

Pheromone Study on Acarid Mites. XII. Characterization of the Hydrocarbons and External Gland Morphology of the Opisthotal Glands of Six Species of Mites (Acari: Astigmata)

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The hydrocarbon components in the opisthotal glands of six species of agriculturally important astigmatid mites were identified. These included n-tridecane, n-tetradecane, n-pentadecane, Z-Δ⁵-tridecene, Z-Δ⁶- and Z-Δ⁷-tetradecene, Z-Δ⁶- and Z-Δ⁷-pentadecene, and Z,Z-Δ^{6,9}-pentadecadiene. Tyrophagus neiswanderi and T. putrescentiae are characterized by abundant quantities of monoenes and dienes, whereas the hydrocarbons of T. similis and Carpoglyphus lactis are predominately n-alkanes, with only minor quantities of the alkenes. Aleuroglyphus ovatus and Rhizoglyphus robini contain only n-tridecane (>97%) and n-tetradecane. Scanning electron microscopy of the opisthotal gland orifice revealed a novel and previously unknown "trapdoor" closure which appears to regulate the release of gland contents.

INTRODUCTION

Astigmatid mites are common pests of a wide variety of stored agricultural commodities, as well as being common components of "house dust" (HUGHES, 1976). Previous studies by Kuwahara and his associates have demonstrated that several astigmatids utilize alarm pheromones. In four species of mites the pheromone is citral (a mixture of (Z)- and (E)-3,7-dimethyl octa-Δ^{2,6}-dienal) (KUWAHARA et al., 1980 a). But in Tyrophagus putrescentiae SHRANK, neryl formate is the major alarm pheromone (KUWAHARA et al., 1975, 1979; MY-YEN et al., 1980) with citral being only a minor component. In all cases, these pheromones have been shown to be produced in the mites paired opisthotal glands (also known as oil glands), and to be discharged in large quantities when the mites are disturbed (KUWAHARA et al., 1979, 1980 b). Other workers have suggested an additional role for these pheromones, namely, the inhibition of molds in the mites environment (COLE et al., 1975; OKAMOTO et al., 1978).

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Although the oxygenated components of the mite opisthonotal gland secretions have been extensively studied, these components are actually of minor importance in the context of the total chemistry of the glands. In the cheese mite, *T. putrescentiae*, for example, the major gland components are *n*-alkanes (C₁₃-C₁₅), the corresponding monoenes, and a pentadecadiene (KUWAHARA et al., 1979). Other species of acarid mites contain similar hydrocarbon mixtures in their glands. These hydrocarbons do not have any alarm pheromone activity, nor do they synergize the activity of citral or neryl formate (KUWAHARA et al., 1979).

In this paper we provide complete identifications for the hydrocarbon components of the opisthonotal glands of six species of astigmatid mites, discuss the possible biosynthetic origin of these hydrocarbons, describe the external morphology of the glands, and discuss the possible ecological significance of the total chemistry of the gland contents.

MATERIALS AND METHODS

Mite species. The following species of mites (all stages) were used in this study: *Tyrophagus putrescentiae* SCHRANK (Shiga, Ibaraki and Tokyo Strain), *T. neiswanderi* JOHNSTON and BRUCE (Ibaraki and Hokkaido Strain), *T. similis* VOLGIN, *Aleuroglyphus ovatus* TROUPEAU, *Carboglyphus lactis* L., and *Rhizoglyphus robini* CLAPAREDE. All mites except *C. lactis* and *R. robini* were reared on dry yeast (KUWAHARA et al., 1979). *C. lactis* was reared on a 1:1 mixture of yeast and sugar (MATSUMOTO, 1965), and *R. robini* was maintained on dry onion powder (KUWAHARA et al., 1985).

Hydrocarbon extraction. At least 10 g of each species of mite were freed of culture medium by saturated saline flotation (MATSUMOTO, 1965), weighed, extracted 3 times by soaking for 3 min in hexane, and the combined hexane washes concentrated in vacuo. The residue was then chromatographed over Wako-gel C-200 Silica Gel, with the hydrocarbons being eluted with hexane. The hexane eluant was analysed for total composition (by gas chromatography), and then alkenes and alkadienes were isolated by chromatography over 5% AgNO₃-Silica Gel using a hexane-diethyl ether gradient.

