

環動昆

報 文

- 伊藤高明：ヒラタキクイムシ *Lyctus brunneus* STEPHENS 雌成虫の産卵行動に及ぼす各種化合物ならびにペルメトリンの影響…………… 1
- 川田 均・牧田光康・津田重典・大坪敏朗・新庄五朗・辻 孝三：ゴキブリ防除用フェニトロチオンマイクロカプセル剤の効力と作用性（英文）…………… 6
- 藤本和義：マダニ類の生態学的研究.8. タネガタマダニ幼・若虫のカナヘビ寄生（英文）…………… 14
- 藤本和義：マダニ類の生態学的研究.9. 3種類の宿主で飼育されたタネガタマダニ幼・若虫の吸血と発育（英文）…………… 25
- 武川 恒・高橋正三：数種ゴキブリの体表ワックスの化学分類学的考察…………… 31

短 報

- 谷川 力・渡辺洋介・中屋文雄・内田明彦・村田義彦：厨芥で飼育した野生ドブネズミの厨芥摂取量と繁殖（英文）…………… 39

資 料

- 辻 英明・種池与一郎：クロゴキブリとヤマトゴキブリの生活史模式図…………… 42

講 演

- 加納六郎：人間の生活環境への昆虫その他の動物のかかわり…………… 44

会 報…………… 49

会員動静

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日本環境動物昆虫学会

ヒラタキクイムシ *Lyctus brunneus* STEPHENS 雌成虫 の産卵行動に及ぼす各種化合物ならびにペルメトリン の影響

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Effects of Non-insecticidal Compounds and Permethrin on Ovipositional Behaviour of Powder-Post Beetles, *Lyctus brunneus* STEPHENS. Takaaki ITO (Takarazuka Research Laboratory, Sumitomo Chemical Co. Ltd., Takarazuka, Hyogo 665, Japan) *Jpn. J. Environ. Entomol. Zool.* 2: 1-5 (1990)

The effects of 25 non-insecticidal compounds and a pyrethroidal insecticide, permethrin, on the ovipositional behaviour of female adults *Lyctus brunneus* were tested. Of the non-insecticidal compounds, only calcium nitrate inhibited both tasting and ovipositional behaviours. This inhibition occurred only when the amount of starch in the treated and untreated wood was equal. The female adults laid more eggs in starch-rich wood than in starch-poor one, whether the wood was treated with calcium nitrate or not. Four other compounds, such as ammonium nitrate and sodium borate, inhibited tasting, which precedes oviposition. Less than a lethal dose of permethrin inhibited oviposition, and this effect was not altered by the starch content of the wood. The results suggested that permethrin could be used as an ovipositional repellent as well as its conventional use as an insecticide.

Key Words: *Lyctus brunneus*, Oviposition, Tasting, Permethrin

殺虫活性のない炭水化物, アミノ酸, 無機試薬等25種化合物ならびにピレスロイド系殺虫剤ペルメトリンの, ヒラタキクイムシ雌成虫の産卵行動におよぼす影響を調べた。25種化合物中, 硝酸カルシウムを含む数種が, 産卵の前行動である tasting を阻害したが, 木材への産卵阻害にまでおよんだのは硝酸カルシウムのみであった。この産卵阻害作用は, 並置した木材のでん粉含量が等しい場合のみ認められ, 異なった場合には硝酸カルシウムの処理の有無に関わらず, でん粉の多い木材に多くの卵を産卵した。一方, ペルメトリンは成虫に対する致死活性を示す濃度以下でも強い産卵阻害作用を示した。この作用はでん粉の多い木材に処理した場合にも認められ, ペルメトリンの接触忌避作用によるものと考えられた。

はじめに

ヒラタキクイムシ, *Lyctus brunneus* STEPHENS, は広葉樹乾材を加害する重要な害虫であるが, 雌成虫は木材表面を咬み, 幼虫の発育に必要なでん粉の多い部分に選択的に産卵するという特異な産卵習性を持つことが知られている(伊藤ら, 1978)。PARKIN(1934)はこの行動をでん粉の多い木材を探す探索行動と考えてtasting行動と呼んだ。

ITO(1983)は, 雌成虫の大顎を切断し木材をtastingできなくすると木材中のでん粉の多少とは無関係に産卵するのでtastingは選択産卵に不可欠であることを認めた。また, ITOら(1981)はピレスロイド系殺虫剤のひとつであるペルメトリンが, 致死施用量以下でも本種雌成虫の産卵を阻害することを認めた。これらの報告から, 殺虫活性のない物質によりtastingを阻害して選択産卵をかく乱させたり, 致死量以下のペルメトリン施用により産卵を阻害する防除方法の可能性が示唆された。

本報告では, 殺虫活性のない各種化合物の雌成虫のtastingと産卵におよぼす影響を調べるとともに, ペルメトリンの産卵阻害様式について若干の検討を行った。

材料および方法

1. 供試昆虫

1978年に青森県八戸市にて採取し, 人工飼料(ITOら, 1980a)により累代飼育中のヒラタキクイムシの, 羽化後4~7日の交尾雌成虫を用いた。

2. 供試化合物

グルコース(無水, 1級), ガラクトース(無水, 特級), D-(+)-キシロース(特級), D-(+)-アラビノース(特級), マルトース(1級), トウモロコシでん粉, L-アルギニン-塩酸塩(特級), L-リジン-塩酸塩(特級) L-アスパラギン酸-ナトリウム(特級), ビルビン酸ナトリウム(特級), ヒドロキシ安息香酸メチル(特級), クエン酸(特級), プロピオン酸ナトリウム(特級), パントテン酸カルシウム(1級), L-アスコルビン酸(特級), リン酸二ナトリウム(12水塩), 硝酸アンモニウム(1級), 硝酸カルシウム(1級), 硫酸アンモニウム(1級), 硫酸カルシウム(1級), 塩化マグネシウム(1級), 塩化カルシウム(2水塩, 1級), (以上和光純薬工業株式会社製), ホウ酸ナトリウム(特級, イシズ薬品工業株式会社), 硫酸マグネ

