ISSN 0915－4698

環動昆

Vol.5 1993

2

日本環境動物昆虫学会

Jpn. J. Environ. Entomol. Zool. 5(2) : 55—58(1993)

New Records of *Boreochlus thienemanni* and *Euryhapsis subviridis* (Chironomidae: Diptera) from Japan

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(Received : March 18, 1993)

日本新記録のユスリカ2種，*Boreochlus thienemanni* と *Euryhapsis subviridis*

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富山県内の山地において *Boreochlus thienemanni* と *Euryhapsis subviridis* を採集した。*Boreochlus* 属はPodonominae亜科に属するが，*B. thienemanni* はこの亜科としては今回初めて日本から記録された。*Euryhapsis* はOrthocladiinae亜科の一属であるが，*E. subviridis* はこの属としては日本で初めての記録であった。両種について再記載を行った。

Boreochlus thienemanni and *Euryhapsis subviridis* are newly recorded from Toyama Prefecture, Japan, and redescribed with illustrations.

Key Words : *Boreochlus thienemanni*, *Euryhapsis subviridis*, Chironomidae, Diptera, New record from Japan

OLIVER and DILLON (1989) listed 8 subfamilies in the family Chironomidae in the Holarctic region. Recent extensive studies (e. g., SASA, 1989; SASA and OKAZAWA, 1992) greatly increased the number of chironomid species in Japan, but 2 subfamilies, Podonominae and Buchonomyiinae, have not been recorded from Japan. In this study, we record a species of *Boreochlus*, Podonominae, and a species of *Euryhapsis*, Orthocladiinae, for the first time in Japan. Since important information on morphology was lacking in previous studies, both species are redescribed based on the specimens collected from Toyama Prefecture.

Boreochlus thienemanni EDWARDS

Boreochlus thienemanni EDWARDS, 1938, Zool. Anz., 122 : 153. BRUNDIN, 1966, Kungl. Svenska Vet. Handl., 11 : 302-3. MAKARCHENKO, 1985, Chironomidae of Far East USSR, 25.

Male. Body length ca. 2.3-2.9 (av. 2.7) mm, wing length 1.5-1.9 (av. 1.7) mm. Body brown to dark brown in color.

Eyes reniform and bare. Antennae (Fig. 1A) with 14 flagellomeres, bending ventrally; antennal ratio (combined length of flagellomeres 13 and 14 divided by combined length of the other flagello-

meres) 0.28-0.30 (av. 0.29); flagellomere 14 small, not completely separated from flagellomere 13, with sensilla chaetica but without plume.

Antepronotum well developed, lobes deeply separated in the middle, with 4-11 (av. 7.1) lateral setae. Scutum with biserial 33-38 (av. 35.8) acrostichals and multiserial 34-50 (av. 39.2) dorsocentrals. Scutellum with 13-16 (av. 14.5) setae in a transverse row. Postnotum bare.

Wing membrane with macrotrichia; venation as in Fig. 1B. Costa extending beyond tip of R_{4+5} and free end reaching wing tip. R_1 short, less than 1/2 as long as strongly curved R_{4+5}. R_{2+3} absent. R_{4+5} ending distal to Cu_1. Cu_2 sinuous, postcubitus extending beyond FCu and longer than anal vein. MCu situated at the middle of distance between arculus and FCu. Venarum ratio (distance between arculus and FCu divided by distance between arculus and RM) 1.17-1.22 (av. 1.19). Radius/Cubitus ratio (horizontal distance between arculus and tip of R_{4+5} divided by horizontal distance between arculus and tip of Cu_1) 1.16-1.20 (av. 1.19). Squama with 7-11 (av. 8.3) setae. Alula with 9-12 (av. 10.8) setae. Anal lobe obtuse.

Tarsus 4 cylindrical and longer than tarsus 5. Anterior and middle legs without spur, posterior legs with a long inner spur. Spur with three prominent, lateral denticles. Comb with 5-7 (av. 6.5) setae. Pseudospurs absent, pulvilli absent. Claws with a long, pale process and a short, spine-like projection at base.

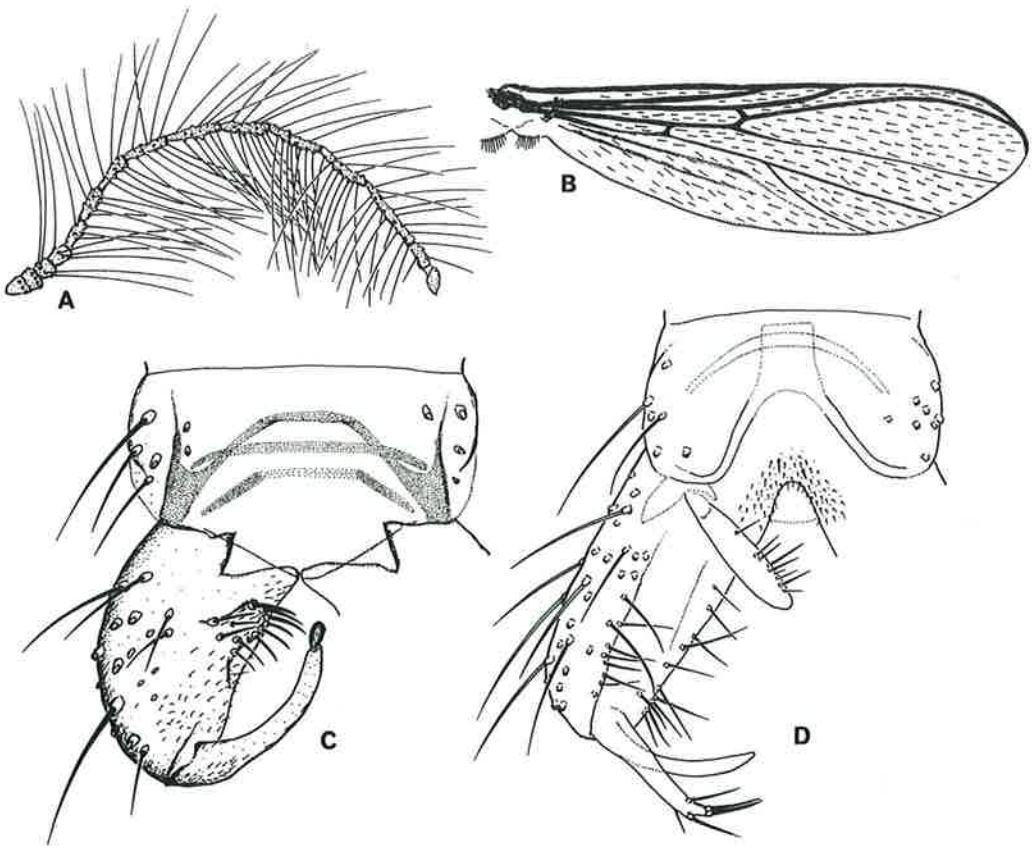

Fig. 1 Males of *Boreochlus thienemanni* (A-C) and *Euryhapsis subviridis* (D). A: Antenna. B: Wing. C: Hypopygium. D: Hypopygium.

