Advances in Natural Science
Vol. **3**, No. **2**, **2010**, pp. 152-160
www.cscanada.net

ISSN 1715-7862 [PRINT]
ISSN 1715-7870 [ONLINE]
www.cscanada.org

The 3rd International Conference of Bionic Engineering

Numerical Characterization of Surface Structures of Slippery Zone in *Nepenthes alata* Pitchers and its Mechanism of Reducing Locust's Attachment[1]

WANG Li-xin[2]
ZHOU Qiang[3]

Abstract: The slippery zone of inner pitchers in carnivorous plant *Nepenthes alata* bears highly specialized structures to serve the functions of trapping insects and restricting escape of preys. Since the surface structures of slippery zone may influence locust's attachment, surface micro-morphologies of the slippery zone were observed with scanning electron microscope (SEM) and scanning white-light interferometer (SWLI) to investigate the micro-morphologies and geometrical dimensions of the surface structures. Attachment force of locust (*Locusta migratoria manilensis*) on the slippery zone was measured with different slanting angles, as well as measured on stainless steel plate for the purpose of comparison. The influence of slippery zone on locust attachment was analyzed based on the viewpoints of micro-morphologies and geometrical dimensions of the surface structures. The slippery zone of *N. alata* pitchers possesses lunate cells and wax crystals with micro-nano dimensions. Measurement results presented that the attachment force of locust on slippery zones is apparently lower than that on stainless steel plates with all the corresponding slanting angles. The surface structures with appropriate geometrical dimensions and physical properties resulted in the significant decrease of attachment force by means of prohibiting locust generating effective mechanical interlock and adhesive attachment. This research probably provides a theoretical foundation for biomimeticing microstructures and function of slippery zone surface to design slippery plates for trapping disaster plague locust and other agricultural pest.

Keywords: slippery zone; *Nepenthes alata*; locust; attachment force; surface structures

1. INTRODUCTION

---

[1] **The authors thank the National Natural Science Foundation of China (Grant No. 50775214) and the Fundamental Research Funds of China Agricultural University for Special Post-graduate Research and Innovation Funding (Grant No. kycx09099) for the financial supports.**

[2] Department of Mechatronic Engineering, China Agricultural University, Beijing 100083, P. R. China

[3] Corresponding author, Department of Mechatronic Engineering, China Agricultural University, Beijing 100083, P. R. China.

* Received 18 May 2010; accepted 15 July 2010

Carnivorous plant *N. alata* grows in nutrient-poor habitats and has evolved special organs namely pitchers that efficiently capture, retain and digest predominantly insects being used as nitrogen and phosphorus source (Elison, 2006). Several parts of the pitchers have been optimized by natural selection to provide different functions, including attracting and trapping insects, preventing escape of prey, digestion and nutrient absorption (Chaveerach et al., 2006; Ellison & Gotelli, 2009). Generally, four parts can typically be distinguished as a leaf-like lid, a collar-like peristome, a slippery zone and a digestive zone. Previous studies have demonstrated that the slippery zone consists of downward-directed lunate cells and irregular epicuticular wax crystals (Gorb et al., 2005; WANG et al., 2009), and the wax crystals are significantly slippery for most insects, especially for insects possessing the attachment system of hairy adhesive pads, so serve the functions of capturing insects and retaining prey by means of reducing the insect attachment (Gaume et al., 2002; Bohn & Federle, 2004; Gaume et al., 2004).

Nowadays, the comprehensive method for controlling agricultural pests is using chemical pesticide, which leads to serious environment pollution. Many researchers are looking for innovative methods to not only effectively control the pests but avoid the pollution. The technology of mechanization of slippery trapping in agricultural pests has been put forward (XU et al., 2005). The crucial point of this technology is creating insects trapping slippery plates, which can make the attachment system of insects lose their normal functions and slide to the collection equipment beneath the slippery plates. Therefore, with the wonderful idea inspired by the slippery zone and the similar bionic method involved in another aspect of biomimeticing engineering (REN et al., 2007; SUN et al., 2009; ZHANG et al.,2007), slippery plates bearing similar surface structures and function can be created. It is necessary to study the geometrical dimensions of surface structures in slippery zone and the attachment force of insects on slippery zone for creating biomimetic surface. Previous studies exclusively focused on the chemical composition and physical properties of the surface structures in slippery zone to explore the mechanism of anti-attachment, and little attention had been paid to the geometrical dimensions of the surface structures and the measurement of insects attachment force.

