Always on the bright side: the climbing mechanism of Galium aparine

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Galium aparine is a herbaceous climbing plant that attaches to host plants mainly via its leaves, which are covered by hooked trichomes. Although such hooks are found on both leaf surfaces, the leaves of G. aparine are mainly positioned upon the leaves of supporting plants and rarely beneath. In order to understand the mechanism underlying this observation, we have studied structural and mechanical properties of single leaf hooks, frictional properties of leaf surfaces, turgor pressure in different leaf tissues and bending properties of the leaves in different directions. Abaxial and adaxial leaf hooks differ significantly in orientation, distribution, structure and mechanical properties. In accordance with these differences, friction properties of leaves depend on the direction of the applied force and differ significantly between both leaf surfaces. This results in a ratchet mechanism. Abaxial leaf hooks provide strong attachment upon the leaves of adjacent plants, whereas adaxial hooks cause a gliding-off from the underside of the leaves of host plants. Thus, the leaves of G. aparine can function as attachment organs, and simultaneously orient themselves advantageously for their photosynthetic function. Further adaptations in turgor pressure or concerning an anisotropy of the flexural stiffness of the leaves have not been found.

Keywords: semi-self-supporter; leaf hooks; attachment; mechanical properties; ratchet mechanism

1. INTRODUCTION

Galium aparine L. is a herbaceous climbing plant from the family Rubiaceae. The species is known as a cosmopolitan weed with a high degree of morphological and physiological plasticity, capable of colonizing a variety of different natural and anthropogenic habitats such as forests, hedgerows, waste grounds and arable fields [1–3]. As typical for climbing species, shoots of G. aparine lose mechanical self-stability during growth and use adjacent plants as support for further elongation growth [3,4]. The attachment of G. aparine to the supporting structures in the surrounding vegetation is mainly provided by the leaves that are arranged in whorls of six (figure 1). This mode of attachment is characteristic of the semi-self-supporting plants, or branch and leaf-angle climbers, which rely on wide-angled branches, leaf petioles or leaves to facilitate climbing. Unlike other climbing plants, this group does not have specialized climbing structures such as twining stems, climbing roots or tendrils to facilitate their scrambling ascending growth habit [5–9]. Goodman reports extremely high breaking strains for stem bases of G. aparine and concludes that the high extensibility is an adaptation to the frequent attachment of G. aparine to support swaying in the wind and therefore straining the stem base of the climber [3].

All aboveground parts of G. aparine plants are covered by hooked trichomes [10]. In G. aparine, attachment is provided mainly by the leaves, therefore it can be hypothesized that the main function of hooks on the leaf lamina, termed ‘leaf hooks’ in this study, is an enhancement of the interlocking with supporting plants. Hooks on fruits or seeds can be considered as an adaptation to epizoochorous dispersal [11,12]. Hooks on shoots increase friction with neighbouring plants [13,14].

Field observations suggest that leaves of G. aparine predominantly attach to the adaxial leaf surface of adjacent plants, and not to the abaxial one. Thus, they are positioned advantageously for light capture, which is important for sufficient photosynthesis. In order to understand the mechanism underlying this observation, we have analysed the structural and mechanical properties of single leaf hooks and frictional properties of leaf surfaces. Preliminary experiments revealed (i) differences in sliding friction between the abaxial and adaxial leaf surfaces, and (ii) an anisotropy of generated friction forces for the two different leaf surfaces. Since sliding friction is influenced by the contact pressure and by the position of hooks, which may be both influenced by turgor pressure, we also measured the cell turgor in different leaf tissues around the insertion of hooks. Additionally, leaf flexural stiffness in different directions was measured as a parameter possibly influencing the contact pressure between leaves and contact surfaces.

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2. MATERIALS AND METHODS

*Galium aparine* plants were collected in July at margins of a woodland path in Stuttgart-Büsnau, Germany, 48°54'50" N, 9°45'50" E.  

