

ACARICIDE-MEDIATED SUPPRESSION OF *PANONYCHUS CITRI* (MCGREGOR) (ACARI: TETRANYCHIDAE) IN PEAR ORCHARDS IN JAPAN

Tetsuo Gotoh¹, *, Tomoko Korenaga¹, Satomi Ikejima¹ and Tsutomu Hoshino²

1. Laboratory of Applied Entomology and Zoology, Faculty of Agriculture, Ibaraki University, Ami, Ibaraki 300-0393, Japan (e-mail: gotoh@mx.ibaraki.ac.jp, *corresponding author); 2. Faculty of Agriculture, Niigata University, Niigata 950-2181, Japan.

ABSTRACT - Population density of the citrus red mite, *Panonychus citri* (McGregor), in Japanese pear orchards remained low until mid-August, even after inoculation of pear leaves with a considerable number of adult female *P. citri* from May onwards. This raised the possibility that pear leaves contain a natural compound that suppresses an increase of *P. citri* populations. The rate of development from larva to adult was significantly lower on leaves collected in July than on leaves collected earlier or later, in several years. The population suppression was caused by molting deterrent activity and ovicidal activity, according to our close observation in the laboratory. To clarify whether a natural pear compound caused this molting deterrence, a methanol crude extract of pear leaves was isolated and added to a newly developed artificial diet, consisting of sodium caseinate, sucrose, levulose, glucose and inositol. The compound extracted from pear leaves resulted in the molting deterrence as observed on pear leaves. Based on infrared and NMR spectral analysis, the compound extracted from pear leaves closely resembled the synthetic acaricide hexythiazox. Furthermore, the LC₅₀ values of the compound extracted from pear leaves for ovicidal activity of *P. citri* eggs and for deterrence of molting to protonymphs were similar to those of hexythiazox. These results strongly suggest that the molting deterrent extracted from pear leaves was in fact hexythiazox, an acaricide in use on pear trees, rather than a natural product. This suggestion becomes even stronger, considering that the molting deterrence was observed in a hexythiazox-spray year, but not in a non-spray year.

Keywords - Acari, Tetranychidae, artificial diet, hexythiazox, molting deterrent, *Panonychus citri*, pear, Japan.

INTRODUCTION

Spider mites such as *Tetranychus urticae* Koch and *Panonychus citri* (McGregor) are serious pests of pear orchards (Gotoh, 1997; Kishimoto, 2002). However, even when pear leaves were inoculated with a considerable number of adult females of *P. citri* at different times from May to September, the population density did not increase on leaves inoculated before mid-August (Gotoh and Kubota, 1997). This could have been due to spraying with acaricides by the farmers. One such acaricide that is often used on pear trees and many other crops is hexythiazox, a synthetic chemical that has excellent ovicidal and molting deterrent activities to various kinds of mites. However, in view of the fact that many plants produce chemicals as defense substances against herbivores (Ujihara *et al.*, 1995; Leskovar and Kipp Boales, 1996; Banaag *et al.*, 1997; Konno *et al.*, 2004; Dabrowski and Seredynska, 2007),

another possibility is that pear leaves contain a natural compound that suppresses an increase in *P. citri* population during spring and summer.

To test the hypothesis that pear plants produce a natural acaricide, we (1) examined seasonal changes in developmental success of juveniles of *P. citri* on pear leaves collected at different times of year to determine whether molting deterrent activity is related to hexythiazox spraying, (2) established a bioassay system to isolate and analyze the compound which has molting deterrent activity derived from pear leaves, and (3) determined LC₅₀ values of the compound derived from pear leaves for ovicidal activity and molting deterrence to *P. citri*.

MATERIALS AND METHODS

Mites - *Panonychus citri* was collected from Japanese holly tree, *Ilex crenata* Thunb., at Chiyoda

(36°09'N, 140°14'E), Ibaraki, on 6 May 1993. The mites were maintained on leaf discs (ca. 16 cm²) of bitter orange (*Citrus aurantium* L.) placed on a water-saturated polyurethane mat in Petri dishes (9 cm in diameter). Mite rearing and all experiments were carried out in a climate-controlled chamber at 25±1 °C and a 16hL8hD photoperiod (Gotoh *et al.*, 2007).

Developmental success - Pear leaves were collected in the same pear orchard of Chiyoda (field-collected pear leaves) at semimonthly or monthly intervals from May 1994 to October 2000 except during leaf-fall periods (Table 1). Ten newly hatched larvae were randomly selected from the stock culture and placed onto each of 6 to 12 leaf discs (ca. 16 cm²) of the field-collected pear leaves. The survivors were counted daily on all leaf discs until the mites reached adulthood. The survival rate from larva to adult was defined as (number of adults emerged/number of larvae inoculated) × 100. Females in teleiochrysalis stage that developed on the above-mentioned leaf discs were individually transferred onto leaf discs made from newly collected pear leaves and kept with two adult males for mating. After initiating oviposition, each female was allowed to lay eggs for 5 days. Hatching and survival of the offspring were checked daily until they reached adulthood.

According to farmer's spraying records, hexythiazox was sprayed on the trees on three occasions during the study period: on 8 July of 1995 and 1997 and on 3 July of 1998. However, we cannot rule out the possibility that the farmer failed to record some sprayings.

Hexythiazox has ovicidal and molting deterrent activities, inhibits hatching and causes lower survival of immature mites hatched from F₁ eggs (Yamada *et al.*, 1987). In a citrus grove, hexythiazox was found to remain active for about 50 days (Yamada *et al.*, 1987).