In this paper, surface structures of slippery zone in *N. alata* pitchers were examined using SEM and SWLI to investigate its morphologies and geometrical dimensions. Attachment force of locust on slippery zone and reference plate (stainless steel plate) with different slanting angles was measured. Based on the morphologies and geometrical dimensions of surface architectures, the mechanism of reducing locust attachment force was analyzed. Such information provides theoretical foundation for biomimeticing the surface structures and function of the slippery zone to design and manufacture slippery plates for trapping plague locusts and other agricultural pest.

2. MATERIALS AND METHODS

2.1 *Nepenthes* specie, locust and stainless steel plate

The carnivorous plant *N. alata* was obtained commercially from Hangzhou, Zhejiang province, continuously cultivated in small greenhouse under controlled conditions at 24-28℃ and 70-90% humidity, and watered daily with rainwater being collected outside our laboratory.

Imagines of locusts (*Locusta migratoria manilensis*) were obtained commercially from Cangzhou, Hebei province and kept in cages with food (various grass species), the temperature and humidity of the cage was maintained 25-30℃ and 35-50%, respectively.

The stainless steel plates (size: 12×10 *cm*) used for the experiment were acquired from market in Beijing, the data of surface roughness (Ra=0.2 *um*) and materials stiffness (Rockwell hardness: 90 HRB) were obtained from the supplier.

2.2 Examination on surface morphologies and structures

Small pieces (about 8×8 *mm*) were cut from the slippery zone of freshly harvested mature pitchers and air-dried for several days. The dried samples were mounted on aluminum holders with conductive carbon double-sided adhesive tape, sputter coated with gold (Bal-Tec SCD005 Sputter Coater; 25mA, 300s; Balzers, Switzerland) and examined with SEM (Hitachi S-3400N, 16KV: Hitachi, Japan). Geometrical dimensions of surface structures in the slippery zone were quantified from digital images with the software belonging to the SEM equipment.

The surface profile of the slippery zone was examined on fresh, untreated samples (about 2 cm^2), cut out with a razor blade and glued with adhesive tape to an Aluminum block. These samples were observed in SWLI (Zygo New View 5000; Zygo Corporation, USA), and the geometrical dimensions of the surface structures in vertical direction can be acquired from the digital images saved by the SWLI.

2.3 Attachment force measurements on slippery zones

To measure the attachment force of locusts on the slippery zone and the reference plate, a force sensor (load cell force transducer, 1-PW4C3, 300g capacity; Hottinger Baldwin Measurement Co., Ltd, Suzhou Jiangsu, P. R. China) was utilized, and fixed on a stand connected to the platform (Fig. 1a). The force sensor was connected to a signal conditioning system, a computer-based data-acquisition system, and a data processing and displaying software. The sampling frequency could be adjusted by the software, and was set at 10 Hz in this experiment.

