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**Instar identification and some notes about the life cycle of *Rhizoecus hibisci*
Kawai & Takagi (Coccoidea: Pseudococcidae)**

Abstract - The root mealybug *Rhizoecus hibisci* Kawai & Takagi is regularly intercepted in The Netherlands on bonsai-trees originating from China. The main host of this quarantine organism is *Serissa* sp. but it has been found on other plant species as well. Observations on a single specimen on *Serissa* plants in a container showed that one generation at 21°C lasts two months starting from the beginning of the first stage whereas on *Nerium* at the same temperature its life cycle is much longer. Males may be present but single female specimens can reproduce parthenogenetically. Examination of slide mounted specimens of mixed stage populations showed the existence of three larval instars. The number of dorsal abdominal ducts, the number of trilocular pores and the number of setae on the posterior margin of the posterior ostiole, appeared to be some of the most important discriminating characters.

Key words: quarantine organism, *Rhizoecus hibisci*, Pseudococcidae, morphology, nymphal stages.

INTRODUCTION

The root mealybug, *Rhizoecus hibisci* Kawai & Takagi (Hemiptera: Pseudococcidae) is a neotenic polyphagous species with three nymphal stages and an adult female stage. Descriptions and illustrations of the adult were provided by Kawai & Takagi (1971), Kawai (1980), Williams & Granara de Willink (1992), Tang (1992) and Williams (1996).

Since its discovery in 1989 *R. hibisci* has been regularly intercepted in The Netherlands on bonsai-trees originating from China. In 1998 it was added to the I/II European quarantine list (Anonymous, 1998). Some members of the genus *Rhizoecus* are polyphagous and some can propagate asexually, living on the roots; several species are regularly recorded as greenhouse pests. Besides, they are hardly detectable if present in low numbers and chemical control is difficult which contributes to a rapid spread with trade of potted plants. Although the genus is well defined, its great uniformity, the slight differences between species and the variability within species, make identification time consuming. There is hardly any knowledge about the

taxonomy of pre-adult stages. Although Hernandez Paz (1965) studied the biology of *R. americanus* (Hambleton) and Snetsinger (1966) the biology of *R. dianthi*, little is known about the biology of *Rhizoecus* species in general and *R. hibisci* in particular, about the predators and parasites, their reproduction and life cycle, mortality factors, phenology and their behaviour in the open.

Serious damage of *R. hibisci* was only noticed once on *Serissa foetida* in a greenhouse in The Netherlands in 1992 (Jansen, 1995) and sometimes damage is rare despite the presence of high numbers. In general root mealybug cause slow plant growth.

MATERIALS AND METHODS

The population development of *R. hibisci* was followed on infected *Serissa*-bonsai plants, which were placed in a transparent plastic chamber through which the activity could be observed (Hernandez Paz, 1965). The population was started with the transmission of one ovisac of a population originating from an imported plant to an uninfected plant.

For the SEM study the different stages of *R. hibisci* were fixed in 3% glutaraldehyde prepared in 0.05 M phosphate buffer at pH 6.8 for one hour at room temperature, washed three times in distilled water and dehydrated in graded series of acetone, critically dried in the critical point dryer, mounted onto the copper stubs and coated by goldpaladium (20 nm thickness) in the sputter coater (Polaron E5100). The stubs were scanned at different angles under the scanning electron microscope (JSM-6300F) at 3 kV in the Botanical Institute of the Agricultural University of Wageningen.

For microscopic slides, specimens were cleared in 10% KOH, ethanol 70%, and acetic acid, stained in a mixture of lignin pink, Essig's aphid fluid and acid fuchsin and mounted in Canada balsam. Measurements were made using an Olympus BX microscope with eyepiece micrometer at magnifications of 100, 200, 400 and 1,000x. All measurements are of slide mounted specimens. Lower or upper extreme measurements, which differ from the normal range, are added in brackets. Body length was measured along the mid dorsal surface, body width at the level of the middle legs. The tarsal measurement does not include the claw. Each illustration is divided longitudinally, with the left half representing the dorsum and the right half the venter.

RESULTS

Life cycle and host plants

A single specimen on *Serissa* plants in containers showed that at 21°C one generation of the females lasts 61 days which is the period between the hatching of eggs of an adult and its descendants. The ovisac has its definitive shape within four days but its compactness increased during an additional four days in which period

most eggs are probably laid. The precise start of egg laying cannot be observed because the eggs are hidden in the ovisac. The first nymph hatched fourteen days after the start of the ovisac and the last one three days later, so the period of increasing compactness of the ovisac is as long as the period of hatching. That means that on average eggs hatch after 9 days. At 21 °C on *Nerium* its life cycle lasts at least one month longer indicating that *Nerium* is a less suitable host.

