Appl. Ent. Zool. **22** (2): 222–228 (1987)

Adhesion of a Leaf Feeding Ladybird *Epilachna vigintioctomaculta* (Coleoptera: Coccinellidae) on a Vertically Smooth Surface

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(Received December 26, 1986)

A leaf feeding Coccinelidae, *Epilachna vigintioctomaculata*, is able to stay and to walk on a vertically smooth surface such as a glass plate in the same manner as on a horizontal one. The insect is able to adhere to the underside of a glass plate and support a load of about five times of its body weight against the force of gravity. A large number of tenent hairs is distributed on the ventral side of the first and second tarsi of the fore-, mid-, and hind-legs in a pattern of a diagonal lattice. The tips of the hairs are bent and expanded in the form of a spatula. Footprints which consisted of a large number of minute droplets were observed on a glass plate on which an insect walked. The distribution of the droplets was of the same pattern as that of the tenent hairs. Thin layer chromatography substantiated the lipidal nature of the droplets. The tenent hairs and the lipids which were probably secreted from the hairs were considered to play important roles on the adhesion of the insect on vertically smooth surfaces against the force of gravity.

INTRODUCTION

It has been known that some species of insects are able to stay and to walk on smooth surfaces such as a window and a glass ceiling against the force of gravity. Mechanisms of the adhesion have been studied since the last century by many investigators using some species of insects. However, there are still controversy about the actual mechanism of the adhesion. Recently, STORK (1980) studied adhesion of *Chrysolina polita* (Coleoptera: Chrysomelidae) and confirmed the cohesive force and surface tension of the adhesive liquid excreted from the adhesive setae played the most important role for adhesion on smooth surfaces. WALKER et al. (1985) studied the adhesive organ of the blowfly, *Calliphora vomitoria* (Diptera: Calliphoridae), and showed tenent hairs projected from the pulvilli and a liquid secretion from the hairs are sufficient to enable successful adhesion to smooth surface by this insect.

Epilachna (*Henosepilachna*) *vigintioctomaculata* (Coleoptera: Coccinellidae) is one of the most serious insect pests of potato and egg plants leaves in Japan. The adults are able to stay and to walk on vertically smooth surfaces such as a glass plate in the same manner as a horizontal one. The present studies were undertaken to know the mechanism of adhesion of this insect on smooth surfaces.

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EXPERIMENTS AND RESULTS

Insects

Adults of *E. vigintioctomaculata* were collected from fields of potato, *Solanum tuberrosum*, egg plant, *Melongena esuculentum*, and wild Solanaceae weeds such as *Solanum nigrum* and *S. americanum* in Tsukuba area. In addition, the eggs and larvae from this insect were also collected from the fields and reared to adulthood on Solanaceae plants in the laboratory under room temperature.

Body weight and weight of burden sustained

Body weight of both sexes, six individuals from each sex, was weighed individually. The mean body weight was 50.4$\pm$2.8 mg for males and 56.6$\pm$3.1 mg for females. Insects to be used for measuring a sustainable weight were tethered with a fine silk thread between the prothorax and mesothorax on the dorsal side. The tethered insects were kept individually in petri dishes with their food plants throughout the experimental period. Each tethered insect then was placed on a glass slide for microscopy cleaned with chromic acid mixture, and known weights were attached by a small piece of Cellotape® onto the thread about 4 to 5 cm from the insect. The weights were hung from the dorsal side of the insect by resting the glass slide on the rim of a 200 m*l* beaker as shown in Fig. 1. The weights were added to the thread until the insect was unable to adhere any longer on the surface by its legs and fell into the beaker.

The experiments on sustainable weights added to the body weight were carried out once a day for five successive days. The number of insects used was eight individuals, four of each sex. The weight sustained was 291.3$\pm$20.5 mg for males, and 299.0$\pm$59.9 mg for females. Thus, the load of which the insect could support against the force of gravity was about five times that of its body weight.

