MEDICAL ENTOMOLOGY

Repellency of Selected Chemicals Against the Bed Bug (Hemiptera: Cimicidae)

CHANGLU WANG, 1,2 LIHUA LÜ, 3 AIJUN ZHANG, 4 and CHAOFENG LIU 5

J. Econ. Entomol. 106(6): 2522–2529 (2013); DOI: http://dx.doi.org/10.1603/EC13155

ABSTRACT In recent years, the common bed bug, Cimex lectularius L. (Hemiptera: Cimicidae), became a major public health concern in urban communities. Bed bugs are notoriously difficult to control, and their bites are not tolerated by most people. The public has an urgent need for materials and methods to reduce bed bug introduction and bites during work, travel, or sleep. A repellent product will help achieve these goals by discouraging and preventing bed bugs from moving to a protected area. We evaluated the repellency of three commercially available insect repellent or control materials and five nonregistered materials with the goal of identifying safe and effective bed bug repellents. The two commercial repellent products that contained 7% picaridin or 0.5% permethrin had little repellency against bed bugs. N,N-diethyl-m-toluamide (DEET), the most commonly used insect repellent, provided a high level of repellency against bed bugs. When a host cue (carbon dioxide) was present, the minimum DEET concentration to repel≥94% of the bed bugs for a 9-h period was 10%. The longevity of repellency of DEET was concentration dependent. At 25% concentration, DEET-treated fabric surface remained highly repellent to bed bugs for a 14-d period. However, DEET has a strong smell and dissolves certain plastic materials. Therefore, we evaluated several odorless, noncorrosive, and potentially effective repellents. Isolongifolenone and isolongifolanone, two natural products and recently reported insect repellents, exhibited strong repellent property against bed bugs but at significantly lower levels than DEET. Three novel potential repellent compounds discovered by Bedoukian Research Inc. (Danbury, CT) exhibited similar level of repellency and longevity as DEET for repelling bed bugs. These nonirritant and odorless compounds are promising candidates as alternatives to DEET for reducing the spread of bed bugs and bed bug bites.

KEY WORDS bed bug, repellent, DEET, natural product, essential oil

Since the late 1990s, bed bugs gradually reemerged as a common urban pest in the United States, Canada, Europe, Australia, and some Asian countries (Boase 2001, Hwang et al. 2005, Gangloff-Kaufmann et al. 2006, Doggett and Russell 2008, Kilpinen et al. 2008, How and Lee 2009, Hirao 2010, Wang and Wen 2011). Once introduced, eliminating bed bugs is both expensive and difficult. Pest control providers charge hundreds to thousands of dollars to control an infestation. The time to eliminate an infestation can take a few months or more, depending on infestation level, complexity of the environment, cooperation from the building occupants, and thoroughness of the treatment procedures. Given these challenges, preventing new bed bug introductions becomes an important issue to many people including residents, travelers,

Insect repellents have long been used for preventing bites from blood-sucking arthropods (see review by Moore and Debboun 2006). DEET (N,N-diethyl*m*-toluamide) is the most successful arthropod repellent in about six decades and has been the mostly widely used active ingredient in topical repellents to protect humans and livestock against variety of arthropods including mosquitoes (Robert et al. 1991, Fradin 1998, Qiu et al. 1998, Schofield et al. 2007, Syed and Leal 2008), biting midges (Harlan et al. 1983, Magnon et al. 1991, Young and Evans 1998), tabanids (Catts 1968), sand flies (Schreck et al. 1982, Coleman et al. 1993, Yaghoobi-Ershadi et al. 2006), black flies (Robert et al. 1992, Kalyanasundaram and Mathew 2006, Tawatsin et al. 2006), horse flies (Blume et al. 1971), chiggers (Lerdthusnee et al. 2003, Kitchen et al. 2009), ticks (Carroll et al. 2005, Zhang et al. 2009), and leeches (Kochhlar et al. 1974, Kumar et al. 1984, Tawatsin et al. 2006, Frances 2006a). The concentration

home care providers, social workers, pest control technicians, and others who may visit bed bug-infested environments. There is an interest for effective and safe repellent materials to help minimize the introduction and spread of bed bugs, and to reduce bed bug hites

 $^{^{\}rm l}$ Department of Entomology, Rutgers University, 93 Lipman Dr., New Brunswick, NJ 08901.

² Corresponding author, e-mail: cwang@aesop.rutgers.edu.

³ Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Tianhe Wushan Road, Guangzhou 510640, China.

⁴ USDA-ARS, Beltsville Agricultural Research Center, 10300 Baltimore Ave., Beltsville, MD 20705.

⁵ Department of Statistics, Purdue University, 250 N. University St., West Lafayette, IN 47907.

of DEET used in a multitude of formulations around the world varies from 5 to 100% (Young and Evans 1998). Some side effects have been reported (Robbins and Cherniack 1986, Clem et al. 1993, Ross et al. 2004). DEET alternatives have always been sought and have been developed over the years as arthropod repellents. Useful repellents include permethrin, IR 3535 (3-[N-acetyl-N-Butyl] aminopropionic acid ethyl ester), p-menthane-3,8-diol, citronella, geraniol, picaridin, isolongifolenone, and isolongifolanone (Moore and Debboun 2006; Zhang et al. 2008, 2009).

