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Research article

Repellency of Naturally Occurring or Related Compounds, DEET, and *Para*-Menthane-3,8-Diol to Bed Bugs (Hemiptera: Cimicidae)

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Abstract

Bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), have become a major health nuisance in the past 20 ysin cities and elsewhere throughout many areas of the world. Few studies have reported on repellent compounds that could reduce their transport in luggage. We evaluated the repellency of six naturally occurring or related compounds used in flavor/fragrance applications or structurally related compounds, para-menthane-3,8-diol, and N,N-diethyl-meta-toluamide (DEET) to bed bugs in a 183 × 183-cm arena . Repellency was assessed using soft-sided polyester lunch bags serving as surrogates of luggage and barrier cloth towels upon which rested untreated lunch bags. We report for the first time repellency of delta dodecalactone, 2-(3, 7-dimethyl-2, 6-nonadien-1-yl)-cyclopentanone (a.k.a. 'methyl apritone'), gamma dodecalactone, and para-menthane-3,8-diol to bed bugs. Propyl dihydrojasmonate, 3-methyl-5-hexyl-2-cyclohexenone, gamma methyl tridecalactone, and DEET are also documented to be repellent to bed bugs. These compounds provided relatively long-term protection. Propyl dihydrojasmonate prevented bed bugs from seeking refuge in treated lunch bags 27 d after treatment, and when applied to cloth towels repelled harborage-seeking bed bugs for 146 d. Methyl apritone blended with 3-methyl-5-hexyl-2-cyclohexenone and delta dodecalactone as an individual compound applied to cloth towels repelled bed bugs for 190 and 276 d, respectively. The above-mentioned compounds, either individually or as blends, may reduce risk of bed bugs seeking harborage in treated suitcases or towels upon which untreated luggage is placed.

Key words: Bed bug, *Cimex lectularius*, repellent, propyl dihydrojasmonate

Bed bugs, *Cimex lectularius* L. (Hemiptera: Cimicidae), have become a significant public nuisance worldwide in the past two decades, and numerous studies have reported various methods of detecting and controlling populations (Reinhardt and Siva-Jothy 2007, Doggett et al. 2012, Vaidyanathan and Feldlaufer 2013). Bed bugs are flightless, nest-dwelling parasites that feed for relatively short periods of time while spending most of their time hidden near the sleeping areas of humans. Active dispersal to nearby rooms or apartments has been documented (Wang et al. 2010, Cooper et al. 2015, Akhoundi et al. 2015, Raab et al. 2016), but long-distance movement to new buildings is likely passive in nature and may occur in luggage and boxes carrying clothing and other personal items (Pinto et al. 2007). In fact, infestation of luggage with bed bugs is a major concern for travelers. Chemical repellents that could be applied to luggage, floors, passage ways along pipes where bed bugs travel, and boxes may be useful in preventing dispersal of bed bugs to new dwellings.

In contrast to the many publications on use of repellents to prevent bites from mosquitoes, other biting insects, and ticks, few have addressed the possible use of repellents for bed bugs (Debbourn et al. 2007). Liu et al. (2014) reported bed bug olfactory sensilla responses to 52 known insect synthetic and botanic repellents, and alarm pheromones of bed bugs have been identified (Levinson et al. 1974, Siljander et al. 2008, Feldlaufer et al. 2010, Liedtke et al. 2011, Gries et al. 2015, Ulrich et al. 2016) . Kumar et al. (1995) reported N,N-diethyl-meta-toluamide (DEET) and *N,N*-diethyl phenyl acetamide (DEPA) to be repellent against bed bugs. Wang et al. (2013) reported efficacy of commercial repellents, including DEET, and natural compounds with repellent activity. Products tested by Wang et al. (2013) included, isolongifolenone, isolongifolanone,

