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Low-temperature SEM studies on genital structures and spermatophores of the grape rust mite *Calepitrimerus vitis* Nalepa, 1905 (Acari, Eriophyoidea)

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(With 11 figures)

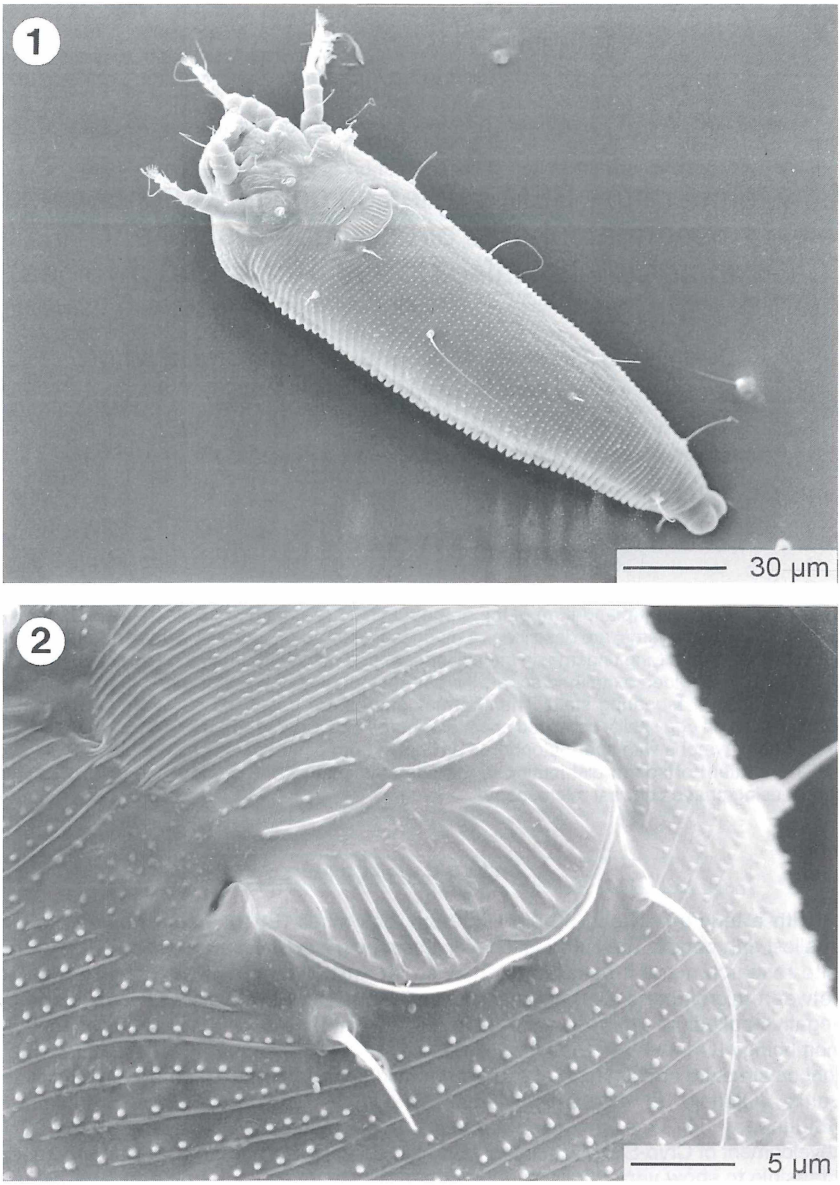
Abstract

Using the Cryo-SEM technique, the external morphology of the genital apparatus of the female (epigynium) and male (epiandrium) of the grape rust mite *(Calepitrimerus vitis* Nalepa, 1905*)*, a common pest in vineyards, are described. A diagnostic feature of the male genital apparatus are the *setae eugenitalis* lying underneath the epiandrium and being absent in the female epigynium. Like in all Eriophyoidea examined so far, the spermatophores of the grape rust mites consist of a base and a disk-bearing stalk. The spermatophores are deposited on all parts of the leaf surface, even on egg shells, collapsed spermatophores and dead grape rust mites. An encircling of quiescent nymphs with spermatophores as known from other species has not been detected yet. The pattern of sperm distribution in the spermathecae of the deutogynes of *C. vitis* was shown using phase contrast microscopy.