Artificial diet - Because of the possibility that a compound in pear leaves could prevent mites from molting from the quiescent stage to the next active stage, a meridic diet was used for mites from the protonymph stage to deutonymph stage. In preliminary experiments, three meridic spider mite diets, KR (Kantaratanakul and Rodrigues, 1979), vdG (van der Geest *et al.*, 1983) and HB (Hare and Bethke, 1988), resulted in low developmental rates from protonymph to deutonymph (Table 3). As a result, we attempted to improve the HB diet, which resulted in the highest developmental rate. The improved composition of the IU diet (Ibaraki University diet) was as follows: sodium caseinate 4,000 mg, sucrose 4,500 mg, levulose 750 mg, glucose 750 mg and inositol 25 mg. The KR, vdG and HB diets were prepared as originally described. The IU diet was prepared as follows: all components were dissolved in approximately 80 ml of distilled water, pH was adjusted to 6.0-6.5 by 0.16N KOH and then the volume was increased to 100 ml by adding distilled water. Thereafter, the diet was autoclaved at 120 °C for 20 min. The diet was offered to the mites through a

parafilm membrane. A piece of parafilm, sterilized by immersion in 70% ethanol, was stretched across the top of a 3 cm in diameter and 2 cm in height sterile Petri dish. One ml of the diet was applied to the membrane and a second piece of sterile parafilm was stretched over the diet and sealed against the side of the Petri dish. Care was taken that no air bubbles were trapped underneath the second membrane. A steep ridge of the Petri dish ("diet container") was surrounded by a piece of wet tissue paper and the diet containers were placed in another Petri dish (9 cm in diameter and 1.5 cm in height) filled with water to confine the mites on the diet area. Twenty newly emerged protonymphs of *P. citri* were randomly selected from the stock culture and placed onto each of 10 diet containers. The survivors and their stages were recorded every day on all diet containers until the mites reached deutonymphs. Deutochrysalis that had not molted to deutonymphs within three days after reaching that stage were scored as "dead." The diets were renewed on the 7th day if experiments were continued more than 7 days.

Bioassay - Ten µl methanol crude extract (0.1 g fresh leaf equivalent/ml, 10%, w/v) from pear leaves collected in 17 July of 1994 and 1998 was added to the artificial diet, and 10 µl methanol was added to the control diet. As the extract became more pure, extract added to the artificial diet was gradually reduced from 10 µl to 1.5 µl.

Protonymphs were used to evaluate the molting deterrent activity of the extracts from all fractions obtained at each purification process. Mites were randomly selected from the stock cultures and then transferred to the diet containers. The survivors and their stages were recorded every day on all containers until the mites reached deutonymphs as mentioned above.

Extraction and analyses - Pear leaves (ca. 1-2 kg) were collected on 17 July 1994, 18 September 1994, and 17 July 1998 at Chiyoda, Ibaraki. Sampling dates were determined on the basis of the results of developmental success experiments on field-collected pear leaves. The collected leaves were rinsed with distilled water, and then the midribs were cut out and discarded. Aliquots of fresh leaves (10 g) were quick frozen in liquid nitrogen, packed in vinyl bags and stored at -20 °C until use.

Eight packets of leaves from -20 °C freezer were put into an eggplant-shaped flask and extracted with 800 ml methanol for 24 h at room temperature. During the extraction, the flask was occasionally shaken. The extract was evaporated to dryness with a rotary vacuum evaporator, yielding a residue of ca. 4 g. The residue was separated according to the scheme shown in Fig. 1. The extract was partitioned into ether, n-butanol and water-soluble portions. The ether portion, whose molting deterrent activity was determined by bioassay, was chromatographed on a silica gel column (Fuji Silysia Chemical Co., Ltd., Aichi, Japan, BW-820MH) and eluted with hexane (80): ethyl acetate (20). Eluates were collected and analyzed by gel filtration (Sephadex, LH-20) and HPLC using a

Table 1. Seasonal changes in developmental success (mean \pm S.D.) of *Panonychus citri* from larva to adult when reared on pear leaves collected at different times of year. Dates in which leaves were possibly sprayed with hexythiazox are typed in bold face and placed in brackets.

