

The insect-trapping rim of *Nepenthes* pitchers

Surface structure and function

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Key words: carnivorous plants, insect aquaplaning, superhydrophilic leaves, *Nepenthes*, peristome

Carnivorous pitcher plants of the genus *Nepenthes* capture prey with a pitfall trap that relies on a micro-structured, slippery surface. The upper pitcher rim (peristome) is fully wettable and causes insects to slip by aquaplaning on a thin water film. The high wettability of the peristome is probably achieved by a combination of hydrophilic surface chemistry, surface roughness and the presence of hygroscopic nectar. Insect foot attachment could be prevented by the delayed drainage of the thin water film between the adhesive pad and the surface. Drainage should be faster for insects with a hairy adhesive system; however, they slip equally on the wet peristome. Therefore the stability of the water film against dewetting appears to be the key factor for aquaplaning. New experimental techniques may help to clarify the detailed function of the pitcher plant peristome and to explore its potential for biomimetic applications.

Introduction

The paleotropic genus *Nepenthes* comprises approximately 90 species of carnivorous plants,¹ all of which use highly specialized pitcher-shaped leaves to capture mainly insect prey.²⁻⁵ The bottom part of each pitcher is filled with a digestive fluid in which the captured prey drowns and subsequently decomposes. The released nutrients are absorbed through multicellular glands on the inner pitcher wall.⁶ The utilization of this additional nutrient source enables pitcher plants to colonize extremely nutrient-poor habitats where other plants struggle to survive.⁷

The pitcher trap consists of several specialized structures (Fig. 1). The inner pitcher wall is divided into a lower glandular and an upper waxy zone (absent in some species). The pitcher rim (peristome) is often conspicuously colored and characterized by a regular pattern of radial ridges. Its inner edge overhangs the pitcher and is densely packed with extrafloral nectaries. The pitcher rim is often elongated upwards to the pitcher lid, forming a distinct "neck". In most species, the lid covers the pitcher opening and thus shelters it from heavy rain, but in some species it is reduced or bent backward.

Like insect-pollinated flowers, pitchers attract visitors by presenting visual and olfactory signals and offering food rewards.

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Submitted: 07/23/09; Accepted: 07/25/09

Previously published online:

www.landesbioscience.com/journals/psb/article/9664

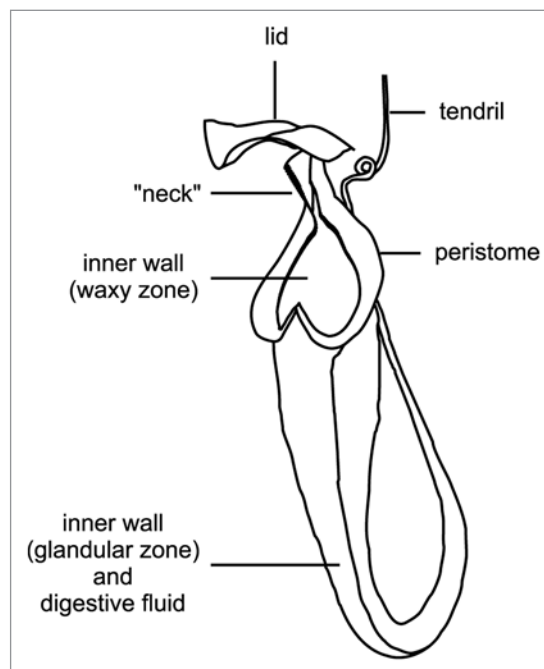


Figure 1. Schematic illustration of a *Nepenthes* pitcher.

For example, upper pitchers of *N. rafflesiana* exhibit distinctive UV reflection patterns⁸ and exude a strong sweet scent.^{3,9,10} A significant part of the prey spectrum consists of anthophilous flying insects, indicating that these pitchers successfully mimic flowers.³ Unlike most flowers, however, pitchers attract large numbers of ants. This is achieved by extrafloral nectaries located on the tendrill, the outer pitcher wall, the underside of the lid and the inner margin of the peristome.^{4,11} Studies on *N. bicalcarata* have shown that the secretion of these nectaries changes during pitcher development: the nectaries on the tendrill and outer wall are mainly active in developing, unopened pitchers while in mature pitchers most nectar is secreted under the lid and on the peristome.^{11,12} Attraction of ants prior to pitcher opening not only provides protection against herbivory for the developing pitchers^{11,13} but also establishes foraging trails of ant visitors which can later fall prey to the pitcher. The subsequent shift of nectar production from the tendrill and outer pitcher surface to the lid and the peristome lures ants into more perilous positions on the pitcher. Our recent study shows that the peristome nectaries of *N. rafflesiana* only start secreting after

