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Original Article

# Anti-termite formulations for soil treatment based on decanoic acid and their efficacy against *Coptotermes formosanus* Shiraki

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## ABSTRACT

Anti-termite formulations based on natural products of no or less toxicity were developed for soil-treatment. One of the formulations was commercialized as First Guard MP. The formulation was composed of, on careful consideration of safety, decanoic acid (n-capric acid, a fatty acid with ten carbons derived from palm oil) as a main active ingredient and of the other natural ingredients including hiba neutral oil and turmeric. An experiment to examine the termiticidal efficacy of the formulation was conducted at the termite field test sites at Kagoshima and Okinawa, where two important kind of subterranean termite species, *Coptotermes formosanus* Sikraki and *Reticulitermes speratus* (Kolbe) inhabit. These termite species cause economically serious damage in Japan. The formulation had performed well for six years at Kagoshima and for three years at Okinawa after the field test had been started. The termiticidal efficacy of decanoic acid was evaluated with *C. formosanus* in our laboratory. The minimum concentration of decanoic acid in soil required for the prevention of termite attack was estimated. Further the rates of disappearance of decanoic acid in the soil treated with the formulation under various conditions were measured.

**Key words:** *Coptotermes formosanus* Shiraki, Anti-termite formulation, Natural products, Decanoic acid, n-Capric acid, Soil-treatment

## Introduction

The use of chemicals for wood protection has been increasing worldwide over the past years. The effect on our health and environment by the use of such chemicals are concerned (Inoue, 1994 ; Endo, 1995). We are also concerned about the global warming by the burning of fossil resources and environmental disruption. The degree of the environmental disruption is passing over the repair capacity inherent in the earth. Most of wood protection chemicals are derived from fossil resources which are said to be exhausted in the not far future. The use of natural products derived from plants would make an important contribution to improve these problems.

Therefore, we have tried to make use of natural products for the development of wood protection chemicals which are no or less toxic and also friendly to environment.

Many substances which have significant termiticidal efficacies have been extracted from wood (Inoue, 1976 ; Carter *et al.*, 1978 ; Yazaki, 1982 ; Yaga and Kinjo, 1985 ; Ohtani *et al.*, 1997).

We have examined the anti-termite efficacy of a large number of plant extracts and found that some fatty acids and fatty acid-esters have significant efficacy against termites (Yoshida *et al.*, 2003 ; Yoshida and Enoki, 1999 ; 2000 ; Yoshida and Igarashi ; 1999). We have found hiba neutral oil also has a certain anti-termite efficacy (Yoshida *et al.*,1998). Taking their safety and the durability of their efficacy into account, we have chosen decanoic acid as an active ingredient and have developed the anti-termite formulation consisted of decanoic acid , wood powder (as a supporting carrier of decanoic acid), hiba neutral oil and turmeric as well as additives (used as food or cosmetic additives) for soil treatment.

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## Materials and Methods

### Anti-termite formulation for soil treatment

***Test compound***

Decanoic acid ; Wako Pure Chemicals Co. Ltd. , purity ; 99%

Hiba neutral oil ; Osaka Organic Chemical Industry Ltd.

Turmeric (powder) ; Tochimoto

Wood powder ; Median diameter ; ca.180 $\mu$m

***The formulations***

Formulation A : Decanoic acid ; 50.0%, Wood powder ; 39.5%, Hiba neutral oil ; 1.0%, Turmeric ; 1.0%, Additives (used for food and cosmetic) ; 8.5%

Formulation B : Decanoic acid ; 10.0%, Wood powder ; 87.5%, Hiba neutral oil ; 0.2%, Turmeric ; 0.2%, Additives (used for food and cosmetic) ; 2.1%

Decanoic acid was the main active ingredient. The wood powder was the supporting carrier of decanoic acid. The additives are used as the thickener at the dilution and for the solidification after soil treatment. The formulation is a powder in form with a light yellow color.

***Field test***

The field test for the anti-termite formulation was carried out according to JWPA Standard No.13 at the termite field test site in Kagoshima Prefecture and Okinawa Prefecture where two important kind of subterranean termite species, *C. formosanus* and *R. speratus* inhabit. These termite species cause economically serious damage in Japan.

***Field test according to JWPA Standard No.13***

Six 45 cm square polyvinyl chloride frames (height 30 cm) with lids but without bottoms were placed at regular intervals on the ground around a pine stump where *C. formosanus* species actively inhabit. One of the six boxes was used as a control to monitor the termite activity.

Two kg of the Formulation A was added to 8 l of water. The mixture was stirred and divided into five equal parts. Each of the five equal parts was uniformly scattered on each soil surface inside the five frames (2 kg/m$^2$) . A formulation layer of about 6 mm thickness was formed over the soil surface. A pile of two bait pine plates (10×10×2 cm) was placed on the center of the soil surface and the five formulation layers inside the six frames. Then the lids were put on the frames.

To encourage initial termite attack, four pine feeder stakes (35×3×3 cm) were driven into the soil around the each box at certain intervals.

Annual visual inspections were conducted and the degrees of termite attack on the bait plates were rated. The feeder stakes and the bait plates seriously damaged by termites were replaced with new ones at every annual inspection to monitor the termite activity at the field test site for the next year.