Introduction

With a length between 80 µm - 500 µm, gall mites (Eriophyoidea) belong to the smallest arthropods. The introduction of the scanning electron microscope (SEM) in gall mite research made it possible to show so far unknown structures of a size ranging between micrometers (µm) and nanometers (nm). The SEM pictures, partly made by negative-collodium impressions, gave insight into new dimensions of eriophyid morphology (Orlob 1966, Eisbein & Proeseler 1967). Subsequent investigations shed light on structure and function of some eriophyid spermatophores, i.e. *Aculus cornutus* Banks (Oldfield *et al.* 1970), *Aceria (Eriophyes) sheldoni* Ewing (Sternlicht & Goldenberg 1971) and *Aceria chondrillae* (Careshe & Wapshere 1974). The development of Cryo-SEM, with integrated high vacuum freezing and sputter unit, made it feasible to show very fragile structures nearly without causing any artefacts.

The grape rust mite *Calepitrimerus vitis* Nalepa, 1905 is a worldwide distributed pest in vineyards. Investigations done by Keifer (1952), Schruft (1962) and Carmona (1978) using light microscopy described eggs, larvae and nymphs. Moreover, they found that two formerly separated species are synonymous representing a summer form (protogyne) and a winter form (deutogyne) of *C. vitis.*



Figs 1-2. *C. vitis* Nal.: 1 - protogyne female, ventral side; 2 - as above, epigynium with the cover flap, the latter without constant number of striae (all SEM images: SEM Laboratory, the University of Basel).

Scientific interest in the biology of *C. vitis* is growing at the same time as this pest starts to cause problems in many regions of viticulture. Hence, the purpose of our study was to get more information about morphological aspects of this vermin. In the present paper the morphology of the genital region of adults and larvae and the structure and position of spermatophores are described. In addition, we examined the spermathecae of inseminated deutogynes of the grape rust mite.

Materials and Methods

The deutogynes mites were obtained by dissecting winter buds [cultivar (= cv) Kerner] at the end of March 1997. To obtain protogynes of *C. vitis* in July 1996 and August 1997, heavily infected leaves (cv. Kerner) from a vineyard of the "Staatliches Weinbauinstitut Freiburg" were collected. To examine the ventral side of the protogynes, a number of mites had to be turned around without damaging them. For this purpose, we brushed off the mites directly onto a drop of glue on a Balzers specimen table using a brushing machine (Juchheim 2300). This way most of the gall mites adhered to the glue with their dorsal side. Numerous specimens of *C. vitis* mounted on four microscope slides are deposited in the Zoologisches Museum Hamburg (ZMH Reg. No. 30/98).

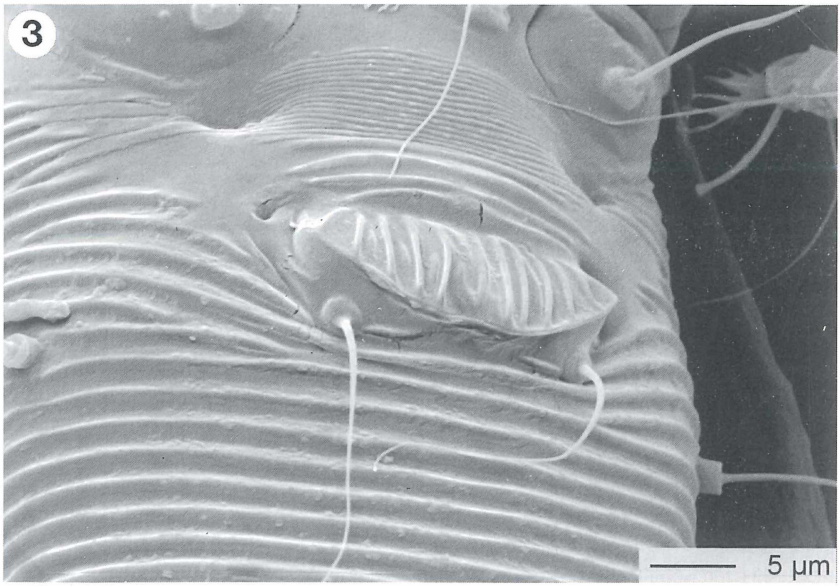
Leaves were cut into pieces of 1-2 cm^2 to examine the spermatophores. The pieces were mounted on a Balzers specimen table using a low-temperature mounting medium and rapidly frozen by plunging them into liquid nitrogen (Müller *et al.* 1986, Guggenheim *et al.* 1991). After cryofixation, samples were transferred to a Balzers cryopreparation unit SCU 020 attached to a JEOL JSM 6400 scanning electron microscope. Sputter coating with 20 nm of gold was carried out in argon gas atmosphere. The samples were examined and photographed at a stage temperature of - 165 °C using an accelerating voltage between 10 and 25 keV. SEM studies were carried out at the SEM Laboratory, University of Basel.