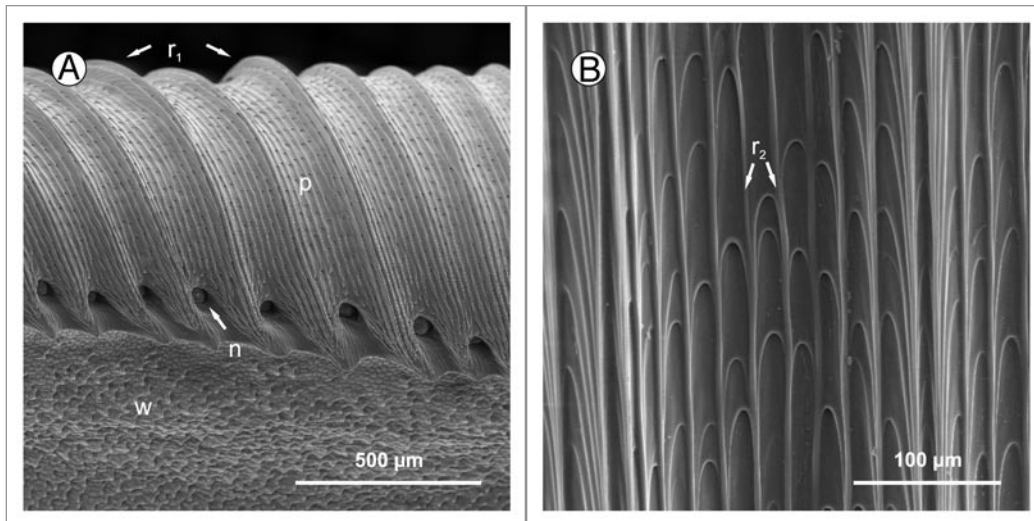


Figure 2. (A) Peristome surface (p) of *Nepenthes alata*, structured by first (r_1) and second order radial ridges. In between the tooth-like projections at the inner edge of the peristome the pores of large extrafloral nectaries (n) can be seen. Below the peristome is the wax-covered inner wall surface (w). (B) The second order ridges (r_2) are formed by straight rows of overlapping epidermal cells.

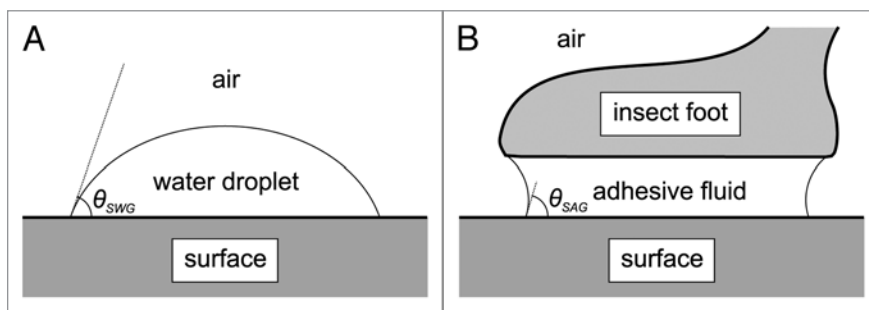


Figure 3. (A) The contact angle θ_{SWG} of a water droplet on a surface provides a measure of the wettability of the surface. (B) Simplified model of the contact of an insect adhesive pad with a surface, mediated by an adhesive fluid.

the pitcher has opened.¹⁰ This ensures that no nectar is wasted before pitcher opening.

Prey is captured by a pitfall mechanism; no moving plant parts are involved in the trapping process.⁷ Insects lose their footing on the specialized, anti-adhesive surfaces of the peristome¹⁴ and/or the inner pitcher wall.^{15–18} Many *Nepenthes* species possess epicuticular wax crystals on the upper part of the inner pitcher wall. The platelet-shaped crystals project perpendicularly from the surface. As a result, the crystals break off very easily, thus contaminating and disabling the insects' adhesive pads.^{15,19} In addition, the micro-rough crystal surface reduces the contact area for insect adhesive pads. The slipperiness of the inner wall surface is further aided by downward-pointing epidermal cells that provide no foothold for insect claws while climbing upwards.²⁰ The anti-adhesive surface on the inner pitcher wall may be more important for the effective retention of captured prey than for the initial trapping.¹⁴ In addition, arthropod prey is also prevented from escaping by the digestive fluid.^{16,21}

The Peristome: Surface Structure, Wettability and Trapping Function

Pitchers of most *Nepenthes* species have a well-developed peristome, the surface of which is characterized by a highly regular microstructure composed of first and second order radial ridges (Fig. 2). The larger first order ridges vary between species in height, shape and spacing. The much smaller second order ridges consist of straight rows of overlapping epidermal cells which form a series of steps towards the pitcher inside. The second order ridges are more uniform among species (Bauer U, unpublished results). This may be a consequence of constraints in cell

development and architecture but it could also mean that their dimension is advantageous for the function of the peristome. The surface of each epidermal cell is smooth and free of epicuticular wax crystals.