シウム(無水, 1級), 硫酸ナトリウム(無水, 1級)(以上関東化学工業株式会社), ペルメトリン(3-フェノキシベンジル 2, 2-ジメチル 3-(2, 2-ジクロロビニル)シクロプロパン-1-カルボキシレート, 純度92.4%, 住友化学工業株式会社製)を用いた。

3. 各種化合物のtastingにおよぼす影響

メチレンブルー0.1%含有蒸留水に各種物質0.5g(ペルメトリンを除く)を加え, 各50ml液を調製した。トウモロコシでん粉液は溶解促進のため60°Cに加熱した。メチレンブルーはtasting痕跡の観察を容易にするため添加した。直径5.5cmの定性用ろ紙(Whatman No.2)を各溶液中に5秒間浸漬し, 24時間風乾したものを処理ろ紙, 同様にメチレンブルーのみの0.1%液に浸漬・風乾したものを無処理ろ紙とした。各ろ紙を2等分し, 図1のように処理および無処理ろ紙の各半円を直径9cmのガラスシャーレ内に貼りつけた。この円板上に直径4.0cm, 高さ2cmのガラスリングを置き, 雌成虫4頭をガラスリング内に放虫し, 25°C, 関係湿度約70%の暗所に置いた。48時間後の処理・無処理ろ紙上のtasting痕跡を以下の基準により記録した。

◎: 無処理ろ紙上に多くの痕跡を認めるが, 処理ろ紙上にはほとんど認めない。

○: 処理ろ紙上にも痕跡を認めるが, 明らかに無処理上の痕跡のほうが多い。

△: 処理, 無処理ろ紙上の痕跡数はほぼ同程度。

×: 処理ろ紙上の痕跡のほうが無処理ろ紙上のそれよりも明らかに多い。

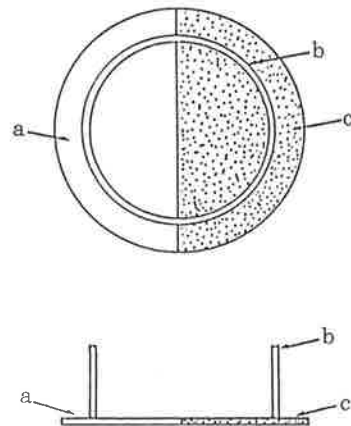


図1 各種化合物を処理したろ紙へのヒラタキクイムシtasting試験(上図: 平面図, 下図: 側面図)
a: 無処理ろ紙, b: ガラスリング, c: 処理ろ紙

実験は6反復行った。

4. 数種化合物の産卵におよぼす影響

熱帯産広葉樹ジェルトン (*Dyera* spp.) から、でん粉含量3.5%および0.9~1.1%の匏屑(厚さ, 0.2mm)を採取し、それぞれを木目を平行に3枚ずつ貼り重ね1.5×1.5cmにしたものを試験体とした(伊藤ら, 1978)。以後でん粉含量の多い方をSベニヤ, 少ない方をNSベニヤと呼ぶ。木材中のでん粉含量はMcCreadyら(1950)の方法に従って測定した。パントテン酸カルシウム, L-アスコルビン酸, 硝酸カルシウム, 硝酸アンモニウム, ホウ酸ナトリウムの各物質0.5gを蒸留水で溶かし各50ml液に調製した。これら水溶液にベニヤを5秒間浸漬処理し, 2枚のガラス板にはさみ90℃にて24時間乾燥した。ろ紙を敷いた内径9cmのガラスシャーレ内に, 処理Sベニヤと無処理Sベニヤまたは処理Sベニヤと無処理NSベニヤを各1枚並置し, 雌成虫3頭を放虫して, 48時間後の各ベニヤへの産卵数を調べた。実験は25℃, 関係湿度約70%, 暗所にて7反復行った。

5. ペルメトリンの産卵におよぼす影響

上記4.で述べたSおよびNSベニヤを15ppmペルメトリンアセトン溶液中に5秒間浸漬処理し24時間風乾した。ろ紙を敷いた内径9cmのガラスシャーレ内に, 以下の組み合わせで処理ベニヤと無処理ベニヤあるいは処理または無処理ベニヤ同士を各1枚ずつ並置し, 雌成虫5頭を放虫して, 48時間後の各ベニヤへの産卵数を調べた。ただしNo.5ではITO(1983)の方法により大顎を切断した雌成虫を用いた。

- No.1: 無処理Sベニヤ / 無処理NSベニヤ
 - No.2: 処理Sベニヤ / 無処理NSベニヤ
 - No.3: 処理NSベニヤ / 無処理Sベニヤ
 - No.4: 処理NSベニヤ / 処理Sベニヤ
 - No.5: 処理Sベニヤ / 無処理Sベニヤ
- 実験は5反復行った。

結果および考察

表1に各種化合物のtastingにおよぼす影響を示した。

表1 ヒラタキクイムシのろ紙へのtastingにおよぼす各種化合物の影響

化合物	tasting への影響 (6反復)					
	1	2	3	4	5	6
グルコース	X	X	△	△	△	X
ガラクトース	△	△	△	△	X	△
D-(+)-キシロース	△	X	X	X	X	X
D-(+)-アラビノース	X	X	X	△	X	X
マルトース	△	△	△	X	X	X
トウモロコシでん粉	X	X	X	X	X	X
L-アルギニン-塩酸塩	○	○	○	△	○	○
L-リジン-塩酸塩	○	○	△	○	○	○
L-アスパラギン酸-ナトリウム	○	○	○	○	△	△
ピルビン酸ナトリウム	○	○	○	○	△	△
ヒドロキシ安息香酸メチル	○	◎	◎	◎	○	○
クエン酸	△	△	△	△	△	△
プロピオン酸ナトリウム	○	X	○	△	○	△
パントテン酸カルシウム	○	○	○	○	◎	○
L-アスコルビン酸	○	○	○	○	○	○
リン酸二ナトリウム	○	○	○	○	○	○
硝酸カルシウム	○	◎	◎	◎	◎	◎
硝酸アンモニウム	◎	◎	◎	◎	◎	◎
硫酸マグネシウム	○	○	△	△	X	△
硫酸アンモニウム	○	○	△	○	○	△
硫酸カルシウム	○	△	○	○	△	○
硫酸ナトリウム	○	○	○	○	△	△
ホウ酸ナトリウム	◎	○	○	◎	○	◎
塩化マグネシウム	△	○	○	○	○	○
塩化カルシウム	○	△	○	○	◎	○