(a) **Hook morphology**

A Leica MZ 12.5 binocular microscope equipped with an integrated digital video-camera Leica ICA (Leica GmbH, Wetzlar, Germany), a Zeiss Axioplan (Jena, Germany) microscope equipped with an Olympus DP12 video camera (Olympus, Japan), a fluorescence microscope (ZEISS Stereo-Lumar V12 with an excitation filter BP 450–490 and an emission filter LP 515), and a cryo-scanning electron microscope Hitachi S-4800 (Hitachi High-Technologies Corp., Tokyo, Japan) equipped with a Gatan ALTO 2500 cryo-preparation system (Gatan Inc., Abingdon, UK) were used to characterize hook morphology. Samples investigated with the fluorescence laser microscope were stained with acridine orange in order to reveal lignified areas of the investigated material. Samples studied in the light microscope were stained with toluidine blue.

(b) **Hook density**

Density of adaxial leaf hooks was measured by counting the number of hooks of the adaxial leaf surface per unit of area in randomly selected areas (two areas of, respectively, 42.7 ± 14.7 mm² per leaf). As abaxial hooks are found in an aligned position on the midrib and on the margins of the abaxial leaf surface, density of abaxial hooks was determined as number of hooks per unit of length. For each leaf, length and total leaf area were determined using the image analysis software SIGMA SCAN PRO v. 5.

(c) **Contact separation force of single hooks**

To measure the pull-off force during contact separation between a single hook and a Kevlar thread loop, a FORT-10 load cell force transducer (10 g capacity, Biopac Systems Ltd., Santa Barbara, CA, USA) combined with a motorized micromanipulator DC3314R with controller MS 314 (World Precision Instruments, Sarasota, FL, USA) was used. Force–time curves were obtained and data were processed using ACQKNOWLEDGE v. 3.7.0 software (Biopac Systems Ltd, Goleta, CA, USA). A Kevlar (Du Pont, Wilmington, USA) thread (12 µm diameter) loop (3–5 mm loop length) was attached to the force transducer. All tests were performed on randomly selected hooks on both sides of whole fresh leaves. Leaves were cut using scissors and fixed on a glass slide with double-sided adhesive tape. The glass slide was then fixed on top of a metal holder with a movable arm. The loop, being wound around the hook, was pulled off at different angles relative to the leaf blade plane (figure 2; according to [11,12]) with the force transducer at a velocity of 200 µm s⁻¹. One hundred single abaxial and adaxial hooks were tested, except for the tests of adaxial hooks pulled at an angle of 45° against the direction of hook orientation, where only 50 single hooks could be measured.

(d) **Sliding friction of leaves**

Friction force was measured by pulling leaves over different artificial surfaces including: VELCRO Vel-Loop; foam plastic Plastoprint (Lehr- und Lernmittel Matthäus Hahn e.K., Altdorf, Germany); Spurr resin moulds of the polymer foam and polishing papers with different asperity size (P100 and P60) (see [15] for moulding preparation.

Figure 1. (a) Tangle of *Galium aparine* shoots in a hedgerow, (b) detail of a flowering shoot.

Figure 2. Diagram of the experimental set-up in contact separation force experiments with single leaf hooks on (a) adaxial and (b) abaxial leaf surfaces. Hooks were pulled with a Kevlar loop at different angles relative to the leaf blade until the hooks gave way or until the loop slipped off. Plus symbols (+) denote traction in the direction of hook orientation; minus (−) symbols denote traction against the direction of hook orientation.
The distance between force transducer (i.e. the holder where the leaf was clamped in) and test substrate was 1.5 mm in case of Spurr resin mouldings, 2.5 mm for foam plastic and 3.3 mm for VELCRO Vel-Loop.

Abaxial and adaxial leaf surfaces were tested both in and against the direction of hook orientation. Force–time curves were recorded and processed using ACQKNOWLEDGE v. 3.7.0 software. With each tested leaf, five test runs were performed at different time intervals after cutting the leaf. For each test run, the mean value of the 10 maximum force peaks of the force–time curve was calculated (figure 4).

(c) Flexural stiffness of leaves
Bending properties of freshly cut leaves were tested in cantilever tests. Using superglue (cyanoacrylate), a thin cotton thread was glued to the tip of a leaf with one end attached to a small vessel made of paper at the other end. A bending force was applied by pipetting a defined amount of water into this recipient in six steps. The leaf deformation was measured after each increase of the bending force. The flexural stiffness, EI, of a uniform beam is the resistance of that beam to curvature and is given by:

$$ EI = \frac{L^3(dF/dY)}{3} $$

where $L$ is the distance between the fixed base and the point where the force was applied and $(dF/dY)$ is the slope of the regression line in the linear elastic region of the force displacement curve [16,17].

