Arthropod-Plant Interactions (2025) 19:91
https://doi.org/10.1007/s11829-025-10190-w

Diamondback moth egg adhesion to cabbage plants: structural, chemical, and mechanical aspects

Dagmar Voigt[1,4] · **Christian Ulrich Baden**[2] · **Anne Jantschke**[3]

Received: 20 July 2025 / Accepted: 17 September 2025 / Published online: 24 October 2025

Abstract

The diamondback moth, *Plutella xylostella*, has developed strategies to overcome the challenging waxy surfaces of plants. Females can lay their eggs on pruinose Brassicaceae plants using a secretion from their colleterial glands, which acts as an egg adhesive. The present microscopic analyses, along with contact angle and force measurements, show that this secretion wets hydrophilic glass surfaces significantly better than hydrophobic ones, forming superthin layers with limited volume. Consequently, the pull-off forces required to remove the eggs are significantly greater on hydrophilic glass (23 mN) compared to hydrophobic glass (2 mN), indicating adhesive strengths of 198 and 29 kPa, respectively. The safety factors, which indicate how many times the weight of the egg (23 µg) corresponds to the pull-off force, are remarkably high: 101,689 for hydrophilic surfaces and 8517 for hydrophobic ones. Egg adhesion to plants varies depending on plant surface structures. Pull-off forces significantly decrease with the increasing number of plant epicuticular wax crystals. For example, safety factors measure 1795 on young adaxial white cabbage leaves and reach as high as 25,461 on the petioles of older Chinese cabbage leaves. This attachment ability is facilitated by the predominantly protein and lipid composition of the egg adhesive, alongside the structural matrices created by plant wax crystals and trichomes embedded within the adhesive. Raman spectroscopy of the untreated solidified egg adhesive reveals characteristic amide I and III bands, a β-sheet structural motif, and the presence of aromatic amino acids phenylalanine and tyrosine, as well as saturated fatty acids. Based on a comprehensive discussion with previous findings, we propose that there is a trade-off between secure egg adhesion and the selection of oviposition sites that match the offspring's preferences and provide enemy-free spaces. Understanding *P. xylostella*'s egg adhesion mechanisms and the characteristics of the adhesive substance may contribute to the improvement of pest control strategies, including physical measures, and the advancement of bioinspired adhesives. Moreover, our study should stimulate future integrative and multidisciplinary research on insect egg adhesives, promoting a comprehensive understanding from various perspectives.

Keywords Contact angle · Egg secretion · Epicuticular plant wax · *Plutella xylostella* · Pull-off test · Raman spectroscopy

Communicated by Heikki Matti Tapio Hokkanen.

✉ Dagmar Voigt
dagmarvoigt@web.de

✉ Anne Jantschke
jantschke@uni-mainz.de

Christian Ulrich Baden
christian.baden@bayer.com

[1] Botany, Faculty of Biology, Technische Universität Dresden, 01062 Dresden, Germany

[2] Bayer AG, Crop Science Division, 40789 Monheim, Germany

[3] AG Biomineralization/Crystallography, Institute of Geosciences, Johannes Gutenberg-Universität Mainz, J.-J.-Becher-Weg 21, 55128 Mainz, Germany

[4] Present Address: 01217 Dresden, Germany

Introduction

Insects face several challenges concerning attachment to the plant surface, including being dislodged, effectively moving on the surface or attaching eggs. One of the main challenges is the presence of epicuticular waxes, which can trigger antibiotic and antixenotic responses in plant-dwelling arthropods (Smith 2005). As reported, the wax crystal coverage on plant surfaces is determined mainly by hydrocarbon composition and is generally anti-adhesive, minimally wettable, and can prevent the tarsal and egg attachment of various arthropod species (e.g., Stork 1980a, b; Jeffree 1986; Juniper 1995; Eigenbrode et al. 1996; Brennan and Weinbaum 2001; Gorb and Gorb 2002, 2006; Müller and Rosenberger 2006; Rahman et al. 2023). The effects of epicuticular plant waxes

can stem from their chemical composition and/or crystal structure. Despite these challenges, some insects have developed the ability to glue their eggs onto these waxy surfaces. For instance, butterflies, such as *Pieris brassicae* L. (Lepidoptera, Pieridae) and aphids like *Brevicoryne brassicae* L. (Hemiptera, Aphididae), attach their eggs to the waxy leaves of Brassicaceae species (Beament and Lal 1957; Ahman 1990). Hessian flies, *Mayetiola destructor* Say (Diptera, Cecidomyiidae), also glue their eggs to wheat leaves *Triticum aestivum* L. (Poaceae) (Kanno and Harris 2000), while leaf beetles *Chrysophtharta* sp. (Coleoptera, Chrysomelidae) and *Opodiphthera* sp. moths (Lepidoptera, Saturniidae) do so on *Eucalyptus* leaves (Myrtaceae) (Ramsden and Elek 1998; Howlett and Clarke 2003; Li et al. 2008). Additionally, lily leaf beetles *Lilioceris merdigera* L. (Coleoptera, Chrysomelidae) lay eggs on the leaves of *Polygonatum multiflorum* (L.) All. (Ruscaceae) (Schmitt 1988). Research has shown that the bond between waxy plant surfaces and insect eggs can be very strong. For example, studies indicated that the maximum adhesion strength can reach up to 271 kPa for asparagus beetles *Crioceris asparagi* L. (Coleoptera, Chrysomelidae) on asparagus (*Asparagus officinalis* L., Asparagaceae) (Voigt and Gorb 2010), and up to 98 kPa for codling moths *Cydia pomonella* L. (Lepidoptera, Tortricidae) on apple fruits (Al Bitar et al. 2012, 2014). Butterflies like *Lycaeides idas* (Masters) demonstrate similar bonding strength on *Lotus nevadensis* (S. Wats.) Greene (Fabaceae; 94 kPa; Fordyce and Nice 2003). These adhesive bonds are believed to withstand significant weathering effects, such as rainfall, wind, and plant vibration (Müller and Rosenberger 2006; Voigt and Gorb 2010). The adhesive strength results from special fluids, further called "egg adhesive" in this manuscript, that covertly surround insect eggs and bond them to the substrate (e.g., Adiyodi and Adiyodi 1976; Hinton 1981; Hilker et al. 2005). These fluids are secreted by specific abdominal glands associated with the female reproductive system (reviewed by Betz 2010) and are primarily proteinaceous (Beament and Lal 1957; Hilker et al. 2005; Li et al. 2008). The strength of the bond between the eggs and the plant can vary greatly depending on the physico-chemical properties of the plant (e.g., Müller and Rosenberger 2006; Hilker and Meiners 2006).

Cabbage plants, in particular, are known for their very low wettability, with water contact angles reaching 148° on *B. oleracea* var. *albiflora* Kuntze (Wang et al. 2023), and between 137 and 151° on other varieties (Holloway 1969a,b). This reduced wettability is due in part to the thick anti-adhesive coverage of epicuticular wax crystals, which consists of two hierarchically arranged layers (Barthlott et al. 1998). The amount of wax can vary by cultivar, ranging from 35 to 50 µg cm^{-2} (Kimura et al. 1987; Znidarcic et al. 2008). The chemical composition of these crystalline surface waxes primarily includes alkanes (C_{20}–C_{33}), alkyl esters (C_{38}–C_{50}), aldehydes (mainly C_{28}–C_{30}), ketones (C_{27}–C_{29}), secondary alcohols, ketols, primary alcohols (C_{22}–C_{30}), fatty acids (C_{14}–C_{30}), and minor triterpenols (α and β-amyrin) (Purdy and Truter 1963; Holloway 1969a; Holloway and Brown 1977; Znidarcic et al. 2008).

Despite the challenges mentioned above, diamondback moths, *Plutella xylostella* L. (Lepidoptera, Plutellidae), complete their life cycle on cabbage plants. Moths are attracted by Brassicaceae host plants through chemical (olfactory and gustatory) and/or physical (tactile and visual) stimuli (Justus and Mitchell 1996; Shelton and Nault 2004; Bukovinsky et al. 2005; Sarfaz et al. 2006; Badenes-Pérez et al. 2014). The female's oviposition behaviour significantly affects the survival of these lepidopterans, as their immature stages have limited mobility (Renwick 1989; Silva and Furlong 2012). A diamondback moth can lay up to 350 eggs, either singly or in groups of 2–16, on various surfaces such as the abaxial and adaxial sides of leaves, as well as on stems and petioles (Hardy 1938; Harcourt 1957; Gupta and Thorsteinson 1960; Rosario and Cruz 1986; Tabashnik and Mau 1986; Talekar and Shelton 1993; Talekar et al. 1994; Furlong et al. 2004; Sarfaz et al. 2005; Zago et al. 2010; Silva and Furlong 2012; Philips et al. 2014; Ang et al. 2016; Rahman 2019; Bhure et al. 2020; Gopika et al. 2021; Saran and Genç 2021; Badenes-Pérez and Heckel 2023).

The secretion that adheres *P. xylostella* eggs to the substrate has been suggested to be a mucopolysaccharide (Justus and Mitchell 1999). Some researchers have argued that waxy plant substrates hinder egg deposition by *P. xylostella*, as surfaces with less wax allow for better egg adhesion (Eckenrode et al. 1986; Uematsu and Sakanoshita 1989; Justus et al. 2000; Zhu et al. 2022a, b). Consequently, *P. xylostella* eggs are easily washed off by rain and sprinkling from more waxy surfaces (Talekar 1986; Annamalai et al. 1988; Wakisaka et al. 1990; Kobori and Amano 2003; Rahman et al. 2019, 2023). Recent studies conducted by Rahman (2019) and Rahman et al. (2019, 2023) have shown that eggs laid on common cabbage plants, *Brassica oleracea* var. *capitata* L., were more vulnerable to rainfall compared to those laid on Chinese cabbage plants, *Brassica rapa* subsp. *pekinensis* (Lour.) Hanelt. Moths tend to lay more eggs in clusters on common cabbage leaves, while they prefer to lay single eggs on Chinese cabbage leaves. It was observed that eggs placed on the second leaf from the apex were less susceptible to rainfall than those laid on the sixth leaf from the apex in *B. oleracea* var. *capitata*. Also, diamondback moths preferred ovipositing on the lower leaves rather than the upper leaves (Rahman et al. 2023). However, rain droplets contacting the leaves caused vibrations that resulted in egg detachment from both adaxial and abaxial leaf surfaces, with a higher removal rate noted from the adaxial leaves compared to the abaxial ones.

These findings raise questions about the strength of *P. xylostella* eggs' adhesion to various plant and control substrates, as well as the role of egg adhesive in this process. Stimulated by the work of Rahman (2019) and Rahman et al. (2019, 2023), we analysed the interaction between eggs, glass, rape and cabbage plant surfaces using digital and cryo-scanning electron microscopy. We examined the wettability of glass and plant surfaces using Aqua Millipore water, along with the surface roughness and wax crystal coverage of the plants. The composition of the untreated egg adhesive was analysed in situ using staining, energy dispersive spectroscopy, and Raman spectroscopy. Furthermore, we measured the forces required to remove *P. xylostella* eggs from both glass and plant surfaces to evaluate the bonding strength of eggs in relation to the substrates.

We wondered if *P. xylostella* egg adhesion differs between (1) differently wettable surfaces, (2) Brassicaceae plant species with varying wax coverage, (3) leaf age, and (4) leaf side. How far can the egg adhesive component analyses help in understanding egg adhesion and egg-substrate interactions? The hypothesis was that the adhesion of diamondback moth eggs correlates with the host plant surfaces and habitats. We aimed to set the present data into a broader context with previous reports on insect egg biology, and particularly, diamondback moth oviposition behaviour and egg dispersal, to explain the impact of rain and sprinkling on diamondback moth eggs. Washing off herbivorous eggs from crop plants can be a promising physical tool in pest control. Moreover, our study should expand the currently limited knowledge about the chemistry and mechanics of insect egg adhesion in relation to functional morphology, ecology, and evolution from a comprehensive perspective.

Materials and methods

All preparations, analyses, and experiments were conducted at a temperature of 22.0 ± 0.29 °C, with a relative humidity of 58.4 ± 3.32%, and a photoperiod of 12 h.

Plants

Cabbage plants *Brassica capitata* var. *alba* 'Brunswijker', *Brassica rapa* subsp. *pekinensis* 'Michihili', and *Brassica napus* 'Licapo' (Kiepenkerl, Bruno Nebelung GmbH, Everswinkel, Germany) were sown and grown in culture substrate COMPO SANA® Qualitäts-Blumenerde ca. 50% weniger Gewicht (COMPO GmbH, Münster, Germany). For the experiments, single plants were placed in Göttinger plant pots with a diameter of 10.0 cm (Lamprecht-Verpackungen GmbH, Göttingen, Germany). The second and fourth fully developed leaves from the apex (adaxial and abaxial) of *B. rapa* subsp. *pekinensis*, as well as the corresponding petioles and stems of *B. capitata* var. *alba* and *B. napus*, were used (Fig. S1).

Glass substrates

Soda-lime glass microscope slides (76 × 26 mm; Gerhard Menzel B.V. & Co. KG, Braunschweig, Germany) were prepared for use by following a thorough cleaning process. First, they were washed with detergent and deionised water. Next, the glass items were immersed in Piranha solution, which consisted of a mixture of sulphuric acid H_2SO_4 and hydrogen peroxide H_2O_2 in a 3:1 ratio, for 12 h. Then, they were rinsed multiple times with Aqua Millipore water and dried immediately with compressed air. This procedure resulted in a hydrophilic glass with a 49° water contact angle (Table 1). To obtain a hydrophobic glass surface with a 110° water contact angle (Table 1), the glass was silanized with 1H,1H,2H,2H-perfluorodecyltrichlorosilane (97%, $C_{10}H_4Cl_3F_{17}Si$, SIH5841.0, ABCR GmbH & Co. KG, Karlsruhe, Germany) as described by Voigt and Gorb (2010).

Insects

A long-term, laboratory-reared, nonresistant diamondback moth population, established in 1957 at Bayer AG, Crop Science Division (Monheim, Germany), was considered. In the *P. xylostella* rearing, we used cylindrical flight cages made from transparent plastic bags framed to allow dim lighting at 23 °C and 50% humidity. Each cage had a diameter of 24 cm and a height of 26 cm, housing 300 to 500 adults that were fed a sugar solution. To encourage egg deposition, we placed black plastic pots, previously dipped in cabbage soup, inside the flight cages. For the larvae, we used the same cage system but provided different species of cabbage, primarily kale, as their food source. During pupation, the larvae utilised a piece of kitchen paper towel or very smooth, thin cloth to cover the tops of the flight cages.

The eggs were stored at 4 °C until use. Subsequent three to five generations were reared on commercially obtained Chinese cabbage leaves of unknown cultivars. Ovipositing females were transferred to potted test plants or glass slides to release their eggs. The glass substrates were placed in 94 and 145 mm diameter polystyrene Petri dishes (15 and 20 mm high, respectively; Greiner Bio-One International GmbH, Frickenhausen, Germany. Short-term (one-generation) moth rearing and individual test plants were kept in separate aerariums (30 × 30 × 30 cm; Aerarium Nets GmbH, Bern, Switzerland).

Table 1 Aqua Millipore water sessile contact angle on glass, Chinese cabbage *Brassica rapa* subsp. *pekinensis*, rape *B. napus*, and white cabbage *B. capitata* var. *alba* (droplet volume 10 µl)

Substrate	Substrate type/age	Substrate site	Contact angle [°]
Glass slides	Hydrophobic		48.5 ± 6.81^{bcd}
	Hydrophilic		109.8 ± 2.18^{bcd}
Chinese cabbage	Old	Adaxial leaf	112.7 ± 5.25^{abcdef}
		Abaxial leaf	111.8 ± 7.92^{abcdef}
		Adaxial petiolus	71.2 ± 16.42^{b}
		Abaxial petiolus	74.1 ± 17.96^{b}
	Young	Adaxial leaf	102.0 ± 13.89^{b}
		Abaxial leaf	105.1 ± 11.67^{bcde}
		Adaxial petiolus	67.8 ± 17.80^{b}
		Abaxial petiolus	66.9 ± 12.26^{b}
Rape	Old	Adaxial leaf	164.8 ± 16.38^{adef}
		Abaxial leaf	161.0 ± 20.64^{adef}
		Stem	149.2 ± 34.05^{abcdef}
	Young	Adaxial leaf	146.6 ± 5.11^{c}
		Abaxial leaf	144.8 ± 10.05^{abcdef}
White cabbage	Old	Adaxial leaf	150.7 ± 3.75^{adef}
		Abaxial leaf	151.2 ± 15.58^{adef}
		Stem	122.4 ± 21.67^{abcdef}
	Young	Adaxial leaf	161.5 ± 16.01^{e}
		Abaxial leaf	166.4 ± 14.50^{abcdef}

Mean ± sd, $n = 10$ per substrate

Different letters (abcdef) indicate statistical differences between means: Kruskal–Wallis one-way ANOVA on ranks ($P \leq 0.001$), followed by pairwise multiple comparison procedures, and Tukey test ($P < 0.05$)

Contact angle measurements

Contact angles of water on glass and plant surfaces were measured at 22.0 ± 0.29 °C and 58.4 ± 3.32% relative humidity, according to Bräuer et al. (2017). Glass slides, turgescent young and old leaves, petioles or stems were ablated from three living plants per species and flat fixed to a holder via double-sided adhesive tape (tesa® extra strong, 05696–00010; tesa SE, Norderstedt, Germany), ensuring the adaxial or abaxial side faced upward. A Research® plus 0.5-10 µl pipette (Eppendorf AG, Hamburg, Germany) was used to apply 10 µl drops of Aqua Millipore water on each test surface type and plant species. The droplets were visualised simultaneously using a horizontally aligned stereo microscope, combined with a 5MP CMOS digital camera and the Vidmess TSO software (Thalheim Spezial Optik, Pulsnitz, Germany). Ten droplets per 20 substrate types (for a total of 200 droplets) were imaged (Table 1). Static contact angles on each surface were measured using the free sessile drop method with ellipse-fitting and the SCA 20 Demo software (DataPhysics Instruments GmbH, Filderstadt, Germany).