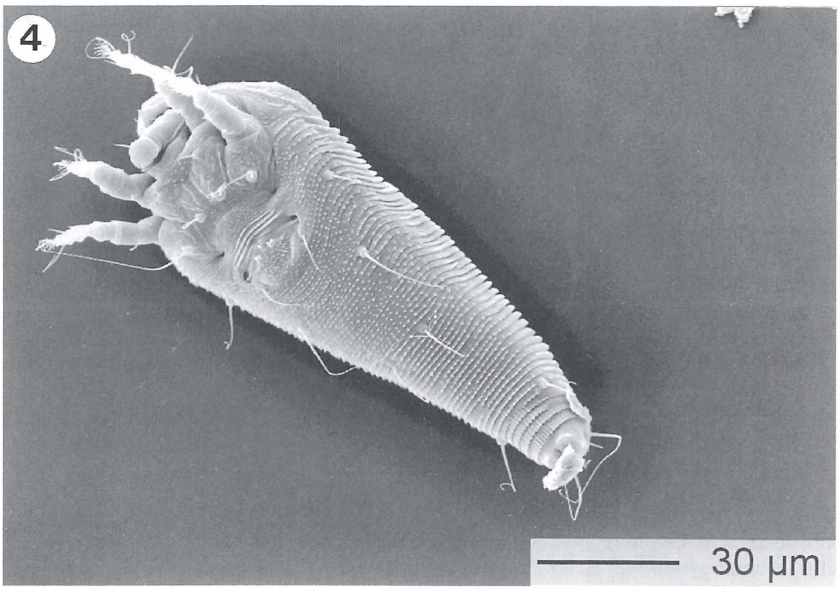
In addition, deutogynes were studied by phase contrast light microscopy. The spermathecae were examined by placing the females in a small drop of distilled water on a microscope slide. By moving the cover slip tenderly, most of the body contents was pressed out so that inseminated or empty spermathecae became visible.