| Date of sampling | n | % Maturity ¹ (range) | Date of sampling | n | % Maturity ¹ (range) |
|--------------------------------|-----|---------------------------------|---|-----|---------------------------------|
| 1994 | | | 1998 | | |
| May 11 | 60 | 86.7 \pm 15.06 a (60-100) | June 15 | 140 | 85.7 \pm 16.97 a (50-100) |
| June 16 | 59 | 85.0 \pm 8.37 a (70-90) | July 1 | 120 | 93.3 \pm 9.85 a (70-100) |
| July 13 | 62 | 11.4 \pm 11.76 b (0-30) | [July 13] | 120 | 20.8 \pm 20.65 b (0-60) |
| Aug. 16 | 62 | 80.3 \pm 14.16 a (60-100) | Aug. 1 | 120 | 81.7 \pm 16.42 a (50-100) |
| Sept. 14 | 50 | 80.0 \pm 10.00 a (70-90) | Aug. 19 | 120 | 85.0 \pm 16.50 a (60-100) |
| Oct. 19 | 60 | 76.7 \pm 27.33 a (30-100) | Sept 15 | 120 | 91.7 \pm 9.37 a (80-100) |
| F _{5,29} ² | | 13.259*** | Oct. 20 | 120 | 79.2 \pm 16.76 a (60-100) |
| 1995 | | | F _{6,77} | | 19.446*** |
| May 18 | 118 | 87.1 \pm 11.58 ab (70-100) | 1999 | | |
| June 3 | 120 | 83.3 \pm 15.57 ab (50-100) | June 22 | 120 | 85.8 \pm 9.96 a (70-100) |
| June 19 | 121 | 94.2 \pm 5.09 a (90-100) | July 2 | 120 | 91.7 \pm 9.37 a (70-100) |
| July 8 | 121 | 95.0 \pm 7.98 a (80-100) | July 12 | 120 | 92.5 \pm 8.66 a (80-100) |
| [July 24] | 121 | 67.7 \pm 28.40 bc (20-100) | Aug. 9 | 110 | 78.2 \pm 17.22 a (50-100) |
| Aug. 2 | 121 | 53.3 \pm 37.01 c (0-100) | Aug. 23 | 120 | 75.0 \pm 20.67 a (40-100) |
| Aug. 19 | 122 | 85.0 \pm 12.48 ab (70-100) | Sept. 15 | 110 | 83.6 \pm 8.09 a (70-100) |
| Sept. 15 | 120 | 64.0 \pm 21.76 bc (30-100) | Oct. 18 | 120 | 82.5 \pm 21.79 a (20-100) |
| Oct. 27 | 120 | 76.7 \pm 11.55 abc (50-100) | F _{6,75} | | 2.497* |
| F _{8,99} | | 5.968*** | 2000 | | |
| 1996 | | | June 14 | 120 | 82.5 \pm 17.65 ab (50-100) |
| July 1 | 120 | 23.3 \pm 35.25 c (0-80) | July 1 | 120 | 94.2 \pm 9.96 a (70-100) |
| July 9 | 120 | 24.2 \pm 34.23 bc (0-100) | July 14 | 120 | 89.2 \pm 11.65 ab (70-100) |
| Aug. 2 | 120 | 60.8 \pm 36.55 ab (0-100) | July 31 | 120 | 66.7 \pm 23.09 b (30-100) |
| Aug. 26 | 120 | 29.2 \pm 34.23 bc (0-80) | Aug. 18 | 120 | 85.8 \pm 13.11 ab (60-100) |
| Oct. 15 | 120 | 84.2 \pm 11.65 a (70-100) | Sept. 14 | 120 | 70.0 \pm 22.56 b (20-100) |
| F _{4,55} | | 8.114*** | Oct. 18 | 120 | 75.8 \pm 25.03 ab (30-100) |
| 1997 | | | F _{6,77} | | 3.719** |
| June 19 | 120 | 90.0 \pm 12.06 ab (60-100) | ¹ Average of 12 replicates except in 1994, which was 6 replicates. Each replicate consisted of ca. 10 individuals. | | |
| June 26 | 120 | 89.2 \pm 9.96 ab (70-100) | ² Data were analysed using one-way ANOVA; *: p<0.05; **: p<0.01; ***: p<0.001. Values in a column followed by the same letter were not significantly different at the 5% level (Tukey-HSD test). | | |
| July 5 | 120 | 94.2 \pm 9.00 a (70-100) | n, Total number of larvae tested. | | |
| [July 11] | 120 | 4.2 \pm 6.69 c (0-20) | | | |
| Aug. 15 | 120 | 81.7 \pm 15.28 ab (50-100) | | | |
| Sept. 16 | 120 | 72.5 \pm 24.91 b (30-100) | | | |
| Oct. 19 | 120 | 84.2 \pm 14.43 ab (60-100) | | | |
| F _{6,77} | | 40.739*** | | | |

Table 2. Number of eggs laid by females of *Panonychus citri* that developed on the field-collected pear leaves during the first five days of the oviposition period, hatchability, juvenile survival rate and offspring sex ratio on pear leaves collected at different dates.

| Date of sampling ¹ | n | No. of eggs per female ² | Hatchability (%) ² | % Survival in immatures ² | % Female offspring ² |
|---------------------------------|----|-------------------------------------|-------------------------------|--------------------------------------|---------------------------------|
| 1998 | | | | | |
| June 15 | 31 | 28.5 ± 6.24 ab | 98.4 ± 2.62 a | 96.7 ± 3.72 a | 73.8 ± 5.36 a |
| July 1 | 25 | 26.6 ± 4.69 abc | 96.4 ± 4.72 a | 94.5 ± 7.13 ab | 74.8 ± 7.14 a |
| July 13 | 28 | 24.1 ± 4.96 bc | 60.4 ± 31.66 b | 57.9 ± 31.03 c | 70.6 ± 18.79 a |
| Aug. 1 | 27 | 30.1 ± 4.45 a | 93.9 ± 7.71 a | 86.6 ± 10.43 b | 72.9 ± 6.90 a |
| Aug. 19 | 23 | 22.9 ± 5.24 cd | 97.4 ± 5.66 a | 90.8 ± 9.65 ab | 72.8 ± 4.85 a |
| Sept 15 | 20 | 19.4 ± 3.08 de | 95.7 ± 4.47 a | 90.9 ± 8.27 ab | 72.6 ± 5.09 a |
| Oct. 20 | 26 | 19.2 ± 3.56 e | 96.9 ± 5.11 a | 91.1 ± 9.20 ab | 68.0 ± 5.91 a |
| F _{6,173} ³ | | 19.881*** | 28.799*** | 18.799*** | 2.239* |
| 1999 | | | | | |
| June 22 | 32 | 26.8 ± 6.15 a | 96.3 ± 4.86 | 91.0 ± 11.67 | 71.8 ± 5.37 |
| July 2 | 28 | 24.6 ± 4.61 a | 96.2 ± 5.12 | 92.6 ± 8.06 | 70.5 ± 5.36 |
| July 12 | 31 | 23.1 ± 6.28 ab | 95.1 ± 7.39 | 90.9 ± 9.29 | 71.7 ± 6.56 |
| Aug. 9 | 19 | 20.9 ± 6.60 bc | 96.7 ± 5.07 | 93.0 ± 9.11 | 71.8 ± 7.22 |
| Aug. 23 | 17 | 21.9 ± 4.10 ab | 96.8 ± 3.65 | 93.4 ± 7.52 | 70.9 ± 3.26 |
| Sept. 15 | 20 | 17.0 ± 5.12 c | 91.8 ± 5.45 | 93.6 ± 5.85 | 69.2 ± 6.54 |
| Oct. 18 | 20 | 20.4 ± 5.05 bc | 94.6 ± 6.81 | 92.5 ± 6.70 | 72.8 ± 5.10 |
| F _{6,160} | | 7.863*** | 1.897ns | 0.146ns | 0.849ns |

