Pheromone Study on Acarid Mites. XIII. Identification of Neryl Formate as an Alarm Pheromone from the Bulb Mite, *Rhizoglyphus robini* (Acarina: Acaridae)

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The alarm pheromone was isolated from a hexane rinse of the bulb mite, *Rhizoglyphus robini*. The main component was identified as neryl formate (3,7-dimethyl-(*Z*)-2,6-octadienyl formate) and two minor components as citral (mixture of (*Z*)- and (*E*)-3,7-dimethyl-octa-2,6-dienal). The alarm pheromone activity of neryl formate was demonstrated against the bulb mite at 1 ppm hexane solution, which was ten times more active than demonstrated against the cheese mite *Tyrophagus putrescentiae*. The activity of citral was weak and demonstrated only at 1,000 ppm solution.

INTRODUCTION

The bulb mite *Rhizoglyphus robini* CLAPAREDE is known as an economically important pest attacking 28 species of 14 families of vegetable crops. Among them Liliaceae crops are most seriously injured by this mite (TOMINAGA, 1963). The mite belongs to sarcoptiformes and possesses a well developed pair of opisthonotal glands (also known as oil glands) in the dorsal part of the abdomen, therefore, some chemicals are suggested to be emitted from the gland.

The bulb mite was also noticed to possess a characteristic smell and to display a distinctive escape behaviour when mites in the rearing vial were squashed or were disturbed by a needle point. The same escape behaviour was reproducible by presenting to mites a filter paper (5 mm × 5 mm) impregnated with a hexane extract of the mites. Similar phenomena have been observed in 3 species of closely related acarid mites; the active compounds of the alarm pheromone have been isolated and their chemical structure has been identified as neryl formate and(or) citral (KUWAHARA et al., 1975, 1979, 1980 a).

Consequently, presence of the alarm pheromone in the bulb mite, possibly as an excretion from the gland, was postulated and the active principle(s) was investigated.

MATERIALS AND METHODS

The bulb mite *Rhizoglyphus robini* colony was maintained by the National Research

Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries in Mie Prefecture. The species was reared at 25°C on a diet of dried onion powder as reported (KUWAHARA et al., 1985). Humidity was maintained by keeping the rearing vials (glass container, 13 cm in diameter $\times$ 7 cm in height) in a loosely covered plastic box, the inner bottom surface of which was covered with water (ca. 1 cm in depth). A small amount of detergent was added to the water to prevent escape of the mite. When the mite population in rearing vials reached its maximum, mite bodies were separated from the culture medium by saline flotation method (MATSUMOTO, 1965).

The mite bodies were dipped into *n*-hexane for 3 min, and were subsequently removed by filtration. The filtrate was, after evaporation of the solvent, transferred to a SiO₂ column (Wako Gel C-200) and eluted with the mixture of solvents indicated later.

A gas liquid chromatogram (GLC) was obtained by a Yanako gas chromatograph model G-180 F, using a FFAP fused silica capillary (0.25 mm id $\times$ 25 m, chemical-bonded, Quadrex) at 100°C and a hydrogen flame ionization detector. Peaks were processed by a SIC intelligent integrator model 7000A. Mass spectra (MS) were measured at low resolution mode by a Hitachi M-80B high resolution mass spectrometer coupled with a Hitachi gas chromatograph (GC/MS) model 367-0200 using a PEG-20M fused silica capillary column (0.25 mm id $\times$ 25 m, chemical-bonded, Gasukuro Kogyo Ltd.).

Alarm pheromone activity was monitored by modifying the method reported (KUWAHARA et al., 1979) as follows: In a test petri dish (10 cm in diameter, bottom surface of which was covered by moistened filter paper), separated mites were introduced. When the mites were scattered evenly on the filter paper, a piece of filter paper (5 mm $\times$ 5 mm) impregnated with a test sample was introduced along with a solvent-treated control. The sample solution for the assay was prepared by dissolving the candidate material in hexane at each ten-fold dilution starting from 1,000 ppm. Activity of a compound was expressed by the lowest concentration (ppm) of the candidate solution impregnated to filter paper against which mites indicated an escaping behaviour.

