Applied Physics A (2020) 126:549
https://doi.org/10.1007/s00339-020-03724-x

Applied Physics A
Materials Science & Processing

T.C. BIOLOGICAL AND BIOMIMETIC MATERIALS

Frictional properties of flower stems in the plant *Hippeastrum reginae* (Amaryllidaceae)

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Received: 19 May 2020 / Accepted: 12 June 2020

Abstract

Plant flower stems covered with three-dimensional (3D) wax prevent access of ants that rob flower nectar and do not contribute to the plant cross pollination. This phenomenon, called *greasy pole syndrome*, is caused by the ant's failure to gain a foothold on slippery waxy stems due to dislodged wax particles. The aim of this study was to analyze frictional properties of 3D wax projections specific to flower stems in *Hippeastrum reginae* by means of a microtribometer. Using the cryo scanning electron microscopy approach, we obtained images of shock-frozen plant surfaces with intact and smeared wax layers. Compared to wax-free stem samples, intact waxy stems showed significantly reduced frictional coefficient. The obtained results allowed us to provide some plausible explanations about the mechanism of the slipperiness of the 3D wax coverage on plant surfaces involved in the greasy pole syndrome. It is most probably based on easy delamination of the layered structure of single wax projections and on finally building of smearing surfaces with presumably lower intrinsic friction between individual layers. This knowledge can potentially contribute to the development of future physical control barriers based on dry lubricants with application in pest control.

Keywords Attachment · Delamination · Frictional coefficient · Greasy pole syndrome · Slippery surface · Wax projections

1 Introduction

Plant surface micro- and nanostructures play an important role in interactions between plants and a variety of environmental factors [1–3]. It is well known that plant surfaces can mediate insect attachment depending on the presence of trichomes, cuticular folds, three-dimensional (3D) epicuticular waxes or fluid coverage (see review by Gorb and Gorb [4]). Especially 3D epicuticular waxes have been repeatedly shown to reduce the attachment ability and impede locomotion of insects (reviewed in Ref. [5]) by making plant cuticles slippery. The protective function of 3D epicuticular waxes coverage against insects has been experimentally supported mainly for plant leaves in representatives of various plant families. However, waxy stems (especially flower stems) have their protective function mainly against ants robbing flower nectar and not contributing to the plant cross pollination. This phenomenon is called *greasy pole syndrome* (GPS) [6–10]. Based on field observations and experiments, it has been previously demonstrated that ants failed to gain a foothold on waxy stems due to dislodged wax crystals and, therefore, fell to the ground.

The effect of wax coverage on stems was explained by the disability of ants to adhere to a wax bloom [8, 11]. Generalist ants do not climb wax-covered stems; this is not necessarily due to their complete incapability of walking on slippery vertical surfaces, but rather due to the fact that additional locomotory efforts are needed to master climbing on slippery stems. Even if ants walk on these surfaces, their locomotion on stems remains cautious and slowed down, and presumably is rather costly from the energetic point of view [9], and that is why under natural conditions they usually select plant stems that have no GPS [10].

Since stems with the GPS are mostly oriented vertically, also frictional forces strongly contribute to the grip and locomotion of ants. Frictional and shear forces under specific geometry of wax projections lead to the breakage of single wax projections, which leads to the slipperiness of the GPS

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surfaces [12]. Indeed, in the previous works we have shown that 3D wax projections on stems have rather specific morphology and may differ from the microstructure of other plant organs even within the same plant species [9, 10].

In the present paper, we examine the frictional properties of 3D wax projections specific to flower stems. As a model system, we selected *Hippeastrum reginae*, the plant which has rather large flower stems (actually stems of inflorescences) that can be easily used in a microtribological experiment. Using experimental measurements by means of a microtribometer, we evaluated frictional coefficients of intact flower stems covered by 3D wax projections and stems with removed 3D wax layers. Furthermore, we applied the cryo scanning electron microscopy (SEM) approach to obtain images of shock-frozen plant surfaces with intact and smeared wax layers. The obtained results allowed us to provide some plausible explanations about the mechanism of the slipperiness of 3D wax coverage on plant surfaces with the GPS and understand the functional role of specific morphology of wax projections involved in GPS.

2 Materials and methods

2.1 Plant species

The royal Knights-star-lily *H. reginae* (L.) Herb. (Amaryllidaceae) is a flowering perennial herbaceous bulbous plant native to Venezuela, Bolivia, Peru and Brazil [13]. The bulbs produce evergreen sessile linear leaves and erected hollow flower stems terminated by umbelliform inflorescences composed of funnel-shaped flowers (Fig. 1a). Many modern commercial cultivars, which are valued for their large ornamental flowers and are widely grown indoor in pots or outside in warm climates, derived from this species [14]. For this study, we used the cultivar “Apple Blossom”.

