In situ visualization of spider mite–plant interfaces

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ABSTRACT

The cosmopolitan and polyphagous two-spotted spider mite Tetranychus urticae (Acari, Tetranychidae) exhibits a remarkable host plant range. A cryo-scanning electron microscopic study of T. urticae in contact with selected hosts was conducted, demonstrating the universal attachment of pretarsi to a variety of plant surface types, including crystalline waxy, glandular, and non-glandular hairy, as well as non-wettable and wettable surfaces. Similar to spiders and geckos, T. urticae bears a dry hairy attachment system. Beside the six empodial hairs (setae), each leg is equipped with four tenent (adhesive) setae with multi-part spatulate terminals (subunits), independent of the developmental stage. The cryo-scanning electron micrographs clearly demonstrate the formation of intimate contact between spatulate tips and substrates. Results are discussed in the context of arthropod attachment and arthropod–plant interactions.

Key words: adhesive setae, ambulacrum, arthropod–plant interactions, attachment, plant surface, spider mite–plant interaction, tarsi

INTRODUCTION

Since cosmopolitan and polyphagous spider mites can cause serious damage and economically important yield loss, their biology and relationships with plants have been comprehensively investigated from the physiological, behavioural, and biochemical perspective. However, although several detailed studies of the spider mite–plant interface have focused on spider mites' mouthparts and the functional morphology of feeding on plants (Liesering, 1960; Hislop and Jeppson, 1976; Brito et al., 1986; Campbell et al., 1990; Beard et al., 2012), direct interactions at the contact points between spider mites and plant surfaces have been rarely considered.

To access plant sap, spider mites must first successfully attach their pretarsi, move and establish on the plant, before setting up the complex silk thread network. Once created the silk web, spider mites mostly walk along the silk threads. The attachment to plant surfaces is considered to be an evolutionary hurdle for arthropods (Southwood, 1986). Previous experimental studies confirm the substantial influence of plant surface structures and physico-chemistry on the attachment ability of insects (e.g., Gorb & Gorb, 2002, 2009; Voigt et al., 2007).
Do plant surface traits also effect the attachment of spider mites?

Pubescent plant species and cultivars have repeatedly been reported to prevent attacks of spider mites and impact their fitness (e.g., Rasmy, 1985; Luczynski, 1988; Nihoul, 1993; Walter, 1996; Grazzini et al., 1997; Fernández-Muñoz et al., 2003; Reddall et al., 2011; Torre Figueiredo et al., 2013). The effect of host plants on spider mites has been previously assumed to cause morphological changes of tarsal bristles on the forelegs (Fritzsche, 1964). Legs and feet (tarsi) have been described in detail, however, predominantly from the taxonomic and evolutionary point of view (West, 1861; Gutierrez, 1985; Gutierrez and Helle, 1985; Alberti & Coons, 1999). The function of acarid legs was reported to influence their shape as well as their sensory structures (Alberti & Coons, 1999). In Tetranychidae, the ambulacrum at the distal end of the pretarsus appears to be exceptional compared to that of other mites; the ambulacral claws are reduced to pads with one pair of solid, compound tenent hairs (adhesive setae), which seem to be very movable instruments rendering lateral support to the empodium (Gutierrez and Helle, 1985). The largest number of different acarine empodium types is found in Tetranychini, in which the empodial claw evolved from a simple claw type to small hair-like processes in Tetranychus sp. (Lindquist, 1985). The development of empodial setae and the evolution of the ambulacrum in spider mites have been proposed to be associated with locomotion on silken strands or webbing structures (Gutierrez and Helle, 1985); however, the authors attribute the synchronous evolution of both tenent and empodial setae to the improvement in locomotion on leaves, and claim further locomotion experiments to advance the understanding of the function of various tarsal appendages in mites.

How ambulacra, eggs, and silk of spider mites adhere to host plant surfaces is not understood so far, and is the subject of the present descriptive study.

MATERIALS AND METHODS

Plants and mites

Exemplary, potted, 15-20 cm high plants at 10-15 leaf-stage of Capsicum annuum L. ‘Yolo Wonder’ (Solanaceae), Cucumis sativus L. ‘Saladin F1’ (Cucurbitaceae), Lycopersicon esculentum L. ‘Harfeuer’ (Solanaceae), Petunia-Hybr. (Solanaceae), and Fragaria chiloensis (L.) Mill. (Rosaceae) were cultivated in a greenhouse and infected with individuals of the two-spotted spider mite *T. urticae* Koch (Acari, Tetranychidae) obtained from a laboratory rearing and kept under a 16-h photoperiod, 23°C, and 60% relative humidity. Plants of the strawberry species *F. chiloensis* were obtained from the stock culture of Hansabred GmbH & Co. KG (Dresden, Germany). The study included a variety of plant species to enable a comparison of a range of plant surface types (Fig. 1).