¹ The sampling dates were the dates when pear leaves were collected to examine the developmental rates from larva to adult. For the oviposition experiment, different leaves were collected at the same field four days later.

² Mean ± S.D.

³ Data were analysed using one-way ANOVA; *: p<0.05; ***: p<0.001; ns: p<0.05. Values in a column followed by the same letter were not significantly different at the 5% level (Tukey-HSD test).
n, number of females tested.

Hewlett-Packard HP1100 apparatus. The biologically active fraction evaluated by bioassay was further purified with a preparative TLC silica gel plate (Wako Chemical Co., Ltd., Osaka, Japan, silica gel 70 F254) using a solvent system of hexane:ethyl acetate, 80:20. After development, eluates with the same *R_f* value on TLC were combined.

Infrared (IR) spectra (KBr disk) of the compound extracted in this study [active ingredient (A.I.) is unknown] and authentic hexythiazox (99.5% A.I.) provided by Nippon Soda Co., Ltd. were obtained with a JASCO Model A-202 instrument (Nihon-Bunkou Co., Ltd, Tokyo, Japan). Nuclear magnetic resonance (NMR) spectra of both chemicals were obtained with a Bruker DMX600 with CD₃OD as the solvent.

Toxicity test (LC₅₀) - To know the biological activity of the purified compound and hexythiazox, ovicidal

and molting deterrent activities of the two chemicals were compared. For testing ovicidal activity, twenty pregnant females of *P. citri* were transferred from the stock culture onto a new leaf disc (ca. 4 cm²) of bitter orange placed on a water-saturated layer of cotton wool in a Petri dish (9 cm in diameter) and allowed to lay eggs for 24 h. Then the mites were removed, and the eggs were counted on each leaf disc. Suspensions (2 mg/cm²) of the compound and hexythiazox were sprayed onto the leaf disc with eggs using a rotary spray tower (Mizuho Scientific Co., Ltd., Nagoya, Japan). After treatment, eggs were kept at 25 °C and a 16hL8hD photoperiod, until egg mortality was assessed after 192 h. Eggs that had not hatched were scored as "dead". Control leaf discs were sprayed with distilled water.

For testing the molting deterrent ability of the compound and hexythiazox against the protonymphs, ten

Table 3. Development and survival of *Panonychus citri* on various artificial diets.

| Diet ¹ | % Individuals developed from protonymph to ² | |
|--------------------------------|---|------------------------|
| | Deutochrysalis | Deutonymph (range) |
| vdG | 10.5 ± 9.85 b | 7.5 ± 8.25 c (0-25) |
| KR | 23.0 ± 14.94 b | 10.0 ± 10.00 c (0-30) |
| HB | 83.5 ± 13.13 a | 61.5 ± 18.11 b (35-90) |
| IU | 95.5 ± 6.85 a | 86.0 ± 6.99 a (80-95) |
| F _{3,36} ³ | 73.866*** | 73.770*** |

¹ vdG: (van der) Geest *et al.* (1983); KR: Kantaratanakul and Rodrigues (1979); HB: Hare and Bethke (1988) (sodium caseinate 8,000 mg, sucrose 9,000 mg, levulose 1,500 mg and glucose 1,500 mg were dissolved and finally measured up to 100 ml by distilled water. The pH was adjusted to 6.0-6.5 by 0.16N KOH); IU: the present study.

² Mean ± S.D. Average of 10 replicates. Each replicate consisted of 20 individuals.

³ Data were analysed using one-way ANOVA; ***: $p < 0.001$. Values in a column followed by the same letter were not significantly different at the 5% level (Tukey-HSD test).

protonymphs of *P. citri* were transferred from the stock culture onto a diet container to which was added either the compound or hexythiazox. The diet containers were put in Petri dishes (9 cm in diameter) filled with water and were kept at 25 °C under a 16hL8hD photoperiod for 92h. Then, molting to deutonymph or later stages was assessed. Mites that failed to molt to deutonymphs were scored as "dead." Six to seven concentrations per chemical with each of four to five replicates were tested. Pooled data for either eggs or nymphs were subjected to Probit analysis and LC₅₀ was estimated (Finney, 1971) using Abbott's correction for natural mortality (Abbott, 1925).