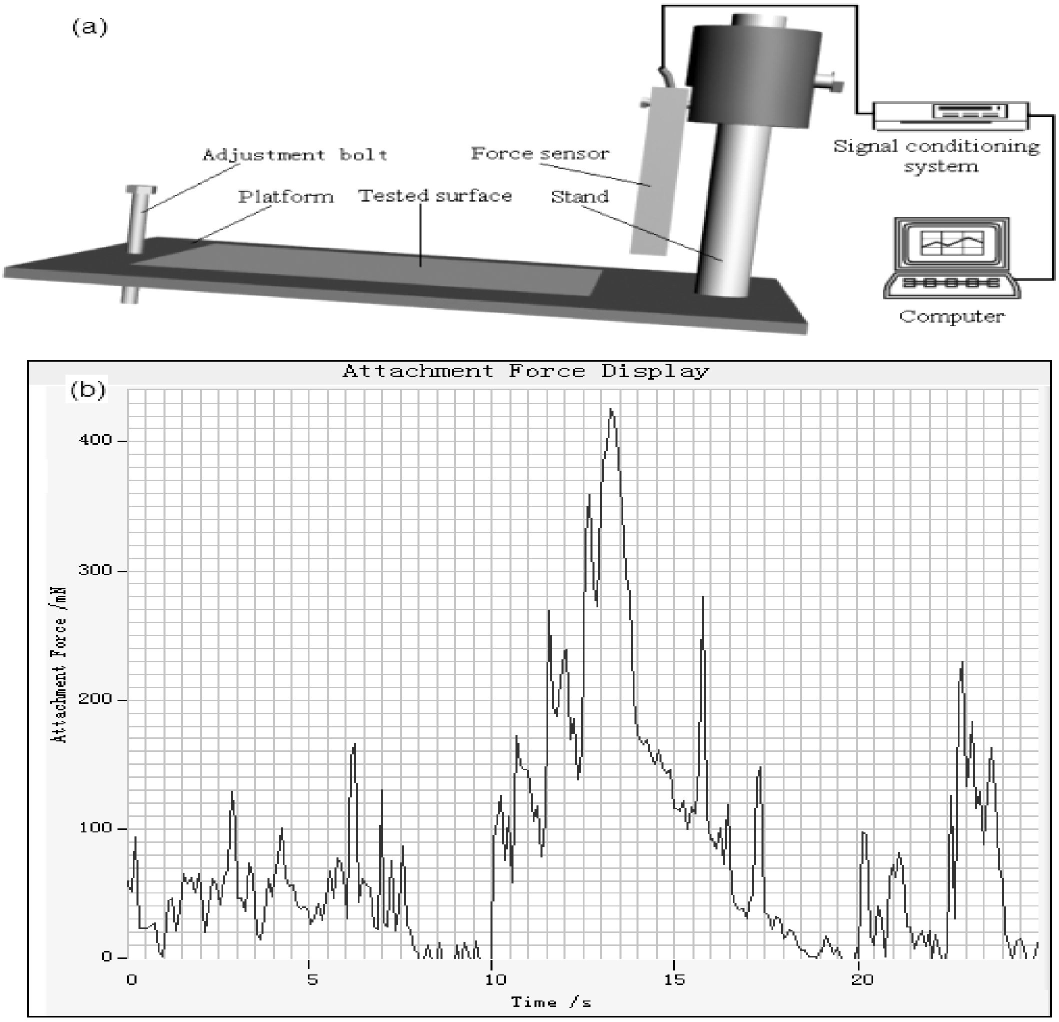


Fig. 1: Attachment force measurement system (a) and a typical attachment force-time curve (b) used for exhibiting the changes of the force in real-time

Prior to experiment, several slippery zones were cut using a sharp razor blade from the *N. alata*, and then connected with double-sided adhesive tape to the platform, the total area of the tested samples is about

10 *cm*^{2}. The corresponding slanting angle was adjusted to 30°, 35°, 40° and 45°, orderly. With a similar method previously introduced in detail (Gorb et al., 2004), experimental locust was cut their wings off and attached to the force sensor (along the load direction) by means of thin thread (about 10 *cm* long) fastened to the position between its neck and thorax, and then was put on the substrate. To make the force sensor precisely obtain the information of the locust attachment force, height of the force sensor was adjusted to make the thin thread can be horizontal to the platform, as well as the position of locust was adjusted and controlled to make the thread lay precisely along the load direction (perpendicular to the load point) of the force sensor. The attachment force we measured was the force generated when locust crawling ahead desperately on the substrate. Force-time curves, which generated by the data processing system, were used to exhibit the changes of the force in real-time (Fig. 1b). For each measurement, the maximal attachment force could be acquired by the data processing software and presented on the display interface. For each surface samples, experiments with 15 locusts (two repetitions per locust) were carried out. For the purpose of comparison, the attachment force of locust was also measured on the reference sample (stainless steel plate) with the same method. All the experiments were carried out under the ambient conditions: temperature about 28°C, relative humidity about 45%.