3-methyl-5-hexyl-2-cyclohexenone, propyl dihydrojasmonate, and gamma methyl tridecalactone. Akhtar and Isman (2016) reported the following eight compounds to be repellent to bed bugs: methyl trans-4-oxo-2-pentenoate, 1-furan-2-yl-2-methylbutan-1-one, (E)-1-hydroxyoct-2-en-4-one, 6, 10-dimethyl-5, 9-undecadien-2-one, furfuryl propionate, 2-butyrylfuran, 1-(furan-2-yl)-pentan-1-ol, and (E)-3-methylhept-3-ene-2,5-dione. We now report for the first time the novel compounds delta dodecalactone, 2-(3,7-dimethyl-2,6-nonadien-1-yl)-cyclopentanone (a.k.a. 'methyl apritone'), and gamma dodecalactone, along with the conventional repellent para-menthane-3,8-diol to be repellent to bed bugs. These chemicals have relatively low volatility, which may result in a longer time-period of protection. Additionally, we report the previously studied compounds propyl dihydrojasmonate, 3-methyl-5-hexyl-2-cyclohexenone, gamma methyl tridecalactone, and DEET to be repellent to bed bugs in a relatively large arena. With the exception of para-menthane-3,8-diol, and DEET, all of the tested compounds occur in nature or are closely related to natural products.

Materials and Methods

Bed Bugs

Bed bugs were from colonies originating in New Haven, CT, and East Hartford, CT, in 2007 and were fed on laboratory rabbits. Male and female unfed adult bed bugs, which were 15- to 45-d old, were used in all experiments. The bed bugs had not fed in the adult stage.

Rabbits were handled in a manner approved by the Animal Care and Use Committee at the Connecticut Agricultural Experiment Station (IACUC # P17-12).

Chemicals Tested

Six naturally occurring or structurally closely related products used in the flavor and fragrance industry, para-menthane-3,8-diol, and DEET were evaluated for efficacy in repelling bed bugs (Table 1). Compounds were tested individually or in combination as blends. The products used in the fragrance industry are considered to be relatively safe to humans and the environment (Ford 1994). Bedoukian Research holds patents/applications on the six naturally occurring or structurally closely related products. These materials have low volatility, which may enhance the time of protection, and were shown in company studies to be repellent to bed bugs in Petri dish assays and to mosquitoes.

Lunch Bag Experiments

Soft-sided luggage bags with their many seams and folds and overall texture are likely refuges for bed bugs (Pinto et al. 2007). We tested soft-sided lunch bags with seams and added a used white cotton sock, worn the day before testing, to evaluate different repellents. The lunch bags with a sock were used to simulate luggage containing recently worn clothing. Soiled clothing has been reported to be attractive to bed bugs (Hentley et al. 2017). Experiments evaluating repellent chemicals applied to lunch bags were conducted in a 183 × 183-cm arena on a light-colored tile floor (Anderson et al. 2009). The temperature was 23°C, and the photoperiod was a 16:8 (L:D) h cycle. Two-sided carpet tape (11.4-cm wide) was placed on the borders of the arena to keep bed bugs from escaping.

To simulate luggage, light blue 20.3 × 15.2 × 15.2-cm Budget Kooler Polyester lunch bags with a white interior and with a black strap (Motivators Promotional Products, Westbury, NY) weighing approximately 55 g were used. A treated bag was placed in one corner of the arena, and an untreated bag was placed in the opposite corner. The treated bag was sprayed on the outside and inside with the repellent. Repellent chemicals diluted in 95% ethanol were allowed to dry for 7.0 h before testing. The control bag was sprayed with 95% ethanol. Quantities of chemical applied to each bag were determined by weighing the spray bottle before and after application. Approximately 12 g of various solutions were applied to the outside, including the seams and strap, and 4.5 g were applied to the inside for a total of 16.5 g. Treatments were replicated twice, except for the single replication of 7.0% methyl apritone + 3.0% 3-methyl-5-hexyl-2-cyclohexenone. The floor of the arena was cleaned with 70% isopropyl alcohol following the completion of each experiment.