Surface roughness measurements

Intact young and old leaves, petioles or stems of three living test plants were ablated and then flat fixed to glass holders using double-sided tape. Surface roughness parameters were measured for an area of 3.26 mm^2 using a digital microscope VHX970F (Keyence Corp., Osaka, Japan). The parameters considered included the arithmetic mean height (Sa), the maximum roughness height (Sz), and the root mean square roughness deviation (Sq). Five sites per 18 substrate types were measured, resulting in a total of 90 measurements (Table S1).

Weighing

The excellence balance XA205 Dualrange (81/0.00001 g; Mettler Toledo GmbH, Greifensee, Switzerland) was used to determine the egg mass. To do this, 10-26 eggs were detached from the polystyrene surface using a 3 mm-wide, 30 mm-long loop of double-sided adhesive tape. The mass of the tape loop was measured before and after the eggs were attached. The number of eggs on the loop was counted,

and the total mass was divided by the number of eggs. This procedure was repeated 20 times.

Microscopy

The 1–3-day-old eggs attached to various substrates were observed using the digital microscope VHX970F (Keyence Corp., Osaka, Japan), which included measurement of egg lengths and widths. Residues of glue on glass slides after egg detachment were stained with Sudanblack B Solution, made from 0.15 g Sudanblack B (CAS number: 4197-25-5, ACROS-Organics™, Fisher Scientific Inc.) in 50 ml 70% Ethanol, Rhodamine B (base 99%, CAS number: 81-88-9, ACROS Organics™, Fisher Scientific Inc.), 0.1 g mixed with 50 ml of ethanol, and Nile blue (Nilblau A, CAS number: 3625-57-8, Sigma Aldrich, Merck KGaA, Darmstadt, Germany) at a concentration of 0.5% in water.

Cryo-scanning electron microscopy (cryo-SEM) was used to examine samples in a frozen state, conducted at an accelerating voltage of 5 kV and a temperature of -100 °C. Fresh-cut plant parts, along with eggs and their adhesive, were mounted on metal holders using polyvinyl alcohol Tissue-Tek® O.C.T.™ compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands). These samples were frozen in the preparation chamber at -140 °C, sublimed at -70 °C to remove ice crystals, and then sputter-coated with platinum (6 nm). Following this, they were transferred to a cryo-SEM SUPRA 40VP-31-79 (Carl Zeiss SMT Ltd., Oberkochen, Germany), which was equipped with the EMITECH K250X cryo-preparation unit (Quorum Technologies Ltd., Ashford, Kent, UK). For element analysis, energy dispersive spectroscopy (EDS) was used in conjunction with cryo-SEM. The Bruker x-flash 6/100 EDS-detector was operated with the Esprit 2.0 software (Bruker Corporation, Billerica, MA, USA) on frozen samples at a working distance of 7-10 mm and an acceleration voltage of 10 kV. Cryo-SEM micrographs were morphometrically analysed using SigmaScan Pro 5.0.0 software (SPSS Inc.).

Raman spectroscopy

Native, untreated eggs attached to hydrophilic glass slides were used (refer to the sections Glass substrates and Insects). Prior to chemical analysis, the eggs were manually removed (pulled off) from the slides to analyse the remaining residues of solidified egg adhesive that had been on the slides for several weeks.

Micro-Raman spectroscopy was conducted by choosing spots with a visible, thin layer of adhesive next to the eggs. Raman maps were collected using a Horiba Yobin Yvon LabRam 800 (HORIBA Jobin Yvon Ltd., Stanmore, Middlesex, UK), a confocal Raman high-resolution spectrometer equipped with a silicon-based CCD detector (Peltier-cooled). The system integrates an upright optical microscope (Olympus BX41; Olympus Corp., Tokyo, Japan) and an automated *x*–*y* stage. Samples were measured using a 100 × long-distance objective (Leica PL FLUOTAR L 100 ×/NA 0.75; Leica Microsystems GmbH, Wetzlar, Germany) with a 532 nm laser excitation and 100% power (approximately 21 mW). All spectra were collected in the range of 200-3350 cm^{-1} using a 300 g mm^{-1} grating and a slit width of 100 µm. The spectrometer was calibrated using the 520.7 cm^{-1} band of a silicon wafer. The Raman map (25 × 35 $µm^2$) was collected with a step size of 1.8 µm (*x*) and 1.7 µm (*y*), an exposure time of 10 s, and two accumulations (Fig. 1). The spectral datasets were imported into the Spectral Imaging software, developed and provided by Matthias Finger based on Matlab (WITec Wissenschaftliche Instrumente und Technologien GmbH, Ulm, Germany). First, a baseline correction was performed using an asymmetric least squares correction (smoothness 5, asymmetry 3.5). The averaged spectrum of the adhesive was obtained from 23 pixels chosen from the measured area (Fig. 1B).

Force tests

Single 24-h-old eggs were tested on both glass and plant substrates. As found in previous studies with the codling moth *C. pomonella*, the egg secretion solidified after 24 h (Al Bitar et al. 2025) and led to results comparable with our earlier research on egg adhesion. Glass slides and turgescent plant parts (young and old leaves, petioles, and stems ablated from living plants) were fixed to a holder using double-sided adhesive tape. Following the methodology of Al Bitar et al. (2012, 2014), a 2 mm wide and 10 mm long double-sided adhesive tape loop was attached to a FORT-10 force transducer (10 g capacity, World Precision Instruments Inc., Sarasota, USA) combined with a Lab-Trax-4/16 (LT4/16-5)-Transbridge 4 M system (World Precision Instruments Inc., Sarasota, USA). The force transducer with the adhesive tape loop was moved vertically up and down using a motorised micromanipulator DC-3 K (Märzhäuser Wetzlar GmbH & Co.KG, Wetzlar, Germany) at a constant speed of 50 µm s^{-1} (Fig. S3). The tape loop was brought into contact with the egg's convex surface and was preloaded with a force of 1.2–26.6 mN. Care was taken to ensure that the tape loop made contact only with the egg surface, not with the glass or plant substrate. The force transducer was then moved upward until the egg detached (i.e., pulled off) from the substrate and adhered to the adhesive tape, while force-time curves were recorded at a rate of 200 samples s^{-1}. In total, 450 single measurements -25 measurements per substrate– were conducted. The contact area of the egg (adhesive) on glass

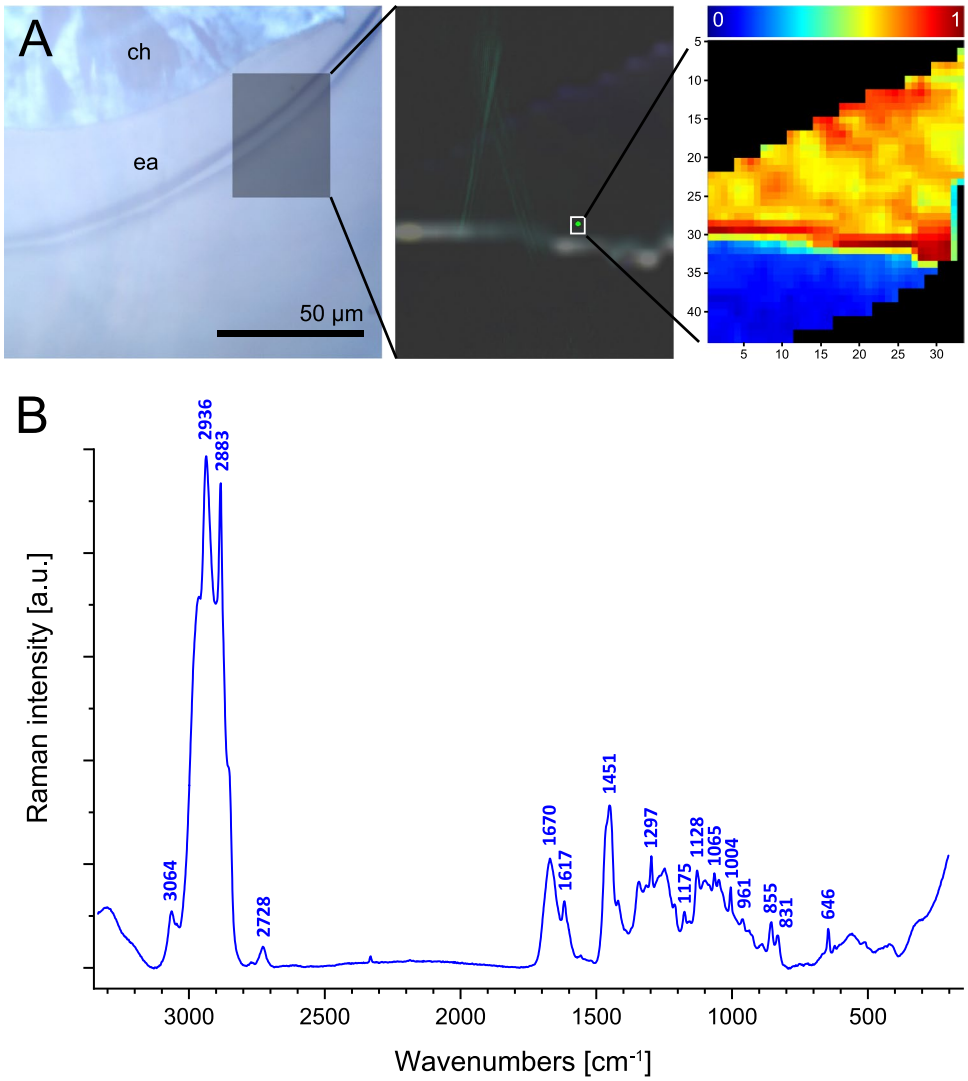

Fig. 1 Chemical analysis of the egg adhesive (Raman spectroscopy). **A** Brightfield image of the secretion layer next to the egg (left). The polarised light image (middle) of the mapping area reveals thicker secretion, which is also visible in the Raman image derived from plotting the normalised Raman intensity at 2925 cm^{-1} (right). **B** Averaged egg adhesive spectrum taken from 23 pixels. The spectrum shows bands characteristic of CH_2 and amide (C=O–NH) groups, indicating the presence of saturated fatty acids and proteins. ch, chorion; ea, egg adhesive (secretion). For detailed information on major compounds present in the egg adhesive, see Table S5

substrates was estimated for all pulled off eggs via digital imaging and analysed with SigmaScan Pro 5 (SPSS, Inc., Chicago, IL, USA).

Statistics

The R *aov* function was utilised to conduct a three-way ANOVA, running on R version 4.4.0 (R Core Team 2021). The three dependent factors and all possible combinations of these factors (i.e., 'interaction terms', up to three-way) were employed to explain the 'response variable'. The *aov*-function was used under the maximum likelihood estimation method, with the error structure assumed to be normally distributed. The factors included substrate type (hydrophilic glass vs. hydrophobic glass vs. *Brassica rapa* subsp. *pekinensis* vs. *B. napus* vs. *B. capitata* var. *alba*), plant site (adaxial leaf vs. abaxial leaf vs. stem/petiole), and leaf age (young vs. old). In addition, one-way ANOVA was applied to evaluate differences between substrate types.

Kruskal-Wallis tests were conducted to assess significant differences in contact angles and surface roughness. Linear regressions were also performed using SigmaPlot 12.0 (Systat Software, San Jose, CA, USA) to investigate the relationship between pull-off force and substrate characteristics.

Results

The egg-substrate interface

The oval-shaped eggs measure 550-600 µm in length, 330-350 µm in width, and 200-220 µm in thickness. They overlap several epidermal cells of the challenging waxy surfaces of their host plants (Figs. 2, 3 and 4). These eggs were often found near leaf veins, trichomes (Fig. 4F-H), and areas of the plant surface that had been abraded of wax (Fig. 4I).

Test plant leaves, petioles, and stems were covered with hierarchically arranged nm- and µm-sized crystalline wax

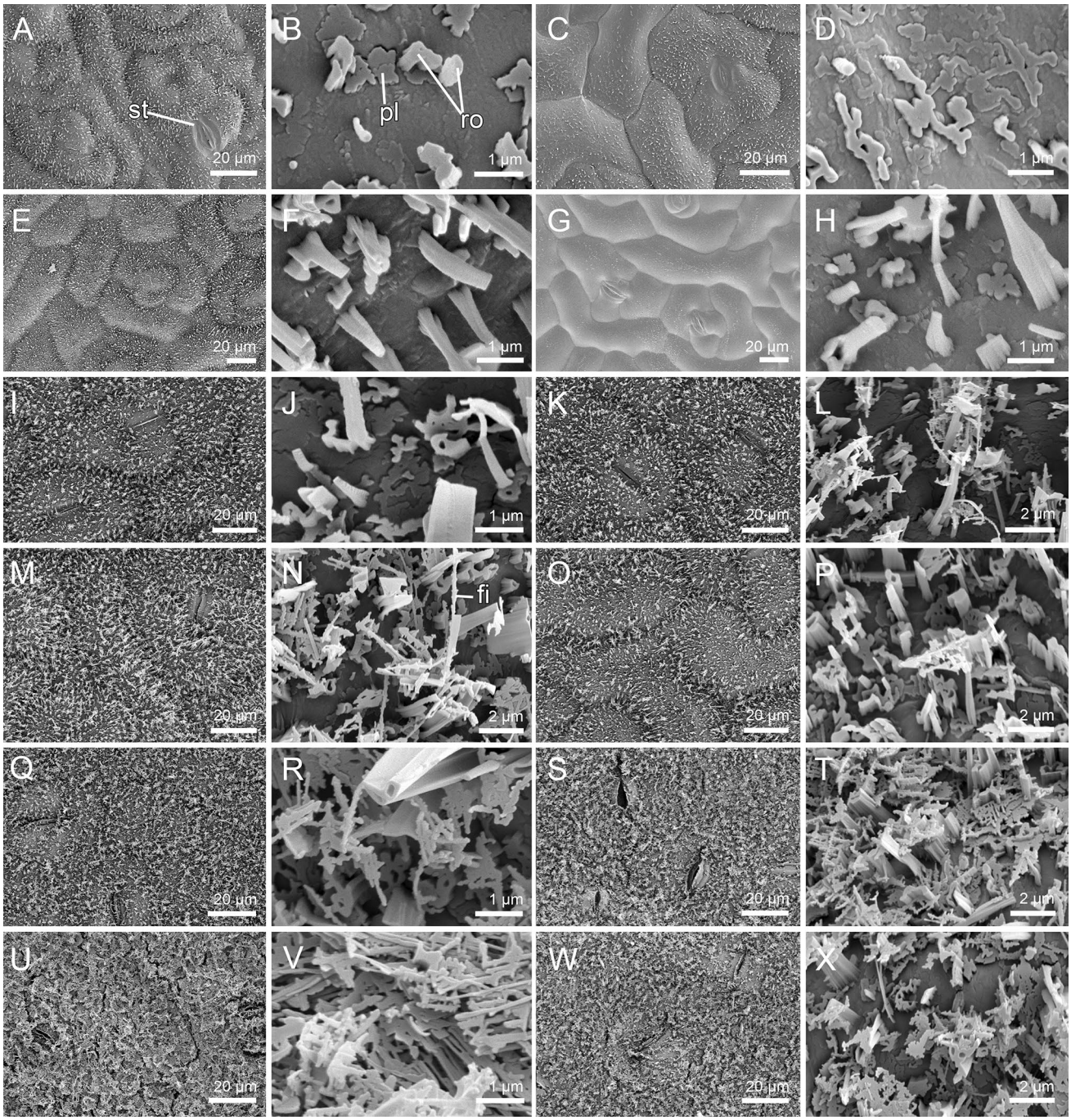

Fig. 2 Cryo-SEM images of leaf surfaces of cabbage plants. A-H. *Brassica rapa* subsp. *pekinensis* 'Michihili', young adaxial (**A**, **B**), young abaxial (**C**, **D**), old adaxial (**E**, **F**), old abaxial (**G**, **H**). **I-P** *B. napus* 'Licapo', young adaxial (**I**, **J**), young abaxial (**K**, **L**), old adaxial (**M**, **N**), old abaxial (**O**, **P**). **Q-X** *B. capitata* var. alba 'Brunswijker', young adaxial (**Q**, **R**), young abaxial (**S**, **T**), old adaxial (**U**, **V**), old abaxial (**W**, **X**). *fi* crystalline wax filaments, *pl* crystalline wax platelets, *ro* crystalline wax rods, *st* stomata

structures, including horizontal, flat platelets (initially developing tubules), robust tubules, rods, and superimposed, fragile filaments that are easily detachable (classification according to Barthlott et al. 1998). The wax coverage differed between plant species, leaf age and leaf side, with interactions between these factors. The number of crystalline wax tubules and filaments increased with leaf ageing (see Fig. 2, Tables S2, S3). Young leaves were more densely covered with waxes abaxially than adaxially. Older leaves exhibited more waxes on the adaxial side and several sites with abraded, altered, or smeared wax crystals, similar to those found on stems (Fig. S4). The wettability properties

