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Original Article

Repellent activity of citrus oils against the cockroaches *Blattella germanica*, *Periplaneta americana* and *P. fuliginosa*

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The repellent efficacy of 17 essential oils against the German cockroach, *Blattella germanica* was examined using a T-tube olfactometer. Five oils repelled *B. germanica* with good efficacy, ranging from 70.0 to 96.7%. Four of these oils, grapefruit, lemon, lime, and orange, were from the citrus family Rutaceae. These citrus essential oils showed similar repellent activity against two more cockroach species, such as *Periplaneta americana* and *P. fuliginosa*. Gas chromatography (GC) and GC-mass spectrometry analyses revealed that the major components responsible for the repellent activity of the citrus oils were limonene, β-pinene and γ-terpinene. Limonene appears to be the main component responsible for the repellent activity rather than β-pinene and γ-terpinene. The repellent efficacy of these components varied with different doses and the cockroach species tested. It is likely that minor components of the oils also contributed to the overall repellent activity of citrus essential oils, except orange oil. The activity of orange oil is almost solely attributed to the activity of limonene. Also, the repellent activity of citrus oil and that of each of the terpenoids makes little difference to the efficacy of a repellant against the three species of cockroaches. © Pesticide Science Society of Japan

Keywords: cockroach, repellent, citrus oils, monoterpene, limonene.

Cockroaches are insects with worldwide distribution that thrive best in warm, humid, lowland areas throughout the Tropics. Of the 4000 species of cockroaches (Dictyoptera: Blattodea) known to exist, approximately 30 species coexist in human habitats and approximately 16 species are associated with human health problems.[1] The cockroach species that are closely associated with human dwellings, food-processing industries, service-rendering facilities and/or occupational environments include *Blattella germanica, Blatta orientalis, Periplaneta australasiae* (Fabricius), *P. americana* (Linnaeus) and *Supella supellectilium* (Serville).[2] Among these, *B. germanica* is the most ubiquitous and also the one most frequently seen in food preparation areas, restaurants, cafeterias, kitchens and toilets. The prevalence of *B. germanica* in human habitats makes this type of cockroach a major nuisance and mechanical transmitter of etiological disease agents.[3]

Cockroaches have long been recognized as potential mechanical vectors of human intestinal parasites and animal pathogens, as well as sources of human allergens. Indeed, it has been found that cockroach antigen is the most common asthma-inducing allergen in children in inner cities.[4,5] Cockroaches are controlled primarily with synthetic organic insecticides in the form of baits, aerosols, foggers, and crack treatments.[6,7] At present, chemical-based methods for cockroach management generally involve repeated applications of residual insecticides (*e.g.*, dichlorvos, chlorpyrifos, propoxur, and pyrethroids),[8] stomach poisons (*e.g.*, hydramethylnon, boric acid, and sulfluramid),[9] and insect growth regulators (*e.g.*, noviflumuron and lufenuron).[10,11] However; several factors have limited the use of synthetic chemicals: the development of natural resistance by cockroaches, and negative effects on the environment and human health in some cases. Consequently, an intensive effort has been made to find alternative repellents, which are environmentally friend and ecologically safe.[12,13)]

Many natural compounds isolated from plants have demonstrated a wide spectrum of biological activities. Among these various kinds of natural substances that have received particu-

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lar attention as natural agents for insect management are essential oils from aromatic and medicinal plants.[12–15] Numerous plants and derivative products, in particular essential oils, have been investigated and described as potentially natural sources of insect repellent.[11,16] Insect repellents are substances that act locally or at a distance, deterring an insect from flying to, landing on or biting human or animal skin. Although the use of synthetic repellents tends to be more effective than natural repellents, the development of insect resistance has restricted the usefulness of synthetic repellents.[17] Thus, plant-based repellents may be comparable to, or even somewhat better than, synthetics, depending on the formula. Many plant essential oils and their components have been shown to have good repellent activity,[18–20] for example, *Piper guineense* (black pepper) seed oil exhibits both insecticidal and repellent activity against the stored product insect pests.[18] Benzene derivatives and terpenoids have also been reported to have insecticidal and repellent activities against *Periplanata americana*.[19] In addition, most plant-based insect repellents currently on the market contain essential oils from one or more of the following plants: citronella (*Cymbopogon nardus*), cedar (*Juniper virginiana*), eucalyptus (*Eucalyptus maculata*), geranium (*Pelargonium reniforme*), lemon-grass (*Cymbopogon excavatus*), peppermint (*Mentha piperita*), neem (*Azadirachta indica*) and soybean (*Neonotonia wightii*).[20)]

Citrus oils, such as grapefruit, lemon, lime and orange, are widely utilized in various industries, including agriculture and household cleaning. Their constituents have been extensively studied and used for various applications; however, it has not been properly addressed whether the monoterpenes in citrus oils can act as insect repellents or whether they could be used in agriculture to replace synthetic insecticides. To test this, we examined the repellent activity of 17 essential oils, including five citrus oils, against household insect pest cockroaches, with special reference to three different species. The major active components of the oils found to have repellent effects were detected by gas chromatography (GC) and GC-mass Spectrometry (MS), and then also tested for their repellent activity.

