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Hierarchical organisation of the trap in the protocarnivorous plant *Roridula gorgonias* (Roridulaceae)

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SUMMARY

The flypaper trap of the protocarnivorous plant *Roridula gorgonias* is known to capture various insects, even those having a considerable body size, by using an adhesive, visco-elastic resinous secretion released by glandular trichomes of different dimensions. However, recent experimental studies have shown that the adhesion of long tentacle-shaped trichome secretion is not as strong as previously assumed. One may then ask why this flypaper trap is so highly effective. In the present study, we compared geometry, flexibility and the adhesive properties of secretion in different sized trichomes. We have analysed the gross morphology of the plant and its surfaces using light and cryo-scanning electron microscopy. Trichomes' stiffness and the adhesive properties of their secretion on different surfaces were measured. A combination of structural and experimental results, presented in this study, let us suggest that *R. gorgonias* represents a three-dimensional trap consisting of three functional hierarchical levels (plant, leaves and trichomes). According to their size, we classified three types of trichomes having a particular arrangement on the leaf. The longest trichomes are more flexible and less adhesive compared with the shortest ones. The latter are 48 times stiffer and their secretion has a 9 times higher adhesive strength. Our data support the hypothesis that the shortest trichomes are adapted to strong, long-term adherence to prey insects, and that the longest trichomes are responsible for initial trapping and entanglement function.

Key words: adhesion, adhesive strength, biomechanics, flypaper plant, insect–plant interactions, plant resin, plant surface, stiffness, trap, trichome.

INTRODUCTION

'Vliegebos', the perennial shrubs of the protocarnivorous genus *Roridula* L. (Roridulaceae), are endemic to the South African Cape Province and show remarkable features in their biology (Carlquist, 1976; Marloth, 1925). They resemble representatives of the carnivorous genus *Drosophyllum* (L.) Link. (Drosophyllaceae) but differ from them by the absence of sessile glands and digestive enzymes (Marloth, 1910; Marloth, 1925; Lloyd, 1934; Lloyd, 1942; Juniper et al., 1989). Nevertheless, *Roridula* plants have been reported very efficiently trapping a noticeable number of insects by means of releasing sticky glandular fluid, making the surface shiny and reflective (Bruce, 1907; Barthlott et al., 2004). The prey range includes insects having considerable size and mass, in particular flying representatives of Hymenoptera, Diptera, Coleoptera, Hemiptera, Lepidoptera and Thysanoptera (mean length of prey=3.55 mm, s.d.=0.57 mm) (Marloth, 1903; Marloth, 1910; Ellis and Midgley, 1996). By contrast, mutualistic mirid bugs from the genus *Pameridea* Reut. (Heteroptera, Miridae) live omnivorously on the plant surface and feed on the captured insects (Reuter, 1907; Dolling and Palmer, 1991; Picker et al., 2004). Plants take up nitrogen from the bugs' faeces through the leaf cuticle (Ellis and Migley, 1996; Anderson and Midgley, 2002; Anderson and Midgley, 2003).

The morphology and anatomy of *Roridula* leaves have been previously comprehensively described (Darwin, 1875; Fenner, 1904; Bruce, 1907; Stehli, 1934; Uphof, 1962). The leaf arrangement on the stem is alternate and spiral, with the highest leaf density on the apex. Sessile, elongated, narrow, triangular leaves are 7–12 cm long and ~0.25 cm wide at the base. They possess a quite cylindrical tip, ending in a single tentacle-shaped trichome. On both adaxial and abaxial intercostal leaf surfaces, single capitate trichomes are sparsely distributed. The adaxial leaf lamina is, in addition, densely covered with non-glandular, unicellular, procumbent, curved, filamentous, pointed trichomes directed distally. The margins of the leaves bear numerous capitate trichomes, which are pointed radially outwards and differ greatly in their length (Fenner, 1904; Bruce, 1907).

The abaxial midvein is densely covered with capitate trichomes varying in size. Glands of midvein trichomes produce a higher amount of secretion than do marginal trichomes. Correspondingly, Bruce (Bruce, 1907) found, in herbarium specimens, a greater proportion of trapped insects adhering to midvein trichomes compared with marginal trichomes. However, Fenner's (Fenner, 1904) observations led to the assumption that marginal trichomes mostly capture flying prey insects, and trichomes on the abaxial midvein are specialised for catching crawling insects.

In spite of trichome variability in size, their structure and shape are very similar (Fenner, 1904; Uphof, 1962). All trichomes consist of a multicellular stalk, which bears, at the tip, a multicellular glandular head releasing a sticky fluid. This water-insoluble, non-volatile secretion has a resinous character and visco-elastic properties (Bruce, 1907; Marloth, 1925; Lloyd, 1934; Voigt and Gorb, 2008). It is partly soluble in ethanol and chloroform but well soluble in acetone. Previous assumptions that this multicomponent substance might be composed of various lipids and terpenes have been recently confirmed by chemical analyses (Simoneit et al., 2008). In *Roridula gorgonias* Planch., no saccharides have been detected. The consistency, similar to a sticky syrup, is caused by a mixture of triterpenoids, as major components, and acylglycerides. However, the composition of an insoluble residue still remains unclear.