Based on present observations and literature data (Ben-Dov, 1994; Williams, 1996) *R. hibisci* has been found on the following host plants: Amaryllidaceae: *Crinum asiaticum*. Apocynaceae: *Nerium oleander*. Araceae: *Dieffenbachia* sp. Bromeliaceae: *Cryptanthus* sp. Commelinaceae: *Dichorisandra thrysiflora*. Cyperaceae: *Carex* sp. Geraniaceae: *Pelargonium* sp. Gramineae: *Hakonechloa macra*. Lythraceae: *Cuphea hyssopifolia*. Malvaceae: *Hibiscus rosa-sinensis*. Maranthaceae: *Calathea makoyana*. Moraceae: *Ficus nitida*. Palmae: *Areca* sp., *Kentia* sp., *Phoenix canariensis*, *P. roebelenii*, *Rhapis excelsa*, *Sabal* sp., Rhamnaceae: *Sageretia theezans*. Rubiaceae: *Serissa foetida* Ulmaceae: *Celtis* sp., *Zelkova serrata*

Egg (Fig. 1)

The number of eggs in ovisacs varied from 11 to 84 (n= 20) on different host plants. The egg is oval in shape, pale yellow in colour and it measures 240-250 microns in length and 140-150 microns in width.

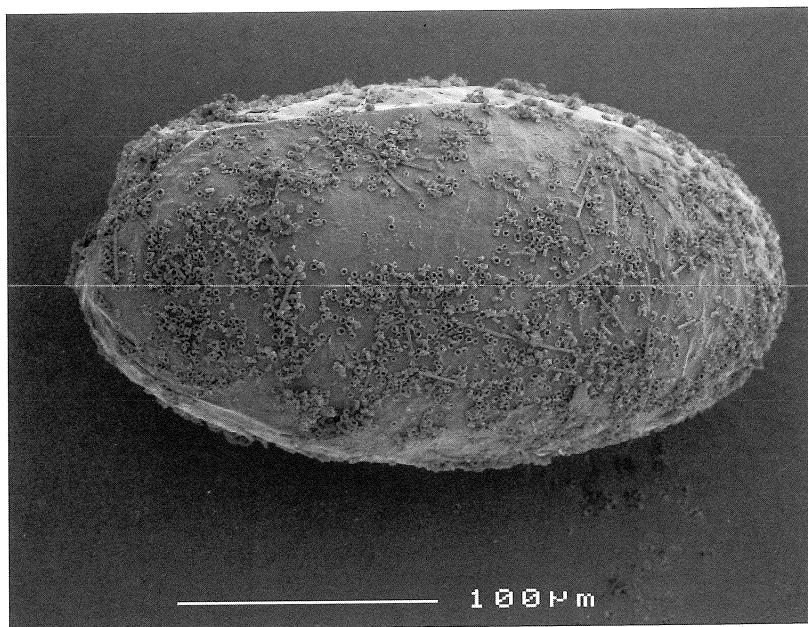


Fig. 1 - SEM photograph of *Rhizoecus hibisci*: egg.

Males

Males were never observed in populations of bonsai-trees during the period 1989-2000. However, both adult males and larvae were observed in high numbers at the roots of a *Serissa* shrub three months after the transmission of an ovisac. Third (prepupa) and fourth (pupa) instar males could be distinguished from female instars, however, no distinguishing characters could be found between the males and females of the first two larval stages. The most striking field characters of the adult male are the yellowish white body, a light brown head, red eyes, the first two segments of the antennae light brown, white legs and the presence of two wings which are dusted with greyish-white wax. The fourth-instar male lives in a white cottony sac, which is elongate in relation to the female ovisac, and its length is about 1.5 mm and its width 0.5 mm.

KEY TO THE FEMALE INSTARS OF *RHIZOECUS HIBISCI*

1. Tritubular ducts present on the first segment of the dorsal abdomen, no bitubular ducts present; body length 275-420 μ m. first instar
- Tritubular ducts absent, bitubular ducts present; body length longer than 450 μ m. 2
2. Posterior ostiole with trilocular pores and setae in posterior margin absent; length 480-720 μ m; dorsal abdomen with four dorsal bitubular ducts second instar
- Posterior ostiole with two or three setae and trilocular pores in posterior margin; length more than 850 μ m; dorsal abdomen with more than four bitubular ducts 3
3. Dorsal abdomen with six bitubular ducts; multilocular disk pores, tubular ducts, circuli and vulva absent; body length 850-1250 μ m third instar
- Dorsal abdomen with more than six bitubular ducts of two sizes; multilocular disk pores and vulva present; circuli 0-2; body length 940-2125 μ m adult female

DESCRIPTION OF NYMPHAL INSTARS

First instar (Figs. 2, 3, 4, 5) (n= 11)

Field characters

Body flesh to pinkish coloured. Dorsal and ventral side with a thin layer of white powdered mealy secretion. The body is oval in shape, flattened ventrally and convex dorsally. Normally they are very active walking around.