Hypopygium as in Fig. 1C. Anal point absent. Gonocoxite ca. 1.5 times as long as width; an inferior lobe conical with about 5-6 stout bristles at apical margin and 2-3 stout, smaller ones at base. Gonostylus slender, slightly shorter than gonocoxite, tapering toward apex; apex with a lamellar megaseta. Sternapodeme broadly arched, without oral projections. Phallapodeme slender, simply rod like, not penetrating into the gonocoxite.

Specimens examined. 5 males mounted on slides and 20 males in ethanol, Daikanjo, Toga Village, Toyama Prefecture, Japan, 5. XII. 1991, T. OKAZAWA.

All specimens were captured while swarming in a spot of sunlight coming through a hole in a canopy of Japanese cedars (*Cryptomeria japonica* D. DON). The swarm was formed 2 m high above a paved road. The collection place was in a narrow, deep valley in which the Toga River runs through from south to north. The altitude was 720 m.

This is the first record of the subfamily Podonominae from Japan. The subfamily shows a circumpolar distribution both in northern and southern hemispheres with expansion into middle and low latitude areas through the mountain ranges, e. g. the Andes and the Rockies. *Boreochlus,* a boreal genus of the subfamily, has been known from discrete areas of the northern temperate zone. Of 7 species of *Boreochlus,* 4 were Nearctic (BRUNDIN, 1966 ; WIRTH and SUBLETTE, 1970), 2 were Oriental (northern Burma, BRUNDIN, 1966) and the remaining one, *B. thienemanni,* Palearctic. *B. thienemanni* was originally described by EDWARDS (1938) from Swedish Lapland and recently recorded from Primorye by MAKARCHENKO (1985).

Euryhapsis subviridis (SIEBERT)

Brillia subviridis SIEBERT, 1979, Aquat, Ins., 1: 167-8.

Euryhapsis subviridis MAKARCHENKO *et al.*, 1988, Spixiana (Suppl.) 14: 130.

Male. Body length ca. 3.8 mm, wing length 1.9-2.3 (av. 2.1) mm. Body whitish in color.

Eyes bare, with dorsomedial extension. Antennae with 13 flagellomeres; antennal ratio 1.3 (length of distal flagellomere divided by combined length of remaining flagellomeres); flagellomere 13 without subapical seta.

Antepronotum well developed, lobes deeply separated in the middle, with 9-10 lateral and 3-4 dorsal setae. Scutum with ca. 40 dorsocentrals; acrostichals absent. Scutellum with ca. 33 setae. Postnotum bare.

Wing membrane with macrotrichia. Costa slightly extending (less than 1/2 length of RM) beyond tip of R_{4+5}. R_1 and R_{3+4} with setae. R_{2+3} ending very close to R_1. Cu_2 almost straight. Postcubitus extending beyond FCu and longer than anal vein. Venarum ratio 1.30-1.40 (av. 1.36). Radius/Cubitus ratio 1.08-1.09 (av. 1.08). Squama with ca. 20 setae. Alula bare. Anal lobe rounded.

Tarsus 4 cylindrical and longer than tarsus 5. Anterior legs with a spur, middle and posterior legs with 2 slender spurs. Comb with ca. 8 setae. Pseudospurs absent. Pulvilli absent. Claws without process at base.

Hypopygium as in Fig. 1D. Anal point absent. Gonocoxite parallel-sided, twice as long as width; superior volsella well developed, elongate and narrow, 1/2 as long as gonocoxite, with setae arising from inner surface; inferior volsella vestigial. Gonostylus bifurcate; apical lobe about 3/4 as long as subapical lobe and about 1/2 length of gonocoxite, with 2 long, pale setae at the end. Anteromedian area of sternapodeme broaden in rectangular plate, without oral projections. Phallapodeme weakly sclerotized, nearly straight.

Specimens examined: 3 males mounted on slides, Sennin Dam, Kurobe River, Toyama Prefecture, Japan, 7. VIII. 1990, T. OKAZAWA.

All specimens were captured by a light trap or a suction tube near lights of a house. The collection place was in the deep valley of the Kurobe River. The altitude was 800 m.

The genus *Euryhapsis* comprises 5 species, and is known from the western Nearctic and Palaearctic regions (OLIVER, 1981; MAKARCHENKO *et al.*, 1988). *Euryhapsis subviridis* was originally described by SIEBERT (1979) from Austria under the name of *Brillia subviridis*. Later, it was recorded from Primorye and Sakhalin, and newly combined with the genus *Euryhapsis* by MAKARCHENKO *et al.* (1988). Antennal ratio of the male differed among the localities. It was 1.3-1.4 in Japanese specimens, 1.5-2.3 in east Russian specimens (MAKARCHENKO *et. al.*, 1988) and 1.25 in an Austrian specimen (SIEBERT, 1979).

Acknowledgements

We thank R. ARAKAWA and M. WATANABE for their assistance in collecting the specimens. We also thank F. KAMIMURA for mounting the specimens on slides.

References

BRUNDIN, L. (1966) Transantarctic relationships and their significance, evidenced by chironomid midges. With a monograph of the subfamilies Podonominae and Aphroteniinae and the austral Heptagyiae. *Kung. svenska Vetensk Akad. Handl.* **11**: 1-472.

EDWARDS, F. W. (1938) A new genus of the subfamily Podonominae. In "*Neuer Beitrag zur Kenntnis der Podonominae (Dipt. Chironomidae)*" (EDWADRS, F. W. and A. THIENEMANN, eds). *Zool. Anz.* **122**: 152-154.

MAKARCHENKO, E. A. (1985) Chironomids of Far East USSR. Subfamilies Podonominae, Diamesinae and Prodiamesinae (Diptera, Chironomidae). 200 pp, Far East Branch, Academy of Sciences of the USSR, Vladivostok. (In Russian).

MAKARCHENKO, E. A., J. J. KIKNADZE and J. E. KERKIS (1988) Morpho-karyological description of *Euryhapsis subviridis* (Siebert) from the South of the Soviet Far East. *Spixiana, Suppl.* **14**: 129-137.

OLIVER, D. R. (1981) Description of *Euryhapsis* new genus including three new species (Diptera: Chironomidae). *Can. Ent.* **113**: 711-722.

SASA, M. (1989) Chironomidae of Japan: Checklist of species recorded, key to males and taxonoic notes. *Res. Rep. Natl. Inst. Environ. Stud.* **125**: 1-177.

SASA, M. and T. OKAZAWA (1992) Studies on the chironomid midges (Yusurika) of Toga-mura, Toyama. Part II. The subfamily Orthocladiinae. In "*Some characteristics of nature conservation within the chief rivers in Toyama Prefecture (The upper reach of Kurobe River)*", pp. 92-204. Toyama Prefectural Environmental Pollution Research center, Toyama.

