Virus-like particles in the tracheal mite *Acarapis woodi* (Rennie)

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(Received 8 August 1990; accepted 10 March 1991)

Summary — Tracheal mite infected honey bees were collected from Scotland and from California. The lysed cells of the body cavity of tracheal mites in the honey bees collected from Scotland harboured numerous 27 nm isometric virus-like particles. These virus-like particles were formed in a crystalline array and were arranged in a hexagonal pattern. No virus-like particles were observed in the tracheal mites of the honey bees collected from California. The significance of these observations in relation to the honey bee tracheal mite resistance is discussed.

honey bee / *Acarapis woodi* / virus-like particle / electron microscopy

INTRODUCTION

The continuing range expansion of tracheal mite *Acarapis woodi* (Rennie) (Acarari, Tarsonemidae) infestation in Canada and the United States has prompted experiments in order to find chemical means of controlling this damaging honey bee disease (Guzman-Novoa and Zozaya-Rubio, 1984; Anonymous, 1986; Gruszka, 1987; Wilson *et al*, 1988; Eischen *et al*, 1989). Chemical control of the tracheal mite is very attractive because of its relatively low cost application and reliability. Unfortunately, consumers are increasingly

aware of environmental pollution and food safety issues associated with nearly 50 yr of synthetic and organic pesticide use. Chemical residue problems will certainly affect the marketability of honey which is harvested from bee hives treated with pesticides. An attempt has also been made to import British honey bees in order to introduce natural tracheal mite resistance to the North American stocks (Morse, 1990). Breeding tracheal mite resistant honey bees is certainly the most desirable approach to reduce the damage caused by tracheal mite infestation. Biological control of tracheal mite infestation,

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may be one of the other attractive alternatives. Because biological control is based on organism interaction, it involves ecological studies of factors that regulate or affect interaction. Attempts have been made during the last 5 yr to find organisms which have the potential of becoming biological control agents in tracheal mites, or are associated with tracheal mites. This paper describes the ultrastructural features of virus-like particles found in the lysed tissue of some tracheal mites.

MATERIALS AND METHODS

Tracheal mite infected honey bees were collected from Buckham, Scotland during the summer of 1985. Infected honey bees were fixed in 8% EM grade glutaraldehyde (Polyscience, Warrington, PA) before being shipped to Canada. Specimens were also collected during 1988–1989 from Vacaville, CA. Tracheae with heavy mite infestation were dissected in saline and transferred to vials containing 4% glutaraldehyde in 0.2 M phosphate buffer, pH 7.4 (Liu *et al.*, 1989). Specimens were fixed overnight at 4 °C, rinsed in the buffer, postfixed in 1% osmium tetroxide in the same buffer, dehydrated in a graded ethanol series, and embedded in Polybed 812 (Polyscience, Warrington, PA). Silver-gray sections were cut on a Sorval MT2 ultramicrotome with glass knives, and mounted on 400-mesh copper grids. Sections were stained with uranyl acetate followed by lead citrate then examined and photographed with a Philips EM 410 or 400 electron microscope operated at an accelerating voltage of 80 Kv.

RESULTS

Of 51 tracheal mites from 17 Scotland honey bees examined, 3 mites from 2 individual bees harboured virus-like particles. Of 75 tracheal mites from 27 California honey bees examined, no virus-like particles were found. In all of the tracheal mites from which virus-like particles were detect-

ed, tissues were extensively lysed, and these mites appeared to be dead before fixation. Residual membranes and large lamellar bodies were also observed (figs 1–3). All of the virus-like particles were packed in crystalline arrays in a hexagonal arrangement (figs 1, 3–4), while scattered single particles were rarely observed. These particles, exclusively found within the mites, were isometric and measured 27 nm in diameter, and were not embedded in protein matrices. None of them had been observed in the tracheal lumen. In 2 of the mites, the entire body cavity appeared to be filled with these virus-like crystalline inclusions (fig 2). At higher magnification the particles consisted of a dense core ringed by an electron-dense coat (fig 4).

DISCUSSION

These virus-like particles found in the tracheal mites bear a close resemblance to some of the honey bee viruses (Bailey, 1976, 1981; Bailey *et al.*, 1980, 1981). The identification of the small isometric virus-like particles is handicapped by the lack of fresh tracheal mites infected by this virus. Ultrastructural appearance of these particles described in the present study resembles several insect picornaviruses, including the P virus of *Drosophila melanogaster* (Teninges and Plus, 1972), and the grasshopper crystalline array virus (Henry and Oma, 1973). These small viruses typically form cytoplasmic crystalline arrays. This type of virus has been described in many invertebrates (Longworth, 1978). Picornaviruses are single-stranded RNA viruses, ranging in size from 22–30 nm (Matthews, 1982). Many small honey bee RNA viruses in Britain, such as black queen cell, acute paralysis and Kashmir viruses, have a dimension of 30 nm. Viruses Y and X are

Fig 1. A lysed cell in the body cavity of a tracheal mite. Virus-like particles are visible (small arrows) among residual membranes. In the cytoplasm there are residual membranes (M) and virus-like particles in the form of paracrystals (x 39 000).

Fig 2. Most of the lysed cells of a tracheal mite are filled with tightly packed virus-like particles in the form of paracrystals (x 45 000).

