JOURNAL OF THE ROYAL SOCIETY Interface

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Cite this article: Szyndler MW, Haynes KF, Potter MF, Corn RM, Loudon C. 2013 Entrapment of bed bugs by leaf trichomes inspires microfabrication of biomimetic surfaces. J R Soc Interface 10: 20130174.
http://dx.doi.org/10.1098/rsif.2013.0174

Received: 23 February 2013
Accepted: 25 February 2013

Subject Areas:
biomimetics

Keywords:
insect, bioinspiration, insect–plant interaction

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rsif.2013.0174 or via http://rsif.royalsocietypublishing.org.

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Entrapment of bed bugs by leaf trichomes inspires microfabrication of biomimetic surfaces

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Resurgence in bed bug infestations and widespread pesticide resistance have greatly renewed interest in the development of more sustainable, environmentally friendly methods to manage bed bugs. Historically, in Eastern Europe, bed bugs were entrapped by leaves from bean plants, which were then destroyed; this purely physical entrapment was related to microscopic hooked hairs (trichomes) on the leaf surfaces. Using scanning electron microscopy and videography, we documented the capture mechanism: the physical impaling of bed bug feet (tarsi) by these trichomes. This is distinct from a Velcro-like mechanism of non-piercing entanglement, which only momentarily holds the bug without sustained capture. Struggling, trapped bed bugs are impaled by trichomes on several legs and are unable to free themselves. Only specific, mechanically vulnerable locations on the bug tarsi are pierced by the trichomes, which are located at effective heights and orientations for bed bug entrapment despite a lack of any evolutionary association. Using bean leaves as templates, we microfabricated surfaces indistinguishable in geometry from the real leaves, including the trichomes, using polymers with material properties similar to plant cell walls. These synthetic surfaces snag the bed bugs temporarily but do not hinder their locomotion as effectively as real leaves.

1. Introduction

Bed bugs are an ancient scourge that have made a dramatic comeback in recent years across the globe, infesting structures such as homes, hotels, schools, movie theatres and hospitals [1–3]. Historical reports describe the trapping of bed bugs in Balkan countries by leaves from bean plants strewn on the floor next to beds [4,5]. During the night, bed bugs walking on the floor would accumulate on these bean leaves, which were collected and burned the following morning to exterminate the bed bugs. The entrapment of bed bugs by the bean leaves was attributed to the action of microscopic plant hairs (trichomes) on the leaf surfaces that would entangle the legs of the bed bugs (figure 1). The decline of bed bug infestations in the 1940s and 1950s following the application of DDT and other potent pesticides legal at the time, and the distraction of the Second World War undoubtedly prevented the 1943 report 'The action of bean leaves against the bedbug' [5] from gaining as much attention as it would have otherwise. While bed bugs have no evolutionary association with bean plants, similar non-glandular trichomes for a variety of plant species have been documented to trap a number of insects in more natural settings [6–13]. This physical entrapment is a source of inspiration in the development of new and sustainable methods to control the burgeoning numbers of bed bugs. A purely physical management method has the additional advantage that it would avoid the problem of pesticide resistance that has been documented extensively for this insect [14–17].

We were motivated to identify the essential features of the capture mechanics of bean leaves to guide the design and fabrication of biomimetic

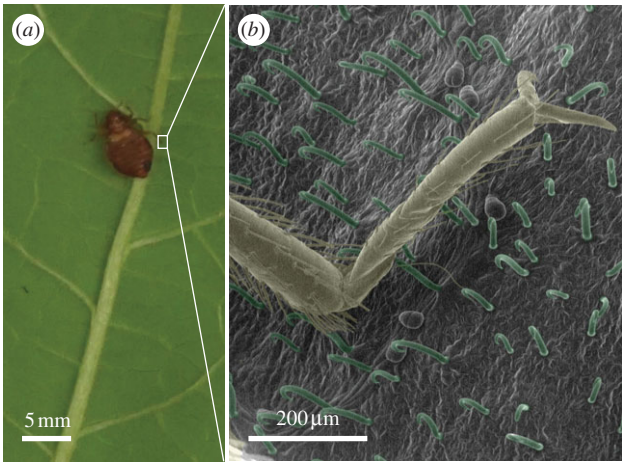

Figure 1. Bed bug standing on a kidney bean leaf. (*a*) Lower magnification image. (*b*) LV-SEM image of a hind leg of a bed bug (yellow) showing its size relative to the microscopic trichomes (green), which surround the tarsi.

surfaces for bed bug trapping. The interaction of bed bug tarsi with the microscopic plant trichomes was evaluated by videography and scanning electron microscopy (SEM). Microfabricated surfaces were generated using a template method and evaluated for hindrance of bed bug locomotion.

2. Material and methods

2.1. Experimental organisms

Kidney beans (*Phaseolus vulgaris* L.) were raised from seeds (Johnny's Seeds, Product 2554). Individual leaves (trifoliate, node ≥ 1) were severed where the base of petiole met the stem, were sealed in bags with moistened paper to remain hydrated prior to experimentation and were used within a few hours.

Bed bugs (*Cimex lectularius* L.) were raised at the University of Kentucky and were not fed within three weeks before use. All bugs used were male adults.

2.2. Imaging techniques

2.2.1. Videography

Digital movies of bugs walking on surfaces were acquired on a Sony HDR CX100 at 30 frames per second with a resolution of 2016 pixels by 1134 pixels (this corresponds to a spatial resolution of 0.1 mm for the field of view used). The camera was positioned in a vertical orientation, viewing the dorsal surface of a bug walking on a horizontally oriented leaf or microfabricated surface. A leaf or its fabricated analogue was placed with the abaxial side (undersurface) facing upward, and a single bug was introduced to the centre of the surface by gently tipping the bug from a glass vial approximately 2 cm above the surface. The abaxial side was used because it usually has a greater density of hooked trichomes than the adaxial side in many species, including the one used [9 11,18,19], although this is not universal [20,21]. All recordings were made at ambient temperature (22 24°C).

2.2.2. Scanning electron microscopy

All SEM imaging was performed on a FEI Quanta 3D FEG Dual Beam SEM (FEI, Hillsboro, Oregon). For low vacuum SEM (LV SEM), entrapped bugs on leaves were prepared by cutting the leaf around the impaled bug to a size approximating the size of an SEM stub, and mounting the leaf piece with its attached bug on the SEM stub with copper tape. In order to confirm and quantify the number of piercing trichomes, every specimen was repeatedly tilted to view underneath the tarsi of all six legs. For some bugs, we forcibly detached impaled bugs from leaves by pulling straight up with forceps, and immediately examined them upside down with LV SEM to look for attached broken trichomes or physical damage to the underside (ventral surface) of the bug legs. LV SEM images were attained at a pressure of 0.6 mbar and 5 kV with water as the ionizing gas. Bugs were still alive and resumed struggling after removal from the LV SEM.

For high vacuum SEM (HV SEM) imaging of replica materials, samples were sputtered with iridium (IBS/e, South Bay Technology, Inc.) with a 60° tilt angle and constant rotation for 4 min (approx. 5 nm iridium). Images of the microstructures were acquired at 5 kV.