3. RESULTS

3.1 Morphologies and geometrical dimensions of surface structures in the slippery zone

The slippery zone of N. alata contributes about a quarter of the pitcher’s height. SEM observation exhibited that slippery zone of the pitchers was covered by plenty of lunate cells and relatively dense and irregular wax layer (Fig. 2a). Each lunate cell (Fig. 2b) corresponded to a single, enlarged overlapping guard cell, forming a crescentic outline with a convex asymmetrical surface and being oriented perpendicularly to the pitcher axis (horizontally) with both ends bent downwards. The wax layer (Fig. 2c) consists of separate, discernible and irregular waxy platelets arranging the surface extremely densely and mostly overlapping each other so that gaps are hardly distinguished, as well as the platelets are located almost perpendicular to both the subjacent layer and the pitcher surface. Geometrical dimensions of waxy crystals (platelets) and lunate cells changed slightly, and the detail information was presented in Table 1.

Information of the surface structures in vertical direction can be obtained from the SWLI examinations. The general surface between lunate cells was undulating and exhibited uniform architectures and significant height difference (Fig.3a). In variation of the height, outside of the lunate cells varied slowly from bottom to top and formed ‘slope’ (Fig. 3b, c), while inside changed sharply from top to bottom and formed ‘precipice’ (Fig. 3b, c). Surface roughness of the slippery zone between lunate cells presented rather small values (about Ra=0.9 *um*), and increased significantly when taking into account the influence of lunate cells (Ra=2.49 *um*, Ry=22.4 *um*, the scope of examined surface is 160×120 *um*).

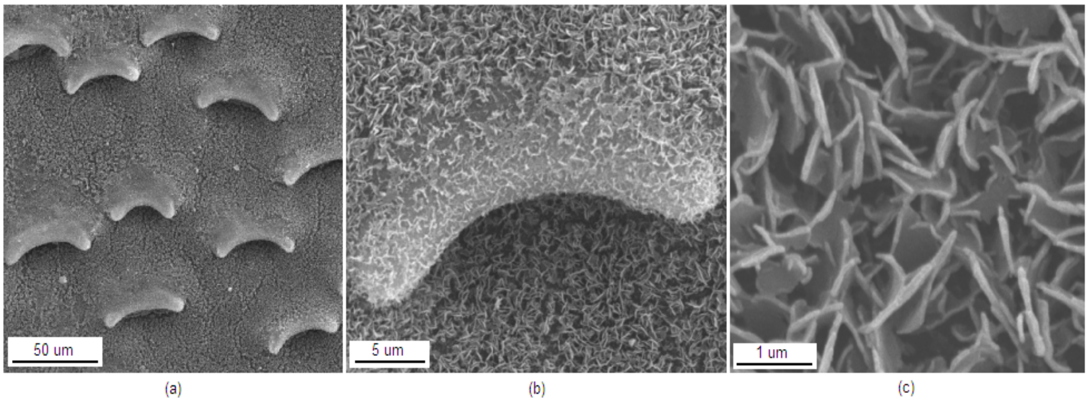
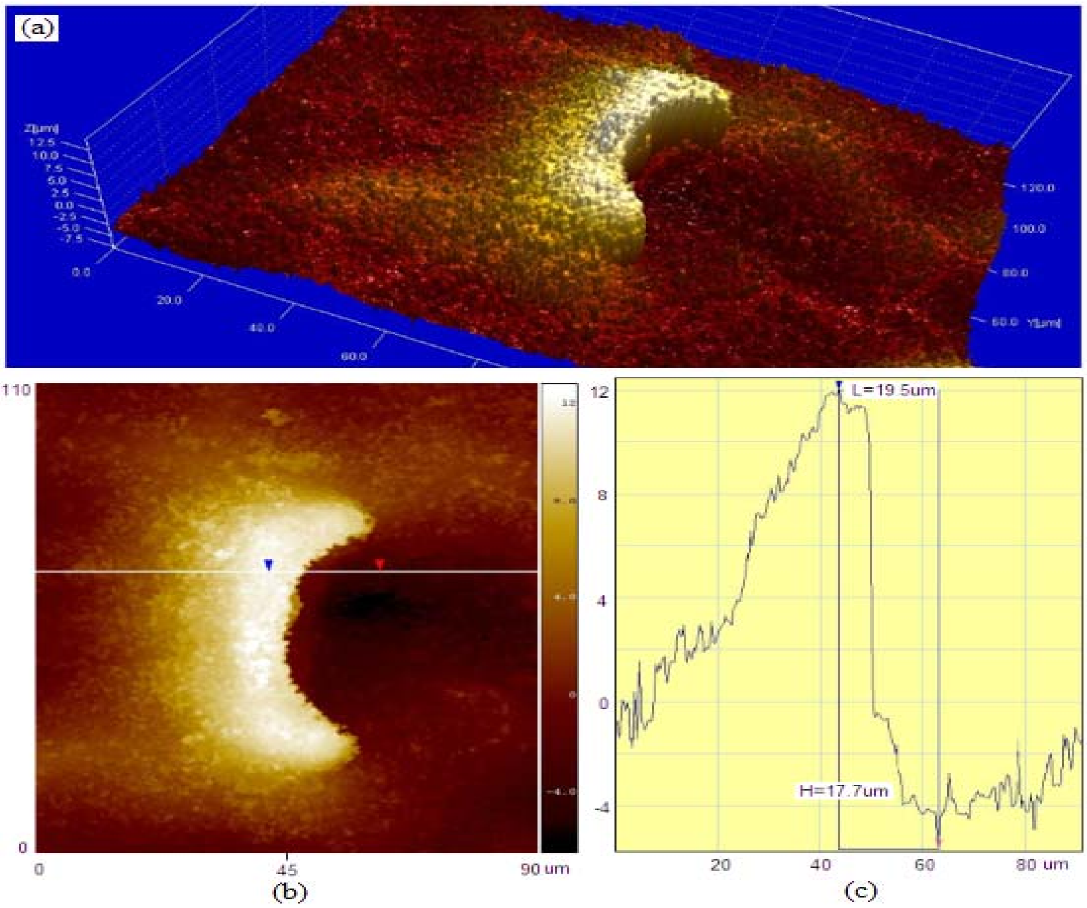


