P ≤ 0.05). There were interactions between CO2 concentration and time exposure (Table 4). Both 15 and 20 g of dry ice in the 2,000 ml flask produced > 98% CO2, and achieved complete bed-bug-egg mortality when the exposure time was ≥ 6 hours. If the gas concentration remained > 90% during the longer exposures, the mortality rate stayed the same.

DISCUSSION

Bed-bug eggs were identified as the life stage most tolerant to carbon dioxide gas, having the lowest mortality in this experiment. The same result in a related species was found by Wang et al. (2012), who reported that Cimex lectularius showed 100% mortality when exposed to 100% CO2 for 7-8 hours. Both nymph and adult bed bugs are more susceptible to CO2 due to the ability of CO2 to relax the closer muscles of insect spiracles, and rapidly decrease their heartbeat. In addition, mobile adult insects have higher carbon dioxide susceptibility due to their higher activity levels than egg stages (How and Lee, 2010).

When using 100% CO2, atmospheric oxygen (O2) levels were reduced much more than usual. O2 is very important in oxidative processes, especially for the production of adenosine triphosphate (ATP). In addition, hypoxic conditions (O2 limitation) cause reduced activity of the motor neurons to the closer muscles of insects (Chapman, 1998). Jones (1974) reported that, at low CO2 levels, the heart can be stimulated and arrested, while high levels of CO2 cause permanent spiracle opening. Under these conditions, insects die from water loss via the spiracles. Carbon dioxide not only causes respiratory effects, but also affects other muscles (McCann and Boettiger, 1961) as well as bioelectrical responses of the nervous system, including inducing the depolarization of neuron systems (Clark and Eaton, 1983). With stress from hypoxic conditions or from high levels of carbon dioxide, insects may also have to deal with increased levels of reactive oxygen species (ROS). ROS can damage cellular proteins and interfere with normal cellular function (Mamidala et al., 2011).

Several researchers have applied modified atmospheres, especially using CO2, to various life stages of psocids, such as Liposcelis bostrychophila and the cigarette beetle, Lasioderma serricorne. The results found that egg stages of both species are also the most tolerant to 100% CO2 even when using high pressure CO2, specifically 15-20 bars

Table 2  Mortality of eggs, nymphs and adult bed bugs when exposed to CO2 from 2.5 g of dry ice for 9 hours.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Bed bug mortality % (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>22.50±20.61</td>
</tr>
<tr>
<td>Nymph</td>
<td>100.00±0.00</td>
</tr>
<tr>
<td>Adult</td>
<td>90.00±8.16</td>
</tr>
</tbody>
</table>

1/ The nymph and adult mortality rates were not significantly different by least significant difference (LSD) test (P ≤ 0.05).
for 60 minutes (Riudavets et al., 2010). The increased tolerance of bed-bug eggs to CO₂ may be caused by the low numbers of aeropyles on their eggs (Chapman, 1998), which would suggest a lower respiratory rate. Due to this low respiratory rate, the metabolism of insect eggs is thought to be low, as well (Riudavets et al., 2010). In keeping with these findings, this study confirms that *C. hemipterus* eggs are the most CO₂-tolerant life stage of the species. However, Wang *et al.* (2012) reported that at still elevated but lower levels of CO₂, the O₂ concentration was lower than normal, which enabled *C. lectularius* eggs to show higher CO₂ tolerance than mobile stages. So, not only does the atmospheric CO₂ level influence insect mortality, by having an effect on insect respiratory systems, it also changes the amount of O₂ available, which negatively impacts insect survival rates again. Mitcham *et al.* (2006) reported that low levels of atmospheric O₂ can cause an insect to go into metabolic arrest, a common strategy to cope with hypoxia. However, insects could also die from these hypoxic conditions. Increasing atmospheric CO₂ has a greater effect on

<table>
<thead>
<tr>
<th>Dry ice (g)</th>
<th>CO₂ concentrations for each exposure time (%)¹/</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>12 hr</th>
<th>15 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>5</td>
<td>82.25</td>
<td>78.35</td>
<td>89.48</td>
<td>86.48</td>
<td>88.00</td>
<td>73.13</td>
</tr>
<tr>
<td>10</td>
<td>96.88</td>
<td>93.20</td>
<td>98.00</td>
<td>84.03</td>
<td>99.23</td>
<td>92.28</td>
</tr>
<tr>
<td>15</td>
<td>97.98</td>
<td>94.70</td>
<td>98.70</td>
<td>96.20</td>
<td>100.00</td>
<td>96.73</td>
</tr>
<tr>
<td>20</td>
<td>99.90</td>
<td>96.95</td>
<td>100.00</td>
<td>94.80</td>
<td>94.95</td>
<td>98.73</td>
</tr>
</tbody>
</table>

¹/ Gas concentration measured by the Bridge Headspace Gas Analyzer (Bridge Analyzers, Inc., CA). Gas concentration ‘Before’ was measured once the solid carbon dioxide had completely sublimated. Insects were introduced to the flask, before the CO₂ was re-measured at the end of exposure time (‘After’).

<table>
<thead>
<tr>
<th>Dry ice (g)</th>
<th>Mortality% for each exposure time ±SD ¹/</th>
<th>3 hr</th>
<th>6 hr</th>
<th>9 hr</th>
<th>12 hr</th>
<th>15 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>5</td>
<td>N/A²/</td>
<td>22.70±21.97b</td>
<td>86.67±16.33a</td>
<td>44.79±66.44b</td>
<td>84.44±13.24a</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>36.60±14.68b</td>
<td>91.67±16.67a</td>
<td>100.00±0.00a</td>
<td>95.83±8.33a</td>
<td>100.00±0.00a</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>49.93±26.12b</td>
<td>100.00±0.00a</td>
<td>100.00±0.00a</td>
<td>100.00±0.00a</td>
<td>100.00±0.00a</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>35.35±17.85b</td>
<td>100.00±0.00a</td>
<td>100.00±0.00a</td>
<td>100.00±0.00a</td>
<td>100.00±0.00a</td>
<td></td>
</tr>
<tr>
<td>Control³/</td>
<td>10.00±8.16</td>
<td>7.50±9.57</td>
<td>25.00±17.32</td>
<td>22.50±17.08</td>
<td>2.50±5.00</td>
<td></td>
</tr>
</tbody>
</table>

¹/ Means within each column followed by the same letters are not significantly different according to the LSD test (*P* ≤ 0.05). Data were adjusted using Abbott’s formula (Abbott, 1925).

²/ Not available.

³/ The average survival rate of eggs in the untreated control was 86.30%
insect mortality than decreasing atmospheric O$_2$, although the mortality rate increases as available O$_2$ decreases. Since higher levels of CO$_2$ should be maintained to kill bed-bug eggs completely, further research using airtight containers to facilitate the CO$_2$ fumigation of mattresses will be needed.

CONCLUSION

Solid dry ice can be used as the source of carbon dioxide for killing the tropical bed bug *Cimex hemipterus* under laboratory conditions, with a room temperature of 25-30 °C. The egg stage was more tolerant to CO$_2$ treatment than nymphal and adult stages. 15 g of solid dry ice in a 2,000 ml flask, which provided a CO$_2$ gas concentration of 96.2% over 6 hours, was the minimum requirement for 100% mortality in 10 bed bug eggs in this study.

REFERENCES