2.2.3. Energy dispersive X ray spectroscopy

Energy dispersive X ray spectroscopy (EDS; 50 mm X MAX, Oxford Instruments, INCA 4.15) was performed using the FEI Quanta 3D FEG Dual Beam SEM (FEI, Hillsboro, OR, USA) on samples at 10 kV with a current of 0.75 nA at a working distance of 8 mm. Elemental mapping was executed over the desired area for 230 s to determine the presence of silicon. Carbon, oxygen, sulfur and iron were also imaged as controls to account for the topography of the sample surface (see the electronic supplementary material, figures S1 and S2). Fabricated surfaces were prepared as described earlier for HV SEM imaging, including sputtering with iridium. Natural leaf controls used the same EDS parameters, but in LV mode at 0.6 mbar and without sputtering.

2.3. Locomotion hindrance of bugs by surfaces

Digital movies were reviewed to identify changes in bug locomotion associated with mechanical interactions with the natural leaf or fabricated surfaces that interrupted normal movements. Incidents of momentary or prolonged struggling by the bug as one or more legs were stuck in place were tabulated. The number of locomotory cycles until a bug experienced a momentary snag and until entrapment by a leaf were counted ($n = 11$ bugs). One locomotory cycle refers to a single step taken by each of the six legs. The number of steps was used rather than time because bugs vary in their walking speed and number of pauses on the surface as most insects [22]. Also, the number of locomotory cycles directly represents the number of opportunities for leg/trichome encounters that can lead to piercing. Each bug, each leaf and each fabricated surface was used only once. One hundred and fifty eight bugs were run on 158 fabricated surfaces and evaluated for entrapment. Twenty eight of those 158 fabricated surfaces were selected for detailed counting of locomotory cycles.

2.4. Retention on leaves after initial entrapment

In order to determine whether a bug could move on a leaf after its initial entrapment, a time series of static images was obtained ($n = 6$ bugs). After entrapment by a leaf surface, an initial photo was immediately taken documenting the location of entrapment (using the same camera described earlier). Subsequent photos were taken after 10, 20 and 30 min. Images were imported into image analysis software (Canvas v. 12, ACD Systems International, USA), stacked, and oriented on the top of one another, lining up the leaf outlines. A circle (4 mm diameter) was centred over each bed bug for each of the four images and the centre to centre distance between these circles was calculated. We estimated that a displacement of approximately 6 mm of the circle centre would result if a pierced bed bug was able to rotate about a single leg impaled at its tip, and therefore a displacement of greater than 6 mm would indicate that the bug was able to free itself during that time interval.

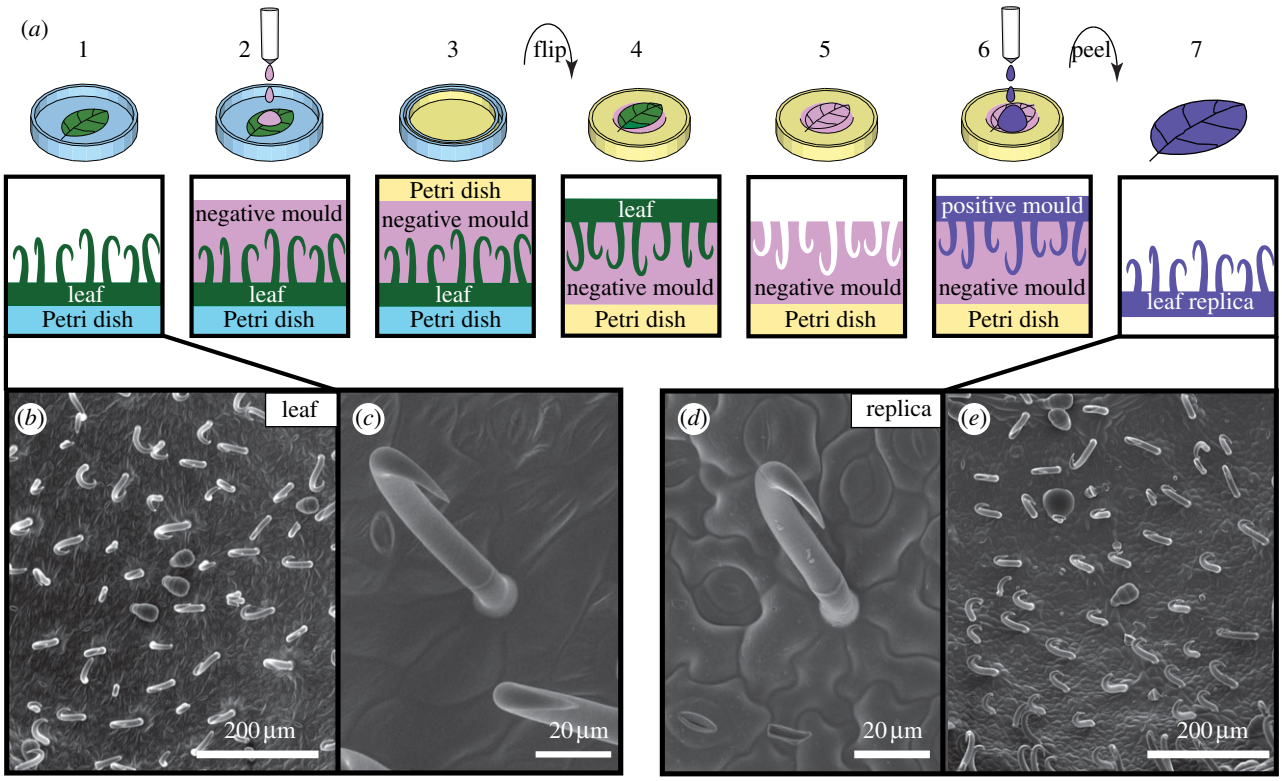

Figure 2. (*a*) Fabrication of biomimetic surfaces from bean leaves. (1–3) A negative moulding material is poured onto a leaf surface, and pressure is applied. (4–6) The leaf is removed, and the negative mould is filled with the positive replica material. (7) The negative mould is removed leaving the replica. (*b*,*c*) LV-SEM images of the bean leaf show the surface density of trichomes and the recurved, sharp trichome tips. (*d*,*e*) SEM images of the replicate materials appear identical to the natural leaves.

2.5. Trichome density

Trichome density (number of trichomes per area of leaf surface) was measured on leaves with impaled bugs, close to the points of capture. Lengths of leaves were measured from base to tip (not including the petiole) to the nearest millimetre. An LV SEM image of each leaf surface was acquired, opened in ImageJ and all trichomes on those images counted. An average of 42 trichomes were counted over an average area of 0.46 mm^2 per leaf ($n = 10$ leaves).