Despite the increased importance of bed bugs in our society, there is only one report on effectiveness of repellents against bed bugs. Kumar et al. (1995) studied the repellency of DEET, diethyl phenyl-acetamide (DEPA), and demethylphthalate (DMP) against *Cimex hemipterus* (F.) by applying the chemical directly onto animal host skin. Both DEET and DEPA were repellent, with DEET being marginally more effective than DEPA.

Using an insect repellent can be a useful method to prevent bed bug bites, and possibly the introduction of bed bugs. Applying a repellent to shoes and pants may reduce the probability of getting bed bugs while a person is visiting an infested area. A repellent may also be applied to luggage, fabric materials, floors, or furniture to reduce the possibility of these objects becoming infested with bed bugs. An ideal bed bug repellent should prevent most of the bed bugs from crossing the treated area and last for at least a few hours or days. In addition, it should be odorless, nonirritating, and not an environmental pollutant. Many natural products and synthetic insecticides are claimed as bed bug repellents; however, there are no scientific data backing the claims. We evaluated the efficacy of several repellent products and chemicals with the aim of identifying effective and safe bed bug repellents. The evaluated materials included: 1) DEET—the most widely used insect repellent, 2) representative commercial products (active ingredients: permethrin and picaridin), 3) two recently reported natural repellent materials—isolongifolenone and isolongifolanone, and 4) three novel potential insect repellents developed by Bedoukian Research Inc. (Danbury, CT).

Materials and Methods

Bed Bugs. A laboratory (Ft. Dix) and three field strains (Essex, Indy, and Irvington) of bed bugs were maintained in plastic containers (47 mm in diameter by 47 mm in height) with folded filter paper as harborages. The laboratory strain had been originally collected from Ft. Dix, NJ, and maintained in glass jars (feeding on Dr. Harlan) since 1973. We obtained this strain from Dr. Harlan in 2009. The Essex, Indy, and Irvington strains were maintained in the laboratory for 6 mo, 2 yr, and 1 mo, respectively. Different experiments used same or different strains of bed bugs based on availability. The bed bugs were fed weekly with defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using Hemotek membrane-feeding sys-

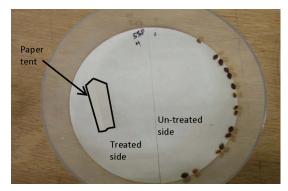


Fig. 1. Petri dish assay set up examining the repellency of candidate materials. Note all bed bugs were resting on the untreated side. (Online figure in color.)

tem (Discovery Workshops, Accrington, 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. In all experiments, 7- to 21-d hungry bed bugs were used.

Chemicals. DEET (97% purity) was purchased from Sigma-Aldrich Co. (St. Louis, MO) and diluted with 95% ethanol (Phamco Products Inc., Brookfield, CT) to desired concentrations. Cutter Advanced Insect Repellent (7% picaridin, United Industries Corporation, St. Louis, MO) and Rest Easy Bed Bug & Insect Control (0.5% permethrin, Eaton, Twinsburg, OH) were purchased from an internet-based vendor. Isolongifolenone was synthesized at Beltsville, MD (Wang and Zhang 2008). Isolongifolanone, chemical A (3-methyl-5-hexyl-2-cyclohexenone), B (propyl dihydrojasmonate), and C (γ -methyl tridecalactone) were provided by Bedoukian Research Inc. Chemical A has a mild peach-herbaceous odor. Chemicals B and C are almost odorless. These three compounds were potentially useful insect repellents based on laboratory assays by the manufacturer.

Petri Dish Assays. Plastic Petri dishes of 11.4 cm diameter by 3.8 cm height were used to quickly evaluate the comparative repellency of the following candidate chemicals: DEET, permethrin, picaridin, isolongifolenone, and isolongifolanone. Filter papers were cut into two equal halves; one half was treated with a repellent using a Potter spray tower at 2.47 mg/cm² or 0.61 gallon/1,000 feet² of ethanol solution. The other half was sprayed with 95% ethanol. A small piece of filter paper was also treated with the same repellent and folded to a tent shape with the treated side facing down. The paper tent was placed on the repellent treated side and the dishes were left uncovered throughout the assay (Fig. 1). In the control dish, one half of the filter paper and the harborage were treated with 95% ethanol. The other half of the filter paper was not treated. In the assay evaluating 2.5% DEET, 7% picaridin, and 0.5% permethrin, 10 Ft. Dix strain bed bugs (fourth-fifth instar nymphs or adult males of unknown age) were released in the center of each dish. The numbers of bed bugs on each side of the dish were recorded at 2 and 24 h after treat-

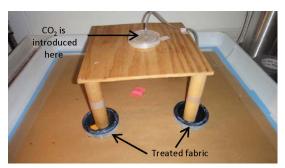


Fig. 2. Arena assay set up examining the repellency of candidate materials. (Online figure in color.)

ment. In the assay evaluating repellency of 5% DEET, isolongifolenone, and isolongifolanone, nine males and six large nymphs of Essex strain bed bugs were released into each Petri dish. The location of bed bugs in each Petri dish was recorded at 3, 5, 9, and 24 h after treatment. Each treatment was replicated four times in both assays. The assays were initiated at \approx 2–5 h into the dark cycle. The experiments were conducted in a room at 22–26°C and a photoperiod of 12:12 (L:D) h cycle.