(f) Cell turgor pressure
The cell turgor pressure was measured in living plants around the midrib of intact leaves using a cell-pressure probe and the pressure compensation method [18]. For this purpose, a silicone oil-filled capillary connected to an oil reservoir was introduced into the cell. After puncturing the cell, the sap penetrates in the capillary and the resulting meniscus (oil–cell sap interface) in the capillary was observed under a binocular microscope. By pushing a remote-controlled piston into the oil reservoir, the meniscus was brought back to the cell wall, thus restoring the original cell turgor. The pressure necessary for pushing the meniscus back to the cell wall was measured with a manometer. This value represents the cell turgor.

(g) Statistics
All statistical analyses were performed with SPSS v. 15.0.1. software (SPSS Inc., Chicago, IL, USA). Contact separation forces in single abaxial and adaxial hooks were compared by one-way analysis of variance (ANOVA) followed by Tukey, Holm-Sidak or Dunns post hoc tests. Friction forces generated by leaves on different artificial surfaces were compared by one-way ANOVA followed by Bonferroni post hoc tests. Friction forces generated by abaxial and adaxial leaf surfaces were compared by Mann–Whitney $U$ tests. The correlation between hook density and total leaf size ($r^2$) was calculated using Microsoft EXCEL 2007.

3. RESULTS
The properties of leaf hooks differ considerably between abaxial and adaxial hooks (figure 5).

(a) Hook structure
On the abaxial leaf surface, hooks are curved towards the leaf base, situated exclusively on the midrib and the leaf margins and continuously lignified (figure 5a,b,f).

On the adaxial leaf surface, hooks are curved towards the leaf tip, distributed evenly over the whole leaf surface and lignified predominantly at their apex (figure 5a–c). Furthermore, abaxial hooks have a larger basal diameter than adaxial ones (figure 5a,d,g). On both leaf surfaces, hooks emerge from circular foundations of epidermal cells. These cellular foundations are larger on the abaxial than on the adaxial leaf surface (figure 5a,d,g). The cones of all leaf hooks are hollow (figure 5e,h).

On both leaf surfaces, the hook density slightly decreases with total leaf size ($r^2 = 0.23$ on adaxial, $r^2 = 0.35$ on abaxial leaf surface). On adaxial leaf surfaces, density of hooks varied from 1.2 to 2.2 mm$^{-2}$. The density of abaxial hooks ranged from 1.2 to 2.3 mm$^{-1}$.

(b) Mechanical properties of single leaf hooks
Tests of the contact separation force of single leaf hooks revealed significant differences in mechanical properties between the abaxial and adaxial hooks; for all tested angles, the force measured in abaxial hooks was larger than that in adaxial ones (figure 6). All tested angles differ significantly from one another except for three pairs: (i) abaxial 90° and 45° against hook orientation; (ii) abaxial 90° against hook orientation and abaxial vertically; and (iii) adaxial vertically and 90° against hook orientation. In abaxial hooks, the largest force was found when hooks were pulled at an angle of 45° against the direction of hook orientation. In adaxial hooks, the largest force was measured when hooks were pulled vertically. Smallest force was found on both leaf sides when hooks were pulled at an angle of 45° in the direction of hook orientation. Tests at an angle of 90° in the direction...
Figure 4. Representative force–time curves obtained during the traction of the abaxial side of a single leaf over the VELCRO Vel-Loop surface in the direction of the orientation of trichome hooks: (a) 150 s and (b) 510 s after cutting from the plant. Force peaks indicate ‘temporary hooking’ of single leaf hooks with the substrate. The 10 maximum force peaks of each test run are numbered according to their absolute values (highest value of the 10 maximum force peaks = 1, lowest value = 10). The speed of motion was 3.1 mm s⁻¹.