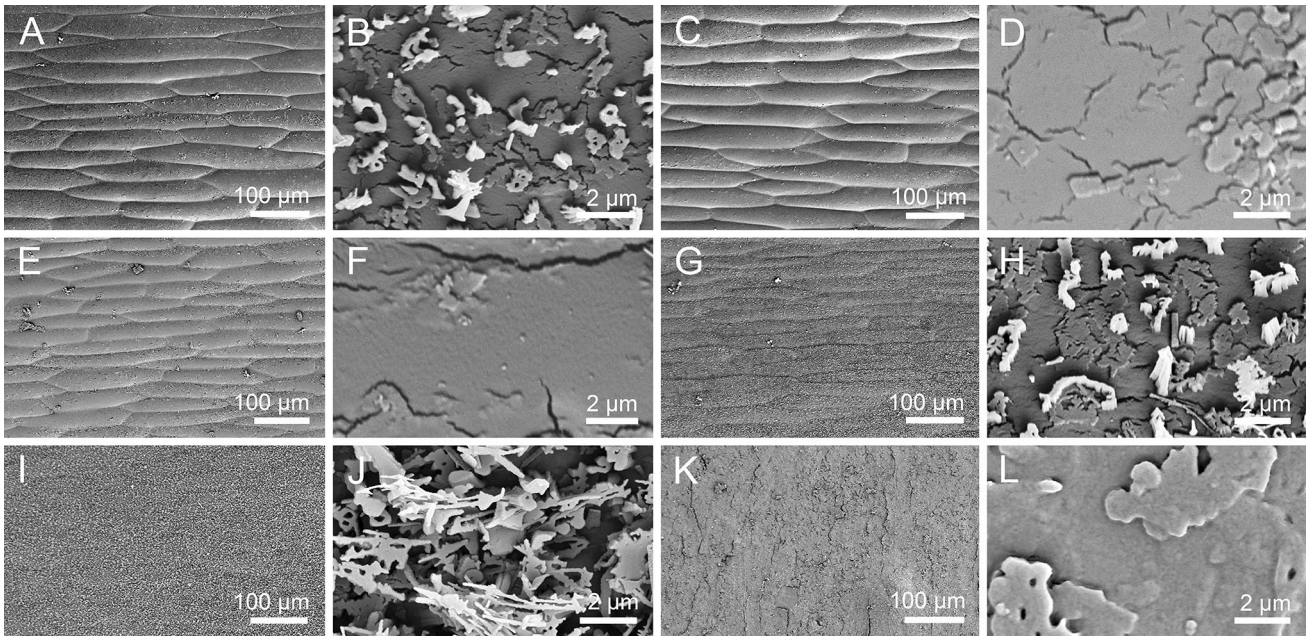

Fig. 3 Cryo-SEM images of petiolus and stem surfaces of cabbage plants, overview (**A**, **C**, **E**, **G**, **I**, **K**) and detail (**B**, **D**, **F**, **H**, **J**, **L**). **A**-**D**. *Brassica rapa* subsp. *pekinensis* 'Michihili', young adaxial petiolus (**A**, **B**), young abaxial petiolus (**C**, **D**), old adaxial petiolus (**E**, **F**), old abaxial petiolus (**G**, **H**). **I**, **J**. *Brassica napus* 'Licapo' stem. **K**, **L**. *B. capitata* var. *alba* 'Brunswijker' stem

of plant surfaces ranged from hydrophobic (with a water contact angle of 68° on young adaxial Chinese cabbage petioles) to predominantly superhydrophobic (with a water contact angle of up to 166° on young abaxial white cabbage leaves; Table 1). Surface roughness (Sa) varied from 13 µm on old adaxial white cabbage leaves to 122 µm on old abaxial Chinese cabbage petioles (Table S1). However, these roughness values should be interpreted with caution due to resolution limits (maximum magnification 2,000x) and unreliable detection of smaller epicuticular wax crystals.

The eggs adhered to the substrate via an apparently small volume of secretion that coated their entire surface, which could be displaced by rinsing with acetone (Fig. 5A-I, M). The eggs adhered firmly to the glass, with an average thickness of the secretion layer measured at 37.8 ± 8.36 nm (mean ± SD, $n = 20$), forming a thin, homogeneous film ranging from 8 to 45 µm in width. A few lamellar filaments protruded at the egg-substrate transition (Fig. 5E-H), gradually thinning towards the edge. In contrast to glass, the eggs appeared to adhere more loosely to cabbage and rape surfaces (Fig. 5J-Q). The egg adhesive adequately wetted the adaxial and abaxial cuticles of Chinese cabbage, forming thin films (Fig. 5K, L). Looser bonds were observed on rape and white cabbage surfaces (Fig. 5N-Q). The less-densely dispersed wax crystals on rape were largely embedded in the egg adhesive (Fig. 5P), though the secretion (egg adhesive) did not form thin films and did not spread widely across the surface. The dense wax coverage on white cabbage (Fig. 5P, Q) led to the formation of long, bulky secretion filaments that frequently detached from the plant surface (Fig. 5P). Irregularly thick patches of secretion covered the anti-adhesive waxy surfaces and were easily removed (Fig. 5Q).

Egg adhesive chemistry

Staining of egg adhesive indicated the presence of various lipids, including triglycerides, lipoproteins, neutral fats, and phospholipids. Sudan black B stained these substances black, while Rhodamine B produced a pink-to-red colouration. Neutral lipids, such as triglycerides, cholesterol esters, and steroids, were stained pink by Nile blue. In contrast, acidic lipids, including fatty acids, chromolipids, and phospholipids, were stained blue by the same dye (Fig. S2).

EDS analysis of the egg adhesive identified several components: carbon (18.6 weight%), nitrogen (2.5 weight%), oxygen (11.3 weight%), sulfur (0.4 weight%), potassium (0.4 weight%), and calcium (0.9 weight%) (Fig. 5R).

Raman spectra of the thin adhesive layer could only be obtained by scanning an area with a 100 × high magnification in a confocal Raman microscope setup (Fig. 1A). The averaged Raman spectrum of the *P. xylostella* egg adhesive,

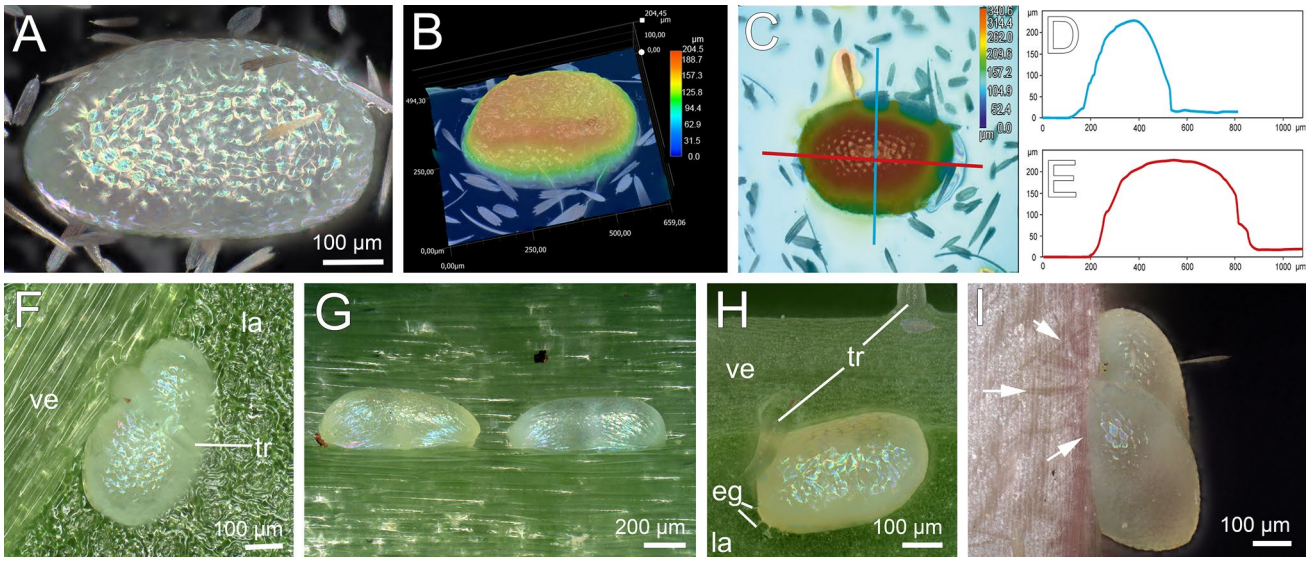

Fig. 4 3D digital imaging of 24-h-old *Plutella xylostella* eggs on a silicon wafer (**A**-**D**), Chinese cabbage *Brassica rapa* subsp. *pekinensis* leaves (**F**, **G**), rape *B. napus* leaf (**H**), and white cabbage *B. capitata* var. *alba* stem (**I**). **A** Top view: adult scales surround the egg. **B**, **C** Topography colour maps. Note the adult scales on the glass surrounding the egg (A-C). They can be covered with egg adhesive and overlaid with eggs. **D** The egg transversal profile, corresponding to the blue line in **C**. **E** The egg longitudinal profile corresponds to the red line in C. Note the different scaling of the x-axes in **D** and **E**. **F** The egg attached to the abaxial vein-lamina transitions at the edge of a trichome base in Chinese cabbage. **G**. Eggs adhering longitudinally to the main leaf vein. **H** The egg position similar to F; however, on an abaxial rape leaf, the egg glue forms a filamentous pattern. **I** Eggs on a white cabbage stem are obviously supported by plant surface sites having abraded waxes (arrows). *eg* egg glue, *la* leaf lamina, *tr* trichome, *ve* vein

derived from the highlighted area in Fig. 1A (total of 23 pixels), is shown in Fig. 1B.

The Raman spectrum of the egg adhesive exhibits characteristic features of both saturated lipids and peptides that partially overlap (see Supplementary Materials, Table S5, for full band assignments) (Rygula et al. 2013; Czamara et al. 2014). The presence of a significant amount of peptides in the adhesive is suggested by the characteristic amide I band at 1670 cm^{-1} and amide III band at 1247 cm^{-1}. The positions of these amide bands indicate a secondary structure consistent with a β-sheet structural motif (Maiti et al. 2004).

This observation is further supported by distinctive amino acid vibrational bands at 1004 cm^{-1} (phenylalanine) and at 855 and 831 cm^{-1} (tyrosine). Additional bands at 3064 cm^{-1} (C–H stretch) and 1617 cm^{-1} (C=C stretch), which are characteristic of aromatic compounds, reinforce this finding. However, the absence of the C–H stretch of the cis-double bond (=C–H), typically observed at 3005 cm^{-1}, and the =C–H bend at 1264 and 974 cm^{-1} suggests that non-saturated fatty acids or other non-saturated hydrocarbons are not likely to be present. Instead, the presence of saturated fatty acids is indicated by specific Raman bands at 2935 cm^{-1} and 2850 cm^{-1} (C–H asymmetric stretch assigned to CH_2), a broad band centred at 1451 cm^{-1} (C–H bend assigned to CH_2), and a strong band at 1297 cm^{-1} (CH_2 twisting). Furthermore, two characteristic C–C stretch bands at 1128 and 1065 cm^{-1}, along with the C–O–O skeletal vibration at 889 cm^{-1}, indicate the presence of saturated fatty acids. The absence of a carbonyl stretch in the range of 1750–1690 cm^{-1} suggests that triglycerides can be excluded (Czamara et al. 2014).

Egg adhesion

The average weight of an egg was measured at 22.8 ± 1.82 µg ($n = 20$). The contact area on hydrophilic surfaces was significantly larger than on hydrophobic glass (0.2 ± 0.02 mm^2 compared to 0.1 ± 0.02 mm^2, respectively; t test, $t = 5.2$, $p \leq 0.001$, $n = 10$).

The highest pull-off forces were observed on hydrophilic glass (22.7 ± 5.59 mN), which were 12 times greater than those on hydrophobic glass and 3 to 57 times higher than those measured on plant surfaces (Figs. 6 and S4, Tables 2 and S4). The high standard deviations corresponding to mean pull-off forces should be noted. It was found that more force was required to detach eggs from Chinese cabbage than from rape or white cabbage, and that egg detachment from older leaves required more force than from younger ones. Additionally, more force was needed to detach eggs from stems and petioles than from leaf laminae. Significant interactions were found between the factors substrate and plant site, plant site and leaf age, as well as between substrate, plant site, and leaf age, as determined by a three-way ANOVA (Table 2). A weak

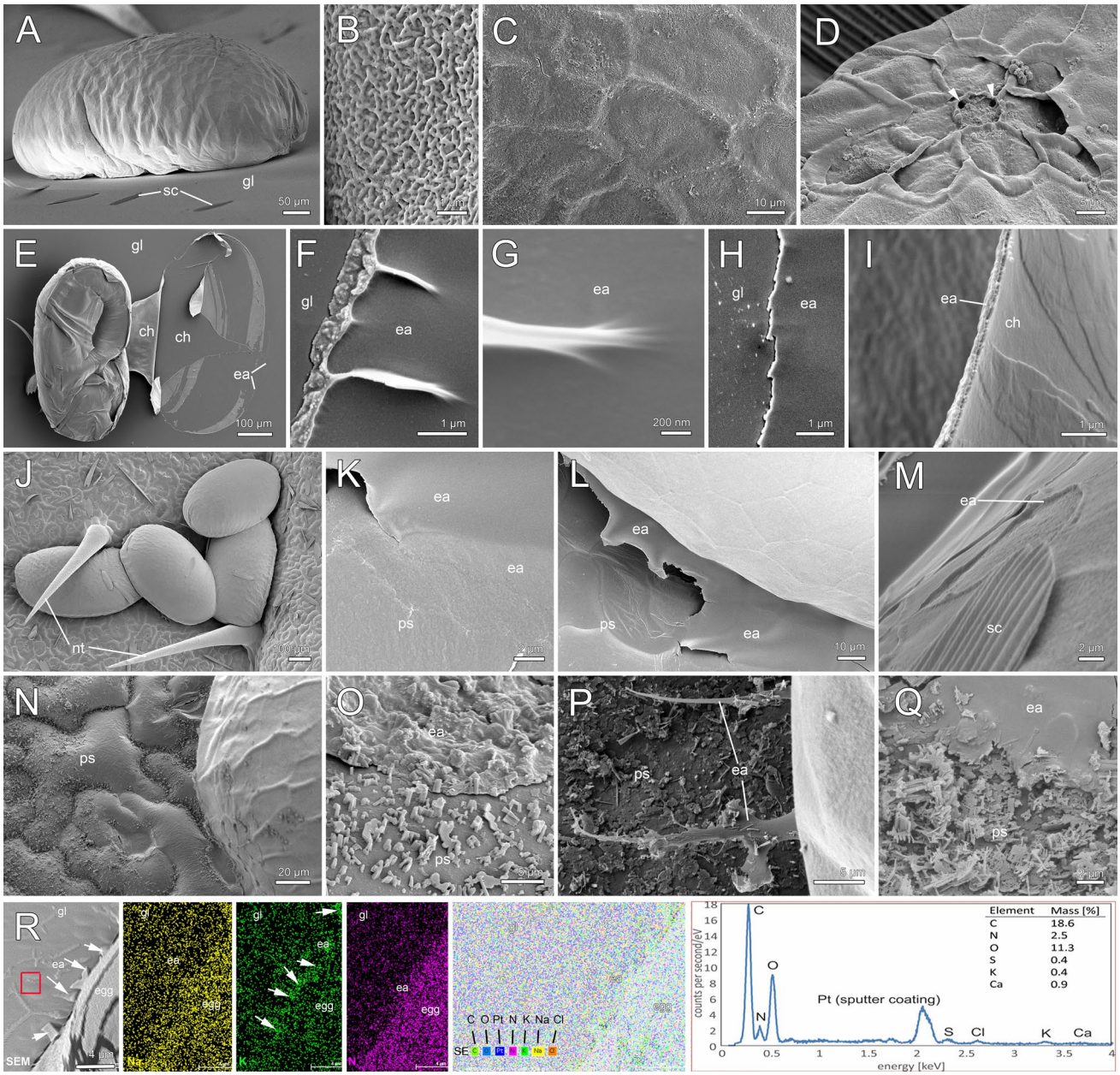

Fig. 5 Cryo-SEM images of *Plutella xylostella* eggs on various substrates. **A**. Lateral view of an egg attached to the hydrophobic glass. **B**. Detail of the corrugated egg chorion surface. **C**, **D**. Egg chorion surface after rinsing with acetone, elucidating the cell pattern (**C**) and micropyle at one egg pole with pores (arrowheads; **D**). **E**. An egg detached from hydrophilic glass; left: the ventral view of the egg; right: the residues left after egg removal. **F**, **G**. Detail of the egg adhesive bulge at the egg edge in contact with lamellar filaments protruding from the thin glue layer and easily detachable, elongated, tapered projections indicating extended fibrils in a fluid matrix (**G**, Detail of **F**). **H** Edge of thin egg adhesive layer on glass. **I** The thin egg glue layer on the chorion, cross-section. **J**–**M** Eggs on Chinese cabbage *Brassica rapa* subsp. *pekinensis*; grouped on the adaxial leaf lamina, close to a vein, between two non-glandular trichomes (**J**). The egg adhesive spreads over the plant surface, forming a thin film (**K**, **L**). The imprint of an adult scale on the egg surface indicates the presence of egg adhesive also on the chorion (M). **N**, **O** The egg edge and secretion on a less wettable adaxial rape *Brassica napus* leaf, close to epidermal cells having abraded wax crystals (**N**). A thicker layer of secretion embeds the less dense wax crystal coverage on the plant surface (**O**). **P**, **Q** Egg adhesive on the anti-adhesive adaxial *Brassica capitata* var. *alba* leaf, forming easily detachable, elongated, tapered projections (**P**) and, close to the egg, sparse, thick patches covering the densely superimposed plant wax crystals. **R** A series of energy dispersive spectroscopy (EDS) images (colour mapping and spectrum) for egg and egg adhesive, elucidating the clear presence of carbon, nitrogen, oxygen, sulfur, potassium, and calcium in the secretion (spectrum). The red square in the left SEM image indicates the site that was sampled for egg adhesive spectroscopy, as shown in the spectrum. The summarising table does not reflect the chlorine peak. Phosphorus, nitrogen, and sodium belong to the main compounds of the chorion (mapping). Arrows point to the small bulge of egg glue at the egg edge in contact with the substrate. *ch* chorion, *gl* glass, *ea* egg adhesive, *nt* non-glandular trichome, *ps* plant surface, *sc* scale