Twenty-five male and female (13 males and 12 females) adult bed bugs placed in a 16-ml clear glass bottle were used in each test. Two pieces of thick blotter paper (l.0 mm in thickness) measuring 1.6 × 4.4 cm and connected at right angles served as a refuge in the glass bottle covered with nylon netting. The unfed bed bugs were held in the bottles for up to 2 wk and not fed before use. The blotter paper, containing the 25 bed bugs, was removed from the bottle and gently placed in the center of the arena at 3:45 to 4:00 pm. Allowing the bed bugs to disperse from the refuge is more natural than placing bed bugs directly on the floor. Numbers of male and female bed bugs resting in or on each bag and elsewhere in the arena were counted the following morning at 8:30 to 9:00 am. Female bed bugs may leave refuges sooner and more frequently than males (Pfiester et al. 2009, Aak et al. 2014). We assessed whether female bed bugs left the refuges more frequently than males. The locations of the treated and untreated bags were switched for each replication. Individual compounds or blends were applied to lunch bags and evaluated for repellency.

Cloth Towel Experiments

We also examined repellents applied to cloth towels upon which an untreated lunch bag was placed. A cloth, with an effective repellent upon which luggage is placed, could prevent bed bugs from entering luggage placed on the floor, luggage racks, or furniture in a room.

White Herringbone cotton dish towels measuring 35.6 × 61 cm (Medline Industries Mundelein, IL) and weighing approximately

Table 1. Compounds tested individually or in blends for repellency to bed bugs

Compound	Origin	Purity (%)	Supplier
DEET	Synthetic	98.5	Sigma–Aldrich, St. Louis, MO
Para-menthane-3,8-diol	Nature Identical	98.3	Takasago, Inc., Ohta-ku, Tokyo, Japan
Delta dodecalactone	Nature Identical	99.9	Wanxiang International, Paramus, NJ
Gamma dodecalactone	Nature Identical	98.0	Mitsui-Soda, Tokyo, Japan
Gamma methyl tridecalactone	Synthetic	99.5	Bedoukian Research, Inc., Danbury, CT
Methyl apritone	Synthetic	99.0	Bedoukian Research, Inc.
Propyl dihydrojasmonate	Synthetic	86.7	Bedoukian Research, Inc.
3-Methyl-5-hexyl-2-cyclohexenone	Synthetic	99.6	Bedoukian Research, Inc.

53.5 g were treated with repellent-solutions and dried for a defined period of time before being placed in a sealed plastic bag. Towels were tested from 0 to as long as 276 d after removal from the sealed bag in the arena described above. Tests were terminated when two or more bed bugs sought refuge in lunch bags resting on the treated towel or when treated towels were effective for a relatively long period of time (i.e., 190 and 276 d). Most treatments were replicated two times, except 3% propyl dihydrojasmonate + 1.0% DEET and 3% propyl dihydrojasmonate + 1.0% *para*-menthane-3,8-diol, which were tested once. *Para*-menthane-3,8-diol is a conventional mosquito repellent, and we wanted to determine whether mixing with propyl dihydrojasmonate might improve efficacy. Mixtures evaluated in our study were tested to evaluate efficacy and provide minimal odor intensity. A treated towel with an untreated lunch bag with a previously worn white sock, as described earlier, was placed in one corner of the arena, and an untreated cloth with an untreated lunch bag and a previously worn white sock was placed in the opposite corner of the arena. Twenty-five unfed adult bed bugs resting on a refuge as described earlier were placed in the center of the arena at 3:45 to 4:00 pm. The number of bed bugs in or on the bags, on or under the towels, or elsewhere in the arena was counted the following morning at 8:30 to 9:00 am as described earlier. We assessed whether female bed bugs left the refuges more frequently than males as discussed earlier. The floor of the arena or beneath the towels was cleaned with 70% isopropyl alcohol each morning following the completion of the experiment. Treated towels were hung open to air in another room between experiments. Individual compounds or blends were evaluated.