***Evaluations of width of the formulation layer***

An experiment to evaluate the width of the formulation layer required to protect the bait wood from termite attack was undertaken at the same field test site in Kagoshima two years after the experiment just above mentioned. The same field test was undertaken at the termite field test site in Okinawa Prefecture one year after the field test in Kagoshima had been started. To 1.6 l of water, 400 g of the Formulation A was added. The mixture was stirred and divided into five equal parts. Each part was uniformly scattered on 20 cm square area of the ground around a pine stump at regular intervals. A pile of two bait pine plates (10×10×2 cm) were placed on the ground untreated with the formulation around the pine stump and also on the center of each of the five formulation layers. Thus the width of the formulation band lying between the edge of the treated soil and the bait wood was 5 cm. Each of the six piles was covered with the same box as that mentioned above **(Fig.1, Fig.2)**.

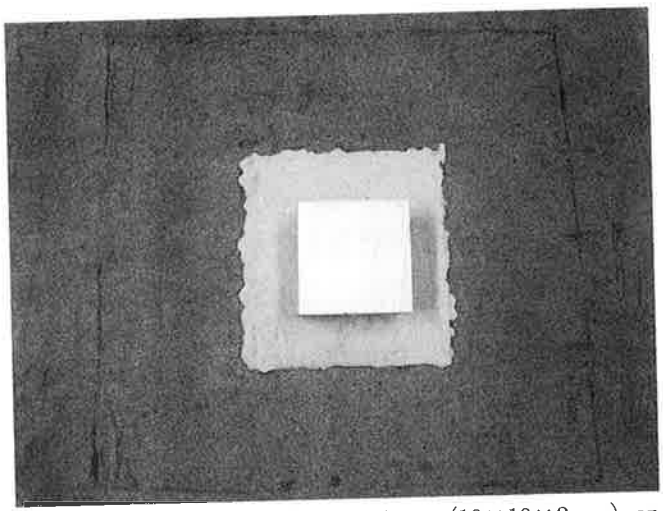

**Fig. 1** A pile of two bait pine plates (10×10×2 cm) on the center of 20 cm square layer or the Formulation A.

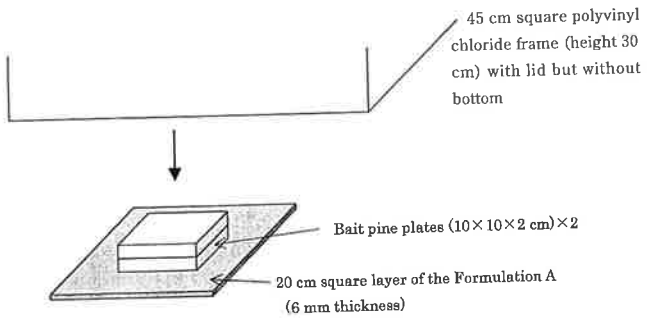


**Fig. 2** Diagram of field test (a pile of two bait pine plates (10×10×2 cm) on the center of 20 cm square layer of the Formulation A).

### Laboratory test

***Termites***

Workers of *C. formosanus* were used. All the termites were collected from a colony that is kept in our laboratory maintained at 28℃.

***Tunneling test***

Formulation A and B were prepared. The tunneling test was carried out in accordance with JWPA Standard No.13.

Sandy loam, sieved through 20 meshes (0.840 mm), was heat-sterilized at 60℃ until its weight became constant. The dried soil was moistened with distilled water (20% w/w). A formulation (3 g) was put in a 100 mlbeaker (inside diameter ; 52 mm) and the beaker was kept at 40℃ in a thermostat with air circulating for 4 weeks as a heat weathering.

About one-half of a glass tube (inside diameter ; 15 mm, length ; 70 mm) was stuffed with the moistened soil and the inner surface of the moistened soil was leveled off with a glass rod. A formulation (353 mg/1.77 $cm^2$ ≒2 $kg/m^2$) exposed to the heat weathering was placed on the inner soil surface in the glass tube. The upper empty space in the glass tube was stuffed with 20%-moistened soil. A 5 mm thick formulation layer was formed and sandwiched in the soil in the glass tube. The glass tube was connected to two glass cylinders at the both ends (joint length ; 10 mm). Wood flakes (about 5 g) were put into one of the two cylinders. About 60 g of soil (ca. 20% moisture content) were put into another one and 200 workers and 20 soldiers were placed on the soil in the cylinder (**Fig. 3**). The assemble unit was incubated at 27℃ and over 70% RH for 3 weeks. Three replicates were prepared for each formulation. After the test duration, the distance of boring (gallery) of the formation layer caused by the termites was measured. If all the termites were dead before the end of the test period, the time elapsed (in days) was recorded.