Fig. 2: SEM images of the slippery zone in *N. alata* pitcher. (a) Overall image of the slippery zone. (b) Specific image of lunate cell. (c) Specific image of epicuticular wax crystals.

Table 1: Geometrical dimension of surface structures in slippery zone of *Nepenthes alata* pitcher

Dimensions	Length (span)	Width	Height	Intervals	Density
Wax crystals	1.12±0.15	90.3±10.7		0.53±0.12	
Lunate cells	52.73±2.95	15.92±1.16	14.58±0.25	80.06±15.71	245.2±16.9

Values are means ± S.D., unit of wax crystals' width and lunate cells' density is *nm* and per mm^2 respectively, the rest is *um*. n of lunate cells' height and density is 8 and 4 respectively, the rest is 40.



(b) (c)

Fig. 3: SWLI images of slippery zone in *N. alata* pitchers. (a) 3D image surrounding the lunate cell, the scanning range is 160×120 *um*. (b, c) 2D image and its profiliograms of slippery zone surrounding lunate cell. The white line in (b) shows the axes for profiles

3.2 Attachment force of locusts on slippery zones

After the measurement experiment, maximal values of the attachment force generated by locusts on slippery zone and stainless steel plats can be acquired from the data processing program. The values presented significant difference according either the different surfaces or the different slanting angles. The attachment force of locusts on slippery zone was apparently smaller (about 200 *mN* lower, depends on the different slanting angles) than that on the reference plate (Fig. 4a). On both of the two surfaces, the attachment force decreases as the increasing of slanting angle, and the greatest extent of reduction is exhibited when the slating angle decreases from 35° to 40° (Fig. 4b).