Statistical analysis - One-way analysis of variance (ANOVA) was used to compare data sets. Means were checked with Tukey-HSD test (SPSS, 2002). To normalize the data, egg number was log-transformed. Egg hatchability, juvenile survival rate, molting rates and offspring sex ratio were arcsine-root transformed (Sokal and Rohlf, 1995).

RESULTS

Developmental success - The developmental rate from larva to adult on pear leaves was significantly lower in July in 1994, 1995, 1997 and 1998 than in other times of the year (Table 1). In 1999 and 2000, the developmental rate was high throughout the year with some fluctuations. The overall developmental rate in 1996 was lower than that in other years, but the reason for this was unclear.

On 13 July 1998 when the developmental rate was low, the hatchability of eggs laid by females that developed on pear leaves and the juvenile survival rate (F₁ generation) were lower than they were on leaves collected at other times (Table 2). In contrast, in July 1999 when the developmental rate was high, egg hatchability and juvenile survival rate were high throughout the year (Table 2).

Thus, pear leaves collected in July in some years conspicuously suppressed juvenile survival of *P. citri* and even reduced egg hatchability and survival rate in the next generation. Hexythiazox spraying was positively related to the lower developmental rate observed on all spraying occasions, except in 1994. However, we suspect that spraying occurred in early July of 1994 but was not recorded (Table 1), because (1) hexythiazox was commonly used in Japanese pear orchards in the early 1990s, (2) an artificial diet to which was added a methanol crude extract from pear leaves collected on 17 July of 1994 had the same molting deterrent activity as that from pear leaves collected on 17 July of 1998, and (3) a discrepancy between the molting deterrence to *P. citri* juveniles and hexythiazox spraying occurred only in 1994.

Hexythiazox was considered to retain residual activity for at least ten days in the pear orchard, because the molting deterrent activity was clearly observed at the 10th day (1998) after spraying, while it was extremely low at the 16th day (1995).

Artificial diet - Protonymphs of *P. citri* successfully developed to deutonymphs on the four artificial diets tested (Table 3). The vdG and KR diets, which were made for *T. urticae*, resulted in lower developmental rates from protonymph to both deutochrysalis and deutonymph than the HB and IU diets, which were made for *P. citri*. The developmental rate from protonymph to deutochrysalis in the HB diet was similar to that of the IU diet, but the rate from deutochrysalis to deutonymph was significantly lower in the former than in the latter ($p < 0.05$, Tukey-HSD test). Therefore, the IU diet was used for bioassay of the compound extracted from pear leaves.

Bioassay of methanol extract from pear leaves - A methanol crude extract of pear leaves collected in July strongly deterred molting of *P. citri* protonymphs (Table 4). When methanol extract originating from frozen pear leaves was added to the artificial diet, most protonymphs

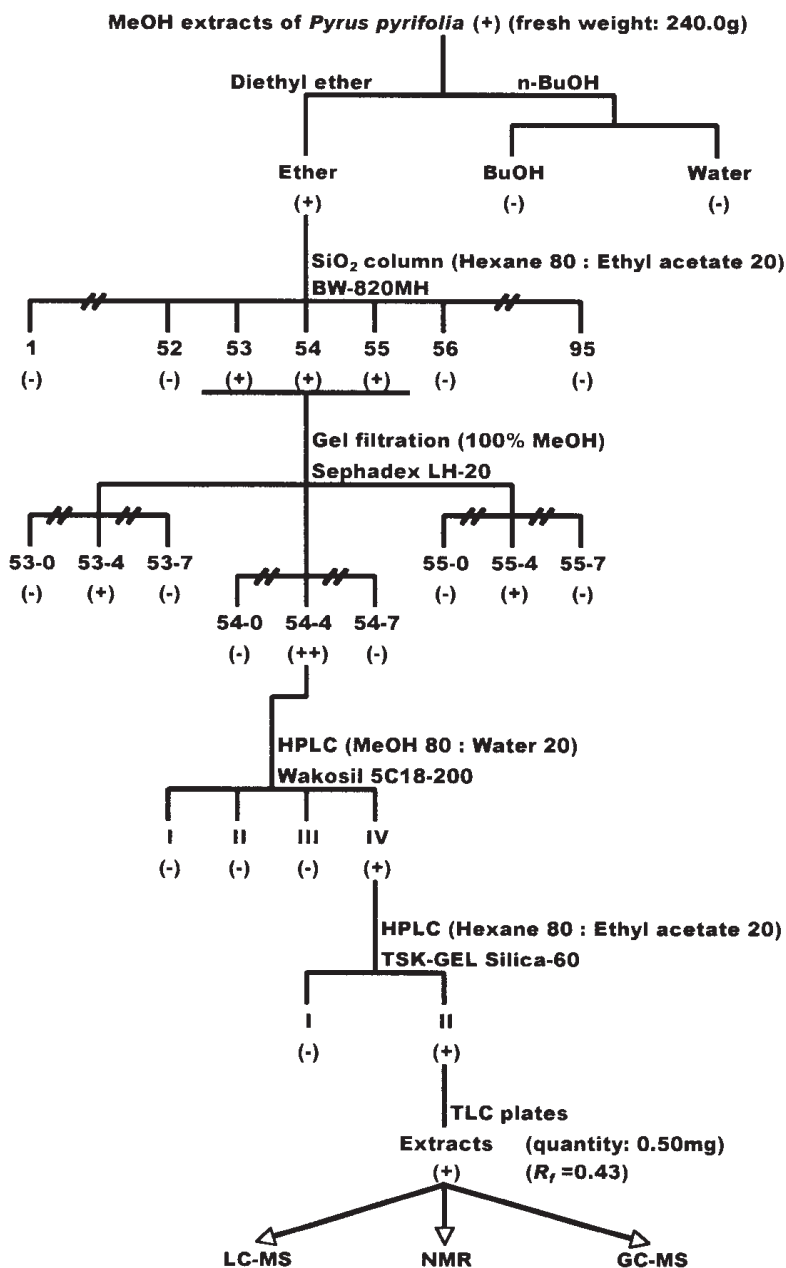


Fig. 1. Fractionation scheme of pear leaf extract and molting deterrent activity to *Panonychus citri* [(++), strong activity; (+), activity; (-), no activity].