Structure of the tarsi

When the ventral side of the tarsi of all three pairs of legs was covered with collodion, the treated insect was unable to stay on a vertical glass plate. Observations of the tarsi with a light microscope and a scanning electron microscope (Hitachi 650, coated with Pt-Pd) showed that the ventral side of both the first and second tarsi of all three pairs of legs is trapezoid in form, but the first tarsus is somewhat more elongated and slender, and the second one is much shorter and wider as seen in Fig. 2A. The areas of tarsi of the fore-, mid-, and hind-legs are somewhat larger in females than those in males.

There are large number of tenent hairs in the ventral side of the first and second tarsi, and the hairs are distributed not at random but in a pattern of diagonal lattice as seen in Fig. 2B. The number of the tenent hairs is approximately 750 to 850 for both tarsi, although the second tarsus is larger to some extent than the first one. The length of the hairs is 70 to 120 μm, and the hairs located at the tip of the tarsus are longer than those located at the base. The tips of the hairs were bent and expanded like a spatula as seen in Fig. 3. The diameter of the tip is about 5 to 10 μm. The edge of the spatulate tip was not a smooth circle.

Footprints

When an insect adhered on the inner surface of a petri dish lid, fine droplets were

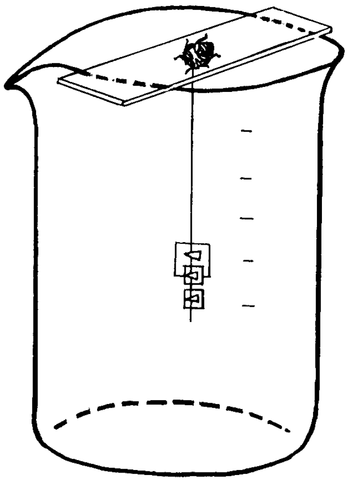

Fig. 1. Method for measurement of a sustainable weight of burden (see the text).

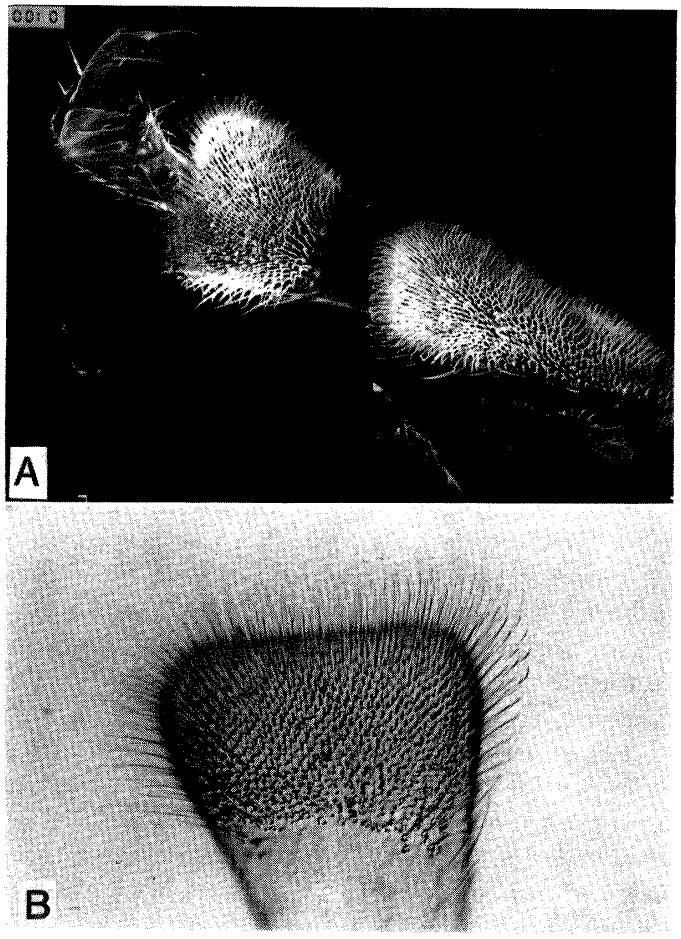

Fig. 2. Ventral view of tarsus. A: hind-leg of female (SEM). B: the second tarsus segment of fore-leg of female.