Arena Assays. Plastic tray arenas (80 by 75 by 5 cm) with brown paper lining the bottom were used (Fig. 2) to evaluate the comparative repellency of selected chemicals when a host cue (carbon dioxide) is present. A layer of fluoropolymer resin (BioQuip products, Rancho Dominguez, CA) was applied to inner walls of the arenas to prevent the bugs from escaping. A piece of folded cardboard and folded fabric was placed at the center of the arena to provide harborages for bed bugs. A plastic ring (13.3 cm in diameter by 6.4 cm in height) was placed around the harborages to confine the bed bugs. Four arenas were placed in a nonventilated room at 24-25°C and a photoperiod of 12:12 (L:D) h cycle. They were served as four replicates. A 26.5 by 6.5-cm wooden stool was placed in each arena. Under the legs of each stool was a 10 cm in diameter by 2.2 cm in height black Climbup Insect interceptor (Susan McKnight Inc., Memphis, TN). An aliquot of 400 µl chemical solution was applied evenly to the fabric tape of each Climbup using a 200 µl pipette, yielding 5.3 mg/cm² or 1.1 gallon/1,000 feet² of chemical solution. The four Climbup interceptors in each arena were treated with four different chemicals with 95% ethanol being used as control.

In each test, bed bugs were released into the center of each arena and confined with a plastic ring. After 1 h and during the dark cycle, treated Climbup interceptors were placed under the stool legs and the rings confining the bed bugs were removed. Carbon dioxide (100% CO₂) was released from a gas cylinder (Airgas East Inc, Piscataway, NJ) to the top of each stool at 100 ml/min to stimulate bed bug activity. CO₂ was a strong stimulant to bed bugs (Wang et al. 2009). The number of bed bugs that fallen into each Climbup was recorded after 2–3 h or at other specified times. After each examination, all of the bugs scattered in the arena

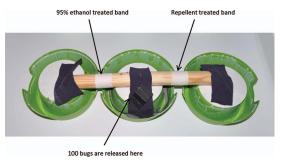


Fig. 3. Triple-bowl assay set up examining the repellency of 25% DEET. (Online figure in color.)

and Climbups were returned to the center of the arena, and confined for another 0.5 h; the room was ventilated to bring down the CO_2 concentration to the same level as the air within the room. The plastic rings confining the bed bugs were removed, and the bed bug numbers were recorded again following the same procedures to evaluate the repellency longevity of the chemicals.

The following comparisons were examined using the arena assay method: 1) comparative repellency of 25% DEET, isolongifolenone, and isolongifolanone at 4, 6, and 9 h after application (Essex strain, 100 male adults per arena); 2) comparative repellency of 5, 10, and 25% DEET at 3, 6, and 9 h after application (Essex strain, 60-70 male adults per arena); 3) repellency of 25% DEET at 1, 7, 14, 21, and 35 d (Indy strain, 50 male adults per arena); and 4) comparative repellency of 25% DEET, chemical A, B, and C (five-legged stools were used) at 0 d (Ft. Dix strain, counts were from 20-h test period) and 15 d (Bayonne strain, counts were from 6-h test period) after application. In the test examining the longevity of 25% DEET, two opposite legs of each stool were sitting on DEET-treated Climbups, whereas the other two legs were sitting on nontreated Climbups.

Triple-Bowl Assays. This experiment was designed to evaluate the efficacy of 25% DEET-treated bands for repelling bed bugs under conditions mimicking the natural environment. The experimental setup consisted of three inverted plastic dog bowl (600 ml in volume and 18 cm in diameter by 64 cm in height) (IKEA, Baltimore, MD), placed next to one another with a wooden rod serving as a bridge between the three bowls (Fig. 3). The inner surfaces of the dog bowls were coated with a layer of fluoropolymer resin to prevent trapped bed bugs from escaping. One piece of filter paper (10 cm in diameter) and a piece of black cloth were placed at both ends of the wooden rod to provide harborages for bed bugs. A piece of cloth was placed at the bottom of the center bowl to allow bed bugs trapped in the bottom to be able to climb back to the harborage located at the wooden rod, whereas bed bugs captured in either of the two side bowls could not return to the harborages associated with the wooden rod. Eight plastic containers, each with 100 Irvington strain bed bugs

(\approx 90% adult males and 10% fourth-fifth instar nymphs), were prepared 1 d before the test. The Irvington strain was selected for this experiment because the strain was only kept in the laboratory for 1 mo and the bugs were very responsive to host cues