The trapping function of the peristome has long been overlooked, probably because of its dependence on wetness.^{14,22} The dry peristome surface provides a secure foothold for insects.^{14,16,22,23} Under humid conditions, however, the peristome becomes extremely slippery, and experimentally obtained capture rates of ants reached more than 80%. A key factor for the slipperiness of the peristome is that its surface is completely wettable.^{14,22} Wettability is determined by the combination of adhesive and cohesive forces in the interaction between water (W), the solid surface (S) and the surrounding gas (G). The contact angle θ_{SWG} of a water droplet on a surface (Fig. 3A) provides a measure of its wettability; according to Young's equation, it is determined by the interfacial tensions γ_{SG} , γ_{SW} and γ_{WG} , denoting the energy per unit area of the solid/gas, solid/water and water/gas interfaces:

$$\cos \theta_{SG} = (\gamma_{SG} - \gamma_{SW}) / \gamma_{WG} \quad (1)$$

A surface is wettable if $\theta < 90^\circ$ and non-wettable if $\theta > 90^\circ$. With regard to water, wettable surfaces are often termed hydrophilic (superhydrophilic if $\theta < 10^\circ$) and non-wettable ones hydrophobic. The interfacial tensions γ_{SW} , γ_{WG} and γ_{SG} determine the spreading coefficient:

$$S_{SWG} = \gamma_{SG} - (\gamma_{SW} + \gamma_{WG}) \quad (2)$$

If $S_{SWG} \geq 0$, a droplet will spread and completely wet the surface; the higher the value of S_{SWG} , the stronger the tendency of the droplet to spread. Most hydrophilic surfaces are also well wettable by non-polar liquids, a phenomenon sometimes referred to as amphiphilicity. A study on *N. alata* has confirmed amphiphilic properties for the peristome; however, contact angles were found to be lower for water than for non-polar liquids.²⁴

Superhydrophilic leaf surfaces have evolved independently in several groups of plants and are often associated with specialized functions such as absorption of water from mist (e.g., Bromeliaceae, some Cactaceae) or underwater growth.²⁵ Wettable leaves are characterized by an absence of epicuticular wax crystals.²⁶ A recent study on superhydrophilicity in the tropical herb *Ruellia devosiana* suggested that in this species high wettability is facilitated by the secretion and spreading of a surfactant from specialized glands.²⁷ It is still unclear whether such a detergent is also present in the peristome nectar of *Nepenthes*; however, high sugar content renders the nectar hygroscopic. The presence of nectar on the peristome has been shown to significantly enhance surface wetting by condensation.²²

Microscopic roughness acts as an enhancer of the general wetting properties of a given surface.²⁸⁻³¹ In other words, micro-roughness increases the wettability of a hydrophilic surface (e.g., in *R. devosiana*²⁷) but decreases the wettability of a hydrophobic surface (e.g., in *Lotus* leaves²⁶). However, *Nepenthes* peristomes are not simply micro-rough; their surface pattern of microscopic ridges and grooves is highly organized and directional (Fig. 2). Water droplets, when placed on a peristome, rapidly spread along these grooves even against the force of gravity, but little or no spreading occurs perpendicularly across the first-order ridges^{14,32} (our own observations). This suggests that, in addition to the absence of wax crystals and presence of hygroscopic nectar, *Nepenthes* peristomes make use of micro-topography and capillary forces to facilitate complete wetting. As a result, the peristome is covered with a continuous thin water film under humid conditions.