observed at the tip of the tenent hairs by using a binocular microscope from the outer surface of the petri dish (Fig. 4). The liquid was seemed to be secreted from the tenent hairs and deposited at the spatulate tips of the hairs. When an insect remained stationary or walked on a clean glass slide, footprints were observed by using a Nomarski's differential interference contrast microscope on the glass. As seen in Fig. 5, the footprints consisted of a large number of fine droplets distributed as a pattern of a diagonal lattice, and the distribution of the droplets was similar in appearance to that of the tenent hairs of the tarsi. Sometimes parallel stripes that consisted of minute droplets and which remained on the glass are also shown in the same figure. These stripes were probably remained on the glass by the insect dragging its legs during secretion of the liquid. The diameter of the droplets was 2 to 4 μm, but sometimes it was as large as 5 to 9 μm. It seems probable that the large droplets were the result of firm and/or long time contact of the tenent hairs with the glass. The droplets were found not only inside of the lid of petri dishes, but also on the bottom of the dishes in which insects were confined.

The droplets on the glass were not volatile, but those remained as long as three

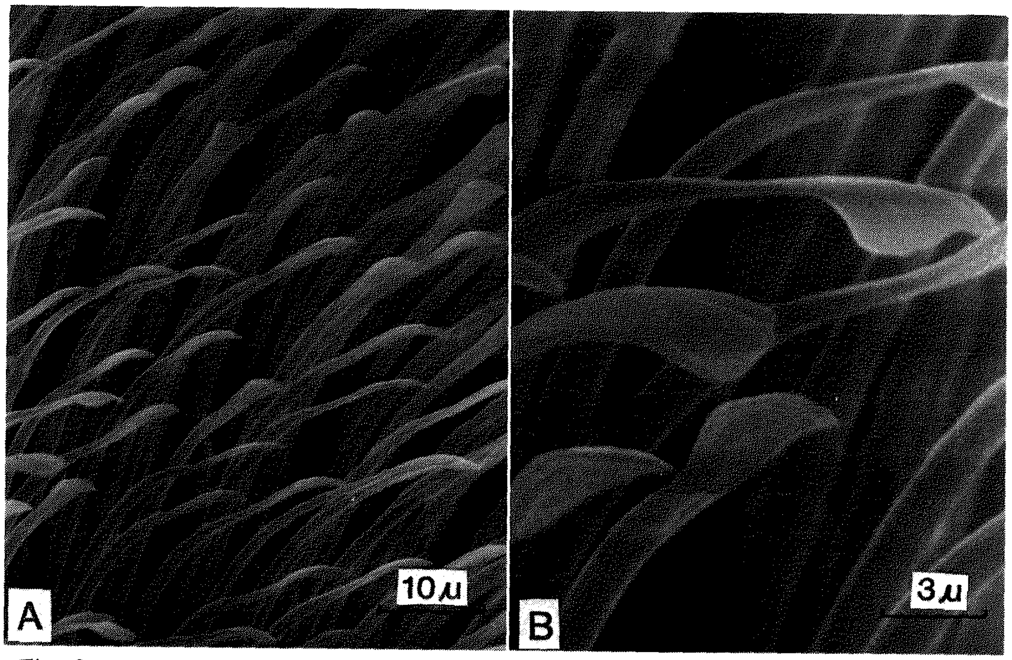

Fig. 3. Tenent hairs of the second segment of a tarsus (hind-leg of female; SEM). A: ×1,500. B: ×5,000.