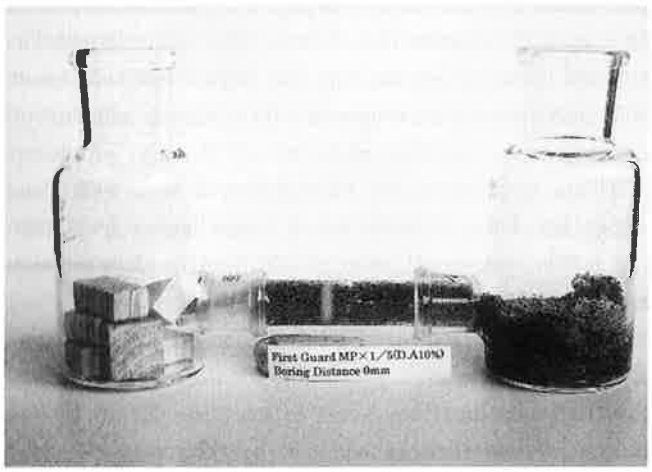


**Fig. 3** Tunneling test with the formulation B.

### Evaluation of termiticidal efficacy of decanoic acid

***Preparation of test soil***

Dried sandy loam (20 g) was placed in a 100 ml beaker (inside diameter ; 52 mm). The soil surface was leveled off by pressing. An acetone solution of decanoic acid (5 ml) was added to the soil in the beaker to give concentrations of 1000 ppm, 500 ppm or 100 ppm (w/w of soil). These concentrations were set to see the minimum termiticidal efficacy of decanoic acid. And 4.2 g of the Formulation A and B was uniformly placed on the soil surface (2 $kg/m^2$) and pressed to be leveled off. The 100 ml beakers were permitted to stand in a 40℃ thermostat for 2 hours and taken out from the thermostat. The decanoic acid-treated soil or the formulation layer on the soil in the 100 ml beakers were moistened with 4 ml or 8 ml of distilled water.

***Termite bioassey***

Ten workers and one soldier were put on the decanoic acid-treated soil (ca. 20% moisture content) or the formulation layer in the beaker (ca. 20% moisture content). The beakers were maintained at 28℃ in the dark. The change of the termites in vitality was visually observed in time-course. Three replicates were prepared for each sample.

### Rates of disappearance of decanoic acid under various conditions

***Preparation of test materials***

Dried sandy loam (30 g) was put in a 100 ml beaker (inside diameter ; 52 mm) and 6 ml of distilled water was added to the soil. After the soil surface was leveled off, the Formulation A (3 g) was placed on the soil surface and 12 ml of distilled water was poured into the beaker. Fifty-four replicate samples were prepared. Fifteen of them were kept in a refrigerator at -20℃ as standard samples.

Nine of them was maintained in a thermometer at 40℃ with air circulating and 15 of them at 28℃. Fifteen of them were placed on the ground under the floor of a house in Kyoto.

***Extraction and analysis***

At the indicated intervals after the beginning of the test, remaining decanoic acid in the formulation was measured. A solution of 10 ml acetone containing 200 mg of ethyl laurate per ml was added to a sample in a 100 ml beaker and acetone (50 ml) was added to the sample. The mixture was vigorously stirred with a glass rod and filtered through a filter paper. Then 300 μl of the filtrate was evaporated under reduced pressure. Trimethylsilanation of decanoic acid was carried out as previously described (Enoki *et al.*, 1981).

Gas chromatography was performed with a Shimadzu GC-14A equipped with a flame ionization detector (FID) and using a glass column packed with 3% OV-101 on chromosorb WAW DMCS. Helium was used as the carrier gas and the oven temperature was programmed from 170 ℃ to 250℃ at 10℃/min. The rate of residual decanoic acid in a sample was estimated from the rate of the peak area of decanoic acid to the peak area of ethyl laurate on GC. Remaining decanoic acid was determined in triplicate.

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## Results and Discussion

### Field test

None of the pine bait plates put on the 45 cm square formulation layers had suffered from termite damage throughout the 6 year test period at the field test site in Kagoshima Prefecture. Also none of 10 cm square pine bait plates placed on the centers of the 20 cm square formulation layers had suffered from termite damage for 4 years at the field test site in Kagoshima Prefecture and for 3 years at the field test site in Okinawa Prefecture. All the bait plates put directly on the untreated soil in the boxes had been seriously attacked and damaged by termites throughout the test duration and were replaced with sound ones at every annual inspection. Thus the 45 cm square formulation layer of 6 mm thickness has proved excellently protective against termite attack throughout a period of 6 years at the termite field test in Kagoshima Prefecture (**Table 1** (a)). Also the band of the formulation of 5 cm width and 6 mm thickness has proved to protect the wood sufficiently from termite attack and damage for at least 4 years in Kagoshima Prefecture (**Table 1** (b)) and for 3 years in Okinawa Prefecture (**Table 2**).

**Table 1** Field test in Kagoshima Prefecture

(a) 45cm spuare layers of the Formulation A (2 kg/m$^2$)

| Years | Treated sites | Control sites |
|---|---|---|
| 1 | No damaged | Damaged |
| 2 | No damaged | Damaged |
| 3 | No damaged | Damaged |
| 4 | No damaged | Damaged |
| 5 | No damaged | Damaged |
| 6 | No damaged | Damaged |

(b) 20cm spuare layers of the Formulation A (2 kg/m$^2$)

| Years | Treated sites | Control sites |
|---|---|---|
| 1 | No damaged | Damaged |
| 2 | No damaged | Damaged |
| 3 | No damaged | Damaged |
| 4 | No damaged | Damaged |

**Table 2** Field test in Okinawa Prefecture

20cm spuare layers of the Formulation A (2 kg/m$^2$)

| Years | Treated sites | Control sites |
|---|---|---|
| 1 | No damaged | Damaged |
| 2 | No damaged | Damaged |
| 3 | No damaged | Damaged |

### Tunneling test

The boring distances in both of the Formulation A and B were 0 mm ~ below 1 mm (**Table 3, Fig 3**). All the termites used for the tunneling tests with the formulations were dead within 21 days of the test duration. Thus the both formulations were ranked as 0 or 1 with 100% mortality (rank 0 : length of tunneling ; 0 cm, rank 1 : length of tunneling ; below 1 cm). These results indicate that even if four fifths of 50% decanoic acid originally present in the formulation have disappeared, the remaining decanoic acid (10%) in the formulation is able to perform well sufficiently against termite attack.