Microscopy

Different stages of *T. urticae* on various plant surfaces were visualized by using a cryo-scanning electron microscope (cryo-SEM) Zeiss Supra 40VP (Carl Zeiss MicroImaging GmbH, Oberkochen, Germany) combined with a cryo preparation unit Emitech K1250x (Quorum Technologies Ltd, Ashford, Kent, UK). Cut pieces of fresh, just fully-developed leaves, occupied
with mites, were mounted on metal holders with Tissue-Tek® O.C. T. Compound (Sakura Fine Technical Co., Ltd., Tokyo, Japan), and then frozen in the cryo preparation unit at −140°C, sputter-coated with a 6-nm–thick layer of platinum, and examined in the frozen state inside the cryo-SEM at −90°C and 5 kV acceleration voltage.

Contact angle measurements

Contact angles of three different fluids (Aqua Millipore water, ethylene glycol, and diiodomethane) were measured on undamaged, adaxial and abaxial surfaces of fresh, just fully-developed leaves. Thus, the free surface energy of the plant surfaces could be estimated according to the OWRK method (Owens and Wendt, 1969; Kaelble, 1970).

For each fluid type and surface type, three 10-µl drops of fluid were dispensed with an Eppendorf Research Plus 0.5-10 µl pipette (Eppendorf AG, Hamburg, Germany) onto the plant surface. Images of sessile drops were taken with a digital camera (1/2 zoll, 5 megapixels, 20 frames per second), mounted on a stereomicroscope, combined with the software TSO-New-Vidmess (Thalheim-Spezialoptik, Pilsnitz, Germany). Obtained images were used to measure contact angles with the software ImageJ (Rasband, W. S., U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2014).

RESULTS AND DISCUSSION

Consistent with previous examinations of mites applying flash freezing (Becker, 2000), the present study demonstrated that flash freezing and cryo-SEM are suitable methods for the analysis of spider mite morphology and, in particular, spider mite–plant interfacial interactions.
Fig. 2. Spider mites *Tetranychus urticae* on leaves of *Fragaria chiloensis*: abaxial side (A, D) and adaxial side (B, C, E-G). A. A silk web attached to non-glandular trichomes, bridging gaps between trichomes and providing walking paths for mites. B. Detail of male pretarsus, demonstrating how small projections of solenidia interlock with silk threads. C. Detail of B. D. An egg loosely situated between non-glandular trichomes. E. An egg laying on the leaf surface, covered with silk threads on its top. Note the small contact site between the egg and the plant surface. F, G. Details of spatulate terminals (here 5 subunits) of tenent (adhesive) setae in contact with a crystalline wax ribbon (F) and with a silk thread (G), which is detectable still under the spatula, indicating the tenuity of the adhesive terminals and their adaptability to surface irregularities. In each panel, arrows point to silk fibres and arrow heads point to interlocking sites of solenidia with silk. Schematic insets indicate plant surface types according to Fig. 1.

*tr*, non-glandular trichomes; *gt*, glandular trichomes; *cw*, epicuticular wax crystals; *ts*, tenent (adhesive) setae; *so*, solenidia with protusions.
Fig. 3. Spider mites *Tetranychus urticae* on leaves of *Fragaria chiloensis*; abaxial side (A, C, E) and adaxial side (B, D, F). A. Lateral view of a female attached to non-glandular plant trichomes. B. Silk threads form contacts with the epicuticular wax film and ribbon-shaped wax crystals on the adaxial leaf surface. C. Detail of female ambulacrum adhering to a non-glandular trichome, oppositionally spreading the flexible empodial and tenent (adhesive) setae. D. Detail of female adhesive setae adhering to the adaxial epicuticular wax film and crystals. E. A dense silk web covering the waxy-pubescent abaxial leaf. Spider mites overcome hindering plant surface structures by creating a protective silk layer, which forms their own substrate for attachment and walking paths. Eggs are loosely arranged on and within the web. F. Detail of a terminal element of a female adhesive seta. The intimate contact between the spatula-shaped tips and the plant surface is clearly visible. The ultra-thin terminals are well adapted for attachment to surface irregularities. In each panel, arrows point to silk fibres, and schematic insets indicate plant surface types according to Fig. 1.

tr, non-glandular trichomes; cw, epicuticular wax crystals; ts, tenent (adhesive) setae; es, empodial setae; so, solenidia with protrusions.
In total, about 500 cryo-SEM micrographs were obtained and evaluated.