- ◎ 無処理ろ紙に多くの痕跡を認めるが, 処理ろ紙上にはほとんど認めない。
- 処理ろ紙上にも痕跡認めるが, 明らかに無処理ろ紙上の痕跡のほうが多い。
- △ 処理, 無処理ろ紙上の痕跡数はほぼ同程度。
- X 処理ろ紙上の痕跡の方が無処理上のそれよりも明らかに多い。

表から明らかのように、炭水化物（グルコースからでん粉まで）処理ろ紙への tasting は、いずれも無処理ろ紙より多く、とくにトウモロコシでん粉において顕著であった。ヒラタキクイムシ雌成虫はグルコースなど炭水化物で処理された木材へ選択産卵することが知られているので（伊藤ら，1978），産卵行動の前行動である tasting がこれら炭水化物を処理したろ紙に多く起こるのは予想された通りである。しかしそれ以外の各種アミノ酸，有機酸またはその塩類，ならびに各種無機塩類の場合，明らかに処理ろ紙への tasting は阻害される傾向にあり，特に硝酸アンモニウムなどで処理されたろ紙では tasting がほとんど認められなかった。これら化合物には何らかの tasting 阻害作用があると考えられたので，さらにそれぞれの産卵への影響を調べた結果を表2に示した。

でん粉含量の多いジェルトンから作製したSベニヤを用いた場合，硝酸カルシウム以外の4化合物では処理・無処理ベニヤ間の産卵数に有意な差は認められず，これら4化合物は tasting を阻害しても産卵は阻害しないと考えられた。一方硝酸カルシウムの場合，処理ベニヤと無処理ベニヤへの平均産卵数は，7反復・2回の試験でそれぞれ6.9と40.0，12.1と67.3であり，ともに危険率5%で有意差ありと判定された。しかし，同処理ベニヤとでん粉含量の少ないNS無処理ベニヤを同一シャーレ内に置くと，処理ベニヤには平均45.6個の産卵があったが，無処理ベニヤには全く産卵が認められず，雌成虫は

表2 ヒラタキクイムシのジェルトンベニヤへの産卵におよぼす数種化合物の影響

実験番号	化合物	使用ベニヤ板 ^{a)}	処理の有無	平均産卵数
1	パントテン酸カルシウム	S	有	26.0
		S	無	24.3
	L-アスコルビン酸	S	有	34.0
		S	無	29.0
	硝酸カルシウム	S	有	6.9*
		S	無	40.0
硝酸カルシウム	S	有	45.6*	
	NS	無	0.0	
硝酸アンモニウム	S	有	23.5	
	S	無	25.0	
2	硝酸カルシウム	S	有	12.1*
		S	無	67.3

* 処理，無処理間の産卵数に有意差あり（5%）。

a) S：でん粉含量3.5%，NS：でん粉含量0.9～1.1%

硝酸カルシウムで処理されていてもでん粉含量の多いベニヤに産卵することが明らかとなった。

ペルメトリンの産卵におよぼす影響を表3に示した。でん粉含量を異にする無処理ベニヤ同士の組み合わせ（No.1）では，産卵は明らかにでん粉含量の多い方に多くなされた。また，でん粉含量の少ない処理ベニヤとでん粉含量の多い無処理ベニヤの組み合わせ（No.3）では，当然のことながら後者に多く産卵が見られた。しかし，処理ベニヤの方がでん粉含量の多い場合（No.2）や，でん粉含量を異にする処理ベニヤ同士の組み合わせ（No.4）では，でん粉含量の多い方への選択産卵は認められなかった。正常な雌成虫をでん粉含量の等しい処理・無処理ベニヤに接触させると，産卵は当然無処理ベニヤに多く行われるが，大顎を切断して tasting できなくした場合（No.5）も，無処理ベニヤへの選択産卵が明らかに認められたので雌成虫は接触によりペルメトリンを感知，処理ベニヤへの産卵を避けていると考えられた。tasting できなくした雌成虫を処理Sおよび無処理Sベニヤへ産卵させた場合には5%水準で有意に無処理ベニヤに多く産卵した。この大顎を切断した個体においても無処理へ選択産卵するという結果から，雌成虫は接触によりペルメトリンを感知して避け，無処理ベニヤに選択産卵したものと考えられる。

ITOら（1981）はペルメトリン，フェニトロチオン，リンデンのヒラタキクイムシ雌成虫の産卵におよぼす影響を比較し，ペルメトリンでは致死以前に産卵阻害作用が出現するが，フェニトロチオンとリンデンではそれが

表3 ヒラタキクイムシのジェルトンベニヤへの産卵におよぼすペルメトリンの影響

組み合わせ番号	供試虫	使用ベニヤ ^{a)}	処理の有無	平均産卵数
1	正常な雌成虫	NS	無処理	6.0*
		S	無処理	44.0
2	同上	NS	無処理	22.4 b)
		S	処理	4.0
3	同上	NS	処理	2.0*
		S	無処理	70.4
4	同上	NS	処理	2.4
		S	処理	7.4
5	左右大顎切断雌成虫	S	無処理	19.8*
		S	処理	5.4

* 産卵数に有意差（5%）あり。

a) S：でん粉含量3.5%，NS：でん粉含量0.9～1.1%

b) 実験反復間における変動大きく有意差認められなかった。

認められないことを報告した。この産卵阻害作用は、DETHIERら(1960)の提唱するlocomotor stimulantによるものと考えられる。本試験でも、ベルメトリン15 ppm液への5秒浸漬処理を行ったベニヤに接触した雌成虫はすべて生存しており、ベニヤ上の歩行、tastingから産卵に至る間にベルメトリンを感知し、処理ベニヤより逃避したものと考えられた。