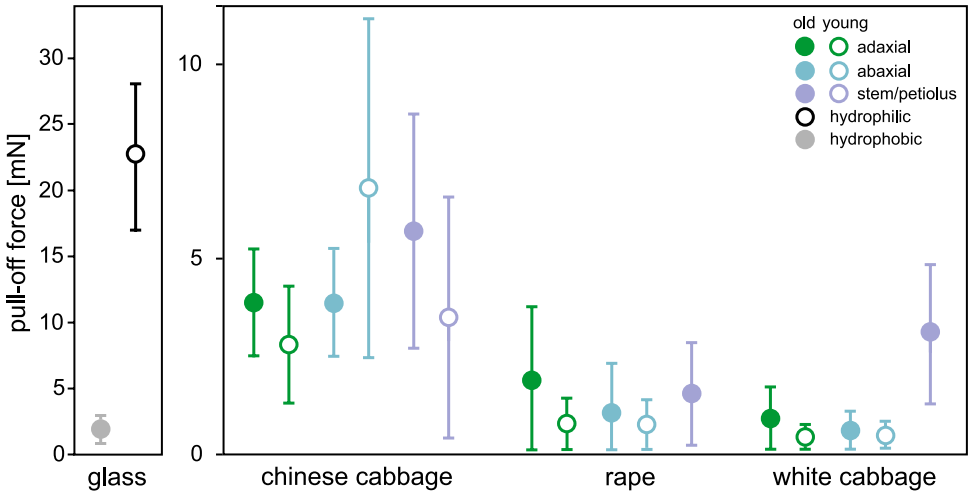

Fig. 6 Forces required to pull off eggs from glass and cabbage surfaces: Chinese cabbage *Brassica rapa* subsp. *pekinensis* leaves and petioli, rape *B. napus* leaves and stem, and white cabbage *B. capitata* var. *alba* leaves and stem. Means and standard deviations; $n=25$ per surface type. See Tables 2 and S4 and Fig. S4 for statistics

Table 2 Three-way ANOVA for pull-off forces obtained in experiments with *Plutella xylostella* eggs attached to glass and plant surfaces (R Core Team 2021)

Factor	Df	Sum of Squares	Mean	*F* value	Probability (*P*)
Substrate	3	5452	1817.5	370.0	<2e-16***
Plant site	3	5506	1835.3	373.6	<2e-16***
Leaf age	1	18	17.5	3.6	0.060 .
Substrate : plant site	4	148	37.0	7.5	7.16e-06***
Substrate : leaf age	2	6	2.8	0.6	0.567
Plant site : leaf age	2	139	69.7	14.2	1.08e-06 ***
Substrate : plant site : leaf age	2	51	25.3	5.2	0.006**
Residuals	432	2122	4.9		

Evaluated factors (and comparisons): Substrate (hydrophilic glass vs. hydrophobic glass vs. *Brassica rapa* subsp. *pekinensis* vs. *B. napus*, vs. *B. capitata* var. *alba*), Plant site (adaxial leaf vs. adaxial leaf vs. stem/petiolus), Leaf age (young vs. old). See Fig. 6 for illustration

Df degrees of freedom

Significance codes: ***0.001, **0.01, *0.05, . 0.1

positive relationship was observed between pull-off forces and surface wettability: pull-off forces = 15.7-(0.1 × contact angle), $R^2=0.2$, $F=3.7$, $p=0.07$; linear regression. It is noteworthy that forces were not related to surface roughnesses: pull-off force = 8.9-(0.02 × Sa), $R^2=0$, $F=0.1$, $P=0.8$; pull-off force = 8.9-(0.003 × Sz), $R^2=0$, $F=0.1$, $P=0.8$; pull-off force = 8.9-(0.01 × Sq), $R^2=0$, $F=0.1$, $P=0.8$; linear regressions. However, the egg pull-off force significantly depended on the number of plant epicuticular wax tubules ($R^2=0.5$, $F=11.6$, $P=0.004$) and filaments ($R^2=0.4$, $F=8.7$, $P=0.011$) but not platelets ($R^2=0.05$, $F=0.7$, $P=0.4$) (Fig. 7).

Considering the maximum pull-off forces on glass and the respective contact areas, the adhesive strength was calculated to be 0.03 N mm^{-2} (28.8 kPa) on hydrophobic glass and 0.20 N mm^{-2} (198.0 kPa) on hydrophilic glass.

Discussion

The present study combined optical, chemical, and mechanical methods to investigate whether the adhesion of diamondback moth eggs differs between artificial and natural surfaces, among three *Brassica* host species, and across different leaf ages.

Raman spectroscopy indicated the presence of various lipids, peptides, amides and aromatic amino acids, confirming the predominantly proteinaceous nature of the moth's egg adhesive.

Unlike the surface properties of the diamondback moth's preferred host plants, the egg adhesive adhered better to less hydrophobic surfaces, as the compounds in the egg adhesive are mainly polar. The limited wettability of plant waxes and a reduced contact area led to a decrease in pull-off forces with an increasing number of epicuticular wax crystals, which diminish as leaves age. The least egg adherence was

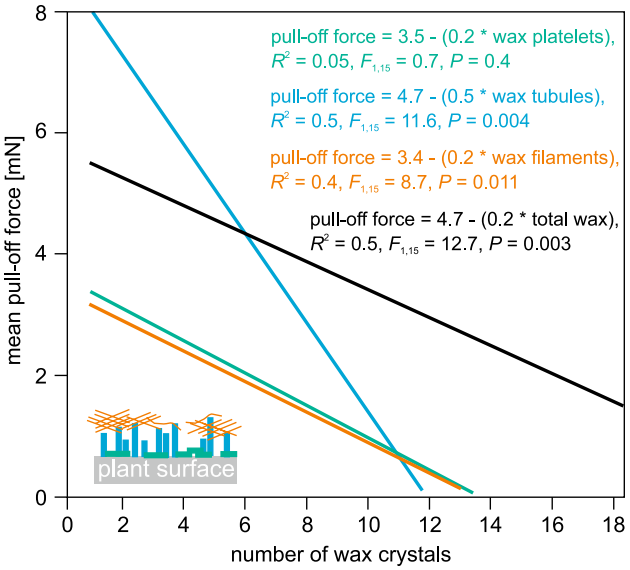

Fig. 7 Relationship between mean egg pull-off force and number of wax crystals per 25 µm^2 plant surface area, separated for wax crystal categories ("platelets"/initially developing tubules), tubules, filaments), and pooled for all categories (total wax); linear regression equations, coefficients, *F* statistics and probability values (*P*)

observed on white cabbage and rape surfaces, while the highest was on the petioles of older Chinese cabbage leaves. Also, the amount of egg adhesive applied by the moths was adapted to the structures of the plant surfaces.

One has to keep in mind that rearing insects predominantly on a single host species and allowing them to lay eggs on artificial substrates for an extended period may have influenced their physiology and behaviour, as well as the results of this study. These effects should be examined in further experiments. Nevertheless, since our observations are consistent with previous studies, the comparisons presented in the Discussion section below seem to be valid.

Given that insect egg adhesion is little understood so far, we will discuss our findings within a broader context, also taking into account previous research regarding the effects of rain impact and sprinkling on moth egg detachment (Rahman 2019; Rahman et al. 2019, 2023). This comprehensive view aims to promote future integrative and multidisciplinary studies on insect oviposition and egg adhesion.

Plant surfaces and oviposition

Various plant cues, potentially both physical and chemical –particularly glucosinolates, isothiocyanates, and surface waxes– stimulate oviposition and feeding in *P. xylostella* (Reed et al. 1989; Justus and Mitchell 1996; Spencer 1996; Renwick 2002; Marazzi et al. 2004; Ang et al. 2014, 2016). Van Loon et al. (1992) reported that a combination of apolar compounds, such as alkanes, and polar glucosinolates, both present in cabbage wax layers, significantly enhances oviposition compared to glucosinolates alone (Spencer 1996). Female butterflies, such as those of *Pieris brassicae*, grasp leaves and 'taste' them with their feet using tarsal chemoreceptors. They attach to leaf margins and bend their abdomens towards the abaxial leaf side after tasting the glucosinolates on the adaxial leaf side, laying their eggs regardless of the properties of the abaxial leaf side (van Loon et al. 1992; Caarls et al. 2023). Indeed, the distribution of chemosensilla on the tarsi differs between *P. xylostella* moths and *P. brassicae* butterflies; however, a chemosensory function in assessing host plant quality has also been suggested for *P. xylostella* tarsi and warrants further investigation (Qiu et al. 1998).

Justus et al. (2000) highlighted the significance of a proper foothold in *P. xylostella* for stabilising the female's body, especially on abaxial or aligned surfaces, which allows abdominal lifts during egg deposition. This background may explain why eggs are often found near leaf veins and trichomes, as well as in areas with abraded wax, because females can effectively grasp these sites, facilitating egg fixation (Fig. 4F-I) (Hardy 1938; Harcourt 1957; Rosario and Cruz 1986; Talekar and Shelton 1993; Rahman 2019; Gopika et al. 2021). Consistent with this observation, Talekar et al. (1994) demonstrated that the number of eggs on Chinese cabbage leaves increased with trichome density.

Intact hierarchically arranged Brassicaceae plant surface structures, composed of wax flat platelets (initial tubules), robust tubules, rods, and superimposed filaments, may function similarly to the anti-adhesive wax layers in carnivorous pitcher plants, *Nepenthes* sp. (Nepenthaceae). Waxes on the pitcher surface have been experimentally shown to consist of mechanically stable lower plates covered with fragile, irregular platelets connected by thin stalks. These upper plates break easily, creating a separation layer between insects and the pitcher's surface. Prey entering the trap loses its foothold due to tarsal contamination with detached waxes and further contact minimisation by the lower waxes (Gorb et al. 2014). In Fig. 5P and Q, detached wax crystals from white cabbage can be seen adhering to egg adhesive. On this substrate, the egg adhesive did not form strong bonds. This observation aligns with previous findings that demonstrate thick wax layers influence diamondback moth oviposition (Justus and Mitchell 1996), indicating that more eggs are deposited on glossy plants and plants with altered waxes (Eckenrode et al. 1986; Uematsu and Sakanoshita 1989; Justus et al. 2000; Ulmer et al. 2002; Zhu et al. 2022a, b). On less waxy leaves, eggs were usually laid singly, whereas clusters were found on waxy plants, such as common cabbage, as clusters create a larger contact area and adhere more efficiently (Rahman 2019).

Previously, more eggs have been observed on younger leaves than on older ones (McCall and Fordyce 2010; Ang

et al. 2014; Badenes-Pérez and Heckel 2023). Various explanations have emerged: (1) higher concentrations of glucosinolates and saponins in younger leaves as compared to older ones (Badenes-Pérez et al. 2014) and (2) lower suitability of older leaves for larval development and survival relative to younger leaves (Larsson and Ohmart 1988; Raupp et al. 1988; Kause et al. 1999; Rodrigues and Pires-Moreira 1999). Additionally, conditions on larger leaves are considered stressful for eggs, as they can become hotter than those on smaller leaves during the day (Potter et al. 2009). However, the impact of epicuticular wax coverage on leaf temperature has been overlooked despite its role in reflecting and protecting against transpiration. Williams (1981) and Bonebrake et al. (2019) noted that oviposition preferences may shift to suboptimal, basal, and senescent leaves if egg mortality is high on apical, more nutritious leaves in specific microclimatic contexts (e.g., sun, heat, wind, drought, or rain).

This study demonstrates that the hierarchical complexity of plant epicuticular wax coverage, i.e., the number of superimposing wax tubules and filaments, increases with leaf ageing (Fig. 2, Table S2). In addition, older leaves often exhibit multiple sites with abraded, altered, or smeared wax crystals, similar to those found on stems (Figs. S4). Previous research indicates that *P. xylostella* can detect subtle differences in phylloplane characteristics even within a single plant, suggesting a combination of stimuli that trigger oviposition (Gupta and Thornsteinson 1960; Tabashnik and Mau 1986; Uematsu and Sakanoshita 1989; Pivnick et al. 1990; Talekar and Shelton 1993; Talekar et al. 1994; Spencer 1996; Spencer et al. 1999; Couty et al. 2006; Ang et al. 2014; Badenes-Pérez et al. 2014; Rahman 2019). These complex plant-moth interactions may explain previous conflicting reports regarding *P. xylostella*'s oviposition preference for adaxial leaves (Harcourt 1957; Rahman 2019; Gopika et al. 2021; Lasota

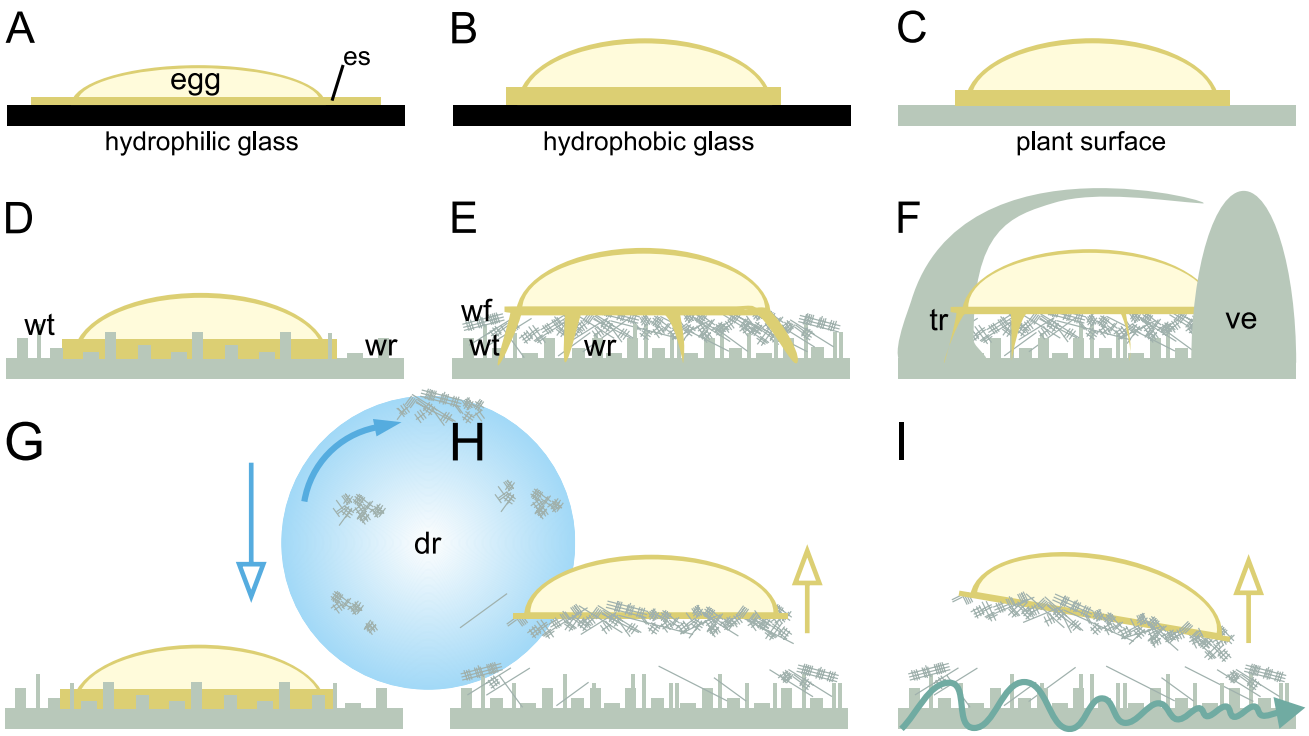

Fig. 8 Hypothetical schematic of *Plutella xylostella* eggs adhering to various substrates. **A** The flat-shaped egg surrounded by the egg adhesive, which spreads and establishes a thin film on the hydrophilic glass. **B** A less flattened egg and a thicker secretion layer on hydrophobic glass. **C** The egg on a rather smooth plant surface, comparable to the situation on hydrophobic glass. **D** The egg on a plant surface covered with solid crystalline wax rods and tubules. The egg adhesive embeds the waxes and contacts the plant cuticle, creating a stable matrix. **E** The egg on a plant surface covered with solid crystalline wax rods, tubules, and superimposed fragile filaments. The secretion cannot contact the plant cuticle, embeds several wax filaments, and forms a few loose lamellar bulks. **F** The same situation as **E**, but the egg fixated between a trichome and the leaf vein, thus resisting environmental influences. **G-I** The impact of rain and irrigation water drops on plant surfaces, including the adhering eggs and suggested interactions. The stable matrix of egg adhesive adhering to the plant cuticle and embedding wax crystals keeps the egg attached to the plant while a rain droplet makes contact with (arrow) and either spreads over the surface or splashes (dotted line arrows) (**G**). Fragile wax filaments and the egg detached and adhered to a rolling-off water drop (**H**). Drop-induced leaf vibration shakes off the egg along with embedded fragile wax filaments, which are separated from the underlying stable wax rods and tubules; the drop either spreads or splashes (**I**). *dr* water drop, *ea* egg adhesive, *tr* trichome, *ve* leaf vein, *wf* crystalline wax filaments, *wr* crystalline wax rods, *wt* crystalline wax tubules

and Kok 1989) versus abaxial leaves (Harcourt 1957; Ang et al. 2014; Gopika et al. 2021), which depend on experimental and environmental conditions, leaf position, leaf age, plant cultivar, and age. It has also been noted that stems can serve as suitable oviposition substrates, with veins and trichomes aiding in egg attachment (Fig. 8F) (Reddy et al. 2004; Sarfaz et al. 2005; Silva and Furlong 2012).

