# Carbon Dioxide Fumigation for Controlling Bed Bugs

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J. Med. Entomol. 49(5): 1076–1083 (2012); DOI: http://dx.doi.org/10.1603/ME12037

**ABSTRACT** We investigated the potential of carbon dioxide ($CO_2$) fumigation as a method for controlling bed bugs, *Cimex lectularius* L. The effect of bed bug developmental stage, temperature, and $CO_2$ concentration on the minimum time to kill 100% of bed bugs was determined. The minimum $CO_2$ concentration lethal to all bed bug stages was ≈30% with 24 h exposure time at 25°C. The minimum fumigation time required to kill 100% of eggs using 100% $CO_2$ at 20, 25, and 30°C were 3, 7, and 8 h, respectively; the minimum fumigation time to kill 100% of adult males/nymphs were 8, 13, and 14 h, respectively. The minimum time to kill 100% of adult males/nymphs using 50 and 70% $CO_2$ at 25°C were 18 and 16 h, respectively. We found that eggs were not completely killed after 24 h fumigation when the $CO_2$ concentration was lower than 80%. Thus, bed bug eggs were more susceptible to 100% $CO_2$ fumigation than nymphs and adult males but more tolerant than nymphs and adult males with lower $CO_2$ concentration (50–80%). There were no significant differences among nymphs, adult males, and adult females in their susceptibility to 100% $CO_2$ fumigation. A 24 h fumigation in sealed 158 liter (42 gallon) heavy duty garbage bags filled 90% full with fabric materials and/or boxes and 1,350 g dry ice per bag was sufficient to kill all stages of bed bugs hidden in the materials at room temperature (23–24°C). Sealed heavy duty garbage bags maintained ≥94% $CO_2$ for at least 24 h. Custom-made double zipper plastic bags (122 × 183 cm) were also used to evaluate the effectiveness of $CO_2$ fumigation for controlling bed bugs. Each bag was filled with fabric and boxes to 50–90% full. Bed bugs were hidden in various locations of each bag. $CO_2$ was introduced into the bags through a $CO_2$ cylinder. $CO_2$ fumigation lasting 24–48 h was sufficient to kill all stages of bed bugs at room temperature, depending on the quantity of materials placed in each bag and whether $CO_2$ was introduced one or two times at the onset. $CO_2$ is an effective alternative to conventional fumigants for eliminating bed bugs hiding in infested household items such as clothing, shoes, books, electronics, sofas, and so forth.

**KEY WORDS** *Cimex lectularius*, fumigation, carbon dioxide, control

Bed bugs (*Cimex lectularius* L. and *Cimex hemipterus* F.) are very difficult pests to manage, in part, because of their widespread resistance to insecticides and cryptic behavior (Romero et al. 2007, Zhu et al. 2011). Bed bugs are not limited to sleeping and resting areas such as beds and sofas, instead virtually anything in the structure is susceptible to infestation. It is not uncommon to find bed bugs in personal items such as electronics, books, pictures, piles of papers, small appliances, furniture, and so forth. Eliminating bed bugs safely and effectively from these types of items is often more challenging than eliminating bed bugs hiding in cracks and holes in furniture or the structure itself.

Current tools and methods for treating bed bug-infested household items include discarding the infested items, hot washing, freezing, hot drying, applying hot steam, placing items in heating chambers, whole structure heating, applying pesticides, or fumigation of infested items with sulfuryl fluoride or dichlorvos (Doggett 2011). Each of these methods or tools has some limitations (Table 1). There is a lack of safe and effective methods for eliminating bed bugs in infested household items.

Modified atmosphere (MA) with high concentrations of carbon dioxide ($CO_2$) has been used in controlling stored product insects (Adler et al. 2000, Navarro 2006, Riudavets et al. 2009, Navarro et al. 2012). Elevated $CO_2$ levels cause insect spiracles to remain open, resulting in death from water loss (Nicolas and Sillans 1989). High $CO_2$ concentration also has toxic effects on the nervous system of insects. The use of MA has advantages over other toxic chemicals in that MA leaves no residue and is less likely to damage the fumigated materials. However, the use of MA is limited because of the requirement for air tight containers, and days or weeks of fumigation time. Herrmann et al. (1999) reported preliminary results on the effect of $CO_2$ fumigation against bed bugs. They found 60% $CO_2$ caused 100% mortality to all bed bug stages within

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**Table 1. Current bed bug control methods and their limitations for treating bed bug infested household items**

| Bed bug control methods and tools | Limitations |
|---|---|
| Hot steam | May damage items such as books, electronics, and furniture |
| Hot washing and laundering | Cannot be used for nonwashable and sensitive items |
| Discarding infested materials | Impractical in many instances |
| Freezers or portable heaters | Large items cannot be treated |
| Whole house heat treatment | Very expensive |
| Insecticide sprays | Will leave residues on treated surface and are often ineffective after dried; may not penetrate into harborages |
| Chemical fumigation | Very expensive and requires extensive training |
| Dichlorvos resin strips | Slow (requires weeks of time) and may corrode iron and steel (Hayes and Laws 1990, Lehnert et al. 2011); may cause discomfort to those with respiratory diseases |