結 論

殺虫活性のない25化合物のうち、硝酸カルシウム、硝酸アンモニウム、ホウ酸ナトリウムなど数種に、ヒラタキクイムシ雌成虫の産卵の前行動であるtastingの阻害作用が認められたが、それが引き続いて産卵阻害にまでおよんだのは硝酸カルシウムのみであった。しかしこの産卵阻害は処理・無処理ベニヤのでん粉含量が等しい場合に限られ、処理ベニヤのでん粉含量が多ければ、処理ベニヤへの産卵が有意に多かった。したがって、硝酸カルシウムは、でん粉含量の多い木材への産卵抑制という実目的には適していないと判断される。一方ベルメトリンは、致死濃度以下の処理でも強い産卵阻害作用があり、木材上でも安定であることから(ITOら, 1980 b), 殺虫剤としてではなく、少量で効果を示す産卵阻害物質として、ヒラタキクイムシの被害防止に利用できる可能性があることが示唆された。

謝 辞

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Efficacy and the Way of Action of Fenitrothion Microcapsules for Cockroach Control

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ゴキブリ防除用フェニトロチオンマイクロカプセル剤の効力と作用性 川田 均¹⁾・牧田光康¹⁾・津田重典¹⁾・大坪敏朗¹⁾・新庄五朗²⁾・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防疫薬事業部)

ポリウレタン膜によってマイクロカプセル化したフェニトロチオンマイクロカプセル剤(フェニトロチオンMC)のゴキブリに対する効力を室内および実地試験によって評価するとともに、ゴキブリに対する効力発現機構について検討を行なった。フェニトロチオンMCは室内、実地いずれの条件においても対照として評価した市販MC剤を上回る効力を示した。MC剤処理面にチャバネゴキブリが接触することによるフェニトロチオンの体表への付着量はほぼ一定であり、これは1頭のチャバネゴキブリを致死させるのに必要かつ十分な量であった。体表に付着したフェニトロチオンの一部はMC粒子の形で附節等に付着しているのが観察されたが、一方この粒子がゴキブリのグルーミング行動によって経口的に摂取され、食毒として作用していることも証明された。したがって、先に著者らが提唱したMCのゴキブリによる破壊による効力発現機構に加えて、食毒機構もフェニトロチオンMCの効力発現にある程度関与していることが明かとなった。

The efficacy of the fenitrothion microcapsules against cockroaches was evaluated, and the mechanism of entry of microcapsules as a stomach poison was studied. Fenitrothion microcapsules showed high performance in both laboratory and field conditions as compared with another microcapsule formulation that is commercially available. The amount of fenitrothion picked-up by German cockroaches after contact with a surface treated with fenitrothion microcapsules was constant irrespective of their contact frequency, being optimally enough for killing them. Some portion of fenitrothion picked up by cockroaches was observed on tarsi of the insect as microcapsule form and was found to act as a stomach poison as ingested orally during body cleaning. Therefore, the oral ingestion of microcapsule particles probably contributes to the effects of this agent, which also acts after the mechanical rupture of the microcapsules when the cockroach touches them ("trampling").

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

Introduction

Fenitrothion has been successfully used for the control of cockroaches in various formulations. However, the modernization of construction and increasing transportation volume and speed have expanded habitats suitable for cockroaches and made the control more difficult. The fenitrothion microcapsules were developed to give improved residual efficacy against cockroaches. Residual efficacy seems to depend on formulation factors of microcapsules such as ^{a)} their strength, ^{b)} the permeability of the capsule wall to the core material, and ^{c)} the active ingredient. Elsewhere, we examined the effect of these factors on the insecticidal efficacy of the microcapsules, finding that the diffusion of core material was negligible and that the main mode of action was the "trampling" of the microcapsules by cockroaches (TSUDA *et al.*, 1987 ; OHISUBO *et al.*, 1987). In this paper, the efficacy of fenitrothion microcapsules against cockroaches is compared with that of another microcapsule formulation that is commercially available. Then the bioavailability of fenitrothion microcapsules to cockroaches is compared with that of an emulsifiable concentrate. In addition, the oral ingestion of the microcapsules by cockroaches is proposed as an additional way of action of the microcapsules.

Materials and Methods

1. Microencapsulation procedure

The microcapsules containing 20% fenitrothion was prepared by interfacial polymerization with polyurethane as the wall material (TSUDA *et al.*, 1987 ; OHISUBO *et al.*, 1987).

2. Efficacy against cockroaches

(1) Residual contact test under the laboratory conditions

a. Residual contact test with repeated contact with a single panel

The microcapsules suspended at different concentration in deionized water were sprayed onto a plywood panel (15×15cm) at the rate of 50ml/m². After the panels were dried for 24 hr at 25 °C, ten German cockroaches, *Blattella germanica* LINNÉ, or six American cockroaches, *Periplaneta americana* LINNÉ were confined to contact with the treated surface for 2 hr and the 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. A microcapsule formulation that contains an organophosphate insecticide and that is commercially available was used as a reference.

b. Residual contact test with use of different panels after storage

A number of panels treated with microcapsules were stored at 40°C, under 100% RH in the dark conditions. At a certain interval, three panels were used in a residual efficacy test. Residual efficacy was examined by the use of panels that insects had not been in contact with.

(2) Field test

The field tests were conducted in the kitchens of two restaurants in Osaka, Japan. At both sites, the dominant cockroach species was the German cockroach. At test site A (area treated, 69m²), a 0.25% suspension (by volume) of fenitrothion microcapsules was sprayed with a standard B&G hand-pressurized sprayer at the rate of 50ml/m² on Jan. 24, 1987. Ten sticky traps without bait were placed in set locations and replaced after 2 or 3 days. The number of cockroaches trapped in each trap per day was counted and the reduction in cockroach popula-

tion was calculated as follows :

$$\text{Reduction in population(\%)} = 100 - \frac{\text{No. of cockroaches after treatment}}{\text{No. of cockroaches before treatment}} \times 100$$

At test site B (area treated, 68m²), a 0.5% suspension of the reference microcapsules containing the organophosphate insecticide was sprayed on Jan. 30, 1987 in the same manner as at test site A. Eight sticky traps were placed at the corners of two rooms and the reduction in the cockroach population was calculated in the same manner as described above.