2.2 Microscopy

Surfaces of plant flower stems and upper (adaxial) side of the leaf were examined using cryo scanning electron microscopy (SEM). Small samples (1 cm × 1 cm) from the middle regions of the plant organs were cut out from living plants, immediately attached mechanically to a small vice on a metal holder and frozen in a cryo stage preparation chamber at − 140 °C (Gatan ALTO 2500 cryo preparation system, Gatan Inc., Abingdon, UK). Frozen samples were sputter-coated with gold–palladium (thickness 6 nm) and studied in the frozen condition in a cryo-SEM Hitachi S-4800 (Hitachi High-Technologies Corporation, Tokyo, Japan) at 3 kV accelerating voltage and − 120 °C temperature. Types of wax projections were identified according to the classification of plant epicuticular waxes proposed by Barthlott et al. [15]. Wax projections were measured from digital images using SigmaScan Pro 5 software (SPSS Inc., Chicago, USA). These data are presented in the text as mean ± SD for $n = 10$.

2.3 Experiment

Frictional measurements were carried out with the microtribometer Basalt-MUST (TETRA GmbH, Ilmenau, Germany). Two-dimensional force detection was accomplished by a metal cantilever (CFn: 22.3 N/m, CFt: 23.1 N/m). The spatial resolution of the system in detection a deflection of the cantilever was 50 nm. The averaged applied normal force was 0.28 mN. The measurement was performed over a sliding distance of 2 mm (in one direction, not reciprocal) at a velocity of 0.50 mm/s in the longitudinal direction of the stem. However, we tested both apical and basal directions. The transversal direction was not tested, as it is not biologically relevant for the GPS. As a contact partner, a glass ball with a diameter of 3 mm was chosen and fixed to the cantilever by cyanoacrylate glue (Ergo 5925 Elastomer, Tagelswangen, Switzerland). The roughness of the glass ball determined by a white light interferometer (NewView, ZygoLOT GmbH, Darmstadt, Germany) was $R_a = 0.006$ µm. The plant surfaces were fixed on metallic sample holders by a double-sided adhesive tape. Since the thickness of the plant samples ranged from 3 to 5 mm and indentation depth was lower than 30 µm, the properties of the tape did not influence the results.

We selected three individual flower stems, which were all tested in a longitudinal direction (along the stem). First, the intact sample (1 cm × 1 cm) of the plant surface was cut off the stem, attached to the tape and the first set of experiments was conducted (10 sliding cycles). Then the sample surface was carefully wiped off using laboratory paper towel, to remove 3D wax projections. During this procedure, the sample turned its appearance from matt to shiny. The second set of experiments was performed on the wax-free sample (10 sliding cycles). From the obtained force-distance curves, the frictional coefficient was calculated and compared for these two different types of samples. To determine whether there is a significant difference between frictional coefficients obtained on different sample types, data were tested using one-way ANOVA (software SigmaPlot 11.0, SPSS Inc., Chicago, USA).

3 Results

3.1 Micromorphology of plant surfaces

Although both studied plant surfaces (flower stem and adaxial leaf side) bear epicuticular wax coverage composed of microscopic 3D wax projections, the micromorphology

