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Plant surfaces with cuticular folds and their replicas: Influence of microstructuring and surface chemistry on the attachment of a leaf beetle

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ABSTRACT

Plant surfaces covered either with epicuticular wax crystals or cuticular folds have been shown to strongly reduce the ability of insects to attach to them. However, the relative impact of surface structuring vs. surface chemistry on insect attachment remains unclear. To understand the mechanisms reducing adhesion of insects on plant surfaces in more detail, we performed traction experiments (i) on plant surfaces covered with cuticular folds of different dimensions, and on their (ii) untreated and (iii) hydrophobized replicas. As a reference, measurements were performed on replicas of smooth plant surfaces and of glass. Traction forces were measured with a highly sensitive force transducer, using tethered male Colorado potato beetles (*Leptinotarsa decemlineata*) as a model insect species. Contact angle measurements with water and diiodomethane were also performed to examine the physicochemical properties of the test surfaces. We found that surface structuring has a strong influence on the magnitude of the attachment force. In contrast, under the chosen experimental conditions, surface chemistry had no significant influence. Our results indicate that attachment of the beetles is reduced solely by the dimensions of the folds, with cuticular folds of about 0.5 μm in both height and width being the most effective. Contrary to the attachment of beetles, the wettability of the surfaces was considerably influenced by both surface structuring and chemistry. These results contribute to a better understanding of plant–insect interactions and the function of microstructured surfaces, and may facilitate the development of biomimetic anti-adhesive surfaces.

1. Introduction

Plants have evolved multifaceted surfaces to cope with different demands. To understand the functions of plant surfaces of different characteristics, different parameters, such as surface structuring and chemistry, have to be taken into account. An overview and classification of the great variety of surface structure is provided by Barthlott and Ehler [1] and in excerpts in the review of Koch et al. [2]. Cuticular folds are a common (sub-)microstructuring found on plant surfaces of different organs, such as leaves and petals. They can originate from different modifications of the epidermis, such as folding of the cuticle or accumulation of pectin beneath the cuticle, or by the cell wall if it is of special shape [2].

Recently it was shown that plant surfaces covered with cuticular folds of different shape and spacing are able to reduce the attachment of the leaf beetle *Leptinotarsa decemlineata* by the same order of magnitude as epicuticular wax crystals [3]. It is widely accepted that wax crystals reduce the attachment of many insects strongly [4–13].

Several hypotheses explaining the mechanism of reducing attachment on certain plant surfaces have been proposed (specified in Refs. [7,9]). (i) Roughness: the real contact area between the insects adhesive pads and the surface is reduced by surface microstructuring [4,8,13–17]. (ii) Chemistry: due to their chemical constituents, plant epicuticular waxes have anti-adhesive properties [11,17,18], which might impair wetting by the insect's adhesive fluid and thereby cause slipperiness [19]. (iii) Contamination: plant epicuticular wax crystals are fragile and might inhibit attachment by contaminating the insect's adhesive pads [4,7,8]. (iv) Wax-dissolving: the adhesive fluid secreted by insect pads might dissolve plant waxes and cause a slippery surface [7,9]. (v) Fluid absorption: the insect's adhesive fluid is absorbed due to capillarity of the microstructures of the waxy plant surfaces [9,14].

The influence of surface roughness on insect adhesion has been analysed in several studies with different foci by performing

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experiments on technical surfaces. Friction experiments (centrifugal method) on replicas of polishing paper of different particle size were performed with insects [15,20,21]. The particle size with the strongest anti-adhesive properties of surfaces tested in the above studies approximately corresponds to the diameter and spacing of the medium cuticular folds investigated in Prüm et al. [3], suggesting a strong influence of the dimensions and spacing of the folds on the insect's attachment.

However, in plant surfaces, the individual impact of on the one hand surface sculpturing and on the other hand surface chemistry on the attachment of insects remain unclear.

Plant waxes are the final layer of the cuticle – the outermost layer of the plant body – and differ in both their chemical composition and structure [22]. Thus, to understand the functional principles of plant surfaces, the physicochemical properties should be considered in addition to the structuring. Gorb and Gorb [18] investigated the physicochemical properties of functional surfaces in the pitchers of a carnivorous plant and proposed a correlation between insect adhesion and the free surface energy, and thereby the wettability, of a surface.

The influence of both architecture and physicochemical properties of a surface on the attachment of the convergent lady beetle (*Hippodamia convergens*) was investigated by Eigenbrode and Jetter [11]. They concluded from friction experiments (using a centrifugal technique) on surfaces covered with wax crystals and with different natural waxes prepared as smooth surfaces that both shape and chemical composition have an influence on insect attachment. Similarly, Gorb and Gorb [17] performed centrifugal friction experiments with a leaf beetle (*Gastrophysa viridula*) on a leaf, on polishing paper and on normal and silanized (hence hydrophobic) glass. The results of this study indicate that both hydrophobicity and surface roughness decrease the attachment force of beetles, with the effect of surface roughness being fourfold that of surface chemistry.

