The attachment strategy of English ivy: a complex mechanism acting on several hierarchical levels

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English ivy (Hedera helix L.) is able to grow on vertical substrates such as trees, rocks and house plaster, thereby attaching so firmly to the surface that when removed by force typically whole pieces of the climbing substrate are torn off. The structural details of the attachment process are not yet entirely understood. We studied the attachment process of English ivy in detail and suggest a four-phase process to describe the attachment strategy: (i) initial physical contact, (ii) form closure of the root with the substrate, (iii) chemical adhesion, and (iv) shape changes of the root hairs and form-closure with the substrate. These four phases and their variations play an important role in the attachment to differently structured surfaces. We demonstrate that, in English ivy, different mechanisms work together to allow the plant’s attachment to various climbing substrates and reveal the importance of micro-fibril orientation in the root hairs for the attachment based on structural changes at the subcellular level.

Keywords: attachment system; Hedera helix; root climber; English ivy; form-structure–function; adventitious roots

1. INTRODUCTION

Biology offers many examples of very efficient adhesion systems that are highly adapted to their respective function and allow for permanent or temporary and reversible adhesion under different environmental conditions with high reliability. Research during the last decade was mainly focused on reversible and permanent adhesion in animals. One main topic was the quantitative analysis of reversible adhesion systems found on the locomotor organs in different groups of animals, e.g. insects, spiders, tree frogs and geckos (Autumn et al. 2000; Gorb 2001; Arzt et al. 2003; Federle et al. 2006; Gorb et al. 2006). In many species, the functionality of the very effective attachment systems is based on an enlargement of the contact area due to contact-splitting by the development of tiny hair-like structures with a thickness of only several nanometres and the van der Waals forces between these structures and the substrate (Arzt et al. 2003).

The second main research topic was centred around permanent adhesion found in marine invertebrates based on the excretion and hardening of various chemical substances (Corne et al. 1997; Silverman & Roberto 2007; Waite 2008; Wang et al. 2008; Sangeetha et al. 2010). In contrast, plants and especially climbing plants, which also have adhesion systems with excellent mechanical properties, are only sparsely studied as to the form–structure–function relationship of their adhesive organs (Endress & Thomson 1976, 1977; Groot et al. 2003; Moloche et al. 2007; Bowling & Vaughn 2008, 2009; Zhang et al. 2008). This holds especially for detailed biomechanical analyses of the strategies of permanent attachment found in climbing plants, which have only recently started to attract more attention (Melzer et al. 2009; Steinbrecher et al. 2009, 2010).

English ivy (Hedera helix L.) is a prominent member of the European flora and grows on tree trunks and building facades. English ivy has been studied in detail with respect to its ecological implications (see Metcalfe 2005), pharmaceutical properties (see Majester-Savornin et al. 1991), ontogenesis (see Rogler & Hackett 1975) and physiological characteristics (see Oberhuber & Bauer 1991). Our study focuses on a detailed analysis of structural and functional aspects of the attachment structures and mechanism of H. helix, and presents a model explaining how English ivy is able to climb on various vertical surfaces up to a height of 30 m (Metcalfe 2005).

During the juvenile phase of its ontogenesis, English ivy develops clusters of adventitious roots, i.e. roots originating from shoots (Tobler 1912). Under the appropriate moisture conditions, these roots can develop into unbranched, 1–15 mm long, attachment roots (figure 1a,b). They squeeze into gaps of the climbing substrate, flatten in the dorsiventral direction, and in a later developmental phase lignify, further enhancing their favourable mechanical properties (Bruhn 1909). The attachment roots excrete a glue-like substance (already described by Malpighi (1679), see also Darwin (1875)) similar to other climbing plants (Groot et al. 2003; Bowling & Vaughn 2008). The chemical composition of the substance was recently the subject of a first analysis (Zhang et al. 2008).

