

Abstract

Insects play a crucial role in our lives. They act as pollinators and sources of nutrition for people and animals alike, thus forming a backbone of virtually all ecosystems on land. However, they can also transmit **diseases** and destroy farm crop yields, necessitating management and control of the behaviour of certain species. Current ways of dealing with insect **pests** mostly rely on **chemical insecticides**, affecting not only the target species with intended consequences, but often also further life forms with unintended consequences: In recent years, the use of chemical insecticides has been linked to global pollinator decline [1] and decline of populations of other non-target organisms such as birds [2]. In humans, the use of insecticides has been associated with elevated risks for developing cancer [3].

Physical mechanisms that merely repel the target species without interruptions to other organisms could provide a **non-toxic alternative** to chemical insecticides. Various plants produce insect repellents based on wax micro- and nanostructures that exhibit specific mechanical and structural properties, such as finely tuned fracture behavior, thereby preventing insect attachment [4]. As England and co-workers showed in 2016, **surface roughness** rather than surface chemistry essentially affects insect adhesion [5]. This exemplifies the fact that for certain functionalities in living Nature, **structure** (physics) is often more important than specific materials (chemistry).

This study aims to investigate the interaction between insects and wax structures found on a selection of plants common in Austria and whether the processes involved could be utilized to **develop non-toxic insect repellents**.

Insect Repelling Mechanisms

There are four effects that may explain the insect repelling nature of certain plant waxes. These effects are described by

- 1) The **Roughness-Hypothesis**
- 2) The **Fluid-Absorption-Hypothesis**
- 3) The **Wax-Dissolving-Hypothesis**
- 4) The **Contamination-Hypothesis**

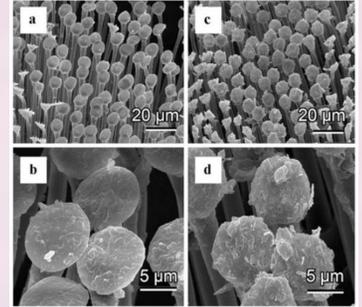


Fig. 4 SEM images of wax contamination on insect adhesion devices after walking on waxy plant surfaces adapted from [7].

Insect Attachment

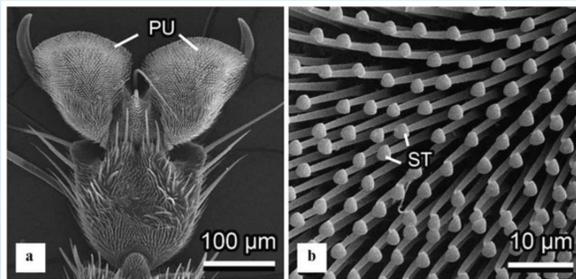


Fig. 1 SEM image of attachment devices on the feet of the hoverfly *Eristalis pertinax* adapted from [4].

Insects use two main mechanisms for adhering to surfaces: On rougher surfaces, they use **claws** on their feet to adhere to protrusions, while on smoother surfaces they utilize **adhesive pads** on their feet, which are specialised attachment devices maximising contact area. These pads can either be smooth and flexible, or covered in hairs called tenent setae. In addition, wet adhesion is employed by insects when adhering to surfaces using the pads by secretion of **adhesion fluids** from the pads.

Plant Wax

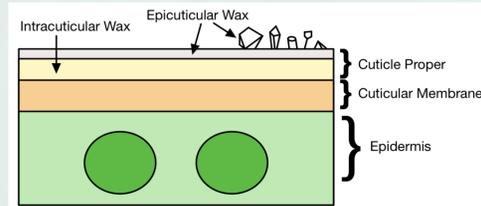


Fig. 2 Schematic drawing of plant cuticle with epicuticular wax layer.

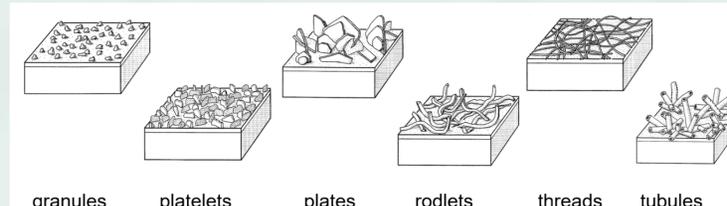


Fig. 3 Examples of different types of epicuticular wax crystals adapted from [6].

Cuticular plant wax comes in different shapes and sizes and fulfils multiple functions in plants. Besides hindering insect attachment, waxy layers inside and on top of the plant cuticle help protect plant cells from excessive **UV radiation** by scattering incoming light, and from drying out by constituting a **transpiration barrier**. In some plants, the structures these waxy layers make up help in keeping the plant clean from dirt via the **Lotus effect**.

In epicuticular waxes, a distinction can be made between thin films only a few nanometers in thickness, and wax structures some 0.5-10 μm thick that are superimposed onto these films. These structures are either continuous layers, crusts, or solitary crystals. **Epicuticular wax crystals** can be classified according to their **structure**, with Barthlott et al. counting 23 different types of crystals [6]. It is these crystals that help plants repel insects by obstructing their attachment in ways that exploit the weaknesses of insect attachment mechanisms.

Experiment



Fig. 5 Epicuticular wax of fruits of *Prunus domestica* dissolved in chloroform.

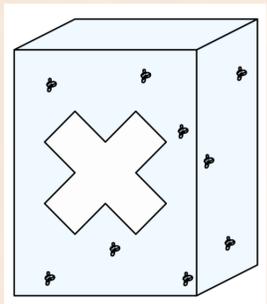


Fig. 6 Sketch of experimental setup.

Plant waxes are organic compounds, and therefore soluble in chloroform, which was used to extract wax from plant surfaces. Designated spots on the walls of a **terrarium** were then coated with the **dissolved wax**, where it was allowed to recrystallize in a dark space at ambient conditions for several weeks.

The **terrarium** with the **coated walls** will be used to **breed insects** in order to observe the interaction of the insects with the terrarium walls, and especially with the spots where wax was applied.

The quality of the waxy deposits on the artificial surface will be assessed after conclusion of the insect interaction experiment by comparison of **electron microscope images** of the artificially coated surfaces and the original plant surfaces.

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