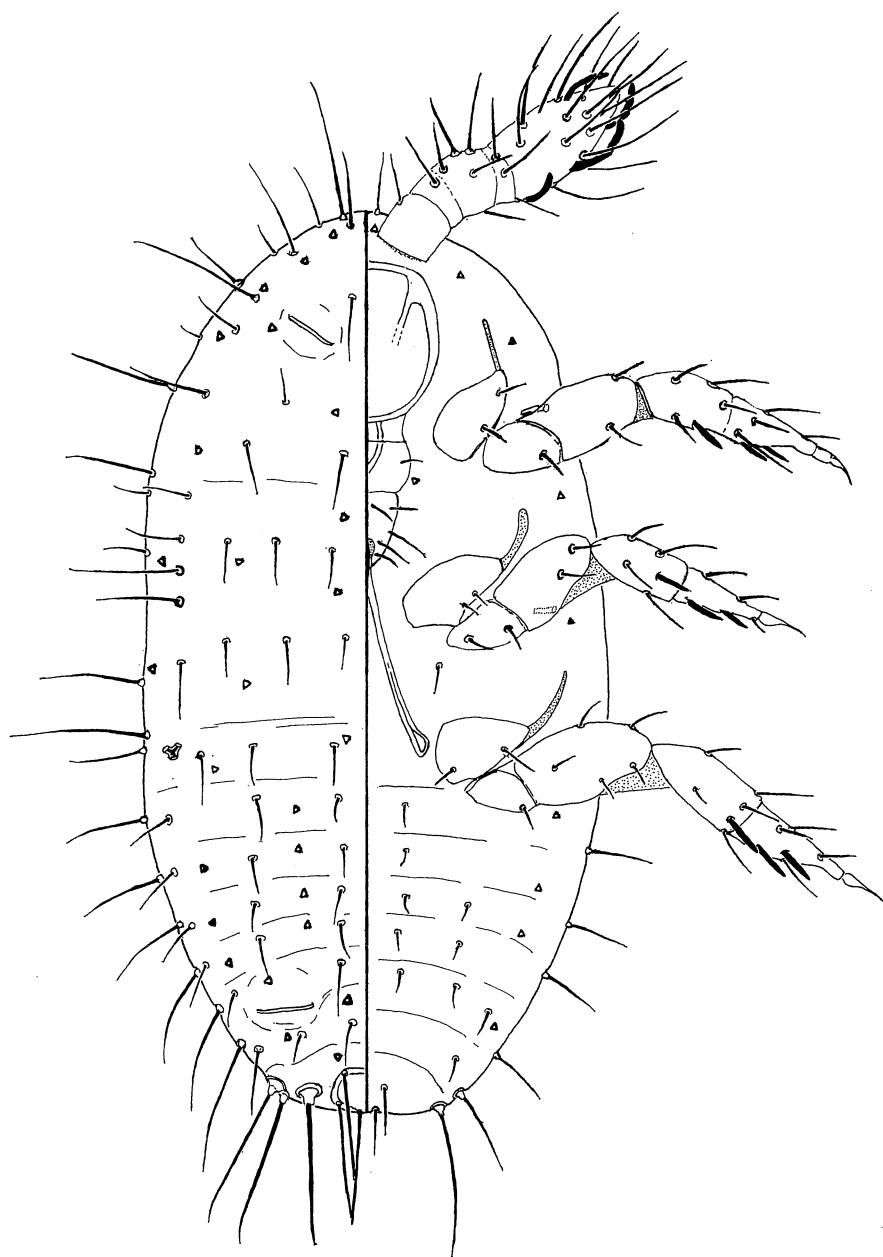


Fig. 2 - First-instar female *Rhizoecus hibisci*. Bleiswijk, 9 XI 2000, The Netherlands, on *Serissa foetida*.