Egg morphometric characteristics observed through three-dimensional digital microscopy and cryo-SEM share similarities with previous reports, confirming the oval egg shape and sculptured surface of the egg (Hardy 1938; Ashwini 2014; Philips et al. 2014; Bhure et al. 2020; Gopika et al. 2021; Saran and Genç 2021).

Egg adhesive

The limited and consistent distribution of adhesive secretion (Fig. 5A-I, M), which covers and surrounds the egg at a distance of 8-45 µm, reflects the oviposition behaviour of the diamondback moth. This behaviour involves probing the substrate with the ovipositor tip, sweeping the ovipositor as the distal abdominal segments bend, lifting the wings, lowering the ovipositor, and making lateral contact with the substrate to release a single egg. As the abdomen moves slightly from side to side, the egg is glued to the substrate while the female is walking; only 14% of oviposition attempts result in successful egg deposition (Harcourt 1957; Justus and Mitchell 1996).

The thin film of egg adhesive, with a few lamellar filaments at the egg-substrate interface, gradually thins to the edge (Figs. 5E-H; 7A, B), suggesting that it has a low-viscosity upon release. This secretion consolidates as it spreads and embeds plant wax crystals, creating a nanometer-thin layer that promotes intermolecular forces and high adhesion (Habenicht 2009).

Rinsing with acetone resulted in the removal of the adhesive from the egg surface. Similar observations have been made for the eggs of the leaf insect *Phyllium philippinicum* Hennemann, Conle, Gottardo, and Bressel (Phasmatodea, Phylliidae), which are related to exteriorly polar and interiorly nonpolar adhesive components (Büscher et al. 2020).

The egg adhesive effectively wetted the hydrophobic adaxial and abaxial cuticles of Chinese cabbage, forming thin films (Figs. 5K, L; 7C). Similar to the egg adhesive of *Cydia pomonella* on waxy apple fruits (Al Bitar et al. 2012, 2014) and the asparagus beetle *Crioceris asparagi* eggs on waxy *Asparagus officinalis* cladophylls (Voigt and Gorb 2010), the diamondback moth egg adhesive covers the sparse, single-layer wax crystals and the cuticle in between, creating stable matrices resistant to removal (Fig. 8D). These properties could be attributed to lipids (Fig. S5), which have been confirmed in egg adhesive by this and previous studies (Hinton 1981; Li et al. 2008). In contrast, the highly hydrophobic, double-layer, and densely wax-covered surfaces of rape and white cabbage were poorly wetted by the *P. xylostella* egg adhesive (Figs. 5N–Q, 7E, F). Evidently, females released a higher volume of secretion to cover the less numerous waxes on rape and to form long, bulky secretion filaments on white cabbage, which were easily and frequently detached. We hypothesise that physical (structural) and chemical effects interact to influence egg detachment from plant surfaces. So far, the majority of identified egg elicitors that induce plant defences are of a non-proteinaceous nature (Hundacker et al. 2021; Caarls et al. 2023). Yet, the primarily proteinaceous nature of insect egg adhesive (Beament and Lal 1957; Amornsak et al. 1992; Burkhart et al. 1999; Jin et al. 2006; Li et al. 2008; Betz 2010), as observed in the present study, may explain the low wetting of highly hydrophobic substrates. This phenomenon could be underpinned by the presence of water-affine mucopolysaccharide compounds in *P. xylostella* egg adhesive, identified by Justus and Mitchell (1999). However, we did not find such a compound in our current analyses.

The portions of carbon (18.6 weight%), nitrogen (2.5 weight%), and oxygen (11.3 weight%) detected by EDS result in ratios of C/N (7.4), C/O (1.6), and N/O (0.2), which differ from those found in the egg glue of the Western conifer seed bug *Leptoglossus occidentalis* Heidemann (Heteroptera, Coreidae) (4.4, 3.4, 0.8, respectively; Sánchez-Hernández et al. 2023). However, the orders from the highest to lowest ratio resemble each other in both moths and bugs. According to Li et al. (2008), the egg adhesive in *P. xylostella* contained less than 45 weight percent protein and did not exhibit strong bands on electrophoresis.

Raman spectroscopy in the present study primarily detected peptides and saturated lipids that partially overlap with each other. Accordingly, Hinton (1981) has stated that some lepidopteran egg glues contain lipids and that inorganic salts are undoubtedly present. We observed a low potassium weight percentage in the EDS spectra, but this was not confirmed by Raman analysis. This discrepancy may be explained by the different ages of the glue material used in both approaches; the material used for EDS was fresher.

While peptides primarily contribute to the inner structure of the glue, lipids mainly aid in wetting different substrates. Amides are common in biological adhesives, forming highly stable bonds in various biomolecules, such as peptides and proteins (Mahesh et al. 2018). Interestingly, the co-presence of amide I and amide III, as identified in *P. xylostella* egg glue, has also been reported in the silk of silkworms, silverfish, and crickets (Walker et al. 2013). The Raman spectrum of diamondback moth egg glue shows several similarities to that of the silk fibroin film and liquid from *Bombyx mori* L. (Lepidoptera, Bombycidae; Monti et al. 2021). Silk production in *P. xylostella* larvae is known to help them overcome

challenging host substrates, escape enemies, and cope with abiotic impacts, e.g., through abseiling. They also create silk puparia (Zhu et al. 2022a, b). Thus, the synthesis of fibroin is likely evolutionarily anchored in this moth species and may influence the chemistry of the egg glue produced by adults. This hypothesis reflects the different silk types and the incorporation of the β-sheet structural motif (Craig 1997). For comparison, a recent proteomic study on spider mite silk revealed that different glands can release similar secretions, as do spider mite silk glands and saliva glands for fibroins (Arai et al. 2025). The material released by salivary glands appears viscous and exhibits adhesive properties. Similarly, fibroin-like sticky substances could be released from the moth's colleterial glands to help fix the eggs in place. The filaments into which the egg adhesive is pulled in Fig. 5F and G may be explained by β-sheet crystals embedded within an amorphous matrix, resulting in polypeptide-based nanocomposites in natural silks, which contribute to the high strength and toughness of spider silk (Chan et al. 2025).

The simultaneous presence of the characteristic aromatic amino acids phenylalanine (Phe) and tyrosine (Tyr), as found in *P. xylostella* egg glue, is widely associated with the formation of L-3,4-dihydroxyphenylalanine (DOPA). DOPA is an o-dihydroxy derivative of the monophenolic amino acid Tyr, shared by phylogenetically different animals. It is well-known that marine bioadhesive systems, such as those found in mussels and tubeworms (Flammang et al. 2009; Lutz et al. 2022), exhibit this property. DOPA contributes to the intermolecular crosslinking of adhesive proteins and their bonding with surfaces, thereby enhancing both cohesive and adhesive strength (Yu et al. 1999; Ooka and Garrell 2000; Power et al. 2010). It is involved in various interactions with other molecules and surfaces, including both covalent and non-covalent bonds. For example, cation-π interactions occur between the benzene rings of aromatic amino acids and the side-chain amine groups of positively charged amino acids, resulting in strong intermolecular interactions (Kamino 2006; Liang et al. 2019). The hydroxyl groups of DOPA can interact with polar surfaces through hydrogen bonds.

Additionally, Tyr exhibits amphiphilic characteristics that allow it to wet both hydrophilic and hydrophobic substrates, including nonpolar waxy plant surfaces. However, since *P. xylostella* egg glue adheres better to less hydrophobic surfaces, we suggest that the glue compounds are predominantly polar in nature. Furthermore, the saturated state of these compounds, as identified in the present study, suggests limited interaction with counterparts but also indicates effective internal crosslinking, potentially mediated by glycosylation (Graham 2018), protein condensation (covalent joining of –H and –OH containing groups on amino acids to form dipeptides or polypeptides), and disulfide (–S–S–) bonds (Hemmerich 2007; Power et al. 2010; Hennebert et al. 2019; Lutz et al. 2022). The latter have been indicated by the S–S stretch in *P. xylostella* egg glue (Table S5).

Another vital process in biological adhesives is the protein phosphorylation of various target amino acids, including Tyr (Flammang et al. 2009; Petrone 2013). This phenomenon is also likely present in *P. xylostella* egg secretion, as suggested by a phosphate stretch in the Raman spectrum (Fig. 1, Table S5). The calcium portion detected by EDS (Fig. 5R) may contribute to Ca^{2+} bridging between molecules, which could enhance both the cohesion and adhesion of secretion. Well-crosslinked materials are also more resistant to weathering (Lutz et al. 2022).

Biological adhesives, such as *P. xylostella* egg glue, are secreted onto the surface as viscous fluids or colloidal suspensions and must solidify to form a load-bearing joint after application (Stewart et al. 2011). In the case of fruit fly pupa glue, glycosylated proteins are believed to enhance solubility at high concentrations, which are necessary for glue release, while β-sheets facilitate nucleation (Monier and Courtier-Orgogozo 2022). Complex coacervation occurs when proteins combine in solution to form soluble aggregates, resulting in the phase separation of liquids into a denser (coacervate) phase and a less dense (equilibrium) phase. This process is *pH*-dependent and typically produces a low-viscosity coacervate with low surface tension. Changes in *pH* or temperature can trigger the subsequent crosslinking of soluble protein aggregates (Waite et al. 2005; Power et al. 2010). This variable, trigger-dependent solidification, may contribute to the considerable standard deviations observed in pull-off forces.

Further research on moth egg glue should consider both general findings and specific characteristics of biological adhesives, including the interesting aspects found in the pupa glue of *D. melanogaster*, where gene sequences undergo rapid evolution and adaptations in glue adhesion to various environmental conditions (Monier and Courtier-Orgogozo 2022). This aspect is particularly relevant, considering that moth eggs are exposed to weathering conditions.

Egg adhesion

The average egg mass was 23 µg, which is less than the previously reported measurement of 57 µg for diamondback moth eggs (Saran and Genç 2021). This difference can be attributed to variations in the moth populations, rearing conditions, and measurement procedures used in the previous and present studies.

The adhesive strength of *P. xylostella* eggs was measured at 29 kPa on hydrophobic glass and 198 kPa on hydrophilic glass. These values are comparable to those of other Lepidoptera, including codling moths *C. pomonella* on waxy apple fruits (up to 98 kPa; Al Bitar et al. 2012, 2014)

and butterflies *Lycaeides idas* on waxy *Lotus nevadensis* (94 kPa; Fordyce and Nice 2003). Differently, asparagus beetle *C. asparagi* eggs exhibited much stronger adhesion on waxy *Asparagus officinalis* than *P. xylostella* eggs on glass, reaching up to 271 kPa (Voigt and Gorb 2010). However, the safety factor (the ratio of pull-off forces to egg weight) of beetle eggs on Asparagus (8650) and *P. xylostella* eggs on hydrophobic glass (8517) were similar. On host plants, the moth egg safety factors ranged from 1795 on young adaxial white cabbage leaves to 25,461 on old Chinese cabbage petioles.

Previous standardised measurements of lepidopteran egg adhesive indicated potentially much higher adhesive strength, such as silkmoth secretion measured between two aluminium plates, resulting in 1.2 MPa for *Bombyx mori* L. (Lepidoptera, Bombycidae; Yoshida and Nagata 1997), 2.0 MPa in *Antheraea yamamai* (Guérin-Méneville), and 3.0 MPa in *Rhodinia fugax* (Butler) (Lepidoptera, Saturniidae; Yago et al. 2001). The adhesive from the egg capsules of the gum moth *Opodiphthera* sp. (Lepidoptera, Saturniidae) was elastic and tacky, resembling a hydrogel, with shear strengths between two wood blocks ranging from 1 to 2 MPa (Li et al. 2008). These higher values compared to those of diamondback moth egg adhesive may be attributed to differences in measurement setups and varying sampling sites.

Interestingly, the adhesive strength of *P. xylostella* eggs on glass is similar to that of *Drosophila melanogaster* Meigen pupae (Diptera, Drosophilidae), fixing their bodies to various substrates by a transparent thin-layered glue (190 kPa on untreated hydrophilic and 21 kPa on hydrophobic surfaces) (Borne et al. 2020; Monier and Courtier-Orgogozo 2022). An average force of 217 mN is required to detach a 1.4 mg weighing pupa from a glass surface, equating to 15,500 times the pupa's weight, i.e., safety factor (Borne et al. 2020). The comparable appearance and performance suggest the employment of similar secretions during different insect life stages and in distantly related lineages, suggesting convergence in substrate adhesion among various phyla.

Our data, which include considerable standard deviations, confirm that the degree to which a *P. xylostella* egg attaches to a plant varies greatly and also depends on the physicochemical characteristics of the plant surface (Fig. 6, Table 1) (Müller and Riederer 2005). Pull-off forces are slightly related to surface wettability and depend significantly on the number of wax crystals (Fig. 7), suggesting both the amphiphilic wetting properties and embedding challenges of surface structures by the limited volume of egg adhesive. The interactions between substrate *x* plant site, plant site *x* leaf age, and substrate *x* plant site *x* leaf age were significant in our pull-off force measurements (Table 2), indicating the plant surface significance for proper egg adhesion. Standard deviations may originate from irregular dispersion and partial alteration of plant surface structures, as well as varying physiological moth fitness.

The significantly larger contact area on hydrophilic glass compared to hydrophobic glass aligns with previous findings by Uematsu and Sakanoshita (1989), which indicated that eggs appeared flatter and formed a larger contact area on less hydrophobic, glossy surfaces than on highly hydrophobic, waxier surfaces (cabbage, broccoli, and rape leaves). A discussed, greater contact areas resulted in higher forces required to detach eggs in the following contexts: (1) from hydrophilic glass compared to hydrophobic glass, (2) from the less waxy Chinese cabbage compared to waxy rape and highly waxy white cabbage, (3) from older leaves compared to younger leaves, and (4) from stems/petioles compared to leaf laminae.

Reliable egg attachment to host plants is crucial for the survival of diamondback moths in the field, as eggs can easily be washed off the leaf surface by rainfall, leading to mortality rates of 56% to over 80% (Harcourt 1957; Annamalai et al. 1988; Wakisaka et al. 1990; Rahman 2019). Corresponding with the relatively low pull-off forces measured in this study, eggs on common cabbage plants have been previously found to be more susceptible to rainfall than those on Chinese cabbage plants in rain simulation experiments conducted by Rahman (2019). Rahman ranked egg susceptibility to simulated rainfall on different parts of common cabbage plants as follows: adaxial leaf surfaces > petioles = abaxial leaf surfaces > stem, which fits well with our force data. In contrast, rain simulations showed no difference in egg susceptibility to rainfall in various parts of Chinese cabbage plants, while we observed a stronger egg adhesion on the younger leaf petioles and the older abaxial lamina. Differences in experimental conditions and cultivars between the previous rain simulations and our pull-off force tests may account for this discrepancy, although the plant ages were nearly comparable.