Statistics

In the experiments where repellent chemicals were applied to lunch bags or to towels upon which untreated lunch bags were placed, bed bugs ended up either in or on the treated lunch bag or towel, the untreated lunch bag or towel, or elsewhere in the arena. The choice of bed bugs between treated and control lunch bags is conditional on a bed bug finding a refuge. Thus, the repellency of a chemical or blend was evaluated as a conditional probability, since only bed bugs finding harborage in a lunch bag or cloth towel provide information on the repellency of a chemical compound (Agresti 2007). A chi-squared test with Yates' correction for continuity, based on bed bugs which entered and stayed in lunch bags or on repellent treated-cloth towels, was used to evaluate repellency of the treated bag or cloth for each experiment (Sokal and Rohlf 1981). The variability in capture rate was accounted for by the Yates' correction when trapped counts were low.

To determine if males or females left the refuge in equal numbers, numbers of each sex remaining on the refuges in the morning were analyzed using Fisher's exact test with Yate's correction for continuity (Sokal and Rohlf 1981).

Results

Five naturally occurring or related compounds individually or in blends and DEET significantly reduced numbers of bed bugs hiding in treated versus the untreated lunch bags (Table 2). The percentage of bed bugs finding harborage in lunch bags varied widely, with an overall average of 61.4%. Significantly fewer bed bugs sought harborage in most treated lunch bags, but two or fewer bed bugs per replication found refuge in lunch bags during the night with the following treatments: 25% propyl dihydrojasmonate, 1.5% propyl dihydrojasmonate + 1% 3-methyl-5-hexyl 2-cyclohexenone, 5.0% and 2.5% 2-(3,7-dimethyl-2,6-nonadien-1-yl)-cyclopentanone(a.k.a.'methylapritone'),2.5%3-methyl-5-hexyl-2-cyclohexenone, 7.0% methyl apritone + 3.0% 3-methyl-5-hexyl cyclohexanone, 5.0% methyl apritone + 2.5% 3-methy-5-hexyl-2-cyclohexenone, 2.5 % methyl apritone + 2.5% 3-methyl-5-hexyl-2-cyclohexenone, 2.5% gamma dodecalactone, and 10% DEET (Experiments 1, 7, 9, 10, 12, 14, 17, 20, 22, and 24). Longer duration protection with highly significant differences recorded between treated and untreated lunch bags of 7–27 d following treatment was noted for 25% propyl dihydrojasmonate, 7.0% methyl apritone + 3.0% 3-methyl-5-hexyl-2-cyclohexenone, 5.0% methyl apritone + 3.0% 3-methyl-5-hexyl-2-cyclohexenone, and DEET (experiments 2, 16, 19, and 26).

Two individual compounds, seven blends, and DEET prevented bed bugs from taking refuge in untreated lunch bags resting on treated cloth towels for 43–276 d following removal of the treated towels from sealed plastic bags (Table 3). Significantly fewer bed bugs sought refuge in untreated lunch bags resting on towels treated with 6.67 % methyl apritone + 3.33% 3-methyl-5-hexyl-2-cyclohexenone + 2.5% tocopherol (used as an antioxidant here, but deemed not necessary for other materials that were more stable) for up to 190 d (experiments 27–36). Towels treated with propyl dihydrojasmonate alone (experiments 37–45) significantly repelled bed bugs for 146 d. As a blend, it significantly prevented bed bugs from resting in untreated lunch bags for 143 d (experiments 46–57), 76 d (experiments 58–64), and 71 d (Experiments 65–70). Delta dodecalactone applied to towels alone (experiments 71–83) and as a blend (experiments 84–93, 94–99, and 100–107) was effective in repelling bed bugs for 91–276 d. Towels treated with 10% DEET were effective for 43 d (experiments 108–113).

A comparison of numbers of males and females remaining on the refuge the morning following placement in the arena showed that males were more likely than females to be found on the refuge. Overall, 20.4% of the males versus 14.6% of the females released were found in the refuge (chi square = 25.71, probability <0.001).