Materials and Methods

1. *Essential oils and terpenes*

The names and sources of the seventeen essential oils used in this study are listed in Table 1. Myrrh, pine needle oil and strawberry oil were from Charabot, France, while the others were purchased from JinArome Co., USA. The terpene compounds used in this study were as follows: β-myrcene (90%) (Sigma, St. Louis, MO), α-pinene (98%), β-pinene (97%), γ-terpinene (97%), and *d*-limonene (97%) (Aldrich, Milwaukee, WI). Benzene (99%) was purchased from Aldrich.

2. *Test insects*

Three species of cockroach, the German cockroach *Blattella germanica*, the American cockroach *Periplaneta americana*, and the smokybrown cockroach *P. fuliginosa*, were initially obtained from the Korean Research Institute of Chemical Technology and then reared in our laboratory for 8 years without exposure to any known insecticides. They were fed on mouse food pellets and water and were kept in cages

Table 1. Names and sources of seventeen essential oils used in this study

Common name	Scientific name	Family	Source
Caraway seed	*Carum carvi*	Umbelliferae	JinArome (USA)
Clary sage	*Salvia sclarea*	Labiatae	JinArome (USA)
Clove leaf	*Eugenia caryophyllata*	Oleaceae	JinArome (USA)
Coriander	*Coriandrum sativum*	Umbelliferae	JinArome (USA)
Eucalyptus	*Eucalyptus globulus*	Myrtaceae	JinArome (USA)
Grapefruit	***Citrus** paradisi*	Rutaceae	JinArome (USA)
Lemon	***Citrus** limonum*	Rutaceae	JinArome (USA)
Lime	***Citrus** aurantifolia*	Rutaceae	JinArome (USA)
Marjoram	*Origanum vulgare*	Labiatae	JinArome (USA)
Myrrh	*Commiphora myrrh*	Burseraceae	Charabot (France)
Orange	***Citrus** sinensis*	Rutaceae	JinArome (USA)
Petitgrain	***Citrus** aurantium*	Rutaceae	JinArome (USA)
Pine needle	*Pinus sylvestris*	Pinaceae	Charabot (France)
Rosemary	*Rosmarinus officinalis*	Labiatae	JinArome (USA)
Spearmint	*Mentha spicata*	Labiatae	JinArome (USA)
Strawberry	*Fragaria ananassa*	Rosaceae	Charabot (France)
Ylangylang	*Cananga odorata*	Annonaceae	JinArome (USA)

(32.0×28.0×22.5 cm) at 25±3°C and 60% RH with a photoperiod of 12 : 12 (L : D) h. For the repellency test, we used 10–20d-old adult female cockroaches since they showed the highest activity to chemicals. To avoid the effects of anesthesia or physical stress that may be caused by handling the cockroaches with tweezers, the insects and their shelters were transferred to repellent test cages by shaking. The number of insects was then adjusted by removing extra insects from the test cage with tweezers.

3. Gas chromatography-mass spectroscopy analysis of essential oils

The citrus oils (grapefruit, lemon, lime, orange and petitgrain) were analyzed by gas chromatography (GC, Agilent Technology 6890N) and gas chromatography-mass spectrometry (GC/MS, Hewlett Packard 7890A/5975C) equipped with a splitless injector. GC analysis was performed using columns of DB-WAX (0.25 mm I.D.×30 m length) and DB-1 (0.25 mm I.D.×30 m length), and nitrogen (N_2) as the carrier gas at a flow rate of 1.0 ml/min. The temperature conditions were as follows: initial temperature of 30°C, ramped at 2°C/min to 180°C for 60 min, while the injector and detector were maintained at 200°C and 210°C, respectively. Spectra were obtained at 70 eV. The components of the oils were identified by comparing their mass spectra to those of authentic samples in a mass spectra library (The Wiley Registry of Mass Spectral Data).

4. Repellent bioassays

The ability of 17 essential oils to repel *B. germanica* was tested using a T-tube olfactometer (ID 9 cm; stem 15 cm; arm length 22 cm; angle between arms 180°) made in our laboratory. Slightly pressurized air that was filtered by charcoal and silica gel before entering the sample container was introduced into the flight chamber through the two tube arms of the T-tube olfactometer at a flow rate of 100 ml/min. A filter paper treated with 10 or 1 μl of essential oils or a monoterpene at the amount found in the various citrus oils was placed in the treated (T) side while the untreated (U) side remained empty. Thus, in this device, the cockroach is offered two choices, *i.e.*, moving towards the smell or moving away from it. Some of the cockroaches stayed before the T-junction, or in another ambiguous region, and they were counted as no-choice. Each oil and compound was tested with forty cockroaches. The experiments were conducted in the dark (to avoid the effect of light) in rooms maintained at 28±3°C with 60±10% RH. The response of an insect was evaluated by recording the chamber in which the insect stayed after 5 min. Oils that effectively repelled *B. germanica* were also tested with the other two cockroaches, *i.e.*, *P. americana* and *P. fuliginosa*. GC and GC/MS analyzed the major components of each essential oil; the major components were then bioassayed at the amounts present in the various citrus oils. The preference ratios in the bioassays were compared using the binominal sign test.[21)]