Root hairs, which represent protrusion of single cells, are found on the surface of the attachment roots (Malpighi 1679; Leitgerb 1858; figure 1c). In order to mediate the attachment between roots and climbing substrates, root hairs need sufficient mechanical strength and the potential for shape modification to form a reliable anchorage with the substrate.
The biomechanical properties of cells depend, to a large degree, on the amount and orientation of the cellulose micro-fibrils in the cell walls. Micro-fibril orientation in cell walls and its significance for mechanical properties attracted a lot of attention in recent years (for concise reviews, see Burgert (2006) and Donaldson (2008)). It could be shown quantitatively that mechanical properties of plant cells and tissues (Mott et al. 2002; Burgert 2006; Salmeén & Burgert 2008) also influence movement of plant cells and organs (Dawson et al. 1997; Burgert et al. 2007; Elbaum et al. 2007, 2008; Fratzl et al. 2008; Burgert & Fratzl 2009) and are related to differences in deformability of fibrils and matrix in the cell wall for mechanical properties and actuation processes.

Investigations of root hairs were the basis for the development of geometrical (Emons & Kieft 1994) and molecular (Galway 2006) models of the deposition of cellulose micro-fibrils. However, the ways micro-fibril angle in the cell walls of the root hairs of H. helix and its alteration during development may influence structural and functional aspects of the attachment process of English ivy have not yet been studied. Based on the structural data of our study, we propose a four-phase model for the attachment mechanism of English ivy.

2. MATERIAL AND METHODS

2.1. Plants and growing conditions

The plant material used in this study was either grown indoors in the greenhouses or laboratories of the Botanic Garden of the University of Freiburg or taken from outdoor populations. Plants were grown indoors in pots and presented with a climbing rack covered with cork as the basic climbing substrate. Some of the young shoots of mature plants were provided with 5 × 10 cm pieces of Mylar foil and glass slides as a climbing substrate. The outdoor plants were sampled in different places in the Botanic Garden Freiburg and the grounds of the Institute for Materials Research II, Karlsruhe Institute for Technology.

2.2. Light and electron microscopy

Root hair samples for light microscopy were stained with a chloride–zinc–iodine solution (6.5 g KI, 10 ml distilled water, 1.3 g I, 20 g ZnCl₂). The images were taken with an Olympus BX61 microscope equipped with an oil immersion objective equipped with an Olympus Dp71 camera, using the differential interference contrast mode. This allows the arrangement and angles of cellulose microfibrils in the cell walls of the root hairs to be analysed.

In order to test lignification, cuttings of established attachment roots were taken and stained with phloroglucin (phloroglucinol in ethanol with 25% hydrochloric acid) which stains lignified tissues red.

For scanning electron microscopy (SEM) imaging, the samples were fast-fixed with methanol, a method that allows fixation with almost no shape distortion (Neinhuis & Edelmann 1996), dried at critical point and sputtered with gold. The SEM analysis was carried out with a SEM LEO 435 VP (Leica, Wiesbaden, Germany) microscope. Samples for ESEM imaging were kept and prepared moist, in a fresh condition. For the ESEM analysis, a Phillips FEI ESEM XL 30FEG microscope at a base pressure of 133 Pa was used.

2.3. Statistics

Each of the 5 per cent groups of fibre orientation angles was tested with the Kolmogorov–Smirnov test for normality. One-way analysis of variance was carried out with a Tukey HSD test as post hoc processing. The two groups of different sections of the root hairs (0–95%) and (95–100%) were compared by a Welch two-sample t-test.

2.4. Tensile testing

For measuring Young’s modulus of the Mylar foil used as a climbing substrate, rectangular pieces with
dimensions 10 × 40 × 0.12 mm (width × length × thickness) were cut. These strips were clamped on each side 5 mm deep into the jaws of a tensile testing machine and tested. The force–displacement data were transferred into Excel 2007 and Young’s modulus was calculated as the slope of the linear elastic part of the stress–strain curve. The tests were carried out with a UPM Instron 4466–10 kN (with a retrofit kit to inspect-DC standard, Hegewald & Peschke Mess- und Prüftechnik GmbH, Nossen, Germany) configured with a 1 kN load cell.