3. Mechanism of entry of fenitrothion applied in microcapsule form

(1) Determination of the amount of fenitrothion trampled and the amount picked up by the insects

An aqueous suspension of microcapsules or emulsion of fenitrothion was applied uniformly to the bottom of 8 cm 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 fenitrothion outside and inside the microcapsules on the treated surface and the amount of fenitrothion picked up by the insects were analyzed as described elsewhere (TSUDA *et al.*, 1987) with deodorized kerosene (Neochizol[®], Chuo Kasei Co. Ltd., Japan) as the extraction solvent. Analysis was done with different dishes which had 1 to 4 times contact by cockroaches with 2 hr contact at each time. All samples were analysed with a gas chromatograph equipped with a flame ionization detector under the following conditions ; glass column, 1.1 m × 3 mm, packed with 3% XE-60 on Chromosorb W (AW, DMCS), 60-80 mesh ; carrier gas, nitrogen, at approximately 60ml/min ; injection temperature, 230°C ; column temperature, 180 °C. The tarsi of the cockroaches were examined under a microscope for adhering microcapsules.

(2) Contact test with mouth-closed cockroaches

An aqueous suspension of microcapsules or

emulsion of fenitrothion was applied to a plywood panel at the rate of 250mg active ingredient/m². On CO₂-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 KT₅₀ (time required for Knock-down of 50% of the cockroaches) was calculated. Mortality was observed after 72 hr.

(3) Observation of microcapsules in crops of the insects

Microcapsules was prepared with ¹⁴C fenitrothion (specific radioactivity, 0.52 mCi/5.4 g fenitrothion). Female German cockroaches were confined to contact with the plywood surface treated with microcapsules at the rate of 1000mg of active ingredient/m² for 2 hr. Then, the insects were buried and frozen in the methyl cellulose paste on a microtome stage. The whole bodies were tape-sectioned and the dried sections (20 μm thick) were brought into contact with X-ray film to obtain autoradiograms. In order to examine the mastication of the microcapsules in the crop, 1 μl of a 2.5% suspension of the microcapsules was orally administrated to insects and autoradiograms were obtained in the same manner as above.

Results and Discussion

Residual efficacy under laboratory conditions

The residual efficacy of microcapsules against the German and American cockroaches is shown in Table 1. The microcapsules on the plywood surface at the rate of 125mg of active ingredient/m² caused 100% mortality to the German cockroach even 8 weeks after treatment. At this dose, the reference microcapsules had significantly less activity at 8 weeks. Nearly twice the dose of fenitrothion microcapsules was needed for American cockroaches to give

Table 1 Residual efficacy of fenitrothion microcapsules against cockroaches by 2 hr confined contact to the plywood surface.

A. *Blattella germanica*

Sample	Dosage (mg a. i./m ²)	% mortality at (weeks after treatment)			
		0	2	4	8
Fenitrothion MC ¹⁾	250	100	100	100	100
	125	100	98	100	100
Reference MC	250	100	98	95	98
	125	100	80	53	20

B. *Periplaneta americana*

Sample	Dosage (mg a. i./m ²)	% mortality at (weeks after treatment)			
		0	2	4	8
Fenitrothion MC	500	100	100	94	94
	250	100	100	94	72
Reference MC	500	100	100	100	61
	250	100	100	67	11

¹⁾ MC, microcapsules

Table 2 Residual efficacy of fenitrothion microcapsules against German cockroach by 2 hr confined contact to the plywood surface which was stored at 40 °C in 100 % RH conditions.

Sample	Dosage (mg a. i./m ²)	% mortality at (months after storage started)			
		0	1	2	3
Fenitrothion MC ¹⁾	125	100	100	100	100
Reference MC	250	100	90	90	80

¹⁾ MC, microcapsules

the same effects as for German cockroaches.

Table 2 shows the residual efficacy of the microcapsules stored at 40 °C and 100 % RH. The fenitrothion microcapsules caused 100 % mortality even after 3 months of storage. On the other hand, the mortality caused by the reference microcapsules with at twice the dose was 80% after 3 months of storage. TSUDA *et al.* (1987) reported that the spontaneous release of fenitrothion from microcapsules was negligible and that the fenitrothion inside microcapsules was stable even after 12 weeks of storage at 25 °C and 40 °C. The long-term efficacy of the fenitrothion microcapsules, there-

fore, seems to arise from the lack of diffusion of the active ingredient from microcapsules.

Field tests

Reduction in the German cockroach population after treatment the kitchens with microcapsules are shown in Fig. 1. More than 90 % reduction was maintained for more than 3 months with fenitrothion microcapsules at the rate of 125mg of active ingredient/m². The residual activity seemed to be more than twice that of the reference microcapsules. The lack of efficacy in the first week compared to the reference microcapsules was probably the result of the lack of quick killing effects. Thus, the

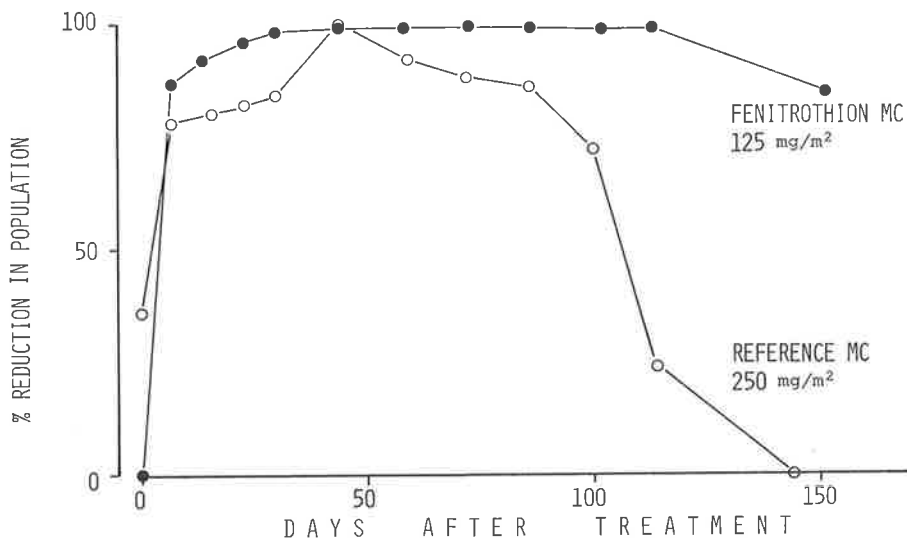


Fig.1 Reduction in population of German cockroaches after treatment with microcapsules in the field.