Based on the conspicuous number, size and mass of trapped insects, the very viscid secretion of *Roridula* is believed to be the strongest glue among all insect-trapping plants (Marloth, 1910; Hartmeyer, 1998; Barthlott et al., 2004). For *R. gorgonias*, we previously measured the pull-off force of secretion droplets in tentacle-shaped trichomes and found that the adhesive strength was 13 kPa (Voigt and Gorb, 2008). On the contrary, the adhesive strength of the viscid secretion in the protocarnivorous tar flower *Befaria racemosa* Venten (Ericaceae) has been previously estimated between 40 and 50 kPa, corresponding to that of commercial flypaper (Eisner and Aneshansley, 1983). Such a difference can be explained by differences in the experimental approach due to the different thickness of the adhesive secretion. Previously, no measurements of the adhesive strength of *Roridula*'s secretion spread between two solid flat surfaces (in an experiment comparable with that done on *B. racemosa* secretion and on commercial adhesives) have been conducted. In our previous study, only secretion of tentacle-shaped trichomes was characterised. However, one can assume differences in consistency and stickiness of adhesive secretion between trichomes of various sizes. Using cryo-scanning electron microscopy (cryo-SEM), we have previously observed that short capitate trichomes release round, apparently more viscous, stable, spherical secretion droplets in contrast to the tentacle-shaped ones that have less viscous ovoid droplets on their tips (Voigt and Gorb, 2008). Assuming a multilevel three-dimensional trap, composed of trichomes of different dimensions, we proposed a functional mechanism of the trap in *R. gorgonias*. Mobile insects may touch and build initial adhesive contact with secretion droplets of long, flexible, tentacle-shaped trichomes. Then, struggling and moving, the insect pulls the droplet into a long fluid filament and becomes more and more entangled in the adhesive secretion, consequently losing energy. Finally, the insect sticks to short trichomes and adheres firmly to the plant surface. However, it remained unclear how different properties of trap subunits contribute to the trapping mechanism of *R. gorgonias*. Are there differences in stiffness and adhesive characteristics between glandular trichomes having a different geometry?

The goal of the present study was to characterise the structure and properties of different trichome types and their combination in the multilevel trapping system of the plant. Distribution, geometry, stiffness and adhesive properties of trichomes were analysed. Using light microscopy and cryo-SEM, the plant surface was examined, and trichome morphometrical variables (length and width) were measured. The trichomes' stiffness, adhesion forces of glandular droplets on smooth hydrophilic and hydrophobic glass surface samples, as well as the adhesion strength of trichome secretion on hydrophilic glass, were experimentally estimated.

MATERIALS AND METHODS

Plants

Between one and three year old seeded *R. gorgonias* were obtained from a private glasshouse culture (Klaus Keller, Augsburg, Germany), kept under laboratory conditions during experiments (23.7±1.7°C, 47.3±10.0% relative humidity, 16 h photoperiod) and fed with wingless adult fruit flies *Drosophila melanogaster* Meigen (Diptera, Drosophilidae; Zoo-Schöniger, Stuttgart, Germany). For experiments, trichomes of the 10–20th leaves below the apex were randomly selected.

Microscopy

Light microscopy observations of living plants were conducted using a stereomicroscope Olympus SZX 12 with a DF PLAPO 1×PF objective (Olympus Corp., Tokyo, Japan). Images were taken using a Nikon Coolpix E995 digital camera adapted to the stereomicroscope with a C-Mount adapter and a MDC 2 relay lens MXA 29005 (Nikon Corp., Tokyo, Japan).

A measuring microscope Mitutoyo MF-U510TH (Mitutoyo Corp., Kanagawa, Japan) was used to estimate the length and width of the stalk and glandular head of the trichomes, used in stiffness measurements. Considering stalk length and width (averaged from measurements at stalk base and stalk tip), trichome aspect ratio was estimated. Differences between obtained morphometrical values in various trichomes were statistically evaluated using SigmaStat 3.1.1® software (Systat Software Inc., Richmond, CA, USA). Both Kruskal–Wallis one-way ANOVA on ranks (for non-normally distributed data) and one-way ANOVA followed by all pairwise multiple comparison procedures (Tukey test) were applied.

To visualise the microstructure of plant surfaces, scanning electron microscopy was applied using a cryo-SEM Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) equipped with a Gatan ALTO 2500 cryo-preparation system (Gatan Inc., Abingdon, UK). Fresh leaf samples were mounted on metal holders using polyvinyl alcohol Tissue-Tek® OCT™ compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands), frozen in the preparation chamber at –140°C, sputter-coated with gold-palladium (6 nm) and examined in a frozen state in the cryo-SEM at 3 kV accelerating voltage and –120°C temperature.

Force measurements

Trichome stiffness

To estimate trichome stiffness, a force cell load transducer (10 g capacity, Biopac Systems, Santa Barbara, CA, USA) combined with a motorised micromanipulator DC3314R with controller MS314 (World Precision Instruments, Sarasota, FL, USA) was used. A steel insect pin (diameter: 100 μm, minucie no. 10, Ento Sphinx s.r.o., Pardubice, Czech Republic) was firmly glued to the force transducer with Loctite® super glue (Henkel Loctite Deutschland GmbH, Munich, Germany). Using a razor blade, a piece of leaf tissue bearing a single trichome was cut out of a living leaf of *R. gorgonias*. The leaf sample, with the trichome oriented horizontally, was mechanically gripped with tweezers attached to a holder. The insect pin was moved up and down with a velocity of ~20 $\mu m\,s^{-1}$. Initially, it was brought into contact with a trichome. Then, the pin was moved down, bending the trichome until contact between the pin and the trichome was lost. Force–time curves were recorded using AcqKnowledge 3.7.0 software (Biopac Systems) at an acquisition sample rate of 100 Hz. They were transformed to force–distance curves, from which slopes ($\mathbf{F}=cd$; where $\mathbf{F}$ is the force recorded, d is the distance travelled by the motor), the trichome spring constant c ($N\,m^{-1}$) was calculated. This parameter provides a measure for the trichome deflection at a certain applied load. For each trichome type (N=3), defined in section 'Plant habitus and trichome types', 10 trichomes (n=10) were used. In total, 30 tests were carried out. Using Kruskal–Wallis one-way ANOVA on ranks followed by all pairwise multiple comparison procedures (Tukey test) differences in the stiffness values between trichome types were evaluated. Additionally, bending behaviour of different trichome types was recorded using a binocular microscope Leica MZ 12.5 with integrated video camera Leica ICA (Leica GmbH, Wetzlar, Germany). Single video frames were superimposed to show, schematically, successive steps of trichome bending under an applied load.