SIEBERT, M. (1979) Two new chironomids (Diptera: Chironomidae) from Germany and Austria. *Aquat. Ins.*, **1**: 165-168.

WIRTH, W. W. and J. E. SUBLETTE (1970) A review of the Podonominae of North America with descriptions of three new species of *Trichotanypus* (Dipt.: Chir.). *J. Kans. entomol. Soc.* **43**: 335-354.

環動昆
第5巻　第2号：59－64(1993)

香料の揮発成分の抗ＭＲＳＡ作用

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（受理：1993年4月5日）

Anti-MRSA Activities of Perfume Vapor Components. Yoshimi YOSHIDA, Kazushi SUGAMOTO, Mosei TOYAMA, Yoshio KATSUDA (Research Laboratory, Dainihon Jochugiku Co. Ltd., Toyonaka, Osaka 561, Japan) and Yoshikazu SAKAGAMI (Osaka Prefectural Institute of Public Health, Osaka 537, Japan). *Jpn. J. Environ. Entomol. Zool.* 5 : 59－64 (1993)

Anti-MRSA (methicillin-resistant *Staphylococcus aureus*) activities of perfume vapor components were studied. As a preliminary test, 27 perfume components were evaluated for their inhibitory effects against the growth of MSSA (methicillin-sensitive *S. aureus*) using the petri dish method. Among them 8 components, peppermint oil, citral, citronellal, L-perilla aldehyde, 1-formyl-2, 4-dimethyl-3-cyclohexene (trivertal), thymol, hinokitiol, and tetrahydrolinalool, almost completely inhibited the colony formation of MSSA at a rate of 10 mg/dish. These components also had strong inhibitory effects against MRSA. No differences in sensitivity of the bacteria to perfume vapor components were observed between MSSA and MRSA. Moreover the inhibitory effects of these components were similarly high in tests where each test space was 50, 100, and 150 times larger than that of a petri dish. Even in a 8 mat-room test in practical use, they were found to be effective with diffusion of vapor components using a fan. These test results suggest that perfume vapor components are useful to prevent the transmission of MRSA in hospitals, although further work including formulation studies would be necessary.

Key Words : MRSA, Methicillin-resistant, Perfume vapor component

香料の揮発成分による抗MRSA作用を検討するため，27種の香料成分につき，ペトリ皿法を用いてMSSAに対する抗菌作用を調べた。peppermint oil，citral，citronellal，L-perilla aldehyde，1-formyl-2，4-dimethyl-3-cyclohexene（以降 trivertal と称す），thymol，hinokitiol，tetrahydrolinalool の8種の香料成分は，添加量10mg／ペトリ皿でMSSAのコロニー形成をほぼ完全に抑制した。これらの香料成分は，MRSAに対しても同様に高いコロニー形成抑制効果を示し，MSSAとMRSA間で感受性差は認められなかった。また，上記8種の香

料成分については，試験の空間容量をペトリ皿法に比べて50倍，100倍および150倍に拡大した条件下（ポット法）でも同様に高い抗菌効果を示した。さらに，実用的な8畳の部屋での試験においてもファンで香料成分を拡散させることによって効果を発現させることが可能であった。製剤化検討を含め，実用化には種々の検討を要するものの，香料の揮発成分の活用は，MRSAの院内感染を防止するうえで有用と考えられる。

はじめに

抗生物質や合成抗菌剤の進歩は，人類に多大な恩恵をもたらしてきた。しかし，その反面，薬剤耐性菌の出現は常に問題となっている。特に，メチシリン耐性黄色ブドウ球菌〔Methicillin-resistant *Staphylococcus aureus*（以降MRSAと略す）〕は有効な治療薬が少なく，これによる院内（病院）感染（起因菌が医療従事者の手指，病院内環境および空中飛沫などを介して発生する感染）は，1961年に，その存在が報告されて以来（STEWART，1961；BARBER，1961；JEVENS；1961），術後感染例や敗血症例など多数知られている。このため院内感染の防止対策がクローズアップされているが，手，指，医療器具等，あるいは院内環境消毒用に供される消毒剤のほとんどは，揮散性がなく，消毒効果が清拭，塗布部位にとどまるので，適用部周辺環境からのMRSAの感染を防止しにくいという問題点を有している。

香料成分の抗菌作用は古くから研究され，その直接接触による作用に関して，すでに数多くの報告がなされている（MARUZZELLA and LICHTENSTEIN，1956；MARUZZELLA and HENRY，1958)。一方，香料の揮発成分の作用については，メチシリン感受性黄色ブドウ球菌〔Methicillin-sensitive *S. aureus*（以降MSSAと略す)〕や大腸菌*Escherichia coli*等に対する抗菌作用に関し若干の報告（INOUYE *et al.*，1983：牛腸，1991）があるのみで，抗MRSA作用についての研究は全く知られていない。本報では，種々の揮散性香料成分について，揮発状態での抗MSSA作用および抗MRSA作用を比較検討し，そのうちのいくつかがMRSAによる院内感染を防止するうえで有用と考えられたのでここに報告する。

材料と方法

1．供試菌株

MSSA：*S. aureus* IFO 12732，MRSA：兵庫医科大学付属病院分離株（メチシリンの最小発育阻止濃度（MIC）が400 μg/mlである株）

2．供試香料成分

香料成分として，peppermint oil，basil oil，sugi oil，lavender oil，armoise morocco oil，tarragon oil，coriander oil，eucalyptus oil，pine oil，citral，citronellal，L-perilla aldehyde，trivertal，thymol，hinokitiol，tetrahydrolinalool，linalool，dehydrolinalool，benzaldehyde，fenchyl acetate，1，4-cineole，vandor B，L-rose oxide，DL-rose oxide，L-carvone，lolitol，3-octanol（以上塩野香料㈱提供）を使用した。なお，各精油の主要構成成分をTable 1に示した。

3．実験方法

（1）ペトリ皿法

滅菌ガラス製ペトリ皿（φ；9 cm×h；2 cm）に，121℃，20分間高圧蒸気滅菌した標準寒天培地（栄研化学製）20 mlを入れ，固化させた。あらかじめSCD培地（日本製薬㈱製）に一白金耳量植菌し，37℃で一晩前培養したMSSAあるいはMRSAを，コロニー数としておよそ1000～2000個／mlになるように滅菌生理食塩液で希釈し，更にその0.1 mlを滅菌コンラージ棒を用いて前記標準寒天平板の表面に均一に塗抹した。Fig. 1に示すように，ペトリ皿の上蓋を裏返しにし，その中央に香料成分を一定量含んだ濾紙（3×3 cm；試料の10%アセトン溶液を100 μl含浸させ，濾紙中の成分含量を10 mgに調整したもの）を載置した。前記標準寒天平板を含むペトリ皿を逆さまにして載せ，これに，同サイズのペトリ皿上蓋をかぶせた。蓋と蓋の合わせ目をビニルテープで封印する