### Evaluation of termiticidal efficacy of decanoic acid

While ten workers and one solider were left on the soil of 1000 ppm, 500 ppm or 100 ppm (w/w of soil) or on the Formulation A or B layer , their health was checked up with visual observation (**Table 4**). All the termites on the soil of 1000 ppm or 500 ppm decanoic acid were seriously weakened or knocked down and stopped walking within one hour and were dead within 3 hours or 6 hours. But the termites on the soil of 100 ppm decanoic acid kept well and walked actively for 3 days. The observation was stopped 3 days after the test had begun. For this result, 500 ppm was the minimum termiticidal efficacy of decanoic acid.

All the termites on the Formulation A or B were dead within one hour. Formulation A keeps higher level than the minimum concentration of 500 ppm to show enough efficacy against *C. formosanus*.

Concerning hiba neutral oil and turmeric, they showed some efficacy against *C. formosanus* for the initial time after the treatment, but they seem to lose the efficacy during the longer test such as field test by their evaporation of active ingredients.

**Table 3** Tunneling test for Formulation A and B against *C. formosanus*

| Sample | Sample No. | Boring distance (mm) | Notes |
|---|---|---|---|
| Formulation A | 1 | 0 | All the termites were dead within 18 days. |
| | 2 | 0 | |
| | 3 | 0 | |
| Formulation B | 1 | 0 | All the termites were dead within 20 days. |
| | 2 | 0 | |
| | 3 | 0.5 | |
| Control | 1 | 50 | Termites have penetrated the soil layer and reached the bait pine flakes within one day. |
| | 2 | 50 | |
| | 3 | 50 | |

**Table 4** Time required for 100% mortality of the termites on soils and formulations containing decanoic acid (D. acid)

| | Formulation A 50% D. acid | Formulation B 10% D. acid | Soil of 1,000 ppm D. acid | Soil of 500 ppm D. acid | Soil of 100 ppm D. acid |
|---|---|---|---|---|---|
| Hours | 0.5 | 0.5 | 3 | 6 | 72> |

**Table 5** Disappearence rate of decanoic acid in the formulation

| Condition | Remaining decanoic acid (%) | | |
|---|---|---|---|
| | 1 year | 2 years | 3 years |
| 40℃ | 12 | ---------- | ---------- |
| 28℃ | 85 | 72 | 62 |
| Temperature under floor at a house in Kyoto | 97 | 95 | 93 |

**Rates of disappearance of decanoic acid in the formulation under various conditions**

The rates of disappearance of decanoic acid in the Formulation A were measured under various conditions (**Table 5**). After one year of incubation at 40℃, only 12 % of the decanoic acid originally present in the formulation remained. Under the incubation at 28℃, the remaining rates of the decanoic acid in the formulation were 85% after one year, 72% after two years and 62% after three years, respectively. After one, two and three years of setting the formulation on the ground under the floor of a house in Kyoto, 97%, 95% and 93% of the originally-existing decanoic acid still remained in the formulation. Thus the disappearance-amounts of decanoic acid in the formulation over the same time interval of each year were very close to each another and decreased gradually with incubation time. The disappearance of decanoic acid is thought to be mainly caused by its evaporation. The estimated concentration of decanoic acid in the formulation after 6 years of incubation of the formulation of 50% decanoic acid at 28℃ or on the ground under the floor of a house in Kyoto is 19.4% $[50(1-0.38\times 2)\times 100/\{50+50(1-0.38\times 2)\}]$ or 46.2% $[50(1-0.07\times 2)\times 100/\{50+50(1-0.07\times 2)\}]$. Therefore 6 years after the formulation of 50% decanoic acid has been sprinkled at a site at 28℃ or on the ground under the floor of a house in Kyoto, the concentration of decanoic acid in the formulation must be grater than 19% or 46%. The values together with the results in the laboratory experiments mentioned above indicate that the Formulation A for soil treatment (2 kg/m$^2$) can perform well against termites for at least 6 years. This presumption based on the results of various experiments in our laboratory is consistent with the results in the field test at the termite field test sites in Kagoshima and Okinawa.

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# デカン酸を主成分とする土壌処理用防蟻剤のイエシロアリに対する防蟻効力


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榎 章郎（近畿大学農学部）



天然物を用いた、環境や人体に対する安全性の高い土壌処理用防剤剤(商品名:ファーストガードＭＰ)を創製した。この製剤は主として有効成分であるデカン酸（カプリン酸：ヤシ油・パーム油より得られる炭素数10の脂肪酸）、ビバ中性油およびウコン等の天然物より構成されている。本製剤を用い、日本で経済的に大きな被害を生じているイエシロアリとヤマトシロアリの活動が活発な鹿児島県と沖縄県の林内で野外試験を行った。鹿児島県では試験開始6年後、沖縄県では試験開始3年後においても効力が持続した。一方、室内試験においてデカン酸の殺蟻効力を調べるとともに、土壌処理剤としてデカン酸を用いた場合の必要最低濃度を決定した。さらに種々の条件下におけるデカン酸の製剤中からの消失速度の測定を行なった。

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Original Article

# Effects of pyriproxyfen-treated targets on the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera : Yponomeutidae)