Adhesion of trichome secretion droplets

The adhesion of single trichomes was measured using the above mentioned load cell force transducer combined with a motorised micromanipulator. A hydrophilic (contact angle of Milli-Q: 45.4±2.29 deg.) or silanised (contact angle of Milli-Q: 110.3±1.11 deg., N=10) glass slide of 5 mm diameter was cleaned (rinsing successively with acetone, ethanol and Milli-Q) and then glued to the force transducer using Loctite® super glue. Fresh leaf samples with the trichome oriented vertically were mechanically gripped with tweezers attached to a solid holder. The slide was moved up and down with a velocity of ~200 μm s^{-1}. It was brought into contact with the adhesive droplet on the trichome tip, preloaded and then withdrawn. The pull-off (adhesion) forces were estimated using recorded force–time curves (see 'Trichome stiffness'). For each trichome type (N=3), 100 measurements (20 trichomes with 5 measurements per trichome) were performed. In total, 300 tests were carried out. For statistical evaluation, pull-off force values of different trichome types, selected at a defined preload level (0.05–0.25 mN), were compared using Kruskal–Wallis one-way ANOVA on ranks followed by all pairwise multiple comparison procedures (Dunn's test). Differences between pull-off force values on hydrophilic and hydrophobic surfaces were estimated using Mann–Whitney rank sum test. The defined preload level (0.05–0.25 mN according to 5–25 mg) resembles the range of loads, which representative struggling prey insects would apply on a single trichome. For example, the body mass of various fruit fly species *Drosophila* sp. ranges between 1 and 1.5 mg (Worthen and Haney, 2002). As force generated by moving (free walking or struggling) insects has been shown to be 5 times higher than in motionless ones (Dai et al., 2002), the force which fruit flies apply on a single trichome may be assumed to be 5–7.5 mg.

Adhesive strength of trichome secretion

The measurements were carried out using a force cell load transducer (100 g capacity, Biopac Systems) in combination with a step motor drive capable of moving the force transducer up and down at a velocity of 2.28 mm s^{-1}. Clean hydrophilic glass slides of 5 mm diameter were glued at their centre to a 100 mm long and 0.01 mm thick polyamid thread, Transfil 70 (Amann and Söhne GmbH & Co. KG, Bönnigheim, Germany), using Loctite® super glue. The free end of the thread was connected to the force transducer. Another glass slide was firmly attached to a holder using double-coated adhesive tape (tesa® handicraft tape, tesa AG, Hamburg, Germany). A droplet of secretion from a single trichome was positioned on the lower slide surface. The slide glued to the thread was placed above the firmly attached one so that the droplet was embedded between the two glass slides. Using a metal weight, this glass–droplet–glass 'sandwich' was preloaded for 30 s. Considering the measured contact areas (see Results), the applied contact pressure varied, depending on secretion viscosity, between 63 kPa in long, 126 kPa in medium and 252 kPa in short trichomes. After preloading, the upper glass slide connected to the force transducer was withdrawn perpendicularly to the plane of the slides. Force–time curves were recorded to estimate the pull-off force. In total, 30 measurements were conducted (N=3 trichome types, n=10 individual trichomes per trichome type). From digital images of glass–droplet–glass 'sandwiches', obtained by means of the stereomicroscope and digital camera, the contact area of droplets between glass slides was estimated using SigmaScan Pro 5 (SPSS, Inc., Chicago, IL, USA) software. Based on obtained values of adhesion force (mN) and contact area (mm^2), the adhesive strength (tenacity) (kPa) of the secretion was calculated. Statistical differences between trichome types were evaluated using Kruskal–Wallis one-way ANOVA on ranks followed by all pairwise multiple comparison procedures (Tukey test).

Since the layer thickness of adhesives has been reported to influence the strength of an adhesive bond (Habenicht, 2002), the thickness of the compressed droplet was estimated to provide the basis for comparing the adhesive strength of secretion. For this purpose, a measuring microscope Mitutoyo (see section 'Microscopy') was used. Thickness was measured after applying the above mentioned contact pressure prior to the pull-off test.

RESULTS

Plant habitus and trichome types

Viewed from the top, the *R. gorgonias* plant appears star-shaped (Fig. 1A) due to its noticeable alternate phyllotaxy (Fig. 1B). The sessile, narrow, triangular leaves bear glandular trichomes, having various lengths and pointing outwards in different directions. Furthermore, a dense coverage of non-glandular, filamentous, curved, distally oriented trichomes is found on the adaxial leaf side (Fig. 1C,D; Figs 2 and 3). All glandular trichomes consist of a multicellular stalk somewhat broadened at the base and slightly tapered, with a multicellular glandular head releasing secretory fluid (Fig. 2). We distinguished three types of glandular trichomes depending on their stalk lengths (Table 1). Morphometric variables of different trichome types are given in Fig. 3B. Type 1: long, inclined, tentacle-shaped trichomes are up to 5 mm long with a mean aspect ratio (length-to-width ratio) of 36.1 (s.d.=3.51) and ovoid glandular heads (Fig. 2E). They are located only on leaf margins at distances of ~5 mm from each other. Between them, smaller capitate trichomes (type 2 and 3) are densely interspersed (Fig. 3A). Type 2: medium-sized, inclined, capitate trichomes are up to 2.4 mm long with a mean aspect ratio of 20.1 (s.d.=4.07) (Fig. 2D). Beside the leaf margins, they occur very sporadically on the abaxial intercostal lamina. Type 3: short capitate trichomes (Fig. 2C) have a maximum length of 0.7 mm. They are ~10 times smaller than trichomes of type 1 and show an ~7.5 times lower aspect ratio. However, the length and width of the glandular head in type 3 did not differ significantly from those in types 1 and 2. Trichomes of type 3 arise mostly perpendicularly to the surface. They densely cover leaf margins, and more prominently, the abaxial midvein.