Table 1　Major components of essential oils

	Essential oil	Major components
1	Peppermint oil	L-Menthol (45-60%), L-Menthone (15-25%)
2	Basil oil	Methyl chavicol, Linalool, Eugenol, Cineole
3	Sugi oil	
4	Lavender oil	Linalyl acetate (35-55%), Linalool (15-20%)
5	Armoise morocco oil	Linalool (60-70%)
6	Tarragon oil	Linalool
7	Coriander oil	Linalool (60-70%), Pinene, Geraniol
8	Eucalyptus oil	1, 8-Cineole
9	Pine oil	α-Pinene, β-Pinene

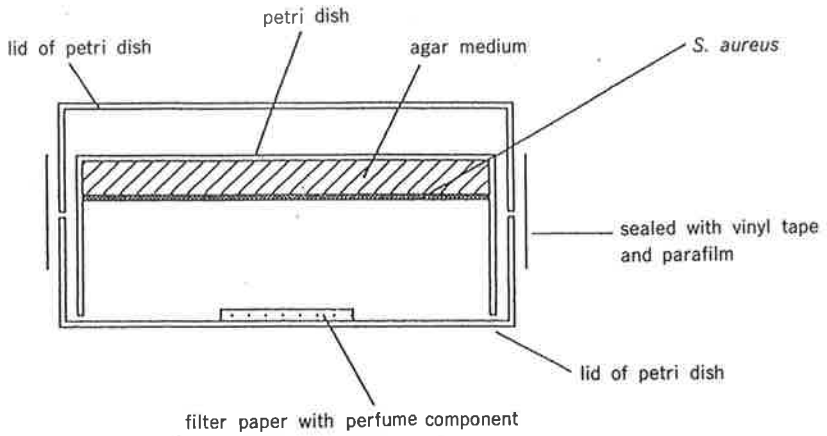

Fig. 1　Petri dish test

と共に，さらにその上をパラフィルムで覆い，揮散香料成分の漏出を防止した。37℃で48時間培養後に形成されたコロニー数を数え，抗菌作用を評価した。別にコントロールとして，香料成分を含まないアセトンを用いて同様に操作した。

（2）ポット法

ペトリ皿に比べて空間容量がそれぞれ，50倍，100倍，150倍になるように，アクリル製円筒（ϕ；20 cm × h；20 cm）を1段，2段，あるいは3段に重ね，その上面及び底面をアルミニウムの板で蓋をして試験を行った（1段：6280 ㎠，2段：12560 ㎠，3段：18840 ㎠）。底面の蓋の中央に，香料成分を各段数に応じてそれぞれ0.5，1.0および1.5 g含んだ滅菌濾紙を載置した。また上面の蓋の内側に，試験菌塗抹ペトリ皿（ペトリ皿に標準寒天培地60～70 mlを入れ，固化後，MSSAあるいはMRSAがコロニーとして100～200個になるように塗抹したもの）を逆さまにして両面テープで貼り付けた。アクリル製円筒の継ぎ目，あるいは蓋との間の隙間は，ビニルテープとパラフィルムで二重にシールし，30℃で48時間培養後に形成されたコロニー数を測定した。

（3）準実地試験

8畳の部屋（高さ2.6 m）の中央の机の上に，ポット法と同様に調製したMSSA塗抹寒天平板を，ペトリ皿の蓋をはずして載置した。部屋の片隅で約2 mの高さから，香料成分含有液（tetrahydrolinalool : thymol : IP solvent＝5 : 2 : 3）を67 mg/hourの速度で，ファン型消臭芳香器具を用いて揮散させた。なお，香料成分としては，基礎試験で最も抗菌効果が高かったthymolと，抗菌効果は中程度ながら匂いの点で緩和なtetrahydrolinalool の混合物を選択し，市販芳香剤の溶剤として一般的なイソパラフィン系のIP solventを用いて各成分が同比率で揮散するように処方調整した。室温を30

℃に保ち，48時間後のMSSAのコロニー形成を，ペトリ皿の蓋を取らずに机の上に載置したコントロールと比較して調べた。

結　果

1．ペトリ皿法

コントロールの寒天培地表面に，MSSAのコロニーが100～200個形成されるような条件で，各種香料成分の増殖抑制効果を調べた。その結果，Table 2 に示すように，香料成分27種（うち精油が９種）のすべてが，添加量10㎎でコロニーの形成を抑制し，コロニー数は，コントロールと比較して，０～48.9%に減少した。精油のうちの peppermint oil と，citral, citronellal, L-perilla aldehyde, trivertal, thymol, hinokitiol および tetrahydrolinalool の計８種が強いコロニー形成抑制作用を示し，MSSAのコロニーは全く形成されなかった。これらの香料成分につき，同様の方法で抗MRSA作用を検討した結果を Table 3 に示す。peppermint oil を除く７種の香料成分で，MRSAのコロニーは形成されず，また，peppermint oil においてもコロニー形成数は，コントロールの17.3％に減少した。

なお，peppermint oil の添加量を５㎎に下げて試験したところ，MSSAに対するコロニー形成数は，コントロールの41.5％で，一方MRSAに対しては，コントロールの47.8%であった。すなわち，添加量と抗菌効果の関係はMSSAとMRSAに対して同傾向であった。他

Table 2　Anti-MSSA activities of perfume vapor components.
10㎎ of each perfume component was added to a petri dish.

No.	Perfume component	(colony number／control) ×100			Mean
1	Peppermint oil	0,	0,	0	0
2	Basil oil	41.3,	45.0,	56.3	48.9
3	Sugi oil	30.8,	36.0,	36.8	34.5
4	Lavender oil	31.5,	41.3,	45.8	39.5
5	Armoise morocco oil	48.0,	48.0,	48.8	48.3
6	Tarragon oil	21.8,	29.3,	30.8	27.3
7	Coriander oil	33.8,	36.0,	34.1	34.6
8	Eucalyptus oil	32.3,	34.5,	39.0	35.3
9	Pine oil	13.5,	14.3,	23.3	17.0
10	Citral	0,	0,	0	0
11	Citronellal	0,	0,	0	0
12	L-Perilla aldehyde	0,	0,	0	0
13	Trivertal	0,	0,	0	0
14	Thymol	0,	0,	0	0
15	Hinokitiol	0,	0,	0	0
16	Tetrahydrolinalool	0,	0,	0	0
17	Linalool	16.1,	20.9,	26.6	21.2
18	Dehydrolinalool	5.7,	19.9,	25.6	17.1
19	Benzaldehyde	34.2,	36.1,	42.7	37.7
20	Fenchyl acetate	0,	1.9,	1.9	1.3
21	1，4-Cineole	33.2,	34.2,	40.8	36.1
22	Vandor B	0,	8.5,	9.5	6.0
23	L-Rose oxide	10.4,	11.4,	23.7	15.2
24	DL-Rose oxide	14.2,	17.1,	34.2	21.8
25	L-Carvone	36.1,	57.0,	65.5	52.9
26	Lolitol	10.5,	16.2,	18.4	15.0
27	3-Octanol	14.6,	22.2,	32.4	23.1