2.6. Microfabrication

Using kidney bean leaf surfaces as templates, we have constructed biomimetic polymeric surfaces (figure 2*a*). Our method for generating biomimetic leaf surfaces is based upon a double moulding process that had been shown to reproduce complex leaf architectures [23–25]. First, a leaf was placed in a Petri dish (100 cm^2 area) with its abaxial (undersurface) side facing upward. The negative polyvinylsiloxane moulding material (President Plus Jet Light Body, Affinis Light Body or Affinis Fast Light Body, Coltene Whaledent, Inc.) was then poured onto the leaf surface and the other side of the Petri dish was placed on the top of the negative moulding material with pressures ranging from 2 to 10 g cm^{-2} during polymerization. The leaf was then peeled off of the negative mould. The negative mould was subsequently filled with a positive moulding material and left to dry overnight prior to removal. A variety of polymeric positive moulding materials were used that replicated the plant microstructures successfully (figure 2*b*–*e*), including Loctite Heavy Duty Quick Set Epoxy (Henkel Corp.), Loctite Epoxy Extra Time (10 : 4 ratio, 1 : 1 ratio) (Henkel Corp.), T88 epoxy (Systems Three Resins, Inc.), Titebond III Wood Glue (Franklin International), Bob Smith Mid Cure 15 min Epoxy (Bob Smith Industries, Inc.) and Bob Smith Slow Cure 30 min Epoxy (Bob Smith Industries, Inc.). The mechanical properties of these polymers span the range expected for the cell walls of the natural plant trichomes (see §4).

Accurate replication of the sharp trichome tips is presumably crucial to facilitate piercing of the bed bug cuticle by synthetic trichomes. The sharpness of trichome tips from both natural and microfabricated surfaces was measured for several representative surfaces to the nearest half pixel (approx. 100 nm) in ImageJ software using SEM images to evaluate whether our synthetic trichomes were sufficiently sharp.

2.7. Incorporation of natural trichome tips into hybrid microfabricated surfaces

In addition to generating completely synthetic surfaces, we were also able to create surfaces that contained some percentage of hybrid trichomes: natural trichome tips incorporated onto synthetic trichome stalks. Synthetic trichome tips are usually indistinguishable from natural trichome tips in SEM (figure 2*c*,*d*), and therefore special analytical techniques are required to unambiguously identify whether a tip is natural or synthetic. EDS was used to reliably identify natural trichome tips by looking for the chemical signature of silicon, which was present in large amounts in the natural trichome tips [20,26] but not in the synthetic polymers used. EDS mapping was used to estimate the percentage of natural tips on 37 of the fabricated surfaces. Analysis of hybrid trichomes was performed by corroborating the elemental silicon maps with the electron image in the INCA software. Four representative areas (each of typical size approx. 0.25 mm^2; figure 2*e*) per surface were analysed and their trichome counts summed (an average of 95 trichomes were analysed per surface). A trichome was deemed 'hybrid' if there were more than 3 pixels with silicon signal that matched with a trichome in the electron image. The percent of hybrid trichomes was calculated from the number of hybrid trichomes (from the Si images) divided by the total number of trichomes (from the electron images). Identical measurements were made on real leaves to serve as a control ($n = 4$ leaves).

If any natural tips were snapped off of leaf trichomes during the generation of a negative mould, broken trichomes should be

visible on the leaf afterwards. To verify this step in the moulding process, we used LV SEM to examine the number of broken trichomes for 30 leaves after moulding. This analysis also used four images acquired on each leaf at different locations. The trichomes on each image were tallied to determine the number of sharp, intact trichome tips and the number of broken tips on the natural leaf surface (an average of 78 trichomes were evaluated per leaf). The data acquired for the four images were summed and the percentage of broken trichomes was calculated for each leaf. These 30 leaves were a subset of the 37 fabricated surfaces that were characterized by EDS (see above). We tested whether there was a significant correlation between the percentage of broken trichomes on the leaves and the percentage of hybrid trichomes on the fabricated surfaces made from those leaves.

The presence of broken natural trichome tips in the negative moulds after leaf removal was evaluated using SEM. The negative polyvinylsiloxane moulds were prepared for this analysis by freeze fracturing to generate a crack without surface deformations and mounted at a 90° angle with silver paint in order to observe the interface. These samples were sputtered (approx. 5 nm iridium) and observed in HV SEM at 5 kV.

2.8. Using the percentage of hybrid trichomes on a microfabricated surface to generate a standard for comparison with natural leaves

A fabricated surface could include both synthetic and hybrid trichomes. If only hybrid trichomes (with natural trichome tips) are capable of snagging or impaling bed bugs, the number of expected locomotory cycles to snag or impale can be estimated from the proportion of trichomes that are hybrid. For example, if half of the trichomes are hybrid, then twice as many cycles might be required to snag a bug compared with a real leaf if only the hybrid trichomes are functioning as natural trichomes. We used a conservative approach in our choice of a standard: the 90th percentile for the number of locomotory cycles that led to entrapment on natural leaves ($n = 11$ bugs). For 28 of the fabricated surfaces characterized by EDS, and therefore had known hybrid percentages, we calculated the expected number of locomotory cycles that would result in entrapment 90 per cent of the time if only the hybrid trichomes are functional:

$$\text{expected number of locomotory cycles} = \frac{\text{90th percentile standard}}{\text{percent hybrid trichomes}}. \quad (2.1)$$

We counted the number of locomotory cycles for a bug running on a fabricated surface until a momentary snag was observed (up to a maximum of 200 cycles or the expected number based on the hybrid percentage, whichever was smaller). This allowed us to compare the performance of the different surfaces in causing difficulties in locomotion.

2.9. Assessing damage to microfabricated surfaces caused by bug walking

Fabricated surfaces would not entrap bugs as effectively if the hooks on the fabricated surfaces simply snapped off without impaling the bed bugs. In order to evaluate whether hooks break when walked on by bugs, three surfaces were examined in SEM both before and after extensive exposure to bug contact during walking. These samples were attached to SEM stubs, and examined under LV SEM for hook number and integrity on four different areas (each with a surface area of 2 mm^2) on each of the three surfaces. The total area of each of the three samples of surfaces examined was approximately the same as the SEM stub: 130 mm^2. The approximate surface contact area for all six tarsi of a single male adult bed bug is 0.15 mm^2. Therefore, 870 locomotory cycles on average would be required for each part of a 130 mm^2 surface to be stepped on once (assuming each 0.15 mm^2 step is on a new area). In order to conservatively ensure that each part of the surface would get stepped on at least once, 10 bugs were placed on each surface (sealed inside a vial) and rotated slowly at 8 r.p.m. (Barnstead Thermodyne Labquake Rotisserie Model C400110) to gently agitate the bugs so that they continued to walk over the surface for 18 h; the total area impacted by 60 bug feet with eight locomotory cycles per minute on average over an 18 h period would be 10 times the area of the surface. After the surfaces had been thoroughly walked on by bugs as described, the same locations on these surfaces were re evaluated in SEM.

2.10. Statistical analyses

All analyses were performed using SAS statistical analysis software (v. 9.2; Cary, NC, USA). When doing linear regressions (using the GLM or general linear models procedure within SAS), graphs of the variables (or the residuals) were visually inspected to ensure that there were no patterns that would violate the underlying assumptions of using a linear regression (such as a nonlinear trend in the data, or heteroscedasticity). The data expressed as percentages (such as percent broken trichomes) were tested for deviation from normality using the 'proc univariate' procedure, and when deviations from normality were significant, they were transformed (using arcsine(square root(X))). Correlation between variables was evaluated using the 'proc corr' procedure. *T* tests used the 'proc ttest' procedure.