Two tests were conducted using triple-bowl devices. In the first test, 100 bed bugs were released into the center bowl at 2 h into the dark cycle. After 15 min of acclimation, two wooden rods were placed horizontally between the bowls to allow bed bugs to cross between the bowls. One wooden rod was wrapped with a 2.5-cm-wide repellent-treated fabric tape (Micropore surgical tape, 3M Health Care, Neuss, Germany). The other rod was wrapped with a 95% ethanol-treated fabric band as control. The chemicals were applied to the bands using the same method as described in the arena assay 1 h before the test. The experiment was conducted in a room at temperature between 27–29°C and lighted with a 25 watt transparent red light bulb. CO₂ (100% concentration) was released from three 5 lb CO2 cylinders each at 100 ml/min to stimulate bed bug foraging movement. Bed bugs would naturally disperse both vertically or horizontally from the center bowl after being stimulated. The three CO₂ release points were ≈ 1.5 m above the test devices. Eight sets of devices were set up in the room. The number of bed bugs found in the two side bowls was counted after 2 h. Once counted, the bed bugs were returned to the center bowl and the wooden rods removed. The room was vented for 10 min using a fan.

A second test was initiated at 8 h after 25% DEET application using exactly the same materials and procedures as in the first test. This test was to determine whether the repellency decreased significantly compared with that observed at 1–3 h after application. The number of bed bugs found in the two side bowls was counted after 2 h. Seven replicates were included in this test.

Statistical Analysis. Repellency indices from Petri dish assays were calculated according to the formula:

Repellency index = $\frac{C-T}{C} \times 100$, where C = the mean numbers of bed bugs on the treated filter paper halves in all control dishes, and T = number of bed bugs ontreated filter paper half in one test dish (Todd 2011). Repellency indices were compared using analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. The bed bug count data in arena assays comparing different chemicals were analyzed using Proc Glimmix based on mixed multinomial model with treatment period as the random effect. The arena assay and the triple bowl assay examining the changes in repellency of 25% DEET were analyzed by using Proc Genmod based on multinomial model with "replicate" as the random effect. All analyses were performed using SAS software (SAS Institute 2009).

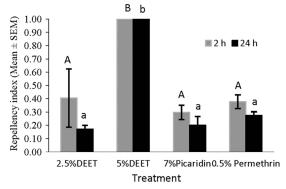


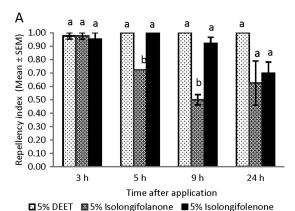
Fig. 4. Repellency of DEET and two commercial insect repellents against bed bugs in Petri dish assays. For each observation period, different letters above the bars of the same observation period indicate significant differences at P=0.05.

Results

Petri Dish Assays. Bed bugs released into center of the dishes soon went under the paper tent harborage if the treatment was not repellent; or stayed along edge of the dish on the nontreated side if the treatment was repellent (Fig. 1). The 2.5% DEET, 7% picaridin, and 0.5% permethrin treatment exhibited low levels of repellency against bed bugs (Fig. 4). Only 5% DEET treatment achieved 100% repellency against bed bugs at 2 and 24 h after application. It was significantly more repellent than 2.5% DEET, 7% picaridin, and 0.5% permethrin (2 h: F = 7.84; df = 3, 12; P =0.0037; 24 h; F = 106.2; df = 3, 12; P < 0.0001). Comparative tests of 5% DEET, isolongifolanone, and isolongifolenone revealed no significant differences in their repellency after 3 h (F = 0.19; df = 2, 9; P = 0.83). Isolongifolanone became significantly less repellent than DEET and isolongifolenone after 5 h ($F = \infty$; df = 2, 9; P < 0.001) and 9 h (F = 62.8; df = 2, 9; P < 0.001). There were no significant differences in their repellency at 24 h (F = 3.48; df = 2, 9; P = 0.08) after application (Fig. 5A).

Arena Assays. Comparative tests of 25% DEET, isolongifolenone, and isolongifolanone showed DEET was the most effective repellent (Fig. 5B). The ratio of the probability of bed bugs passed DEET-treated band vs. that passed isolongifolanone-treated band was 0.042 (P < 0.0001). The ratio of the probability of bed bugs passed DEET-treated band vs. that passed isolongifolenone-treated band vs. that passed isolongifolenone-treated band was 0.028 (P < 0.0001). Individual comparisons at 6 and 9 h showed DEET was significantly more repellent than isolongifolenone and isolongifolanone (P < 0.05). However, the 25% DEET treatment did not completely prevent bed bugs from passing the treated surface. Among the bed bugs found in Climbups, 1% of them were found in 25% DEET-treated Climbups both at 6 and 9 h after treatment.

In concentration-repellency relationship assays, all tested concentrations exhibited significant repellency at 3 and 6 h after application (Fig. 6). At 9 h, the repellent effect of 5% DEET became insignificant (P = 100)



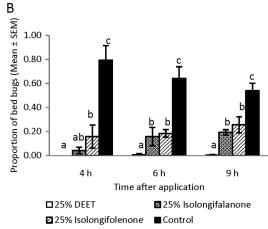


Fig. 5. Repellency of DEET and two recently patented insect repellent materials against bed bugs: (A) petri dish assay, (B) arena assay. Different letters above the bars of the same observation period indicate significant differences at P = 0.05.

