Introduction

A resurgence in populations of the bedbug, *Cimex lectularius* Linnaeus, has been reported in many areas of North America, Australia and Europe (Boase, 2001; Hwang et al., 2005; Potter, 2005; Harlan, 2006; Doggett, 2007; Pinto et al., 2007). Their increase has been documented in apartment houses, shelters for homeless people, rooming houses, hotels, single-family residences and other facilities in both urban and rural areas. The proliferation of both *C. lectularius* and *Cimex hemipterus* (Fabricius) has been attributed to increased travel, the exchange of used bedding and furniture, limited availability of effective and approved insecticides, insecticide resistance (Doggett, 2007; Romero et al., 2007) and the cyclical nature of the species (Harlan et al., 2008).

Bedbugs are characterized by their obligate bloodfeeding, absence of wings, traumatic insemination (Johnson, 1942;...
Materials and methods

Trap description

The trap was devised using the inverted bottom of a high-density polyethylene cat-feeding dish (35.0 cm long, 18.4 cm wide, 6.7 cm high) (Van Ness Lightweight Double Diner; Van Ness Pet Supplies, Clifton, NJ, U.S.A.) (Fig. 1). A single layer of Pellon® fusible featherweight interfacing, made of 80% polyester and 20% nylon fabric (≥ 10 µm diameter) (Jo Ann Fabric, Bristol, CT, U.S.A.) was glued to the outer sides of the dish to provide a rough surface over which bedbugs could crawl and ascend to the top of the trap. A thermal lure taken from a Dragonfly® mosquito trap manufactured by BioSensory, Inc. (Putnam, CT, U.S.A.) was placed horizontally on the inverted bottom of the dish and fastened with screws. The thermal lure consisted of a flexible heater with integral thermistor mounted on an injection-moulded chassis 8.9 cm in diameter and 25.4 cm in length, with associated microprocessor control logic to measure ambient temperature and produce surface temperatures of 37.2–42.2 °C. The temperatures on the floor immediately adjacent to the base and at the lip of the heat-activated trap were 0.40–0.46 °C and 1.33 °C above ambient temperature, respectively. A power cord exited along the side of the heater tube. The height of the trap was 15.2 cm. Compressed CO₂ was released at rates of 50–400 mL/min (mL of water displaced by CO₂/unit of time) into the trap. A strip of Agilex Flavors and Fragrances Icon gel (Agilex Flavors and Fragrances, Inc., Somerville, NJ, U.S.A.) in BioSensory, Inc. plastic housing (Dragonfly® octenol lure EPA Reg. No, 70909-3) was placed in the bottom of the thermal lure. The following chemical substances were impregnated into the gel: 33.0 µg propionic acid; 0.33 µg butyric acid; 0.33 µg valeric acid; 100 µg octenol, and 100 µg L-lactic acid. The lure was manufactured by Agilex Flavors and Fragrances, Inc. The quantities impregnated into the gel were based on mixtures of these chemicals that elicited an orientation response in Triatoma infestans Klug (Barrozo & Lazzari, 2004a, 2004b). Traps with and without heat, chemical lure and CO₂ were evaluated in the laboratory and field.

Laboratory procedures

Bedbug colony. A colony of bedbugs was established from nymphs and adults collected from an apartment in New Haven, CT, U.S.A. The colony was maintained at room temperature (~ 23 °C), kept at ~ 75% relative humidity (RH) with 16 h of light/day, and fed on a laboratory rabbit (Davis, 1956). Rabbits were handled in a manner approved by the Animal Care and Use Committee at the Connecticut Agricultural Experiment Station.

Trap testing within the bedbug arena. A square area measuring 183 cm per side was established on a light-coloured linoleum floor in a room without windows in the insectary at the Connecticut Agricultural Experiment Station’s Lockwood Farm in Hamden, CT, U.S.A. The temperature in the room was ~ 23 °C. Lighting was L:D 16:8 h. The borders of the arena were established by placing 4.8-cm-wide, two-sided carpet tape on the floor. Bedbugs which attempted to leave the arena either were repelled by or became stuck on the tape. Adults that had not fed in the adult stage were used in all experiments. Five females and five males were placed together in 16-mL clear glass bottles, 2–10 days after moulting into adults. In each jar a substrate for the bedbugs was provided by two strips of thick filter paper cut half-way and placed together at right angles (Usinger, 1966). Most females are autogenous (i.e. able to lay eggs without taking a bloodmeal in the adult stage) (Davis, 1964). Males and females were allowed to mate and most females were allowed to lay eggs before their response to
the traps was tested. For each experiment, the filter paper substrate, on which five female and five male bedbugs were resting, was removed from the bottle and placed on the floor in the middle of the arena at an equal distance of 86.4 cm from each of the two traps being tested. The bedbugs were placed in the arena immediately before the person exited the room. Numbers of bedbugs caught in each trap were recorded the following morning.