Preparation of dithiomethylethers. Alkenes and alkadienes (ca. 0.1 mg) were dissolved in ca. 200 μ l of dimethyldisulfide (Wako Chemical Co.), one small crystal of iodine added (ca. 50 μ g), and the mixture stirred at room temperature (20–22°C) for 8 hr. Two ml of hexane were added, the solution washed twice with 1 ml of saturated sodium thiosulfate, and then dried over anhydrous sodium sulfate. The dry solution was concentrated to dryness with nitrogen, and re-dissolved in ca. 100 μ l of hexane for subsequent analysis.

Synthesis of Z,Z-A^{6,9}-Octadecadiene. Z,Z-A^{9,12}-Octadecadienol (99% pure), derived from a lithium aluminum hydride reduction of methyl linolate, was converted to its tosylate in benzene using tosyl chloride and pyridine. The tosylate (syrup, 3 g) without further purification was then reduced to the hydrocarbon with an excess of lithium aluminum hydride in ether to give 5% of the desired product. The pure diene (50 mg, 3%) was obtained by chromatography over a silica gel column, and shown to be 99+ % pure by GC-MS (M⁺; m/z 250).

Analytical methods. Percent compositions and isomeric and stereochemical purity of isolated hydrocarbons were obtained by isothermal (60°C or 80°C) capillary gas chromatography using a Yanaco Model G-180F Gas Chromatograph equipped with

a 25 m by 0.25 mm FFAP Bonded Phase capillary column (0.25 μm thickness, Quadrex) and a flame ionization detector. Retention times were compared to *n*-alkane standards and to authentic Δ^6 and Δ^7 alkenes prepared by Wittig reactions. Data were stored and integrated using an SIC intelligent integrator Model 7000A.

Mass spectra were obtained by GC-MS on a Hitachi Model 367-0200 Gas Chromatograph interfaced to a Hitachi M-80B High Resolution Mass Spectrometer operated in the low resolution mode. The GC was equipped with a 50 m by 0.25 mm OV-1 Bonded Phase capillary column. All mass spectral analyses utilized temperature programming at a rate of 5°C/min.

Proton Nuclear Magnetic Resonance spectra were obtained using a JEOL FX-100 NMR spectrometer. Samples were dissolved in high purity CDCl_3 and TMS was used as the internal standard.

Infrared spectra were obtained as thin films on NaCl plates using a JASCO Spectrometer (Japan Spectroscopic Co.). Polystyrene was used as the external standard.

SEM of external gland morphology. Mites were fixed in either Bouin's, Carnoy's, or Champy's mixture, depending on mite species. After routine ethanol dehydration and critical point drying, the specimens were sputter coated with gold, mounted on double-stick tape, and then observed using a Hitachi Model S-4308 Scanning Electron Microscope.

RESULTS

The percent composition of the hydrocarbons from the opisthotal glands of the six species of mites examined in this study are listed in Table 1. *Tyrophagus neiswanderi* and *T. putrescentiae* have similar compositions, with alkenes and alkadienes being the major components, and *n*-alkanes being minor components. In contrast, the hydrocarbons of *T. similis* are dominated by the *n*-alkanes, with only low amounts of the monoenes and no dienes. A similar *n*-alkane rich profile was found for *Carpoglyphus lactis*. The remaining two species contain only *n*-alkanes in the hydrocarbon fraction of their gland secretions.

When olefins were present, the location of the double bonds was readily evident from an examination of the EI-Mass spectra of their methylthioether derivatives (FRANCIS and VELAND, 1981) (Fig. 1, Table 2). These locations were found to be the same in all species (Table 1). Except for *Z*- Δ^5 -tridecene, all monoenes occur as mixtures of *Z*- Δ^6 - and *Z*- Δ^7 -positional isomers. The pentadecadiene was shown to be a single compound, *Z,Z*- $\Delta^{6,9}$ -pentadecadiene. Abundant parent ions and strong alpha-cleavage fragment ions were present in all cases. Inasmuch as the pentadecadiene is a symmetrical molecule, only one monoaddition product was obtained from the methylthiolation reactions. Under the reaction conditions that we used, this was the major product. The stereochemistry of all double bonds was clearly *cis* (*Z*) from infrared and chromatographic data, with no evidence of any *trans* (*E*) components being found.