24 h, suggesting $CO_2$ fumigation may be a viable approach for eliminating bed bugs from infested household items. The objectives of the current study were to: 1) determine the lethal exposure time and minimum lethal $CO_2$ concentration to kill bed bug eggs, nymphs, and adults; 2) determine the effect of temperature on the effectiveness of fumigation; and 3) determine the efficacy of containerized $CO_2$ fumigation for control of bed bugs.

## Materials and Methods

**Insects.** A laboratory and two field strains of *C. lectularius* were maintained in plastic containers (47 mm diameter, 47 mm tall) with folded filter paper as harborages. The two field strains (Jersey and Indy) were collected 1 wk and 2 yr before this study, respectively. The laboratory and the Indy strains were fed weekly with defibrinated rabbit blood using an artificial membrane feeding system (Hemotek Ltd., England, United Kingdom). The bed bugs were kept at 23–26°C, 24–48% relative humidity (RH), and a photoperiod of 12:12 (L:D) h environment. Before the experiment, bed bug eggs of 0–8 d old along with the paper substrate were carefully cut out and were transferred into 3.7 cm diameter, 1.0 cm tall plastic petri dishes. Bed bug nymphs and adult males that were fed 3–9 d previously were transferred into the same dishes as the eggs or into separate dishes. Bed bug adult females were placed in separate dishes when used in experiments. The dish lids and bottoms had 1.25 cm diameter openings covered with fine nylon screens to allow for air exchange. The closed dishes were wrapped using Parafilm Sealing Sheets (Bemis Flexible packing, Neenah, WI) to hold the lids and the bottoms together during the experiments. Preliminary tests indicate there were no significant differences among the laboratory strain and two field strains in their mortality response to $CO_2$ fumigation. Therefore, we used the laboratory strain in most experiments. The Indy strain was used in a few experiments when not enough laboratory strain bed bugs were available.

**Fumigation Containers.** Four types of fumigation containers were used in this study to evaluate the effect of high $CO_2$ concentrations on bed bug survival (Fig. 1). Pyrex Erlenmeyer flasks (2000 ml volume; VWR International, LLC, Bridgeport, NJ) were used for evaluating the effect of 100% $CO_2$ fumigation. Ziploc double zipper bags (3.7 liter volume) (SC Johnson, Racine, WI) were used for evaluating 50–90% $CO_2$. Heavy duty garbage bags (158 liter volume, 3 mm thickness; Husky, PolyAmerica, Grand Rapids, TX) were used for evaluating the dry ice treatment. Buganator fumigation bags (122 × 183 cm, 3.5 mm thickness) with double zippers (Protect-A-Bed, Chicago, IL) were used for evaluating the $CO_2$ gas treatment. Each Buganator bag has two 3 cm diameter openings for connecting to a hose or power source.

**Experiment 1: Minimum Lethal $CO_2$ Concentration.** Mixed air containing 19.38 and 29.86% $CO_2$ (Airgas East Inc, Piscataway, NJ) were obtained to evaluate the effect of elevated $CO_2$ concentration on bed bug eggs, nymphs, and adult males. Ten bed bug third to fifth instar nymphs, 10 adult males and 11–31 eggs were placed in each petri dish. Ziploc double zipper bags were used as fumigation container (Fig. 1). The bags were checked for leakage before being used. Each dish was placed in a separate bag and affixed to middle portion with tape. Each bag was filled with mixed air of known $CO_2$ concentration or natural atmosphere. Three bags were tested for each concentration. The bags were suspended from racks in an incubator (model I36VL, Percival Scientific, Inc. Perry, IA) at 25°C. A running fan located at the ceiling of the incubator kept the bags moving during the fumigation period, which prevented $CO_2$ from possible settling inside the bags. After 24 h, the bags were removed from the incubator and the dishes were taken out from the bags. They were blown briefly with a table fan to remove high $CO_2$ levels inside the dishes. The dishes were placed in a laboratory at 23.4 ± 0.1°C and a photoperiod of ≈12:12 (L:D) h cycle. Bed bug mortality and egg hatch was observed daily for up to 10 d after fumigation.

**Experiment 2: Minimum Lethal Exposure Time to Kill Bed Bugs With 100% $CO_2$.** Thirty grams dry ice pellets were placed in each 2,000 ml flask. A rubber stopper was loosely placed on top of each flask. After sublimation was complete, the flasks were immediately sealed tightly using rubber stoppers. Because $CO_2$ is the densest component in the natural atmosphere, the sublimation process would create a 100% $CO_2$ environment inside the flasks.