**The attachment system**

The tetranychid tarsus is equipped with anisotropically arranged setae (solenidia) with distally...

(Figs. 2-5). In total, about 500 cryo-SEM micrographs were obtained and evaluated.

**Fig. 4.** Immature stages of *Tetranychus urticae* on the abaxial leaf surfaces of *Cucumis sativus* (A, C) and *Capsicum annuum* (B, E), and the adaxial leaf surface of *Lycopersicon esculentum* (D, F). A, D, F. Nymphs and larva attached to space between non-glandular and glandular trichomes without obvious hindrance. B. A larva situated closely to the main leaf vein. C. Tenent (adhesive) setae form contact with epidermal cell irregularities close to a stomata. E. Spatulae of tenent setae attached to silk threads. In each panel, arrows point to silk fibres, and schematic insets indicate plant surface types according to Fig. 1.

*tr*, non-glandular trichomes; *gt*, glandular trichomes; *ts*, tenent setae; *es*, empodial setae; *so*, solenidia with protusions.
pointing, prominent 1.5-µm long projections. These small projections are aligned at an angle of about 20° to the axis of solenidia and obviously provide interlocking sites with the silk web fibres (Fig. 2B, C). The ambulacrum, which arises terminally from the pretarsus, is composed of four tenent (adhesive) and six empodial setae. No differences in the shape or numbers of ambulacral structures were observed among legs, among stages, or between sexes.

The 18.5-µm long and 0.4-µm wide empodial setae are oriented oppositely to the tenent setae. They obviously support the interlocking of *T. urticae* with the plant surface, and seem to stabilize the stance (Fig. 3C, 4C). Close contact between the empodial setae and the plant surfaces was not observed.

Tenent setae are terminally broadened and flattened to form spatulae, indicating that they provide an adhesive function by forming intimate contact with plant surfaces (Figs. 2F, G; 3C, D, F; 4C; 5C, D). Previous theoretical approximations predicted that spatulae must be an essential feature for intimate contact formation between the attachment pads and substrates and thus for

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**Fig. 5.** Abaxial leaf surfaces of *Petunia*-Hybr. (A, C) and *Cucumis sativus* (B, D). A. Nymphs of *Tetranychus urticae* attached without obvious hindrance to spaces between glandular trichomes of *Petunia*-Hybr. B. Silk threads adhering to the knobby surface of a non-glandular trichome of *C. sativus*. C. Detail of the pretarsus of a nymph attached to the leaf surface of *Petunia*-Hybr., and adhering to the knobby surface of a non-glandular trichome of *C. sativus* (D). In each panel, arrows point to silk fibres, and schematic insets indicate plant surface types according to Fig. 1.

*tr*, non-glandular trichomes; *gt*, glandular trichomes; *ts*, tenent (adhesive) setae; *es*, empodial setae; *so*, solenidia with protusions.
generation of strong adhesive forces in beetles and geckos (Persson and Gorb, 2003). *Tetranychus urticae* exhibit a hairy attachment system, forming multiple contact points with substrates, like many species of spiders, flies, beetles, and geckos (Ruibal and Ernst, 1963; Gorb and Beutel, 2001; Arzt et al., 2003; Varenberg et al., 2010). Thus, hairy attachment systems evolved independently, not only in representatives of the insect orders Diptera, Coleoptera, Dermaptera, Megaloptera, the reptilian order Squamata, and the arachnid order Araneae, but also the arachnid order Trombidiformes (Acari, Acariformes).

Flexible tarsal adhesive setae of hairy attachment systems have been reported to perform particularly well on rough substrates because, by dividing the contact zone into microscopic subunits, multiple-point contact formation, controllable detachment and increased adhesion, as well as certain self-cleaning effects are achieved (e.g., Gorb et al., 2002; Federle, 2006). A single adhesive seta in *T. urticae* appears to be a unit of three to five ultra-thin, tape-shaped, superimposed setal compartments (subunits), which are packed in parallel, conjoined at their bases, and slightly separated from each other at their tips (Figs. 2F, G; 3C, D, F; 4C, E; 5C, D). The shape of spatulate terminals in *T. urticae* (Fig. 2F, G; 3F) resembles those found in *Philodromus cespitum* Walckenaer (Araneae, Philodromidae) (Wolff and Gorb, 2012) and *Gecko gecko* L. (Squamata, Geckonidae) (Ruibal and Ernst, 1963), but differs from those found in many other spiders (Wolff and Gorb, 2012), beetles (Stork 1980), and flies (Niederegger et al., 2002).