のいくつかの香料成分についても同様で，従って，これらの香料の揮発成分は，MSSAとMRSAに対して同時に高い抗菌作用を示し，両者間に有意な感受性差は認められなかったといえる。

2．ポット法

容積が127 cm³のペトリ皿に対し，容量が約50倍大きいアクリル製円筒（6280 cm³）を用いて同様に香料の揮発成分の作用を調べた。Table 4 に示すように，8種の香料成分すべてが，添加量500 mg（ペトリ皿法での薬量の50倍）で，MSSAに対し，コロニーの形成を完全に抑制した。また，MRSAに対しても，同様に供試した8種の香料成分のすべてにおいて，コロニーの形成が全く観察されなかった。Table 2 ～Table 4 の結果から，香料の揮発成分のMSSAとMRSAに対する抗菌作用に

Table 3　Anti-MRSA activities of perfume vapor components. 10 mg of each perfume component was added to a petri dish.

No.	Perfume component	(colony number／control) ×100 Mean			
1	Peppermint oil	1.5	3.8	33.0	17.3
10	Citral	0,	0,	0	0
11	Citronellal	0,	0,	0	0
12	L-Perilla aldehyde	0,	0,	0	0
13	Trivertal	0,	0,	0	0
14	Thymol	0,	0,	0	0
15	Hinokitiol	0,	0,	0	0
16	Tetrahydrolinalool	0,	0,	0	0

Table 4　Anti-MSSA and anti-MRSA activities of perfume vapor components by pot test I. 500 mg of each perfume component was added to a single pot(φ : 20 cm×h : 20 cm, 6280 cm³).

No.	Perfume component	(colony number／control)×100 MSSA			MRSA		
1	Peppermint oil	0,	0,	0	0,	0,	0
10	Citral	0,	0,	0	0,	0,	0
11	Citronellal	0,	0,	0	0,	0,	0
12	L-Perilla aldehyde	0,	0,	0	0,	0,	0
13	Trivertal	0,	0,	0	0,	0,	0
14	Thymol	0,	0,	0	0,	0,	0
15	Hinokitiol	0,	0,	0	0,	0,	0
16	Tetrahydrolinalool	0,	0,	0	0,	0,	0

Table 5　Anti-MSSA activities of perfume vapor components by pot test II. 1.0 g or 1.5 g of each perfume component was added to a pot, double(φ : 20 cm×h : 40 cm, 12560 cm³) or triple (φ : 20 cm×h : 60 cm, 18840 cm³) layers, respectively.

No.	Perfume component	(colony number／control)×100 double			triple		
1	Peppermint oil	0,	0,	0	0,	0,	0
10	Citral	0,	0,	0	0,	0,	0
11	Citronellal	0,	0,	0	0,	0,	0
12	L-Perilla aldehyde	0,	0,	0	0,	0,	0
13	Trivertal	0,	0,	0	0,	0,	0
14	Thymol	0,	0,	0	0,	0,	0
15	Hinokitiol	0,	0,	0	0,	0,	0
16	Tertrahydrolinalool	0,	0,	0	0,	0,	0

感受性差が認められないことが確認されたので，以降の試験はMSSAについてのみ実施した。アクリル製円筒を2段，あるいは3段に重ね，容器容量と香料成分添加量を更に2倍，3倍に拡大した試験の結果をTable 5 に示す。8種の香料成分のいずれも，MSSAのコロニー形成を完全に抑制し，容器容量を増大しても有効であることが明らかとなった。

3．準実地試験

実地により近い実験系として，8畳の部屋で，ファン型の消臭芳香器具を用いて香料成分を揮散させた。香料成分として基礎試験で高い抗菌効果が認められたtetrahydrolinalool とthymol（5 : 2）の混合物を選択し，これにイソパラフィン系溶剤のIP solventを30%加えた製剤を使用した。48時間後の揮散量は，3.2 g（香料成分として2.2 g）で，ポット法の場合と比較すると容積あたりの香料成分量は1/500以下で非常に低いものであったが，机の上に上蓋をとって載置した寒天平板上にMSSAのコロニーは形成されなかった。一方，蓋をかぶせたコントロールの寒天平板上には，MSSAのコロニーの形成が認められた。今後，製剤化研究を含め，実用場面に即した種々の実験系で検討を重ねていく予定である。

考　察

香料成分の抗菌作用に関する報告には，香料成分を直

接接触させる方法と，香料成分を寒天培地あるいは液体培地から離して作用させる揮発状態での方法とがある。MARUZZELLA and BRAMNICK (1961) は，香料成分を液体培地に添加したとき，citral を含む数種の香料成分において，Penicillin-resistant *S. aureus* の方が，Penicillin-sensitive *S. aureus* よりも抵抗性が高いことを報告している。一方著者らの香料の揮発成分による試験結果では，citral を含む8種の香料成分がMSSAとMRSAに対し同等の高い抗菌作用を示し，両者間に感受性差が認められなかった。供試した菌株の違いのほか，試験方法の差異（直接接触と揮発状態での作用）も関連しているものと思われる。

本試験でMSSAとMRSAに強い抗菌作用を示した citral, citronellal, L-perilla aldehyde, trivertal, thymol, hinokitiol, tetrahydrolinalool のような香料成分はすべてアルデヒド基，もしくは水酸基を有する化合物である。一方，INOUYEらの報告（1983）によれば，octylaldehyde や allyl isothiocyanate の揮発成分が，MSSAに対して高い抗菌作用を示し，また，牛腸（1991）の報告では，citral, citronellal, benzaldehyde などのアルデヒド類が揮発状態での作用で大腸菌 *E. coli* に有効であったとされているが，いずれもMRSAに対しては試験されていない。香料の揮発成分による作用では，供試菌の種類にかかわらず，概してアルデヒド基の役割が大きいこと，また，これ以外にも抗菌効果のすぐれた構造骨格が存在することが示唆される。なお，Table 2 に示すように，供試した精油のうち peppermint oil のみが強い抗菌性を示したが，その主要構成成分である L-menthol と L-menthone にはMSSAに対する抗菌作用が見られず（未発表データ），peppermint oil の活性の本体は不明である。他の精油は概してMSSAに対して抗菌性が弱かったが，これらはTable 1 に示すように主に linalool や cineole, terpene 系等から成り，抗菌性の高い thymol や citral 等を多く含まない組成のためであったと推定される。

香料の揮発成分のMSSAあるいはMRSAに対する増殖抑制作用は，容器容量と薬量をペトリ皿法に比べて50倍，100倍および150倍に拡大しても認められ，更に8畳の部屋での実用的な試験でもファンを用いて香料の揮発成分の拡散を図れば有効であった。本試験から，香料の揮発成分が院内感染防止用消毒剤として有用であることが明らかとなった。また最近，消毒剤が十分に使えない部位，例えば，医師や看護婦の鼻粘膜からもMRSAが検出されている状況を考えると，作用の緩和な香料の揮発成分の活用はMRSA対策上好ましいといえる。今後更に，製剤化研究も含め，実際の場に近い実験系で検討を続けていく予定である。

引用文献

BARBER, M. (1961) Methicillin resistant staphylococci. *J. Clin. Pathol.* **14** : 385-393.