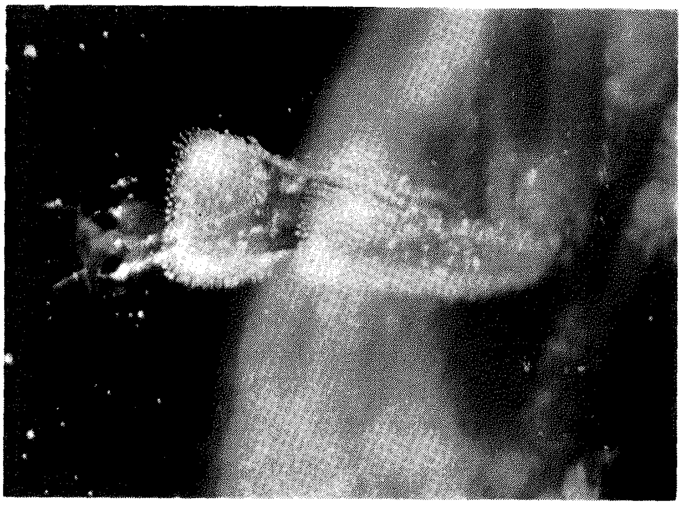

Fig. 4. Tarsus of a mid-leg adhering to the inside of the lid of a petri dish. Upper round parts of the first and second segments were in contact with the glass (right mid-leg of female).

days without significant evaporation under laboratory conditions (about 25°C). The droplets were not soluble in water, but soluble in certain organic solvents such as *n*-hexane and methanol. When the droplets were exposed under I_2 vapor, a yellowish brown color developed.

TLC of the liquid

Adults of *E. vigintioctomaculata*, about 50 individuals, were confined in a glass pot

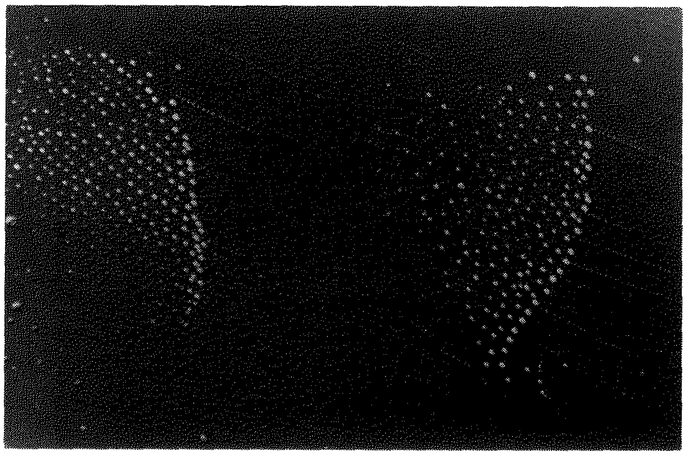

Fig. 5. The footprints of an insect on a glass plate.

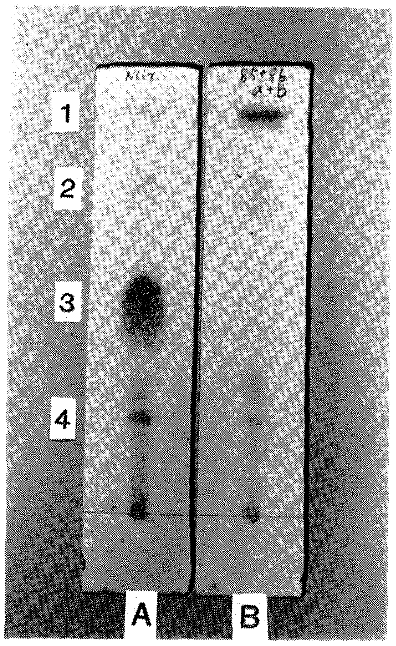

Fig. 6. Chromatograms. A: a developed TLC plate of a standard lipid mixture. B: a developed TLC plate of *n*-hexane extracts from footprints. 1: hydrocarbons, 2: triglycerides, 3: fatty acids, 4: cholesterol.

(11.5 cm diameter $\times$ 12 cm depth) with their food plants, and the pot was covered with a petri dish. The insects were allowed to walk freely inside of the pot. Footprints that remained inside of the petri dish were washed with *n*-hexane once a day. The extracts were pooled for further analysis. The total number of insects used was about 10,700 from September 1985 to January 1986.