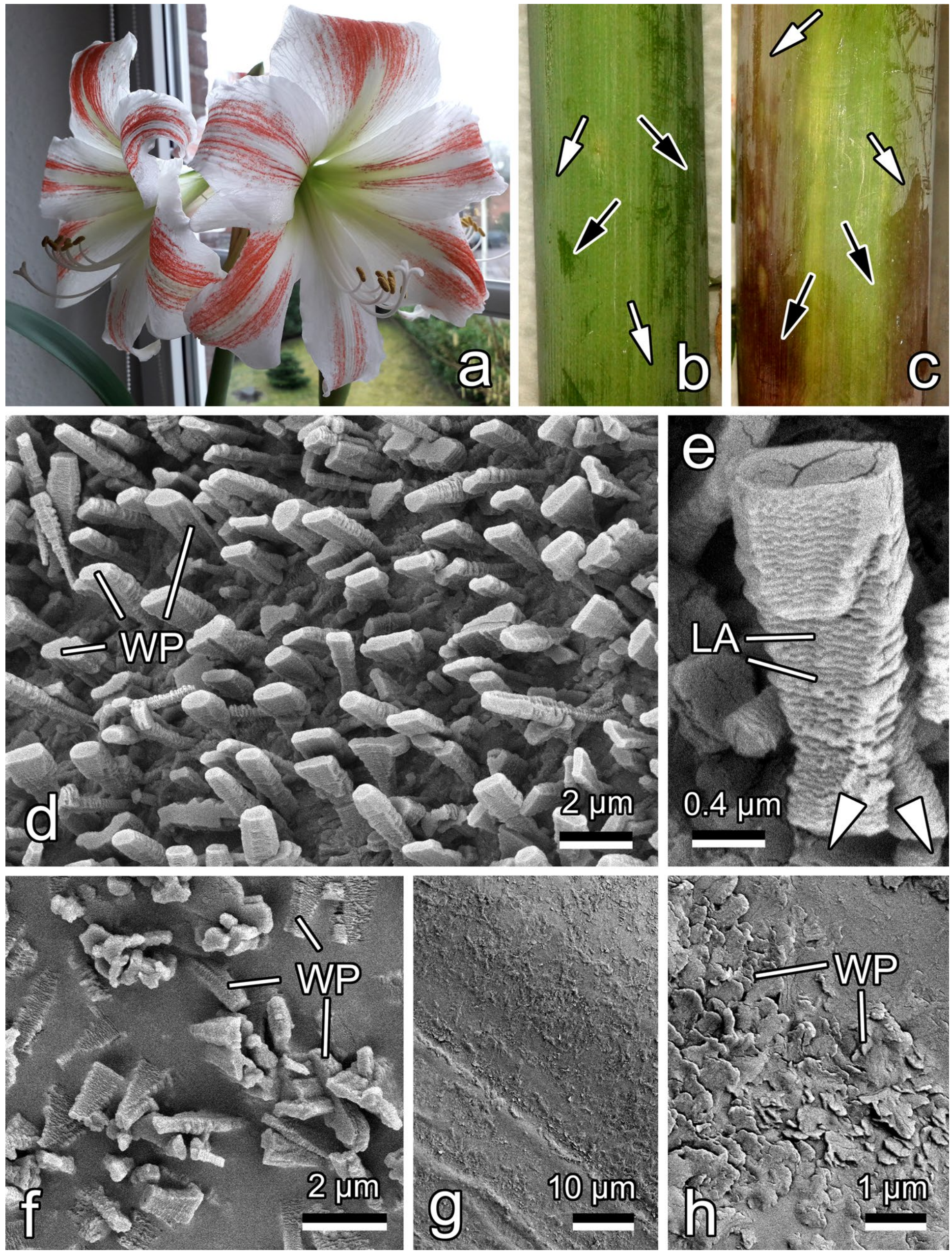

Fig. 1 **a** *Hippeastrum reginae* flowers with the leaf and flower stems. **b**, **c** Two individual flower stems with 3D wax coverage (matt appearing sites, white arrows) and with some damages in the 3D wax coverage (glossy appearing sites, black arrows). **d**, **e** 3D wax projections of intact coverage of the flower stem and an individual wax projection with clearly seen layered structure and characteristic damage failure (arrowheads) due to the delamination between layers (**e**). **f** 3D wax projections peeled off by Scotch™ tape. **g**, **h** 3D wax projections of the upper (adaxial) surface of the leaf. LA, layers constituting wax projections; WP, wax projections

of the wax structure differs between the surfaces. On the leaf having rather shiny appearance, flat, irregularly-shaped, thin platelet-like projections (length: 0.59 ± 0.09 µm; width: 0.34 ± 0.09 µm; thickness: 0.02 ± 0.00 µm) almost completely (density: 6.5 µm^{-2}) cover the surface like appressed scales (Fig. 1g, h). In the pruinose flower stem, the cuticle is overlayed by regularly distributed (density: 0.6 µm^{-2}), separate, transversely ridged rodlets protruding perpendicular from the surface (Fig. 1d). These projections are of variable length (2.02 ± 0.84 µm) and have different, but rather regular, shapes and dimensions (large variable: 0.68 ± 0.14 µm; small variable: 0.32 ± 0.06 µm) of a cross section. Perpendicular to their longitudinal axis, the rodlets show typical alternating furrows and ridges clearly indicating the layered structure (layer thickness: 28.28 ± 3.04 nm) of these projections (Fig. 1e). Delamination between layers causes characteristic damage and exfoliation of the rodlets (Fig. 1e).