Discussion

While bed bugs are not known to transmit disease to their human hosts, the bites of bed bugs can affect the health and well-being of those who have been bitten. Effects vary from simple skin irritation from the 'transient' bite, to more serious secondary skin infections and illness for people that have a more significant exposure or who may have underlying health issues, and even to mental illness from the fear of being 'infested' by these insects (Goddard and deShazo 2009). With these factors in mind, the use of repellent chemicals may be helpful in preventing bed bug bites and their spread to new locales. Application to luggage, fabrics upon which luggage is placed, and clothing such as trousers, socks, shoes, and shirts may reduce risk of exposure of persons, who may come into contact with bed bugs. Be they travelers staying overnight in hotel rooms, social workers, health officials, pest control operators, apartment dwellers and their visitors, and maintenance workers, who become exposed to bed bugs when working or visiting infested rooms or hallways.

We report six naturally occurring or structurally related products used in the flavor and fragrance industry, para-menthane-3,8-diol, and DEET individually or as blends to be relatively repellent to bed bugs. We believe these products, based on our studies, show promise as repellents, which could be applied to luggage, clothing, bed sheets, or barrier cloths upon which luggage, clothing, or boxes are stored, or even furniture fabrics. They may even be usefully applied to wooden surfaces, such as floors, bed posts, clothing bureaus, and drawers, and to pipes in wall voids connecting apartments that are used by bed bugs to disperse to new dwellings, but additional studies are needed to confirm effectiveness on these various substrates.

Table 2. Number of bed bugs seeking refuge overnight in lunch bags treated with repellents

Experiment no.	Treatment (g)	Days after application of repellent	Replicates	Total bed bugs				Chi-square	Probability
				Released	In lunch bags		In arena		
					Treatment	Untreated			
1	25% Propyl dihydrojasmonate (17.6)	0.3[a]	2	50	0	25	25	23.04	<0.001
2	25% Propyl dihydrojasmonate (17.6)	27	2	50	2	22	26	15.04	<0.001
3	12.5% Propyl dihydrojasmonate (19.0)	0.3	2	50	6	31	13	15.57	<0.001
4	5.0% Propyl dihydrojasmonate (19.4)	0.3	4	100	9	41	50	19.22	<0.001
5	2.5% Propyl dihydrojasmonate (19.6)	0.3	2	50	6	17	27	4.35	0.037
6	0.625% Propyl dihydrojasmonate (19.4)	0.3	2	50	8	16	26	2.04	0.153
7	1.5% Propyl dihydrojasmonate + 1% 3-methyl-5- hexyl-2-cyclohexenone (19.0)	0.3	2	50	3	26	21	16.69	<0.001
8	2.5% Gamma-methyl tridecalactone (18.9)	0.3	2	50	5	21	24	8.77	0.003
9	5.0% Methyl apritone[b] (19.0)	0.3	2	50	2	39	9	31.61	<0.001
10	2.5% Methyl apritone (19.0)	0.3	2	50	2	33	15	25.71	<0.001
11	2.5% Methyl apritone (19.0)	42	2	50	12	28	10	5.63	0.018
12	2.5% 3-Methyl-5-hexyl-2-cyclohexenone (19.2)	0.3	2	50	2	33	15	25.71	<0.001
13	2.5% 3-Methyl-5-hexyl-2-cyclohexenone (19.2)	14	2	50	6	35	9	19.12	<0.001
14	7.0% Methyl apritone + 3.0% 3-methyl-5-hexyl-2-cyclohexenone (16.3)	0.3	1	25	1	22	2	17.39	<0.001
15	7.0% Methyl apritone + 3.0% 3-methyl-5-hexyl-2-cyclohexenone (16.3)	3	1	25	0	20	5	18.05	<0.001
16	7.0% Methyl apritone + 3.0% 3-methyl-5-hexyl-2-cyclohexenone (16.3)	7	1	25	0	19	6	17.05	<0.001
17	5.0% Methyl apritone + 2.5% 3-methyl-5-hexyl-2-cyclohexenone (18.9)	0.3	2	50	3	30	17	20.49	<0.001
18	5.0% Methyl apritone + 2.5% 3-methyl-5-hexyl-2-cyclohexenone (18.9)	3	2	50	3	30	17	20.49	<0.001
19	5.0% Methyl apritone + 2.5% 3-methyl-5-hexyl-2-cyclohexenone (18.9)	7	2	50	0	27	23	25.04	<0.001
20	2.5% Methyl apritone + 2.5% 3-methyl-5-hexyl-2-cyclohexenone (19.1)	0.3	2	50	0	35	15	33.03	<0.001
21	2.5% Methyl apritone + 2.5% 3-methyl-5-hexyl-2-cyclohexenone (19.1)	7	2	50	8	26	16	8.5	0.004
22	2.5% Gamma-dodecalactone (19.6)	0.3	2	50	2	30	18	22.78	<0.001
23	2.5% Gamma-dodecalactone (19.6)	7	2	50	7	24	19	8.26	0.004
24	10% DEET (17.9)	0.3	3	75	2	58	15	50.42	<0.001
25	10% DEET (17.9)	3	3	75	4	55	16	42.37	<0.001
26	10% DEET (17.9)	7	3	75	11	46	18	20.28	<0.001