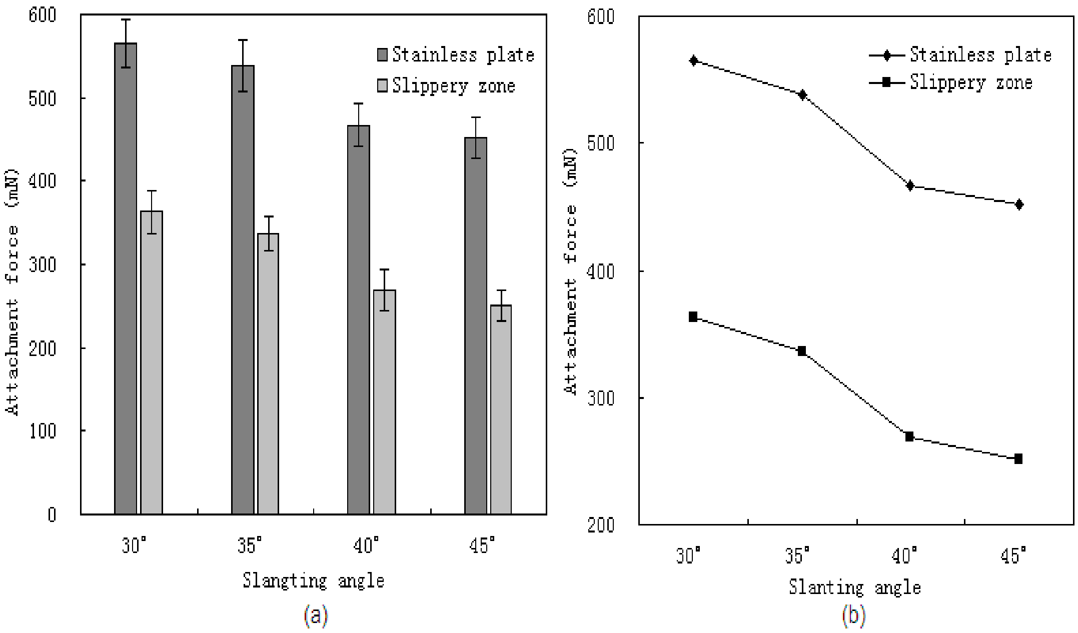


Fig. 4: Maximal attachment force generated by locusts on slippery zone and stainless steel plate with different slanting angles. (a) Presenting the maximal values. (b) Exhibiting the decreasing trend.

4. DISCUSSIONS

In the present study, based on the SEM and SWLI observations of surface structures in the slippery zone, we analyze why the slippery zone of *N. alata* can significantly reduce the attachment force of locust.

During natural evolution, insects have developed highly specialized organs, namely claws and pads, to effectively attach themselves to varieties of substrates when attaching and locomoting (Betz, 2002; Beutel & Gorb, 2001). Mechanical interlock can be generated by claws interacting with surface irregularities of macroscopically rough substrates (DAI et al., 2002), whereas adhesive attachment depends on the pads, including smooth flexible pads and hairy adhesive structures, to maximize the contact area and guarantee the acquisition of sufficient attachment force on smooth surface (Voigt et al., 2008). Locust attachment system depends on the smooth adaptable pads and rigid claws to generate adhesive attachment and mechanical interlock, with which can effectively attach to various substrates during staying, climbing and jumping when taking food or escaping from being hunted. Presumably, the slippery zone reduces the locust attachment force via restricting the adhesive attachment and the mechanical interlock.

In *Nepenthes* plants, the slippery zone was comprehensively regard to serve the functions of trapping and especially in restraining prey via decreasing the insects attachment. Several hypotheses have been put forward to explain the anti-attachment properties of slippery zone.

(1) Wax crystals are responsible for surface roughness of the slippery zone which results in the reduction of contact area between pads and substratum.

(2) Wax crystals are easily detachable structures that may contaminate insect attachment pads.

(3) The adhesive fluid secreted by insect pads interacts with wax crystals and generates slippery substratum.

(4) The capillarity of the structure in waxy surface leads the adhesive fluid secreted by insect pads to be absorbed by the wax crystals (Gorb et al., 2005; Gaume et al., 2002; Scholz et al., 2010). However, all the hypotheses only emphasized the influence of the wax crystals on pads.