The maximum width (1.5 µm) and thickness (35.2 nm; n = 10; males and females pooled together) of spatulae in *T. urticae* are smaller than those in the chrysomelids *Leptinotarsa decemlineata* Say (9 µm wide, 210 nm thick) and *Gastrophysa viridula* DeGeer (4 µm wide, 190 nm thick) (Voigt et al., 2012). Narrower spatulae than those of *T. urticae* have been described for several spiders (0.28 to 1.02 µm wide) (Wolff and Gorb, 2012), and the dipteran *Calliphora vicina* Rob.-Des. (0.5 to 1.4 µm wide) (Niederegger et al., 2002). The reptile *G. gecko* bears spatulae (200 nm wide) (Ruibal and Ernst, 1963; Persson and Gorb, 2003) that are approximately a third the thickness of those of *T. urticae*. Particularly thin spatula tips are known to adhere properly by mainly molecular interactions to various substrates (e.g., Persson and Gorb, 2003). Such tips resemble thin film-like structures and behave viscoelastically in contact with the surface during attachment (Eimüller et al., 2008). The material at the terminal of each spatula 'flows', which leads to an increase in the contact area between the spatula and the substrate and to an enhanced adaptability to the local topography of the surface (Eimüller et al., 2008). In the present study, the contact area of a single spatula-shaped terminal of *T. urticae* in contact with a rather smooth plant surface was roughly estimated to be 1.4 µm² (Fig. 2F, G; 3F). Thus, a single adhesive setae comprised e.g. of three terminal spatula-shaped subunits spans a contact area of 4.2 µm². Accordingly, a single leg equipped with four adhesive setae, each comprised of three terminal spatula-shaped subunits, results in a contact area of 16.8 µm². Considering all eight legs and all adhesive setae are in contact with a flat surface, a female *T. urticae* may achieve a total contact area of 134.4 µm². Previous force measurements with *T. urticae*, using a micro-displacement detector, resulted in a pull-off force of 8.2 µN on glass (Mizutani et al., 2006); therefore, an overall lateral tenacity of 0.06 µN*µm⁻² can be estimated for spatula-shaped terminals of tenent setae in a female *T. urticae*. This value corresponds to that reported for hairy pulvilli of dipteran flies (0.06-0.13 µN*µm⁻²; Gorb et al., 2001), but it is lower than the estimates
for single adhesive setae/setules in *Evarcha arcuata* Clerck (Araneae, Salticidae) (0.22 µN*µm⁻²; Kesel et al., 2003) and in *G. gecko* (0.10 µN*µm⁻²; Autumn et al., 2000).

In contrast to the observations of Mizutani et al. (2006), no tarsal adhesive secretion was observed for *T. urticae* in the present cryo-SEM studies, however cryo-SEM has been proved to be a suitable technique for the visualization of fluid biological samples and insect footprints (Gorb et al., 2007). Thus, like spiders and geckos (Niederegger and Gorb, 2006), spider mites seem to rely on “dry” hairy attachment devices.

Note that the flexibility and high aspect ratio of adhesive setae ( shaft length : width = 26.0 : 0.4 µm) may result in small peeling angles and high crack resistance during attachment and detachment processes, which in turn might positively affect the attachment ability of spider mites (Varenberg et al., 2010).

**Access to plant surfaces**

The tremendous host plant range of spider mites suggests that their attachment devices would have great adaptability, because plant surfaces provide a remarkable diversity of surface structures and physico-chemical properties (reviewed by Koch et al., 2008).

Estimation of the free surface energy (FSE) via contact angle measurements is a method to evaluate physico-chemical properties, which give information about the hydrophobicity, polarity, and heterogeneity of surfaces. FSE is a measure of the energy necessary to change the surface to form contact with counterparts; it equals the reversible work that must be done to increase the interface, and it characterises molecular interactions and intermolecular forces occurring at the surface. Interactions are split in polar and disperse portions: i.e., those between dipoles (e.g., hydrogen bonds) and those due to fluctuations in the load distribution within the molecules, respectively. Previous studies with dock leaf beetles showed that low FSE (hydrophobicity, large disperse portion) leads to a decrease in attachment force of beetles on different substrates (Gorb & Gorb, 2009). In the present study, FSE ranged between a minimum of 15 mN*m⁻¹ in abaxial leaves of *F. chiloensis* to a maximum of 49 mN*m⁻¹ in adaxial leaves of *Petunia*-Hybr. (Table 1). The latter plant species was characterised by the highest polar and lowest disperse component of FSE, whereas the opposite was observed for *C. sativus*. The high FSE of *Petunia*-Hybr. indicates that this species has well-wettable leaves; however, it didn’t show particular effect on the attachment of spider mites. Despite the large range of FSE values in the six plant species examined, *T. urticae* were found to attach ambulacra, eggs, and silk to all tested plant surfaces without any obvious differences. This indicates that attachment was independent of FSE.