Before the fumigation treatment, the flasks were placed in a 30°C incubator to bring the temperature of the flasks containing 100% $CO_2$ to the same temperature as the incubator. Bed bugs were prepared as in the previous experiment. Bed bug eggs and mobile stages were placed in different dishes. Each dish contained 10 males and 10 nymphs, or 14–29 eggs. One


**Fig. 1.** Fumigation containers used in the study. (A) A 2,000 ml glass flask with a petri dish containing bed bugs; (B) A 3.7 liter Ziploc bag with a petri dish containing bed bugs; (C) A 158 liter (42 gallon) heavy duty garbage bag filled with fabric materials and three $CO_2$ sensors; (D) A Buganator fumigation bag ≈50% full of fabric materials and boxes, a laptop computer, and three $CO_2$ sensors for recording $CO_2$ concentrations. (Online figure in color.)

dish was placed into each flask and the flask was immediately resealed using a rubber stopper. Preliminary tests showed eggs were killed within 2–4 h and mobile stages were killed after 7–9 h fumigation at 30°C. Therefore, we used 2.25, 3, 4, 5, 6, 7, and 8 h fumigation time for eggs and 7, 8, and 9 h fumigation time for nymphs/males. The fumigation time in the control was 8 h for eggs and 9 h for nymphs/males. For each exposure time, three flasks were used. After fumigation, the bed bug dishes were taken out of the flasks and placed in a laboratory incubator at 23.4 ± 0.1°C and a photoperiod of 12:12 (L:D) h cycle for observation of mortality daily for 14 d.

**Experiment 3: Effect of 100% $CO_2$ Fumigation on Bed Bugs at Different Temperatures.** Similar to Experiment II, bed bug eggs, nymphs, and adults were subjected to 100% $CO_2$ fumigation at 25 and 20°C using 2,000 ml flasks. In the 25°C test, each dish contained 8–15 eggs, 10 nymphs and 10 males or 10 females. We included females in this experiment to evaluate whether there are any differences among nymphs, males, and females. The fumigation time evaluated was 5, 6, 7, 8, and 9 h for eggs and 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 h for nymphs, males and females. The eggs and mobile stages in the control were removed from flasks containing natural atmosphere after nine and 15 h, respectively. For each exposure time, three flasks were used. The egg hatches were observed daily until 3 d after no more eggs hatched.

In the 20°C test, each dish contained 10 nymphs and 5 males, or 10–31 eggs. The fumigation time was 7, 8, 9, 10, and 11 h for eggs and 12, 13, 14, and 15 h for nymphs/males. The eggs and nymphs/males in the control were removed from flasks containing natural atmosphere at 11 and 15 h, respectively. For each exposure time, three flasks were used. The egg hatches were observed daily until 3 d after no more eggs hatched.

**Experiment 4: Effect of 50–90% $CO_2$ Fumigation on Bed Bugs.** The relationship between $CO_2$ concentration (49.32 and 71.38%) and time required to kill 100% of the bed bugs were examined at 25°C. Nymphs/males were treated with 49.32 and 72.38% $CO_2$. The fumigation times evaluated were 10, 12, 14, 16, and 18 h for 49.32% $CO_2$, and 10, 14, and 16 h for 71.38% $CO_2$. Ten nymphs and 10 males were placed in each dish. One dish was placed in each Ziploc bag as in Experiment I. Eggs were only treated with 71.38% $CO_2$. The treatment time was 12, 14, and 16 h. Ten to 23 eggs were placed in each dish. Each exposure time was replicated three times.

Because the above test resulted in very low egg mortality, we further tested the effect of $CO_2$ fumigation on eggs using higher $CO_2$ (78.96 and 89.64%) concentration, higher temperature (30°C), and longer exposure time (24 h). Each dish contained 12–34 eggs. Each $CO_2$ treatment was replicated three times.

**Experiment 5: Efficacy of $CO_2$ Fumigation Against Bed Bugs in Garbage Bags.** This test was intended to determine whether dry ice can be used as $CO_2$ source for controlling bed bugs buried in household items. Heavy duty 158 liter (42 gallon) sized garbage bags were filled with pillows, clothing, and fabric sheets (including one water proof fabric mattress cover) to