In line with our findings on pull-off forces, eggs laid on older common cabbage leaves were less susceptible to rainfall than those on younger ones. In Chinese cabbage, egg susceptibility to rainfall was unaffected by leaf age (Rahman 2019; Rahman et al. 2019). However, pull-off forces were higher on younger abaxial leaves compared to older ones, and higher on older adaxial leaves and petioles compared to younger ones. Rahman also demonstrated that eggs laid in the leaf veins of common cabbage plants were less easily dislodged by rainfall than eggs on other parts of the plant (Rahman 2019). Eggs laid singly were more easily detached by water droplets compared to clustered eggs. These aspects have often been overlooked in force experiments, yet they represent valuable questions for future research that need to be addressed. Also, the bond between *P. xylostella* eggs and leaves weakens with ageing (Rahman 2019), likely due to the effects of time and the expansion of particularly young

leaves, as observed for *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on cotton (Kyi et al. 1991), which is an interesting point to be included in further egg adhesion experiments. Furthermore, the egg adhesives of various herbivorous insects contain elicitors that also contribute to inducing plant defences against the eggs or hatching larvae. This fact suggests that plants have not only evolved physical and chemical mechanisms to prevent insect attachment but also receptors that can recognise certain substances in the egg adhesive, adding another dimension to this topic (Hilker and Fatouros 2015; Hundacker et al. 2021; Caarls et al. 2023; Hilker et al. 2023).

The present results allow further integration with previous data, as discussed in the following paragraphs.

Knowledge integration: egg adhesion and the influence of weather

Insect eggs are exposed to various abiotic environmental factors, including radiation, wind, and precipitation. Rainfall is a significant mortality factor in *P. xylostella* eggs, which are often washed or shaken off, inflecting plants by the impact of raindrops (Annamalai et al. 1988; Kobori and Amano 2003). Water sprinkling can also dislodge eggs and larvae from leaves, especially from those with thick wax layers (Harcourt 1957; Nakagome and Kato 1974; Eckenrode et al. 1986; Talekar 1986; Wakisaka et al. 1990; Rahman 2019).

Raindrops have diameters of several millimetres and reach mean fall velocities of approximately 9-10 m s^{-1}, exerting considerable forces upon impact (Gatlin et al. 2015; Serio et al. 2019). The dynamics of drop impact involve several variables, including force, drag, pressure, surface wettability, and stress distributions, which are influenced by drop size, shape, and release height (Cheng et al. 2022). Depending on the material properties of the substrate, drops may bounce, splash, or spread, transferring kinetic energy when they strike leaf surfaces. Bhosale et al. (2020) described the complex elastic responses of leaves to raindrop impacts, including bending, torsion, and flapping (secondary torsion). Leaves behave similarly to damped harmonic oscillators, reaching amplitudes of several centimetres for large, thin leaves at a velocity of up to 50 m s^{-1} (Gart et al. 2015; Kim et al. 2021; Gilet and Tardrist 2025).

When raindrops hit a pruinose plant surface, part of the wax bloom can be removed (Kimura et al. 1987; Rahman 2019). Fragile wax filaments (dendrites) may fracture (Baker and Hunt 1986) and adhere easily to the surface of the water droplet, potentially washing off moth eggs along with the wax bloom due to the low contact area of the egg adhesive with the rough, waxy leaf coverage (Fig. 8G and H). This phenomenon resembles the self-cleaning Lotus effect described by Barthlott and Neinhuis (1997). On hydrophobic plant surfaces, water droplets bead up (splashing). They may dislodge spores and tiny objects, such as arthropod eggs (Davies 1961; Soto et al. 2014). Additionally, Kim et al. (2021) noted that a vortex ring forms during raindrop impact, which can carry micron-sized spores away from infested leaf surfaces.

The initial phase of drop impact causes a hydraulic shock that lasts for nanoseconds, during which the water mass is abruptly halted, resulting in a change in momentum for the molecules involved (Soto et al. 2014). A shock wave travels through the deforming drop at the sound velocity, exerting pressure as a result. The second phase of impact (crashing time), which lasts for milliseconds, reduces the drop's velocity to zero, applying dynamic pressure to the affected area. This phase is crucial for energy conversion (Soto et al. 2014; Roth-Nebelsick et al. 2022). Given that a drop can impact at speeds of up to 40 m s^{-1} and a drop sound velocity of 1480 m s^{-1}, the pressures involved can exceed 1 MPa, making the elastic responses of leaves essential during these impacts (Roth-Nebelsick et al. 2022). These pressures, along with the resulting stresses and leaf deflections, can overcome the adhesive strength of *P. xylostella* eggs (which ranges from 0.4 to 6.8 mN on host plants and can be as high as 198 kPa on hydrophilic glass), potentially detaching the eggs through washing-off or shaking-off (Fig. 8). The moth's preference for ovipositing on stems, veins, and alongside trichomes (Rahman 2019; present study) appears to be not only a strategy to avoid rain but also a means to escape high pressures and leaf deflections caused by drop impact. Stems and petioles are more stable structures that are less likely to bend or flap compared to leaf laminae. Millimetre-sized trichomes can significantly influence drop impact by absorbing energy and suppressing splashing (Nasto et al. 2019). In a study on Pieridae butterflies, Peters et al. (2024) observed that oviposition away from leaves can also lower the risk of eggs being harmed by plant leaf cell death and hypersensitive responses.

Knowledge integration: ecological-evolutionary perspective

Insect oviposition has been viewed as a complex tradeoff influenced by various factors that can sometimes be contradictory. These factors include egg-killing plant traits (Griese et al. 2021), host plant range, clutch size, host quality, predation and parasitism risks, larval mobility, host-finding capability, microclimate, and more (Janz 2002). Ovipositing females face several selection pressures related to their fitness, energy-efficient metabolism, and behaviour.

Importantly, female host plant preferences do not always align perfectly with larval performance. Most insect larvae, including those of diamondback moths, prefer and perform better on younger leaves (although more exposed to abiotic impacts), which tend to have higher concentrations of

defense chemicals, more nutrients, and water; they are softer and less fibrous than mature or senescent leaves ("plant-age hypothesis") (Scheirs and De Bruyn 2002; Moreira et al. 2016). For neonate *P. xylostella*, waxy surfaces of *Brassica oleracea* var. *capitata* provide a suitable substrate, causing them to spend significantly more time palpating, biting, and spinning silk (Eigenbrode et al. 1991). The "preference-performance hypothesis" suggests that oviposition preference should align with host suitability. Female phytophagous insects tend to select host plants that optimise the fitness of their progeny (Jaenike 1978; Price 1990; Gripenberg et al. 2010), as demonstrated in experiments with *P. xylostella* by Zhang et al. (2012). Uematsu and Sakanoshita (1989) noted that the oviposition site preference of *P. xylostella* is not linked to factors that affect egg attachment; instead, these factors influence preferences prior to egg release. As a result, glossy surfaces that facilitate stronger egg adhesion are not necessarily favoured over waxy ones by ovipositing *P. xylostella* (Talekar and Shelton 1993). Conversely, eggs are primarily concentrated in the lower leaf stratum of waxy cabbage (Ang et al. 2014), supporting the food supply for hatching larvae that have limited mobility (Silva and Furlong 2012). The preference for pruinose surfaces for laying eggs may also be attributed to the effect that eggs deposited on waxier Brassicaceae induce little or no plant cell death and do not trigger hypersensitive responses (Fatouros et al. 2012; Griese et al. 2021; Bassetti et al. 2022). However, Rahman et al. (2023) observed fewer eggs on waxy plant parts that wash away easily with rain, with these inadequate substrates causing moths to cluster the eggs together. The authors concluded that females may alter their behaviour to enhance egg survival when suitable oviposition substrates are lacking. Cryo-SEM images further suggest that the volume of egg adhesive can be adjusted to meet these requirements; specifically, the thicker the epicuticular wax layers, the more prominent the egg adhesive (Fig. 5E–O).

Placing eggs on pruinose substrates may provide enemy-free space for eggs and offspring, as many predators and parasitoids are hindered by epicuticular plant waxes (e.g., Eigenbrode 2004). Shorter egg incubation times can mitigate egg loss caused by rain impact, which can be counterbalanced by high fecundity. Similar to apple moths *C. pomonella* (Wood 1965; Aghdam et al. 2009; Blomefield and Giliomee 2009; Saeed et al. 2019; Saran and Genç 2021) and asparagus beetles *C. asparagi* (Witt and Edwards 2002), *P. xylostella* eggs usually hatch 2-6 days after oviposition (Harcourt 1957; Rosario and Cruz 1986; Liu et al. 2002; Gowri and Manimegalai 2016; Bhure et al. 2020; Gopika et al. 2021), with a female laying an average of 150 eggs and a maximum of up to 350 eggs throughout its lifespan (Gunn 1917; Marsh 1917; Tsedeler 1931; Harcourt 1957; Gopika et al. 2021). Unlike asparagus beetles, diamondback moths can establish large populations (Bigger and Fox 1997; Ferenca and Tamutis 2017), which helps cushion egg losses considerably.

Conclusions

Oligophagous diamondback moths lay their eggs on anti-adhesive plant surfaces, which are covered with epicuticular wax crystals. However, these oviposition sites do not always correspond to substrates with the strongest egg adhesion.

The greatest force required to remove eggs was observed on hydrophilic glass, indicating an adhesive strength of 198 kPa and a certain polar affinity of the egg adhesive. The viscous egg adhesive forms nanometer-thin films on smooth and less structured surfaces, embedding the epicuticular waxes. This adhesive primarily consists of proteins and lipids, which could inspire the development of advanced biomimetic adhesives for use on anti-adhesive substrates in extreme environments (related to previous descriptions of diamondback moths' habitats). Environmental influences on egg adhesion require further detailed investigation.

By understanding the attachment and adhesive properties of eggs, we can develop more effective strategies for plant protection, thereby enhancing pest control. This study gives us at least three starting points for further exploration. The first is to modify plant surface structures (such as waxes and trichomes) through breeding techniques to increase their tolerance to diamondback moths. The second option is to alter the formulation of plant protection products to incorporate mechanisms that interfere with egg adhesion, thereby enhancing their efficacy. The third approach focuses on physical plant protection measures, including mechanical removal of herbivorous eggs from plants by mechanical impact, such as sprinkling drops and plant vibrations.

Comprehensive and integrative research approaches will provide deeper insights into the evolution and ecology of insect oviposition and egg adhesion.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11829-025-10190-w.

Acknowledgements The first author thanks Thomas Thieme and Christoph Buuk (BTL Bio-Test Labor GmbH Sagerheide, Sanitz, Germany) for bringing diamondback moths to our attention. The team of DataPhysics Instruments GmbH (Filderstadt, Germany) granted access to the SCA 20 Demo software. We are highly grateful for the reviewers' constructive comments.

Author contributions D.V. devised the project, prepared the materials, performed biophysical measurements, microscopic analyses, and evaluations, and received project funding. C.U.B. provided the insect stock population and rearing information. A.J. conducted and evaluated Raman spectroscopy. D.V. wrote and edited the manuscript. All authors have approved the final version of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. This study was partially supported by tax funds allocated in the budget approved by the Landtag of the Free State of Saxony, Germany, to DV, project "Biomimetic surfaces for pollutant separation (BIOS)".

Data availability All data needed to evaluate the conclusions in the paper are present in the article and/or the Supplementary Materials.

Declarations

Conflict of interest The authors declare no competing interests.

References

Adiyodi KG, Adiyodi RG (1976) Morphology and cytology of the accessory sex glands in invertebrates. Int Rev Cytol 43:353–398. https://doi.org/10.1016/S0074-7696(08)60072-8

Aghdam HR, Fathipour RG, Rezapanah M (2009) Temperature-dependent development and temperature thresholds of codling moth (Lepidoptera: Tortricidae) in Iran. Env Entomol 38:885–895. https://doi.org/10.1603/022.038.0343

Ahman I (1990) Plant-surface characteristics and movements of two *Brassica*-feeding aphids, *Lipaphis erysimi* and *Brevicoryne brassicae*. Symp Biol Hung 39:119–125

Al Bitar L, Gorb SN, Zebitz CPW, Voigt D (2012) Egg adhesion of the codling moth *Cydia pomonella* to various substrates: I. leaf surfaces of different apple cultivars. Arthropod-Plant Interact 6:471–488. https://doi.org/10.1007/s11829-012-9198-z

Al Bitar L, Gorb SN, Zebitz CPW, Voigt D (2014) Egg adhesion of the codling moth *Cydia pomonella* to various substrates: II. Fruit surfaces of different apple cultivars. Arthropod-Plant Interact 8:57–77. https://doi.org/10.1007/s11829-013-9288-6

Al Bitar L, Zebitz CPW, Gorb SN, Voigt D (2025) Egg adhesion of the codling moth *Cydia pomonella* L. (Lepidoptera, Tortricidae) to synthetic substrates. J Appl Entomol: submitted.

Amornsak W, Noda T, Yamashita O (1992) Accumulation of glue proteins in the developing colleterial glands of the silkworm *Bombyx mori*. J Sericult Sci Jpn 61:123–130

Ang GCK, Silva R, Maxwell SL, Zalucki MP, Furlong MJ (2014) Contrary effects of leaf position and identity on oviposition and larval feeding patterns of the diamondback moth. Entomol Exp Appl 151:86–96. https://doi.org/10.1111/eea.12172

Ang GCK, Zalucki MP, Furlong MJ (2016) Temporal changes in olfactory and oviposition responses of the diamondback moth to herbivore-induced host plants. Entomol Exp Appl 160:28–39. https://doi.org/10.1111/eea.12458

Annamalai S, Itô Y, Saito T (1988) Population fluctuations of the diamondback moth, *Plutella xylostella* (L.) on cabbages in *Bacillus thuringiensis* sprayed and non sprayed plots and factors affecting within-generation survival of immatures. Res Popul Ecol 30:329–342. https://doi.org/10.1007/BF02513253

Arai Y, Takeda N, Komoto S, Murakami H, Voigt D, Suzuki T (2025) Dual roles of salivary proteins in feeding and silk fiber coating in the spider mite *Tetranychus urticae*. Commun Biol: https://doi.org/10.1038/s42003-025-08885-0

Ashwini SB (2014) Biology and management of *Plutella xylostella* L. (Plutellidae: Lepidoptera) and *Spodoptera litura* F. (Noctuidae: Lepidoptera) on cabbage. PhD Thesis. Department of Agricultural Entomology, College of Agriculture, Raichur University of Agricultural Sciences

Badenes-Pérez FR, Heckel DG (2023) Intraspecific and interstage similarities in host-plant preference in the diamondback moth (Lepidoptera: Plutellidae). Horticulturae 9:39. https://doi.org/10.3390/horticulturae9010039

Badenes-Pérez FR, Gershenzon J, Heckel DG (2014) Insect attraction versus plant defense: young leaves high in glucosinolates stimulate oviposition by a specialist herbivore despite poor larval survival due to high saponin content. PLoS ONE 9:e95766. https://doi.org/10.1371/journal.pone.0095766

Baker EA, Hunt GM (1986) Erosion of waxes from leaf surfaces by simulated rain. New Phytol 102:161–173. https://doi.org/10.1111/j.1469-8137.1986.tb00807.x

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta 202:1–8. https://doi.org/10.1007/s004250050096

Barthlott W, Neinhuis C, Cutler D, Ditsch F, Meusel I, Theisen I, Wilhelmi H (1998) Classification and terminology of plant epicuticular waxes. Bot J Linn Soc 126:237–260. https://doi.org/10.1006/bojl.1997.0137

Bassetti N, Caarls L, Bukovinszkine'Kiss G, El-Soda M, van Veen J, Bouwmeester K, Zwaan BJ, Schranz ME, Bonnema G, Fatouros NE (2022) Genetic analysis reveals three novel QTLs underpinning a butterfly egg-induced hypersensitive response-like cell death in *Brassica rapa*. BMC Plant Biol 22:140. https://doi.org/10.1186/s12870-022-03522-y

Beament JWL, Lal R (1957) Penetration through the egg-shell of *Pieris brassicae* (L.). Bull Entomol Res 48:109–125. https://doi.org/10.1017/S0007485300054134

Betz O (2010) Adhesive exocrine glands in insects: morphology, ultrastructure, and adhesive secretion. In: von Byern J, Grunwald I (eds) Biological adhesive systems from nature to technical and medical application. Springer, Wien, pp 111–152

Bhosale Y, Esmaili E, Bhar K, Jung S (2020) Bending, twisting and flapping leaf upon raindrop impact. Bioinspir Biomim 15:036007. https://doi.org/10.1088/1748-3190/ab68a8

Bhure KY, Pandya HV, Naikwadi BV (2020) Biology of diamondback moth, *Plutella xylostella* (Linnaeus), (Lepidoptera: Plutellidae) of cabbage under laboratory condition. J Entomol Zool Stud 8:1627–1631

Bigger DS, Fox LR (1997) High-density populations of diamondback moth have broader host-plant diets. Oecologia 112:179–186. https://doi.org/10.1007/s004420050298

Blomefield TL, Giliomee JH (2009) Development rates of the embryonic and immature stages of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), at constant and fluctuating temperatures. Afr Entomol 17:183–191. https://doi.org/10.4001/003.017.0208

Bonebrake TC, Guo F, Dingle C, Baker DM, Kitching RL, Ashton LA (2019) Integrating proximal horizon threats to biodiversity and conservation. Trends Ecol Evol 34:781–788. https://doi.org/10.1016/j.tree.2019.04.001

Borne F, Kovalev A, Gorb S, Courtier-Orgogozo V (2020) The glue produced by *Drosophila melanogaster* for pupa adhesion is universal. J Exp Biol 223:jeb220608. https://doi.org/10.1242/jeb.220608