inoculated successfully reached deutochrysalis. However, the molting rate from deutochrysalis to deutonymph was strongly affected by the amount of extract: the molting rate was only 5.6% when 5% methanol extract (0.05 g fresh leaf equivalent/ml) was added to the artificial diet and 0% when 10% methanol extract was added (Table 4). In contrast, when added methanol extract originated from pear leaves collected in September, no influence on molting was observed regardless of the concentration added to the artificial diet (Table 4).

IR and NMR analyses of the compound - The IR spectrum of the compound was sufficiently consistent with that of hexythiazox over an entire range of wave number, including the fingerprint region (data not shown). Furthermore, the ¹³C- and ¹H-NMR spectra of the isolated compound were also superimposable on those of hexythiazox. The ¹³C-NMR spectra are depicted in Fig. 2. As shown in Fig. 3B, these signals were assigned to hexythiazox to C1-C17.

Table 4. Development and survival of *Panonychus citri* on artificial diet containing various concentrations of pear extracts obtained from leaves collected in July and September, 1994.

| Concentration of pear extracts ¹ | Replicate (no. of protonymphs inoculated) | % Individuals developed from protonymph to ² | |
|---|---|---|------------------------|
| | | Deutochrysalis | Deutonymph (range) |
| Leaves collected in July | | | |
| 1% | 5 (106) | 96.3 ± 6.20 | 79.7 ± 7.03 a (71-88) |
| 2% | 5 (106) | 97.3 ± 4.07 | 52.1 ± 7.00 b (41-58) |
| 5% | 5 (105) | 89.6 ± 5.93 | 5.6 ± 7.71 c (0-19) |
| 10% | 5 (109) | 85.3 ± 11.14 | 0.0 ± 0.00 c (0-0) |
| Leaves collected in September | | | |
| 1% | 5 (105) | 95.2 ± 3.14 | 74.8 ± 8.81 a (63-79) |
| 2% | 5 (102) | 98.9 ± 2.36 | 71.9 ± 7.30 ab (62-79) |
| 5% | 5 (108) | 90.0 ± 12.00 | 78.6 ± 10.99 a (63-92) |
| 10% | 5 (120) | 84.9 ± 15.44 | 70.4 ± 6.79 ab (64-82) |
| Check (containing 10 µl methanol) | | | |
| 0% | 5 (91) | 93.2 ± 5.19 | 83.6 ± 4.74 a (76-89) |
| $F_{8,36}$ ³ | | 1.915ns | 88.785*** |

¹ Mites were reared on the IU artificial diet containing pear extracts of 1-10% (W/V).

² Mean ± S.D.

³ Data were analysed using one-way ANOVA; ***: $p < 0.001$; ns: not significantly different at the 5% level. Values in a column followed by the same letter were not significantly different at the 5% level (Tukey-HSD test).

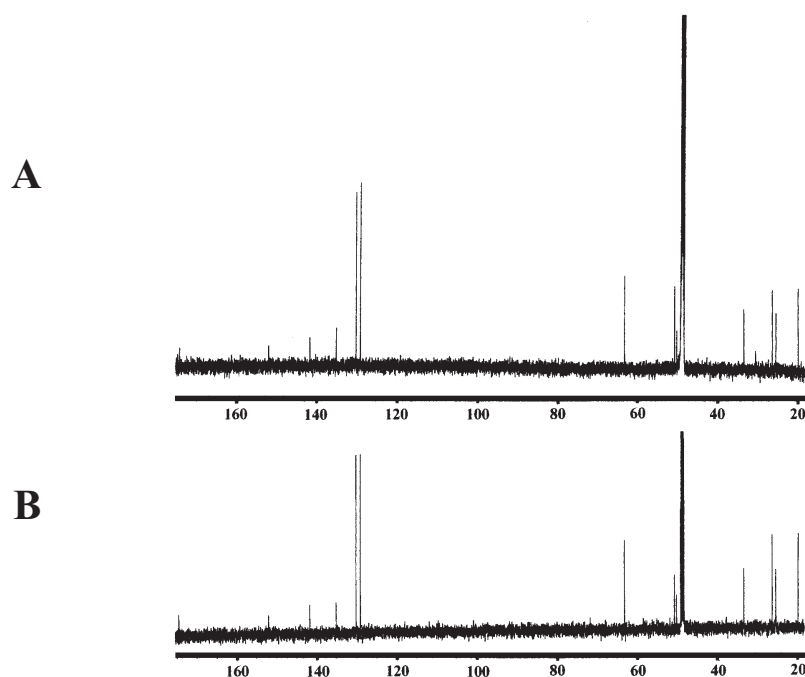
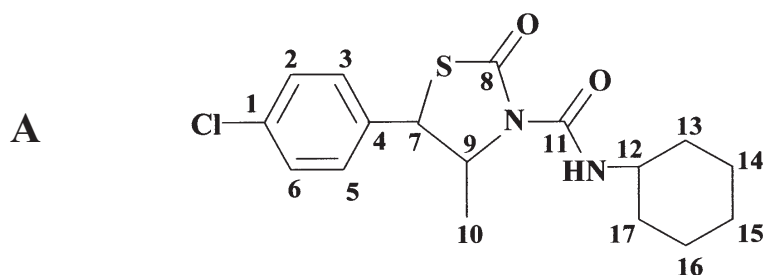


Fig. 2. Comparison of the ¹³C-NMR spectra of the compound from pear leaves (A) with authentic hexythiazox (B), which were dissolved in CD₃OD.