Each experiment was set up as follows. One trap served as an untreated control without CO₂, heat or chemical lure. A treated trap emitted CO₂, heat and/or chemical lure (Table 1). The treatment trap was placed in one corner of the arena and the control trap was placed in the corner directly opposite it. The traps were rotated to each corner over 4 or 8 days and nights of testing. The long axis of each trap faced the centre of the arena. Emission of CO₂ was directed towards the centre of the arena.

Two control experiments were undertaken. The first was to test for possible effects of air movement caused by release of CO₂ through a small orifice. The catch of bedbugs in a trap emitting compressed CO₂ at 400 mL/min was compared with the catch in a trap emitting compressed air at 400 mL/min. In the second experiment, the traps which had been used as the treatment and control traps were switched round in order to determine if the physical features of the trap influenced the trap-catch.

Field sites and trapping procedures

Unoccupied high-rise apartment without furniture. A one-bedroom apartment on the sixth floor of a 109-unit, 15-storey high-rise apartment building, which had been designed for elderly and disabled people and built in 1996 in New Haven, CT, U.S.A., was reported to be infested with bedbugs on 3 June 2007. The mattresses and fabric furniture were steam-cleaned, and obvious harbourage sites, baseboards and the floor were treated with deltamethrin (Suspend SC; Bayer Environmental Science, Montvale, NJ, U.S.A.), 6% pyrethrin and 60% piperonyl butoxide (ExciteR; Sandersville, GA, U.S.A.) (6% pyrethrin) and Chlorfenapyr (Phantom; BASF Corporation, Research Triangle Part, NC, U.S.A.). The furniture and all belongings of the resident were removed and the resident was relocated before the experiments began. The floor area of the bedroom and living room measured 4.0 × 2.9 m and 4.6 × 2.5 m, respectively.

The efficacy of traps with and without chemical lures and CO₂ was tested in the apartment from 16 August to 3 September 2007. Two traps were used per day (one trap was placed in the bedroom and the other in the living room). All traps were heated. Only the trap in the bedroom had a chemical lure. The two traps were operated without CO₂ in the living room and bedroom for 15 and 14 days, respectively. CO₂ was added to the traps in the living room and bedroom for 4 and 5 days, respectively.

From 4 September to 5 October 2007, traps with and without heat, chemical lure and CO₂ were evaluated on 19 different days (Table 2). Three trapping stations were established in the living room and another three were set up in the bedroom. The three sampling sites were 2.7–4.0 m apart in the living room and 2.4–3.4 m apart in the bedroom. Traps were placed randomly at each trapping station on each day, set up between 08.00 hours and 09.00 hours, eastern daylight time, U.S.A., and operated for 24 h. Captured bedbugs were transferred from each trap into a labelled, wide-mouthed, clear plastic 540-ml container and killed by freezing or by submersion in 70% alcohol. Bedbugs were later identified to stage of development (Usinger, 1966).

Unoccupied high-rise apartment with furniture. Trapping was conducted in a seventh-floor, one-bedroom apartment in the building described above. This apartment had been sprayed with the same chemical pesticides as the apartment described above. The tenant had relocated but had left all the furniture in the apartment. One trap with or without heat and with chemical lure was placed in the bedroom next to the bed and another trap with heat only was placed in the living room next to the sofa or in the