Results and Discussion

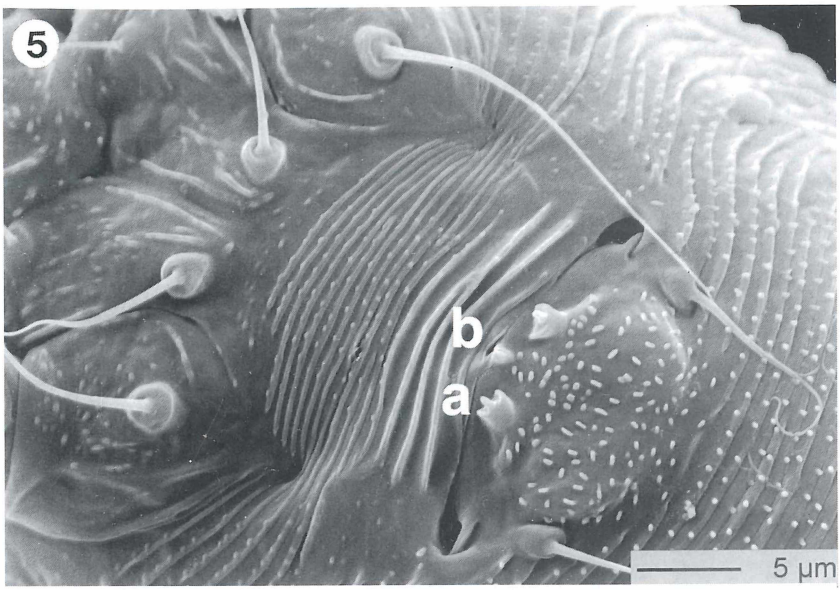
FEMALE GENITAL REGION. The external genital region of adult Eriophyoidea is located in both sexes near the coxae of the second extremity on the ventral ophisthosome (Fig. 1). In females, the external genital region (epigynium) consists of a half-moon formed flap covering the progenital chamber (Fig. 2). The cover flap possesses a series of clearly visible longitudinal striae. The number of these striae is not constant between species, and should not be used for taxonomy because intraspecific variability is possible (Schliesske 1978). Protogynes and deutogynes of *C. vitis* normally bear 12 striae but there are deviations from 8 up to 13. The shape of the distal part of the genital flap of deutogynes is more pointed than in protogynes (Fig. 3). The cover flap in both forms has no microtubercles. But in contrast to the ventral ophistosoma of the protogynes of *C. vitis,* which is covered heavily by them, the ventral side in deutogynes has a reduced number of microtubercles and small ridges on the posterior annuli.

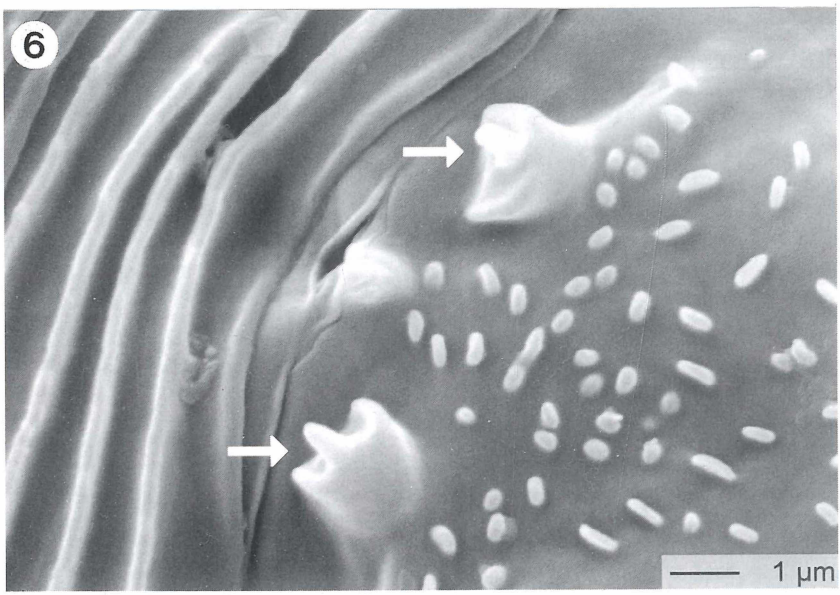
MALE GENITAL REGION. The external genital region of males (epiandrium) of *C. vitis* consists of a transverse slightly curved crevice, its lateral ends showing an abrupt bend