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## ABSTRACT

The concept of sterility induction using attractive targets treated with a juvenoid to control the diamondback moth, *Plutella xylostella*, was simulated in the laboratory. Adults of both sexes were brought into tarsal contact for 10 min with a commercially available plastic tape coated with pyriproxyfen, 2-[1-methyl-2-(4-phenoxy)ethoxy] pyridine, (Lano®, Sumitomo Chemical Co. Ltd.). Only when both partners in a couple had been treated was there a reduction in egg production to 50% compared with untreated controls. However, egg viability was virtually unaffected. Similarly, topical doses of pyriproxyfen in acetone (10 $\mu$g in 0.5 $\mu$l) were only effective when both sexes in a mating couple had been treated. However, in the case of females treated within 1 hour following emergence, egg production was reduced to around 50% of control following mating with untreated males. Tarsal contact with oil/acetone formulations of pyriproxyfen on aluminium foil surfaces was more effective than other treatments, causing reduction in egg numbers and egg viability. Increasing concentrations of pyriproxyfen above 0.04 mg/cm$^2$ did not increase effectiveness, although the time of exposure was important, 1 hour exposure being more effective than 10 min. The most effective formulation used was in sesame oil. A direct effect of pyriproxyfen on the viability of eggs treated less than 24 hours after oviposition was demonstrated by dipping them in an aqueous EC formulation, which induced 99% mortality by preventing eclosion of the fully developed larva within the egg.

Present results suggest that appropriate formulations of pyriproxyfen for use on attractive targets for contamination of adults and eggs should provide effective control of the diamondback moth in glasshouse horticulture.

**Key words:** Juvenoid, Yellow-Target, Sterilization, Diamondback moth

## Introduction

In a recent study (Oouchi, 2005) pyriproxyfen, a photostable juvenoid was an effective chemosterilant for the diamondback moth, *Plutella xylostella* (Linnaeus). It disrupted reproduction in the female moth and produced a variety of effects at critical points of every life stage. Hargrove and Langley (1990) formulated pyriproxyfen for use on visually attractive targets for control of the tsetse fly, *Glossina morsitans*. The technique was expected to replace conventional spraying of residual insecticides. This use of pyriproxyfen prompted development of target devices for the control of the greenhouse whitefly, *Trialeurodes vaporariorum* in 1990 (Oouchi and Langley, 2005) and *Bemisia tabaci* (Nakamura *et al.*, 1994).

Targeting the larval stages of herbivorous insects such as *P. xylostella* using a juvenoid is inappropriate since it leads to production of supernumerary larval instars with consequent increase in crop damage. Hence, in the present study an attempt has been made to investigate the effects of topical dosing of adults or exposure by tarsal contact to different formulations of pyriproxyfen on candidate target surfaces. *Inter alia* the effects of direct treatment of recently laid eggs on their viability has also been investigated. The aim has been to produce a formulation of pyriproxyfen for presentation on visually and olfactorily attractive targets that will sterilize adults or recently laid eggs in glasshouse horticultural systems.

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## Materials and Methods

### Insects

An insecticide-susceptible strain of the diamondback moth, *P. xylostella*, was obtained from a mass culture maintained at 25℃, 14 : 10 L : D photo-regime and 65% RH by Sumitomo Chemical Co., Ltd..

### Insecticide (Juvenoid)

Technical grade pyriproxyfen, 2-[1-methyl-2-(4-phenoxy phenoxy) Ethoxy] pyridine (97.2% ai), dissolved in acetone was used for topical application. Ready-made formulations (Sumitomo chemical Co. Ltd.) were used in other experiments ; e.g. eggs were dipped into aqueous dilutions of a 10% emulsifiable concentrate (EC) of pyriproxyfen, while a commercially available yellow tape impregnated with pyriproxyfen (2 g ai/$m^2$) (Lano®, Sumitomo chemical Co., Ltd.) was used in tarsal contact experiments with adult insects.

Further tarsal contact experiments were conducted in a Petri dish (9.5 cm in diameter) using aluminium foil coated with canola oil in acetone (1 : 50 V/V) containing different concentrations of pyriproxyfen in order to establish dose/response relationships. One ml of each oil formulation was spread on the foil (9.5 cm in diameter) to give concentrations of 0.04, 0.1 and 0.4 mg/$cm^2$ pyriproxyfen. Higher concentrations of oil in acetone than 1 : 50 (V/V) proved lethal to the insects. Therefore this same concentration was used also to test the efficacy of peanut oil and sesame oil as vehicles for the entry of pyriproxyfen into the insect body.

### Topical treatment of adults

Individual adult males and females emerged less than 24 hours ago were dosed topically on ventral abdomen with 10 μg pyriproxyfen in 0.5 μl acetone. Treated adults of both sexes were allowed access to untreated mates. Numbers of eggs produced and their viability were monitored for 8 days under laboratory conditions at 27℃ with a 15:9 L:D at 65% RH. Five pairs of insects were used in each experiment.

### Dipping treatment of eggs

A dose response relationship was determined by permitting five adult couples to oviposit for 24 hours on fresh cabbage leaves placed in a clear plastic cups (9 cm in diameter) . Eggs deposited were dipped for 10 seconds into aqueous dilutions of the EC containing 6.25, 25 and 100 ppm of pyriproxyfen. They were air-dried, stored in plastic containers and egg hatch plus numbers of dead and living larvae were recorded 6 days post treatment. Controls were untreated. All experiments were carried out under laboratory conditions at 25℃.

