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Microstructure of Faecal Pellets and Silk of the Two Spotted Spider Mite, *Tetranychus urticae* (Tetranychidae: Acarina)

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점박이응애 분비물의 미세구조

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ABSTRACT

Asthma and allergic rhinitis due to outdoor spider mites are major health problems worldwide. The sensitization route to spider mites has not yet been well elucidated. We examined the microstructure of faecal pellets and silk of the two-spotted spider mite, *Tetranychus urticae*, using a field emission scanning electron microscope. Black and white pellets of the two-spotted spider mites faeces contain a large amount of plant pigment waste products. Black faecal pellets are strawberry-shaped. White faecal pellets are silken threads. These pellets are likely to be the source of allergens of relevant mites because desiccated faeces particles probably disintegrate and become incorporated into dust particles more readily than whole bodies or encased internal organs. We conclude that the importance of spider mites in respiratory allergy needs emphasis.

Key words : Microstructure, faecal pellets, silk, two-spotted spider mite, *Tetranychus urticae*

INTRODUCTION

All spider mites are outdoor phytophagous mites causing significant damage to fruit leaves throughout the world, and belong to the Suborder Prostigmata of Order Acarina (Lee *et al.* 1987, Bolland *et al.* 1998a, b). Epidemiological studies have demonstrated that spider mites are important allergens in the development of work-related asthma and rhinitis in fruit-cultivating farmers. Several case reports of occupational allergy to spider mites have been reported (Reunala *et al.* 1983, Delgado *et al.* 1994, Kim and Kim 2002, Kim *et al.* 2006).

Korean spider mites (Family Tetranychidae) are known to be from four genus encompassing nine species, i.e., *Panonychus citri* (citrus red mite), *Panonychus ulmi* (European red mite), *Tetranychus urticae* (two-spotted spider mite), *T. kanzawai*, *T. vienensis*, *Oligonychus karamatus*, *O. perditus*, and *O. ununguis* (Lee *et al.* 1987). The two-spotted spider mite, *T. urticae* is known to be the most import mite which damages pear and apple leaves in Korea (Lee 1999). Recently, The two-spotted spider mite is a common sensitizing allergen in the general population of adults, and sensitization to this mite may play a role in the manifestation of asthma and symptoms during adulthood (Jee *et al.* 2000, Kim *et al.* 2002, Kim *et al.* 2006).

There have been a few reports of occupational asthma caused by the two-spotted spider mite in fruit farmer or greenhouse workers (Delgado *et al.* 1994, Delgado *et al.* 1997, Jee 1999, Kim *et al.* 1999a, b, Kim *et al.* 2006). These findings suggest that many cases of occupational asthma thought to be intrinsic of allergens derived from spider mites.

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The microstructure of faecal pellets and silk of spider mites have not yet been thoroughly studied. The objective of the present study was to describe the fine structure of *T. urticae* by using light microscope scanning (SEM).

MATERIALS AND METHODS

The strain of *T. urticae* was obtained from Department of Biology, Chonbuk University in Jeonju, Korea. The mites were reared on the kidney bean plant in the laboratory under the conditions of 25°C, 70% relative humidity (RH) and the long-day condition of 16 h light : 8 h dark (16L : 8D) photoperiod. Adult mites were kept on detached leaves of bean.

Specimens were anesthetized with CO_2 and examined under a dissecting light microscope. Specimens were gently removed and fixed in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde buffered with 0.1 mol/L phosphate buffer at pH 7.4. Postfixation was done in 1% osmium tetroxide (OSO_4) in the same buffer. Several washes in 0.1 mol/L phosphate buffer were done following fixation.

For scanning electron microscopic examination, the specimens were dehydrated in ascending concentrations of ethanol from 30 to 100%. The specimens were transferred to hexamethyldisilazane and were allowed to air dry. All specimens were coated to a thickness of approximately 20 nm with gold-palladium alloy using a sputter coater and they were examined on a Hitachi S-800 (Hitachi, Tokyo, Japan) filed emission scanning electron microscope operated with accelerating voltages of 20 kV.