Results and Discussion

1. Repellency of essential oils

When cockroaches were used in a blank test with the T-tube olfactometer, there was no difference in their choice of arm, which indicates that the olfactometer did not have any bias. When screening the seventeen selected essential oils, only five oils showed significant repellent activity in the order of grapefruit (96.7%), lemon (92.9%), lime (86.7%), orange (71.4%) and clove leaf (70.0%) against *B. germanica* at 10 μl (Table 2). Based on the significant repellent activity, except clove oil, the four essential oils from the citrus group were chosen for further experiments with *B. germanica*. Although petitgrain is also a citrus oil, it did not show significant activity; ylangylang was attractive rather than repellent. Citrus oils at 10 μl repelled *B. germanica* adults more significantly than that of 1 μl, indicating the dose-dependant effect of essential oil towards the pest insect species.

We subjected the citrus oils that repelled *B. germanica* to further tests and found that at 10 μl they also significantly repelled *P. americana* and *P. fuliginosa* (Table 3). Grapefruit oil was the most effective repellent of all the oils tested against the three different species of cockroaches with repellent activity of 96.7, 90.3, and 82.4% for *B. germanica*, *P. americana* and *P. fuliginosa*, respectively. The lemon and lime oils repelled *P. americana* (85.7 and 83.3%, respectively) slightly less effectively than they repelled *B. germanica* (92.9 and 86.7%, respectively), and were even less effective against *P. fuliginosa* (72.0 and 70.6%, respectively). While orange oil mildly repelled *P. americana* (70.0%, similar to its effect on *B. germanica*), its effect against *P. fuliginosa* was not significant (62.5%). Indeed, all the citrus oils tested repelled *B. germanica* and *P. americana* better than *P. fuliginosa*. Of these three kinds of cockroaches, it has been shown that *P. fuliginosa* is relatively tolerant to low temperatures,[22)] which could be linked to the observed difference in repellent activity. This difference could of course be due to other different physiological/biochemical characteristics.[11)]

Essential oils have been utilized as insecticides, antifeedants, and repellents with a wide variety of target insects[12–19,23–27)]; however, one study that tested 96 herb extracts found that while Japanese mint oil had a strong repellent effect against *B. germanica*, the essential oils of grapefruit, lemon, and lime did not show strong repellent activity.[28)] This is in contrast with our results, but the previous study was performed by a classical assay method based on the behavior of cockroach groups, and this may have been affected by the chemical factors associated with aggregation. By contrast, we used a T-tube olfactometer, in which the cockroaches had to move either toward or away from the test oil, and therefore, our method could detect the intrinsic activity of essential oils.

The T-tube olfactometer may have contributed to the unexpectedly large number of no-choices. We judged the result as no-choice when the cockroaches stayed in the T-junction or in

Table 2. Olfactory response of *Blattella germanica* to the seventeen essential oils[a)]

Essential oils	Dose (μl/filter paper)	Olfactory response			%	*P*
		Treated Side (T)	Untreated Side (U)	No choice		
Caraway seed	10	10	15	15	60.0	n.s.
	1	15	11	14	42.3	n.s.
Clary sage	10	13	15	12	53.6	n.s.
	1	11	15	14	57.7	n.s.
Clove leaf	10	9	21	10	70.0	<0.04
	1	11	18	11	62.1	n.s.
Coriander	10	18	9	13	33.3	n.s.
	1	13	10	17	43.5	n.s.
Eucalyptus	10	16	14	10	46.7	n.s.
	1	15	12	13	44.4	n.s.
Grapefruit	10	1	29	10	96.7	<0.0001
	1	10	23	7	69.7	<0.05
Lemon	10	2	26	12	92.9	<0.0001
	1	8	21	11	72.4	<0.05
Lime	10	4	26	10	86.7	<0.0001
	1	12	23	5	65.7	<0.05
Marjoram	10	15	10	15	40.0	n.s.
	1	14	9	17	39.1	n.s.
Myrrh	10	12	15	13	55.5	n.s.
	1	15	13	12	46.4	n.s.
Orange	10	8	20	12	71.4	<0.04
	1	10	18	12	64.3	n.s.
Petitgrain	10	17	10	13	37.0	n.s.
	1	14	15	11	51.7	n.s.
Pine needle	10	16	11	13	40.7	n.s.
	1	10	17	13	63.0	n.s.
Rosemary	10	11	17	12	60.7	n.s.
	1	19	15	6	44.1	n.s.
Spearmint	10	9	16	15	64.0	n.s.
	1	12	18	10	60.0	n.s.
Strawberry	10	16	11	13	40.7	n.s.
	1	14	13	13	48.1	n.s.
Ylangylang	10	19	7	14	26.9	<0.03
	1	14	15	11	51.7	n.s.