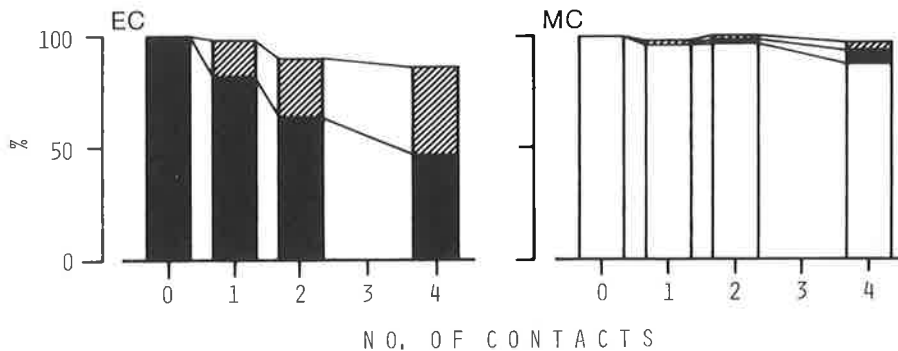


Fig.2 Changes in the trampling rate of the fenitrothion microcapsules and the amount of fenitrothion picked up by cockroaches. MC, microcapsules ; EC, emulsifiable concentrate. □ Fenitrothion inside MC on the surface, ▨ Fenitrothion picked up by insects, ■ Fenitrothion outside MC on the surface.

simultaneous use of fenitrothion microcapsules and another insecticide with high knock-down activity such as a pyrethroid may be desirable, because the "flushing activity" of pyrethroids (SHINJO *et al.*, 1981) would improve the chance of insects coming contact with the surfaces treated with fenitrothion microcapsules.

Trampling and picking up of microcapsules by cockroaches

Figure 2 shows the changes in the amount of fenitrothion outside the microcapsules and the amount that picked up by the cockroaches after 1-4 times of contact by the insects. Fenitrothion on the treated surface decreased as the times of contact increased. The decrease in the amount of fenitrothion on the surface was greater in emulsifiable concentrate than microcapsules. Both decreases seemed to occur when

Table 3 Amount of fenitrothion picked up by single German cockroach during 2 hr of contact with glass surfaces¹⁾ treated with different formulations.

Sample ²⁾	Amount ($\mu\text{g}/\text{insect}$) picked up during each 2 hr of contact			
	1 st	2 nd	3 rd	4 th
MC	2.1	2.2	2.5	2.7
EC	39	24	18	15

¹⁾ Dosage is 2.6 mg of active ingredient per Petri dish (8 cm in diameter)

²⁾ MC, Microcapsule; EC, Emulsifiable concentrate.

the insects carried away the fenitrothion on their body. Table 3 shows the changes in the mean amount of fenitrothion recovered from the body surface of single insects after 1-4 contacts. The amount found on the insect was almost constant with different numbers of contacts. However, with emulsifiable concentrate, the amount picked up during first contact, was largest and the amount then decreased as the numbers of contacts increased. It seemed that an excess was picked up in case of emulsifiable concentrate (the amount of fenitrothion required for 100% mortality is around $1 \mu\text{g}/\text{insect}$). The total amount of fenitrothion picked up by a single cockroach during four times of contacts was more than 10 times when the emulsifiable concentrate was used. The improvement of residual activity with the microcapsules may partly arise from the "mechanical release control", regulated by the strength of microcapsules. Figure 3 is a photograph of fenitrothion microcapsule particles on the tarsus of a German cockroach after contact with plywood panel treated with the microcapsules. Some portion of fenitrothion picked up by insects seems to have been swallowed in microcapsule form. SAKURAI *et al.* (1982) suggested the importance of massive ingestion of the diazinon microcapsules into digestive tracts caused by grooming of cockroaches. The presence of two main modes of entry, dermal penetration of fenitrothion by mechanical rupture of microcapsules with "trampling" and ingestion of

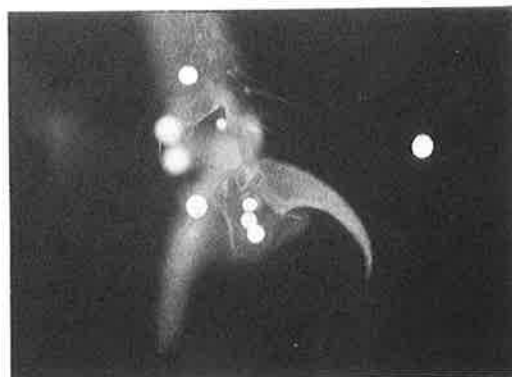


Fig. 3 Microcapsules observed on the tarsus of a German cockroach after contact with plywood surface treated with fenitrothion microcapsules.

microcapsules, is suggested in fenitrothion microcapsules.

Efficacy against mouth-closed cockroaches

The KT_{50} and mortality caused by two forms of fenitrothion with which mouth-closed cockroaches are confined to contact are shown in Table 4. In the case of the microcapsules, the knock-down was later and mortality was slightly lower when cockroaches had closed mouths than with untreated control, while the differences in the efficacy of the emulsifiable concentrate were slighter. The delay in knock-down with fenitrothion microcapsules was greater than with diazinon microcapsules (SAKURAI *et al.*, 1982). These results suggest that ingestion of the microcapsules, which occurs during grooming behavior of cockroaches, is one route by which the fenitrothion enters the insect body.

Table 4 Knock-down and mortality of German cockroach by contact with plywood surface treated with different formulations of fenitrothion.

Sample ¹⁾	KT ₅₀ (min.) -	Mortality (%)
	Mouth-closed ²⁾ Normal	
MC	1500- 96	290-100
EC	91-100	69-100

¹⁾ MC, Microcapsule; EC, Emulsifiable concentrate.