3.2 Frictional properties

On the intact (3D wax covered) stems, in spite of strong fluctuation of the force within one sliding cycle, frictional forces were rather low in comparison to the samples with removed wax (Fig. 2a). Strong fluctuations within individual sliding cycles can be explained by the presence of some course roughness caused by the shape of underlying epidermal cells.

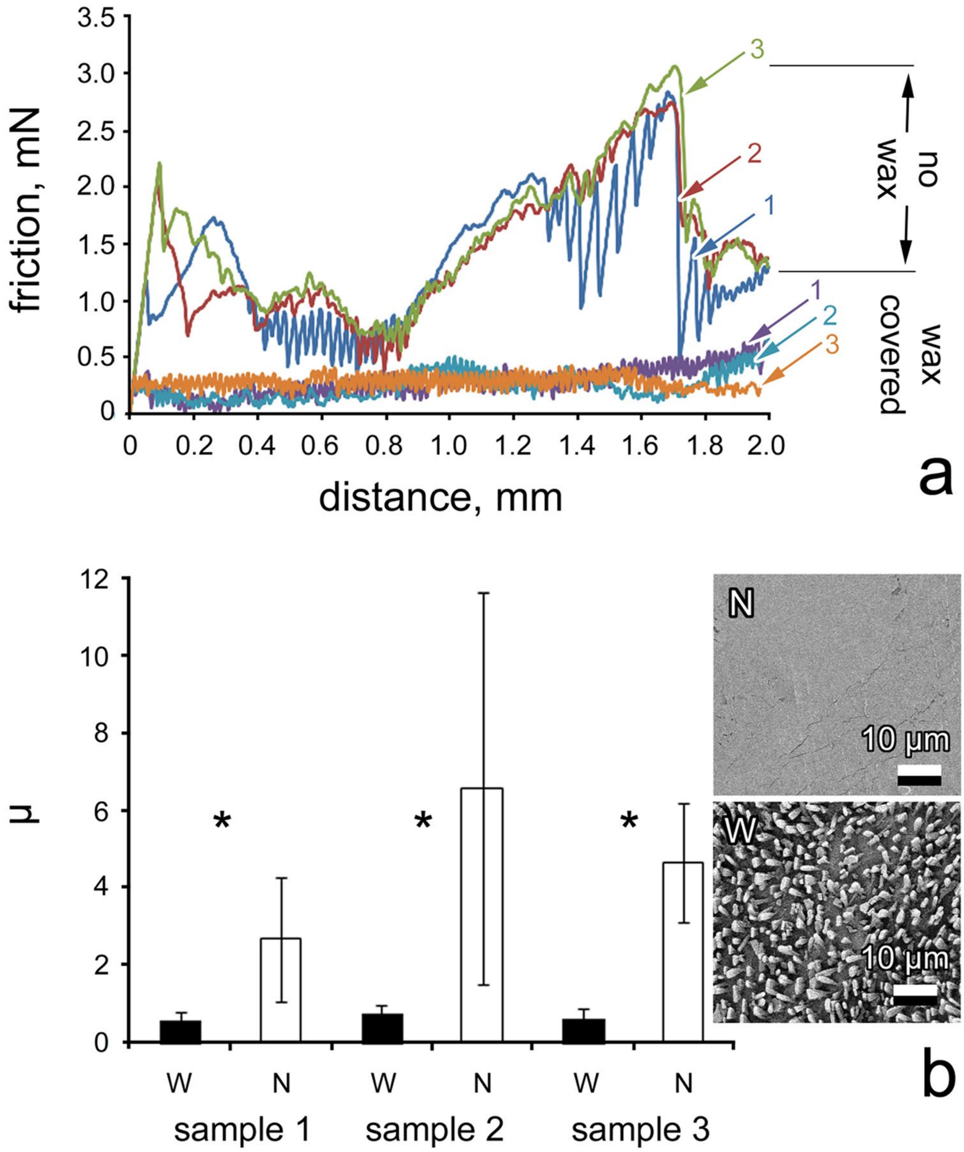

Fig. 2 Frictional properties of flower stems in *Hippeastrum reginae.* **a** Force-distance diagrams of three first subsequent sliding cycles (same direction) on the intact stem covered by 3D wax projections (wax covered) and of those on the stem with wiped off wax projections (no wax). **b** Frictional coefficients (μ) obtained for 10 sliding cycles on three individual intact flower stems (*W*) and on the same stems with removed wax coverage (*N*). Asterisks indicate statistically significant differences between the two different conditions of each sample ($p < 0.001$, one-way ANOVA). Insets show an intact sample (*W*) and sample with removed wax coverage (*N*)