RESULTS

The body is compact; both unsegmented and long, hairlike setae. The legs are four pairs of walking legs (Fig. 1, A).

The egg surface may be smooth and egg shape varies from round to onion-like. The egg surface is covered by silken threads (Fig. 1, B).

Mite faecal pellets have black pellet and white pellet (Fig. 2, A). The faeces are roughly spherical and have a relatively smooth surface. The surface of the faeces is covered by silken threads. White faecal pellet is covered by a thin secretion (Fig. 2, A). Black faecal pellet is strawberry-shaped (Fig. 2, B). Surface of the black faecal pellet is covered by a thin secretion. The black and white pellets contain a large amount of plant pigment waste products.

Silk spinning is fibrillar in structure (Fig. 3), consisted of a

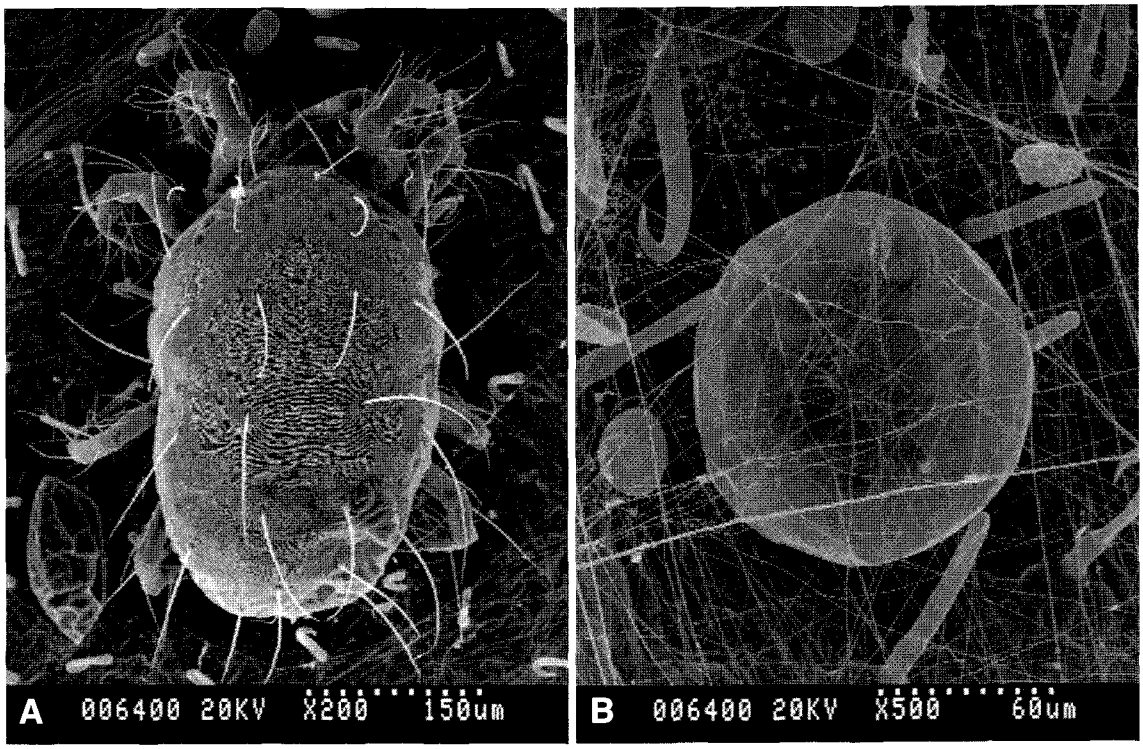

Fig. 1. A, Quiescent female of *Tetranychus urticae*, with strands web spun over her (Scale bar, 150 μm). B, Egg of *Tetranychus urticae* on bean leaf (Scale bar, 60 μm).