### Table 1. Number of bedbugs captured in traps placed in the laboratory arena.

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Age, days*</th>
<th>Treatment†</th>
<th>Total bedbugs</th>
<th>Released</th>
<th>Trap 1</th>
<th>Trap 2</th>
<th>Not captured</th>
<th>χ²</th>
<th>P</th>
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<tr>
<td>1</td>
<td>23–40</td>
<td>50 CO₂</td>
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<td>40</td>
<td>31†</td>
<td>2</td>
<td>7</td>
<td>23.8</td>
<td>&lt;10⁻⁵</td>
</tr>
<tr>
<td>2</td>
<td>31–40</td>
<td>0</td>
<td>50 CO₂</td>
<td>40</td>
<td>4</td>
<td>28</td>
<td>8</td>
<td>16.5</td>
<td>&lt;10⁻⁴</td>
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<tr>
<td>3</td>
<td>22–34</td>
<td>100 CO₂</td>
<td></td>
<td>80</td>
<td>60</td>
<td>8</td>
<td>12</td>
<td>38.3</td>
<td>&lt;10⁻⁹</td>
</tr>
<tr>
<td>4</td>
<td>22–31</td>
<td>300 CO₂</td>
<td></td>
<td>40</td>
<td>26</td>
<td>6</td>
<td>8</td>
<td>11.28</td>
<td>&lt;10⁻³</td>
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<tr>
<td>5</td>
<td>2–17</td>
<td>400 CO₂</td>
<td></td>
<td>80</td>
<td>47</td>
<td>21</td>
<td>12</td>
<td>9.19</td>
<td>&lt;10⁻²</td>
</tr>
<tr>
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<td>16–28</td>
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<td>0</td>
<td>80</td>
<td>60</td>
<td>7</td>
<td>13</td>
<td>40.4</td>
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<td>26–36</td>
<td>400 CO₂</td>
<td>400 air</td>
<td>40</td>
<td>26</td>
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<tr>
<td>8</td>
<td>23–32</td>
<td>400 CO₂, H, L</td>
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<td>30</td>
<td>5</td>
<td>5</td>
<td>16.46</td>
<td>&lt;10⁻⁴</td>
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<tr>
<td>9</td>
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<td>H</td>
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<td>40</td>
<td>12</td>
<td>13</td>
<td>15</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
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<td>&lt;10⁻³</td>
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<tr>
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<td>10–19</td>
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<td>10</td>
<td>12</td>
<td>18</td>
<td>0.04</td>
<td>0.83</td>
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</tbody>
</table>

*Days as an unfed adult.
†Numbers indicate flow rate (mL/min) of released gas in trap (CO₂ or air).
H, heated trap; L, chemical lure; 0, no attractant.
‡Counts in bold are significantly (P < 0.05) larger than the catch in the other trap.
Statistical analysis

The chi-squared test with Yates’ correction for continuity (Conover, 1980) was used to evaluate all laboratory experiments and several of the field experiments. To compare the relative efficacy of traps with or without CO₂, chemical lure and heat, each of the 19 trapping dates from 5 September through 5 October 2007 was ranked from 1 to 6 according to the numbers of bedbugs caught (1 = least, 6 = most). When two or more trap catches were tied, ranks were averaged. Friedman non-parametric two-way analysis of variance was used to analyse the data (Conover, 1980).

Results

Laboratory experiments

Although all types of treated traps captured bedbugs, only traps with CO₂ emissions of 50–400 mL/min caught significantly (P < 10⁻²–10⁻⁹) more bedbugs (Experiments 1–6; Table 1). The attractiveness of CO₂ was also demonstrated by the capture of significantly (P < 0.01) more bedbugs in the trap expelling 400 mL CO₂/min compared with the trap emitting 400 mL compressed air/min (Experiment 7). When the traps that were normally designated as treatment and control traps, respectively, were switched (Experiment 2), the treated trap emitting 50 mL CO₂/min caught significantly (P < 0.001) more bedbugs than the control trap. These latter two experiments demonstrated that bedbugs were attracted to CO₂ and not the movement of gas forced from a small orifice, and that the physical features of the two traps were similar.

Field experiments

Unoccupied high-rise apartment without furniture. Traps with heat and with or without the chemical lure were tested without CO₂ on 29 trap-days and with CO₂ on 9 trap-days. The numbers of bedbugs captured were 656 and 5898 in traps without and with CO₂, respectively. The numbers of bedbugs of all feeding stages captured were significantly greater in traps with CO₂ (χ² = 15 942, d.f. = 1, P < 10⁻⁹). Similarly, when feeding stages were segregated into early-, mid- and late-stage nymphs and adults, the numbers captured were significantly greater in traps emitting CO₂ (first and second instars: χ² = 13 220, d.f. = 1, P < 10⁻⁶; third instars: χ² = 1 259, d.f. = 1, P < 0.001; fourth and fifth instars: χ² = 1109, d.f. = 1, P < 10⁻⁶; adults: χ² = 522, d.f. = 1, P < 10⁻⁶).