Trichome spring constants

Spring constant values varied significantly between trichome types (Fig. 4A). The lowest spring constant was measured in long, tentacle-shaped trichomes (median: 0.02 N m^{-1}, min.–max.: 0.02–0.03 N m^{-1}). Medium-sized trichomes had an ~3.8 times higher (median: 0.08 N m^{-1}, min.–max.: 0.02–0.10 N m^{-1}) and short ones ~48 times higher (median: 0.95 N m^{-1}, min.–max.: 0.20–1.60 N m^{-1}) spring constant. Video recordings of the bending behaviour of trichomes showed bending angles (between the tip and base of the trichome at the largest deflection) of 40–65 deg. in long, 50 deg. in medium and up to 90 deg. in short trichomes. Whereas long and medium trichomes bent at approximately midway along their stalks, short ones bent at their bases (see inset in Fig. 4A).

Adhesive strength of glandular secretion

Both pull-off force of the secretion droplet measured directly on the trichome and the adhesive strength of secretion differed significantly depending on the trichome type (Fig. 4B,C). No statistical difference was found in pull-off force values of different trichome droplets when compared on hydrophilic and hydrophobic glass (Mann–Whitney rank sum test, long: T=7430, P=0.302;

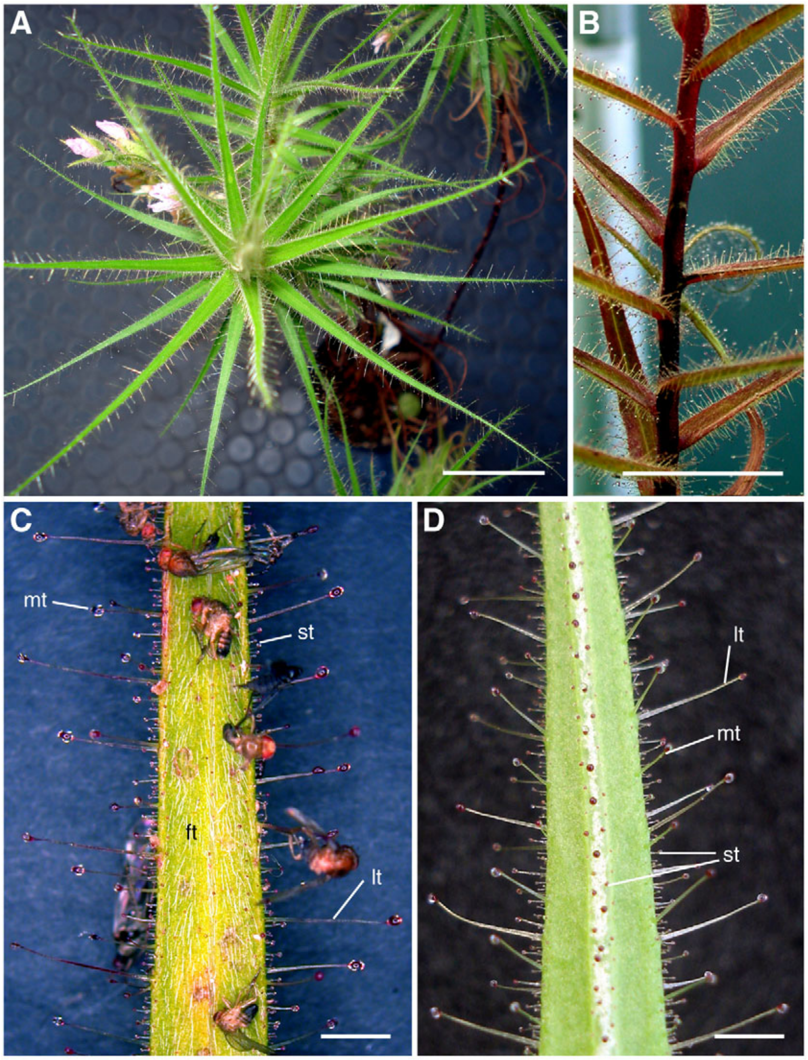

Fig. 1. Digital images of *Roridula gorgonias*. (A) Plant branch with an alternate leaf arrangement (top view). (B) Same as A (side view). (C) The adaxial leaf surface densely covered with non-glandular filamentous trichomes (ft) pointing to the leaf tip. (D) The abaxial leaf surface bearing a concave midvein covered with short capitate (st) trichomes of different length. Leaf margins are densely covered with short capitate trichomes and moderately dispersed medium-sized capitate trichomes (mt) and long tentacle-shaped trichomes (lt) C, D. Scale bars: A and B=30 mm; C and D=3 mm.

medium: T=2257, P=0.147; short: T=268, P=0.147). The secretion of long and medium-sized trichomes, withdrawn from the glass surface, took the shape of fluid filaments, pulled up to 5 cm long. During a further increase of distance between the glass slides, the filaments snapped back, relaxed and recovered to the initial ovoid shape. By contrast, the secretion of short trichomes built thicker and shorter (maximum 1 cm long) filaments, seldom completely recovering to their spherical shape after snapping back from contact with the substrate. In this case, a meniscus or short filament-like residue on the droplet surface remained visible for a certain time.

The contact area, covered with the secretion compressed between two glass slides after applying pressure, was 0.6 mm^2 for short, 1.2 mm^2 for medium and 2.4 mm^2 for long trichomes. The adhesive strength of secretion reached significantly higher values in the case of short trichomes (median: 156.2 kPa, min.–max.: 29.70–427.68 kPa). They were ~6 times higher than in medium-sized trichomes (median: 24.5 kPa, min.–max.: 0.77–314.53 kPa) and ~9 times higher than in long trichomes (median: 17.5 kPa, min.–max.: 0.39–112.21 kPa). The secretion layer thickness differed significantly between 0.11 mm (median, min.–max.: 0.11–0.12 mm) in long trichomes and 0.13 mm (medians) in medium-sized and short ones (min.–max. 0.11–0.16 mm for medium-sized and 0.12–0.18 mm for short) (Kruskal–Wallis one-way ANOVA on ranks followed by all pairwise multiple comparison procedures Tukey test, $H_{2,29}$=17.350, $P\leq$0.001).