[a]Tests conducted overnight following treatment 7 h earlier.

[b]2-(3,7-Dimethyl-2,6-nonadien-1-yl)-cyclopentanone.

Table 3. Number of bed bugs seeking refuge overnight in untreated lunch bags placed on cloth towels treated with a repellent

Experiment	Treatment		Day[a]	Replicates	Total bed bugs				Chi-square	Probability
	Towel 1 (g)	Towel 2			Released	Towel 1	Towel 2	Arena		
27	6.67% Methyl apritone +	0	1	2	50	0	27	23	25.04	<0.001
28	3.33% 3-methyl-5-hexy-	0	7	2	50	0	30	20	28.03	<0.001
29	2-cyclohexenone + 2.5%	0	16	2	50	0	31	19	29.03	<0.001
30	tocopherol (10g)	0	22	2	50	0	28	22	26.04	<0.001
31		0	30	2	50	0	32	18	30.03	<0.001
32		0	61	2	50	0	31	19	29.03	<0.001
33		0	89	2	50	2	27	21	19.86	<0.001
34		0	114	2	50	7	21	22	6.04	0.014
35		0	154	1	25	0	12	13	10.08	0.001
36		0	190	1	25	0	17	8	15.06	0.014
37	5.0% Propyl dihydrojas-	0	1	2	50	0	30	20	28.03	<0.001
38	monate (5)	0	4	1	25	0	7	18	5.14	0.023
39		0	11	2	50	0	27	23	25.04	<0.001
40		0	24	2	50	0	36	14	34.03	<0.001
41		0	32	2	50	0	32	18	30.03	<0.001
42		0	54	2	50	0	35	15	23.04	<0.001
43		0	88	2	50	0	28	22	26.04	<0.001
44		0	118	1	25	0	10	15	8.10	0.004
45		0	146	1	25	2	12	11	5.79	0.016
46	5.0% Propyl dihydrojas-	0	1	2	50	0	32	18	30.03	<0.001
47	monate + 2.5% 3-methyl-	0	6	1	25	0	16	9	14.06	<0.001
48	5-hexyl-2-cyclohexenone	0	13	2	50	0	28	22	26.04	<0.001
49	(7.5)	0	20	1	25	0	11	14	9.09	0.003
50		0	28	2	50	0	30	20	28.03	<0.001
51		0	35	2	50	0	30	20	28.03	<0.001
52		0	63	2	50	0	28	22	26.04	<0.001
53		0	93	2	50	1	27	23	22.32	<0.001
54		0	101	2	50	0	30	20	28.03	<0.001
55		0	113	2	50	0	26	24	24.04	<0.001
56		0	143	2	50	0	34	16	32.03	<0.001
57		0	192	1	25	4	12	13	3.06	0.080
58	3.0% Propyl dihydrojas-	0	1	1	25	1	14	10	9.60	0.002
59	monate + 1.0% DEET (4)	0	7	1	25	0	12	13	10.08	0.001
60		0	13	1	25	0	14	11	12.07	0.001
61		0	21	1	25	0	13	12	11.08	0.001
62		0	34	1	25	0	11	14	9.09	0.003
63		0	76	1	25	1	13	11	8.64	0.003
64		0	104	1	25	4	12	9	3.06	0.080
65	3.0% Propyl dihydrojas-	0	1	1	25	0	14	11	12.07	0.001
66	monate + 1.0% para-	0	8	1	25	0	16	9	14.06	<0.001
67	menthane-3,8-diol (4)	0	16	1	25	0	12	13	10.08	0.001
68		0	29	1	25	0	13	12	11.08	0.001
69		0	71	1	25	1	18	6	13.47	<0.001
70		0	99	1	25	4	12	9	3.06	0.080
71	5.0% Delta dodecalactone	0	1	2	50	0	31	19	29.03	<0.001
72	(5)	0	3	1	25	0	12	13	10.08	0.001
73		0	8	1	25	0	13	12	11.08	0.001
74		0	17	2	50	0	28	22	26.04	<0.001
75		0	29	2	50	0	35	15	33.03	<0.001
76		0	58	2	50	0	25	25	23.04	<0.001
77		0	88	2	50	0	28	22	26.04	<0.001
78		0	121	2	50	1	34	15	29.26	<0.001
79		0	161	2	50	0	26	24	24.04	<0.001
80		0	189	1	25	0	16	9	14.06	<0.001
81		0	217	1	25	0	13	12	11.08	0.001
82		0	245	1	25	0	14	11	12.07	0.001
83		0	276	1	25	0	14	11	12.07	0.001
84	3.0% Delta dodecalactone +	0	1	2	50	0	38	12	36.03	<0.001
85	1.0% DEET (4)	0	6	2	50	0	27	23	25.04	<0.001
86		0	15	2	50	0	30	20	28.03	<0.001
87		0	21	2	50	0	29	21	27.03	<0.001
88		0	29	2	50	1	25	24	20.35	<0.001