牛腸　忍 (1991) 各種合成香料の蒸気状態での抗菌機構 *J. Antibact. Antifung. Agents* **10** : 511-515.

INOUYE, S., H. GOI, K. MIYAUCHI, S. MURAKI, M. OGIHARA and Y. IWANAMI (1983) Inhibitory effect of volatile constituents of plants on the proliferation of bacteria. *J. Antibact. Antifung. Agents* **11** : 609-615.

JEVENS, M. P. (1961) "Carbenin"-resistant staphylococci. *Br. Med. J.* **1** : 124-125.

MARUZZELLA, J. C. and M. B. LICHTENSTEIN(1956) The in vitro antibacterial activity of oils. *J. Am. Pharm. Assoc. Sci. Ed.* **45** : 378-381.

MARUZZELLA, J. C. and E. BRAMNICK (1961) The antibacterial properties of perfumery chemicals. *Soap Perfum. Cosmet.* **35** : 743-745.

MARUZZELLA, J. C. and P. A. HENRY (1958) The in vitro antibacterial activity of essential oils and oil combinations. *J. Am. Pharm. Assoc. Sci. Ed.* **47** : 294-296

STEWART, G. T. (1961) Changes in sensitivity of staphylococci to methicillin. *Br. Med. J.* **1** : 863-866

環動昆
第5巻　第2号：65—72（1993）

Insecticidal Characteristics of Cyphenothrin Microcapsules for Cockroach Control

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(Received : June 23, 1993)

ゴキブリ防除用シフェノトリンマイクロカプセルの殺虫特性　川田　均[1]・大坪敏朗[1]・津田重典[2]・安部八洲男[1]・辻　孝三[1]　1）住友化学工業株式会社宝塚総合研究所農業科学研究所，2）住友化学工業株式会社農業化学品質管理室

ポリウレタン膜によってマイクロカプセル化したシフェノトリンマイクロカプセル剤（シフェノトリンMC）のゴキブリに対する効力を評価するとともに，ゴキブリに対する効力発現機構をフェニトロチオンMCと比較検討した。各種粒径，膜厚のカプセルを用いたスクリーニングによって選択されたシフェノトリンMCの最適処方のカプセル強度はフェニトロチオンMCのそれに比べ小さく，より破壊されやすいカプセル設計になった。その結果，マイクロカプセル化することにより，吸収面における残効は，シフェノトリン乳剤に比べ大幅に向上するのに対し，非吸収面においては乳剤とほぼ同等となった。また，フェニトロチオンMCにおいて確認されたカプセル粒子の経口的な摂取による効力の発現は，シフェノトリンMCにおいては主要因ではなく，カプセルの機械的な破壊による有効成分への被暴が主要因であることが示唆された。シフェノトリンMCとフェニトロチオンMCにおけるこの違いは，有効成分の殺虫特性の違いが原因であると考えられた。

The efficacy against cockroaches and the mode of action of the cyphenothrin microcapsules (MC) were studied in comparison with the fenitrothion MC. The strength of the insecticidally optimum formulation of cyphenothrin MC was less than that of fenitrothion MC, namely the capsule particles became more fragile than fenitrothion MC. The residual efficacy of cyphenothrin was improved on the absorptive surface by microencapsulation. The major factor in toxication with cyphenothrin MC was found not to be oral intake of capsule particles and digestion in the crop, which was demonstrated in fenitrothion MC as a mode of entry of active ingredient, but the exposure to insecticide by destruction of capsule walls with cockroach trampling.

Key Words : Microcapsule, Cyphenothrin, *Blattella germanica*, *Periplaneta americana*

Introduction

Cyphenothrin is a synthetic pyrethroid structurally related to phenothrin, namely cyphenothrin has a cyano group at the α-position of the alcohol constituent of phenothrin. After the discovery of cyphenothrin, many compounds having cyano groups in the α-position of their alcohol constituent have been developed (OHNO *et al.*, 1976 ; ITAYA *et al.*, 1983). Most pyrethroids for recent agricultural use have the α-cyano groups and a dihalovinyl chrysanthemic acid or non-cyclopropanecarboxylic acid moiety for enhancement of insecticidal activity and photostability. Cyphenothrin, on the contrary, lacks a dihalovinyl group in the acid part and is less photostable than pyrethroids for agricultural use. Cyphenothrin is characterized by its higher toxicity against cockroaches than permethrin. Cyphenothrin microcapsules (MC) were developed to give cyphenothrin improved insecticidal activities against cockroaches. Encapsulation of insecticides with proper particle size and wall thickness not only reinforces the biological activity but also gives them new characteristics in their modes of action as previously reported for fenitrothion MC (KAWADA *et al.*, 1990). In this paper, we report on the insecticidal characteristics and mode of action of cyphenothrin MC in comparison with that of fenitrothion MC.

Materials and Methods

Microencapsulation procedure

The microcapsules containing 10% cyphenothrin ((*RS*) − α −cyano-3-phenoxybenzyl (1 *R*)-*cis, trans*-chrysanthemate) or 20% fenitrothion (*O, O*-dimethyl *O*-4-nitro-m-tolyl phosphorothioate) were prepared by interfacial polymerization using polyurethane as a wall material (TSUDA *et al.*, 1987 : OHTSUBO *et al.*, 1987).

Measurement of strength of the microcapsules

An aqueous suspension of microcapsules was applied uniformly to the bottom of 8-cm diameter Petri dishes at a rate of 2.5 mg of the active ingredient per dish. Ten German cockroaches were confined to contact with the treated surface for 2 hr. The amount of active ingredients outside and inside the microcapsules on the treated surface were analyzed as described elsewhere (TSUDA *et al.*, 1987) with deodorized kerosene (Neochiozol®, Chuoo Kasei Co. Ltd., Japan) as the extraction solvent. Cyphenothrin was analyzed by HPLC with a fixed wavelength (230nm) ultraviolet detector. The column was a Sumipax ODS A-212 (15 cm × 6 mm φ). Fenitrothion was analyzed with a gas chromatograph with a flame photometric detector under the following conditions : glass column, 1.1 m × 3 mm φ, packed with 3 % XE-60 ON Chromosorb W (AW, DMCS) 60-80 mesh ; carrier gas, nitrogen ; injection temperature, 230℃ ; column temperature, 180℃.