**Table 2. Efficacy of tanked $CO_2$ fumigation against bed bug eggs in Buganator plastic bags (121 × 183 cm)**

| Vacuuming/ adding $CO_2$ | Fumigation time (h) | Contents in bags | Amount of materials in each bag | No. of bags tested | Location of bed bugs in each bag | Mean hatch rate (total no. of eggs) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Treated | Control |
| Once | 24 | Fabric | 50% full | 3 | Top, center | 0 ± 0% (44) | 93 ± 7% (17) |
| Once | 25.5 | Fabric | 90% full | 1 | Top, center, bottom | 42 ± 2% (42) | 100 ± 0% (51) |
| Once | 25.5 | Fabric + boxes | 90% full | 1 | Top, center, bottom | 60 ± 3% (40) | 100 ± 0% (51) |
| Twice | 24 | Fabric | 90% full | 1 | Top, center, bottom | 1 ± 1% (60) | 89 ± 7% (34) |
| Twice | 24 | Fabric + boxes | 90% full | 1 | Top, center, bottom | 20 ± 7% (35) | 89 ± 7% (34) |
| Twice | 48 | Fabric | 50% full | 1 | Top, center, bottom | 0 ± 0% (69) | 100 ± 0% (50) |
| Twice | 48 | Fabric + boxes | 90% full | 2 | Top, center, bottom[a] | 0 ± 0% (96) | 100 ± 0% (91) |
| Twice | 72 | Boxes | 90% full | 1 | Top, center, bottom | 0 ± 0% (66) | 95 ± 3% (89) |
| Twice | 72 | Fabric + boxes | 90% full | 1 | Top, center, bottom | 0 ± 0% (72) | 95 ± 3% (89) |

The room temp during the experiments was 22 to 24°C.
[a] The bed bug dishes at the "bottom" location were wrapped in water proof fabric.

≈90% full (22 kg materials) (Fig. 1c). Three petri dishes containing bed bugs were placed in each bag at three locations: top, center, and bottom. In the 900 g dry ice treatment, each dish contained 10–22 (average 15.6) eggs, 10 nymphs, and 10 males. Each dish was placed into a cotton sock to minimize air exchange inside the dishes. Nine hundred grams dry ice pellets total were spread near bottom, at center, and at the top of the fabric pile in each bag. Dry ice was not placed in the control bags. Each bag was sealed by tightly tying cotton string around the top of the bag to minimize air exchange between the air in the bags and the air outside the bags during experiments. The bags were placed in a laboratory with the sealed opening directed upward. After 24 h, the bed bug dishes were taken out of the bags and ventilated briefly using a table fan, and then placed in a laboratory for observation of egg hatch or mortality of nymphs/males daily for up to 10 d. The mean room temperature during the 24 h fumigation period was 23.6 ± 0.1°C. The treatment and the control were replicated three times.

The above test did not result in 100% mortality of the bed bugs. We further tested $CO_2$ fumigation using 1,350 g dry ice per bag. Each dish contained 10–15 eggs, 5 nymphs, and 5 males. Three dishes were placed in each bag. The treatment was replicated six times and the control was replicated four times. The room temperature during the fumigation period was 23.0–23.9°C. Other procedures were the same as in the 900 g dry ice treatment.

Three 0–100% $CO_2$ sensors ($CO_2$Meter, Ormond Beach, FL) were placed in three separate bags, each with 1,350 g dry ice, to record $CO_2$ concentrations every 10 min for 2 d (Fig. 1c). The first two bags contained 22 kg (per bag) fabric materials (pillows, clothing, and sheets). The third bag contained a mixture of fabric materials and two cardboard boxes that were filled with empty round plastic (5.5 cm diameter, 4 cm tall) containers. All bags were ≈90% full. One sensor was placed near the bottom of the fabric pile, one was placed at the center, and one at the top. The sensor at the center location was wrapped in water proof fabric to simulate the most challenging situations where $CO_2$ penetration may be slow.

**Experiment 6: Efficacy of $CO_2$ Fumigation Against Bed Bugs in Custom-Made Fumigation Bags.** Buganator (122 × 183 cm) bags were used to evaluate the efficacy of $CO_2$ fumigation for eliminating bed bugs buried in household items. These bags have resealable double zippers and can accommodate much larger items than the 42 gallon garbage bags. Preliminary tests showed the amount of materials, plasticity of the items (soft or hard), and preparation method (vacuuming to remove air in the filled bag before adding $CO_2$) would affect the final $CO_2$ concentration in the bag. Therefore, we tested the effectiveness of various preparations (Table 2). The fabric materials included clothing, bed sheets, pillows, mattress covers, and socks. The boxes included cardboard and Styrofoam boxes (total volume of the boxes in each bag was 5.9 $m^3$). Two or three dishes containing bed bug eggs (10–36 eggs/dish) were placed in each bag. In bags with fabric materials only, the dishes were placed on the top, the center, and bottom of the fabric pile. In bags with boxes, the "center" dish was placed inside one of the boxes. Only the egg stage was tested because it was much less susceptible to $CO_2$ fumigation (<80% $CO_2$) than nymphs and adults based on results from Experiment 4.