Figure 5. Leaf hooks of *Galium aparine*. (a) Lateral view of a leaf: hooks on the abaxial surface (AB) of the leaves are curved towards the leaf base (LB), hooks on the adaxial surface (AD) are curved towards the leaf tip (LT). (b) Distribution of hooks: adaxial hooks (AD) are distributed evenly over the surface area, abaxial hooks (AB) occur exclusively on midrib and leaf margins (cryo-scanning electron microscope (SEM) micrograph). (c) Adaxial hook, stained with acridine orange, revealing the lignification of the tip in fluorescence microscopy. (d) Adaxial hook, SEM. (e) Freeze fracture at the base of an adaxial hook. (f) Fluorescence of an entirely lignified abaxial hook, stained with acridine orange. (g) Abaxial hook, cryo-SEM micrograph. (h) Abaxial hook, stained with toluidine blue; scale bar, 100 μm.
of hook orientation was not possible, because the Kevlar loop slipped-off the hooks without interlocking. This is also the reason why adaxial hooks could only be tested 50 times at an angle of 45° in the direction of hook orientation.

(c) Frictional properties of the leaves
In accordance with the observed differences in orientation, distribution, structure and mechanical properties of the hooks, friction properties of leaves (i) depended on the direction of the applied force, and (ii) differed significantly between the abaxial and adaxial leaf surfaces. When leaves were pulled over a test substrate against the direction of hook orientation 150 s after leaf cutting, the generated friction forces were minimal, ranging below the noise level of the testing device (<5 mN) (values not shown here). By contrast, when leaves were pulled in the direction of hook orientation, considerable friction forces were generated (figure 7). When tested in the direction of the hook orientation, friction of the abaxial leaf surface was significantly higher than that of the adaxial surface for all artificial test substrates, with the exception of foam plastic where no significant differences were found. The largest differences between the abaxial and adaxial leaf surfaces were recorded when leaves were pulled over a mould of foam plastic. In this case, mean friction force generated by the abaxial leaf surface was 6.6 times higher than that by the adaxial leaf surface (figure 7).

Mean values of friction force generated by adaxial leaf surfaces ranged from 8.8 ± 1.8 mN on the foam plastic mould to 21.9 ± 13.4 mN on the foam plastic itself (figure 7). Friction forces generated by the abaxial leaf surfaces ranged from 33.3 ± 15.1 mN on the foam plastic to 71.4 ± 24.6 mN on the VELCRO Vel-Loop (figure 7).

Friction forces generated by pulling leaves over different test substrates decreased with time after cutting, and the number of tests per single leaf (figure 8). Tests performed 330 s after leaf-cutting resulted in 58 ± 22%...
(abaxial leaf surface), and 59 ± 20% (adaxial leaf surface) of the force values obtained 150 s after cutting (figure 8, data pooled for VELCRO Vel-Loop and Spurr resin moulds of both polishing papers and foam plastic) (figure 8).

In the majority of the tested leaves, several abaxial hooks were damaged after completion of the fifth friction test. A few abaxial hooks had broken tips or were entirely pulled out of the tissue (figure 9a,b). Other failure modes were not observed. Failure of adaxial hooks was not observed.

(d) Leaf mechanics
Values of turgor pressure measured in epidermal cells above the midrib, in the parenchyma above the mid rib, and the parenchyma surrounding the vascular bundles scatter over a wide range from 0.1 to 6.4 bar. This holds also, when only values measured in a single leaf at a particular time after leaf-cutting are compared, where neither daily variation of the turgor nor differences between single leaves can be the origin of the wide variation of pressure values. Furthermore, values measured in hooks (2.11 and 3.48 bar, n = 2) and in the epidermal cells of the cellular foundations (2.25 ± 0.98 bar, n = 7) did not differ significantly.

Leaves of *G. aparine* did not differ significantly in their flexural stiffness when force was applied in different directions. Values obtained when leaves were bent downwards range from 0.06 to 0.45 Nmm⁻² (n = 24), and values obtained when leaves were bent in the opposite direction range from 0.06 to 0.49 Nmm⁻² (n = 24).

4. DISCUSSION
Abaxial and adaxial leaf hooks of *G. aparine* differ considerably in their position on the leaf surface, orientation, size, lignification and mechanical properties (measured here as contact separation force). These differences result (i) in significant differences of the friction properties of the abaxial and adaxial leaf surfaces and (ii) in a pronounced friction anisotropy of both leaf surfaces.