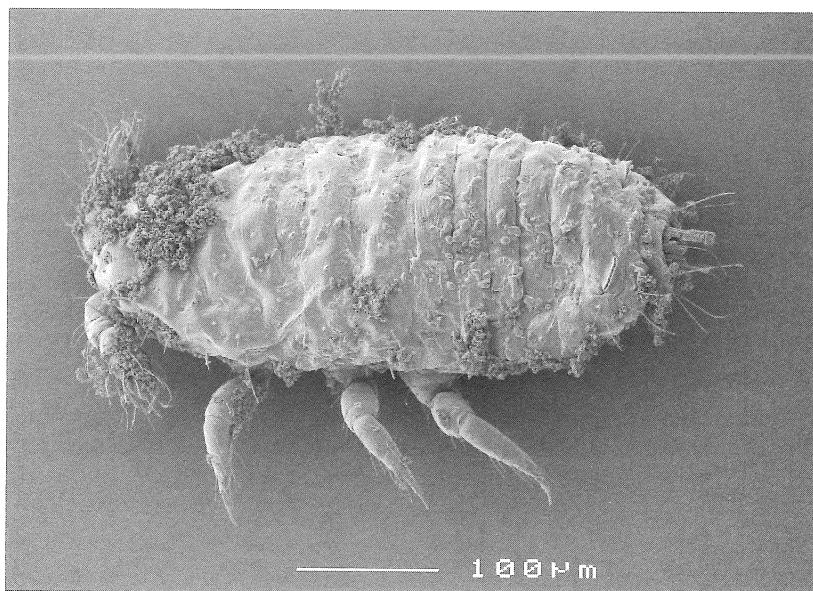


Fig. 3 - SEM photograph of *Rhizoecus hibisci*: first-instar female R39.

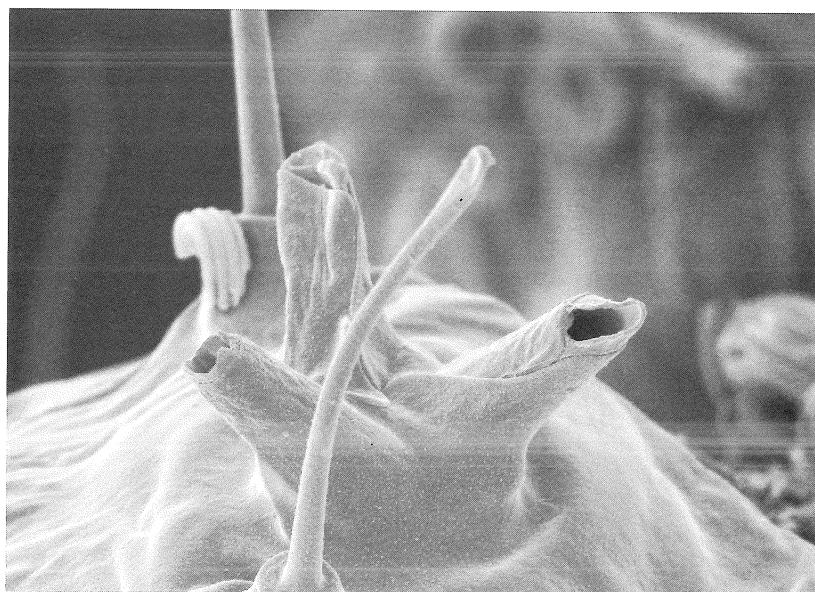


Fig. 4 - SEM photograph of *Rhizoecus hibisci*: tritubular duct first-instar female.

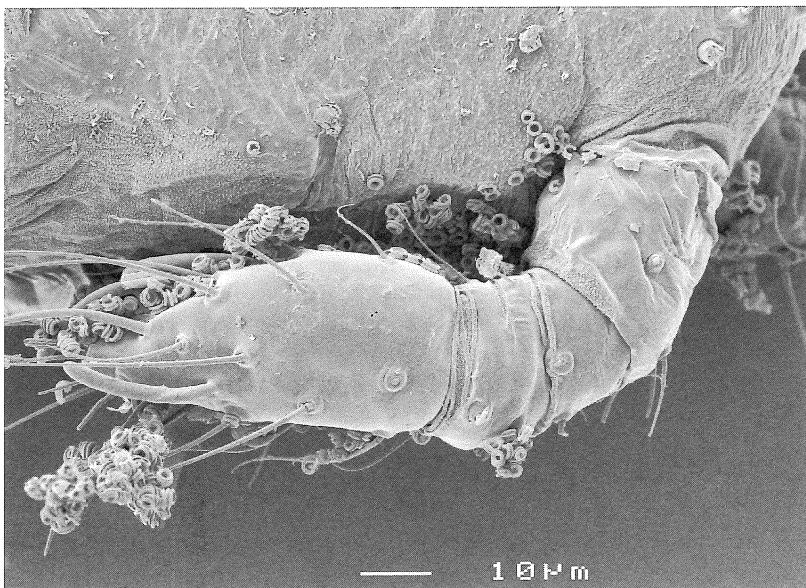


Fig. 5 - SEM photograph of *Rhizoecus hibisci*: antenna first-instar female.