0.17). Overall, the repellency of 5% DEET was significantly lower than 10% DEET (P < 0.001). There were no significant differences in repellency between 10%

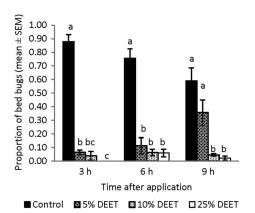


Fig. 6. Relationship between concentration and repellency of DEET against bed bugs in arena assays. Different letters above the bars of the same observation period indicate significant differences at P = 0.05.

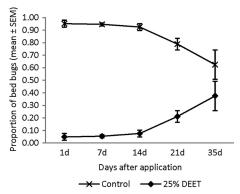


Fig. 7. Longevity of the repellency of DEET against bed bugs in arena assays.

DEET and 25% DEET (P=0.14). Longevity tests of 25% DEET showed its repellency started to decrease significantly after 21 d (P=0.003; Fig. 7). The percentage of bed bugs (mean \pm SEM) found in 25% DEET treatment at 1, 7, 14, 21, and 35 d were 5 \pm 2, 5 \pm 1, 8 \pm 3, 21 \pm 5, and 38 \pm 12%, respectively. At 35 d, the 25% DEET repellency was insignificant (P=0.19). Chemical A, B, and C exhibited similar level of repellency as DEET at 0 d and 15 d (P>0.05) (Fig. 8).

Triple-Bowl Assays. Bed bugs actively moved to the wooden rods once they were placed between the bowls. The vast majority of the bugs exhibited avoidance behavior when they reached the treated bands. No avoidance behavior was observed when bed bugs reached the control bands. In the first test (1–3 h after DEET application), the mean number of bed bugs appeared in the 25% DEET and the control side were 0.25 \pm 0.3 and 41.4 \pm 4.3, respectively. The DEET treatment side had an average 97 \pm 1% less bed bugs compared with the control side. In the second test (8–10 h after DEET application), the mean number of bed bugs appeared in the 25% DEET and the control side were 1.3 \pm 0.6 and 34.0 \pm 3.5, respectively. The

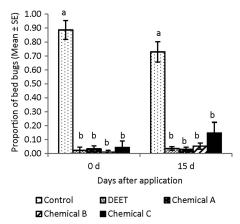


Fig. 8. Repellency of DEET and three potential insect repellents against bed bugs in arena assays. Different letters above the bars of the same observation period indicate significant differences at P = 0.05.

DEET treatment side had an average $94 \pm 3\%$ less bed bugs compared with the control side. There was no significant differences in the repellency measured at 3 and 10 h (P = 0.14).

Discussion

This is the first study addressing repellents for *Cimex lectularius* L. We found DEET and three compounds from Bedoukian Research Inc. are effective repellents against bed bugs. At 25% or higher concentration, DEET can prevent >94% bed bugs from crossing the treated area for at least 8 h under high pest pressure (i.e., hungry bed bugs and a strong host cue were present). The findings suggest that applying a repellent to luggage, shoes, or clothing could be an effective method to avoid bed bug infestations by home visitors, pest control technicians, travelers, and other personnel who need to visit or work in bed bug-infested environments.

We used three test methods to evaluate the repellent properties of candidate compounds. The Petri dish assay method provides a simple and fast method for screening large numbers of compounds. It is a more robust method than that introduced by Todd (2011), which does not contain harborages in the dishes. In that setup, bed bugs may randomly rest anywhere in the control dish, making it difficult to calculate the repellency index. In our Petri dish assays, 68 and 89% of the bed bugs stayed under the harborages in the control dishes at 2 and 24 h. Therefore, the repellency indices were more readily separated between treatments. Because there was not a host cue present in the Petri dish assays, the minimum effective concentration of chemical was much lower than that obtained from the arena assays. The arena assays mimic the field conditions where bed bugs from the floor need to climb a vertical substrate to reach the host. The drawback of this method is that the number of bed bugs falling into the Climbups was smaller than the number of bugs that reached the top of the interceptors because not all bugs reaching the top of the interceptors fell into the traps. It was not clear how many bed bugs crossed the treated fabric. The triple-bowl assays most closely mimic the natural conditions. While bed bugs can cross the wooden rods back and forth, once they fall to the bottom of the bowls, they cannot climb back. Most of the bugs (77% in the first test and 94% in the second test) in the side bowls were found at bottom of the bowls.

In several tests, we used same bed bugs repeatedly over time to determine the longevity of repellency. It is not clear whether bed bugs became less sensitive after previous exposure as shown in mosquitoes (Stanczyk et al. 2013). The repellency measured at a later time might be a combination of aging effect and changes in bed bug sensitivity. However, there is no evidence to believe prior exposure would affect the comparative repellency of the evaluated chemicals. From field application standpoint, the test design reflected the effectiveness of the repellents when bed bugs were continuously present. This repellency in-

formation is important to users who need to stay in an infested environment continuously for more than a few hours.