### Exposure of adults by tarsal contact with yellow target tape impregnated with pyriproxyfen

Adults emerging within 24 hours were separated individually into clear plastic cups (220 ml) to prevent spontaneous mating. These insects were used to test the effects of treating males or females alone or of treating both sexes prior to mating. Adults of precisely known age up to 12 hours post-emergence were obtained by allowing the first few adults to emerge from a batch of 400 pupae of the same age. Two hundred of these pupae were then enclosed individually in plastic tubes (120 mm × 5 mm in diameter) and refrigerated at 6℃ for 3-4 days. Upon returning to a higher temperature (19-24℃) adult emergence occurred spontaneously in about 85% of the pupae. Hence, females of known age were available for further experiments.

Insects were exposed individually for 10 min to Lano® tape lining the inner walls, bottom and screw top of a 30 ml glass vial. After treatment, each couple (untreated male × treated female, treated male × untreated female, treated male × treated female) was held individually in a clear conical plastic cup (220 ml, 6.5 cm in diameter at open end) containing a yellow stick-on paper rectangle (5.0 cm × 7.5 cm) impregnated with the juice from two young pressed radish leaves to provide an oviposition surface. Water was supplied on moistened filter paper but no food was supplied. The experimental room temperature varied from 20-24℃. Total numbers of eggs produced were recorded daily for 6-7 days and egg viability was monitored for 5 days following oviposition.

### Tarsal contact with oil formulations on aluminium foil

Five adult females were exposed individually to treated aluminium foils for 1, 10 or 60 min in a Petri dish before mating with untreated males. Foils were prepared with 0.04, 0.1 and 0.4 mg pyriproxyfen/$cm^2$. Pyriproxyfen was formulated in canola, sesame or peanut oil. After treatment, 5 adult couples were placed in a cage (14 cm × 14 cm × 14 cm) covered with nylon net containing young radish leaves and moistened filter paper. Egg production was recorded daily and egg hatch was monitored for 5 days following oviposition. Larval viability was monitored for a further 7 days.

## Results

### Topical treatment of adults

Results (**Table 1**) show that females treated with 10 μg pyriproxyfen in 0.5 μl acetone produced fewer and less viable eggs than controls and oviposition terminated prematurely on day 5. Untreated females mated to treated males produced only slightly fewer and slightly less viable eggs than untreated controls, although initiation of

**Table 1** Mean numbers of eggs produced by couples of *P. xylostella* in which one mate was treated topically with 10$\mu$g of pyriproxyfen (five replicates)

| Treated | Days following treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Total |
| ♀ | 12. 3 | 23. 4 | 10. 1 | 11. 9 | 2. 4 | − | − | − | 59. 8 |
| | (65. 8) | (37. 1) | (17. 4) | (15. 4) | (0) | | | | (39. 3) |
| ♂ | 0. 0 | 10. 0 | 36. 7 | 20. 0 | 2. 0 | 9. 5 | 4. 0 | 2. 0 | 94. 2 |
| | (−) | (100. 0) | (91. 7) | (80. 0) | (90. 5) | (46. 2) | (50. 0) | (50. 0) | (83. 5) |
| Control | 32. 0 | 30. 4 | 29. 4 | 15. 8 | 0. 0 | 6. 0 | 4. 0 | − | 139. 7 |
| | (98. 0) | (93. 9) | (91. 1) | (86.5) | (87. 1) | (80. 1) | (25. 0) | | (90. 0) |

( ) ; % eclosion of eggs produced each day after treatment
Note : Statistical analysis was not available since egg numbers were aggregated as total.

**Table 2** Egg and larval mortality of *P. xylostella* following treatment of eggs on cabbage leaf by dipping in EC solution of pyriproxyfen less than 24 hours after oviposition[a] (n=3)

| Treatment | Conc. | No. of eggs | | No. of larvae | |
|---|---|---|---|---|---|
| | (ppm) | Total | Dead | Dead [b] | Alive |
| Pyriproxyfen | 6. 25 | 146 | 12 | 131 | 3 |
| | 25 | 151 | 20 | 131 | 0 |
| | 100 | 120 | 33 | 87 | 0 |
| Control | − | 115 | 0 | 11 | 104 |

[a] Observations were made 6 dys after treatment.
[b] Larvae were dead soon after their eclosion.

oviposition appeared to be delayed by 24 hours.

### Treatment of eggs on cabbage leaf by dipping

**Table 2** shows that the hatchability of treated eggs was only reduced slightly but there was some indication of a dose/dependent effect. However, the overwhelming effect was the rapid and almost 100% lethality induced among larvae at the moment of eclosion even at the lowest concentration tested.

### Tarsal contact of adults with pyriproxyfen-treated yellow tape

Results shown in **Table 3** indicate that treated females were unaffected with respect to egg production, although egg viability was reduced, the greatest effect being on day 1 following treatment. Treated males had no effect upon their untreated mates, which were little different from untreated controls. However, where both male and female were treated there was seemingly a clear reduction in the numbers of eggs produced (**Fig.** 1A), although egg viability was unaffected. Nevertheless, differences of total egg numbers were insignificant either by Scheffé's multiple range test among the treatment or by *t*-test with control, $p < 0.05$.

**Figure 2** shows the effect of exposure of the adult female to pyriproxyfen treated tape upon the fully developed larva within one of her eggs, which fails to complete eclosion.