Non-wettable, crystalline waxy, and glandular hairy plant surfaces are known to prevent the attachment of numerous arthropods (e.g., Gorb & Gorb, 2002, 2009; Voigt et al., 2007). In particular, glandular plant trichomes have been reported to affect spider mites’ performance and host preference, as well as to enhance plant resistance against spider mites (Nihoul, 1993; Grazzini et al., 1997; Torre Figueiredo et al. 2013). On the tomato *Lycopersicon hirsutum* f. *glabratum* Humb & Bonpl. (Solanaceae), mites became quickly entrapped in the exudate of glandular hairs and died before reaching the deutonymphal stage (Rasmy, 1985). Luczynski (1988) observed the contamination of tarsi by exudates on strawberry leaves, whereas Gugole Ottaviano et al. (2013) didn’t find clear effects of glandular trichomes on the performance of *T.*
Table 1. Free surface energy (FSE), its disperse and polar components, and contact angles (mean±SD) of Aqua Millipore water, diiodomethane, and ethylene glycol measured on adaxial and abaxial leaf surfaces of the various plant species used in Cryo-SEM studies with *Tetranychus urticae* (n=3 droplets per fluid and surface, droplet volume 10 µl). See Figs. 2-5 for images of the plant surfaces.

| Plant species* | Capsicum annuum | Cucumis sativus | Fragaria chiloensis | Lycopersicon esculentum | Petunia-Hybr. |
|----------------|-----------------|-----------------|---------------------|------------------------|--------------|
|               | abaxial         | adaxial         | abaxial             | adaxial               | abaxial      | abaxial | abaxial | 
| Contact angle [°] |                 |                  |                     |                       |              |
| Aqua Millipore | 70.3±6.8        | 102.5±14.7      | 107.3±9.1           | 121.7±2.8             | 146.2±3.6    | 105.6±4.4 | 137.6±15.7 | 101.7±7.8 | 51.1±6.9  | 48.4±3.9  |
| Diiodomethane  | 49.6±18.0       | 75.1±5.1        | 67.3±4.9            | 66.2±2.6              | 99.5±12.0    | 69.7±4.8  | 84.8±8.9  | 70.6±2.4  | 65.5±20.0 | 80.3±12.1 |
| Ethylene glycol| 63.9±6.8        | 74.9±17.1       | 90.3±4.0            | 83.6±6.9              | 121.9±2.9    | 83.7±2.4  | 106.4±14.7| 91.7±11.2 | 69.8±8.9  | 58.7±13.9 |
| FSE [mN*m⁻¹]   | 33.1            | 20.7            | 23.4                | 31.5                   | 14.8         | 23.5      | 19.7      | 18.9      | 42.1      | 49.0      |
| Total           |                 |                  |                     |                       |              |
| Disperse component |             |                  |                     |                       |              |
| Polar component | 20.1            | 19.0            | 23.4                | 30.7                   | 12.2         | 23.4      | 18.3      | 17.5      | 7.7       | 4.4       |
|                 | 13.0            | 1.6             | 0                   | 0.9                    | 2.6          | 0.1       | 1.5       | 1.4       | 34.4      | 44.6      |

* Both abaxial and adaxial leaf surfaces were examined.
urticae on various strawberry cultivars. In contrast, smooth-leaved cotton plants were occupied with fewer mites than pubescent ones (Reddall et al., 2011).