Table 3. *Continued*

Experiment	Treatment		Day[a]	Replicates	Total bed bugs				Chi-square	Probability
	Towel 1 (g)	Towel 2			Released	Towel 1	Towel 2	Arena		
89		0	36	2	50	3	21	26	12.04	<0.001
90		0	43	1	25	0	18	7	16.06	<0.001
91		0	63	1	25	0	14	11	12.07	0.001
92		0	91	1	25	1	15	9	10.56	0.001
93		0	98	1	25	1	10	14	5.82	0.016
94	2.5% Delta dodecalactone +	0	1	2	50	0	26	24	24.04	<0.001
95	2.5% 3-methyl-	0	6	2	50	0	20	30	18.05	<0.001
96	5-hexyl-2-cyclohexenone	0	16	2	50	0	19	31	17.05	<0.001
97	(5)	0	40	2	50	1	36	13	14.78	<0.001
98		0	67	2	50	1	34	15	29.26	<0.001
99		0	104	2	50	0	29	21	27.03	<0.001
100	3.0% Delta dodecalactone +	0	1	2	50	0	33	22	31.03	<0.001
101	1.0% para-menthane-	0	6	2	50	0	36	14	34.03	<0.001
102	3,8-diol (4)	0	13	2	50	0	37	13	35.03	<0.001
103		0	20	2	50	0	30	20	28.03	<0.001
104		0	28	2	50	0	27	23	25.04	<0.001
105		0	35	2	50	1	33	16	28.26	<0.001
106		0	53	2	50	2	33	15	25.71	<0.001
107		0	91	1	25	2	12	11	5.79	0.016
108	10% DEET (10)	0	1	2	50	0	32	18	30.03	<0.001
109		0	7	2	50	1	27	22	22.32	<0.001
110		0	16	2	50	0	26	24	24.04	<0.001
111		0	23	2	50	1	31	18	26.28	<0.001
112		0	43	1	25	0	15	10	13.07	<0.001
113		0	51	2	50	16	17	17	0.00	1

[a]Treated towel removed from sealed plastic bag on day 0 and tested that evening. The following day, the towel was hung in another room until tested in the bed bug arena at a later date. This procedure was repeated until testing was stopped.