Slide mounted characters

BODY: Dorsoventrally flat, oval, membranous, 275-420 μ m long (in mounted specimens up to 340 due to shrivelling) and (110)135-165 μ m wide, Ratio of body width to width of clypeus 2.8-3.5.

DORSUM: Multilocular disc pores and tubular ducts absent. One pair of tritubercular pores on the margin of abdominal segment I, 5-6 μ m wide. Posterior ostiole with no trilocular pores and no setae in posterior margin, inner edges of lips not sclerotized. Anterior ostiole often barely perceptible. Anal ring about 26-29 μ m wide with six setae, each about 33-43 μ m long; anal lobe setae 45-55 μ m long. Abdomen with slender setae in six rows, on segment I-VI 13-17 μ m long; segment VII-VIII 18-21 μ m long. Thoracic setae 13-20 μ m long; setae on head 15-20 μ m long. Trilocular pores on abdominal segment I: 4. Marginal setae on thorax up to (21)40-58 μ m long; on abdomen up to (22)26-38 μ m long.

VENTER: Antennae with five segments and total length of 85-100 μ m. Length of segment I: 23-30 μ m; length segment V: 50-58 μ m, total length of segment II-IV 15-20 μ m, each segment can be recognised by a weak sclerotization but whereas the outward side is clearly divided, the inward side is more or less undivided. Legs well developed, total length 185-213 μ m; without tibiotarsal sclerosis; hind femur 48-55 μ m long; hind tibia 38-43 μ m long; hind tarsus 52-59 μ m long; hind claw 20-23 μ m long;

hind coxa about 42-45 μ m long; hind trochanter + femur 65-75 μ m long, hind tibia + tarsus 90-108 μ m long, claw slender, 19-23 μ m long. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.35-1.44. Ratio of lengths of hind tibia to tarsus 0.63-0.78; Ratio of lengths of hind tibia to femur 0.7-0.88. Abdominal marginal setae up to 40 μ m long, thoracic marginal setae up to 58 μ m long. Setae on abdomen in six rows, on segment I-VII 8-15 μ m long, on segment VIII 13-17 μ m long. Labium 48-53 μ m long, ventral side with three setae alongside groove; width clypeus 45-48 μ m; Ratio of body width to width of clypeus 2.8-3.8. Cephalic plate not visible. Trilocular pores as follows: abdominal segment I: 4; II: 2; Posterior spiracle with long muscle plate; length 17-18 μ m, peritreme 5-6 μ m wide.

Second instar (Figs.6, 7) (n= 11)

Field characters

Body flesh to pinkish coloured. Dorsal and ventral side with a layer of white powdered mealy secretion. The body is oval in shape, flattened ventrally and convex dorsally.

Slide mounted characters

BODY: Dorsoventrally flat, oval, membranous, 480-720 μ m long and 180-360 μ m wide. Ratio body width to width of clypeus 4.4-6.0.

DORSUM: Posterior ostiole with no trilocular pores and no setae in posterior margin, inner edges of lips unsclerotized. Anal ring about 35-40 μ m wide with six setae, each about 37-43(48) μ m long; anal lobe setae 58-70 μ m long. Abdomen with slender setae, on segment I-VI 10-25 μ m long; segment VII-VIII 16-30 μ m long. Setae on head 16-34 μ m long; thoracic setae 9-45 μ m long. Segment I with (9) 12-16 trilocular pores. Number and position of bitubular ducts are as follows: one in midfrontal position on head, two on the submarginal position of the mesothorax; four on abdomen from which two on margin of segment II and the other on margin of segment VII.

VENTER: Multilocular disc pores and eyes absent. Antennae with five segments 125-135 μ m long; segment I: (27)32-38 μ m; length segment V: 58-64 μ m, segment II-IV (31)35-40 μ m, weakly sclerotized at their outer side and more pronounced at their inner side; interantennal width 26-37 μ m.