3. Results

3.1. Mechanism of bed bug entrapment by bean leaves

As a bed bug walked on a leaf, entanglement of any legs by trichomes caused a visible change in its walking behaviour. We have identified two discrete categories of entanglement: (i) a momentary snag of a leg with the bug able to break away (usually within a second; electronic supplementary material, movie S1) and (ii) a more lengthy and irreversible snag in which a visibly struggling bug is unable to pull away and is therefore considered 'trapped' by the leaf (see the electronic supplementary material, movie S2). It was usually impossible to see details of the trichome–bed bug interaction *in situ* using light microscopy because the trichomes are very small (approx. 10 μm in diameter and 50–100 μm high) and were often underneath the tarsi. In order to visualize the actions of the trichomes that corresponded to entrapment, we examined live bed bugs on leaves using LV-SEM after recording their entrapment. Every bug entrapped by a leaf had at least one piercing on one leg by a trichome ($n = 18$ bugs). 'Piercing' was defined as a clear and unambiguous penetration of the insect cuticle by the trichome tip (figure 3*a–c*); tilting the specimen was usually required for such confirmation because piercing generally occurred on the underside of the foot. The same legs that appeared irreversibly snagged on the leaves in the movies of the struggling bugs were confirmed as pierced in LV-SEM. Therefore, we conclude that piercing is necessary for entrapment. Occasionally, some legs were hooked by the trichomes (figure 3*d*), and we inferred that this hooking could lead to momentary snags.

Bed bugs were entrapped fairly quickly when walking on kidney bean leaves. Typically, a bug showed a visible momentary snag after only six locomotory cycles (one locomotory cycle

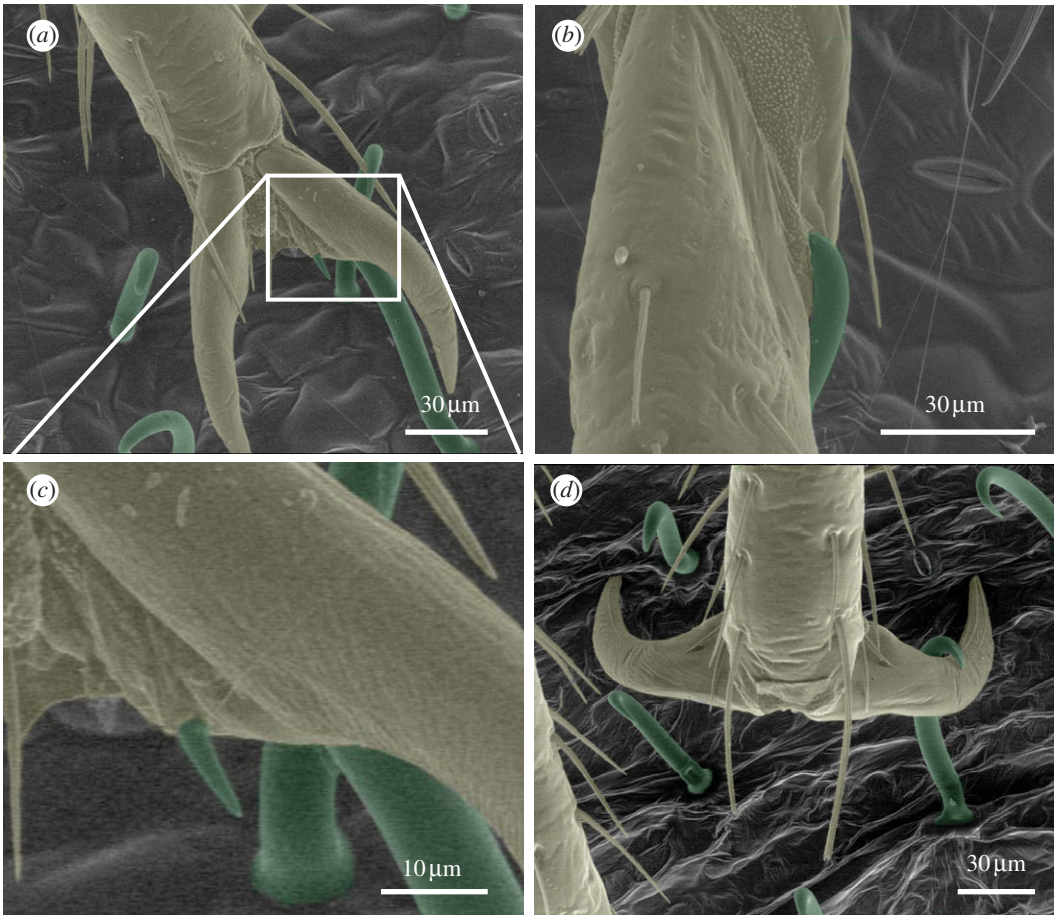

Figure 3. LV-SEM images of bed bug legs (yellow) on bean leaf surfaces with hooked trichomes (green). (*a*) Piercing under a pretarsal claw leads to entrapment of a bug by a leaf. (*b*) Piercing occasionally occurs at a tarsal intersegmental membrane, also causing entrapment of a bug. (*c*) Higher magnification of piercing from (*a*). (*d*) In contrast, hooking causes momentary snagging of a bug leg.

refers to a single step taken by each of the six legs; median = 6 cycles, range 0 13 cycles, '0' means that the bug displayed snagging behaviour immediately on introduction to the surface, $n = 11$ bugs). Bugs were typically entrapped only after nine cycles (median, range 0 39 cycles, $n = 11$ bugs), with 90 per cent of the bugs entrapped after 19 locomotory cycles (therefore, 19 locomotory cycles is the 90th percentile standard for equation (2.1)). This means that a bed bug was usually entrapped within seconds after placement on a leaf. Bed bugs continued to struggle after being pierced by a trichome, and the struggling movements often led to more piercings of the bug on the same or additional legs. Examination of all legs of a set of entrapped bugs in LV-SEM showed an average of 3.8 piercings/bug (range 1 7 piercings/bug, $n = 6$ bugs, 36 legs). The most common location for piercing was underneath the pretarsal claws (figure 3*a*,*c*; 61% of the 23 piercings on the six bugs). The other common location on the legs where piercing occurred was in the intersegmental membrane between the first and second tarsal subsegments (figure 3*b*; 30% of the 23 piercings on the six bugs; tarsal subsegments were counted from proximal to distal).

Additional piercings can occur because the trichomes are of sufficient density that all legs are surrounded by trichomes (figure 1*b*; average 106 trichomes mm^{-2}, s.d. = 51, $n = 10$ leaves); this trichome density is comparable to that reported in the literature for this species [19]. The lengths of these leaves ranged from 69 to 123 mm (base to tip, not including petiole); although there was an overall trend towards a decrease in trichome density with increasing leaf length, trichome density is extremely variable and was not significantly related to leaf length for this sample size ($r^2 = 0.36$, $n = 10$, regression line $y = \quad 1.4x + 244$; slope of regression line is not significant at the $p = 0.05$ level); and therefore, leaf length was not included in other statistical analyses.

The average displacement of a bug 30 min after entrapment on a bean leaf was only 3.2 mm (range 1.4 9.9 mm; $n = 6$ bugs), consistent with rotation in place around a pierced leg (approx. 6 mm, see above). Impaled bugs struggled, but were only rarely able to generate enough force to pull free of a piercing trichome (by breaking the trichome or ripping the insect cuticle), and usually immediately got recaptured on the leaf.