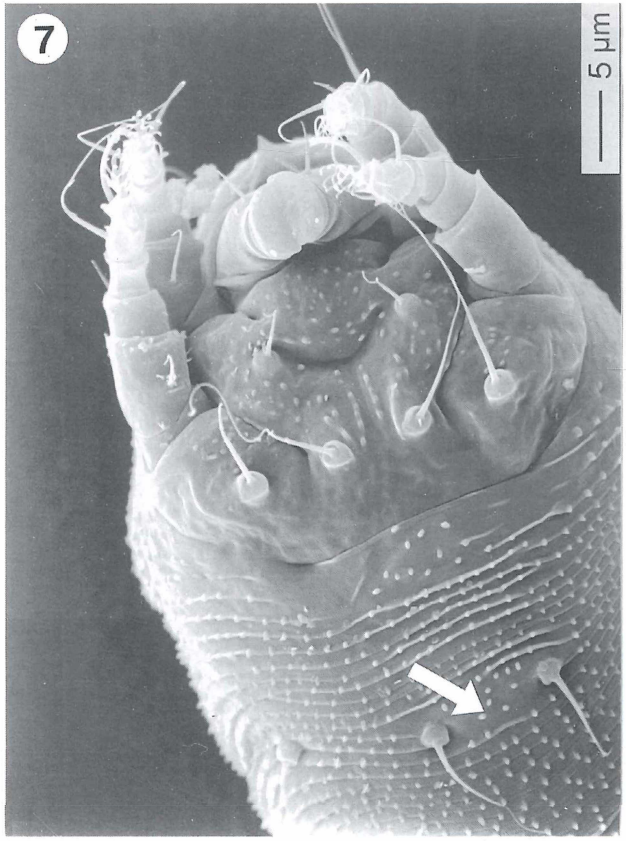


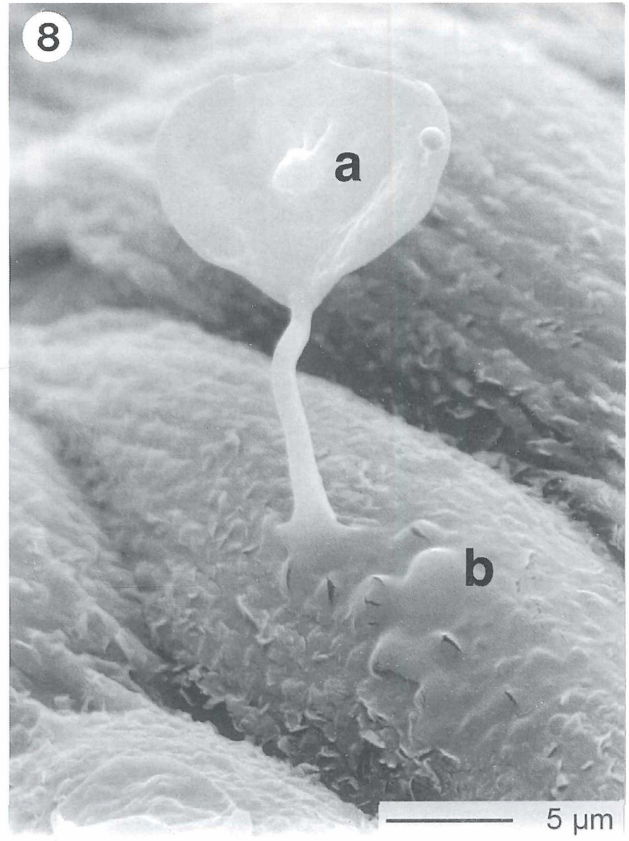
Figs 3-4. *C. vitis* Nal.: 3 - deutogyne, epigynium with the cover flap, ventral view; 4 - male, ventral side.





Figs 5-6. *C. vitis* Nal.: 5 - male, epiandrium, ventral, a - setae eugenitalis, b - gonopore; 6 - as above, the (very short) setae eugenitalis in detail (arrows).





Figs 7-8. *C. vitis* Nal.: 7 - nymph, future genital region (arrow), ventral; 8 - spermatophore of *C. vitis* on a leaf of *Vitis vinifera*: a - head with the sperm sack, b - the stalk and the base with an affix substance.