Figure 2. Structural evidence for different phases in the attachment process of English ivy. (a) Tip of a root hair with spherical excrescences, which are most probably the containers of the gluing substance excreted by the plant in phase 3 of the attachment process. Scale bar, 5 μm. (b–d) Formation of the spoon-like shape at the tip of root hairs. Images of different root hairs taken at different developmental stages during the shape change. Scale bar, 10 μm. (b) Tip of a fresh root hair, ESEM image. (c) Tip of a root hair in the process of drying showing the first indications of a spoon-shaped tip, SEM image. (d) Tip of a dried root hair in completed spoon-like configuration, SEM image. (e) Single root hair, demonstrating the fibre orientation, SEM image. Scale bar, 20 μm. (f) Differential interference contrast picture of a stained single root hair. The darker lines visible on the root hair show micro-fibril orientation. The picture is a montage of 11 micro-images of one root hair, the lines drawn in black indicate the borders of the individual images. Scale bar, 50 μm. (g) Bar plot diagram of the mean fibre angle orientation versus the relative position on the longitudinal axis over the entire relative length of the root hair in steps of 5% ((0–5%), (5–10%), ... (95–100%)). The error bars represent standard deviations. The diagram demonstrates the steep increase in cellulose micro-fibril angles in the last 5% of root hair length; number of tested root hairs: n = 11, pooled data. The box colours indicate two groups with significantly different cellulose micro-fibril angles with a p-value of 7.023 × 10⁻⁸ (Welch two-sample t-test; for details, see §2). The blue group consists of all 5% steps from the base of the root hair to 95% relative length of the root hair length. The orange group consists of the apical 5% of root hair length including the tip of the root hairs.
3. RESULTS

Fastened attachment roots of *H. helix* are squeezed into gaps of the climbing surface and are covered in the contact regions with the climbing substrate with a multitude of unicellular root hairs. Free growing, non-fastened attachment roots also develop root hairs proximal from the root’s calyptras, i.e. root caps (figure 1b,c). Established attachment roots tested positive for lignin. The root hairs of the attachment roots excrete a glue-like substance on smooth as well as on structured surfaces (for details see below). On glass surfaces where the attachment is not enduring, the plant eventually detaches but leaves glue stains on the glass substrate that correspond to the pattern of root hairs on the detached contact area of the roots. In contrast to glass, Mylar foil, a smooth, inert and pliant synthetic material with a Young modulus of 3.2 ± 0.6 GPa (mean ± s.d.; n = 40) is accepted as a climbable surface (figure 3a). The distribution of glue ‘threads’ found at attached root hairs on Mylar foil (figure 3a magnification) matches the pattern of roundish excrescences found on the root hairs of not yet attached attachment roots (figure 2a).

In unfastened root hairs, a shape change takes place with increasing age of the root hairs. Initially, young root hairs represent turgescence cylinders with rounded tips and a length from 20 to 400 μm and 12.8 ± 1 μm diameter (mean ± 1 s.d.; n = 59; figure 2e). With increasing age the root hairs, owing to loss of water, begin to shrink and change shape. Two distinct types of shapes are observed. They resemble either grooved shoehorns (figure 3a) or spirally curled flattened cylinders (figure 3d). Both types end in spoon-shaped tips (figure 2b–d). The formation of these spoon-shaped tips could be observed during the drying of an intact living root hair in an ESEM chamber.