Table 5. LC₅₀ (ppm; mg l⁻¹) values in *Panonychus citri* eggs sprayed with and in *P. citri* protonymphs reared on artificial diet containing the compound extracted from pear leaves (pear compound) and the synthetic chemical (hexythiazox).

| Chemical | n | LC ₅₀ (95% CL) | Slope ± S.E. | R ² | χ ² | df |
|-------------------|-------|------------------------------|---------------|----------------|----------------|----|
| Egg | | | | | | |
| Pear compound | 1,260 | 9.232 (8.219-10.369) | 2.852 ± 0.116 | 0.9935 | 6.334 | 4 |
| Hexythiazox | 1,206 | 7.748 (6.836-8.781) | 2.658 ± 0.054 | 0.9984 | 1.357 | 4 |
| Protonymph | | | | | | |
| Pear compound | 187 | 0.077 (0.056-0.104) | 2.622 ± 0.322 | 0.9432 | 9.399 | 4 |
| Hexythiazox | 363 | 0.032 (0.025-0.042) | 1.657 ± 0.195 | 0.9352 | 6.918 | 5 |

n, Total number of eggs or protonymphs tested.



B

| Carbon | Compound | Hexythiazox |
|--------|----------|-------------|
| 1 | 135.2 | 135.2 |
| 2 | 129.2 | 129.2 |
| 3 | 130.3 | 130.3 |
| 4 | 141.8 | 141.8 |
| 5 | 130.3 | 130.3 |
| 6 | 129.2 | 129.2 |
| 7 | 50.4 | 50.4 |
| 8 | 174.5 | 174.5 |
| 9 | 63.4 | 63.4 |
| 10 | 20.0 | 20.0 |
| 11 | 152.1 | 152.1 |
| 12 | 51.0 | 51.0 |
| 13 | 33.7 | 33.7 |
| 14 | 25.6 | 25.6 |
| 15 | 26.6 | 26.6 |
| 16 | 25.6 | 25.6 |
| 17 | 33.7 | 33.7 |

Fig. 3. Chemical structure of hexythiazox (A) and the chemical shifts (δ_c) of the compound from pear leaves and those of authentic hexythiazox (B) were given in ppm relative to the residual solvent peak (CD₃OD, 49.0 ppm).

LC₅₀ values in eggs and nymphs - In eggs, the LC₅₀ value for ovicidal activity of the compound extracted from pear leaves was 9.232 ppm (mg l⁻¹), which was similar to the value (7.748 ppm) for hexythiazox (Table 5). The LC₅₀ value for molting deterrence for the compound (0.077 ppm) and hexythiazox (0.032 ppm), were similar (Table 5).

DISCUSSION

The survival and development of arthropod herbivores are known to be influenced by secondary chemicals (Ryan, 1990; Roininen *et al.*, 1993; Bernays and Chapman, 1994) and plant extracts (Rasikari *et al.*, 2005; Wang *et al.*, 2007). For example, in *Hydrangea serrata* (Thunb.) in Kyoto, Japan, the phylloidalin content was highest in July when the plants flowered (Ujihara *et al.*, 1995). In Ibaraki, Japan, the *Tetranychus kanzawai* Kishida population crashed in June when hydrangea flowered without much year-to-year variation (Gotoh and Gomi, 2000). Although they were not examined on the seasonal variation in the content of phylloidalin, these results suggest that the drastic decline in survival of *P. citri* juveniles in July (Table 1) is caused by chemicals contained in pear leaves. However, the cause of year-to-year variation in effects on juvenile survival rate in *P. citri* has remained unknown till now.

The artificial diet described in this study allowed development from protonymph to deutonymph in *P. citri* with survival rates of more than 80% at each trial, but it failed to allow completion of development. Van der Geest *et al.* (1983) showed that *T. urticae* could complete its life cycle on an artificial diet. This diet is presently considered to be the best for spider mites. However, in our study, *P. citri* did not grow well on this diet, possibly because the optimal concentrations of major nutrients (sugar and sodium caseinate) were 4 to 10 times higher for *P. citri* than for *T. urticae* (Hare and Bethke, 1988).

In our bioassay experiments, the biologically active fraction closely resembled hexythiazox based on IR and NMR spectra (Figs. 2, 3). Hexythiazox inhibits hatching and molting, but it does not kill adult mites (Yamada *et al.*, 1987; Yamamoto *et al.*, 1995). These activities are consistent with the results obtained in this study on developmental success and hatchability of the F₁ eggs reared on field-collected pear leaves (Tables 1, 2).

Assuming that hexythiazox was sprayed in early July of 1994, we conclude that the compound extracted from pear leaves is hexythiazox. At the beginning of the study, we hypothesized that observed decreases in mite populations on pear leaves in summer were caused by a naturally produced molting deterrent. The present results lead us to reject this hypothesis.