**Table 4** shows that treatment of adult females 0−1 hour following emergence reduced significantly their fecundity at day 1, 2 and 3 (Scheffé's test, $p < 0.05$), although egg viability was less affected with respect to untreated controls. Total egg production from the female of 0−1 hour treatment was also significantly less than that of controls (*t*-test, $p < 0.05$, see **Fig. 1**B).

Adult females treated 3 or 6 hours after emergence seemed to be less affected when compared to untreated controls (**Fig. 1**B). However, treatment of both sexes even 12−24 hours after emergence reduced significantly egg production on average 50% with respect to controls

**Table 3** Mean egg numbers (±SE) per female and percent mortality recorded daily after exposure by tarsal contact of male and/or female adult *P. xylostella* to yellow tape coated with pyriproxyfen (Lano®) within 24 hours following eclosion (n=5)

| Treated | Days after treatnment | | | | | | Total[A] |
|---|---|---|---|---|---|---|---|
| mate | 1 | 2 | 3 | 4 | 5 | 6[1] | |
| ♀ | 4.8±4.0 | 26.4±11.0 | 42.0±11.6 | 15.2±12.7 | 3.2±2.2 | 1.0±0.7 | 92.4±4.3 |
| | (4.8±4.8) | (29.6±23.9) | (11.5±4.9) | (6.7±6.7) | (33.3±16.7) | (0) | (12.5±6.3) |
| ♂ | 6.2±6.2 | 51.8±23.1 | 35.6±16.9 | 7.8±3.3 | 7.2±4.0 | 0.6±0.6 | 108.6±17.0 |
| | (9.7±iv) | (6.5±1.8) | (11.9±9.6) | (0) | (5.9±5.9) | (0) | (4.7±1.5) |
| ♀×♂ | 12.0±6.6 | 20.6±8.9 | 5.8±4.9 | 6.0±6.0 | 4.8±4.8 | 2.0±2.0 | 51.2±23.3 |
| | (17.0±13.8) | (3.5±1.7) | (0) | (20.0±iv) | (5.9±5.9) | (70.0±iv) | (5.3±4.6) |
| Control | 20.0±11.8 | 26.8±10.2 | 25.4±11.5 | 13.2±4.9 | 14.0±9.6 | 2.0±1.3 | 101.4±8.8 |
| | (3.0±1.8) | (2.7±2.7) | (1.1±0.7) | (1.4±1.4) | (7.2±6.0) | (0) | (3.1±1.1) |

[1] All females were dead on day 6. iv; invalid due to egg production from single ♀
Egg mortality ; figures in bracket (±SE). Eggs on day 5 were treated as dead.
[A] Total egg numbers were insiginificant among the treatments (Sceffe's multiple range test, $p<0.05$, see Fig. 1A for *t*-test comparison to untreated control).

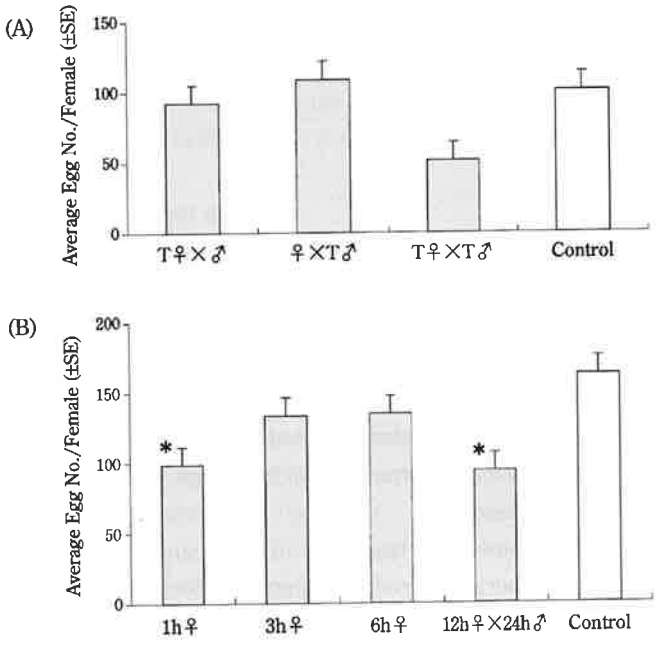


**Fig. 1** Total numbers of eggs (±SE) produced by *P. xylostella* adult females exposed by tarsal contact for 10 min to pyriproxyfen treated yellow tape (n=5) : A) within 24 hours following emergence (each treatments did not differ from control by *t*-test, $p < 0.05$), B) at precise times following emergence. T = treated (for A). Controls (for A and B) were untreated.
* ; significantly different when compared to Control (*t*-test, $p < 0.05$).