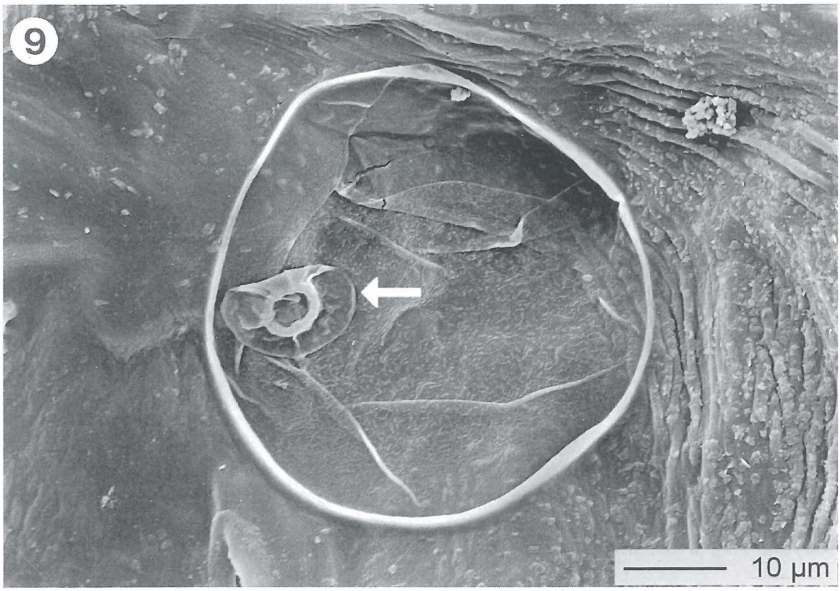
of 180° (Fig. 4 and Fig. 5). Through this, a thin flap is formed that does not open backwards like the female cover flap but forwards. The little openings on both sides of the crevice possibly originated by distorting the outer ends. The margins of the epiandrium are less thickened and close the progenital chamber. Medially a gonopore is located which connects the *ductus ejaculatoris* and *vas deferens* to the testis (Nuzzaci and Alberti 1996). The typical annuli of Eriophyidae are interspaced between the *setae genitalis* of the epiandrium. Instead of annuli this area is covered by many microtubercles.

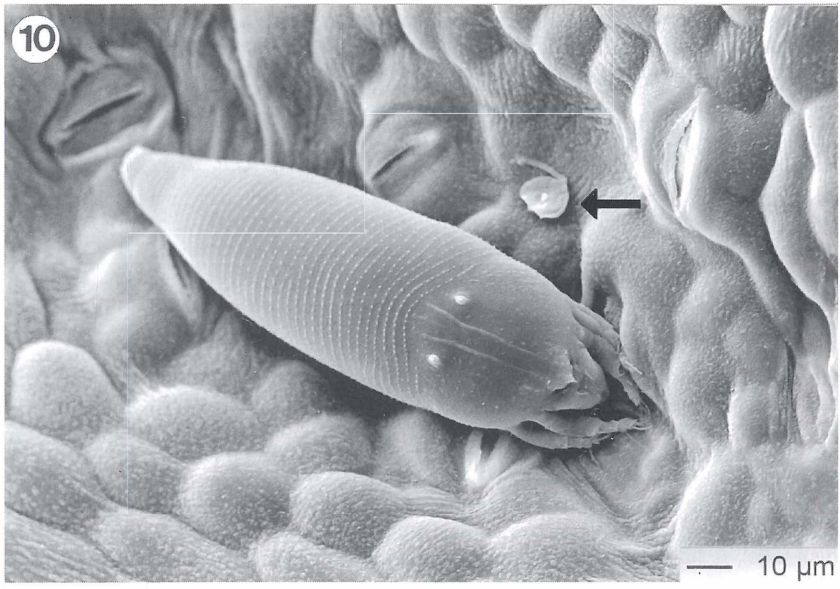
Shortly posterior to the crevice the *setae eugenitalis* insert. These setae are absent in females and constitute minute stumps fairly rising above the border of the bases (Fig. 6). They possibly represent sensorial organs, which help to determine the deposition sites of spermatophores or support the deposition itself. Females and males of *C. vitis* possess *setae genitalis*, lying beneath the genital openings. Also larvae and nymphs have *setae genitalis* (Fig. 7). Lindquist (1996) questioned that these setae are real genital setae, but coxisternal setae of the third, reduced pair of legs.

LARVAE AND NYMPHS. Larvae and nymphs have no genital opening (Fig. 7). An indication for future genital structures in nymphs of *C. vitis* is the absence of annuli between the *setae genitalis* and a reduced number of microtubercles. Between prosoma and ophistosoma, the juveniles have a distinct slit ventrally, which disappears with the ecdysis. This is possibly a rudimentary division of both body parts.

SPERMATOPHORES. The existence of spermatophores to transfer male genetic material is known from many mite families. There is a great diversity in spermatophore structure and the mode of its deposition between the families, which reflects the adaptation to various environmental conditions (Witte 1991).