In fresh, turgescant root hairs differential interference contrast microscopy and SEM revealed that the cellulose micro-fibrils in the walls of the root hairs are not arranged in a crosswise pattern but possess a single main spiral micro-fibril orientation (figure 2e,f). Cellulose micro-fibrils run at an almost constant angle spiralling around the longitudinal direction for most of the root hairs. Subdividing the root hairs into 20 equally spaced sections, the angle between the main longitudinal axis of the root hair and the cellulose micro-fibrils is constant for over 95 per cent of the root hair length, varying between 35.5° and 46°, and shows no significant difference between the sections (Tuley HS1D). The mean value of the micro-fibril angle for the basal 95 per cent of the roots hairs is 41° ± 1° (mean ± s.d.; 170 samples from 11 root hairs). In the apical 5 per cent of the root hair, the micro-fibril angle increases abruptly at the tip to 55° ± 6° (mean ± s.d.; 15 samples; figure 2f,g). A Welch two-sample t-test between the fibre angles of the first group (0–95% root hair length) and the second group (95–100% root hair length) yields a significant difference with a p-value of 7.023 × 10⁻⁸. For further details, we refer to the electronic supplementary material of our study.

Attachment roots that were not anchored were studied via SEM microscopy. They show turgescant root hairs and both types of shapes of dried root hairs, with the proportions depending on the developmental age of the root hairs. Free root hairs that are not attached with their tips to a supporting surface often twist during the drying out process (figures 1c and 2e). Root hairs that are attached to a supporting surface have no degree of freedom to twist. They therefore either form grooved shoehorns, if attached over (nearly) their entire length to a flat supporting surface. If attached initially only with their tip in gaps of a structured surface, the root hairs curl up and shorten during the drying out process, which causes the described pulling of the root to the support. Roots attached to Mylar foil exhibit only root hairs of the *grooved shoehorns*-like form, while in sections through attached ivy roots on cord mainly root hairs showing the *spiral curls* shape can be found anchored within the cavities of the substrate.

4. DISCUSSION

The first attachment phase is the initial contact formation of the root attachment system—clusters of attachment roots—of *H. helix* with the climbing substrate. There exist two possibilities in which part of the English ivy will first perceive contact. The first possibility is that the longest root tip makes the initial contact as it always points towards the climbing substrate. The second possibility is that the root hairs that have been present already on free growing, non-fastened attachment roots are the first root structures coming into contact with the climbing substrate as they protrude outwards from the growing root, but only in the region proximal to the calyptra. The lengths of the attachment roots differ strongly; figure 1b shows examples for a rather short specimen. In combination with the random structure of the rough, natural climbing surfaces that English ivy usually grows on, we neither have direct evidence as to which of the two possibilities is more likely nor know what the exact signal ‘announcing’ contact to the substrate is and how it may be mediated to initiate the second attachment phase.

This initial contact triggers the second phase, the structural adaptation of the attachment root system to the topology of the climbing substrate. It is also the onset for the lignifications of the attachment roots (Bruhn 1909). The first reaction to contact formation is an enlargement of the contact area between the attachment root and the climbing substrate, which apart from enhancing the form closure also brings more of the root hairs in contact with the climbing substrate. The second reaction leads to an improved and enduring connection of the attachment root and the substrate owing to the incorporation of lignin and an increase in diameter of the root, which interlocks the mechanically stabilized root within the bigger gaps of the climbing substrate.
Figure 3. Adaptation for smooth and structured climbing substrates. (a) Root hair attached to Mylar foil. Strands of glue can be seen to brace the root hair against the substrate, SEM image. Scale bar on overview 10 μm; scale bar on inset 5 μm. (b) Fresh root hair after first contact with the substrate, schematic drawing. (c) Dried root hair with strands of glue braiding it against the substrate, schematic drawing. (d) Spirally curled flattened root hair, SEM image. (e) Fresh root hair growing into a cavity in the substrate, schematic drawing. (f) Drying root hair anchored to protrusions inside the cavity, pulling the attachment root towards the substrate, schematic drawing.