Efficacy against cockroaches

1. Residual contact test under the laboratory conditions

The microcapsule concentrates were suspended with deionized water and were sprayed onto a plywood panel or an overlaid plywood panels (15 × 15 cm) at the rate of 50 ml for each square meter. After the panels were dried for 24 hr at 25℃, ten German cockroaches, *Blattella germanica* or four American cockroaches, *Periplaneta americana* with an even sex ratio were confined to contact with the treated surface for 2 hr. Knockdown of insects was observed and KT_{50}, that is the time required to cause 50% knockdown, was calculated by Bliss' probit method. The cockroaches were then transferred into a clean plastic cup with water and food, and mortality was observed after 72 hr. The panels were stored at room temperature at 60% RH. Residual efficacy was examined with the same manner

as described above by using the same panel.

2. Voluntary contact test under simulated field conditions

The microcapsule concentrates were suspended with deionized water and were sprayed onto a plywood panel or an overlaid plywood panel (15 × 15 cm) in the manner described above. After drying for 24 hr at 25℃, four plywood and four overlaid panels were placed on the corner of a stainless tray (2.5 × 1.25 m) alternately, and the equivalent number of panels were placed on the opposite corner (Fig. 1). Food (CE-2, Oriental Yeast Co., Ltd.) and water were placed on the same corners where the panels were arranged. Fifty German cockroaches, or ten American cockroaches, with an even sex ratio were accustomed to triangular wood shelters one or two days before tests and the shelters were placed on the center of the tray. Number of knocked-down or dead insects was counted at 1 or 2 day intervals up to 7 days. All insects were collected on the 7 th day and the same procedure was repeated.

Mode of action of cyphenothrin microcapsule

1. Contact test with mouth-closed cockroaches

An aqueous suspension of microcapsule or emulsion of cyphenothrin was applied to a plywood panel at the rate of 50 ml for each square meter. On CO_2-anesthesia, the mouths of the German cockroaches were closed with paraffin to make them unable to ingest orally. The insects were confined to contact with the treated surface for 2 hr. Knock-down was observed at certain intervals and the KT_{50} was calculated. Mortality was observed after 72 hr.

2. Definite time Contact test

An aqueous emulsion of cyphenothrin or fenitrothion was applied to a glass surface at a certain dose from 5 mg to 40 mg for each square meter. Ten German cockroaches were confined to contact with the surface for a certain duration from 15 to 240 min. Mortality was observed after 72 hr.

3. Topical application of insecticides

A 0.5 μl aqueous emulsion of cyphenothrin or fenitrothion was applied orally or dermally

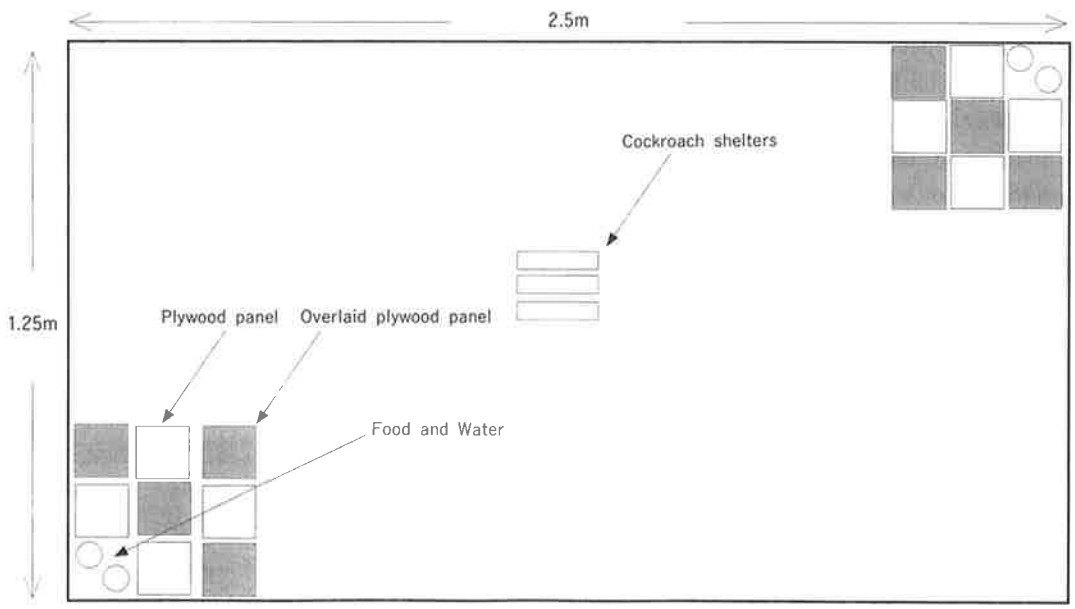

Fig. 1 Arrangement of panels in voluntary contact test under simulated field conditions.

(to sternal mesothorax) to male American cockroaches. Knock-down was observed at certain intervals and the KT_{50} was calculated. Mortality was observed after 72 hr.

4. Measurement of the amount of cyphenothrin and fenitrothion picked up by the insects

An aqueous suspension of microcapsules of cyphenothrin or fenitrothion was applied uniformly to the bottom of 9-cm diameter Petri dishes or to round-cut plywood panels (9 cm in diameter) in the Petri dishes at the rate of 2.5 mg of the active ingredient per dish. Ten German cockroaches were confined to contact with the treated surface for 2 hr. After 2 hr insects were collected and were immersed in a 20 ml acetone solution for 24 hr to extract the active ingredients picked up by the insects and amount per insect was calculated. Analysis was done under the same conditions as described above. The same procedures were repeated using the same dishes.

Results and Discussion

Strength of microcapsules against the cockroach trampling

The amount of active ingredients released out of microcapsule walls by cockroach trampling for 2 hr is shown in Table 1. The percentage of active ingredient out of microcapsule wall was nearly 10 times higher in cyphenothrin MC than that in fenitrothion MC. The strength of capsule wall is, therefore, thought to be higher in fenitrothion MC than cyphenothrin MC.

Residual efficacy under laboratory and simulated field conditions

The residual efficacy of microcapsules against the German cockroaches is shown in Table 2-A. Cyphenothrin MC maintained high mortality on the plywood surface for more than 16 weeks. On the contrary, killing activity rapidly decreased in cyphenothrin EC in less than 2 weeks. On the non-absorptive surface, however, the residual activity is almost the same between two formulations. The results for the American cockroach (Table 2-B) show the same tendency as for the German cockroach. That is to say, encapsulation of cyphenothrin can improve the activity on an absorptive surface.

Efficacy under the simulated field conditions is shown in Table 3. High activity was maintained for more than 20 weeks against the German cockroach at the rate of 62.5 mg/m^2 as active ingredient. In this test insects were allowed to come into contact with insecticide-treated plywood panels voluntarily. The test conditions for MC formulations, therefore, seem to be harder in the case of confined contact than this test because the more the frequency of contact the higher the rate of destruction of microcapsule particles.