After all materials were placed in each bag, a vacuum pump (model HB-124BS, Ho Lee Co. Ltd., Taiwan, Republic of China) was attached to the bag via tubing to remove as much air as possible from the bag (Fig. 1d). The vacuuming time lasted for 4–6 min. Immediately after vacuuming, $CO_2$ was introduced into the bag from a 50 lb $CO_2$ cylinder (Airgas East Inc., Piscataway, NJ) via a connecting hose at 20–25 psi for 4–6 min until the bag was fully inflated. The vacuuming/$CO_2$ introduction process was conducted two times in a row in some preparations as shown in Table 2. Repeating the vacuuming-injecting $CO_2$ allowed for achieving higher $CO_2$ concentrations in the bag. Once finished vacuuming and introducing $CO_2$, the hose connected to the bag was sealed with a pair of nitrile gloves. After the desired fumigation time (24–48 h) was reached, the bag was opened and the dishes were taken out of the bags and kept in a laboratory for observation of egg hatch. The temperature of the laboratory was 22–24°C.

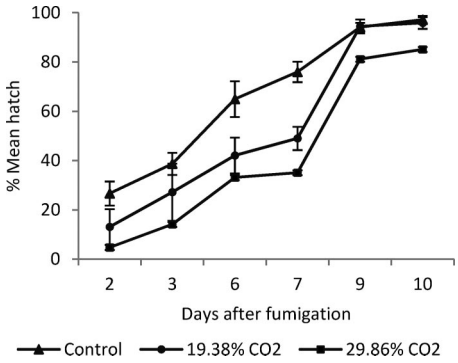


**Fig. 2.** Effect of 24 h exposure to elevated $CO_2$ levels on bed bug eggs (mean % hatch and SEM) at 25°C.

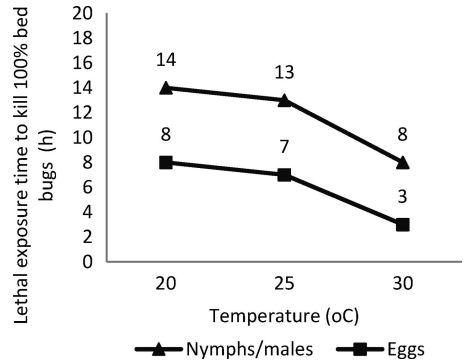


**Fig. 3.** Relationship between temperature and lethal time to kill bed bugs by 100% $CO_2$. The maximum time required to kill all tested insects in the three replicates is shown.

Three 0–100% $CO_2$ sensors were placed in each bag to record $CO_2$ concentrations in bags over time (Fig. 1d). The three bags were ≈50, 60, and 75% full with a mixture of fabric materials and two boxes. Each bag was vacuumed and $CO_2$ was added once. Three sensors were placed in each bag: at bottom of fabric pile, inside a box on top of the fabric pile, and the center of the fabric pile. The sensor in the center was wrapped in water-proof fabric. The sensors recorded $CO_2$ concentrations every 10 min.

To determine the final $CO_2$ concentrations in bags that were 90% full, three $CO_2$ sensors were placed in a bag that contained a mixture of fabric materials and boxes. The sensor locations were: bottom of clothing pile, inside a Styrofoam box with the lid slightly open, and inside a box. The sensor in the box was wrapped in water-proof fabric. The bag was vacuumed and $CO_2$ added two times.

**Data Analysis.** Mortality data were corrected using Abbott's (1925) formula. One-way analysis of variance (ANOVA) was used to compare mortalities between the treatment and the control in all experiments. All analyses were performed using SAS software (SAS Institute 2009).

## Results

**Minimum Lethal $CO_2$ Concentration.** After 24 h fumigation at 25°C, 19.38% $CO_2$ did not cause significant mortality to bed bug eggs or nymphs/males (Fig. 2). The 29.86% $CO_2$ treatment caused significant mortality to eggs ($F = 5.52$; df = 2, 6; $P = 0.044$) and nymphs/males ($F = 12.57$; df = 2, 6; $P = 0.01$) with mean corrected mortality being 12.5 ± 4.1% for eggs and 28.8 ± 7.8% for nymphs/males. Thus, the minimum lethal $CO_2$ concentration to bed bugs was ≈30% when exposed for 24 h at 25°C.

**Relationship Between Temperature and Lethal Time to Kill Bed Bugs by 100% $CO_2$.** The minimum required time to kill 100% of test insects was negatively correlated with temperature (Fig. 3). Eggs were always more susceptible to 100% $CO_2$ fumigation than nymphs/males at the three temperatures tested. At 20°C, the mean egg hatch rate was 4.2 ± 4.2% after 7 h fumigation. No eggs survived in 8, 9, 10, and 11 h treatments while all eggs in the control hatched. Nymphs/males suffered 95.6 ± 4.4% mortality in the 12 h treatment and 100% mortality in the 14 and 15 h treatments. There was no mortality of nymphs and males in the control.