DISCUSSION

The flypaper trap of *R. gorgonias* has three-dimensional architecture, consisting of functional units at various hierarchical levels (Fig. 5). As major levels, we define (1) the whole plant possessing a star-shaped leaf arrangement, (2) sticky leaves densely covered with glandular trichomes and (3) trichomes of different size, mechanics and adhesive properties that build three different functional subunits. There are three trichome types described here: long, medium-sized and short capitate trichomes (see section ‘Plant habitus and trichome types’). Although trichomes have a similar basic structure (multicellular stalk with glandular head releasing a sticky fluid), they differ distinctly in their dimensions and mechanical properties. We assume different functional roles for different levels and various trichome types in the trapping process (Fig. 5), as suggested by previous authors (Bruce, 1907; Voigt and Gorb, 2008).

Translucent secretion droplets against the background of red coloured glands probably reflect both visible and ultraviolet light similar to flypaper traps of the carnivorous plants *Drosophyllum lusitanicum* Link (Drosophyllaceae), *Drosera binata* Labil and

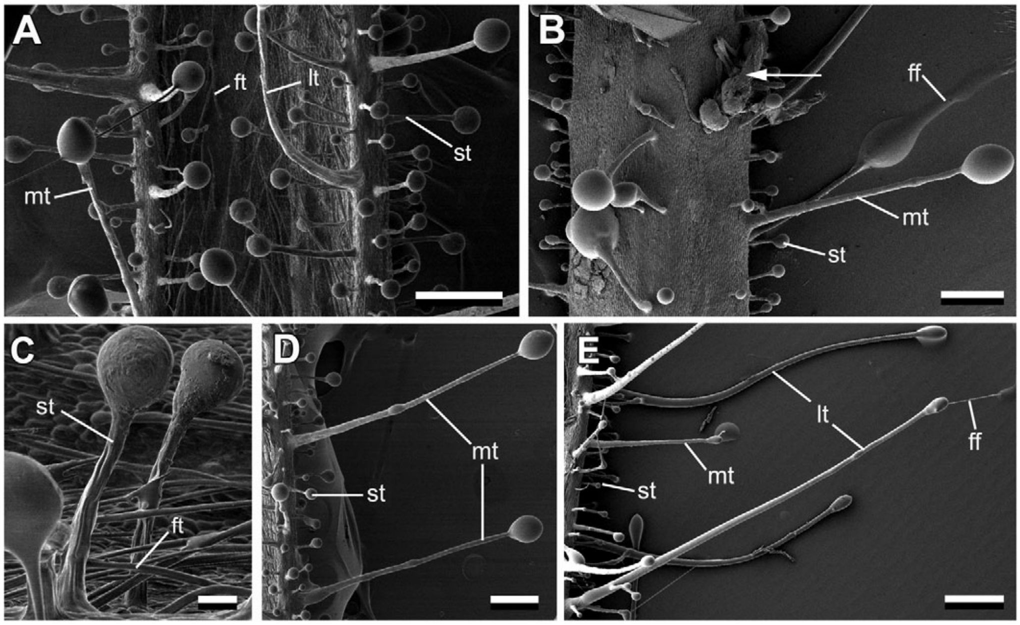

Fig. 2. Cryo-scanning electron microscopy micrographs of the leaf surface of *Roridula gorgonias*. (A) Adaxial leaf side densely covered with non-glandular filamentous trichomes pointing to the leaf tip and short capitate trichomes. Leaf margins are somewhat rolled-up due to the preparation procedure. (B) Abaxial leaf side having short capitate trichomes along the mid vein. (C) Detail of the adaxial leaf side showing filamentous trichomes and short capitate ones releasing round secretion droplets. (D) Detail of the leaf surface with medium-sized capitate trichomes situated at the leaf margin and exuding ovoid secretion droplets; short trichomes are visible. (E) Detail with long tentacle-shaped trichomes, situated at the leaf margin and releasing elongated ovoid secretion droplets; medium-sized and short capitate trichomes are visible. Arrow points to a captured fruit fly. lt, long tentacle shaped trichomes; mt, medium-sized capitate trichomes; st, short capitate trichomes; ft, non-glandular filamentous trichomes; ff, fluid filament. Scale bars: A, B, D, E=500 μm; C=50 μm.

Drosera capensis L. (Droseraceae) (Joel et al., 1985; Glossner, 1992). As ultraviolet light of wavelengths 330–370 nm is the most attractive for the majority of insect species (Menzel, 1979), prey is strongly attracted to these glistening plant surfaces, which are noticeable from considerable distances.

First trapped by the mesh of leaves and trichomes, insects try to free themselves by powerfully moving their legs and wings. However, even if they manage to break one contact with a leaf, they will touch another lower leaf of the cascade-like system of alternating leaves and be captured. Among the three-dimensionally arranged trichome coverage, the insect initially touches prominent, long trichomes. Once in contact with fluid droplets, insects may struggle intensively to free themselves. These attempts cause the trichome stems to bend and pull secretion droplets into viscous fluid filaments. After rupturing, filaments snap back and secretion continues to adhere to the insect's body. Thereby, insects then contact trichomes located below the canopy of long trichomes and become entangled by the even more viscous and strong secretion of the medium-sized and short trichomes. During this procedure, they apply enormous force trying to overcome the long viscid filaments and thus lose a lot of energy. For example, for blowflies *Calliphora vicina* Rob.-Des. (Diptera, Calliphoridae), the work that had to be applied to retract an adhering tentacle-shaped trichome from the insect cuticle to a distance of 1.5 mm has been previously estimated as 0.18 J (Voigt and Gorb, 2008). This is the work necessary to lift 1.8 kg to a height of 1 cm.

Finally, prey insects will move less, contacting numerous densely arranged short capitate trichomes thus being caught by strongly viscous secretion and adhering firmly to the plant surface.