[a)] n.s.: not significant

a marginal region by dividing three equal regions between arms. Although we optimized the conditions based on the preliminary examinations, we could not reduce the numbers of no-choice. It could be possible that some of the cockroaches became insensible to effects of the tested oils after having been exposed to them for 5 minutes under dark conditions.

Plant essential oils as cockroach repellents have been studied less than possible attractants and insecticides. Our observation of the repellent activity of citrus oils against three species of cockroach suggests that citrus oils may serve as an effective component in a commercial repellent against household insect pests like cockroaches.

2. *Gas chromatography/mass spectroscopy analysis of essential oils*

The components of the four effective citrus oil repellents and

Table 3. Olfactory response of *Periplaneta americana* and *P. fuliginosa* to the four essential oils

Essential oils	Olfactory response									
	Periplaneta americana			%	*P*	*Periplaneta fuliginosa*			%	*P*
	Treated Side (T)	Untreated Side (U)	No choice			Treated Side (T)	Untreated Side (U)	No choice		
Grapefruit	3	28	9	90.3	<0.0001	6	28	8	82.4	<0.0001
Lemon	4	24	12	85.7	<0.0001	7	18	15	72.0	<0.05
Lime	5	25	10	83.3	<0.0001	10	24	6	70.6	<0.05
Orange	9	21	10	70.0	<0.05	9	15	16	62.5	n.s.

the non-repellent citrus oils (petitgrain) were analyzed by GC and GC/MS (Table 4). The major components were found to be limonene, α-pinene, β-pinene, β-myrcene, γ-terpinene, benzene, linalool and linalyl acetate, but the proportion of each component varied with respect to the different oils. The most abundant monoterpene in all citrus oils was limonene, which was found to be 93.8, 92.4, 61.3, 47.7, and 2.5% from the orange, grapefruit, lemon, lime, and petitgrain oils, respectively. Petitgrain had very low amounts of limonene and did not contain β-myrcene or benzene; instead, it had large amounts of linalool and linalyl acetate, which were absent from the other oils. This may explain why petitgrain lacks significant repellant activity, unlike the other citrus oils (Table 2); petitgrain was thus excluded from further study. Unlike the most effective repellent citrus oils, orange oil lacks β-pinene, γ-terpinene and benzene; this may partly explain why it is less repellent than the others. Although benzene is not a terpene compound, it was present at relatively high proportions in lemon and lime oils.

We observed that grapefruit and orange oils shared the same general composition (Type I) while lemon and lime oils shared a different general composition (Type II). Thus, the four oils were divided into two groups to examine the repellent activity of each major monoterpene component.

3. *Repellent effect of monoterpenes against Blattella germanica*

We tested the repellent efficacy of the monoterpenes and benzene against *B. germanica* using the olfactometer. The monoterpenes were tested with the amounts found in 10 μl of the four citrus oils, which were determined on the basis of GC and GC/MS data (Table 4). We compared the repellent efficacy of the individual monoterpenes against the efficacy of all citrus oils tested at 10 μl (Table 5). With regard to Type I oils, limonene comprised more than 90%, and showed repellent efficacy of 71.0 and 72.4% at doses of 9.24 μl and 9.38 μl/filter paper, which were equivalent to the amounts in 10 μl of the original grapefruit and orange oils, respectively. No significant repellent activity was observed for other monoterpenes, such as α-pinene, β-pinene and γ-terpinene, and benzene at doses found in 10 μl of grapefruit and orange oils. β-Myrcene had no repellent activity either, but had weak attractant activity at doses of 0.23 μl and 0.25 μl/filter paper. Based on these results and the finding that the activity of limonene was almost identical to that of orange oil, the repellent activity of orange oil was considered to be solely attributable to limonene. Since the repellent activity of grapefruit oil was higher than that of limonene alone, minor components were likely to have an additive or synergistic effect on the repel-

Table 4. Ratios of major components of five *Citrus* oils identified by GC and GC/MS

Major Component	RT[a]	*Citrus* spp. oil (%)				
		Grapefruit	Lemon	Lime	Orange	Petitgrain
α-Pinene	0.16	0.69	2.11	2.40	0.75	0.20
β-Pinene	5.27	0.27	13.52	11.66	—	2.50
β-Myrcene	7.05	2.32	1.41	1.11	2.53	—
Limonene	8.04	92.35	61.30	47.68	93.79	2.50
γ-Terpinene	9.43	0.19	4.23	3.40	—	2.00
Benzene	10.23	0.22	8.47	18.29	—	—
Linalool	18.00	—	—	—	—	23.0
Linalyl acetate	21.22	—	—	—	—	50.0

[a] Retention time: min.