In the present study, the surface structuring and chemistry of plant surfaces were considered separately by fabricating replicas of plant surfaces with cuticular folds. We selected plant surfaces with cuticular folds of three different shapes: medium cuticular folds, high cuticular folds and hierarchical surfaces with cuticular folds (according to Ref. [3]). Additionally, one smooth plant surface and plain glass slides were chosen as a reference. Traction experiments with tethered Colorado potato beetles (*L. decemlineata*; Coleoptera: Chrysomelidae) were performed on (i) untreated and (ii) hydrophobized epoxy resin replicas and (iii) the respective original surface. Subsequently, data were compared within the groups and between the untreated replicas of the different surface structures. Physicochemical properties were examined by static contact angle measurements with water and diiodomethane on all the surfaces investigated.

2. Materials and methods

2.1. Beetles

For traction experiments, *L. decemlineata*, with adhesive pads of the hairy type (setae), was used as a model insect species. This leaf beetle has been used as the model insect species in several studies dealing with traction experiments, and its attachment devices are well described [3,20,23–25]. Some of the beetles were collected from organic potato fields in the Kaiserstuhl area near Freiburg and others were obtained from the Julius Kühn Institut in Darmstadt. The insects were kept in a terrarium on their host plant, *Solanum tuberosum*, using a day–night regime of 16L:8D (Osram Lumilux Daylight 865 lamp, 58 W). For the experiments, only male beetles (11–14 mm in length, body mass 90 – 140 mg) were used (see also Refs. [3,26]).

2.2. Surfaces investigated

We focused on three plant surfaces investigated in Prüm et al. [3], showing surfaces with cuticular folds of different shape (medium cuticular folds: *Hevea brasiliensis*, adaxial leaf surface (ad); high cuticular folds: *H. brasiliensis*, abaxial leaf surface (ab); hierarchical surface with cuticular folds: *Litchi chinensis*, abaxial leaf surface) and on one plant species with a smooth leaf surface (*Magnolia grandiflora*, adaxial leaf surface). All plant specimens were collected in the Botanic Garden of the University of Freiburg. Plant samples were freshly picked at the day of experimentation and kept in a vase (twigs with leaves, *L. chinensis* and *H. brasiliensis*) or in a closed box (individual leaves, *M. grandiflora*) until the start of the individual experiment to avoid dehydration artefacts. As a reference surface we used glass slides (76 × 50 mm, Plano GmbH, Wetzlar, Germany), cleaned with ethanol and distilled water before use. In addition to the plant surfaces and glass, both their untreated replica and a replica covered with a hydrophobizing agent were analysed.

2.3. Preparation of replicas

Replicas of all surfaces described above were prepared largely following the two-step replication process described in detail by Koch et al. [27] and Schulte et al. [28]. Negative moulds were prepared from the silicone elastomer President light body (PLB; Coltène® Whaledent AG, Altstätten, Switzerland; using the automatic mixing device). PLB was stored in a freezer at −18 °C to extend the handling time. The plant surfaces were gently rinsed with water to remove dust and quickly dried with pressurized air. They were then cut into pieces of approximately 3 × 3 cm, covered with PLB (within 5 min after cutting to avoid artefacts by drying) and immediately pressed down upon gently with a Petri dish. After polymerization (approximately 15 min), the plant surface was carefully peeled off.

After a latency of at least 12 h, the positive mould was produced using epoxy resin (Epoxy Resin L & Hardener S, both Toolcraft, Conrad Electronic SE, Hirschau, Germany). The two components were mixed (mixing ratio of resin to hardener of 10:4), put on a shaker at 100 rpm for approximately 2 min and subsequently carefully applied to the negative mould to avoid bubbles. After a curing time of 24 h at room temperature, the replicas were peeled off the negative mould. For each surface type investigated, at least eight different pieces of leaf were replicated to allow independent results in traction experiments and contact angle measurements. Accordingly, the reference substrate glass was replicated from different glass slides (76 × 50 mm; Plano GmbH, Wetzlar, Germany) using the same moulding technique. Each replica was quality-checked for artefacts by means of bubbles at the surface of either the moulds or the replicas using a stereo microscope, and replicas identified as being deficient in relevant areas were discarded. To hydrophobize the replica, specimens were covered with an antispread (E2/200 FE 60, Dr. Tillwich GmbH, Werner Stehr, Horb (Ahldorf), Germany), a commercially available agent for surface hydrophobization which has been used in a number of studies [27,29,30]. The thickness of the applied film is specified to be approximately 10 nm (manufacturer's data).

2.4. Scanning electron microscopy

The morphology of the plant surfaces and their replicas was analysed using scanning electron microscopy (SEM). For SEM analysis of the plant surfaces, leaf samples were dehydrated in metha-