The herbaceous species *G. aparine* is a typical leaf-angle-climber (cf. [7]). When growing individuals reach a certain size and loose mechanical self-stability, attachment to supporting structures is provided mainly by the leaves. Owing to the differences in attachment properties between the abaxial and adaxial leaf surfaces, leaves of *G. aparine* interlock mainly with their abaxial surfaces upon the leaves of neighbouring plants. With a leaf (or several leaves) positioned in this manner, the action of a force moving *G. aparine* towards its support (e.g. induced by wind) results in a further rapprochement of the two plants because in this direction, the abaxial surface of the *G. aparine* leaf generates very low friction forces and glides on its contact surface. By contrast, when such a leaf of *G. aparine* is exposed to a force tending to pull it away from its support, the generated friction forces are much higher and the abaxial hooks provide strong attachment by interlocking with their contact surface. The opposite happens when a leaf of *G. aparine* clings with its adaxial surface beneath another leaf. In this case, the adaxial surface glides easily off the contact surface but resists further slipping-in because of the orientation of the adaxial hooks. With this ratchet mechanism acting differently on both leaf surfaces, the leaves of *G. aparine* are always positioned upon the leaves of the neighbouring supporting plants and do not get attached underneath them. Therefore, the leaves of *G. aparine* can function as attachment organs, and simultaneously orient themselves advantageously for their photosynthetic function: they are ‘always on the bright side’.

The attachment via abaxial leaf surfaces corresponds with the observed position of abaxial hooks on the slightly bent-downward leaf margins and the protruding midrib, as these are the leaf parts primarily in contact with the different surfaces of the supporting plants. Furthermore, the attachment via the abaxial leaf surfaces is consistent with the significantly higher mechanical stability of abaxial hooks providing attachment compared with mechanical stability of adaxial hooks. Owing to the curvature of their hooks, the latter only provide resistance against further sliding in *G. aparine* leaves that are positioned beneath the leaves of supporting plants.

Under natural conditions, each single *G. aparine* plant interlocks with its leaves, acting like ratchets, with several surrounding plants. Therefore, a strong attachment of leaves to supporting plants may cause considerable tension stresses in basal stem parts, when supporting plants sway in different directions. This consideration further supports previous conclusions of Goodman, who has reported an unusually high extensibility of basal stem parts of *G. aparine* and discussed its functional
significance as a strategy to minimize the effects of a moving support [3]. Although a decrease of hook density with total leaf size is observed, there seems to be no alteration of the functioning as attachment organs in full-grown leaves. It would be interesting to analyse in further studies how many hooks are necessary to attach a Galium aparine plant to different supporting structures.

Field observations show that the attachment mechanism of Galium aparine leaves acts on a wide variety of different natural contact surfaces such as leaves, shoots and even flowers and inflorescences of many different plant species. This is consistent with our results obtained on different artificial contact surfaces with a wide range of surface roughness and stiffness. The ability to interlock with a high diversity of contact surfaces may also be one of the properties enabling Galium aparine to successfully colonize structurally very different habitats, such as hedgerows, waste land or arable fields, and its nearly worldwide establishment as a weed in different crops [1,2]. Whether the observed decrease of friction forces generated by leaves at different times after cutting can be attributed to a drop of leaf turgor pressure or to a fatigue owing to leaf hook damage, or to a combination of both, cannot be concluded here. In order to answer this question, a quantitative analysis of leaf hook damage after friction tests and a study simultaneously analysing turgor pressure and friction properties in different leaves is necessary. If a decrease of turgor pressure turned out to be the main process underlying the variation of friction properties of Galium aparine leaves after cutting, this could be one of the factors restricting the distribution of the species to habitats with a favourable water supply as previously described by Auge & Mahn [1].

The hypothesis of further structural adaptation of Galium aparine leaves for optimizing the attachment properties via (i) turgor pressure differences in different tissues around the insertion of leaf hooks, or (ii) an anisotropy of the flexural stiffness of the leaves is not confirmed. Our results suggest that the ‘ratchet mechanism’, leading to the attachment of Galium aparine leaves upon and not beneath different contact surfaces, can mainly be attributed to the different structural and mechanical properties of the abaxial and adaxial leaf hooks, resulting in a pronounced anisotropy of the friction properties of the leaf surfaces.

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