We forcibly detached impaled bugs from leaves, and immediately examined them upside-down in LV-SEM to look for attached broken trichomes or physical damage to the underside (ventral surface) of the bug legs. In eight out of nine cases, we were able to identify at least one broken trichome still attached to the bug (figure 4), and in the remaining case, there was evidence of damage (leaking haemolymph in the pierced location). Therefore, we are able to confirm piercings from damage on the undersides of bug tarsi. Bugs that had been momentarily snagged but not entrapped by leaves never exhibited any evidence of piercing when examined using LV-SEM; presumably their legs had only been hooked.

3.2. Microfabrication and characterization of biomimetic surfaces

The bean leaves entrapped bed bugs so quickly and effectively that a logical starting place for microfabrication of an entrapping surface for bed bug control was to faithfully

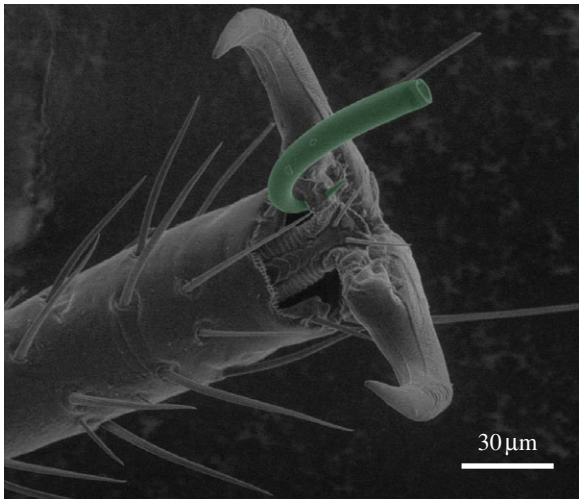

Figure 4. Underside of a bed bug tarsus showing a dangling broken trichome (highlighted in green) as evidence of piercing. Note that the trichome stalk is hollow.

reproduce the leaf trichomes with the relevant surface density and orientation. The hardest challenges in the microfabrication of the high aspect ratio trichomes on a replicate bean leaf surface are to accurately reproduce the sharp tips and the recurved shapes.

Microfabricated surfaces were generated with indistinguishable trichome geometry and hook point sharpness seen in natural leaves (figure 2*b*–*e*). The kidney bean trichomes had an average tip sharpness of 220 ± 35 nm (mean $\pm$ 1 s.d., $n = 16$ trichomes from 16 different leaves) and the synthetic replicas had an average tip sharpness of 230 ± 50 nm (mean $\pm$ 1 s.d., $n = 27$ from 27 different synthetic surfaces); tip sharpness is not significantly different between the real trichomes and the synthetic replicas (t-test; $t = 0.69$, d.f. $= 41$, $p = 0.49$). Therefore, our method is accurately duplicating the geometry of the microstructures on the natural surfaces.

Bugs were run on fabricated surfaces for a number of locomotory cycles that exceeded the maximum number observed for entrapment on natural leaves (greater than 39 locomotory cycles, see above). Occasionally, bugs were temporarily hooked on our fabricated surfaces (45 out of 158 bugs on 158 surfaces; electronic supplementary material, movie S3), but they were never pierced (0 out of 158 bugs). We examined the undersurfaces of bug tarsi in LV-SEM for 14 bugs which had been momentarily snagged on a fabricated surface for evidence of piercing or damage. No evidence for damage or piercing was ever seen on bug tarsi that was consistent with what we observed from damage by natural leaves (figure 4).

Serendipitously, we discovered that natural trichome tips would sometimes be retained in the negative mould material (figure 5) and become incorporated into fabricated surfaces (figure 6). This allowed us to generate surfaces that had trichomes with natural piercing hook tips attached to synthetic stalks (hybrid trichomes). The natural tips were usually indistinguishable in appearance from synthetic tips when viewed in SEM (figure 6*b*). Therefore, to reliably identify natural trichome tips, EDS was used to look for silicon, which was present in large amounts in the natural trichome tips (figure 6*a*,*c*) [20,26] but not in the synthetic polymers used (figure 6*b*). The percentage of natural tips ranged from 0 to 68 per cent in the 37 fabricated surfaces that were characterized by EDS mapping (median of 16% natural tips; figure 6*d*). In the controls, the percentage of natural tips measured on real kidney bean leaves using identical methods ranged from 77 to 100 per cent (median of 94%, $n = 4$ leaves). EDS was not used to evaluate the specific trichome tips from the microfabricated surfaces used for measuring sharpness of the tips (see above); and therefore, an unknown small percentage of them were probably hybrids with natural tips. This suggests that natural tips incorporated into hybrid trichomes were not dulled during the process, and that the fully synthetic replicas were as sharp as the natural trichomes.

Any natural trichome tips incorporated into fabricated surfaces must have snapped off the natural leaf; and therefore, broken trichomes should be visible on the natural leaf after being used to generate the negative mould. There was a significant correlation between the percentage of broken tips on a leaf surface evaluated using LV-SEM (range 0 95% broken tips, $n = 30$ leaves) and the percentage of the number of hybrid trichomes (range 0 100%) on the fabricated surface made from that particular leaf estimated using EDS mapping ($r = 0.76$, $n = 30$ leaves, $p < 0.0001$, correlation performed on arcsine-squareroot transformed percentages). This is further corroborative evidence for our interpretation of the incorporation of natural trichome tips in fabricated surfaces.

The number of locomotory cycles before a bed bug exhibited a momentary snag while running on a fabricated surface was always greater than measured on natural leaves. However, a fabricated surface could include both synthetic and hybrid trichomes. If the hybrid trichomes (with natural tips) are able to hook or pierce the bed bugs, but the completely synthetic trichomes are not, fabricated surfaces with a larger percentage of hybrid trichomes should interfere more with bed bug locomotion than fabricated surfaces with fewer hybrid trichomes. Therefore, the number of locomotory cycles expected to generate a snag or entrapment could be predicted by correcting a conservative standard (19 locomotory cycles, the 90th percentile for entrapment on natural leaves) for the percentage of hybrid trichomes estimated using EDS (equation (2.1)). Only four out of 28 bugs showed a momentary snag within the number of locomotory cycles that would be expected to result in entrapment 90 per cent of the time for their particular surface: these four bugs showed snags after a median of 84 locomotory cycles (on four surfaces ranging from 8 to 29% hybrid trichomes). Therefore, even the hybrid trichomes with natural tips are not snagging or entrapping bed bugs as efficiently as the trichomes on the natural leaves.

If the bugs were not entrapped by fabricated surfaces because they were able to break the synthetic or hybrid trichomes, broken trichomes should be evident on the fabricated surfaces after bugs ran on them. We compared SEM images both before and after bugs were confined on surfaces for many hours. Not a single broken trichome was observed out of the several hundred trichomes viewed, suggesting that neither synthetic nor hybrid trichomes are breaking when the bugs are walking on them.