The *n*-hexane extracts were concentrated under reduced pressure. About 20 mg of a pale yellow oily liquid was obtained. This oily liquid was dissolved in a small amount of *n*-hexane and chromatographed on a silica gel plate (Precorted TLC plate SILG-25, Macherey-Nagel, 10 cm $\times$ 2 cm) with a mixture of *n*-hexane 70: ether 30: acetic acid 1 as the developing solvent. Color was developed by heating about 110°C after spraying the plate with a chromic acid mixture. A typical chromatogram of a standard lipid mixture and the extracts of secretory material is shown in Fig. 6.

As seen in Fig. 6, the chromatogram of the extracts from the secretory material showed a typical lipid-like pattern. *Rf* values suggested the presence of hydrocarbons, triglycerides, fatty acids, alcohols and other substances. Color development in the position of the hydrocarbons clearly showed an overlap of multiple substances.

DISCUSSION

It is known that if the surface on which insects walk is rough in nature, they can hold it by their tarsal claws. However, if the surface is too smooth for the claws to grip, they use their adhesive organs generally consist of a large number of tenent hairs moistened with a liquid secretion. The mechanism by which insects can climb on a smooth surface such as a window has been controversial. Some workers have considered that the tenent hairs act as suckers, being held to the surface by atmospheric pressure, and that the small amount of liquid served merely to make the union between the tips of the hairs and surface airtight. Others have believed that the secretion as a sticky liquid and that it is the cohesive properties of this liquid which holds the insect to the smooth surface. These results have been reviewed by WIGGLESWORTH (1972) and NACHTIGALL (1974).

Recently, STORK (1980) studied adhesion of *Chrysolina polita* (Coleoptera: Chrysomelidae) on glass and perpex, and he concluded that the cohesive forces and surface tension on a thin layer of liquid secreted from the adhesive setae as the most likely modes of adhesion on smooth surfaces. More recently, during the course of the present experiments, WALKER et al. (1985) studied adhesion of the blowfly, *Calliphora vomitoria* (Diptera: Calliphoridae), on a smooth surface. They showed that the footprints of the blowfly remained on the glass, and concluded that the surface tension of the liquid secreted under tenent hairs was sufficient to enable successful adhesion to a smooth surface. They found that the liquid was a lipid, and showed on its some compositions.

The tarsus of *Epilachna vigintioctomaculata* is composed of four segments, and the third segment is so small that it is not in contact with the surface when walking. A pair of branched claws at the tip of the fourth segment does not play a role for adhesion on a smooth surface. Insects whose ventral surfaces of the first and second tarsi of all three pairs of legs were covered with collodion failed to adhere on the smooth surface indicating that the tenent hairs distributed on the tarsi played an important role for adhesion.

Fine droplets were found at the tips of the tenent hairs, and footprints which consisted of a large number of fine droplets were also found on a glass plate after an insect had walked on it. The distribution of the droplets found on the glass was in a pattern of a diagonal lattice, which coincided with that of the tenent hairs. These findings suggest that the liquids were secreted from the spatulate tips of the hairs.

The facts that the droplets were relatively non-volatile, insoluble in water but soluble in organic solvents such as *n*-hexane and methanol, and their extracts yielded *Rf* values to known standards of lipids by TLC, strongly suggested that the secretory material is lipoidal in nature. Further support was provided by the color response of secretory material when exposed in iodine vapor indicating the presence of unsaturated lipids.

The tips of the tenent hairs were bent and expanded as a spatula, and the edge of the spatulate tip was not so smooth as to make airtight when in contact with a smooth surface. It seems likely that the liquid deposited in the spatulate tips played a key role in order for this insect to adhere tightly on a smooth surface. The chemical composition of the liquid was not clearly demonstrated in this study. However TLC showed it to be a complex mixture of lipids consisting predominantly hydrocarbons, wax esters, and triglycerides. The mechanism of the adhesion will be discussed when the chemical composition of the secretory materials is clarified.

ACKNOWLEDGEMENTS

The present experiments were carried out primarily at the laboratories of the National Institute of Agro-Environmental Sciences, Tsukuba, Japan. I wish to express my sincere thanks to the members of the Division of Entomology of the Institute for the use of their facilities. Thanks are also due to Dr. J. N. Kaplanis for his critical reading of the manuscript and comments.

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