The Eriophyoidea also possess spermatophores. They all have the same basic structure, consisting of a base attached to the substratum, a slender stalk standing upright, and a disk-like "head" containing the sperm reservoir with a sperm droplet in its centre. The spermatophores of *C. vitis* are in accordance with this fundamental structuring (Fig. 8). The average diameter of the slightly oval disk is about 12,5 µm x 9,3 µm and its size is similar to the spermatophore disk of *Phyllocoptruta oleivora* Ashmead, *Novophytoptus sp.* or *Eryophyes pyri* Pagenstecher (Oldfield *et al.* 1970). The sperm reservoir, a sack of 2 µm in width, lies in the centre. In the case of the rust mite *Aculus cornutus* Nalepa & Trouessart this sack contains about 50 spermatozoa (Oldfield and Newell 1972). The back of the spermatophore disk shows bulging walls. There is always the same pattern, a circle which reaches out from the middle to the top of the disk. From this circle radial arms lead away to the thickened border of the disk. These thickenings are thought to stabilise the spermatophore and keep it in an upright position. However, the disk does not lie in the plane of the surface as reported by Oldfield and Michalska (1996), but is slanting up. This might facilitate the reception of sperm by the epigynium of females, pushing down the head of the spermatophores. Sternlicht and Griffiths (1974) described a somewhat thinner stalk near the head as a flexible "neck" so that the disk touches the substratum when the female of *Eriophyes sheldoni* Ewing visits the spermatophore. After the female leaves, the spermatophore returns to an upright position. The stalk of the spermatophore of *C. vitis* has an average length of 8.5 µm. Like other members of the family of Eryophyidae, the diameter of the spermatophore disk (12.5 µm) is larger than the length of the stalk. In contrast to the Eryophyidae, for





Figs 9-10. *C. vitis* Nal.: 9 - spermatophore (arrow) of *C. vitis* on an empty egg shell of this species; 10 - quiescent nymph on the lower leaf side of *Vitis vinifera*. Beside the nymph lies a spermatophore (arrow).

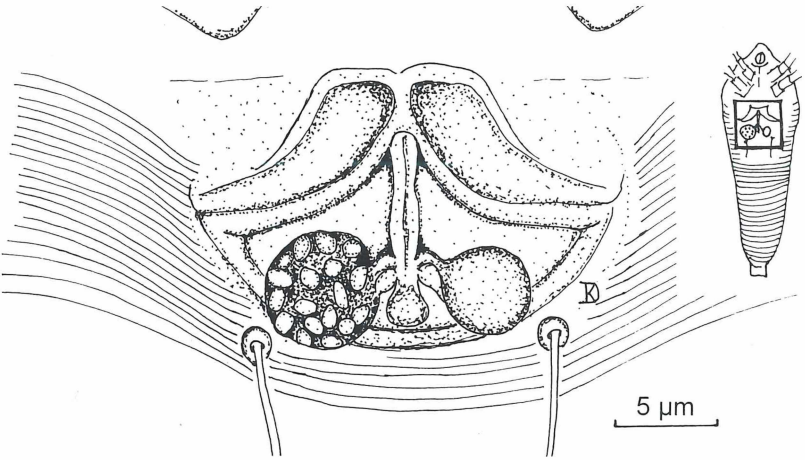


Fig. 11. *C. vitis* Nal.: deutogyne, ventral. The left spermatheca with spermatozoa, the right one is empty [the females from dissected winter buds (cv. Kerner). Drawn from phase contrast].

which relatively short stalks and wide disks seems to be a characteristic feature, the stalk of the phytoptid *Novophytoptus sp.* is substantially longer than the diameter of the spermatophore disk. The base of the stalk is connected to the substratum by a viscous drop of liquid. It is assumed that this "glue" is a quick-drying substance (Sternlicht & Griffiths 1974) which sticks to parts of the adjunct leaf surface of the grapevine, as can be seen in Fig. 8.