4. Discussion

Plants exhibit extraordinary abilities to entrap insects using a variety of mechanisms [27]. Glandular trichomes on a plant

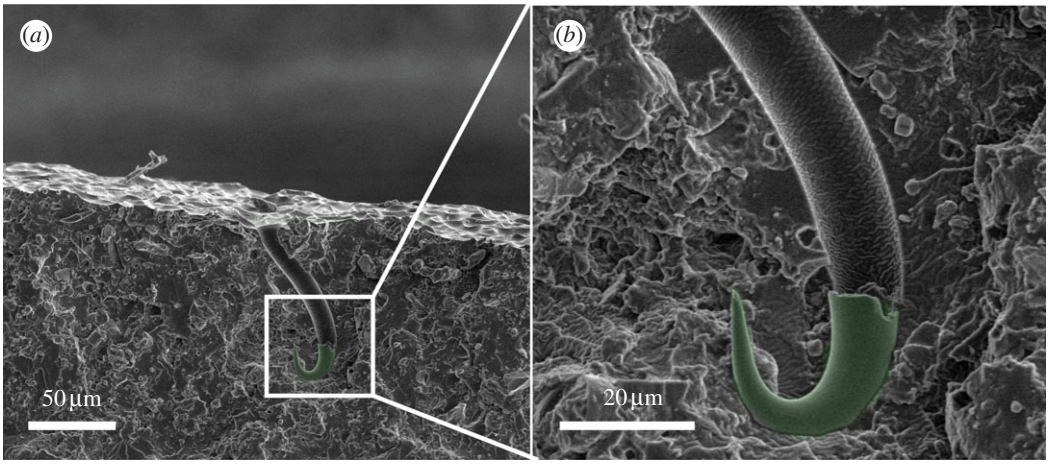

Figure 5. SEM image of a cross section of negative moulding material showing an embedded natural trichome tip (highlighted in green) that broke off the natural leaf trichome during moulding. Natural tips similar to this were sometimes incorporated into hybrid microfabricated surfaces.

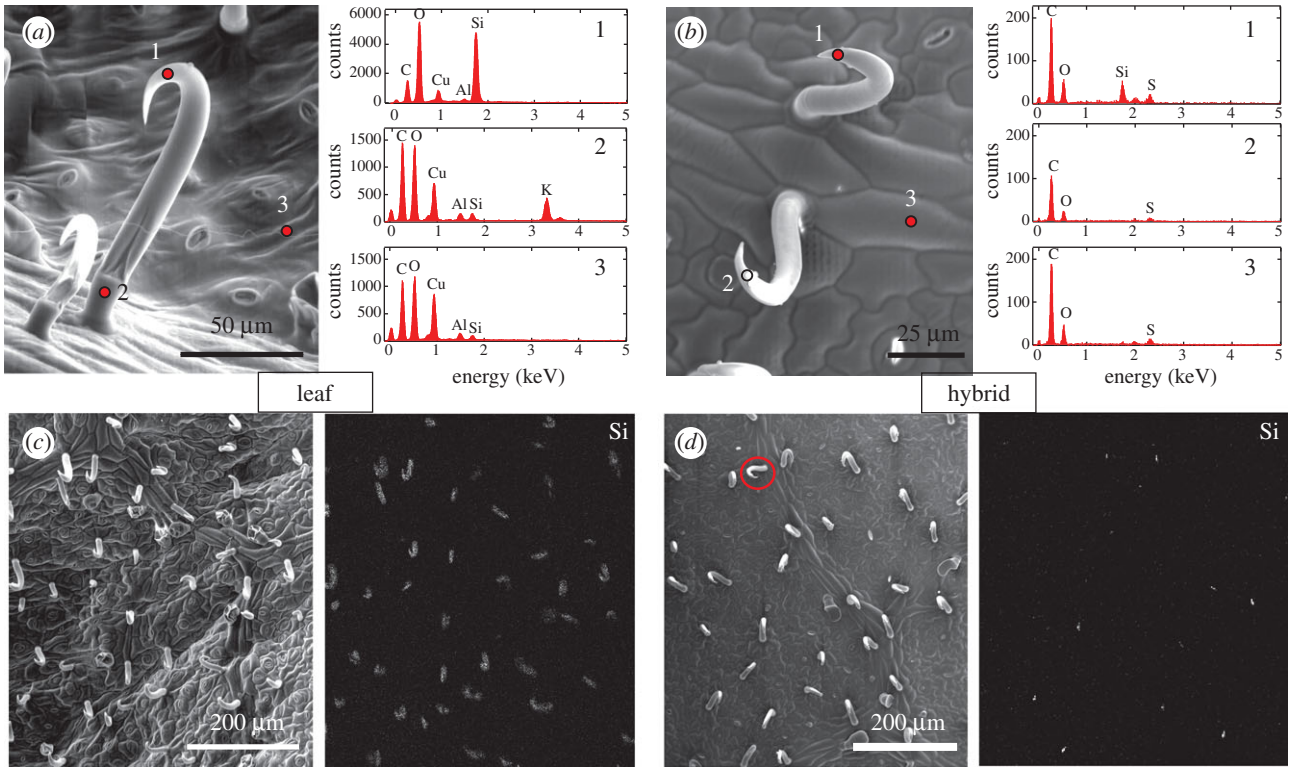

Figure 6. Discrimination between natural (hybrid) and synthetic trichome tips on microfabricated surfaces using elemental analysis was possible using energy dispersive X-ray spectroscopy (EDS). (*a*) An LV-SEM image of a trichome on a natural bean leaf surface and the locations of EDS spectra are identified. The trichome tip (*a*1) shows a strong silicon signature compared with the base (*a*2) and leaf surface (*a*3). (*b*) An SEM image showing both a hybrid trichome (*b*1) and non-hybrid trichome (*b*2) and their corresponding EDS spectra showing the presence or the absence of detectable silicon, respectively. (*c*) EDS mapping of trichomes on the leaf surface showing the presence of silicon on the natural trichomes. (*d*) Representative synthetic surface showing examples of natural trichome tips incorporated into the polymer surface (indicated by the presence of silicon) along with an example of a fully synthetic trichome (indicated by a circle).

surface may exude sticky or poisonous chemicals, while non-glandular trichomes, which do not secrete chemicals, may impale, entangle or impede the locomotion of insects by physical interactions [6, 11]. Non-glandular trichomes from a variety of plant species have been reported to impale aphids, bees, ants, leafhoppers, flies and some other insects [6 13], but there is no information about the mechanical properties of these impaling trichomes, how long it takes for an insect to become impaled, whether an impaled insect can ever escape, and little quantification of the locations of piercing. However, these studies provide compelling justification for developing a more mechanistic description of this physical entrapment.

A serious insect pest that is vulnerable to this method of attack is the bed bug, a species that has made a recent global resurgence. Bed bugs are entrapped quickly and effectively after walking only a few steps on the leaf of a kidney bean plant, despite the lack of any evolutionary association between bed bugs and kidney bean plants. We have identified the mechanism of entrapment as the physical impaling

of bed bug tarsi by the leaf trichomes. This is a mechanism different from a Velcro type of entanglement; the trichomes are more analogous to barbless fishhooks in their mechanical action. Furthermore, we have established that there are two primary locations on a bed bug's body that are mechanically vulnerable to piercing by these trichomes: underneath the pretarsal claws and at the intersegmental membrane between tarsal subsegments. Intersegmental membrane is thinner cuticle, and therefore should be pierced more easily, but this is the first identification of the mechanical vulnerability of the underside of the pretarsal claws. Specification of the vulnerable locations on bed bug tarsi is required to design an effective piercing method for bed bug entrapment and control.