35 nm in size. The very common cloudy wing particle virus is 17 nm in size (Bailey, 1981). The size of these honey bee virus particles is either larger of smaller than the virus particles observed in the tracheal mites. Due to a lack of thin-section electron microscope studies, it is impossible to make a direct comparison between the honey bee viruses and the virus-like particles in the tracheal mites, concerning the size of the virus particles and the pattern of cytoplasmic crystalline arrays. In addition, the large number of virus crystals found in the tracheal mites is always associated with cellular debris, which is an indication that these particles replicated themselves within the cells of tracheal mites. Therefore, it is unlikely that these particles are

honey bee viruses ingested by the tracheal mites.

Viruses of any kind have not previously been described in the tracheal mite. Because these virus-like particles were found in the extensively lyzed cells, it could indicate that they are lethal to the tracheal mites. These particles were found among cellular debris of the lyzed cells, which is an indication of the multiplication that took place in the cells and accumulated in the cytoplasm. All of these virus-like particles were found in the mites from honey bees collected in Scotland. None have been observed in the mites from the honey bees collected in California. Because of the sample size the extent of the infection in the tracheal mite population within the

Fig 3. Most of the lyzed cells contain a large number of lamellar bodies (LB) and virus-like particles (x 39 000).

Fig 4. Higher magnification view of the virus-like particles which have an electron-dense core (arrow) and an electron-dense coat (arrowheads). Note that these particles are arranged in a hexagonal pattern (x 105 000).

cotland honey bees it not certain. Since these virus-like particles have not been observed in the tracheal mites from the California honey bees, it is quite possible that the British honey bees are more resistant to tracheal mite infestation due to the presence of a viral disease in the tracheal mite population. The tracheal mite population in the British honey bees may be subjected to viral disease regulation. An extensive survey is underway to determine the incidence and pathogenicity of these virus-like particles, in order to demonstrate the above postulation.

ACKNOWLEDGMENTS

The author wishes to thank members of the Bee Biology Facilities, University of California, Davis Campus, for their hospitality and to M Mobus, North Scotland Agricultural College, for collecting specimens in Scotland. This work was supported by the work transfer program, Agriculture Canada.

Résumé — Des particules de type viral chez l'acarien *Acarapis woodi* (Rennie). Des abeilles (*Apis mellifera* L) parasitées par l'acarien des trachées, *Acarapis woodi* (Rennie) (Acari, Tarsonemidae), ont été prélevées à Aberdeen (Écosse) en 1985 et à Vacaville (Californie) en 1988-1989. Les trachées des abeilles fortement parasitées ont été disséquées dans du liquide physiologique et transférées dans des récipients contenant 4% de glutaraldéhyde dans un tampon phosphate à 0,2 mol.l⁻¹, pH 7,4. Les trachées renfermant des acariens ont été laissées dans ce milieu toute une nuit à 4 °C puis rincées dans le même tampon et fixées au tétraoxyde d'osmium à 1% dans le même tampon durant 1 h. Elles ont été ensuite déshydratées progressivement par l'alcool éthylique et montées dans du Polybed. Des coupes minces

ont été réalisées au microtome, colorées à l'acétate d'uranyle et au citrate de plomb, puis examinées en microscopie électronique (Philips EL 400) à 80 kV. Les *Acarapis* des abeilles venant d'Écosse renfermaient dans les cellules lysées des particules de type viral. Ces particules isométriques de 27 nm se présentaient sous forme cristallisée dans une disposition hexagonale. Il n'y avait pas de particules de type viral chez les *Acarapis* des abeilles provenant de Californie. Il est possible que ces abeilles soient plus résistantes à *A woodi* à cause de la présence d'une virose chez ce dernier.

Acarapis woodi / particule de type viral / microscopie électronique

Zusammenfassung — Virus-ähnliche Partikel bei der Tracheenmilbe *Acarapis woodi*. Von Tracheenmilbe befallene Honigbienen wurden 1985 in Aberdeen, Schottland, und 1988/1989 in Vacaville, Kalifornien, gesammelt. Tracheen von mit Milben schwer befallenen Bienen wurden in Insekten-Salzlösung präpariert und in Gläschen mit 4% Glutaraldehyd in 0.2 M Phosphatpuffer, pH 7.4, übertragen. Die mit Milben besetzten Tracheen wurden über Nacht bei 4 °C in dieser Fixierlösung belassen, dann mit demselben Puffer gespült und anschließend in 1% Osmiumtetroxid in demselben Puffer für 1 h nachfixiert. Die Präparate wurden dann stufenweise in Äthylalkohol dehydriert und in Polybed eingebettet.

Mittels Glasmesser hergestellte Dünnschliffe wurden mit Uranylazetat und Bleizitrat gefärbt und unter einem Philips EM 400 Elektronenmikroskop bei einer Beschleunigungs-Spannung von 80 kv untersucht. Einige der Tracheenmilben aus Bienen, die in Schottland gesammelt worden waren, enthielten Virus-ähnliche

Partikel in aufgelösten Zellen (Fig. 1, 3, 4). Diese 27 nM messende isometrischen Virus-ähnlichen Partikel befanden sich in kristalliner Anordnung mit hexagonalem Muster. In den Milben, die aus in Kalifornien gesammelten Bienen stammten, wurden keine Virus-ähnlichen Partikel gefunden. Es wird vermutet, daß ein Zusammenhang zwischen diesem Befund und der erhöhten Milbenanfälligkeit der Bienen in Kalifornien bestehen könnte.

Honigbiene / *Acarapis woodi* / Virus-ähnliche Partikel / Elektronen-Mikroskopie

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