Chi-squared tests showed that traps that included CO₂ (P < 10⁻⁹, traps 1, 3, 4), heat (P < 10⁻², traps 1, 2, 3) or a chemical lure (P < 10⁻⁶, traps 1 and 5) captured more bedbugs of all
feeding stages than the unbaited trap (trap 6) (Table 2). The nonparametric two-way analysis of variance evaluation of the total catch in six different traps over 19 trapping days showed that trap type was highly significant ($P < 10^{-7}$) (Table 2). With the exception of first and second instars, where differences in trap catches were not significant, significant differences were noted for all stages. However, traps with CO$_2$ alone or heat alone did not catch significantly greater numbers of adult bedbugs compared with the non-baited control trap. The trap with heat alone did not capture significantly more early or late instar bedbugs than the non-baited trap. The trap with CO$_2$, heat and the chemical lure (trap 1) captured 52.1% of all bedbugs, but only caught significantly more fourth and fifth instar nymphs than all other traps. Otherwise, the catches in trap 1 did not differ significantly from those of trap 3, which contained CO$_2$ and heat. A chi-squared analysis comparing the numbers of bedbugs captured in each room did not show significant differences at the $P = 0.05$ level.

The total numbers of bedbugs collected for each trapping date (pooling all six traps) followed an exponential decline over the trapping period (Fig. 2). The numbers trapped declined from over 1000 bedbugs on 4 September to 14 bedbugs on 5 October 2007. This same exponential decline was true for first-, second- and third-stage nymphal instars (exponential decline = 0.13/day, $R^2 = 0.67$) and older insects (fourth and fifth instars and adults: exponential decline = 0.10/day, $R^2 = 0.46$).

**Unoccupied high-rise apartment with furniture.** Two first or second instar nymphs and 17 third instar or larger nymphs and adults were collected over one 17-day period in the bedroom with a trap containing a chemical lure with or without heat. In the living room or kitchen, where the trap contained heat only, one early instar and seven third instar or larger nymphs and adults were collected over the same 17-day period. A trap with CO$_2$ placed in the bedroom for 3 days captured 50 bedbugs (11 adults, 19 fourth and fifth instars, nine third instars, and 11 first and second instars) and a trap containing CO$_2$ placed in the living room for 1 day and in the kitchen for 1 day captured four bedbugs, (one adult, one fourth instar, one third instar, and one first or second instar). Significantly more bedbugs were captured in CO$_2$-baited traps ($\chi^2 = 210.1$, d.f. = 1, $P < 10^{-9}$).

**Occupied apartment.** Four bedbugs (three and one fourth instars) and 29 bedbugs (23 first and second instars, four third instars, two adults) were collected during the 2 nights the trap had been placed on the floor next to the head of the bed where the person slept. One fourth instar nymph was filled with fresh blood. The others had not recently fed on blood.

**Discussion**

Bedbugs have been reported to be attracted to human breath and CO$_2$ (Marx, 1955), to heat (Rivnay, 1932; Sioli, 1937; Aboul-Nasr & Erakey, 1967) and to chemical odours (Rivnay, 1932; Aboul-Nasr & Erakey, 1968). *Triatoma infestans*, another human biting hemipteran, was reported to be attracted to CO$_2$, 1-octen-3-ol and short-chain fatty acids, such as propionic, butyric and valeric acids, mixed with L-lactic acid (Barrozo & Lazzari, 2004a, 2004b). The described trap was based upon the previously reported attraction of bedbugs to CO$_2$ and heat, and to their possible attraction to chemicals that initiated positive responses in *T. infestans*.

CO$_2$ was the most effective bait for attracting bedbugs to traps in laboratory and field studies. In the laboratory, traps emitting CO$_2$ invariably captured significantly more unfed bedbugs (2–40 days after molting) than traps without CO$_2$, even at concentrations of 50mL/min, a concentration significantly below the 15L/h (250mL/min) expired by a resting human (Leff & Schumacker, 1993). In field studies in two different unoccupied infested apartments, traps with CO$_2$ were similarly superior to traps without CO$_2$.