Permethrin is used as an effective repellent against a variety of biting insects by the U.S. military (McCain and Leach 2006). It effectively repels mosquitoes, sand flies, black flies, and ticks (Lindsay and Mc-Andless 1978, Mercier et al. 2003). However, it exhibited low repellency against bed bugs at the commonly used rate. Similarly, Moore and Miller (2006) reported no significant repellency against bed bugs from several pyrethroid insecticides: λ-cyhalothrin, bifenthrin, and deltamethrin. Romero et al. (2009) found low level repellency from deltamethrin treatment. Thus, it is plausible that pyrethroids would not be good candidates as bed bug repellents. Picaridin has been shown to be as good as or better than DEET formulations for repelling mosquitoes (Frances 2006b). However, it only slightly repelled bed bugs. We tested 45% DEET using arena assays and found 100% repellency was never achieved in preliminary assays. These results suggest that bed bugs are more tolerant to insect repellents compared with some other blood-sucking arthropods.

Some essential oils were reported having repellent properties against blood-sucking insects. Among them, white cedar oil and peppermint oil were most repellent against mosquitoes (Barnard 1999). In a different study, we evaluated repellency of two essential oil-based bed bug control products using the arena assay method: EcoRaider (Reneotech Inc., North Bergen, NJ) and Bed Bug Patrol (Nature's Innovation Inc., Buford, GA). EcoRaider contains 1% cedar oil and Bed Bug Patrol contains 1% peppermint oil. Both these two products did not exhibit significant repellency against bed bugs (Singh and Wang, unpublished data). Based on these findings, it is unlikely that low concentration essential oils will be useful as bed bug repellents.

Isolongifolenone is a relatively new natural repellent material. Zhang et al. (2008, 2009) found it was equally or more repellent than DEET against two mosquitoes (Aedes aegypti (L.) and Anopheles stephensi Liston), blacklegged tick (Ixodes scapularis Say), and lone star tick (Amblyomma americanum (L.)) in laboratory assays. In the current study, this compound exhibited strong repellency against bed bugs but at significantly lower levels than DEET. Because it is natural product, it has high potential to be used as an alternative to DEET against bed bugs.

The comparable performance of the three chemicals from Bedoukian Research Inc. and the traditional DEET repellent is encouraging. These relatively new chemicals could be safer alternative repellents for preventing bed bug infestations than DEET. Increasing the band width from 2.5 to 7.5 cm did not improve the repellency in our preliminary studies. Thus, the 2.5-cm-wide bands were used in all repellent tests and we expect this width would be sufficient for personal protection under field conditions. These results imply that applying a narrow band of repellent may significantly reduce the probability of obtaining bed bugs

while a human host is staying in a bed bug-infested room. This method could also be used to reduce the spread of bed bugs from an infested room to surrounding units in multiunit dwellings while waiting for treatment.

Acknowledgments

We thank Marcus Kwasek and Narinderpal Singh for technical assistance, Bedoukian Research Inc., for providing study materials and partial financial support to this project. We also appreciate the critical review from Narinderpal Singh, Richard Cooper, and two anonymous reviewers for reviewing an earlier draft of the manuscript. This is New Jersey Experiment Station Publication No. D-08-08117-03-13.

References Cited

- Barnard, D. 1999. Repellency of essential oils to mosquitoes (Diptera: Culicidae). J. Med. Entomol. 36: 625–629.
- Blume, R. R., R. H. Roberts, J. L. Eschle, and J. J. Matter. 1971. Tests of aerosols of DEET for protection of livestock from biting flies. J. Econ. Entomol. 64: 1193–1196.
- Boase, C. 2001. Bedbugs-back from the brink. Pestic. Outlook 8: 159-62.
- Carroll, J. F., J. A. Klun, and M. Debboun. 2005. Repellency of DEET and SS220 applied to skin involves olfactory sensing by two species of ticks. Med. Vet. Entomol. 19: 101–106.
- Catts, E. P. 1968. DEET-impregnated net shirt repels biting flies. J. Econ. Entomol. 61: 1765.
- Clem, J. R., D. F. Havemann, M. A. Raebel, D. R. De Almentero, and C. Guevremont. 1993. Insect repellent (N,N-diethyl-m-toluamide) cardiovascular toxicity in an adult. Ann. Pharmacother. 27: 289–293.
- Coleman, R. E., L. L. Robert, L. W. Roberts, J. A. Glass, D. C. Seeley, A. Laughinghouse, P. V. Perkins, and R. A. Wirtz. 1993. Laboratory evaluation of repellents against four anopheline mosquitoes (Diptera: Culicidae) and two phlebotomine sand flies (Diptera: Psychodidae). J. Med. Entomol. 30: 499–502.
- Doggett, S. L., and R. C. Russell. 2008. The resurgence of bed bugs, *Cimex* spp. (Hemiptera: Cimicidae) in Australia, pp. 407–425. *In* W. H. Robinson and D. Bajomi (eds.), Proceedings of the 6th International Conference on Urban Pests, 13–16 July 2008, Budapest, Hungary. OOK-Press Kft., Pápai út 37/a, Hungary.
- Fradin, M. S. 1998. Mosquitoes and mosquito repellants: a clinician's guide. Ann. Int. Med. 128: 931-940.
- Frances, S. P. 2006a. Chapter 16: efficacy and safety of repellents containing DEET, pp. 311–325. In M. Debboun, S. P. Frances, and D. Strickman (eds.), Insect Repellents: Principles, Methods, and Uses. CRC: Boca Raton, FL.
- Frances, S. P. 2006b. Chapter 18: picaridin, pp. 337–340. In M. Debboun, S. P. Frances, and D. Strickman (eds.), Insect Repellents: Principles, Methods, and Uses. CRC: Boca Raton, FL.
- Gangloff-Kaufmann, J., C. Hollingsworth, J. Hahn, L. Hansen, B. Kard, and M. Waldvogel. 2006. Bed bugs in America: a pest management industry survey. Am. Entomol. 52: 105–106.
- Harlan, H. J., C. E. Schreck, and D. L. Kline. 1983. Insect repellent jacket tests against biting midges (diptera: Culicoides) in Panama. Am. J. Trop. Med. Hyg. 32: 185–188.
- Hirao, M. 2010. Recent resurgence of bedbug and its management. Med. Entomol. Zool. 61: 211–221.