²⁾ Mouths of insects were closed with paraffin.

Observation of microcapsules in crops of the insects

A whole-body autoradiogram of a German cockroach that came into contact with a surface treated with ¹⁴C-labeled fenitrothion microcapsules is shown in Fig.4. Microcapsule particles orally ingested were found in the crop. Figure 5 shows ¹⁴C-labeled fenitrothion microcapsules in the crop of a German cockroach 30 minutes after oral administration (Fig. 5-A) and the release of fenitrothion into the crop at 150 minutes (Fig. 5-B). Bloating of the crop, which can occur commonly in toxication process by organophosphate (HOPKINS, 1961), is visible in this figure. Mastication of microcapsules in the crop may release the fenitrothion inside the wall of the microcapsules, allowing it to act as a stomach poison.

Conclusion

The fenitrothion microcapsules showed higher residual effects than the reference microcapsules used and the possibility of use as a strong weapon for cockroach control was suggested.

The insufficiency of the residual efficacy of emulsifiable concentrate may be due mainly to its absorption into the treated surfaces and also to its vaporization or degradation. Microencapsulation of insecticides is often used to prevent these problems. It was suggested, in

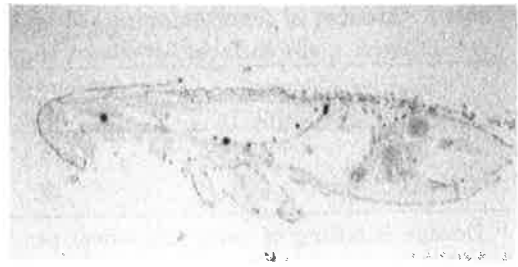


Fig. 4 Whole-body autoradiogram of a German cockroach that came into contact with plywood surface treated with ¹⁴C-labeled fenitrothion microcapsules. The black spots on the crop wall are microcapsules.

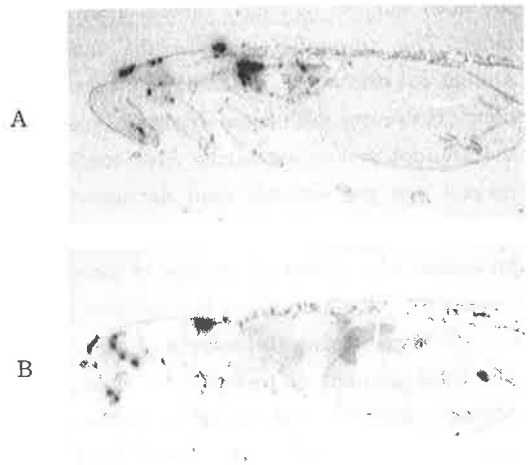


Fig. 5 Whole-body autoradiogram of a German cockroach given ¹⁴C-labeled microcapsules orally. A, 30 min after administration; B, 150 min after administration.

our experiments, that the carrying-away of the insecticides by the insects could not be ignored as another factor that could reduce efficacy. With fenitrothion microcapsules, however, the amount of active ingredients carried away was in suitable level required for killing insects. That is, microencapsulation made the fenitrothion bioavailability to the cockroaches optimum.

Oral ingestion of the microcapsules and their mastication in the crop were found to be one route by which the fenitrothion microcapsules cause the death to the cockroaches.

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Ecological Studies on Ixodid Ticks. 8. Parasitism by Immature Stages of the Tick *Ixodes nipponensis* (Acarina: Ixodidae) of the Lizard *Takydromus tachydromoides* (Lacertilia: Lacertidae)

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マダニ類の生態学的研究. 8. タネガタマダニ幼・若虫のカナヘビ寄生 藤本和義 (埼玉医科大学寄生虫学教室)

カナヘビに対するタネガタマダニ幼・若虫の寄生調査を1982年から1988年にかけて、埼玉県南西部の一丘陵地で行った。同時に実験室内において、カナヘビに幼・若虫を吸血させ、寄生部位の選択や寄生密度の影響について調べた。丘陵地でのタネガタマダニ幼・若虫の寄生率の年変動は比較的小さかった。幼虫の寄生は春から秋までみられたが、春の寄生率は若干低かった。若虫の寄生は春から夏にみられたが、秋にはまれであった。カナヘビ上での幼・若虫の寄生数の分布パターンは負の2項分布によく適合し、これは生息地内での幼虫の分布とカナヘビの行動によって生じると思われた。幼・若虫の寄生部位はカナヘビの前脚とその周辺部に限定された。幼虫の多数寄生によるカナヘビや幼虫自身に対する影響はほとんど観察されなかった。しかし若虫の多数寄生はカナヘビの死亡率を高め、若虫の脱皮成功率を低下させた。

Annual and seasonal changes, the characteristics of infestation patterns, and the attachment sites of immature *Ixodes nipponensis* KITAOKA et SAITO that fed on the lizard, *Takydromus tachydromoides* (SCHLEGEL) were studied in a hilly region in the southwestern part of Saitama Prefecture during 1982-1988. Laboratory experiments were done on the preferred attachment sites and the effects of the infestation density of larval and nymphal ticks. The infestation levels of immature *I. nipponensis* on the lizards were stable during the study period. The larval ticks were active from spring to autumn, but their numbers were slightly lower in spring. Nymphal ticks were abundant in spring and summer, but scarce in autumn. Immature ticks were found on the lizards in a negative binomial distribution. This pattern appeared to arise from the distribution of larval ticks and by the behavior of host lizards in their habitat. The attachment sites of larval and nymphal ticks on the host lizards were almost always on the forelimbs and nearby regions. Damage to the host lizards and to larval ticks themselves was rarely observed even in heavy infestations. However, heavy infestations by nymphal ticks caused the increased mortality of the hosts and lowered the percentage of nymphal ticks that dropped from the hosts and molted.