DEPOSITION OF SPERMATOPHORES. Males of *C. vitis* are not very selective when choosing a deposition site for spermatophores. These are deposited on the lower side of the vine leave, on elevations like leaf veins, on depressions, as well as on unprotected spots on the surface. The surface property does not seem to be very important for deposition because spermatophores can be found both on coarse and smooth areas as well as on stomata. An irregular deposition behaviour is confirmed by the observation that the spermatophores are deposited on empty egg shells (Fig. 9), on collapsed old spermatophores and even on dead, dried grape rust mites. Many eggs of *C. vitis* have been found on the upper side of the leaves of *Vitis vinifera.* Hence, spermatophores should also be expected there, even though the surface has so far not been scanned.

Michalska and Boczek (1991) report an interesting phenomenon in relation to deposition sites of different Eriopyidea. Males of *Acalitus essigi* Hassan, *Vasates fockeui* Nal. & Trt., *V. robiniae* Nal., and *V. allotrichus* Nal. deposited the spermatophores around quiescent nymphs. They had little or no interest in larvae, quiescent larvae or active nymphs because in these stages, insemination is still not possible. A very close circle of spermatophores around the quiescent nymphs, achieves a maximum reproductive success, because after ecdysis the virgin females showed

interest in spermatophores. In 10 scanned quiescent deutonymphs of *C. vitis*, we did not find nymphs encircled with spermatophores. However, some quiescent nymphs had one or two spermatophores beside the body which could also be by chance (Fig. 10). Further studies including more quiescent nymphs of *C. vitis* will hopefully resolve this question.

STORAGE OF SPERMATOZOA. Eriophyoid females have two spermathecae under the epigynium. In these spermathecae sperm mass from the spermatophores is stored. The examination of the spermathecae showed an interesting pattern of sperm mass distribution (Oldfield & Michalska 1996). Eriophyoidea from dicotyledonous plants (Eryophyidae and Diptilomiopidae) always store sperm in a single spermatheca as did the diapaused deutogynes of *C. vitis* on *Vitis vinifera* (Fig. 11). In contrast, gall mite species of monocotyledonous plants (Eriophyidae and Phytoptidae) store sperm in both spermathecae. From 100 investigated females of *C. vitis* 54 had filled the right spermatheca only and 46 had filled the left one. It thus seems that there is no preference for either side. Nevertheless there are exceptions because in one case both spermathecae were inseminated. The sperm-filled spermathecae are globose or oval and have a granular structure inside, which is caused by the spermatozoa. The diameter ranges between 5 µm and 7 µm whereas the size of the empty ones is normally about 30% less. There the phase-contrast microscope renders a more homogenous inner appearance.

Zusammenfassung

Anhand von Kryo-REM Untersuchungen wird der äußere Geschlechtsapparat der Weibchen (Epigynium) und Männchen (Epiandrium) der Kräuselmilbe *(Calepitrimerus vitis)*, eines Schädlings im Weinbau, beschrieben. Dabei fallen bei den Männchen vor allem die unterhalb des Epiandriums liegenden *setae eugenitalis* auf, welche den Weibchen fehlen. Larven und Nymphen besitzen zwar keine Geschlechtsöffnung, die vorhandenen *setae genitalis* geben jedoch Hinweis auf den Sitz der späteren Genitalregion. Wie bei allen bislang untersuchten Eriophyoidea, bestehen die Spermatophoren bei Kräuselmilben aus einer Basis, einem Stiel und einem daraufsitzenden Teller. Dieser Teller scheint bei *C. vitis* nicht parallel zur Blattoberfläche, sondern schräg in die Höhe gerichtet zu sein. Die Spermatophoren werden auf alle Teile der Blattoberfläche abgesetzt, sogar auf Eihüllen, kollabierte Spermatophoren und eingetrocknete Kräuselmilben. Ein Einkreisen ruhender Nymphen mit Spermatophoren, wie von anderen Arten beschrieben, konnte bislang nicht beobachtet werden. Bei den Deutogynen (Winterform) von *C. vitis* wurden mittels lichtmikroskopischer Untersuchungen die Spermatheken (*receptacula seminis*) sichtbar gemacht. Es bestätigt sich hierbei die Beobachtung an Deutogynen anderer Arten, daß normalerweise immer nur eine Spermathek mit Spermien gefüllt ist, während die andere leer bleibt.

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