ACKNOWLEDGEMENTS

We thank H. Takahara for kind guidance on extraction and analyses of the pear leaf compound, A. Yamamoto and R. Hatano for providing authentic hexythiazox, K. Oyama for kind suggestion on the pear leaf compound, and Y. Kitashima, K. Arai and S. Yaguchi for kind assistance with the experiments.

REFERENCES

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Banaag, A., H. Honda and T. Shono. 1997. Effects of alkaloids from yam, *Dioscorea hispida* Schluskel, on feeding and development of larvae of the diamond-back moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Appl. Entomol. Zool.* 32: 119-126.
- Bernays, E. A. and R. F. Chapman. 1994. Host-plant selection by phytophagous insects, Chapman & Hall, New York, 312 pp.
- Dabrowski, Z. T. and U. Serebinska. 2007. Characterization of the two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) response to aqueous extracts from selected plant species. *J. Plant Prot. Res.* 47: 113-124.
- Finney, D. J. 1971. Probit analysis, 3rd ed., Cambridge Univ. Press, London, 383 pp.
- Geest, L. P. S. van der, Th. C. Bosse and A. Veerman. 1983. Development of a meridic diet for the two-spotted spider mite *Tetranychus urticae*. *Entomol. Exp. Appl.* 33: 297-302.
- Gotoh, T. 1997. Annual life cycles of populations of the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) in four Japanese pear orchards. *Appl. Entomol. Zool.* 32: 207-216.
- Gotoh, T. and K. Gomi. 2000. Population dynamics of *Tetranychus kanzawai* (Acari: Tetranychidae) on hydrangea. *Exp. Appl. Acarol.* 24: 337-350.
- Gotoh, T. and M. Kubota. 1997. Population dynamics of the citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae) in Japanese pear orchards. *Exp. Appl. Acarol.* 21: 343-356.
- Gotoh, T., S. Abe, Y. Kitashima and S. Ehara. 2007. Divergence in host range and reproductive compatibility in three strains of *Oligonychus gotohi* Ehara (Acari: Tetranychidae). *Internat. J. Acarol.* 33: 7-13.
- Hare, J. D. and J. A. Bethke. 1988. Egg production and survival of the citrus red mite on an artificial feeding system. *Entomol. Exp. Appl.* 47: 137-143.
- Kantaratanakul, S. and J. G. Rodriguez. 1979. Nutritional studies in *Tetranychus urticae* II. Development of a meridic diet. pp. 405-411. *In: Rodriguez, J. G.*

- (Ed.). Recent Advances in Acarology, Vol. I. Academic Press, New York. 631 pp.
- Kishimoto, H. 2002. Species composition and seasonal occurrence of spider mites (Acari: Tetranychidae) and their predators in Japanese pear orchards with different agrochemical spraying programs. *Appl. Entomol. Zool.* 37: 603-615.
- Konno, K., C. Hirayama, M. Nakamura, K. Tateishi, Y. Tamura, M. Hattori and K. Kohno. 2004. Papain protects papaya trees from herbivorous insects: role of cysteine proteases in latex. *Plant J.* 37: 370-378.
- Leskovar, D. I. and A. Kipp Boales. 1996. Azadirachtin: potential use for controlling lepidopterous insects and increasing marketability of cabbage. *HortScience* 31: 405-409.
- Rasikari, H. L., D. N. Leach, P. G. Waterman, R. W. Spooner-Hart, A. H. Basta, L. K. Banbury and P. I. Forster. 2005. Acaricidal and cytotoxic activities of extracts from selected genera of Australian Lamiaceae. *J. Econ. Entomol.* 98: 1259-1266.
- Roinien, H., J. Vuorinen, J. Tahvanainen and R. Julkunen-Tiitto. 1993. Host preference and allozyme differentiation in shoot galling sawfly, *Euura atra*. *Evolution* 47: 300-308.
- Ryan, C. A. 1990. Protease inhibitors in plants. Genes for improving defenses against insects and pathogens. *Annu. Rev. Phytopathol.* 28: 425-449.
- Sokal, R. R., F. J. Rohlf. 1995. *Biometry*, 3rd ed. W.H. Freeman and Company, New York. 887pp.
- SPSS 2002. *SPSS 11.5J Brief Guide*. SPSS Inc., Tokyo, 160 pp (in Japanese).
- Ujihara, M., M. Shinozaki and M. Kato. 1995. Accumulation of phylloidalin in sweet leaf plants of *Hydrangea serrata* and its neutrality in the defense against a specialist leafmining herbivore. *Res. Popul. Ecol.* 37: 249-257.
- Wang, Y. N., G. L. Shi, L. L. Zhao, S. Q. Liu, T. Q. Yu, S. R. Clarke and J. H. Sun. 2007. Acaricidal activity of *Juglans regia* leaf extracts on *Tetranychus viennensis* and *Tetranychus cinnabarinus* (Acari: Tetranychidae). *J. Econ. Entomol.* 100: 1298-1303.
- Yamada, T., M. Kaeriyama, N. Matsui and H. Yoneda. 1987. Development of a new miticide, hexythiazox. *J. Pesticide Sci.* 12: 327-335.
- Yamamoto, A., H. Yoneda, R. Hatano and M. Asada. 1995. Laboratory selection of populations in the citrus red mite, *Panonychus citri* (McGregor), with hexythiazox and their cross resistance spectrum. *J. Pesticide Sci.* 20: 493-501.