The described trapping strategy enables the plant to catch even vigorous prey insects of considerable body size and mass. Indeed, it has been previously reported that *R. gorgonias* can trap large

Table 1. Morphometrical variables of the glandular trichomes of *Roridula gorgonias*

Variable	Trichome type			Statistics
	Long, tentacle-shaped	Medium, capitate	Short, capitate	
Stalk length (mm), l_s	**4.17**^{a} (3.299–5.024)	**1.37**^{b} (0.994–2.406)	**0.40**^{c} (0.312–0.726)	$H_{2,29}$=25.812, $P\leq0.010$, Tukey, $P<0.05$
Base width (mm), w_{tb}	**0.16**^{a}±0.021	**0.10**^{b}±0.015	**0.10**^{b}±0.015	$F_{2,29}$=29.938, $P\leq0.001$, Tukey, $P<0.05$
Tip width (mm), w_{tt}	**0.08**^{a}±0.005	**0.05**^{b}±0.009	**0.08**^{b}±0.010	$F_{2,29}$=52.724, $P\leq0.010$, Tukey, $P<0.05$
Aspect ratio, l_s:$w_{mean(tb,tt)}$	**36.06**^{a}±3.517	**20.09**^{b}±4.068	**4.82**^{c}±1.409	$F_{2,29}$=236.885, $P\leq0.001$, Tukey, $P\leq0.05$
Length of glandular head (mm), l_g	**0.33**^{a}±0.030	**0.25**^{b}±0.063	**0.29**^{a,b}±0.056	$F_{2,29}$=6.189, $P\leq0.006$, Tukey, $P<0.05$
Width of glandular head (mm), w_g	**0.17**^{a} (0.133–0.193)	**0.13**^{a} (0.106–0.209)	**0.22**^{b} (0.177–0.339)	$H_{2,29}$=19.977, $P\leq0.001$, Tukey, $P<0.05$

Median (minimum–maximum) for not-normally distributed data and means ± s.d. for normally distributed data are presented. Different letters indicate statistical differences between values in the line. *F*, statistic value of one-way ANOVA. *H*, statistic value of Kruskal–Wallis one-way ANOVA on ranks. See Fig. 3B for definition of measured parameters.

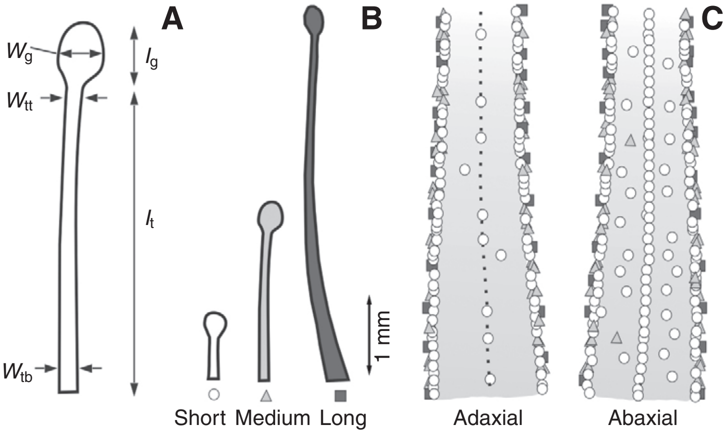

Fig. 3. (A) Scheme showing measured morphometrical parameters (for values see Table 1). (B) Three types of trichomes. (C) The arrangement of trichomes on the adaxial and abaxial leaf side in *Roridula gorgonias* (A). w_g, width of the glandular head in the medium; w_{tt}, width at trichome stalk tip; w_{tb}, width at trichome stalk base; l_g, length of glandular head; l_t, length of trichome stalk.

representatives of orders Hymenoptera, Diptera, Coleoptera, Hemiptera and Lepidoptera (Marloth, 1903; Marloth, 1910).

At the trichome level, three hierarchical sublevels contribute to the trapping efficiency. Although having similar multicellular structure and shape, different trichome types show distinct differences in dimension, stiffness and adherence, corresponding to their function in the trapping process. Stalks of long tentacle-shaped and medium-sized trichomes are highly flexible: their low spring constant corresponds to that of commercial, highly sensitive, microcantilevers used in Atomic Force Microscopy (AFM) (0.006–0.03 $\mathrm{N\,m^{-1}}$, Biolever BL-RC 150VB, Olympus Corp., Tokyo, Japan) whereas 48 times stiffer short capitate trichomes are comparable with glass springs, previously used in studies for estimating insect adhesion (Gorb et al., 2000). A load of 1 g would result in a deflection of 10 mm in long trichomes, 1 mm in medium-sized and 0.1 mm in short ones.

These very flexible trichomes may act like springs, which repeatedly bend and relax under the load of struggling insects, intercepting and damping the insects' force. By contrast, less flexible short trichomes finally immobilise and hold the prey insects.

The adherence of trichome's secretion varies significantly, depending on trichome type, but is similar on substrates with different surface energy. Thus, one may assume that the hydrophobic cuticle of prey insects should not interfere with *Roridula*'s trapping success. The smaller the trichome, the stronger is the stickiness of the secretion. This effect seems to correlate with an increase in the viscosity of secretion, because droplets of shorter trichomes remain thicker under the same applied pressure.

The secretion of *R. gorgonias* has been described as a mutual solution of mainly triterpenoids, acyl glycerides and undefined mushy glue (Simmoneit et al., 2008). Considering the latter as an elastomeric substance (Marloth, 1925; Loyd, 1934; Voigt and Gorb, 2008), the secretion composition is qualitatively comparable with that of commercial pressure-sensitive adhesives (PSA). PSA are visco-elastic polymers, comprising mixtures of rubber, resins and oil (Dahlquist, 1969; Creton, 2003). For instance, a combination of about 66% resinous gums, 30% fatty acids and 4% natural rubber has been recommended for coating fly paper or vermin traps (Dahlquist, 1969). Although natural rubber is known to have a strong self-adhering ability, rubber mixed in the right proportion with resins as tackifiers (particularly polyterpenes) makes an adhesive with excellent tack and a good balance between adhesiveness and cohesive strength. Additionally, oils (glycerol esters) are used to replace resin fractions thus avoiding crystallisation problems. They affect plasticising and lowering of the elastic modulus of PSA over a long period of time in contact (Dahlquist, 1969).