The third phase is the chemical adhesion to the surface, which is also likely to be triggered by the initial contact in the first phase. The congruence of the ‘threads’ of glue found between root hairs and substrate with the pattern of the roundish excrescences on unattached root hairs (figures 2a and 3a) suggests that these structures are the source of the glue and not as stated by Zhang et al. (2008) the trichomes of the ivy shoot. One may hypothesize that the shear between climbing substrate and the root hairs occurring during the attachment process causes opening of the roundish excrescences and release of the glue. Our data show that chemical adhesion alone can only secure enduring root attachment on certain smooth surfaces, such as Mylar foil but not on glass. This finding contrasts with results for Ficus pumila (Groot et al. 2003), which has attachment roots that adhere on glass. This suggests a chemical reaction of the excreted glue of H. helix with the polymer surfaces. Further studies of the excretion process are planned and shall contribute to a better understanding of this process and will help to compare the glue excreted by English ivy with glues found in the attachment systems of other climbing plants (Groot et al. 2003; Bowling & Vaughn 2008).

The fourth phase of the attachment process is brought about by a passive shape change of the root hairs of the attachment roots, driven by the loss of water. This shape change initiated by the drying of the root hairs is actuated by the different deformability of cellulose microfibrils and matrix in the cell wall of the root hairs (see Burgert & Fratzl 2009). The stresses occurring during desiccation mainly deform the soft matrix, while the stiff cellulose micro-fibrils generate the shape change of the shrinking root hair. Our studies suggest that the different shapes found in dried root hairs are caused by an interaction between the internal structure of the root hairs and the structure of the climbing substrate. In all dried root hairs, the formation of a spoon-shaped tip (figure 2c) can be observed, most probably owing to the sharp increase in micro-fibril angle in the apical 5 per cent of the cell wall of the root hairs. On smooth surfaces, the climbing roots develop root hairs that are oriented mainly perpendicular to the root and excrete the gluing substance. After a hair has reached its full length, it flattens owing to turgor loss and the excreted glue hardens. In the following drying process caused by the micro-fibril arrangement of the root hairs, the rims and tip of the root hairs start to bend upwards, thereby bracing the root hairs at the glue spots against the surface of the substrate (figure 3b,c) and also forming a sterically constrained version of the spoon-shaped tip. This tension bracing provides additional resistance of the root hairs and of the entire attachment system against shear forces occurring because of the mechanical loads to which the plant is subjected.

On highly structured substrates, the root hairs grow into gaps and fissures where they excrete the glue and secure the first attachment. In addition to the first stable attachment provided by this adhesive substance, the curling of the root hairs caused by the micro-fibril arrangement in the root hair cell wall further anchors them to protrusions within the gaps while drying out. The formation of the spoon-shaped tip can be seen as an additional way to secure attachment, which will anchor the root hair at protrusions and undercuttings of the structured surface even if the chemical adhesion fails. The ongoing shortening of the attached root hairs during the drying process pulls the whole attachment root towards the substrate and thereby tightens the attachment (figure 3e,f).
The shape change of root hairs induced by loss of water during the drying process is another example of a precise actuation of plant parts mediated by the orientation of cellulose micro-fibrils in the cell walls and initiated by changes in moisture content. In contrast to other examples (see Dawson et al. 1997; Elbaum et al. 2007), in the case of English ivy, the actuated shape change does not act at a tissue or organ level but on the protrusions of single cells, thus adding another facet to the understanding of the form–structure–function relations caused by the orientation of cellulose micro-fibrils. In comparison with permanent attachment systems known from animals (see Silverman & Roberto 2007; Waite 2008; Wang et al. 2008; Sangeetha et al. 2010), the use of a passively actuated shape change of an inactive (dead) organ part, i.e. the shape change of the root hairs as occurring in the last phase of the attachment process in English ivy, seems to be limited to the plant kingdom and represents a new aspect in form–structure–function relationships of biological attachment systems.

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