Legs well developed, without tibiotarsal sclerosis, total length 255-289 μ m; hind femur 65-78 μ m long; hind tibia 45-55 μ m long; hind tarsus 44-50 μ m long; hind claw slender (18)21-22(25) μ m long; diagonal length hind coxa (53)55-60 μ m; Hind trochanter + femur 88-100 μ m long, hind tibia + tarsus 92-108 μ m long. Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 0.97-1.08. Ratio of lengths of hind tibia to tarsus 0.8-1.13. Ratio of lengths of hind tibia to femur 0.64-0.81. Partly with two rows of marginal setae; abdominal ones up to 25-48 μ m long, longest on

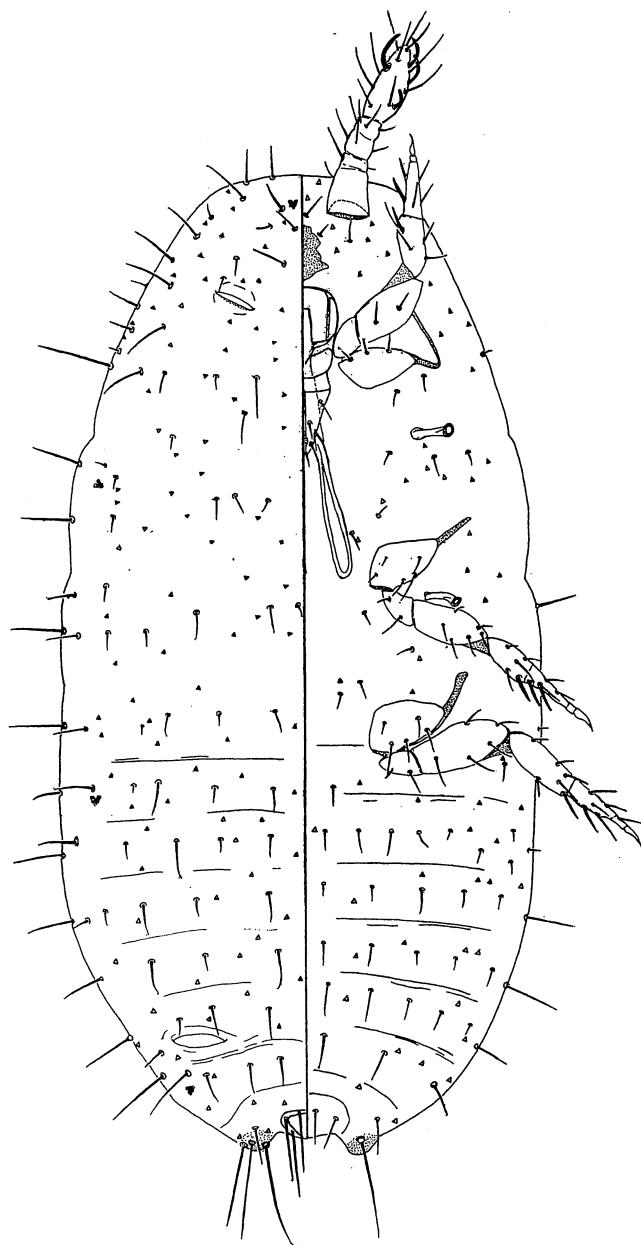


Fig. 6 - Second-instar female *Rhizoecus hibisci*. Bleiswijk, 7 XI 2000, The Netherlands, on *Serissa foetida*.

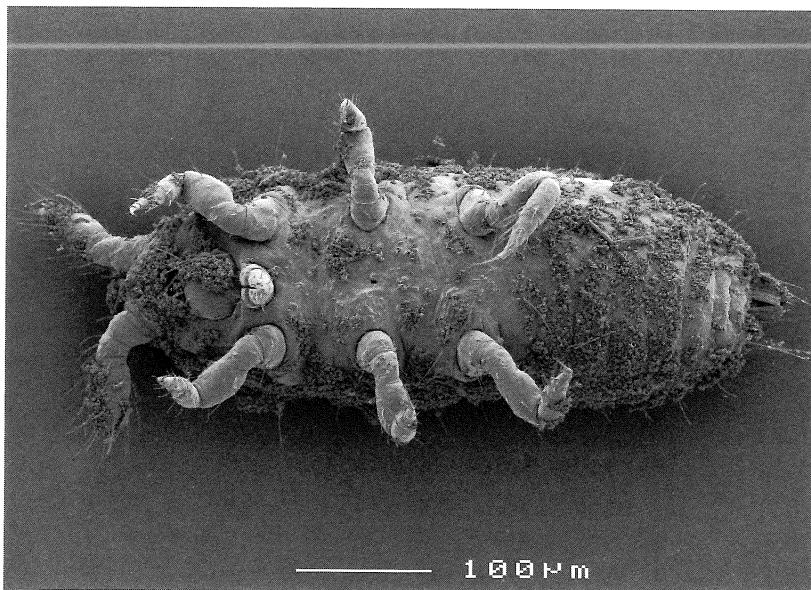


Fig. 7 - SEM photograph of *Rhizoecus hibisci*: second-instar female.

thorax up to 55 μ m. Setae on abdominal segment I-VII 10-20 μ m long, on segment VIII 15-33 μ m long. Labium 62-72 μ m long; width of clypeus 55-70 μ m; Ratio of body width to width of clypeus 4-6.0. Cephalic plate waved, triangular. Posterior spiracle with long muscle plate; 20-26 μ m long, peritreme 9-11 μ m wide.