Inspired by the impressive entrapment of bed bugs by bean leaves using a purely physical mechanism, we microfabricated surfaces that duplicated the geometry of the natural trichomes on a leaf surface. The moulding process generated synthetic trichomes that were indistinguishable from the natural trichomes, with the proper aspect ratio and sharpness of tips, arranged with the same density, orientation and height seen on the natural leaves. One way to duplicate the mechanical properties of the natural surface is to match both the geometry and the material properties of the synthetic surface to the natural leaf. Various epoxies and glues with different hardening rates and resin : hardener ratios were used in order to generate artificial trichomes with mechanical properties that span the largely uncharacterized properties of natural trichomes. For example, epoxies have Young's moduli (tensile) in the range 0.8 4.2 GPa [28 32] compared with 0.1 70 GPa for plant cell walls [33 37]. In a rare example of mechanical testing of trichomes, individual bending tests resulted in a range of 2 23 GPa for Young's modulus (flexural) for hooks from fruits of *Galium aperine* [38]; these hooks are substantially larger than the trichomes in this study. Therefore, materials used to generate our synthetic surfaces overlap in material properties with plant cell walls. However, it should be noted that there is tremendous variability in plant material properties over orders of magnitude [37]. Both our natural and synthetic trichomes had a large breaking strain, as indicated by their ability to bend completely over and elastically return to an upright orientation without breaking. In addition, the curved portions of both the natural and synthetic trichomes are able to straighten and elastically return to their curved configuration when pulled from the negative mould; freeze-fracture of the negative moulds showed none of the damage or ripping that would be caused by a non-straightened hook pulling through the material (figure 5). Therefore, qualitatively the natural and synthetic trichomes are showing similar mechanical behaviour in this regard.

Although natural trichome tips are able to pierce bed bugs as they walk on bean leaves, the same trichome tips are unable to pierce bed bugs when incorporated into hybrid fabricated surfaces that position these trichome tips at the natural and relevant orientation and height. This surprising result occurs despite the unchanged sharpness of the tips and the similarity of the material properties of the synthetic compounds to those of plant cell walls, and suggests that the bending or twisting behaviour of the trichome stalks is crucial to guide piercing and is not yet exhibited by our fabricated surfaces. Because structural properties are a function of both geometry and material properties, it can be challenging to simultaneously satisfy geometrical and mechanical requirements of these complex natural surfaces. For example, our synthetic trichomes are entirely solid, while only the tips but not the stalks of the kidney bean trichomes are solid (figure 4; also documented for hooked trichomes on French bean leaves [10] and on plant burrs [39,40]). Therefore, even if the material properties and external dimensions were the same in the natural and synthetic trichomes, the flexural and torsional stiffnesses would be greater for the stalks of the solid synthetic trichomes because of the increase in the second moment of area for a solid compared with a hollow cylinder [41,42]. The second moment of area, I, of a solid cylinder is related to the outer radius, r_o, as

$$I = \frac{\pi r_o^4}{4}, \tag{4.1}$$

and I for a hollow cylinder with an inner radius, r_i, is

$$I = \frac{\pi(r_o^4 \quad r_i^4)}{4}. \tag{4.2}$$

For example, a trichome stalk with an outer diameter of 10 μm and a wall thickness of 400 nm (figure 4; $r_o = 5$ μm and $r_i = 4.6$ μm) would have a second moment of area approximately 3.5 times as great as an otherwise identical but solid stalk. Both flexural and torsional stiffness (of a bending and twisting cylinder, respectively) are directly proportional to the second moment of area. Thus, the tip of a more flexible hollow natural trichome could more readily skitter along the cuticle of a bug's surface until the sharp point ended up in a crevice or pit, leading to piercing, while a stiffer solid synthetic trichome may simply bend away. Our ability to attach natural trichome tips to synthetic trichome stalks has allowed us to unambiguously identify the importance of the mechanical behaviour of the trichome stalks in facilitating piercing of bed bugs. The next generation of microfabricated surfaces for insect control by physical methods will require direct measurements of the relevant mechanical properties of natural trichomes.

5. Conclusions

Bed bugs walking on bean leaves are trapped within seconds by the sharp microscopic trichomes on the leaf surfaces. We have documented that these trichomes pierce the cuticle of bed bug tarsi at specific mechanically vulnerable locations, resulting in entrapment. We were able to generate synthetic surfaces that accurately mimicked the geometry, orientation and arrangement of the trichomes. Hybrid surfaces in which natural microscopic trichome tips were attached to synthetic stalks allowed separate evaluation of the mechanical properties of the different parts of the trichome in bed bug entrapment. These fabricated surfaces snagged the bed bugs but did not hinder their locomotion as effectively as real leaves, suggesting that future development of surfaces for bed bug entrapment must incorporate mechanical characteristics of whole trichomes. With bed bug populations skyrocketing throughout the world [1,2], and resistance to pesticides widespread [2,3,17], bioinspired microfabrication techniques have the potential to harness the bed bug-entrapping power of natural leaf surfaces using purely physical means.

This research was supported by the National Science Foundation (CHE 1057638). Imaging and EDS were performed at the Laboratory for Electron and X ray Instrumentation (LEXI) at UCI. Rollins, Inc. supported our bed bug rearing programme. The paper was improved by constructive criticism from three anonymous referees.