However, in the present study T. urticae attached successfully to wettable and non-wettable plant surfaces as well as to hairy, glandular hairy, crystalline waxy, and non-structured (‘smooth’) plant surfaces (Figs. 2-5, Table 1), which indicates a universal attachment ability providing access to a broad variety of host plants. Mites were also found adhering via tenent setae to leaf surface in spaces between trichomes (Figs. 4A, F, 5A) and to trichomes themselves (Figs. 3A, C, 5D). A similar strategy was previously described for oak aphids (Kennedy, 1986). This raises the possibility that, compared with larger animals, smaller animals such as mites and aphids might escape from hampering plant surface structures by avoidance and attachment between them or by utilizing them as attachment sites. Furthermore, T. urticae can cover apparently any surface with their silk due to its proteinaceous, wetting, surfactant-like nature (Figs. 2A, 3B, E, 5B; Hazan et al., 1975). Thus, 90-nm wide (single) to 10-µm wide (bundled) silk threads adhere also to wax crystals, knobby trichomes, and surface irregularities. Silk webs may enclose entire plants and protect mites against predators, rain and wind, but also offer attachment sites and optimized walking paths for mites (Figs. 2A–C, 3E, 4E; Le Goff et al., 2012). Silk may surround the 130.2-µm wide eggs (Figs. 2E, 3E). However, for all plant surface types examined, eggs were also found loosely laying on the plant surface or positioned between trichomes (Fig. 2D, E). In contrast to other arthropods (e.g., Asparagus beetles; Voigt and Gorb, 2010), no glue between the egg and plant surface was visualized by cryo-SEM in the present study.

It is well known that several factors affect spider mite host plant selection, performance on host plants, and the formation of host races: for example, olfactory discrimination, other behavioural characteristics, as well as physiological and genetic factors (e.g. Gotoh et al., 1993; Navajas, 1999; Agrawal, 2000). Nevertheless, to overcome the first hurdle and gain initial access to host plants, mites have to apply their ambulacra. That is why the present cryo-SEM study was carried out. The obtained images demonstrate the formation of intimate contacts between tenent setae and a variety of substrates, which would be expected to result in remarkable attachment ability on a broad range of plant surfaces. Future biomechanical studies may help in the understanding of direct spider mite–plant surface interactions, although the tiny samples are challenging for experimental approaches. Since T. urticae doesn’t bear claws, but only setae of defined number and dimensions, it is considered to be an ideal model species for the exploration of the distinct functions of tarsal adhesive setae in living systems, excluding hooking effects of ambulacral terminals with substrates.

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REFERENCES

Agrawal, A. A. (2000) Host-range evolution: adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology*, 81: 500–508.

Alberti, G. and L. B. Coons (1999) Acari: Mites. In: Harrison, F. W., Foelix, R. F. (eds.) Microscopic anatomy of invertebrates. Vol. 8c, Chelicerate Arthropoda, Chapter 6. Wiley-Liss, Inc., pp. 515–521.

Arzt, E., S. Gorb and R. Spolenak (2003) From micro to nano contacts in biological attachment devices. *PNAS*, 100: 10603–10606.

Autumn, K., Y. A. Liang, S. T. Hsieh, W. Zesch, W. P. Chan, T. W. Kenny, R. Fearing and R. J. Full (2000) Adhesive force of a single gecko foot-hair. *Nature*, 405: 681–685.

Beard, J. J., R. Ochoa, G. R. Bauchan, W. C. Welbourn, C. Pooley and A. P. G. Dowling (2012) External mouthpart morphology in the Teucrialpidae (Tetranychoidae): *Raoiella* a case study. *Experimental and Applied Acarology*, 57: 227–255.

Becker, H. (2000) Mites get frozen, photographed, and identified. *Agricultural Research*, October 2000, 4–7.

Brito, R. M., V. M. Stern and F. V. Sances (1986) Physiological response of cotton plants to feeding of three *Tetranychus* spider mite species (Acari: Tetranychidae). *Journal of Economic Entomology*, 79: 1217–1220.

Campbell, R. J., R. L. Grayson and R. P. Marini (1990) Surface and ultrastructure feeding injury to strawberry leaves by the twospotted spider mite. *HortScience*, 2.5: 948–951.

Eimüller, T., P. Guttmann and S. Gorb (2008) Terminal contact elements of insect attachment devices studied by transmission X-ray microscopy. *Journal of Experimental Biology*, 211: 1958–1963.

Federle, W. (2006) Why are so many adhesive pads hairy? *Journal of Experimental Biology*, 209: 2611–2621.

Fernández-Muñoz, R., M. Salinas, M. Álvarez and J. Cuartero (2003) Inheritance of resistance to two-spotted spider mite and glandular leaf trichomes in wild tomato *Lycopersicon pimpinellifolium* (Jusl.). *Journal of the American Society for Horticultural Science*, 128: 188–195.

Fritzsche, R. (1964) Pflanzenschädlinge, Bd. 3, Milben. p. 141, Neumann Verlag, Radebeul.