***Third instar* (Figs. 8, 9, 10) (n=18)**

Field characters

Body flesh to pinkish coloured. Dorsal and ventral side with a thick layer of white powdered mealy secretion. The body is elongate oval in shape, flattened ventrally and convex dorsally.

Slide mounted characters

BODY: Dorsoventrally flat, oval, membranous, 850-1250 μ m long and 340-550 μ m wide. Ratio of body width to width of clypeus 5.9-7.2.

DORSUM: Multilocular disc pores and tubular ducts absent. Posterior ostiole with (2)3-4 trilocular pores and 1-3 setae in posterior margin, inner edges of lips unsclerotized. Anal ring about 40-48 μ m wide with six setae, each about (38)51-58(62) μ m long; anal lobe setae (65)70-85 μ m long. Abdominal surface with slender setae; on head 13-45 μ m

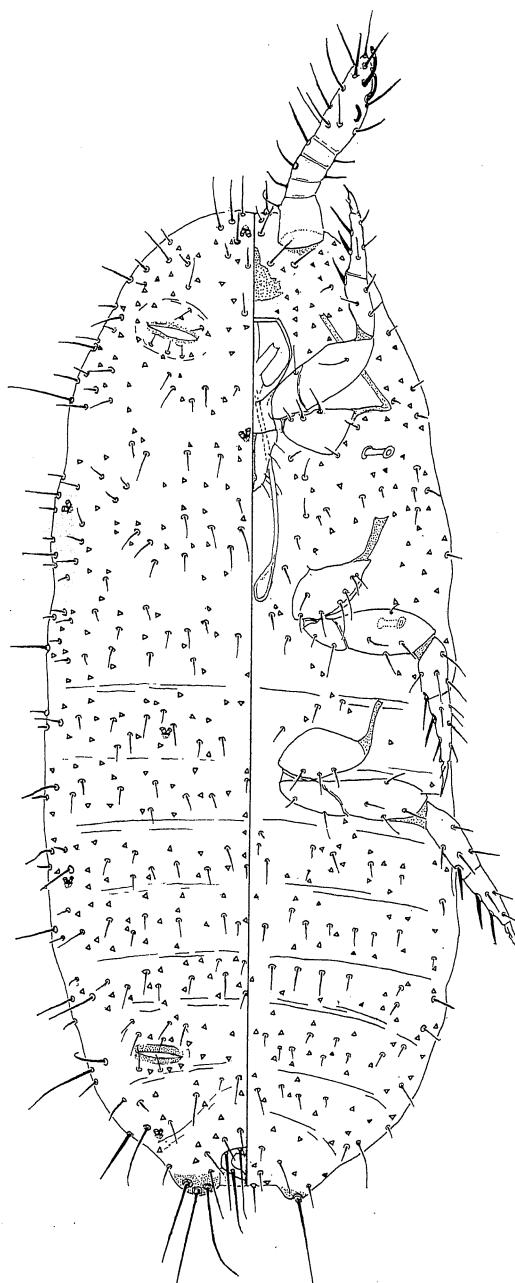


Fig. 8 - Third-instar female *Rhizoecus hibisci*. De Kwakel, 16 X 2000, The Netherlands, on *Serissa foetida*.