References

1. Saenz VL, Booth W, Schal C, Vargo EL. 2012 Genetic analysis of bed bug populations reveals small propagule size within individual infestations but high genetic diversity across infestations from the eastern United States. *J. Med. Entomol.* **49**, 865–875. (doi:10.1603/ME11202)
2. Harlan JH. 2007 Bed bug control: challenging and still evolving. *Outlooks Pest Manag.* **18**, 57–61. (doi:10.1564/18apr04)
3. Potter MF, Rosenberg B, Henricksen M. 2010 Bugs without borders: defining the global bed bug resurgence. *Pest World Sept./Oct.*, 8–20.
4. Potter MF. 2011 The history of bed bug management—with lessons from the past. *Amer. Entomol.* **57**, 14–25.
5. Richardson HH. 1943 The action of bean leaves against the bedbug. *J. Econ. Entomol.* **36**, 543–545.
6. Southwood R. 1986 Plant surfaces and insects: an overview. In *Insects and the plant surface* (eds B Juniper, R Southwood), pp. 1–22. London, UK: Edward Arnold Ltd.
7. Eisner T, Eisner M, Hoebeke ER. 1998 When defense backfires: detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *Proc. Natl Acad. Sci. USA* **95**, 4410–4414. (doi:10.1073/pnas.95.8.4410)
8. Levin DA. 1973 The role of trichomes in plant defense. *Q. Rev. Biol.* **48**, 3–15. (doi:10.1086/407484)
9. Riddick EW, Wu Z. 2010 Lima bean–lady beetle interactions: hooked trichomes affect survival of *Stethorus punctillum* larvae. *Biocontrol* **56**, 55–63. (doi:10.1007/s10526-010-9309-7)
10. Johnson B. 1953 The injurious effects of the hooked epidermal hairs of french beans (*Phaseolus vulgaris* L.) on *Aphis craccivora* Koch. *Bull. Entomol. Res.* **44**, 779–788. (doi:10.1017/S000748530002472X)
11. Jeffree CE. 1986 The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In *Insects and the plant surface* (eds B Juniper, R Southwood), pp. 23–64. London, UK: Edward Arnold Ltd.
12. Cardoso MZ. 2008 Herbivore handling of a plant's trichome: the case of *Heliconius charithonia* (L.) (Lepidoptera: Nymphalidae) and *Passiflora lobata* (Killip) Hutch. (Passifloraceae). *Neotrop. Entomol.* **37**, 247–252. (doi:10.1590/S1519-566X2008000300002)
13. Gilbert LE. 1971 Butterfly-plant coevolution: has *Passiflora adenopoda* won the selectional race with Heliconiine butterflies? *Science* **172**, 585–586. (doi:10.1126/science.172.3983.585)
14. Romero A, Potter MF, Potter DA, Haynes KF. 2007 Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? *J. Med. Entomol.* **44**, 175–178. (doi:10.1603/0022-2585(2007)44[175:IRITBB]2.0.CO;2)
15. Yoon KS, Kwon DH, Strycharz JP, Hollingsworth CS, Lee SH, Clark JM. 2008 Biochemical and molecular analysis of deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). *J. Med. Entomol.* **45**, 1092–1101. (doi:10.1603/0022-2585(2008)45[1092:BAMAOD]2.0.CO;2)
16. Zhu F, Wigginton J, Romero A, Moore A, Ferguson K, Palli R, Potter MF, Haynes KF, Palli SR. 2010 Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. *Arch. Insect Biochem.* **73**, 245–257.
17. Mamidala P *et al*. 2012 RNA-Seq and molecular docking reveal multi-level pesticide resistance in the bed bug. *BMC Genomics* **13**, 6. (doi:10.1186/1471-2164-13-6)
18. Stenglein SA, Arambarri AM, Vizgarra ON, Balatti PA. 2004 Micromorphological variability of leaf epidermis in Mesoamerican common bean (*Phaseolus vulgaris*, Leguminosae). *Aust. J. Bot.* **52**, 73–80. (doi:10.1071/BT02083)
19. Pillemer E, Tingey W. 1976 Hooked trichomes: a physical plant barrier to a major agricultural pest. *Science* **193**, 482–484. (doi:10.1126/science.193.4252.482)
20. Dahlin RM, Brick MA, Ogg JB. 1992 Characterization and density of trichomes on three common bean cultivars. *Econ. Bot.* **46**, 299–304. (doi:10.1007/BF02866628)
21. Bauer G, Klein MC, Gorb SN, Speck T, Voigt D, Gallenmuller F. 2011 Always on the bright side: the climbing mechanism of *Galium aparine*. *Proc. R. Soc. B* **278**, 2233–2239. (doi:10.1098/rspb.2010.2038)
22. Tourtellot MK, Collins RD, Bell WJ. 1991 The problem of movelength and turn definition in analysis of orientation data. *J. Theor. Biol.* **150**, 287–297. (doi:10.1016/S0022-5193(05)80428-X)
23. Koch K, Schulte AJ, Fischer A, Gorb SN, Barthlott W. 2008 A fast, precise and low-cost replication technique for nano- and high-aspect-ratio structures of biological and artificial surfaces. *Bioinspir. Biomim.* **3**, 046002. (doi:10.1088/1748-3182/3/4/046002)
24. Schulte AJ, Koch K, Spaeth M, Barthlott W. 2009 Biomimetic replicas: transfer of complex architectures with different optical properties from plant surfaces onto technical materials. *Acta Biomater.* **5**, 1848–1854. (doi:10.1016/j.actbio.2009.01.028)
25. Koch K, Bhushan B, Barthlott W. 2009 Multifunctional surface structures of plants: an inspiration for biomimetics. *Prog. Mater. Sci.* **54**, 137–178. (doi:10.1016/j.pmatsci.2008.07.003)
26. Perry CC. 1986 A study in silicification in the macrohairs of the grass *Phalaris canariensis* L. In *Insects and the plant surface* (eds B Juniper, R Southwood), pp. 345–346. London, UK: Edward Arnold Ltd.
27. Eisner T. 2003 *For the love of insects*. Cambridge, MA: Harvard University Press.
28. Wetzel B, Haupert F, Zhang MQ. 2003 Epoxy nanocomposites with high mechanical and tribological performance. *Compos. Sci. Technol.* **63**, 2055–2067. (doi:10.1016/S0266-3538(03)00115-5)
29. Zheng S, Ashcroft IA. 2005 A depth sensing indentation study of the hardness and modulus of adhesives. *Int. J. Adhesion Adhes.* **25**, 67–76. (doi:10.1016/j.ijadhadh.2004.02.004)
30. Lilleheden L. 1994 Mechanical properties of adhesives in-situ and in bulk. *Int. J. Adhesion Adhes.* **14**, 31–37. (doi:10.1016/0143-7496(94)90018-3)
31. Burst N, Adams DO, Gascoigne HE. 2011 Investigating the thin-film versus bulk material properties of structural adhesives. *J. Adhesion* **87**, 72–92. (doi:10.1080/00218464.2011.538326)
32. Katnam KB, Comer AJ, Stanley WF, Buggy M, Young TM. 2012 Investigating tensile behaviour of toughened epoxy paste adhesives using circumferentially notched cylindrical bulk specimens. *Int. J. Adhesion Adhes.* **37**, 3–10. (doi:10.1016/j.ijadhadh.2012.01.007)
33. Vincent JFV. 1999 From cellulose to cell. *J. Exp. Biol.* **202**, 3263–3268.
34. Vincent JFV. 1990 *Structural biomaterials*. Princeton, NJ: Princeton University Press.
35. Hiller S, Bruce DM, Jeronimidis G. 1996 Micro-penetration technique for mechanical testing of plant cell walls. *J. Texture Stud.* **27**, 559–587. (doi:10.1111/j.1745-4603.1996.tb00094.x)
36. Gibson LJ, Ashby MF, Harley BA. 2010 *Cellular materials in nature and medicine*. Cambridge, UK: Cambridge University Press.
37. Gibson LJ. 2012 The hierarchical structure and mechanics of plant materials. *J. R. Soc. Interface* **9**, 2749–2766. (doi:10.1098/rsif.2012.0341)
38. Gorb EV, Popov VL, Gorb SN. 2002 Natural hook-and-loop fasteners: anatomy, mechanical properties, and the attachment force of the jointed hooks of the *Galium aparine* fruit. In *Design and nature: comparing design in nature with science and engineering* (eds CA Brebbia, L Sucharov, P Pascolo), pp. 151–160. Southampton, UK: WIT Press.
39. Bowling AJ, Maxwell HB, Vaughn KC. 2008 Unusual trichome structure and composition in mericarps of catchweed bedstraw (*Galium aparine*). *Protoplasma* **233**, 223–230. (doi:10.1007/s00709-008-0006-7)
40. Gorb E, Gorb S. 2002 Contact separation force of the fruit burrs in four plant species adapted to dispersal by mechanical interlocking. *Plant Physiol. Biochem.* **40**, 373–381. (doi:10.1016/S0981-9428(02)01381-5)
41. Vogel S. 2003 *Comparative biomechanics: life's physical world*. Princeton, NJ: Princeton University Press.
42. Gere JM, Timoshenko SP. 1997 *Mechanics of materials*, 4th edn. Boston, MA: PWS Publishing Company.