At 25°C, one of 39 eggs hatched in the 6 h treatment. No eggs survived in the 5, 7, 8, and 9 h treatments. The mean hatch rate in the control was 91.7 ± 8.3%. Nymphs, males, and females exhibited significant mortality in the 6 h treatment and nearly 100% mortality after 8–10 h. One male, 1 female, and 4 nymphs showed signs of movement after 13 h fumigation, but did not recover. All of the insects died in the 14–15 h treatments without showing any signs of movement after being removed from the flasks.

At 30°C, only 18.3 ± 9.8% of the eggs hatched in the 2 h 15 min treatment. No eggs hatched in the 3, 4, 5, 6, 7, and 8 h treatments. The mean egg hatch rate in the control was 92.5 ± 5.3%. Nymphs/males suffered 96.7 ± 3.3% mortality in the 7 h treatment and 100% mortality in the 8 and 9 h treatments. The mean nymph/male mortality in the control was 1.7 ± 1.7%.

**Effect of 50–90% $CO_2$ Fumigation on Bed Bugs.** The minimum exposure time required to kill 100% of nymphs and males at 25°C using 49.32 and 71.38% $CO_2$ were 18 and 16 h, respectively (Fig. 4). In contrast, 16 h exposure to 71.38% $CO_2$ only resulted in slightly lower egg hatch rate (83.0 ± 1.7%) compared with the control (97.8 ± 2.2%) ($F = 25.5$; df = 1, 4; $P = 0.01$). Using 24 h treatment time and higher $CO_2$ concentrations at 30°C resulted in higher egg mortality. The mean corrected mortality in 78.96 and 89.64% $CO_2$ treatments were 75.6 ± 12.3 and 100 ± 0%, respectively (Fig. 5). No additional eggs hatched after 11 d. The mean egg hatch rate in the control was 93.9 ± 3.3%.

**Efficacy of $CO_2$ Fumigation in Heavy-Duty Garbage Bags.** In the test using 900 g dry ice per bag, all eggs were killed. The mean egg hatch rate in the control was 98.1 ± 1.9%. Three males (30%) and 6 nymphs (60%) located at the "top" location in one treated bag survived. The mean nymph/male mortality in the control was 3.9 ± 2.0%.

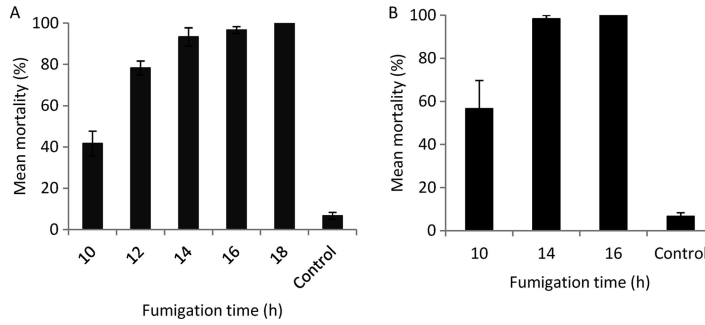


**Fig. 4.** Effect of $CO_2$ fumigation on bed bug mortality (mean ± SEM) of nymphs and adult males: (A) 49.32% $CO_2$; (B) 71.31% $CO_2$.

All eggs, nymphs, and males died in the 1,350 g dry ice treated bags. Bed bug nymphs and males showed no signs of movement after the 24 h fumigation treatment. The mean egg hatch rate in the control was 94.6 ± 4.7%. The mean nymph/male mortality in the control was 1.7 ± 1.7%.

$CO_2$ sensors in each bag recorded similar peak levels of $CO_2$, with the top location reaching the peak concentration at the slowest speed (Fig. 6). The average $CO_2$ concentrations within each bag plateaued at ≈5 h and were maintained at ≥94% after 24 h.

**Efficacy of $CO_2$ Fumigation in Custom-Made Fumigation Bags.** All bed bug eggs were killed after 24 h fumigation when each bag was vacuumed, $CO_2$ was introduced once, and the bag was 50% full of materials (Table 2). When the bags were 90% full, it required the bags to be vacuumed and injected with $CO_2$ two times, and a 48 h fumigation period to successfully kill all bed bug eggs.

When vacuumed and $CO_2$ was introduced once, it took 190, 210, and 310 min, respectively, to reach stable $CO_2$ concentrations in the center of the Buganator bags filled 50, 75, and 90% with fabric materials (Fig. 7). The final $CO_2$ concentration was negatively correlated with the volume of the bag contents. Each Buganator bag was able to keep constant, high $CO_2$ concentrations. In a bag 90% full of fabric materials, cardboard boxes, and Styrofoam boxes, the mean final $CO_2$ concentration measured was 66 ± 2% (Fig. 8). Wrapping the $CO_2$ sensor tightly inside water proof fabric slowed down $CO_2$ penetration, but only slightly reduced the final $CO_2$ concentration.