Table 5. Repellency of major components of four *Citrus* oils against female adults of *B. germanica* in T-tube olfactometer

Compound	Dose (μl/filter paper)[a]	No. of insect in			%[b]	*P*[c]
		Treated side	Untreated side	No choice		
Type I						
Grapefruit	10.00	1	29	10	**96.7**	<0.0001
β-Myrcene	0.23	20	13	7	39.4	n.s.
Limonene	9.24	9	22	9	71.0	<0.03
α-Pinene	0.07	15	14	16	48.3	n.s.
β-Pinene	0.03	12	11	17	47.8	n.s.
γ-Terpinene	0.02	10	12	18	54.5	n.s.
Benzene	0.02	13	16	12	55.2	n.s.
Orange	10.00	8	20	12	**71.4**	<0.04
β-Myrcene	0.25	12	8	20	40.0	n.s.
Limonene	9.38	8	21	11	72.4	<0.02
α-Pinene	0.08	16	14	8	46.7	n.s.
Limonene+β-myrcene (9.4+0.3 μl)	10.00	9	17	14	65.4	n.s.
Limonene+α-pinene (9.4+0.1 μl)	10.00	11	19	10	63.3	n.s.
Limonene+β-pinene (9.4+0.1 μl)	10.00	9	18	13	66.7	n.s.
Limonene+γ-terpinene (9.4+0.1 μl)	10.00	8	17	16	68.0	n.s.
Limonene+benzene (9.4+0.1 μl)	10.00	15	16	9	51.6	n.s.
Limonene+A[d] (9.4+0.6 μl)	10.00	9	19	12	67.9	n.s.
Type II						
Lemon	10.00	2	26	12	**92.9**	<0.0001
α-Pinene	0.21	10	20	10	66.7	n.s.
β-Pinene	1.35	6	16	18	72.7	<0.01
β-Myrcene	0.14	12	4	24	25.0	<0.02
Limonene	6.13	10	21	9	67.7	n.s.
γ-Terpinene	0.42	8	22	10	73.3	<0.02
Benzene	0.85	20	16	4	44.4	n.s.
Lime	10.00	4	26	10	**86.7**	<0.0001
α-Pinene	0.24	14	18	8	56.3	n.s.
β-Pinene	1.17	10	26	4	72.2	<0.01
β-Myrcene	0.11	12	4	24	25.0	<0.03
Limonene	4.77	11	20	9	64.5	n.s.
γ-Terpinene	0.34	13	18	9	58.1	n.s.
Benzene	1.83	18	16	6	47.1	n.s.

Table 5. (Continued)

Compound	Dose (μl/filter paper)[a]	No. of insect in			%[b]	P[c]
		Treated side	Untreated side	No choice		
Limonene+α-Pinene (6.1+0.2 μl)	10.00	8	14	18	63.6	n.s.
Limonene+β-Pinene (6.1+1.4 μl)	10.00	3	28	9	90.3	<0.0001
Limonene+β-Myrcene (6.1+0.2 μl)	10.00	8	16	16	66.7	n.s.
Limonene+γ-Terpinene (6.1+0.4 μl)	10.00	6	22	12	78.6	<0.0003
Limonene+Benzene (6.1+0.9 μl)	10.00	7	14	19	66.7	n.s.
Limonene+α-Pinene+β-Pinene (6.1+0.2+1.4 μl)	10.00	9	16	15	64.0	n.s.
Limonene+α-Pinene+β-Myrcene (6.1+0.2+0.1 μl)	10.00	11	18	11	62.1	n.s.
Limonene+α-Pinene+γ-Terpinene (6.1+0.2+0.4 μl)	10.00	7	15	18	68.2	n.s.
Limonene+β-Pinene+β-Myrcene (6.1+1.4+0.1 μl)	10.00	4	22	14	84.6	<0.001
Limonene+β-Pinene+γ-Terpinene (6.1+1.4+0.4 μl)	10.00	5	23	12	82.1	<0.001
Limonene+β-Myrcene+γ-Terpinene (6.1+0.1+0.4+3.4)	10.00	6	21	13	77.8	<0.006

[a] The mixtures of monoterpenes were made up to 10 μl with ethanol before applying to filter paper. [b] Olfactory response (%)=Untreated/(Untreated+Treated)*100. [c] Sign test to evaluate differences from 50 : 50 response (treated and untreated arms, N=40, P<0.05, P<0.01 and P<0.001). n.s., not significant. [d] Mixture of minor monoterpenes except limonene found in 10 μl of original oils.

lency of limonene; however, several artificial cocktails of monoterpenes did not reproduce the activity of grapefruit oil (Table 5), and the additive or synergistic components could not be identified in this study.