The *cis*, *cis*-methylene interrupted structure of the pentadecadiene was confirmed by synthesizing a model diene (*Z,Z*- $\Delta^{6,9}$ -octadecadiene) and comparing its 100 MHz NMR spectrum to that of the pentadecadiene isolated from the mites. The chemical shifts and splitting patterns for vinylic and allylic protons (around $\delta 5.3$ ppm and $\delta 2.8$ ppm, respectively) of the model diene and mite hydrocarbon were identical.

Examination of the external morphology of the opisthotal glands of *A. ovatus*,

Table 1. Percent composition of hydrocarbon components from the opisthonotal glands of six species of astigmatid mites

Mites species	C ₁₃	Δ^5 -C _{13:1}	C ₁₄	Δ^6 -C _{14:1}	Δ^7 -C _{14:1}	C ₁₅	Δ^6 -C _{15:1}	Δ^7 -C _{15:1}	$\Delta^{6,9}$ -C _{15:2}
<i>Aleuroglyphus ovatus</i>	97.49	— ^b	2.51	—	—	—	—	—	—
<i>Carpoglyphus lactis</i>	90.63	0.62	0.60	—	—	5.71	2.44	—	—
<i>Rhizoglyphus robini</i>	97.50	—	2.50	—	—	—	—	—	—
<i>Tyrophagus neiswanderi</i> A ^a	24.57	0.62	1.26	1.07	0.43	11.10	34.36	19.80	6.79
<i>T. neiswanderi</i> B	21.80	0.60	1.13	1.08	0.23	11.61	37.23	21.01	5.31
<i>T. putrescentiae</i> B	16.42	0.67	1.31	1.06	0.23	18.38	35.24	20.83	5.86
<i>T. putrescentiae</i> C	12.74	2.26	0.71	1.44	0.40	4.02	41.19	23.80	13.44
<i>T. putrescentiae</i> D	18.12	0.70	1.27	0.83	0.45	11.87	40.44	23.56	2.76
<i>T. similis</i>	95.20	0.37	2.91	—	—	1.34	0.18	—	—

^a A: Hokkaido strain, B: Ibaraki strain, C: Shiga strain, and D: Tokyo strain.

^b Not detected.