Compared to stainless steel plate, the slippery zone surface can significantly reduce the locust's attachment force, and the surface architectures of slippery zone, including wax crystals and lunate cells, probably contribute to the reduction. Previous laboratory experiments have demonstrated that insects bearing rigid claws and hairy adhesive pads are not able to attach or walk well on surface of slippery zone, and this phenomenon presumably results from the contamination of wax crystals on pads [6, 19]. But the contamination seems to be merely suitable for the pads bearing hairy adhesive structures, since there is no obvious wax crystal being discovered on smooth flexible pads after insect walking on slippery zone of *N. alata* pitchers (Scholz et al., 2010). In *N. alata*, geometrical dimensions (Table 1) of wax crystals and the cavities formed by adjacent crystals are distinctly smaller than the claws tip diameter of locust, so the slippery surface can not supply effective anchorage point for locust to grasp. Besides, wax crystals of the slippery zone surface bear the mechanical properties of fragility and brittleness, as well as easily to detach from the beneath layer and break into tiny pieces (Gord et al., 2005), so it can preclude locust from utilizing them as steady sites to engender the mechanical interlock. The detached wax crystals form numerous ‘tiny slippery plates’ between the pads and the lower layer surface, which can restrict the increase of contact area and make locust slide to the beneath zone.

The geometrical characteristics of lunate cells covering the slippery zone probably prohibit the increase of real contact area. When locust attach to the slippery surface, each pad occupies a large number of lunate cells, and these lunate cells with convexities and asperities probably indicate that the slippery zone is very difficult to be duplicated. According to it there is no sufficient contact area to engender effective adhesive force for locust’s attachment. Besides, the wax crystals sparsely covering in the lunate cells probably contribute to the further reduction of the contact area. Pendant hoods with crescentic outline covering the downward-directed lunate cells presumably protect the surface of lunate cells from being scraped by claws and prohibit the generation of the mechanical interlock.

Previous studies demonstrated that the surface roughness with appropriate range (from 0.3 to 3.0 um) could reduce the attachment force of insects strongly (Peressadko & Gorb, 2004), and the surface roughness of slippery zone possesses appropriate values (about Ra=0.9 *um*, and Ra=2.49 *um* when taking into account the lunate cells) to decrease the locust’s attachment force. Besides the surface roughness, materials stiffness also can influence the attachment of locust (Wang et al., 2009: 30; Wang et al., 2009: 54). In order to allow locust attachment to or locomotion on, the substrate stiffness should exhibit appropriate value (neither too high nor too low), otherwise the substrate will prevent claws from penetrating into or make the claws slide over its surface. The slippery zone does not appear to allow effective claws interlocking because the wax crystals from upper layer are too fragile to supply sufficient stiffness.

5. CONCLUSIONS

In summary, the slippery zone of *N. alata* pitchers can significantly decrease the locust’s attachment force, and the reduction results from the special surface structures and appropriate geometrical dimensions of slippery zone. Wax crystals possess apparently smaller dimensions than the tip diameter of locust’s claw, as well as properties of fragility and brittleness, therefore no steadily anchorage point can be provided for locust to generate effective mechanical interlock. Geometrical characteristics of lunate cells probably prohibit the increase of contact area, and lead to the failure of forming effective adhesive attachment. Wax crystals and lunate cells result in appropriate surface roughness of the slippery zone, which contributes to the strongly decrease of locust attachment force. These conclusions supply insights into the biomimeticing surface structures and function of slippery zone to design slippery plates for trapping plague locusts and other agricultural pest.

REFERENCES

Betz O. (2002). Performance and adaptive value of tarsal morphology in rove beetles of the genus Stenus (Coleoptera, Staphylinidae). *Journal of Experimental Biology, 205*, 1097-1113.

Beutel R, Gorb S N. (2001). Ultrastructure of attachment specialization of hexapods (Arthropoda): evolutionary patterns inferred from a revised ordinal phylogeny. *Journal of Zoological Systematics and Evolutionary Research, 39,* 177-207.

Bohn H F, Federle W. (2004). Insect aquaplaning: *Nepenthes* pitcher plants capture prey with the peristome, a fully wettable water-lubricated anisotropic surface. *Proceedings of the National Academy of Sciences,* USA, *101*, 14138–14143.