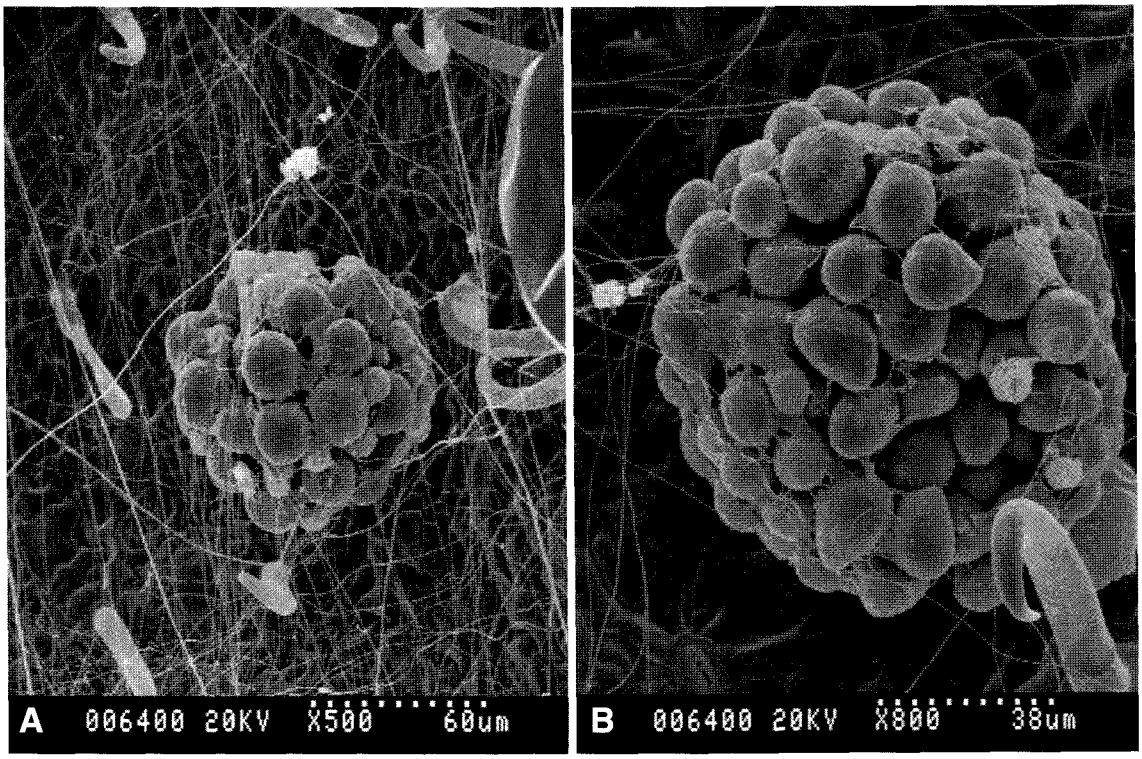

Fig. 2. A, Black faecal pellet of *Tetranychus urticae*, anchored to the leaf surface by silken threads (Scale bar, 60 μm). B, Surface of the black faecal pellet covered by a thin secretion (Scale bar, 38 μm).

few strands of silk threads (Fig. 3, B, C, D). Spider mites' silk threads are similar to the spider silk threads.

DISCUSSION

Asthma and allergic rhinitis due to indoor house dust mites and outdoor spider mites are major health problem worldwide. Potential sources of relevant mites allergens in dust particles include whole bodies, cast skins, egg castings, secretions, and feces.

In Korea, predominant species among domestic mites were *Dermatophagous farinae* (American house dust mite), *D. pteronyssinus* (European house dust mite), and *Trophagous putrescentiae* (Ree 1997). House dust mite faeces have been reported as a major source of indoor allergens (Tovey *et al.* 1981, Park *et al.* 2000, Jeong *et al.* 2002). Two species of the mite genus *Dermatophagoides*, *D. farinae* and *D. pteronyssinus*, are important sources of house dust allergens. House dust mites can produce a variety of materials that may contain significant allergenic activity.