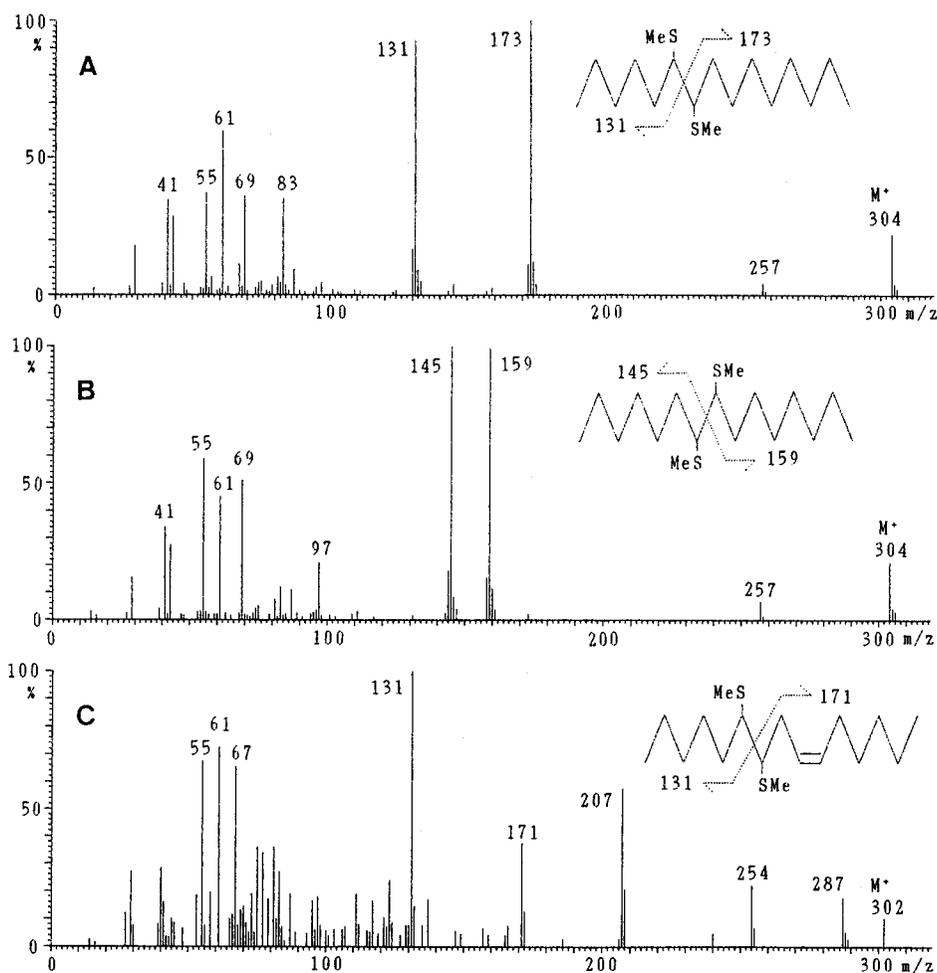


Fig. 1. EI-mass spectra of the dithiomethylethers of *Z*- Δ^6 -pentadecene (A), *Z*- Δ^7 -pentadecene (B), and *Z,Z*- $\Delta^{6,9}$ -pentadecadiene (C).

C. lactis, and *T. putrescentiae* showed that the glandular reservoir opens to the exterior through a half-moon shaped pore of ca. 2.5 to 3.0 μm diameter (Fig. 2). This pore is provided with a novel cuticular "hinged trapdoor" which apparently serves to regulate the egress of the glandular secretions. The "hinge" portion of the structure is situated in all cases latero-posteriad to the longitudinal axis of the mite, thus ensuring that upon depression of the "trapdoor" the glandular components will be discharged over the rear part of the mites body. In all three of the species examined here, long, prominent setae are present in this region (Fig. 2) which could either aid in the mechanical transfer of the released secretion onto another organism (predator?), or in increasing the evaporative release of the chemicals for semiochemical functions.

Table 2. Diagnostic EI-mass spectral ion fragments for dithiomethylethers of alkenes and alkadienes from five species of astigmatid mites

Olefin	Dithiomethylether		
	M ⁺	Ion A ^a m/z (rel. abundance)	Ion B ^a
Δ^5 -C ₁₃ :1	276 (10)	117 (65)	159 (50)
Δ^6 -C ₁₄ :1	290 (20)	131 (85)	159 (85)
Δ^7 -C ₁₄ :1	290 (25)	145 (85)	145 (85)
Δ^6 -C ₁₅ :1	304 (22)	131 (92)	173 (100)
Δ^7 -C ₁₅ :1	304 (21)	145 (100)	159 (99)
$\Delta^{6,9}$ -C ₁₅ :2	302 (10)	131 (100)	171 (38)

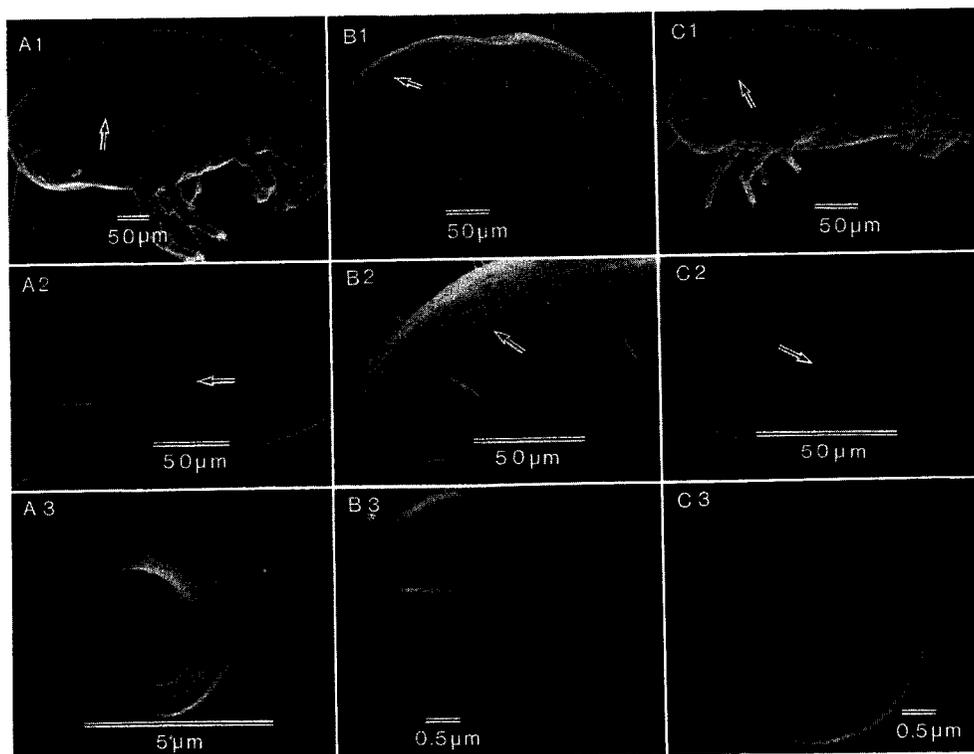
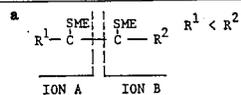