Bräuer P, Neinuis C, Voigt D (2017) Attachment of honeybees and greenbottle flies to petal surfaces. Arthropod-Plant Interact 11:171–192. https://doi.org/10.1007/s11829-016-9478-0

Brennan EB, Weinbaum SA (2001) Stylet penetration and survival of three psyllid species on adult leaves and waxy and de-waxed juvenile leaves of *Eucalyptus globulus*. Entomol Exp Appl 100:355–363. https://doi.org/10.1046/j.1570-7458.2001.00883.x

Bukovinsky T, Potting RP, Clough Y, van Lenteren JC, Vet LEM (2005) The role of pre and post-alighting detection mechanisms in the response to patch size by specialist herbivores. Oikos 109:435–446. https://doi.org/10.1111/j.0030-1299.2005.13707.x

Burkhart CN, Stankiewicz BA, Pchalek I, Kruge MA, Burkhart CG (1999) Composition of the louse sheath. J Parasitol 85:559–561

Büscher T, Lohar R, Kaul M-C, Gorb SN (2020) Multifunctional adhesives on the eggs of the insect *Phyllium philippinicum* (Phasmatodea: Phylliidae): solvent influence and biomimetic implications. Biomimetics 5:66. https://doi.org/10.3390/biomimetics5040066

Caarls L, Bassetti N, Verbaarschot P, Mumm R, van Loon JJA, Schranz ME, Fatouros NE (2023) Hypersensitive-like response induced by cabbage white butterflies is specifically induced by molecules from egg-associated secretions in *Brassica* plants. Front Ecol Evol 10:1070859. https://doi.org/10.3389/fevo.2022.1070859

Chan NJ, Lentz S, Gurr PA, Tan S, Schultebeyring M, Rosenfeldt S, Schenk A, Scheibel T, Qiao GG (2025) Robust β-sheet peptide reinforced polymer fibers. Small Sci. https://doi.org/10.1002/smsc.202500115

Cheng X, Sun T-P, Gordillo L (2022) Drop impact dynamics: impact force and stress distributions. Ann Rev Fluid Mech 54:57–81. https://doi.org/10.1146/annurev-fluid-030321-103941

Couty A, van Emden H, Perry JN, Hardie JIM, Picket JA, Wadham LJ (2006) The roles of olfaction and vision in host-plant finding by the diamondback moth *Plutella xylostella*. Physiol Entomol 31:134–145. https://doi.org/10.1111/j.1365-3032.2006.00499.x

Craig CL (1997) Evolution of arthropod silks. Annu Rev Entomol 42:231–267. https://doi.org/10.1146/annurev.ento.42.1.231

Czamara K, Majzner K, Pilarczyk M, Kochan K, Kaczor A, Baranska M (2014) Raman spectroscopy of lipids: a review. J Raman Spectrosc 46:4–20. https://doi.org/10.1002/jrs.4607

Davies RR (1961) Wettability and the capture, carriage and deposition of particles by raindrops. Nature 191:616–617. https://doi.org/10.1038/191616a0

Eckenrode CJ, Dickson MH, Lin J (1986) Resistance in crucifers to diamondback moth and other lepidopterous pests. In: Talekar NS, Griggs TG (eds) Diamondback moth management, Proceedings of the first international workshop. Asian Vegetable Research and Development Center, Tainan, Taiwan, pp 129–136

Eigenbrode SD (2004) The effects of plant epicuticular waxy blooms on attachment and effectiveness of predatory insects. Arthr Struct Dev 33:91–102. https://doi.org/10.1016/j.asd.2003.11.004

Eigenbrode SD, Espelie KE, Shelton AM (1991) Behavior of neonate diamondback moth larvae (*Plutella xylostella* (L.)) on leaves and on extracted leaf waxes of resistant and susceptible cabbages. J Chem Ecol 17:1691–1704. https://doi.org/10.1007/BF00984697

Eigenbrode SD, Castagnola T, Roux M-B, Steljes L (1996) Motility of three generalist predators is greater on cabbage with glossy leaf wax than on cabbage with a wax bloom. Entomol Exp Appl 81:335–343. https://doi.org/10.1046/j.1570-7458.1996.00104.x

Fatouros NE, Lucas-Barbosa D, Weldegergis BT, Pashalidou FG, van Loon JJA, Dicke M, Harvey JA, Gols R, Huigens ME (2012) Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. PLoS ONE 7:e43607. https://doi.org/10.1371/journal.pone.0043607

Ferenca R, Tamutis V (2017) New data of common Asparagus beetle *Crioceris asparagi* (Linnaeus, 1758) (Coleoptera, Chrysomelidae) in Lithuania. Liet Entomol Draugijos Darb 1:13–16

Flammang P, Lambert A, Bailly P, Hennebert E (2009) Polyphosphoprotein-containing marine adhesives. J Adhes 85:447–464. https://doi.org/10.1080/00218460902996358

Fordyce JA, Nice CC (2003) Variation in butterfly egg adhesion: adaptation to local host plant senescence characteristics? Ecol Lett 6:23–27. https://doi.org/10.1046/j.1461-0248.2003.00389.x

Furlong MJ, Shi Z-h, Liu S-S, Zalucki MP (2004) Evaluation of the impact of natural enemies on *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) populations on commercial *Brassica* farms. Agric Forest Entomol 6:311–322. https://doi.org/10.1111/j.1461-9555.2004.00228.x

Gart S, Mates JE, Magaridis CM, Jung S (2015) Droplet impacting cantilever: a leaf-raindrop system. Phys Rev Appl 3:044019. https://doi.org/10.1103/PhysRevApplied.3.044019

Gatlin PN, Thurai M, Bringi VN, Petersen W, Wolff D, Tokay A, Carey L, Wingo M (2015) Searching for large raindrops: a global summary of two-dimensional video disdrometer observations. J Appl Meteorol Climatol 54:1069–1089. https://doi.org/10.1175/JAMC-D-14-0089.1

Gilet T, Tadrist L (2025) Leaf oscillation and upward ejection of droplets in response to drop impact. Phys Rev Fluids 10:053601. https://doi.org/10.1103/PhysRevFluids.10.053601

Gopika R, Sonkamble MM, Bantewad SD (2021) Biology and morphometry of diamondback moth, *Plutella xylostella* on cabbage. Pharma Innov J 10:427–430

Gorb S, Gorb E (2002) Attachment ability of the beetle *Chrysolina fastuosa* on various plant surfaces. Entomol Exp Appl 105:13–28. https://doi.org/10.1046/j.1570-7458.2002.01028.x

Gorb S, Gorb E (2006) Do plant waxes make insect attachment structures dirty? Experimental evidence for the contamination hypothesis. In: Herrel A, Speck T, Rowe NP (eds) Ecology and biomechanics. CRC Press, Taylor & Francis, Boca Raton, pp 147–162

Gorb E, Böhm S, Jacky N, Maier L-P, Dening K, Pechook S, Pokroy B, Gorb S (2014) Insect attachment on crystalline bioinspired wax surfaces formed by alkanes of varying chain lengths. Beilstein J Nanotechnol 5:1031–1041. https://doi.org/10.3762/bjnano.5.116

Gowri G, Manimegalai K (2016) Biology of diamondback moth, *Plutella xylostella* (lepidoptera: plutellidae) of cauliflower under laboratory condition. Int J Fauna Biol Stud 3:29–31

Graham LD (2018) Biological adhesives. In: Wnek GE, Bowlin GL (eds) Encyclopedia of biomaterials and biomedical engineering, vol 1, 2nd edn. Taylor & Francis, Boca Raton, pp 236–253

Griese E, Caarls L, Bassetti N, Mohammadin S, Verbaarschot P, Bukovinszkine'Kiss G, Poelman EH, Gols R, Schranz ME, Fatouros NE (2021) Insect egg-killing: a new front on the evolutionary arms-race between brassicaceous plants and pierid butterflies. New Phytol 230:341–353. https://doi.org/10.1111/nph.17145

Gripenberg S, Mayhew PJ, Parnell M, Roslin T (2010) A meta-analysis of preference-performance relationships in phytophagous insects. Ecol Lett 13:383–393. https://doi.org/10.1111/j.1461-0248.2009.01433.x

Gunn D (1917) The small cabbage moth (*Plutella maculipennis* Curtis). Union South Africa Dep Agric Bull 8:1–10

Gupta PD, Thorsteinson AJ (1960) Food plant relationships of the diamondback moth (*Plutella maculipennis* (Curt.)). II. Sensory regulation of oviposition of the adult female. Entomol Exp Appl 3:305–314. https://doi.org/10.1111/j.1570-7458.1960.tb00459.x

Habenicht G (2009) Kleben. Grundlagen, Technologien, Anwendungen. Aktualisierte Auflage, Vol 6. Springer-Verlag, Berlin, Heidelberg

Harcourt DG (1957) Biology of the diamondback moth, *Plutella maculipennis* (Curt.) (Lepidoptera: Plutellidae), in Eastern Ontario. Part II. Life-history, behaviour, and host relationships. Can Entomol 89:554–564. https://doi.org/10.4039/Ent89554-12

Hardy E (1938) *Plutella maculipennis*, Curt., its natural and biological control in England. Bull Entomol Res 29:343–372. https://doi.org/10.1017/S0007485300026274

Hemmerich S (2007) Protein sulfation. In: Lajtha A, Banik N (eds) Handbook of neurochemistry and molecular neurobiology. Springer-Verlag, Berlin, Heidelberg, pp 283–302

Hennebert E, Gregorowicz E, Flamman P (2019) Involvement of sulfated biopolymers in adhesive secretions produced by marine invertebrates. Biol Open 7:bio037358. https://doi.org/10.1242/bio.037358

Hilker M, Fatouros NE (2015) Plant responses to insect egg deposition. Ann Rev Entomol 60:493–515. https://doi.org/10.1146/annurev-ento-010814-020620

Hilker M, Meiners T (2006) Early herbivore alert, insect eggs induce plant defense. J Chem Ecol 32:1379–1396. https://doi.org/10.1007/s10886-006-9057-4

Hilker M, Stein C, Schröder R, Varama M, Mumm R (2005) Insect egg deposition induces defence responses in *Pinus sylvestris*: characterisation of the elicitor. J Exp Biol 208:1849–1854. https://doi.org/10.1242/jeb.01578

Hilker M, Salem H, Fatouros NE (2023) Adaptive plasticity of insect eggs. Ann Rev Entomol 68:451–469. https://doi.org/10.1146/annurev-ento-120120-100746

Hinton HE (1981) Biology of insect eggs, vol I–III. Pergamon Press, Oxford

Holloway PJ (1969a) Chemistry of leaf waxes in relation to wetting. J Sci Food Agric 20:124–128. https://doi.org/10.1002/jsfa.2740200214

Holloway PJ (1969b) The effects of superficial wax on leaf wettability. Ann Appl Biol 63:145–153. https://doi.org/10.1111/j.1744-7348.1969.tb05475.x

Holloway PJ, Brown GA (1977) The ketol constituents of *Brassica* epicuticular waxes. Chem Phys Lett 19:1–13. https://doi.org/10.1016/0009-3084(77)90077-9

Howlett BG, Clarke AR (2003) Role of foliar chemistry versus leaf-tip morphology in egg-batch placement by *Chrysophtharta bimaculata* (Olivier) (Coleoptera, Chrysomelidae). Aust J Entomol 42:144–148. https://doi.org/10.1046/j.1440-6055.2003.00331.x

Hundacker J, Bittner N, Weise C, Brohan G, Varama M, Hilker M (2021) Pine defense against eggs of an herbivorous sawfly is elicited by an annexin-like protein present in egg-associated secretion. Plant Cell Environ 45:1033–1048. https://doi.org/10.1111/pce.14211

Jaenike J (1978) On optimal oviposition behavior in phytophagous insects. Theor Popul Biol 14:350–356. https://doi.org/10.1016/0040-5809(78)90012-6

Janz N (2002) Evolutionary ecology of oviposition strategies. In: Hilker M, Meiners T (eds) Chemoecology of insect eggs and egg deposition. Blackwell Verlag GmbH, Berlin, Vienna, pp 349–376

Jeffree CE (1986) The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In: Juniper BE, Southwood TRE (eds) Insects and the plant surface. Edward Arnold Publishers Ltd., London, pp 123–164

Jin Y, Chen YL, Jiang Y, Xu M (2006) Proteome analysis of the silkworm (*Bombyx mori* L.) colleterial gland during different development stages. Arch Insect Biochem Physiol 61:42–50. https://doi.org/10.1002/arch.20095

Juniper BE (1995) Waxes on plant surfaces and their interactions with insects. In: Hamilton RJ (ed) Waxes: chemistry, molecular biology and functions. The Oily Press, Dundee, UK, pp 157–174

Justus KA, Mitchell BK (1996) Oviposition site selection by the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). J Insur Issues 9:887–898. https://doi.org/10.1007/BF02208976

Justus KA, Mitchell BK (1999) Reproductive morphology, copulation, and inter-populational variation in the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Int J Ins Morphol Embryol 28:233–246. https://doi.org/10.1016/S0020-7322(99)00027-6

Justus KA, Lloyd MD, Mitchell BK (2000) Oviposition by *Plutella xylostella* (lepidoptera: plutellidae) and effects of phylloplane waxiness. J Econ Entomol 93:1152–1159. https://doi.org/10.1603/0022-0493-93.4.1152

Kamino K (2006) Barnacle underwater attachment. In: Smith A, Callow J (eds) Biological adhesives. Springer, Berlin Heidelberg, pp 145–166

Kanno H, Harris MO (2000) Physical features of grass leaves influence the placement of eggs within the plant by the Hessian fly. Entomol Exp Appl 96:69–80. https://doi.org/10.1046/j.1570-7458.2000.00680.x

Kause A, Ossipov V, Haukioja E, Lempa K, Hanhimäki S (1999) Multiplicity of biochemical factors determining quality of growing birch leaves. Oecologia 120:102–112. https://doi.org/10.1007/s004420050838

Kim J, Lee D, Park S, Clark JM, Lee SH (2021) Characterization of nit sheath protein functions and transglutaminase-mediated crosslinking in the human head louse, *Pediculus humanus capitis*. Parasites Vectors 14:425. https://doi.org/10.1186/s13071-021-04914-z

Kimura T, Fujimura T, Araya E (1987) Overwintering of the diamondback moth, *Plutella xylostella* Linne, in Aomori Prefecture. 3. Mortality of the second instar larvae under the snow. Annu Rep Soc Plant Prot North Jpn 38:141–142. https://doi.org/10.11455/kitanihon1966.1987.141

Kobori Y, Amano H (2003) Effect of rainfall on a population of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Appl Entomol Zool 38:249–253. https://doi.org/10.1303/aez.2003.249

Kyi A, Zalucki MP, Titmarsh IJ (1991) An experimental study of early stage survival of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on cotton. Bull Entomol Res 81:263–271. https://doi.org/10.1017/S0007485300033526

Larsson S, Ohmart Csiro CP (1988) Leaf age and larval performance of the leaf beetle *Paropsis atomaria*. Ecol Entomol 13:19–24. https://doi.org/10.1111/j.1365-2311.1988.tb00329.x

Lasota JA, Kok LT (1989) Seasonal abundance of imported cabbageworm (Lepidoptera: Pieridae), cabbage looper (Lepidoptera: Noctuidae), and diamondback moth (Lepidoptera: Plutellidae) on cabbage in southwestern Virginia. J Econ Entomol 82:811–818. https://doi.org/10.1093/jee/82.3.811

Li D, Huson MG, Graham LD (2008) Proteinaceous adhesive secretions from insects, and in particular the egg attachment glue of *Opodiphthera* sp. moths. Arch Ins Biochem Physiol 69:85–105. https://doi.org/10.1002/arch.20267

Liang C, Strickland J, Ye Z, Wu W, Hu B, Rittschof D (2019) Biochemistry of barnacle adhesion: an updated review. Front Mar Sci 6:565. https://doi.org/10.3389/fmars.2019.00565

Liu S-S, Chen F-Z, Zalucki MP (2002) Development and survival of the diamondback moth (Lepidoptera: Plutellidae) at constant and alternating temperatures. Environ Entomol 31:221–231. https://doi.org/10.1603/0046-225X-31.2.221

Lutz TM, Kimna C, Casini A, Lieleg O (2022) Bio-based and bio-inspired adhesives from animals and plants for biomedical applications. Mater Today Bio 13:100203. https://doi.org/10.1016/j.mtbio.2022.100203

Mahesh S, Tang K-C, Raj M (2018) Amide bond activation of biological molecules. Molecules 23:2615. https://doi.org/10.3390/molecules23102615

Maiti NC, Apetri MM, Zagorski MG, Carey PR, Anderson VE (2004) Raman spectroscopic characterization of secondary structure in natively unfolded proteins: α-synuclein. J Am Chem Soc 126:2399–2408. https://doi.org/10.1021/ja0356176

Marazzi C, Patrian B, Städtler E (2004) Secondary metabolites of leaf surface affected by sulphur fertilization and perceived by the cabbage root fly. Chemoecol 14:87–94. https://doi.org/10.1007/s00049-003-0265-x

Marsh HO (1917) Life history of *Plutella maculipennis*, the diamondback moth. J Agric Res 10:1–13