Recently, Spider mites have been reported as a major source of outdoor allergens (Reunala *et al.* 1983, Delgado *et al.* 1994, Delgado *et al.* 1997, Kim and Kim 2002, Kim *et al.* 2006). Three species of the mite family Tetranychidae, *Panonychus citri*, *P. ulmi*, *Tetranychus urticae* are important sources of outdoor phytophagous mites (Lee *et al.* 1987, Cho *et al.* 1995, Lee 1999). Kim *et al.* (1999) described approximately 17% of farmers with work-related asthmatic symptoms exhibited an isolated positive skin response to spider mites and that about 9% of farmers were sensitized only to spider mites. These findings suggest that many cases of asthma thought to be intrinsic of allergens derived from outdoor allergens. Spider mite allergens have two peaks in late spring and mid-fall and are minimal during other periods, particularly in winter (Kim *et al.* 1999a). Their asthmatic symptoms were aggravated seasonally, especially in summer and early fall, which are the seasons when the levels of the two-spotted mite densities on the pear leaves are at their highest (Astarita *et al.* 1994, Jee *et al.* 2000). Hot, dry weather is conducive to rapid dispersal of old skin and faecal pellets. Spider mites can produce a variety of materials such as whole bodies, cast skins, egg castings, secretions, and feces.

The two spotted mites faecal pellets are black and white pellets contain a large amount of plant pigment waste products (Fig. 2). Mothes and Seitz (1981a) assumed that these