Fig. 2. Scanning Electron Micrographs of the "hinged trapdoor" cuticular closure of the external pore of the opistonotal glands of *Aleuroglyphus ovatus* (A1-A3) *Carpoglyphus lactis* (B1-B3), and *Tyrophagus putrescentiae* (C1-C3). Arrows in each case indicate gland opening in cuticle.

DISCUSSION

Although the mites that we have studied are all pests of stored products and agricultural crops, like most other astigmatid mites, their ancestors were probably fungivores (O'CONNOR, 1979). Indeed, even though at least 34 genera in 10 families have invaded human stored products or homes (as "house dust") (HUGHES, 1976), only a few species within each taxon have successfully done so, and the majority of the other species within each genus are still present in their native habitats. Even those species that have invaded stored products still have feral populations (O'CONNOR, 1979). Any consideration of the role(s) of the opisthotal gland and its secretions on the population ecology of these mites must thus take into account this evolutionary history.

O'CONNOR (1979) notes that astigmatid mites of stored products fall into four major ecological groups: (1) mites associated with specific and ephemeral resources that are patchily distributed in either space or time; (2) mites associated with widespread field resources; (3) mites associated with mammals, and; (4) mites associated with birds. The mites that we have studied fall into two (possibly three) of these ecological groupings. *Tyrophagus* spp. (grassland soil and litter) and *Rhizoglyphus* spp. (soil, roots, and tubers) are members of group 2, *Carpoglyphus* spp. (rotting fruits and vegetation, dried fruits, honeycombs) are members of group 1, and *Aleuroglyphus* spp. (stored grains, mice burrows, and voles nests) are ecologically associated with group 1 and possibly group 3 (HUGHES, 1976).

The secretions of astigmatid opisthotal glands are best characterized as dilute solutions of oxygenated terpenes in hydrocarbon solvents. The exact role of the solvent in these mixtures is not clear, however, since the hydrocarbons show no mite alarm pheromone activity nor do they act as behavioral synergists for the terpenes. Very similar glandular mixtures are well known from a diversity of other arthropods, however, and have frequently been characterized as defensive secretions (BLUM, 1981). Although little direct evidence exists to confirm that the mite opisthotal gland secretions are primarily defensive, circumstantial evidence is suggestive.

Two lines of evidence suggest that the hydrocarbons we have identified derive from the opisthotal glands. First, when the mites are disturbed, they immediately release large quantities of these hydrocarbons (along with the oxygenated terpenes) into the atmosphere (KUWAHARA et al., 1979). The opisthotal glands are the only glands in the mites large enough to produce hydrocarbons in the quantities observed. Second, direct sampling from the pore of the opisthotal glands of single *Tyrophagus putrescentiae* using glass microcapillaries, with subsequent gas chromatographic analysis, revealed small, but measurable quantities of the pentadecene (unpublished data).

The function of such hydrocarbons has been discussed by several workers. BLUM and BRAND (1972) suggested that a possible role for such low molecular weight hydrocarbons is to temporarily disrupt the olfactory acuity of the receiving organisms antennal receptors. Other workers (reviewed in BLUM, 1981) have argued that the primary role of hydrocarbons in defensive secretions is to act as a "biosolvent" to facilitate the transport of noxious chemicals across the predators cuticle. These roles (and other roles not yet defined) are not of course mutually exclusive, and would seem to be readily applicable to mites as well as to insects. Little hard evidence exists to substantiate these hypotheses for any predator-prey system, but they are at least reasonable hypotheses worthy of future testing. As McMURRY (1984) notes, most astigmatid species are com-

monly considered to have such a high reproductive potential that they would seldom be held in check by predators. It is not inconceivable, however, that part of the reason that predators are ineffectual is that the mites opisthotal gland secretions are effective anti-predator agents.