Additionally, in the wax-free sample, strong stick-and-slip behavior was detected. This is due to the presence of rather a large contact area caused by the smooth surface of epidermal cells and by the soft cellular nature of underlying fresh plant material. On the intact stems, both frictional force and stick-and-slip were strongly reduced. Results of the measurements obtained on three individual stems clearly show a statistically significant difference between plant surfaces with wax ($\mu = 0.1$–0.3) and without wax ($\mu = 2.0$–9.0) (Fig. 2b) (stem 1: $F = 50.4$; stem 2: $F = 200.6$; stem 3: $F = 39.4$, $p < 0.001$ for all, one-way ANOVA).

The present study shows that flower stem surfaces covered by wax projections can strongly reduce friction. The cryo-SEM inspection of intact plant surfaces after frictional experiments (Fig. 3) shows that the mechanism of slipperiness is based on easy delamination of the layered structure of single wax projections (Fig. 3c) and on finally building of smearing surfaces with presumably lower intrinsic friction between individual layers (Fig. 3a, b, d, e).

4 Discussion

In *Hyppeastrum*, one mechanism that limits self-pollination is self-incompatibility, when seeds are only produced by pollination from other plants. Furthermore, the plant generally releases its pollen about two days before its stigma is receptive, making cross-pollination more likely [16]. Pollinators include humming birds and moths. All these facts indicate that ants with their nectar-robbing activity and low ability to cross-pollination are not welcomed by this plant. Also our cryo-SEM results showing strong differences in