Gorb, S. N. and R. G. Beutel (2001) Evolution of locomotory attachment pads of hexapods. *Naturwissenschaften*, 88: 530–534.

Gorb, S. N., R. G. Beutel, E. V. Gorb, Y. Jiao, V. Kastner, S. Niederegger, V. L. Popov, M. Scherge, U. Schwarz and W. Vötisch (2002) Structural design and biomechanics of friction-based releasable attachment devices in insects. *Integrative and Comparative Biology*, 42: 1127–1139.

Gorb, S., E. Gorb and V. Kastner (2001) Scale effects on the attachment pads and friction forces in syrphid flies (Diptera, Syrphidae). *Journal of Experimental Biology*, 204: 1241–1243.

Gorb, E. V. and S. N. Gorb (2002) Attachment ability of the beetle *Chrysolina fastuosa* on various plant surfaces. *Entomologia Experimentalis et Applicata*, 105: 13–28.

Gorb, E. and S. Gorb (2009) Effects of surface topography and chemistry of *Rumex obtusifolius* leaves on the attachment of the beetle *Gastrophysa viridula*. *Entomologia Experimentalis et Applicata*, 130: 222–228.

Gorb, S. N., D. Voigt and E. V. Gorb (2007) Visualisation of small fluid droplets on biological and artificial surfaces using the cryo-SEM approach. In: Méndez-Vilas, A., Díaz, J. (eds.) Modern Research and Educational Topics in Microscopy. pp. 812–819, Formatex.

Gotoh, T., J. Bruin, M. W. Sabelis and S. B. J. Menken (1993) Host race formation in *Tetranychus urticae*: genetic differentiation, host plant preference, and mate choice in a tomato and cucumber strain. *Entomologia Experimentalis et Applicata*, 68: 171–178.

Grazzini, R., D. Walters, J. Harmon, D. J. Hesk, D. Cox-Foster, J. Medford, R. Craig and R. O. Mumma (1997) Inheritance of biochemical and morphological characters associated with two-spotted spider mite resistance in *Pelargonium x hortorum*. *Journal of the American Society for Horticultural Science*, 122: 373–379.

Gugole Ottaviano, M. F., N. E. Sánchez, M. F. Roggiero and N. M. Greco (2013) Performance of *Tetranychus urticae* and *Neoseiulus californicus* on strawberry cultivars and assessment of the effect of glandular trichomes. *Arthropod-Plant Interactions*, 7: 547–554.
Gutierrez, J. (1985) 1.1.4 Systematics. In: Spider mites. Their biology, natural enemies and control. Volume 1 A (eds., Helle, W. and M. W. Sabelis), pp. 75–90, Elsevier Science Publishers, Amsterdam.

Gutierrez, J. and W. Helle (1985) 1.1.5 Evolutionary changes in the Tetranychidae. Volume 1 A (eds., Helle, W. and M. W. Sabelis), pp. 91–107, Elsevier Science Publishers, Amsterdam.

Hazan, A., A. Gertler, A. S. Tahori and U. Gerson (1975) Spider mite webbing III. Solubilization and amino acid composition of the silk protein. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 51: 457–462.

Hislop, R. G. and L. R. Jeppson (1976) Morphology of mouthparts of several species of phytophagous mites. Annals of the Entomological Society of America, 69: 1125–1135.

Kaelble, D. H. (1970) Dispersion-polar surface tension properties of organic solids. Journal of Adhesion, 2: 66–81.

Kennedy, C. E. J. (1986) Attachment may be a basis for specialization in aphids. Ecological Entomology, 11: 291–300.

Kesel, A. B., A. Martin and T. Seidl (2003) Adhesion measurements on the attachment devices of the jumping spider Evarcha arcuata. Journal of Experimental Biology, 206: 2733–2738.

Koch, K., B. Bushan and W. Barthlott (2008) Diversity of structure, morphology and wetting of plant surfaces. Soft Matter, 4: 1943–1963.

Le Goff, G. J., T. Hance, C. Detrain, J.-L. Deneubourg, A.-C. Maillieux (2012) The locomotor activities on sites covered by silk produced by related and unrelated spider mites in Tetranychus urticae (Acari: Tetranychidae). Comptes Rendus Biologies, 335: 226–231.

Liesering, R. (1960) Beitrag zum phytopathologischen Wirkungsmechanismus von Tetranychus urticae Koch (Tetranychidae, Acari). Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 67: 524–542.