With regard to Type II oils, limonene at doses of 6.13 and 4.77 μl/filter paper (equivalent to the contents in 10 μl lemon and lime oils, respectively) was not repellent (Table 5). Instead, β-pinene at doses found in 10 μl of lemon and lime oils and γ-terpinene at the dose found in 10 μl lemon oil were significantly repellent (72.7, 72.2, and 73.3%, respectively). As observed in Type I oils, β-myrcene showed attractant rather than repellent activity at doses of 0.14 and 0.11 μl, respectively, which are equivalent to their contents in 10 μl of original lemon and lime oils. α-Pinene appeared as a weak repellent, but the activity was not statistically significant. Benzene also did not show any significant repellent activity. Benzene was thus excluded from further study.

Since neither of the components alone showed repellency comparable to the original oils, the repellent activity of Type II oils is likely to be exerted by the additive or synergistic effects of multiple constituents; therefore, we tested the various combinations of monoterpenes.

As shown in Table 5, enhanced repellency was observed when β-pinene was mixed with limonene at the same ratio as in the original oil. The addition of limonene to γ-terpinene also increased repellent activity, although the additional effect was less than that of β-pinene and limonene mixture. The mixture of β-pinene and limonene showed repellency of 90.3%, almost comparable to that of original lemon and lime oils. The addition of a third component to this mixture was not effective in terms of increased repellent activity in every case tested, and thus, we concluded that the blend of β-pinene with limonene at a definite mixing ratio is responsible for the repellent activity of lemon and lime oils.

In a previous study, it was shown that β-pinene had good insecticidal activity against female *B. germanica* in the contact toxicity test.[27] It has also been reported that *d*-limonene in various essential oils has insecticidal activity against various pests.[29] *d*-Limonene is also known to inhibit the growth of offspring from the oothecae of *B. germanica*, and such intrinsic toxicity is most likely associated with the repellent effects.[30] While *d*-limonene at high concentrations has been observed to repel various pests, including *B. germanica*,[29,31] we found here that a lower dose of *d*-limonene that does not show repellency alone can repel cockroaches when mixed with β-pinene or γ-terpinene. These findings agree with the results of previous reports, in which essential oil constituents had a synergistic effect against adults of *Lasioderma sericorne*.[32] The high repellency of grapefruit oil could not be explained by the mixture of known components in this study. Since the activity of grapefruit components in doses less than 1% was not examined, further study is required to clarify the minor components with additive/synergistic effects.

4. *Repellency of citrus oil monoterpenes against other cockroach species*

We also examined the repellent efficacies of the major monoterpene components in citrus oils against *P. americana* (Table 6) and *P. fuliginosa* (Table 7). The components that repelled *B. germanica*, such as limonene, with an amount

Table 6. Repellency of major components of four *Citrus* oils against female adults of *P. americana* in T-tube olfactometer

Compound	Dose (μl/filter paper)[a]	No. of insect in			%[b]	P[c]
		Treated side	Untreated side	No choice		
Type I						
Grapefruit	10.00	3	28	9	**90.3**	<0.001
β-Myrcene	0.23	9	23	8	71.9	<0.01
Limonene	9.23	8	22	10	73.3	<0.01
α-Pinene	0.07	12	19	10	61.3	n.s.
β-Pinene	0.03	14	13	12	48.1	n.s.
γ-Terpinene	0.02	13	15	12	53.6	n.s.
Orange	10.00	9	21	10	**70.0**	<0.05
β-Myrcene	0.25	—	—	—	—	n.t.
Limonene	9.38	8	23	9	74.2	<0.01
α-Pinene	0.08	13	16	14	55.2	n.s.
Limonene+β-myrcene (9.4+0.3 μl)	10.00	6	16	8	72.7	<0.03
Limonene+α-pinene (9.4+0.1 μl)	10.00	12	15	13	55.6	n.s.
Limonene+β-pinene (9.4+0.1 μl)	10.00	11	16	13	59.3	n.s.
Limonene+γ-terpinene (9.4+0.1 μl)	10.00	12	16	12	57.1	n.s.
Limonene+benzene (9.4+0.1 μl)	10.00	15	18	7	54.5	n.s.
Limonene+A[d] (9.4+0.6 μl)	10.00	8	20	12	71.4	<0.02
Type II						
Lemon	10.00	4	24	12	**85.7**	<0.001
α-Pinene	0.21	10	21	9	67.7	<0.05
β-Pinene	1.35	10	20	10	66.7	<0.05
β-Myrcene	0.14	10	21	9	67.7	<0.05
Limonene	6.13	8	21	11	72.4	<0.01
γ-Terpinene	0.42	9	21	10	70.0	<0.02
Lime	10.00	5	25	10	**83.3**	<0.001
α-Pinene	0.24	10	24	6	70.6	<0.05
β-Pinene	1.17	2	24	14	92.3	<0.001
β-Myrcene	0.11	11	23	6	67.6	<0.05
Limonene	4.77	8	20	12	71.4	<0.05
γ-Terpinene	0.34	11	20	9	64.5	n.s.