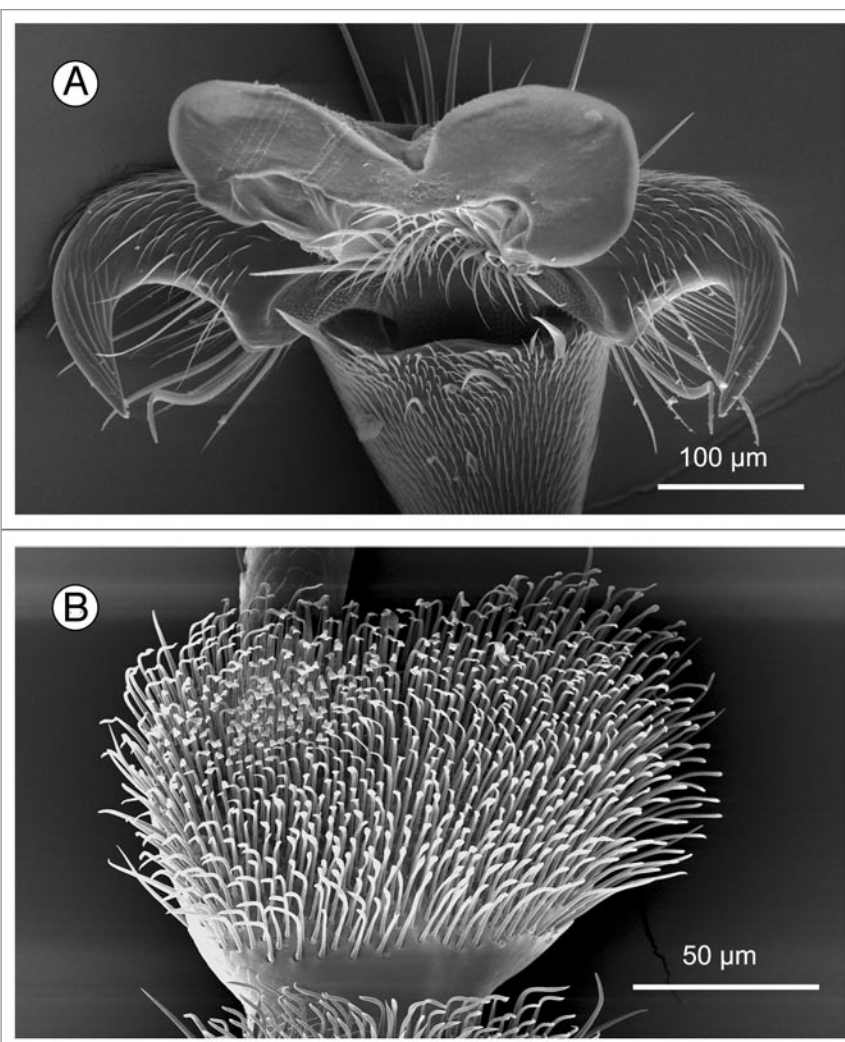


Figure 4. Two different designs of adhesive pads are found in insects. (A) Smooth pad of an ant (*Oecophylla smaragdina*). (B) Hairy pad of a beetle (*Gastrophysa viridula*).

Effect of Peristome Water Films on Insect Adhesion

It has been shown that water films on the peristome disable insect adhesive pads while the anisotropic surface topography prevents interlocking of claws when an insect slips into the pitcher.¹⁴ The exact mechanism of how the water films disrupt pad attachment is still unclear; however, some predictions can be made from what is known about insect adhesion. Insects cling to (dry) smooth surfaces by means of either smooth or hairy adhesive pads (Fig. 4). The contact between the pad and the surface is mediated by an adhesive fluid³³⁻³⁵ (Fig. 3B). Adhesive pads with a watery secretion fail when they are submerged in water, as experiments with frogs have shown.³⁶ This may be due to the loss of capillary bridges between the foot and the surface when the watery toe pad mucus fuses with the surrounding water. In insects, however, the main component of the adhesive fluid is a water-in-oil emulsion which is likely to be stable under water.³⁴

When an insect steps onto the wet peristome, its adhesive pads need to make contact with the surface in order to avoid slipping

Table 1. Running performance of ants and beetles on a wet *Nepenthes* sp. peristome.

Insect species	n	Captured	Not captured
Smooth adhesive pads:			
Carpenter ant (<i>Camponotus</i> sp.)	20	18	2
Hairy adhesive pads:			
Dock beetle (<i>Gastrophysa viridula</i>)	13	13	0
Harlequin ladybird (<i>Harmonia axyridis</i>)	16	16	0

or falling. This process can be split up into three phases: (1) penetrating the water surface, (2) bringing the pad close to the surface by squeezing out the subjacent water layer and (3) dewetting and establishing the contact. Phase (1) is dependent on the wettability of the insect foot which is determined by the interfacial tension between the water film on the peristome and the oily adhesive fluid covering the insect's adhesive pad. The interfacial tension of vegetable oil has been shown to be in the same order of magnitude against water and air.³⁷

$$\gamma_{AG} \approx \gamma_{AW} \quad (3)$$

Hence, the contact angle θ_{AWG} of a water droplet (W) on the surface of the adhesive pad (A) should be approximately 90°:

$$\cos \theta_{AWG} = (\gamma_{AG} - \gamma_{AW}) / \gamma_{WG} \approx 0 \quad (4)$$