RESULTS AND DISCUSSION

The hexane extract of medium-free mites (10 g) after evaporation of the solvent was transferred to a SiO₂ column (5 g) and eluted successively with 50 ml of each of the following solvents: a mixture of hexane and EtOAc (1:0), (20:1), (10:1) and (5:1), and MeOH. The active fraction was recovered in the (20:1) eluate. The fraction was again chromatographed on a column (5 g) successively eluting it with 50 ml portions of a mixture of Et₂O and hexane (1:20), (1:10), (1:1) and (0:1). The activity was recovered in the (1:20) and (1:10) fractions. GLC of the former fraction consisted of one peak (*t_R* by FFAP: 15.10 min) and the latter of two peaks (*t_R* 15.68 min and 20.31 min).

The former fraction gave the largest fragment ion by GC/MS at *m/z* 136 (10%, M⁺—HCOOH) and base ion at 69, along with the following diagnostic ions: *m/z* 41 (18%), 93 (12%), 121 (3.5%). The MS was identical to one derived from neryl formate (3,7-dimethyl-(*Z*)-2,6 octadienylformate) and also to that reported by KUWAHARA et al. (1975). The retention time of neryl formate by GLC coincided with that of the substance isolated. The compound was thus identified as neryl formate.

The shorter- t_R peak (15.68 min) of the latter fraction gave by GC/MS the largest fragment ion at m/z 134 (5.7%, $M^+ - H_2O$), the base ion at m/z 69, and the following diagnostic ions: m/z 41 (93%), 79 (15%), 81 (13%), 84 (22%), 94 (22%) and 109 (22%). The results were completely identical to those from neral (3,7-dimethyl-(*Z*)-2,6-octadienal) under the same condition and similar to those reported (KUWAHARA et al., 1980 a). The longer- t_R peak (20.31 min) of the fraction indicated M^+ ion at m/z 152, base ion at m/z 69, and the following diagnostic ions: m/z 41 (82%), 81 (11%), 84 (17%), 94 (9.6%), 109 (6.5%), 123 (4.8%) and 137 (2.7%). The MS was identical with that of geranial (3,7-dimethyl-(*E*)-2,6-octadienal) analyzed at the same condition and also similar to that reported (KUWAHARA et al., 1980 a). GLC of both peaks gave completely identical retention times to each component of citral, respectively, using a FFAP capillary column.

As a result, the alarm pheromone components of the bulb mite were identified as neryl formate (73.0%), neral (12.8%) and geranial (14.2%), as shown in Fig. 1. The presence of nerol (3,7-dimethyl-(*Z*)-2,6 octadienol) and geraniol (3,7-dimethyl-(*E*)-2,6-octadienol) was also detected in the GLC, though in small amounts. Bioassay results are listed in Table 1. Neryl formate brought about an alarm pheromone activity when a filter paper impregnated with 1 ppm solution was presented to tested mites. The activity was 10 times stronger than was shown against the cheese mite (KUWAHARA et al., 1975). Commercially available citral (the ratio of the two components is almost the same as the isolated citral) only indicated activity at 1,000 ppm. No synergistic enhancement of the activity was observed for a natural mixture of neryl formate and citral. Therefore, the major alarm pheromone component of the mite was concluded to be neryl formate. This is the second case in which neryl formate has been found to function as the major alarm pheromone component of acarid mites. Because of its high activity and the economic importance of the mite, the compound

Fig. 1. Typical gas liquid chromatogram of mite hexane extract, using a FFAP CB capillary column at 100°C. NF: neryl formate, Nal: neral, Gal: geranial, Nol: nerol, Gol: geraniol, A: *n*-tridecane, B: *n*-tetradecane, C: unknown, D: *n*-pentadecane, E and F: unknown.