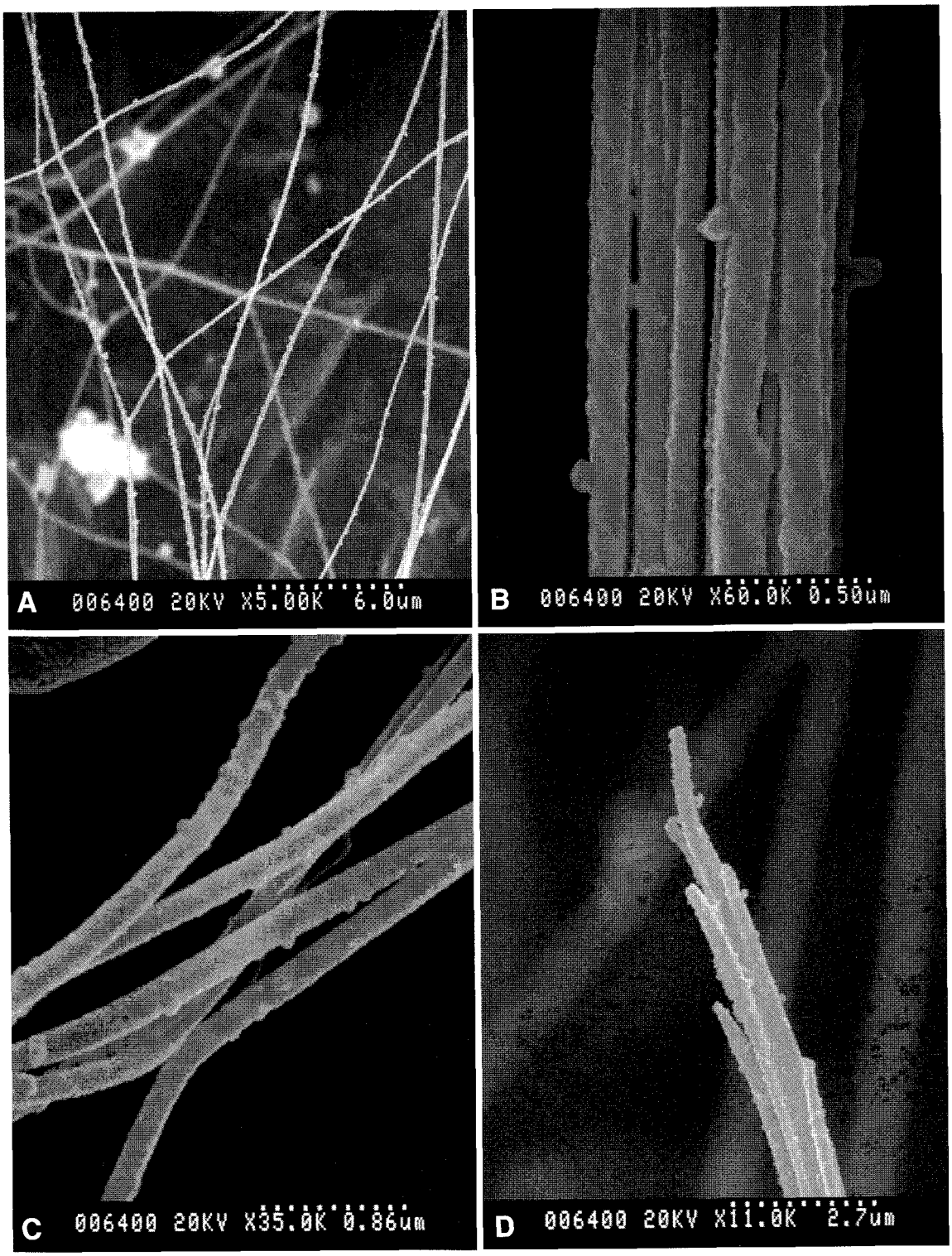

Fig. 3. Scanning electron micrographs of silk. A, Filament structures at low magnification (scale bar, 6 μm). B, C, D, Filament structures at higher magnification (B, scale bar, 0.5 μm; C, scale bar, 0.86 μm; D, scale bar, 2.7 μm).

cells phagocytize thylakoid granules and starch and that after degradation of these products, low molecular components are released by these cells and used by other cells for the formation of storage material such as glycogen and protein. The black and white pellets, which are formed by the phagocytes and consisting mainly of food residues, also contain a certain amount of guanine (McEnroe 1961, Helle and Sabelis 1985). Hallas *et al.* (1993) suggest that much of the guanine in house

dust is derived from exogenous sources. Since guanine, an essential nucleic component, is also an important constituent of excreted nitrogenous waste in mites, as *D. farinae* and *D. pteronyssinus* living in house dust, it could serve as an indicator of house dust-mite activity. Its concentration in dust samples reflects the quantity of mite pellets; these fecal pellets are known to be a major source of allergens (Tovey *et al.* 1981, Quoix *et al.* 1993).

Males and females of *T. urticae* possess five and one unpaired gland. The five paired glands are silk glands, dorsal podocephalic glands, anterior podocephalic glands, tracheal organs, and coxal organs. The unpaired gland is the tracheal gland. Spider mite silks have been characterized as elongation and alignment of globules and interactions among globules promoted by physical shear, leading to fibrillar structure. Silk threads may be composed of mucous material, glycoproteins, and liquid material which is added by the coxal organs (Mothes and Seitz 1981b). Silk threads use for nests, form sperm webs, attachment discs, dispersal, and hold egg. The main function of this silk is probably to protect eggs from mite predators, man of which are voracious egg feeders (Helle and Sabelis 1985).

Many types of silk have been characterized, including cocoon silk fibroin from the silkworm, *Bombyxn mori*, and dragline silk from the spider *Nephila clavipes*. Silkworm cocoon silk contains two structural proteins, the fibroin heavy (~350 KDa) and the sericin light (~25 KDa) chains. Silk fibre formation involves shear and elongational stress acting on the fibroin solution (up to 30% wt/vol.) in the gland, causing fibroin in solution to crystallize. Elongational flow orients the fibroin chains, and the liquid is converted into filaments (Vollrath and Knight 2001, Jin and Kaplan 2003, Kim and Shin 2005). Spider mites' silk threads whose physical properties are similar to those of the spider.

The sensitization route to spider mites has not yet been well elucidated. Spider mites allergens, however, are believed to be inhaled; therefore, mites faeces are likely to be the source of relevant mites allergens because desiccated faecal particles probably disintegrate to become incorporated into dust particles more readily than chitinous body parts and encased internal organs. These faeces, whose physical properties are similar to those of house dust mites, would seem to be a very effective way of carrying proteins to the nasal mucosa.

In conclusion, we suggest that the two spotted spider mites are the most potent producers of outdoor environment aeroallergens.

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