## Discussion

We showed that using dry ice or tanked $CO_2$ and thick plastic bags can effectively kill bed bugs hidden in household items after 24 or 48 h treatment at room temperature. The minimum required time to kill 100% of bed bugs is affected by the room temperature, amount of materials placed in a bag, and quantity of $CO_2$ introduced into each bag. It is also possible the physiological status of the bed bugs (such as nutritional status, days after molting) may affect the bed bug susceptibility to $CO_2$ fumigation. We did not try to control the exact age of the bed bugs or feeding status of the bed bugs when conducting the experiments. This may have resulted in large variances and irregular time-mortality response patterns.

Eggs were more susceptible to 100% $CO_2$ than mobile stages but much less susceptible than mobile stages at 50–80% $CO_2$ concentrations. Although we did not test females using 50–80% $CO_2$ concentrations, it is predicted that females will have similar mortality response as males and nymphs as shown in

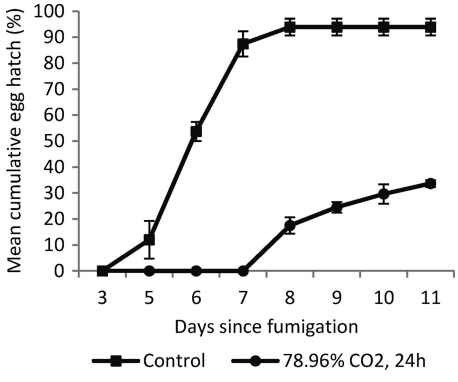


**Fig. 5.** Effect of 78.96% $CO_2$ fumigation on bed bug eggs (mean % hatch and SEM) at 25°C.

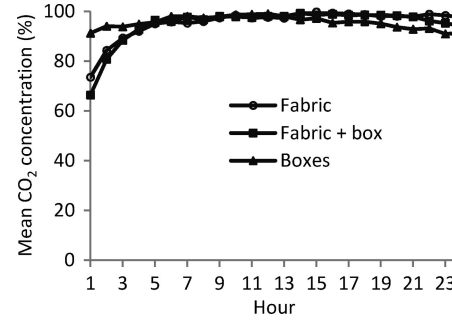


**Fig. 6.** Mean $CO_2$ concentration in heavy-duty garbage bags that were 90% full. In total, 1,350 g of dry ice pellets were placed near the bottom, the center, and near the top of the contents in each bag. Three $CO_2$ sensors were placed in each bag.

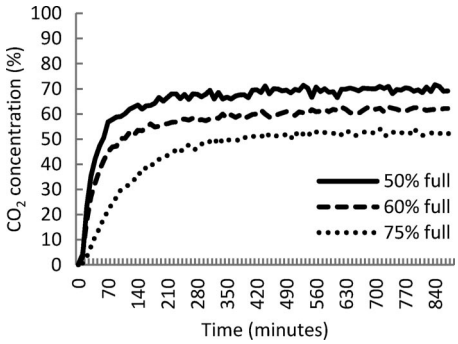


**Fig. 7.** $CO_2$ concentration measured at the center of Buganator bags that were filled with different amounts of fabric materials. The bag was vacuumed and injected with $CO_2$ once. The $CO_2$ sensor was wrapped inside a piece of water proof fabric.

the 100% $CO_2$ treatment. The higher sensitivity to 100% $CO_2$ in eggs is interesting. Presumably, insect eggs have lower metabolism rate than the mobile stages and is the most tolerant stage regardless of the $CO_2$ concentration being used (Riudavets et al. 2010). The different relative susceptibility to various $CO_2$ levels suggests modified air with 0% oxygen is more lethal to eggs than the mobile stages. However, when low level oxygen is present, eggs can survive better than nymphs and adults.

Compared with other insects studied, bed bugs are more sensitive to $CO_2$ fumigation than Formosan subterranean termite (*Coptotermes formosanus* Shiraki) (Delate et al. 1995), Oriental cockroach (*Blatta orientalis* L.) (Gannon et al. 2001), German cockroach (*Blattella germanica* (L.) (C.W., unpublished data), red flour beetle (*Tribolium castaneum* (Herbst)) (Bailey and Banks 1975), rusty grain beetle (*Cryptolestes ferrugeneius* (Stephens)) (Mann et al. 1999), and several other stored product pests (Annis 1987). The relatively short required fumigation time (1–2 d) makes $CO_2$ fumigation a promising technique for eliminating bed bugs from infested household items. The materials and procedures involved in $CO_2$ fumigation are relatively simple and inexpensive. Larger sizes of Buganator bags are available for treating large household items such as sofas or mattresses. The Buganator bags were able to maintain a constant high $CO_2$ concentration throughout the fumigation period without the need for adding $CO_2$ during fumigation. Sealed garbage bags were not as air tight as the Buganator bags. However, when a large enough amount of dry ice was placed in each garbage bag, high concentrations of $CO_2$ ($\geq$94%) were maintained for at least 24 h, which is sufficient for complete control of bed bugs at room temperature.