Our studies using repellent-treated lunch bags as surrogates of luggage and cloth towels as barriers are the first to show repellency of delta dodecalactone, methyl apritone, gamma dodecalactone, and para-menthane-3,8-diol to bed bugs, except Bedoukian (2013) in a patent application reported methyl apritone to be repellent to bed bugs in petri dish assays. The first three compounds have been reported in patent applications to be repellent to mosquitoes (Takken et al. 2015, Bedoukian 2016). These are novel compounds, and extensive bioassay studies have not been reported previously. In contrast to DEET (Gupta and Bhattacharjee 2007), these materials do not have unpleasant odors and do react with plastics or synthetic rubber. These compounds show relatively long lasting effectiveness, probably due, in part, to their low volatility. The 5% delta dodecalactone-treated towel was effective for 276 d, the longest period of time that a towel effectively repelled bed bugs. Methyl apritone and gamma-dodecalactone applied to lunch bags were effective overnight against bed bugs, and when methyl apritone was mixed with 3-methyl-5-hexyl-2-cyclohexenone, treated lunch bags were repellent for 7 d and treated towels were effective for 190 d. *Para*-menthane-3,8-diol combined with propyl dihydrojasmonate was effective on cloth towels for 53 d.

The previously reported bed bug repellents propyl dihydrojasmonate, 3-methyl-5-hexyl-2-cyclohexenone, and gamma methyl tridecalactone by Wang et al. (2013) were also effective in our relatively larger arena studies. Propyl dihydrojasmonate was the longest lasting (27 d) product applied to lunch bags. The compound 3-methyl-5-hexyl-2-cyclohexenone, when combined with methyl apritone or propyl dihydrojasmonate, provided relatively long-lasting repellency for up to 190 d on cloth towels.

DEET and *para*-menthane-3-8-diol are well-known mosquito repellents (Debboun et al. 2007). DEET applied to lunch bags and to cloth towels was effective against bed bugs for 7 and 43 d, respectively, and when mixed with propyl dihydrojasmonate or delta dodecalactone to assess synergism, effectiveness on cloth towels was 76 d or longer. Our studies show these compounds to be effective when applied to lunch bags and cloth towels and confirm the earlier reports of repellency of DEET to bed bugs (Kumar et al. 1995, Wang et al. 2013).

The natural product compounds tested may be good alternatives to DEET. Many were effective for much longer periods of time than DEET, and some, such as delta dodecalactone, may offer opportunities to blend with DEET to produce a more effective product. Similarly, the known mosquito repellent para-menthane-3,8-diol (Strickman 2007) may be combined with propyl dihydrojasmonate or delta dodecalactone to produce a product that has relatively long-lasting repellency against bed bugs. Further studies are needed to determine effectiveness and costs of different mixtures applied to a variety of substrates (wooden and tile floors, different types of rugs and upholstery, and plastic and copper tubing used in the construction of apartment buildings). Cost-effective and relatively long-lived repellents would greatly reduce risk of acquiring bites, reduce spread of bed bugs, and help reduce anxiety of those workers who often work in environments infested with bed bugs as well as travelers staying overnight in hotel rooms.

Female bed bugs may leave refuges more frequently than males (Pfiester et al. 2009, Aak et al. 2014). In our studies, more males remained on the refuges the morning after their placement in the arena, suggesting that females may leave more frequently in search of food or new refuges as reported previously.

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