Table 1. Bioassay results of active components from acarid mites against *R. robini*

Compound	Concentration (ppm)	Activity
Neryl formate	1.0	active
	0.1	inactive
Citral ^a	1,000	active
	100	inactive
Isopiperitenone ^b	1,000	active
	100	inactive
2-Hydroxy-6-methylbenzaldehyde ^c	1,000	inactive

^a A mixture of neral (35%) and geranial (65%).

^b Alarm pheromone identified from *Tyrophagus similis* (KUWAHARA et al., 1987).

^c Compound identified from extract of *Acarus siro*, whose biological function is obscure (CURTIS et al., 1981)

might be a useful tool to use in new applications for plant protection.

Citral has also been identified from closely related acarid mites, *Calpoglyphus lactis* and *Lardoglyphus konoi* as a major component of the alarm pheromone and also from, *Aleuroglyphus ovatus* and *Dermatophagoides farinae*, possibly as a defense compound (KUWAHARA et al., 1980b). The cheese mite also contained a small amount of citral (MY-YEN et al., 1980). Citral is thus recognized as a common component of the opisthonotal gland which is found in all acarid mites.

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REFERENCES

- CURTIS, R. F., A. HOBSON-FROHOCK, G. R. FENWICK and J. M. BERREEN (1981) Volatile components from the mite *Acarus siro* L. in food. *J. Stored Prod. Res.* **17**: 197–203.
- KUWAHARA, M., M. TAKAI and K. FUJIMOTO (1985) Simple rearing of the bulb mite, *Rhizoglyphus robini*, and test methods of acaricides. *Syokubutsu-boueki* **39**: 68–70 (in Japanese).
- KUWAHARA, Y., S. ISHII and H. FUKAMI (1975) Neryl formate: Alarm pheromone of the cheese mite, *Tyrophagus putrescentiae* SCHRANK (Acarina, Acaridae). *Experientia* **31**: 1115–1116.
- KUWAHARA, Y., H. FUKAMI, S. ISHII, K. MATSUMOTO and Y. WADA (1979) Pheromone study on acarid mites. II. Presence of the alarm pheromone in the mold mite, *Tyrophagus putrescentiae* SCHRANK (Acarina: Acaridae) and the site of its production. *Jap. J. Sanit. Zool.* **30**: 309–314.
- KUWAHARA, Y., H. FUKAMI, S. ISHII, K. MATSUMOTO and Y. WADA (1980 a) Pheromone study on acarid mites. III. Citral: isolation and identification from four species of acarid mites, and its possible role. *Jap. J. Sanit. Zool.* **31**: 49–52.
- KUWAHARA, Y., K. MATSUMOTO and Y. WADA (1980 b) Pheromone study on acarid mites IV. Citral: composition and function as an alarm pheromone and its secretory gland in four species of acarid mites. *Jap. J. Sanit. Zool.* **31**: 73–80.
- KUWAHARA, Y., K. AKIMOTO, W. S. LEAL, H. NAKANO and T. SUZUKI (1987) Isopiperitenone: a new alarm pheromone of an acarid mite, *Tyrophagus similis* (Acarina; Acaridae). *Agric. Biol. Chem.* **51**: 3441–3442.
- MATSUMOTO, K. (1965) Studies on the environmental factors for breeding of grain mites. IV. Digestive

- enzymes of the grain mites *Carpoglyphus lactis*, *Aleuroglyphus ovatus*, and *Tyrophagus dimidiatus*. *Jap. J. Sanit. Zool.* **16**: 86–89.
- MY-YEN, L. T., K. MATSUMOTO, Y. WADA and Y. KUWAHARA (1980) Pheromone study on acarid mites. V. Presence of citral as a minor component of the alarm pheromone in the mold mite *Tyrophagus putrescentiae* (SCHRANK, 1781) (Acarina: Acaridae). *Appl. Ent. Zool.* **15**: 474–477.
- TOMINAGA, T. (1963) Studies on the biology and the control method of the bulb mites, *Rhizoglyphus echinopus* FUMOZE and ROBIN, attacking shallot (*Allium bakeri*). Report of the Fukui Agricultural Experiment Station, No. 1, pp. 1–79.