Characteristic features of PSA are the instantaneous adhesion with touch contact, the appreciable force of separation and the high cohesive strength, facilitating interfacial separation without residues on the substrates (Habenicht, 2002). The contact pressure, required for immediate adhesion to the secretion of *R. gorgonias*, is generated by insects slightly touching the plant exudates. Similar to PSA, the good initial adhesive contact formation ability of tentacle-shaped trichome secretion may be achieved by its low viscosity and therefore high deformability, permitting good conformation to the prey surface profile (Dahlquist, 1969; Soboleva et al., 2009). High wettability, adaptability to the substrate and rather low thickness of the adhesive layer have been previously reported to be the key factors for successful adhesion of PSA. The excellent wettability of various surfaces by secretion droplets of *R. gorgonias*' tentacle-shaped trichomes has been previously visualised using cryo-SEM (Voigt and Gorb, 2008). Extendible fluid filaments, into which the secretion is pulled under applied force, and the ability of the fluid to relax immediately after contact breakage, demonstrates its elasticity and deformability. These characteristics of the secretion are comparable with that of commercial filament tapes thus enabling the secretion to provide sufficient adhesive strength. PSA, used for heavy-duty bundling or packaging, offer a phenomenal tensile strength due to lineally aligned, embedded filaments of fibre-forming materials in the visco-elastic matrix (Dahlquist, 1969; Derail and Marin, 2009). Assuming the presence of analogical structures in the secretion of *R. gorgonias*, this plant adhesive should also be strongly capable of energy dissipation and have damping ability similar to commercial PSA.

However, the ideal rheological state for good tack in PSA has been found to dictate that the elasticity should be high in the time range selected for bonding and low in the time range selected for separation (Dahlquist, 1969; Feldstein, 2009). For less fluid adhesive secretion, such as produced by short trichomes (to which prey insects finally stick), it has been shown that high viscosity results in high bonding strength (Habenicht, 2002). Although such adhesives, lacking a fast relaxation mechanism, will not extensively wet the surface and establish a rather small contact area, they may provide much resistance to separation on a long time scale (Kendall, 2001). In *R. gorgonias*, poor wetting and reduced contact area are compensated for by the dense coverage of the surface with short capitate trichomes. This provides many contact points and strong

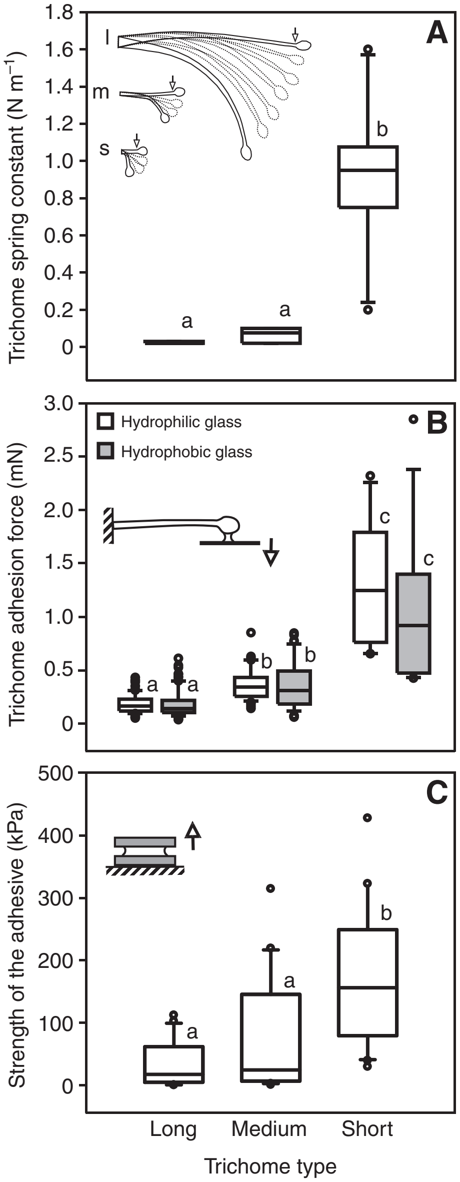

Fig. 4. Box-and-Whisker diagrams of the measured trichome stiffness, adhesion force of the secretion droplet and adhesive strength of secretion depending on the type of glandular trichomes in *Roridula gorgonias*. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. Different lower cases indicate statistical differences between trichome types. Insets show the type of experiment. Arrows point to the direction of movement. (A) Trichome stiffness defined by the spring constant, i.e. the slope (Hooke's line) of obtained force–distance curves (Kruskal–Wallis one-way ANOVA on ranks, $H_{2,29}=22.243$ with $P\leq 0.001$ and Tukey test, $P<0.05$). Inset shows diagrams of trichome bending behaviour obtained from video recordings. Trichome types: l, long; m, medium; s, short. (B) Adhesion force of single adhesive droplets to glass surfaces (Kruskal–Wallis one-way ANOVA on ranks, for hydrophilic glass: $H_{2,139}=80.810$; for hydrophobic glass: $H_{2,156}=54.668$, both with $P\leq 0.001$ and Dunn's test, $P<0.05$). (C) Adhesive strength of secretion measured between two hydrophilic glass surfaces (Kruskal–Wallis one-way ANOVA on ranks, $H_{2,29}=25.668$ with $P\leq 0.001$ and Tukey test, $P<0.05$). Contact pressure applied before pull-off measurements: 1.9 kPa for 30 s.

bonding ability even with non-smooth captured prey. Simultaneously, high adhesion is ensured due to the combination of stiff backing (short stem) and viscid secretion.