Although the presence of the opisthotal glands and their associated external pores are well known in all stages of mites in both the Astigmata and Cryptostigmata (KRANTZ, 1978), detailed ultrastructural studies of these glands seem not to have been previously conducted. Our finding of the novel "hinged trapdoor" closures on the mites cuticular surface was unexpected, but considering that acarid mites are extremely prone to desiccation (KNULLE, 1984), such closures make evolutionary sense. Further ultrastructural studies will be required to ascertain whether the "trapdoors" are operated by neuromuscular or hydrostatic mechanisms. Concomitant behavioral studies on the stimuli eliciting the opening and closure of the "trapdoor" are also needed.

If as we have argued, the astigmatid opisthotal gland secretions are an evolutionary response to environmental hazards, then one would expect the mites to biosynthesize some or all of the glandular constituents. Although we have no direct evidence on this question, we note that the three *Tyrophagus* spp. and *Aleuroglyphus ovatus* were all cultured on the same medium, yet they have very different hydrocarbon profiles (Table 1), thus strongly suggesting that they biosynthesize their hydrocarbons. The carbon number profile and location of double bonds in all of these astigmatid hydrocarbons are readily explainable if these hydrocarbons, like insect hydrocarbons, are derived from common fatty acids by elongation-decarboxylation or chain shortening-decarboxylation pathways (ROELOFS and BJOSTAD, 1984). A test of this hypothesis must await future experimentation.

Chemical ecology studies of mites are still relatively rare, but our work, and that of others, clearly indicate that chemicals almost certainly play an important role in the population dynamics of these organisms. Although the technical difficulties involved in studying the ecology of such small organisms is formidable, their ecological importance is so great as to justify the effort. Indeed, we expect future studies to uncover many new features (such as the "hinged trapdoor" reported in this paper) that result from the very demands of being small and that such findings will greatly increase our overall knowledge of acarine chemical ecology.

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REFERENCES

- BLUM, M. S. (1981) *Chemical Defenses of Arthropods*. Academic Press, New York.
BLUM, M. S. and J. M. BRAND (1972) Social insect pheromones: their chemistry and function. *Am.*

- Zool. **12**: 553-576.
- COLE, L., M. BLUM and R. RONGADORI (1975) Antifungal properties of the insect alarm pheromones citral, 2-heptanone, and 4-methyl-3-heptanone. *Mycologia*. **67**: 701-708.
- FRANCIS, G. W. and K. VELAND (1981) Alkylthiolation for the determination of double-bond positions in linear alkenes. *J. Chromatog.* **219**: 379-384.
- HUGHES, A. M. (1976) The mites of stored food. In *Tech. Bull.* No. 9, Her Majesty's Stationery Office, London.
- KNULLE, W. (1984) Water vapor uptake in mites and insects: an ecophysiological and evolutionary perspective. In *Acarology VI*. Vol. 1 (D. A. GRIFFITHS and C. E. BOWMAN, ed.). Ellis Horwood Ltd., Chichester, U.K., pp. 71-82.
- KRANTZ, G. W. (1978) *A Manual of Acarology*. 2nd ed. O.S.U. Book Stores, Inc., Corvallis, Oreg.
- 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* SHRANK (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* SHRANK (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., H. FUKAMI, S. ISHII, 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.
- 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.
- MCMURTY, J. A. (1984) A consideration of the role of predators in the control of acarine pests. In *Acarology VI*. Vol. I (D. A. GRIFFITHS and C. E. BOWMAN, eds.). Ellis Horwood, Ltd., Chichester, U.K., pp. 109-121.
- 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* (SHRANK, 1781, Acarina: Acaridae). *Appl. Ent. Zool.* **15**: 474-477.
- O'CONNOR, B. M. (1979) Evolutionary origins of astigmatid mites inhabiting stored products. In *Recent Advances in Acarology*. Vol. I (J. G. RODRIGUEZ, ed.). Academic Press, New York, pp. 273-278.
- OKAMOTO, M., K. MATSUMOTO, Y. WADA and H. NAKANO (1978) Studies on antifungal effect of mite alarm pheromone citral. I. Evaluation of antifungal effect of citral. *Jap. J. Sanit. Zool.* **29**: 255-260.
- ROELOFS, W. and L. BJOSTAD (1984) Biosynthesis of lepidopteran pheromones. *Bioorg. Chem.* **12**: 279-298.