McCall AC, Fordyce JA (2010) Can optimal defence theory be used to predict the distribution of plant chemical defences? J Ecol 98:985–992. https://doi.org/10.1111/j.1365-2745.2010.01693.x

Monier M, Courtier-Orgogozo V (2022) *Drosophila* glue: a promising model for bioadhesion. Insects 13:734. https://doi.org/10.3390/insects13080734

Monti P, Taddei P, Freddi G, Asakura T, Tsukada M (2021) Raman spectroscopic characterization of *Bombyx mori* silk fibroin: Raman spectrum of silk I. J Raman Spectrosc 32:103–107. https://doi.org/10.1002/jrs.675

Moreira LF, Teixeira NC, Santos NA, Valim JOS, Maurício RM, Guedes RNC, Oliveira MGA, Campos WG (2016) Diamondback moth performance and preference for leaves of *Brassica oleracea* of different ages and strata. J Appl Entomol 140:627–635. https://doi.org/10.1111/jen.12294

Müller C, Riederer M (2005) Plant surface properties in chemical ecology. J Chem Ecol 31:2621–2651. https://doi.org/10.1007/s10886-005-7617-7

Müller C, Rosenberger (2006) Different oviposition behaviour in chrysomelid beetles, characterisation of the interface between oviposition secretion and the plant surface. Arthropod Struct Dev 35:197–295. https://doi.org/10.1016/j.asd.2006.06.001

Nakagome T, Kato K (1974) Studies of forecasting of occurrence on diamondback moth, *Plutella xylostella* (L.), 1: on the seasonal prevalence of adults and larvae. Res Bull Aichi-Ken Agric Res Cent Ser B Hortic 6:19–24

Nasto A, Brun PT, Hosoi AE (2019) Drop impact on hairy surfaces. Phys Rev Fluids 4:064004. https://doi.org/10.1103/PhysRevFluids.4.064004

Ooka AA, Garrell RL (2000) Surface-enhanced raman spectroscopy of DOPA-containing peptides related to adhesive protein of marine mussel, *Mytilus edulis*. Biopolymers 57:92–102. https://doi.org/10.1002/(SICI)1097-0282(2000)57:2%3c92::AID-BIP6%3e3.0.CO;2-4

Peters DH, Greenberg LO, Fatouros NE (2024) Oviposition strategies in Pieridae butterflies and the role of an egg-killing plant trait therein. Ecol Evol 14:e11697. https://doi.org/10.1002/ece3.11697

Petrone L (2013) Molecular surface chemistry in marine bioadhesion. Adv Coll Interface Sci 195–196:1–18. https://doi.org/10.1016/j.cis.2013.03.006

Philips CR, Fu Z, Kuhar TP, Shelton AM, Cordero RJ (2014) Natural history, ecology, and management of diamondback moth (Lepidoptera: Plutellidae), with emphasis on the United States. J Integr Pest Manag 5:1–11. https://doi.org/10.1603/IPM14012

Pivnick KA, Jarvis BJ, Slater GP, Gillott C, Underhill EW (1990) Attraction of the diamondback moth (Lepidoptera: Plutellidae) to volatiles of oriental mustard: the influence of age, sex, and prior exposure to mates and host plants. Environ Entomol 19:704–709. https://doi.org/10.1093/ee/19.3.704

Potter K, Davidowith G, Woods HA (2009) Insect eggs protected from high temperatures by limited homeothermy of plant leaves. J Exp Biol 212:3448–3454. https://doi.org/10.1242/jeb.033365

Power AM, Klepal W, Zheden V, Jonker J, McEvilly P, von Byern J (2010) Mechanisms of adhesion in adult barnacles. In: von Byern J, Grunwald I (eds) Biological adhesive systems. Springer, Vienna, pp 153–168

Price PW (1990) The plant vigor hypothesis and herbivore attack. Oikos 62:244–251. https://doi.org/10.2307/3545270

Purdy SJ, Truter EV (1963) Constitution of the surface lipid from the leaves of *Brassica oleracea* var. *capitata* (Winnigstadt) I. Isolation and quantitative fractionation. Proc R Soc B-Biol Sci 158:536–543. https://doi.org/10.1098/rspb.1963.0062

Qiu Y-T, van Loon JJA, Roessingh P (1998) Chemoreception of oviposition inhibition terpenoids in the diamondback moth *Plutella xylostella*. Entomol Exp Appl 87:143–155. https://doi.org/10.1046/j.1570-7458.1998.00316.x

R Core Team (2021) R, a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org

Rahman M, Zalucki M, Furlong M (2019) Diamondback moth egg susceptibility to rainfall: effects of host plant and oviposition behaviour. Entomol Exp Appl 167:701–712. https://doi.org/10.1111/eea.12816

Rahman M, Zalucki M, Furlong M (2023) Diamondback moth egg susceptibility to rainfall: effects of leaf surface properties on the direct and indirect impact of simulated rain. Entomol Exp Appl 171:704–715. https://doi.org/10.1111/eea.13338

Rahman M (2019) Survival of diamondback moth eggs and neonates: the effects of rainfall. PhD Thesis, School of Biological Sciences, The University of Queensland, Australia

Ramsden N, Elek J (1998) Life cycle and development rates of the leaf beetle *Chrysophtharta agricola* (Chapuis) (Coleoptera: Chrysomelidae) on *Eucalyptus nitens* at two temperature regimes. Aust J Entomol 37:238–242. https://doi.org/10.1111/j.1440-6055.1998.tb01577.x

Raupp M, Werren JH, Sadof C (1988) Effects of short-term phenological changes in leaf suitability on the survivorship, growth, and development of gypsy moth (Lepidoptera: Lymantriidae) larvae. Environ Entomol 17:316–319. https://doi.org/10.1093/ee/17.2.316

Reddy GVP, Tabone E, Smith MT (2004) Mediation of host selection and oviposition behaviour in the diamondback moth *Plutella xylostella* and its predator *Chrysoperla carnea* by chemical cues from cole crops. Biol Control 29:270–277. https://doi.org/10.1016/S1049-9644(03)00162-2

Reed DW, Pivnick KA, Underhill EW (1989) Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella*, present in three species of Brassicaceae. Entomol Exp Appl 53:277–286. https://doi.org/10.1111/j.1570-7458.1989.tb03575.x

Renwick JAA (1989) Chemical ecology of oviposition in phytophagous insects. Experientia 45:223–228. https://doi.org/10.1007/BF01951807

Renwick JAA (2002) The chemical world of crucivores: lures, treats and traps. Entomol Exp Appl 104:35–42. https://doi.org/10.1007/978-94-017-2776-1_4

Rodrigues D, Pires-Moreira GR (1999) Feeding preferences of *Heliconius erato* (Lepidoptera – Nymphalidae) in relation to leaf age and consequences for larval performance. J Lepid Soc 53:108–113. https://doi.org/10.5281/zenodo.16581916

Rosario C, Cruz C (1986) Life cycle of diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Puerto Rico. J Agric Univ Puerto Rico 70:229–234. https://doi.org/10.46429/jaupr.v70i4.7099

Roth-Nebelsick A, Konrad W, Ebner M, Miranda T, Thielen S, Nebelsick JH (2022) When rain collides with plants – patterns and forces of drop impact and how leaves respond to them. J Exp Bot 73:1155–1175. https://doi.org/10.1093/jxb/erac004

Rygula A, Majzner K, Marzec KM, Kaczor A, Pilarczyk M, Baranska M (2013) Raman spectroscopy of proteins: a review. J Raman Spectrosc 44:1061–1076. https://doi.org/10.1002/jrs.4335

Saeed S, Jaleel W, Naqqash MN, Saeed Q, Zaka SM, Sarwar ZM, Ishtiaq M, Qayyum MA, Sial MU, Ul-Aine Q, Batool M, Khan KA, Ghramh HA, Hafeez M, Ansari MJ, Sharma GK (2019) Fitness

parameters of *Plutella xylostella* (L.) Lepidoptera; Plutellidae) at four constant temperatures by using age-stage, two-sex life tables. Saudi J Biol Sci 26:1661–1667. https://doi.org/10.1016/j.sjbs.2018.08.026

Sánchez-Hernández E, Martín-Ramos P, Niño-Sánchez J, Diez-Hermano S, Álvarez-Taboada F, Pérez-García R, Santiago-Aliste A, Martín-Gil J, Diez-Casero JJ (2023) Characterization of *Leptoglossus occidentalis* eggs and egg glue. Insects 14:396. https://doi.org/10.3390/insects14040396

Saran C, Genç H (2021) Determination of biological properties of *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera, Plutellidae). ÇOMÜ Zir Fak Derg (COMU J Agric Fac) 9:21–30. https://doi.org/10.33202/comuagri.899370

Sarfaz M, Dosdall LM, Keddie BA (2005) Evidence for behavioural resistance by the diamondback moth, *Plutella xylostella* (L.). JEN 129:340–341. https://doi.org/10.1111/j.1439-0418.2005.00969.x

Sarfaz M, Dosdall LM, Keddie BA (2006) Diamondback moth-host plant interactions: implications for pest management. Crop Prot 25:625–539. https://doi.org/10.1016/j.cropro.2005.09.011

Scheirs J, De Bruyn L (2002) Integrating optimal foraging and optimal oviposition theory in plant-insect research. Oikos 96:187–191. https://doi.org/10.1034/j.1600-0706.2002.960121.x

Schmitt M (1988) The Criocerinae: biology, phylogeny and evolution. In: Jolivet P, Petitpierre E, Hsiao TH (eds) Biology of Chrysomelidae. Kluwer Academic Publishers, Alphen aan den Rijn, pp 475–479

Serio MA, Carollo G, Ferro V (2019) Raindrop size distribution and terminal velocity for rainfall erosivity studies. A review. J Hyrol 576:210–228. https://doi.org/10.1016/j.jhydrol.2019.06.040

Shelton AM, Nault BA (2004) Dead-end trap cropping: a technique to improve management of the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). Crop Prot 23:497–503. https://doi.org/10.1016/j.cropro.2003.10.005

Silva R, Furlong MJ (2012) Diamondback moth oviposition: effects of host plant and herbivory. Entomol Exp Appl 143:218–230. https://doi.org/10.1111/j.1570-7458.2012.01255.x

Smith CM (2005) Plant resistance to arthropods. Molecular and conventional approaches. Springer, Berlin

Soto D, De Larivière AB, Boutillon X, Clanet C, Queré D (2014) The force of impacting rain. Soft Matter 10:4929. https://doi.org/10.1039/C4SM00513A

Spencer JL (1996) Waxes enhance *Plutella xylostella* oviposition in response to sinigrin and cabbage homogenates. Entomol Exp Appl 81:165–173. https://doi.org/10.1111/j.1570-7458.1996.tb02028.x

Spencer JL, Pillai S, Bernays EA (1999) Synergism in the oviposition behaviour of *Plutella xylostella*: sinigrin and wax compounds. J Insur Issues 12:483–500. https://doi.org/10.1023/A:1020914723562

Stewart RJ, Ransom TC, Hlady V (2011) Natural underwater adhesives. J Polymer Sci B 48:757–771. https://doi.org/10.1002/polb.22256

Stork NE (1980a) Experimental analysis of adhesion of *Chrysolina polita* (Chrysomelidae: Coleoptera) on a variety of surfaces. J Exp Biol 88:91–107. https://doi.org/10.1242/jeb.88.1.91

Stork NE (1980b) Role of waxblooms in preventing attachment to brassicas by the mustard beetle *Phaedon cochleariae*. Entomol Exp Appl 28:100–107. https://doi.org/10.1111/j.1570-7458.1980.tb02992.x

Tabashnik BE, Mau RFL (1986) Suppression of diamondback moth (Lepidoptera: Plutellidae) oviposition by overhead irrigation. J Econ Entomol 79:189–191. https://doi.org/10.1093/jee/79.1.189

Talekar NS (1986) Diamond moth management. Proceedings of the first international workshop. Asian Vegetable Research and Development Center, Tainan, Taiwan

Talekar NS, Shelton AM (1993) Biology, ecology, and management of the diamondback moth. Annu Rev Entomol 38:275–301. https://doi.org/10.1146/annurev.en.38.010193.001423

Talekar NS, Liu S-H, Chen C-L, Yiin Y-F (1994) Characteristics of oviposition of diamondback moth (Lepidoptera: Yponomeutidae) on cabbage. Zool Stud 33:72–77

Tsedeler OE (1931) Cabbage moth (*Plutella maculipennis* Curt.) in connection with cultural mustard. J Exp Agrochem South-East 9:165–195

Uematsu H, Sakanoshita A (1989) Possible role of cabbage leaf wax bloom in suppressing diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) oviposition. Appl Entomol Zool 24:253–257. https://doi.org/10.1303/aez.24.253

Ulmer B, Gillot C, Woods D, Erlandson M (2002) Diamondback moth, *Plutella xylostella* (L.), feeding and oviposition preferences on glossy and waxy *Brassica rapa* (L.) lines. Crop Prot 21:327–331. https://doi.org/10.1016/S0261-2194(02)00014-5

van Loon JJA, Blaakmeer A, Griepink FC, van Beek TA, Schoonhoven LM, de Groot AE (1992) Leaf surface compound from *Brassica oleracea* (Cruciferae) induces oviposition by *Pieris brassicae* (Lepidoptera: Pieridae). Chemoecol 3:39–44. https://doi.org/10.1007/BF01261455

Voigt D, Gorb SN (2010) Egg attachment of the asparagus beetle *Crioceris asparagi* to the crystalline waxy surface of *Asparagus officinalis*. Proc R Soc B-Biol Sci 277:895–903. https://doi.org/10.1098/rspb.2009.1706

Waite H, Andersen NH, Jewhurst S, Sun C (2005) Mussel adhesion: finding the tricks worth mimicking. J Adhes 81:297–317. https://doi.org/10.1080/00218460590944602

Wakisaka S, Tsukuda R, Nakasuji F (1990) Effects of natural enemies, rainfall, temperature and host plants on survival and reproduction of the diamondback moth. In: Talekar NS (ed) Diamondback moth and other crucifer pests: proceedings of the second international workshop. AVRDC, Tainan, pp 1–25

Walker AA, Church JS, Woodhead AL, Sutherland TD (2013) Silverfish silk is formed by entanglement of randomly coiled protein chains. Insect Biochem Mol Biol 43:572–579. https://doi.org/10.1016/j.ibmb.2013.03.014

Wang B, Wang J, Yu C, Luo S, Peng J, Li N, Wang T, Jiang L, Dong Z, Wang Y (2023) Sustained agricultural spraying: from leaf wettability to dynamic droplet impact behavior. Glob Chall 7:2300007. https://doi.org/10.1002/gch2.202300007

Williams EH (1981) Thermal influences on oviposition in the montane butterfly *Euphydryas gillettii*. Oecologia 50:342–346. https://doi.org/10.1007/BF00344974

Witt ABR, Edwards PB (2002) Aspects of the biology, distribution and host range of *Crioceris* sp. (col.: Chrysomelidae: Criocerinae), a potential biological control agent for *Asparagus asparagoides* in Australia. Biol Control 23:56–63. https://doi.org/10.1006/bcon.2001.0991

Wood TG (1965) Field observations on flight and oviposition of codling moth (*Carpocapsa pomonella* (L.)) and mortality of eggs and first-instar larvae in an integrated control orchard. N Z J Agric Res 8:1043–1059. https://doi.org/10.1080/00288233.1965.10423735

Yago M, Mitamura T, Abe S, Hashimoto S (2001) Adhesive strength of glue-like substances from the colleterial glands of *Antheraea yamamai* and *Rhodinia fugax*. Int J Wild Silkmoths Silk 6:11–15

Yoshida K, Nagata M (1997) Adhesive strength of the glue substances in the colleterial glands of the silkmoth *Bombyx mori*. J Seric Sci Jpn 66:453–456

Yu M, Hwang J, Deming TJ (1999) Role of $_{L}$-3,4-dihydroxyphenylalanine in mussel adhesive proteins. J Am Chem Soc 121:5825–5826. https://doi.org/10.1021/ja990469y

Zago HB, Barros R, Torres JB, Pratissoli D (2010) Egg distribution of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and the parasitism by *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Neotrop Entomol 39:241–347. https://doi.org/10.1590/s1519-566x2010000200015

Zhang P-J, Lu Y-b, Zalucki MP, Liu S-S (2012) Relationship between adult oviposition preference and larval performance of the diamondback moth, *Plutella xylostella*. J Pest Sci 85:247–252. https://doi.org/10.1007/s10340-012-0425-2

Zhu J-Y, Xiang Z-W, Yu W-Y, Zhang S-Z, Wang W-W, Liu T-X (2022a) *Pieris rapae* larvae facilitate the oviposition of *Plutella xylostella* by spinning silk on waxy host plants. Entomol Gen 42:641–648. https://doi.org/10.1127/entomologia/2022/1379

Zhu JY, Xiang ZW, Zhang SZ, Wu L-N, Xian T (2022b) Adaptations of *Plutella xylostella* adult females and larvae to waxy host plants. J Pest Sci 95:203–214. https://doi.org/10.1007/s10340-021-01366-3

Znidarcic D, Valic N, Trdan S (2008) Epicuticular wax content in the leaves of cabbage (*Brassica oleracea* L. var. *capitata*) as a mechanical barrier against three insect pests. Acta Agric Slov 91:361–370. https://doi.org/10.2478/v10014-008-0016-3