Table 6. (Continued)

Compound	Dose (μl/filter paper)[a]	No. of insect in			%[b]	P[c]
		Treated side	Untreated side	No choice		
Limonene+α-Pinene (6.1+0.1 μl)	10.00	5	18	17	78.2	<0.005
Limonene+β-Pinene (6.1+1.4 μl)	10.00	6	18	16	75.0	<0.05
Limonene+β-Myrcene (6.1+0.1 μl)	10.00	6	17	17	73.9	<0.05
Limonene+γ-Terpinene (6.1+0.4 μl)	10.00	5	18	17	78.3	<0.01
Limonene+α-Pinene+β-Pinene (6.1+0.2+1.4 μl)	10.00	6	21	13	77.8	<0.01
Limonene+α-Pinene+β-Myrcene (6.1+0.2+0.1 μl)	10.00	8	22	10	73.3	<0.01
Limonene+α-Pinene+γ-Terpinene (6.1+0.2+0.4 μl)	10.00	6	24	10	80.0	<0.001
Limonene+β-Pinene+β-Myrcene (6.1+1.4+0.1 μl)	10.00	6	19	15	76.0	<0.01
Limonene+β-Pinene+γ-Terpinene (6.1+1.4+0.4 μl)	10.00	6	24	10	80.0	<0.001
Limonene+β-Myrcene +γ-Terpinene (6.1+0.1+0.4 μl)	10.00	5	25	10	83.3	<0.001

[a] The mixtures of monoterpenes were made up to 10 μl with ethanol before applying to filter paper. [b] Olfactory response (%)=Untreated/(Untreated+Treated)*100. [c] Sign test to evaluate differences from 50 : 50 response (treated and untreated arms, N=40, P<0.05, P<0.01 and P<0.001). n.t., not tested; n.s., not significant. [d] Mixture of minor monoterpenes except limonene found in 10 μl of original oils.

equivalent to the content in 10 μl Type I oils, and β-pinene and γ-terpinene equivalent to the contents in Type II oils, also repelled *P. americana*. In contrast to *B. germanica*, a small amount of β-myrcene equivalent to the contents in Type I or II oils showed significant repellent activity against *P. americana*. γ-Terpinene was active at the dose equivalent to the content in 10 μl lemon oil, but was inactive at the dose equivalent to that in lime oil, which was similar to that of *B. germanica*.

As for *B. germanica*, limonene appeared to be mainly responsible for the repellency of grapefruit oil, but again, its high activity was not reproducible with any combinations of its monoterpene components. The mixture of limonene and β-myrcene had almost the same activity as limonene or β-myrcene alone, and no additive/synergistic effect was observed. By contrast, the repellency of orange oil was mostly explained by limonene, which was also similar to *B. germanica*.

The highest contents of limonene in lemon and lime oils suggested that the repellency of these two oils was also mainly explained by limonene. The addition of α-pinene or γ-terpinene to limonene slightly enhanced the repellency, but the addition of α-pinene plus γ-terpinene had no further significant enhancement. The highest repellent efficacy (83.3%) was observed when limonene was mixed with γ-terpinene plus β-myrcene, and this was almost equivalent to the activity of the original oils of lemon and lime. The effect of β-myrcene is intriguing, because its addition to limonene caused hardly any increase in repellency, but caused a considerable increase when mixed with a combination of limonene and γ-terpinene. This may suggest the occurrence of complicated synergistic effects of monoterpenes. With respect to *P. americana*, one study demonstrated that limonene showed no repellent effect against nymphs of *P. americana*, but benzene derivatives such as eugenol and safrole, and a monoterpene α-pinene were active.[19] The discrepancy between earlier studies and the current result may be due to the different conditions concerning both insects and the different experimental methods, but it should be mentioned that the repellency of a single component may be less important if the repellency is exerted by the interaction of multiple components.

The repellent efficacy of the major monoterpene components of citrus oils against *P. fuliginosa* is shown in Table 7. As with *B. germanica* and *P. americana, P. fuliginosa* were also repelled by the Type I-oil-equivalent amount of limonene, and Type II-oil-equivalent amounts of α-pinene and β-pinene; however, for *P. fuliginosa*, Type II oil-equivalent amounts of limonene and α-pinene were also active, which was the case for *P. americana*, but not for *B. germanica*. Conversely, like *B. germanica* but not *P. americana*, β-myrcene was inactive against *P. fuliginosa*.