Key Words: Lizard tick, *Ixodes nipponensis*, *Takydromus tachydromoides*, Attachment sites, Seasonal changes, Infestation level

Introduction

There are many species of ticks that infest reptiles. According to HOOGSTRAAL and ARSCHLIMANN (1982), immature forms of about ten *Ixodes* species feed on reptiles in their immature stages, as well as on birds and mammals. Two species of *Ixodes* are parasitic on lizards in Japan: *I. asanumai* KITAOKA (all stages) (HAYASHI and HASEGAWA, 1983) and *I. nipponensis* (immature stages) (FUJITA and TAKADA, 1979). Many ecological studies have been done on *I. asanumai* fed on the lizard *Eumeces okadae* STREINER (HAYASHI and HASEGAWA, 1983, 1984a, 1984b; HAYASHI *et al.*, 1984). A few short studies of *I. nipponensis* parasitic on lizards have been reported (FUJITA and TAKADA, 1979; FUJIMOTO *et al.*, 1986, 1987). There is no quantitative information about the host-parasite relationship between *I. nipponensis* and its host lizard. The purpose of this paper is to clarify the annual and seasonal changes, distribution patterns, attachment sites, and effects of infestation density of immature *I. nipponensis* fed on the lizard *T. tachydromoides*.

Materials and Methods

Field observations

Field surveys were done at two sites, Minowada and Hatoyama, in a hilly region in the southwestern part of Saitama Prefecture (36°00'N and 139°20'E). These two sites were covered by secondary forest composed of deciduous trees (mainly *Konara* oak *Quercus serrata* THUNB.) and *Hinoki* cypress, *Chamaecyparis obtusa* ENDL.

The lizards, *T. tachydromoides*, were collected during their active season (April to November) from 1982 to 1988 in Minowada and from 1984 to 1988 in Hatoyama. Collection

in both Minowada and Hatoyama was the path about 1000 m long. The lizards were captured by hand and marked with oil color paint so that they would not be counted if recaptured within a short period. The snout-hindlimb length, sex, and presence or absence of parasitic ticks were recorded for each captured lizard, which was then released. The developmental stage, number, and location of attached ticks on lizards collected were noted. Some of the lizards infested with ticks in 1982 and 1983 were taken back to the laboratory and kept individually in plastic containers (28×17×18 cm) at 25-28°C, with a light-dark cycle of 16:8 hr. Engorged ticks that dropped from the lizards were identified and laboratory experiments.

Feeding experiments with larval and nymphal ticks were done under the same laboratory conditions as above. The experiments were done to identify the preferred attachment sites, and the effects of the number of infesting ticks on tick development and host survival. Unfed ticks were placed on the back of a lizard with a brush with four infestation densities, from 10 to 100 per lizard, for larvae and three infestation densities, from 5 to 20 per lizard, for nymphs. Positions and the number of ticks were recorded two or three days after the attachment. To clarify the effects of tick infestation density on the development of the ticks themselves, engorged or semiengorged ticks that had dropped from lizards were placed in Petri dishes (3 or 4.5 cm in diameter) the bottoms of which were covered with wet filter paper, and the ticks were reared in a incubator at 25°C, with a light-dark cycle of 16:8 hr. After molting was completed, the percentage of molting successes was found for different tick infestation densities.

The body length (Idiosoma) of the molted ticks was also measured under a stereoscopic microscope and compared for the different infection densities.

Ticks used in these experiments were obtained from the rearing of engorged females fed on the ears of domestic rabbits. The host lizards were collected from Minowada and Hatoyama. German cockroaches, *Blattella germanica* LINNÉ, were given as food to the lizards every day.

Results and Discussion

Annual and seasonal fluctuations of infestation levels

The host lizards, *T. tachydromoides* began to appear in late March or early April and disappeared in late November or early December. Active ticks were observed throughout the active season of the host lizards.

Figure 1 shows annual changes in the infestation density of immature *I. nipponensis* per lizard. The annual densities of nymphal ticks in Minowada ranged from 0.55 to 2.23, with a mean for the entire survey period of 1.32. The densities of nymphal ticks in both Minowada and Hatoyama fluctuated around the same level. The densities of larval ticks were different at the two survey sites. The densities of larval ticks in Minowada ranged from 1.14 to 1.96, with a mean of 1.51, and in Hatoyama, these densities were from 0.47 to 0.69, with a mean of 0.58. In both survey sites, the range of fluctuations in the density of the larval ticks was less than that of the nymphal ticks. These results suggest that the population density of *I. nipponensis* in these study sites is relatively stable.

Figure 2 shows seasonal changes in the percentage of infested lizards. The seasonal patterns of tick occurrence were the same each year and the same in the two study sites. Larval ticks were found from spring to

autumn, but the percentage of parasitism was low in spring. It seemed that the larval ticks found in spring are mainly overwintering ones, although some are larvae newly hatched that spring. Nymphal ticks were abundant in spring to summer, but scarce in autumn. It seemed that the nymphal ticks found spring and summer include both overwintering nymphs and those newly molted that spring. However, the low density of the nymphal ticks in autumn cannot be fully explained by the density of the preceding stage, (the larval ticks). BELOZEROV (1982) reports that ticks have a diapause that synchronizes the different stages of their development with seasonal changes in the climate. The low density of nymphal ticks in autumn reported here may also arise from this diapause.

The seasonal occurrence of immature *I. nipponensis* on the host lizards is similar to that of *I. ricinus* (LINNAEUS) (BAUWENS *et al.*, 1983), but different from that of *I. asanumai*, whose larvae disappear in autumn (HAYASHI and HASEGAWA, 1984a). This suggests that the life cycle of *I. nipponensis* resemble to that of *I. ricinus* more than to that of *I. asanumai*.

Infestation patterns on lizards

Figure 3 shows the frequency distribution of the number of ticks per lizard in three cases, larvae in April to June and September to November, and nymphs in April to June. In all cases, the ratio of the variance to the mean number of ticks per lizard was significantly greater than 1 (F-tests, $P < 0.001$). Thus, the distributions were not of the Poisson series and could be approximated by the negative binomial series, a variety of clumped distributions (in all cases, χ^2 -tests, $P > 0.4$). Clumped distributions (negative binomial distributions) of ticks on hosts have been reported by BAUWENS *et al.* (1983) and HAYASHI and HASEGAWA (1984b) for lizards