- How, Y.-H., and C.-Y. Lee. 2009. Survey of bed bugs in infested premises in Malaysia and Singapore. J. Vector Ecol. 35: 89-94.
- Hwang, S. W., T. J. Svoboda, I. J. De Jong, K. J. Kabasele, and E. Gogosis. 2005. Bed bug infestations in an urban environment. Emerg. Infect. Dis. 11: 533–538.
- Kalyanasundaram, M., and N. Mathew. 2006. N,N-diethyl phenylacetamide (DEPA): a safe and effective repellent for personal protection against hematophagous arthropods. J. Med. Entomol. 43: 518–525.
- Kilpinen, O., K.-M.V. Jensen, and M. Kristensen. 2008. Bed bug problems in Denmark, with a European perspective, pp. 397–399. In W. H. Robinson and D, Bajomi (eds.), Proceedings of the 6th International Conference on Urban Pests, 13–16 July 2008, Budapest, Hungary. OOK-Press Kft., Pápai út 37/a, Hungary.
- Kitchen, L. W., K. L. Lawrence, and R. E. Coleman. 2009. The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. J. Vector Ecol. 34: 50-61.
- Kochhlar, R. K., R. S. Dixit, and C. I. Somaya. 1974. A critical analysis of 'DEET' as a repellent against arthropods of public health importance and water leeches. Indian J. Med. Res. 62: 125–133.
- Kumar, S., S. Prakash, R. K. Sharma, S. K. Jain, M. Kalyanasundaram, R. V. Swamy, K. M. Rao, and C.A.B.A. November C1-Medline. 1984. Field evaluation of three repellents against mosquitoes, black flies & land leeches. Indian J. Med. Res. 80: 541–545.
- Kumar, S., S. Prakash, and K. M. Rao. 1995. Comparative activity of three repellents against bedbugs *Cimex hemipterus* (Fabr.). Indian J. Med. Res. 102: 20–23.
- Lerdthusnee, K., N. Khlaimanee, T. Monkanna, S. Mungviriya, W. Leepitakrat, M. Debboun, and R. E. Coleman.
 2003. Development of an in vitro method for the evaluation of candidate repellents against *Leptotrombidium* (Acari: Trombiculidae) chiggers. J. Med. Entomol. 40: 64-67
- Lindsay, I. S., and J. M. McAndless. 1978. Permethrintreated jackets versus repellent-treated jackets and hoods for personal protection against black flies and mosquitoes. Mosq. News 38: 350.
- Magnon, G. J., L. L. Robert, D. L. Kline, and L. W. Roberts. 1991. Repellency of two DEET formulations and Avon Skin-So-Soft against biting midges (Diptera: Ceratopogonidae) in Honduras. J. Am. Mosq. Control Assoc. 7: 80–82.
- McCain, W. W., and G. L. Leach. 2006. Chapter 13: repellents used in fabric: the experience of the U.S. military, pp. 261–273. *In M. Debboun*, S. P. Frances, and D. Strickman (eds.), Insect Repellents: Principles, Methods, and Uses. CRC: Boca Raton, FL.
- Mercier, P., P. Jasmin, and A. Sanquer. 2003. Prevention of sand fly attack by topical application of a permethrin/ pyriproxyfen combination on dogs. Vet. Ther. 4: 309–316.
- Moore, S. J., and M. Debboun. 2006. Chapter 3: history of insect repellents, pp. 3–29. In M. Debboun, S. P. Frances, and D. Strickman (eds.), Insect Repellents: Principles, Methods, and Uses. CRC: Boca Raton, FL.
- Moore, D. J., and D. M. Miller. 2006. Laboratory evaluations of insecticide product efficacy for control of *Cimex lectularius*. I. Econ. Entomol. 99: 2080–2086.
- Qiu, H., H. W. Jun, and J. W. McCall. 1998. Pharmacokinetics, formulation, and safety of insect repellent N,N-diethyl-3-methylbenzamide (DEET): a review. J. Am. Mosq. Control Assoc. 14: 12–27.
- Robbins, P. J., and M. G. Cherniack. 1986. Review of the biodistribution and toxicity of the insect repellent N,N-