Mode of action of cyphenothrin microcapsule

Knock-down activity of cyphenothrin MC and fenitrothion MC against the German cockroaches whose mouth parts were closed with paraffin to make them unable to ingest orally, and normal

Table 1 The amount of active ingredients released out of capsule walls by 2 hr contact of the German cockroaches. Numbers in parentheses are 95% fiducial limits.

Samples	Amount of active ingredient after contact		%
	Total amount treated	Amount released	
Cyphenothrin MC	2.464 (2.451−2.478)	0.435 (0.260−0.610)	17.7
Fenitrothion MC	2.542 (2.511−2.572)	0.045 (0.018−0.071)	1.75

ones, is shown in Fig. 2. With fenitrothion MC, knock-down time was delayed in mouth-closed cockroaches but delay in knock-down was not so much in fenitrothion EC. With cyphenothrin MC, on the contrary, delay in knock-down time was not significant in both formulations. These results suggest that insecticide, dermally taken, plays a main role in toxication and that orally taken does not, with cyphenothrin MC. This must be caused by the lesser oral toxicity of pyrethroids. Table 4 shows the differences in LD_{50} values of cyphenothrin and fenitrothion against the American cockroach. Relative effectiveness of dermal toxicity to oral toxicity in cyphenothrin (Oral LD_{50} /Dermal LD_{50} = 6.85) was larger than that in fenitrothion (Oral LD_{50} /

Table 2 Residual efficacy of cyphenothrin microcapsules against cockroaches by 2 hr confined contact to various surfaces.

A. German cockroach, *Blattella germanica*

Sample	Surface material	% Mortality (weeks after treatment)					
		0	2	4	8	12	16
Cyphenothrin MC	plywood	100	100	100	100	100	93.3
	Overlaid plywood	100	100	100	100	100	100
Cyphenothrin EC	plywood	60.0	40.0	50.0	13.3	—	—
	Overlaid plywood	100	100	100	100	100	100

B. American cockroach, *Periplaneta americana*

Sample	Surface material	% Mortality (weeks after treatment)					
		0	2	4	8	12	16
Cyphenothrin MC	plywood	100	—	100	87.5	75.0	56.3
	Overlaid plywood	100	100	100	100	100	100
Cyphenothrin EC	plywood	100	—	18.8	—	—	—
	Overlaid plywood	100	100	100	100	100	100

1) MC, Microcapsule ; EC, Emulsifiable concentrate
2) Dosage is 125 mg/m^2 as active ingredient

Table 3 Residual efficacy of cyphenothrin microcapsules against cockroaches under simulated field conditions.

A. German cockroach, *Blattella germanica*

Dosage (mg/m^2)[1)]	% Mortality (weeks after treatment)					
	0	4	8	12	17	20
62.5	100	100	100	100	88.0	84.0
31.25	100	98.2	98.2	90.2	42.0	—

B. American cockroach, *Periplaneta americana*

Dosage (mg/m^2)	% Mortality (weeks after treatment)					
	0	8	16	26	32	40
62.5	100	100	100	100	100	100
31.25	100	100	100	100	90.0	90.0

1) Active ingredient

Dermal LD_{50} = 2.03). These data appear to explain the difference in modes of action between pyrethroids and organophosphates.

As shown in Table 1, the strength of fenitrothion MC was almost ten times larger than that of cyphenothrin MC, that is to say cyphenothrin MC is more easily destroyed by cockroach trampling than fenitrothion MC. The amount of cyphenothrin and fenitrothion picked up by German cockroaches were analyzed under two different coditions : cyphenothrin MC and fenitrothion MC were put onto the plywood and glass surfaces at the same dosage, one an absorptive and the other a non-absorptive surface. The results are shown in Fig. 3. The amount of cyphenothrin picked up was higher with non-absorptive surface and lower with absorptive surface as compared to that of fenitrothion. These results indicate that active ingredient released out of the capsule wall, which is destroyed by trampling of cockroaches, is rapidly absorbed in the substratum, and the more easily the capsule wall is broken the more the amount of active ingredients absorbed into the substratum. The differences in residual activities of

Table 4 Differences in LD_{50} values of cyphenothrin and fenitrothion against the American cockroaches by different admnistration methods.

A. Cyphenothrin

Dosage[1] (μg/male)	KT_{50} (min.) - % Kill	
	Dermal	Oral
0.1	>150 - 0.0	>150 - 0.0
0.2	>150 - 50.0	>150 - 0.0
0.4	>150 - 100	>150 - 0.0
0.8	39.8 - 100	>150 - 20.0
1.6	46.2 - 100	>150 - 60.0
3.2	36.5 - 100	67.7 - 90.0
6.4	35.0 - 100	23.5 - 100
LD_{50}	0.20	1.37

1) Active ingredient

B. Fenitrothion

Dosage[1] (μg/male)	KT_{50} (min.) - % Kill	
	Dermal	Oral
1	>3600 - 0.0	>3600 - 0.0
2	>3600 - 0.0	>3600 - 0.0
4	>3600 - 40.0	>3600 - 0.0
8	258.0 - 95.0	>3600 - 45.0
16	162.0 - 100	>3600 - 75.0
32	150.0 - 100	174.0 - 100
64	120.0 - 100	138.0 - 100
LD_{50}	4.39	8.92

1) Active ingredient

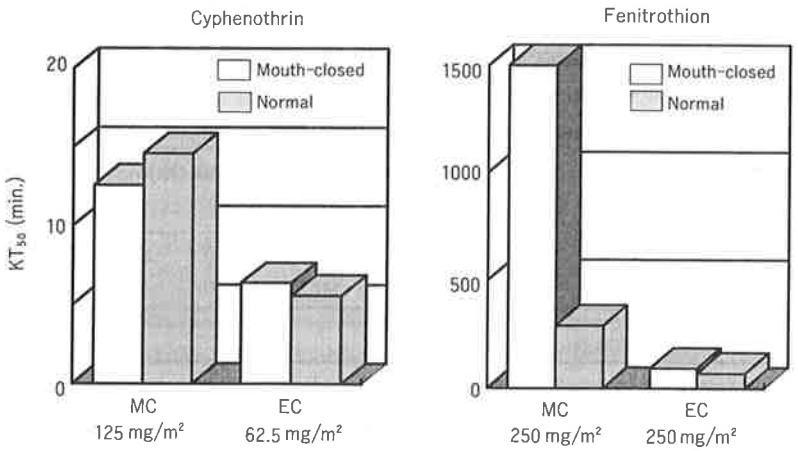

Fig. 2 Knockdown activity of cyphenothrin and fenitrothion against the mouth-closed and the normal German cockroach by contact with plywood surfaces treated with microcapsules (MC) and emulsifiable concentrates (EC).