Lindquist, E. E. (1985) External anatomy. In: Spider mites. Their biology, natural enemies and control. Volume 1 A (eds., Helle, W. and M. W. Sabelis), pp. 3–28, Elsevier Science Publishers, Amsterdam.

Luczynski, A. (1988) Chemical and morphological factors of resistance against the twospotted spider mite (Tetranychus urticae Koch) in beach strawberry Fragaria chiloensis (L.) Duchesne. Ph. D. Thesis, Univ. of British Columbia, 152 p.

Mizutani, K., K. Egashira, T. Toukai and J. Ogushi (2006) Adhesive force of a spider mite, Tetranychus urticae, to a flat smooth surface. JSME International Journal, 49: 539–544.

Navajas, M. (1999) Host plant associations in the spider mite Tetranychus urticae (Acari: Tetranychidae): insights from molecular phylogeography. J. Bruin, L. P. S., van der Geest, M. W. Sabelis (eds) Ecology and Evolution of the Acari, pp. 163–174.

Niederegger, S., S. Gorb and Y. Jiao (2002) Contact behaviour of tenent setae in attachment pads of the blowfly Calliphora vicina (Diptera, Calliphoridae). Journal of Comparative Physiology A, 187: 961–970.

Niederegger, S. and S. N. Gorb (2006) Friction and adhesion in the tarsal and metatarsal scopulae of spiders. Journal of Comparative Physiology A, 192: 1223–1232.

Nihoul, P. (1993) Controlling glasshouse climate influences the interaction between tomato glandular trichome, spider mite and predatory mite. Crop Protection, 12: 443–447.

Owens, D. K. and R. C. Wendt (1969) Estimation of the surface free energy of polymers. Journal of Applied Polymer Science, 13: 1741–1747.

Persson, B. N. J. and S. Gorb (2003) The effect of surface roughness on the adhesion of elastic plates with application to biological systems. Journal of Chemical Physics, 119: 11437–11444.

Rasmy, A. H. (1985) The biology of the two-spotted spider mite Tetranychus urticae as affected by resistant solanaceous plants. Agriculture, Ecosystems and Environment, 13: 325–328.

Reddall, A. A., V. O. Sadras, L. J. Wilson and P. C. Gregg (2011) Contradictions in host plant resistance to pests: spider mite (Tetranychus urticae Koch) behaviour undermines the potential resistance of smooth-leaved cotton (Gossypium hirsutum L.). Pest Management Science, 67: 360–369.

Ruijbal, R. and V. Ernst (1963) The structure of the digital setae of lizards. Journal of Morphology, 117: 271–294.

Southwood, R. (1986) Plant surfaces and insects—an overview. In: Insects and the plant surface. (eds, Juniper, B. and R. Southwood), pp. 1–22, Edward Arnold Publishers, London.

Stork, N. E. (1980) A scanning electron microscope study of tarsal adhesive setae in the Coleoptera. Zoological Journal of the Linnean Society, 68: 173–306.
Torre Figueiredo, A. S., J. T. Vilela Resende, R. G. Ferreira Morales and A. P. Santos Gonçalves (2013) The role of glandular and non-glandular trichomes in the negative interactions between strawberry cultivars and spider mite. *Arthropod-Plant Interactions, 7*: 53–58.

Varenberg, M., N. M. Pugno and S. N. Gorb (2010) Spatulate structures in biological fibrillar adhesion. *Soft Matter, 6*: 3269–3272.

Voigt, D., E. V. Gorb and S. N. Gorb (2007) Plant surface-bug interactions: *Dicyphus errans* stalking along trichomes. *Arthropod-Plant Interactions, 1*: 221–243.

Voigt, D. and S. N. Gorb (2010) Egg attachment of the asparagus beetle *Crioceris asparagi* to the crystalline waxy surface of *Asparagus officinalis*. *Proceedings of the Royal Society B, 277*: 895–903.

Voigt, D., A. Schweikart, A. Fery and S. Gorb (2012) Leaf beetle attachment on wrinkles: isotropic friction on anisotropic surfaces. *Journal of Experimental Biology, 215*: 1975–1982.

Walter, D. E. (1996) Living on leaves: Mites, tomenta, and leaf domatia. *Annual Review of Entomology, 41*: 101–114.

West, T. (1861) The foot of the fly; its structure and action: elucidated by comparison with the feet of other insects. *Transactions of the Linnean Society of London, 23*: 393–421.

Wolff, J. O. and S. N. Gorb (2012) Comparative morphology of pretarsal scopulae in eleven spider families. *Arthropod Structure & Development, 41*: 419–433.