The required $CO_2$ fumigation time to kill 100% of bed bugs was negatively correlated with the environmental temperature and negatively correlated with the $CO_2$ concentration in the bags. We tested under the most challenging conditions where each fumigation bag was 90% full and both soft, hard, and water proof materials were included. $CO_2$ sensors wrapped in water proof fabric recorded similar final $CO_2$ concentration as those nonwrapped sensors, indicating water proof materials can be effectively treated, but will require longer fumigation time than nonwater proof fabric materials.

When a smaller volume of material is placed in each Buganator bag, bed bugs may be killed faster because higher $CO_2$ concentration can be achieved. In environments where the temperature is lower than 23°C, a heating fan can be placed inside the Buganator bag to maintain a warm temperature. Each Buganator bag is equipped with a reattachable power cable that can be connected to one of the round openings. We placed a fan heater (model FH03D, Ningbo Dongji Electronic Technology, Co., China) in a Buganator bag that was 70% full of boxes and a few pieces of fabric. The heating fan increased the temperature inside the bag by at least 12°C. In a bag that was 80% full of fabric materials, the heating fan increased the temperature by at least 4°C. Larger volumes of tightly packed fabric materials were more difficult to warm up than rigid materials (e.g., boxes).

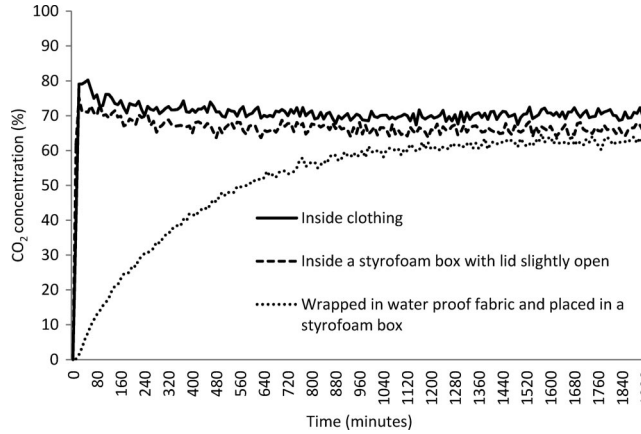


**Fig. 8.** $CO_2$ concentration measured at different locations within a bag filled 90% full with fabric materials and boxes. The bag was vacuumed and injected twice with $CO_2$ from a $CO_2$ cylinder.

Potential risks of $CO_2$ fumigation using dry ice or tanked $CO_2$ include injury from direct contact with dry ice and increased $CO_2$ levels when adding dry ice or tanked $CO_2$ into bags or during aeration after fumigation. The risk of asphyxiation can occur when many garbage bags are used in a small room. Transporting dry ice or $CO_2$ cylinders may also present safety risks. These risks can be easily prevented by wearing gloves, ventilating the rooms during fumigation, and ventilating the vehicle when transporting dry ice. It is recommended that individuals who want to use this technique obtain proper training to ensure safe and effective use of the technique. $CO_2$ fumigation should not be used as a whole house treatment because it is very difficult, if not impossible, to reach lethal $CO_2$ concentration for extended period of time that is necessary to kill all bed bug stages. It should also be noted that releasing $CO_2$ into the atmosphere contributes to the green house effect. Nevertheless, compared with other treatment methods currently used for treating bed bug-infested household items, $CO_2$ fumigation is unlikely to damage the treated items, very inexpensive, and leaves no residue.

In conclusion, $CO_2$ fumigation in plastic containers offers an effective, affordable, and fast way to treat bed bug-infested household items, many of which cannot be treated readily by other techniques. It can be used as part of an integrated bed bug management program. The fumigation process may increase the $CO_2$ levels in a closed room to an injurious level. Proper ventilation, especially during the introduction of $CO_2$ and ventilation of the bag, is required to prevent the risk of accidental asphyxiation.

## Acknowledgments

We thank Marcus Kwasek, Moneen Jones, and Vincenzo Averello for technical assistance. Susan McKnight provided suggestions on using dry ice as a fumigation tool. Protect-A-Bed and U.S. Department of Agriculture Northeastern Integrated Pest Management Center provided funding for this project. Kurt Saltzmann and Richard Cooper reviewed an earlier draft of this paper. This is New Jersey Experiment Station Publication D-08-08117-01-12.

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*Received 21 February 2012; accepted 28 June 2012.*