- diethyl-m-toluamide (DEET). J. Toxicol. Environ. Health 18: 503–525.
- Robert, L. L., I. Schneider, and R. A. Wirtz. 1991. DEET and permethrin as protectants against malaria-infected and uninfected Anopheles stephensi mosquitoes. J. Am. Mosq. Control Assoc. 7: 304–306.
- Robert, L. L., R. E. Coleman, D. A. Lapointe, P. J. Martin, R. Kelly, and J. D. Edman. 1992. Laboratory and field evaluation of five repellents against the black flies *Prosimulium mixtum* and *P. fuscum* (Diptera: Simuliidae). J. Med. Entomol. 29: 267–272.
- Romero, A., M. F. Potter, and K. F. Haynes. 2009. Behavioral responses of the bed bug to insecticide residues. J. Med. Entomol. 46: 51–57.
- Ross, E. A., K. A. Savage, L. J. Utley, and I. R. Tebbett. 2004. Insect repellant interactions: sunscreens enhance DEET (N,N-diethyl-m-toluamide) absorption. Drug Metab. Dispos. 32: 783–785.
- SAS Institute. 2009. SAS/STAT user's guide, version 9.2. SAS Institute Cary, NC.
- Schofield, S., M. Tepper, and R. Gadawski. 2007. Laboratory and field evaluation of the impact of exercise on the performance of regular and polymer-based DEET repellents. J. Med. Entomol. 44: 1026–1031.
- Schreck, C. E., D. L. Kline, and B. N. Chaniotis. 1982. Evaluation of personal protection methods against phlebotomine sand flies including vectors of leishmaniasis in Panama. Am. J. Trop. Med. Hyg. 31: 1046–1053.
- Stanczyk, N. M., J.F.Y. Brookfield, L. M. Field, and J. G. Logan. 2013. Aedes aegypti mosquitoes exhibit decreased repellency by DEET following previous exposure. PLoS ONE 8: e54438. (doi:10.1371/journal.pone.0054438).
- Syed, Z., and W. S. Leal. 2008. Mosquitoes smell and avoid the insect repellent DEET. Proc. Natl. Acad. Sci. U.S.A. 105: 13598–13603.
- Tawatsin, A., U. Thavara, U. Chansang, P. Chavalittumrong, T. Boonruad, P. Wongsinkongman, J. Bansidhi, and M. S.

- Mulla. 2006. Field evaluation of deet, Repel Care, and three plant based essential oil repellents against mosquitoes, black flies (Diptera: Simuliidae) and land leeches (Arhynchobdellida: Haemadipsidae) in Thailand. J. Am. Mosq. Control Assoc. 22: 306–313.
- Todd, R. 2011. Chapter 9-Repellents for protection from bed bugs: the need, the candidates, safety challenges, test methods, and the chance of success, pp. 137–150. In G. E. Paluch and J. R. Coats (eds.), Recent developments in invertebrate repellents. ACS Symposium Series, American Chemical Society: Washington, DC.
- Wang, C., and X. Wen. 2011. Bed bug infestations and control practices in China: implications for fighting the global bed bug resurgence. Insects 2: 83–95.
- Wang, S., and A. Zhang. 2008. Facile and efficient synthesis of isolongifolenone. Org. Prep. Proced. Int. 40: 405–410.
- Wang, C., T. Gibb, G. W. Bennett, and S. McKnight. 2009. Bed bug attraction to pitfall traps baited with carbon dioxide, heat, and chemical lure. J. Econ. Entomol. 102: 1580–1585.
- Yaghoobi-Ershadi, M. R., A. A. Akhavan, E. Jahanifard, H. Vatandoost, G. Amin, L. Moosavi, A. R. Zahraei Ramazani, H. Abdoli, and M. H. Arandian. 2006. Repellency effect of Myrtle essential oil and DEET against *Phlebotomus papatasi*, under laboratory conditions. Iran. J. Public Health 35: 7–13.
- Young, G. D., and S. Evans. 1998. Safety and efficacy of DEET and permethrin in the prevention of arthropod attack. Mil. Med. 163: 324.
- Zhang, A., J. F. Carroll, S. Wang, and J. A. Klun. 2008. Method for preparing isolongifolenone and its use in repelling arthropods. USA Patent No. 7,378,557 B1.
- Zhang, A., J. A. Klun, S. Wang, J. F. Carroll, and M. Debboun. 2009. Isolongifolenone: a naturally occurring and easily synthesized sesquiterpene that repels ticks and deters feeding by mosquitoes. J. Med. Entomol. 46: 100–106.

Received 1 April 2013; accepted 8 August 2013.