In the two-way analysis of variance of total numbers of bedbugs collected in all stages in six different types of traps in an infested uncluttered apartment, the trap with CO$_2$, heat and a chemical lure (trap 1) captured significantly more bedbugs than all other types of trap except the trap with CO$_2$ and heat (trap 3). The addition of heat and possibly a chemical lure added to CO$_2$ seemed to have a synergistic effect. Adult bedbugs were equally attracted to traps 1, 3 and 5 (lure only). We are unable to explain why baited traps did not catch significantly greater numbers of first and second instar nymphs than the non-baited trap.

Bedbugs have been reported to detect temperatures that are $\geq 1$ °C higher than ambient from a distance of 1–5 cm, but they apparently do not respond to temperature differences of $\leq 0.5$ °C (Rivnay, 1932; Sioli, 1937). The trap baited with heat only in field tests (trap 2) did not catch significantly more bedbugs than the unbaited trap. In laboratory tests, traps with heat caught significantly more relatively older adult bedbugs (22–44 days, Experiment 10), but not younger bedbugs (15–24 days, Experiment 9), but 38.3% of the released bedbugs did not enter either trap. The heating element of the trap produced temperatures of 37.2–42.2 °C, but the temperature near the base of the trap was $< 0.5$ °C above ambient. The temperatures produced by the trap may have been insufficient to attract bedbugs crawling on the floor and thus many adults were not attracted to either trap. However, the 1.3°C increase in temperature at the lip of the trap.
trap may have been attractive to bedbugs that ascended the sides of the trap and thus encouraged larger numbers to fall into the trap.

All the bedbugs captured in the unoccupied apartments were probably seeking a host. Blood was not observed in any of the 9468 bedbugs captured in our traps. Our trapping did not begin until 30 days after the tenants had moved out of the apartments. Nymphal development following repletion has been reported to range from 5.18 days in first-stage instars to 7.81 days in fifth-stage instars at 23 °C, and eggs are initially laid by adult females an average of 5.38 days after engorgement at 23 °C (Johnson, 1942). Thus, all nymphs had had time to digest their food and moult, and, in the case of adults, lay eggs and begin seeking a bloodmeal during the trapping period in the unoccupied apartments. Mellanby (1939) reported pitfall traps to be comprised of 77.9% unfed and 22.1% blood-fed bedbugs in an animal room where bedbugs fed on rats. We captured one bedbug containing blood during the trapping period in the unoccupied apartment. This type of trap may be useful in detecting infestations, especially early infestations, which can be difficult and time-consuming to detect by manual surveillance.

Pyrethroid insecticides repel cockroaches and elicit dispersal behaviour (Ross & Cochrane, 1992). We placed traps at two field sites that had previously been treated with a pyrethroid insecticide, which may have been irritating to bedbugs and thus influenced our trapping results. However, pyrethroid insecticides are not known to repel bedbugs or to increase their dispersal (Cooper & Harlan, 2004). Furthermore, our laboratory studies, which showed bedbugs to be attracted to traps baited with CO₂, were conducted in a room in which pyrethroids had never been used.

Baited traps may be useful for reducing the numbers of host-seeking bedbugs, thereby reducing the number of bites on humans. Our trapping on 19 different days in an unoccupied apartment resulted in an exponential decline in bedbug catch over 31 days. This reduction is likely to be primarily a result of trapping rather than normal mortality. Bedbugs survive without feeding for relatively long periods; at 25 °C, first and second instar nymphs kept under 90% RH had mean lifespans of 45.7 and 53.8 days, respectively, and adult males and females had mean lifespans of 85.2 and 69.4 days, respectively, under the same conditions (Johnson, 1942).

The attractiveness of CO₂ to bedbugs may have been enhanced by the addition of the volatile chemicals tested in this study, as has been reported for other biting insects (Vale & Hall, 1985; Takken & Kline, 1989; Van Essen et al., 1994; Barrozo & Lazzari, 2004b) and by the addition of heat, which has previously been shown to be an attractant to bedbugs (Rivnay, 1932; Sioli, 1937; Aboul-Nasr & Erakey, 1967). We believe this type of trap has utility in studying the ecology of bedbugs in dwellings occupied and unoccupied by humans.

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