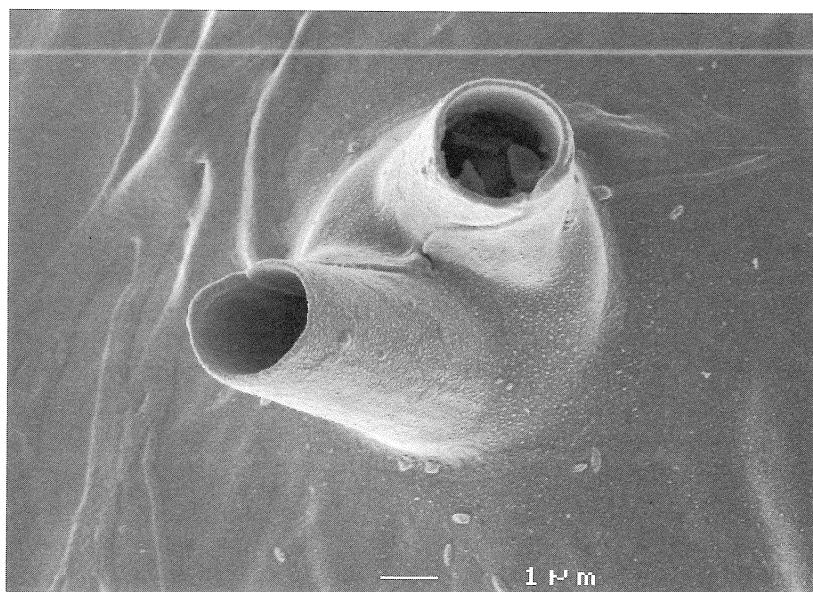


Fig. 9 - SEM photograph of *Rhizoecus hibisci*: bitubular duct third-instar female.

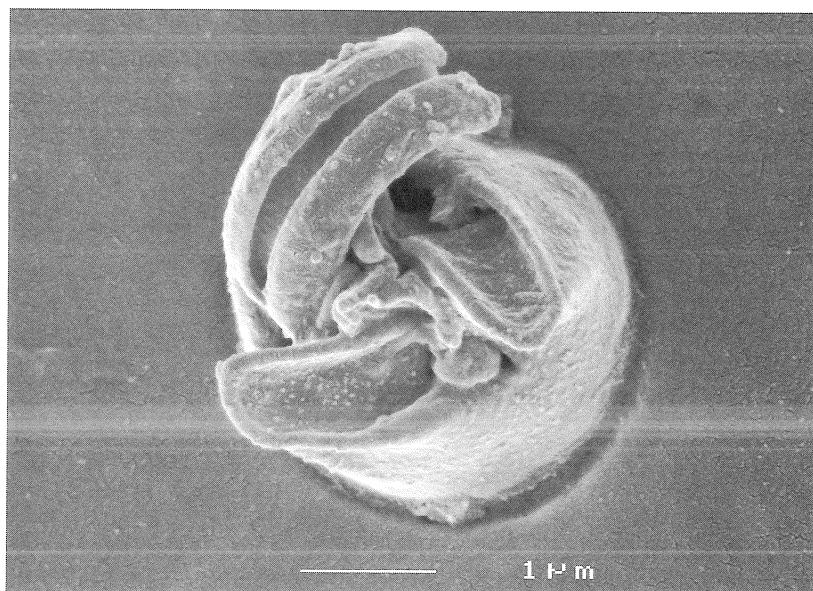


Fig. 10 - SEM photograph of *Rhizoecus hibisci*: trilocular pore third-instar female.

long; on thorax 11-30 μ m long; on abdominal segment I-VII 9-35 μ m long, Segment I with 21-35 trilocular pores. Number and position of bitubular ducts are as follows: one in midfrontal position on head, 3 (4-8) on thorax, one in median position between prothorax and metathorax and two in the submarginal position of the metathorax; 6 on abdomen: two in submedian position of segment I, two in submargin of segment III (seldom on segment II) and two on submargin of segment VII.

VENTER: Antennae with five segments; length of segment I: 35-48 μ m; length segment V: (67)73-85 μ m, segment II-IV 50-60 μ m, total length (140)150-180 μ m, well sclerotized. Legs well developed, without tibiotarsal sclerosis; femur (90)98-108 μ m long; tibia 67-81 μ m long; tarsus 82-96 μ m long; claw 24-27 (30) μ m long; hind coxa (70)75-87 μ m long; Hind trochanter + femur 115-155 μ m long, hind tibia + tarsus (135)150-170 μ m long, Ratio of lengths of hind tibia + tarsus to hind trochanter + femur 1.07-1.38. Ratio of lengths of hind tibia to tarsus 0.78-0.88; Ratio of lengths of hind tibia to femur 0.68-0.76. Cephalic plate waved, triangular. Interantennal width 48-60 μ m long. Abdominal marginal setae (25)40-52(63) μ m long. Marginal setae on thorax (40)55-65 μ m long. Setae on abdomen as follows: on segment I-VII 9-25 μ m long, on segment VIII (18)25-30 μ m long. Labium 70-87 μ m long, width clypeus 65-80 μ m; Ratio of body width to width of clypeus 6.1-7.1. Circuli absent. Cephalic plate in stained specimens well visible. Posterior spiracle with long muscle plate; greatest length 30-35 μ m, peritreme 12-15(17) μ m wide.

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