Of the monoterpene components in Type I oils, limonene showed the highest repellency against *P. fuliginosa*. In contrast to *B. germanica* and *P. americana*, the activity of limonene was higher than the original grapefruit and orange oils, suggesting that other minor components have some masking effect on the repellency by limonene. Supporting this statement, the addition of any monoterpene component to limonene lowered the repellency compared to limonene alone. Repellent activity of only 75% was observed when limonene was mixed with all other monoterpene components found in 10 μl of original oils. Such a masking effect also appeared to occur with repellency by type II oils. Each monoterpene com-

Table 7. Repellency of major components of four *Citrus* oils against female adults of *P. fuliginosa* in T-tube olfactometer

Compound	Dose (μl/filter paper)[a]	No. of insect in			%[b]	P[c]
		Treated side	Untreated side	No choice		
Type I						
Grapefruit	10.00	6	26	8	**81.3**	<0.001
β-Myrcene	0.23	16	16	8	50.0	n.s.
Limonene	9.23	4	24	12	85.7	<0.001
α-Pinene	0.07	15	18	7	54.5	n.s.
β-Pinene	0.03	12	15	13	55.6	n.s.
γ-Terpinene	0.02	13	12	15	48.0	n.s.
Orange	10.00	8	20	12	**71.4**	<0.05
β-Myrcene	0.25	—	—	—	—	n.t.
Limonene	9.38	4	24	12	85.7	<0.001
α-Pinene	0.08	13	14	13	**51.9**	n.s.
Limonene+β-Myrcene (9.4+0.3 μl)	10.00	8	12	20	60.0	n.s.
Limonene+α-pinene (9.4+0.1 μl)	10.00	10	20	10	66.7	n.s.
Limonene+β-pinene (9.4+0.1 μl)	10.00	9	18	13	66.7	n.s.
Limonene+γ-terpinene (9.4+0.1 μl)	10.00	18	12	10	40.0	n.s.
Limonene+benzene (9.4+0.1 μl)	10.00	12	14	14	53.8	n.s.
Limonene+A[d] (9.4+0.6 μl)	10.00	8	24	8	75.0	<0.01
Type II						
Lemon	10.00	10	24	6	**70.6**	<0.05
α-Pinene	0.21	9	21	10	70.0	<0.05
β-Pinene	1.35	9	20	11	69.0	<0.05
β-Myrcene	0.14	15	16	9	51.6	n.s.
Limonene	6.13	6	23	11	79.3	<0.01
γ-Terpinene	0.42	6	20	14	76.9	<0.01
Lime	10.00	7	18	15	**72.0**	<0.05
α-Pinene	0.24	9	21	10	70.0	<0.05
β-Pinene	1.17	4	18	18	81.8	<0.01
β-Myrcene	0.11	16	16	8	50.0	n.s.
Limonene	4.77	7	23	10	76.7	<0.05
γ-Terpinene	0.34	14	14	12	50.0	n.s.

Table 7. (Continued)

Compound	Dose (μl/filter paper)[a]	No. of insect in			%[b]	P[c]
		Treated side	Untreated side	No choice		
Limonene+α-Pinene (6.1+0.2 μl)	10.00	6	22	12	78.6	<0.01
Limonene+β-Pinene (6.1+1.4 μl)	10.00	4	18	18	81.8	<0.01
Limonene+β-Myrcene (6.1+0.1 μl)	10.00	9	17	14	65.4	n.s.
Limonene+γ-Terpinene (6.1+0.4 μl)	10.00	5	16	19	76.2	<0.05
Limonene+α-Pinene+β-Pinene (6.1+0.2+1.4 μl)	10.00	6	23	11	79.3	<0.01
Limonene+α-Pinene+β-Myrcene (6.1+0.2+0.1 μl)	10.00	8	15	17	65.2	n.s.
Limonene+α-Pinene+γ-Terpinene (6.1+0.2+0.4 μl)	10.00	8	21	11	72.4	<0.05
Limonene+β-Pinene+β-Myrcene (6.1+0.1+1.4 μl)	10.00	9	14	17	60.9	n.s.
Limonene+β-Pinene+γ-Terpinene (6.1+1.4+0.4 μl)	10.00	6	24	10	80.0	<0.001
Limonene+β-Myrcene+γ-Terpinene (6.1+0.1+0.4 μl)	10.00	14	12	14	46.2	n.s.

[a] The mixtures of monoterpenes were made up to 10 μl with ethanol before applying to filter paper. [b] Olfactory response (%)=Untreated/(Untreated+Treated)*100. [c] Sign test to evaluate differences from 50 : 50 response (treated and untreated arms, $N=40$, $P<0.05$, $P<0.01$ and $P<0.001$). n.s., not significant. [d] Mixture of minor monoterpenes except limonene found in 10 μl of original oils.

ponent with significant activity showed higher, or almost equal, at least, repellency, compared to original lemon and lime oils. Experiments using a mixture of monoterpenes showed that the addition of α-pinene, β-pinene and γ-terpinene to limonene had no increasing effect. By contrast, the addition of β-myrcene considerably decreased the repellency, and was likely to be the major masking principle. This also demonstrates an example of a complicated interaction among monoterpene components in essential oils, as β-myrcene alone apparently showed a neutral effect in terms of attraction and repellency.

Concluding Remarks

This study shows that citrus oils have repellent efficacy against *B. germanica*, *P. americana* and *P. fuliginosa*. The repellent efficacy was largely due to their main monoterpene component, limonene, but other major components may also contribute to the repellent efficacy of these oils. Further studies examining whether these minor monoterpene components act additively or synergistically on various cockroach species are in progress.

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