Equation 4 suggests that a foot pad should easily penetrate the water surface, because attractive or repulsive forces scale with $\cos \theta_{AWG}$. However, this conclusion may be less reliable for insects with hairy adhesive pads where the general wetting properties are enhanced by topography so that small differences between γ_{AG} and γ_{AW} could lead to significant forces.

Phase (2) is strongly influenced by the load on the foot which depends on the insect's body mass, geometry and motion, and the slope of the peristome. The time to squeeze out the water film underneath the pad depends on its thickness and viscosity. Some *Nepenthes* species secrete copious amounts of concentrated nectar from their peristome nectaries.^{10,11} As sugar solutions have a higher viscosity than water, the nectar could inhibit drainage and thus assist insect aquaplaning.³⁸ On the other hand, the ridges on the peristome surface form channels that should enhance drainage.³⁹

Once the water film between the pad and the surface has become very thin ($\sim 0.1 \mu\text{m}$), complete removal of the water by dewetting becomes possible.^{40,41} To make direct contact, the adhesive fluid covering the foot needs to displace the thin layer of water. For simplification, we treat the adhesive pad as a piece of solid material with the same surface energy as the adhesive fluid. Spontaneous dewetting will only occur if the spreading coefficient S_{SWA} for water in between the pad and the surface is negative. From equation 2 it follows that

$$S_{SWA} = \gamma_{SA} - (\gamma_{SW} + \gamma_{WA}) = S_{SWG} - \gamma_{SG} + \gamma_{SA} + \gamma_{WG} - \gamma_{WA}$$

Since $\gamma_{WA} \approx \gamma_{AG}$ (equation 3) and $S_{SAG} = \gamma_{SG} - (\gamma_{AG} + \gamma_{AS})$

$$S_{SWA} \approx S_{SWG} - S_{SAG} - 2\gamma_{AG} + \gamma_{WG} \quad (5)$$

where S_{SAG} is the spreading coefficient of the adhesive fluid on a dry peristome surface. The wettability of the peristome for water appears to be higher than that for hydrophobic oils,²⁴ i.e., $S_{SWG} - S_{SAG} > 0$. Therefore, and since the surface tension of water is likely to be at least twice as high as that of the hydrophobic pad secretion ($\gamma_{WG} \approx > 2\gamma_{AG}$), S_{SWA} is likely to be positive. This suggests that no dewetting should occur between the insect pad and the peristome and that the lubricating water film should remain stable.

We tested this prediction and the relative importance of water drainage versus surface wettability by comparing the performance of ants (smooth adhesive pads) and beetles (hairy adhesive pads) on a wet peristome. If drainage was the limiting factor, beetles should adhere better since their hairy pads should drain more easily than smooth pads (diameter of individual hair contacts only ca. $5 \mu\text{m}^{42}$). We placed individual insects on the outer surface of a pitcher and observed one single visit of the wet peristome for each insect. Table 1 shows that both beetles and ants were captured efficiently, suggesting that not drainage but the difficulty of de-wetting is the main barrier for adhesion, consistent with the above prediction.

Conclusions and Outlook

The high wettability of the *Nepenthes* peristome is achieved by a combination of hydrophilicity, surface micro-topography and secretion of hygroscopic nectar. The stability of water films on the peristome is probably the key factor that prevents insect adhesion. To reach a quantitative understanding of the function of the peristome, more detailed data on insect adhesion and on the surface chemistry of the peristome are needed. The fabrication of high-resolution surface replicas⁴³ might make it possible to separate experimentally between the effects of surface chemistry and topography. Moreover, modern micro/nanofabrication techniques provide a tool to create artificial surfaces that mimic the topography of the peristome. Similar to biomimetic substrates inspired by the superhydrophobic surface of the Lotus leaf,²⁶ these "peristome mimics" could be used for a variety of applications, such as water-lubricated surfaces or anti-fogging coatings on mirrors, lenses, windows and screens that prevent droplet formation.⁴⁴

Acknowledgements

Our research is financially supported by an external research studentship of Trinity College Cambridge to U.B. and a research grant of The Leverhulme Trust to W.F.

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