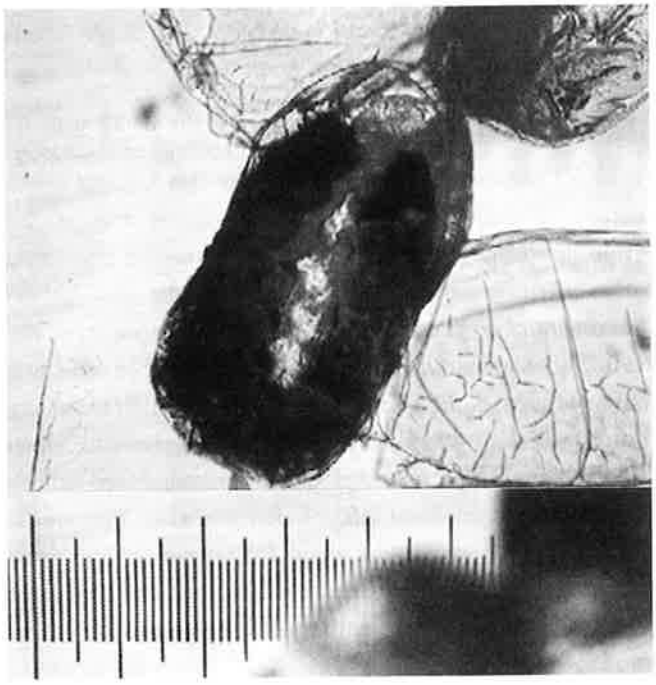

**Fig. 2** Larva of *P. xylostella* showing disruption of eclosion within an egg laid by a female 4 days after exposure for 10 min to pyriproxyfen treated yellow tape (photographed 8 days after oviposition, size; ×100, Scale; @=10 μm).

**Table 4** Daily egg production and percent egg mortality following tarsal exposure to yellow tape (Lano®, see text) of female adult *P. xylostella* at different times following eclosion (n=5)

| Feamle(♀)[1] | Days after treatment (mean±SE) | | | | | | Total[D] |
|---|---|---|---|---|---|---|---|
| Age(h) | 1[A] | 2[B] | 3[C] | 4 | 5 | 6 | |
| 0−1 | 19.6±4.1[a] | 69.6±5.6[a] | 8.4±1.9[a] | 1.0±0.3 | 0 | − | 98.6±5.1 |
| | (9.5±5.4) | (10.2±4.7) | (8.3±3.5) | (0) | (0) | | (9.9±4.4) |
| 3 | 18.4±10.0[b] | 84.2±14.4[b] | 25.0±6.6[b] | 4.4±3.7 | 1.6±0.9 | − | 133.6±17.2 |
| | (12.5±8.8) | (8.0±2.8) | (23.0±7.8) | (3.5±3.5) | (0) | | (28.1±18.1) |
| 6 | 43.2±2.3[c] | 59.4±7.5 | 26.8±9.6 | 5.6±2.9 | 1.4±1.0 | − | 135.0±14.3 |
| | (3.7±1.1) | (6.7±3.5) | (6.3±2.1) | (16.6±10.5) | (0) | | (6.1±1.3) |
| 12 | −[2] | 70.0±8.3[c] | 20.8±8.9 | 3.0±1.5 | 0.2±0.2 | − | 94.0±11.4 |
| | | (9.9±2.3) | (0) | (44.1±28.0) | (33.3±33.3) | | (8.7±1.8) |
| Control[3] | 87.3±1.7[d] | 33.3±16.2 | 39.3±2.0[c] | 2.7±0.9 | 0 | − | 162.7±18.5 |
| | (2.5±0.7) | (1.3±0.7) | (2.4±1.4) | (0) | (0) | | (2.4±0.8) |

−, All females were dead at day 5. Egg mortality ; figures in brackets (±SE).
Egg on day 5 were treated as dead.
[1] Males less than 10 hours old were paired with treated females except for 12 hours old females where males were 24 hours old at the time of mating.
[2] No data were collected on day 1 and were accumulated for 2 days.
[3] n=3.
[A−C] Different letters on day 1, 2 and 3 were significant between the treatments (Scheffe's multiple range test, $p < 0.05$ ; no letter indicates in significant difference among the treatments).
[D] Total egg numbers did not differ between the treatments by scheffé's, $p<0.05$. See Fig. 1B for *t*-test comparison to untreated control.

(*t*-test, $p < 0.05$, see **Fig. 1**B) though differences detected by Scheffé's test ($P < 0.05$) were insignificant among the treatments.

### Tarsal contact treatment with aluminium foil coated with pyriproxyfen in vegetable oil formulations

In experiments to test the effects of different concentrations of pyriproxyfen presented in oil on an aluminium foil no dose response relationship was seen. Surprisingly, the lowest concentration tested (0.04 mg/cm$^2$) had the greatest effect, reducing the numbers of eggs produced daily and reducing the viability of those that were produced with respect to the two higher doses (**Fig. 3**) and the untreated controls (see **Fig. 4**). Hence, in further experiments the lowest concentration was used. As shown in **Fig. 4** an exposure time of 1 min to 0.04 mg/cm$^2$ had little effect on egg production, although egg viability was reduced with respect to controls. However, increasing exposure times reduced both egg production and viability: the greatest effect was obtained with a 60 min exposure when both parameters fell below 20% of control values (**Fig. 4**). Ten minutes exposure was almost as effective since egg viability fell from 50% on day 1 to zero by day 4.

A comparison between the effects of the canola oil formulation and formulation in two other vegetable oils is shown in **Fig. 5**. It appears that sesame oil was superior to either of the other two in terms of suppressing egg production and egg viability after day 1. Peanut oil was equally good at suppressing egg production, although the viability of eggs that were produced remained high.

## Discussion

When applied topically to adult female *P. xylostella*, within 24 hours following emergence, pyriproxyfen caused a 50% reduction in egg production and the viability of eggs produced was also reduced with respect to untreated controls. Treatment of males, which were then mated to untreated females, was less effective, causing only a slight reduction in egg numbers and viability.

Tarsal contact with a commercially available, pyriproxyfen-impregnated tape (Lano®) had little effect when either the male or the female were exposed before