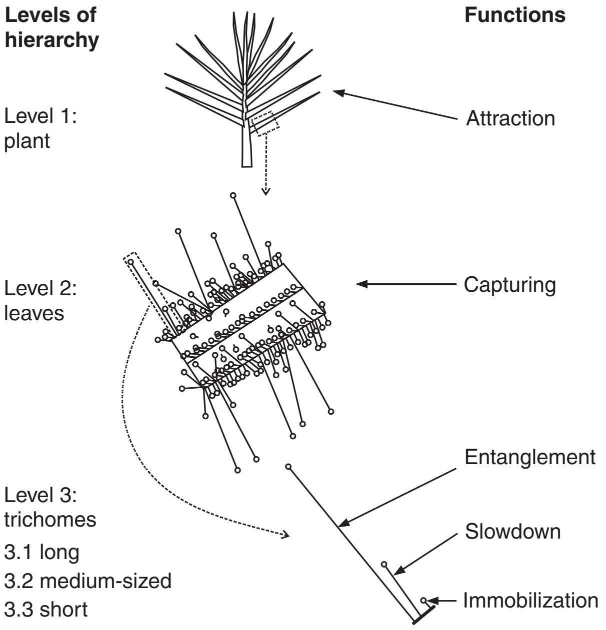

Fig. 5. Hypothetical diagram of functional units in the three-dimensional hierarchical trap of *Roridula gorgonias*. Three main levels (1–3) and three sublevels (3.1–3.3) are assumed to work in concert, interacting with the prey and fulfilling five main functions in the trapping process.

Because in PSA, both the tack and the complex tensile modulus vary identically with the composition of rubber–resin mixtures (Dahlquist, 1969), differences in adhesive properties of secretion between trichome types of *R. gorgonias* are probably caused by variations in the proportions of similar components. A larger amount of tacky resins in the secretion of short trichomes could explain its enormous adhesive strength of about 156 kPa. This is 9 times higher than in long and 6 times higher than in medium-sized trichomes.

Commercial PSA seem to adhere much more strongly than the secretion of *R. gorgonias* at a comparable adhesive layer thickness. Probe tack tests with standard rubber tack compound representative of acrylic PSA, used in common applications, namely poly(2-ethylhexyl acrylate) with 2% acrylic acid (PEHA-AA), resulted in a debonding strength of 1.3 MPa at a 1 mm s^{-1} separation rate, a 0.07–0.1 mm adhesive layer thickness and 900 kPa applied contact pressure (Lakrout et al., 1997).

Nevertheless, compared with viscid secretion of the protocarnivorous tar flower *B. racemosa*, the adhesive strength of which ranges between 40 and 50 kPa on glass and corresponds to that of the commercial flypaper Tanglefoot (The Tanglefoot, Grand Rapids, MI, USA) (Eisner and Aneshansley, 1983), the short trichome secretion produced by *R. gorgonias* is ~4 times stickier. This confirms previous arguments that the adhesive secretion of *R. gorgonias* is extremely viscid and is the strongest glue among all plant flypapers (Hartmeyer, 1998), although in experiments on applied contact pressure with *B. racemosa* (4–9 kPa) and Tanglefoot (16–33 kPa), the secretion layer thickness (1.3 mm) and the pulling velocity (0.5 mm s^{-1}) differed from the conditions used in our experiments with *R. gorgonias* (contact pressure: 1.9 kPa, secretion layer thickness: 0.13 mm, pulling velocity: 2.3 mm s^{-1}).

The operation of the *R. gorgonias* flypaper trap is based on the interaction of different hierarchical units (plant architecture, leaves, glandular trichomes) specialised in their stiffness and adhesive properties of secretion (Fig. 5).

According to previous observations, initially, the prey contacts and adheres to prominent, long, tentacle-shaped trichomes, whose less viscous secretion effectively wet the insect cuticle (Voigt and Gorb, 2008). Consequently, insects unsuccessfully try to free themselves by struggling, thereby pulling plant secretion into long fluid filaments, in which they gradually become entangled. Our present results allow us to suggest more detailed trichome functions. As long trichomes are flexible they partially support insect movements in any direction thus causing their further entanglement and contact formation with further, medium-sized trichomes. Additionally, trichomes may contribute to damping the insect's movements. We assume prey slowdown to be the main function of tendentiously stiffer medium-sized trichomes. Compared with long trichomes, medium-sized ones release stronger adhesive secretion, causing prey insects to adhere more closely to the plant's surface. Their secretion can also extend into filaments, allowing insects some degree of movement and contact with short stiff trichomes releasing a highly viscous secretion. Numerous contact points with short trichomes result in final immobilisation of prey insects and their long-term adherence to the plant surface. Theoretically, an adult syrphid fly could adhere sufficiently even to a single medium-sized trichome, considering an average fly body mass of 60 mg (Gorb et al., 2001) and a maximum adhesion force of plant secretion droplets to hydrophobic glass of 0.84 mN. Because the maximum adhesion force of secretion in short trichomes reaches values up to 2.85 mN, a fly may be glued to a single trichome with ~4.8 times its own body mass. By contacting many trichomes, belonging to three different, hierarchically arranged types, the combined interaction results in a very strong adhesion of insects to the plant surface.

Conclusion and outlook

Not only the presence of adhesive secretion but rather the multilevel, three-dimensional arrangement of leaves and trichomes enables *R. gorgonias* plants to effectively capture a large number of prey insects. Moreover, the specialisation in trichome dimensions, stiffness and adhesion properties also contribute to trapping success. *R. gorgonias*' trap is a highly effective mechanism. Because properties of plant adhesive secretion are comparable with that of commercial PSA, its chemical characterisation and the three-dimensional hierarchical organisation not only contribute to a further understanding of the trapping mechanism in this plant species but may also inspire innovations in commercial PSA and pest management by applying adhesive traps.

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