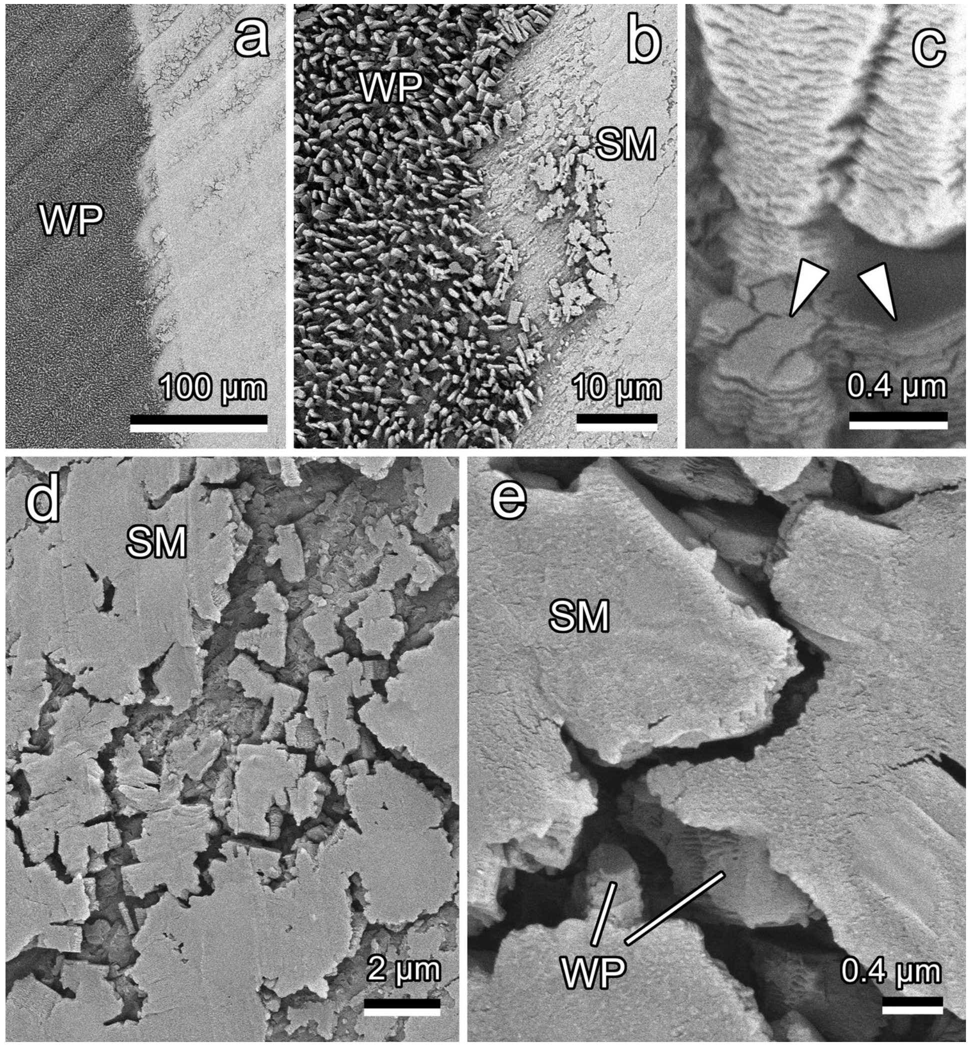

Fig. 3 *Hippeastrum reginae* with damages of 3D wax coverage on flower stems. **a**, **b** A smeared track in the 3D wax coverage (right side of the image). **c** Fractured single wax projection due to the delamination between layers (arrowheads). **d**, **e** Smeared track in the 3D wax coverage at high magnifications. SM, wax projections smeared into a wax layer; WP, individual wax projections

the structure of 3D wax coverage between leaves and flower stems of *H. reginae* indicate strong specialization of stem wax projections.

It has previously been found that ants could readily walk on the wiped (with removed epicuticular wax coverages), but not intact, stems of the plants *Anethum graveolens* L. (Apiaceae), *Dahlia pinnata* Cav. (Asteraceae), and *Tagetes patula* L. (Asteraceae) [9]. Flower stems of *Tulipa gesneriana* with prominent microstructured wax were avoided by ants during their foraging behavior [10]. According to a great number of previous experimental studies with many insect and plant species (reviewed in Ref. [5]), 3D epicuticular wax coverage usually reduces insect attachment using different mechanisms, such as (1) reduction of real contact area, (2) contamination of insect adhesive organs, (3) adsorption of fluid from insect pads, (4) hydroplaning, and (5) formation of separation layer [17, 18]. The present microtribological experiment shows for the first time that surfaces covered by flake-like wax nanoparticles of flower stems can drastically reduce friction and act as a kind of dry lubricant, which mechanism of slipperiness is based on easy delamination of layers and lower intrinsic friction between individual layers. Such dry (or solid) lubricants in engineering are powder-like materials that reduce friction between two surfaces without the need for a liquid medium. The two most known dry lubricants are graphite and molybdenum disulfide, which low-frictional characteristics are attributed to a layered structure at the nanostructural or molecular level with weak bonding between layers. Such layers are able to easily slide relative to each other with minimal applied force, thus giving them their low frictional properties [19].

Recent study by Salerno et al. [20] investigated the effect of surfaces covered by kaolin nanoparticles on the reduction of attachment ability in two economically important polyphagous insect pests characterized by different types of attachment devices: the Southern green stink bug *Nezara viridula* and the Mediterranean fruit fly *Ceratitis capitata*. The insect traction was heavily affected by kaolin particle film in both tested species. Further SEM analysis of the tarsal attachment devices in these insects after walking on kaolin-contaminated surfaces demonstrated strong contamination by the kaolin nanoflakes. Kaolin particle films consist of flat flake-like nanoparticles that can easily erode and contaminate insect tarsal attachment devices, which in turn can interfere with its adhesion to the surface. Such a mechanism of action of kaolin particle film in reducing insect adhesion is similar to that described in the contamination-hypothesis proposed previously to explain the effect of epicuticular plant waxes on reducing insect attachment [17]. However, the data from the present study show that strongly flattened structure of kaolin particles also might enable their action as a dry lubricant in addition to the contaminating effect. This knowledge can potentially contribute to develop future physical control barriers based on dry lubricants against pest insects, particularly relevant owing to the need to reduce the negative impacts of pesticides on the environment and human health.

5 Conclusions

Microtribological experiments with *H. reginae* showed that flower stem surfaces covered by 3D waxes strongly reduce friction compared to wax-free stems. The cryo-SEM examination of intact plant surfaces after the experiments revealed delamination of the layered structure of single wax projections (transversely ridged rodlets). Based on the obtained results, we suggest that the mechanism of slipperiness is most likely based on easy damage and exfoliation of the rodlets. This resulted in finally building of a flake-like smearing stratum presumably acting as a dry lubricant.

Acknowledgements Nils Heide (Kiel University, Germany) helped with frictional measurements.

Author contributions EVG: investigation; data curation; formal analysis; writing—original draft; writing—review and editing. SNG: conceptualization; methodology; resources; supervision; validation; visualization; writing—original draft; writing—review and editing.

Funding Open access funding enabled and organized by Projekt DEAL.

Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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