Chaveerach A, Tanomtong A, Sudmoon R, Tanee T. (2006). Genetic diversity among geographically separated populations of *Nepenthes mirabilis. Biologia, 3*, 295-298.

DAI Z D, Gorb S N, Schwarz U. (2002). Roughness-dependent friction force of the tarsal claw system in the beetle *Pachnoda marginata* (Coleoptera, Scarabaeidae). *Journal of Experimental Biology*, *205*, 2479–2488.

Ellison A M, Gotelli N J. (2009). Energetics and the evolution of carnivorous plants—Darwin's 'most wonderful plants in the world'. *Journal of Experimental Botany, 1*, 19-42.

Ellison A M. (2006). Nutrient limitation and stoichiometry of carnivorous plants. *Plant Biology, 8*, 740-747.

Gaume L, Gorb S, Rowe N. (2002). Function of epidermal surfaces in the trapping efficiency of *Nepenthes alata* pitchers. *New Phytologist*, *156*, 476–489.

Gaume L, Perret P, Gorb E. (2004). How do plant waxes cause flies to slide? Experimental tests of wax-based trapping mechanisms in three pitfall carnivorous plants. *Arthropod Structure & Development, 33*, 103–111.

Gorb E, Hass K, Henrich A, Enders S, Barbakadze N, Gorb S. (2005). Composite structure of the crystalline epicuticular wax layer of the slippery zone in the pitchers of the carnivorous plant *Nepenthes alata* and its effect on insect attachment. *Journal of Experimental Biology, 208*, 4651-4662.

Gorb E, Kastner V, Peressadko A, Arzt E, Gaume L, Rowe N, Gorb S. (2004). Structure and properties of the glandular surface in the digestive zone of the pitcher in the carnivorous plant Nepenthes ventrata and its role in insect trapping and retention. *Journal of Experimental Biology*, *207*, 2947-2963.

Peressadko A G, Gorb S N. (2004). Surface profile and friction force generated by insects. *Proceeding of the First International Conference Bionik* 2004, Hannover, Germany, 257-263.

REN L Q, WANG S J, TIAN X M, HAN Z W, YAN L N, QIU Z M. (2007). Non-smooth morphologies of typical plant leaf surfaces and their anti-adhesion effects. *Journal of Bionic Engineering, 4*, 33-40.

Scholz I, Buckins M, Dolge L, Erlinghagen T, Weth A, Hischen F, Mayer J, Hoffmann S, Riederer M, Riedel M, Baumgartner W. (2010). Slippery surfaces of pitcher plants: Nepenthes wax crystals minimize insect attachment via microscopic surface roughness. *Journal of Experimental Biology*, *213*, 1115-1125.

SUN G, FANG Y, CONG Q, REN L Q. (2009). Anisotropism of the Non-smooth surface of butterfly wing. *Journal of Bionic Engineering*, *6*, 71-76.

Voigt D, Schuppert J M, Dattinger S, Gorb S N. (2008). Sexual dimorphism in the attachment ability of the Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) to rough substrates. *Journal of Insect Physiology, 54,* 765–776.

WANG L X, ZHOU Q, CHENG X T. (2009). Sliding effect test of locust on surface of slope plates. *Journal of Jiangsu University (Natural Science Edition), 30,* 232-235.

WANG L X, ZHOU Q, XU S Y, ZHENG Y J. (2009). Investigation of the sliding friction behaviors of locust on slippery plates. *Chinese Science Bulletin, 54*, 4549-4554.

WANG L X, ZHOU Q, ZHENG Y J, XU S Y. (2009). Composite structure and properties of pitcher surface of carnivorous plant *Nepenthes* and its influence on insect attachment system: A Review. *Progress in Natural Science,19*, 1557-1664.

XU R Q, ZHOU Q, WANG S M. (2005). Technical development on mechanical control of locust. *Transactions of the Chinese Society for Agricultural Machinery, 36*, 165-168.

ZHANG Y, ZHOU C H, REN L Q. (2007). Biology coupling characteristics of mole crickets' soil-engineering components. *Journal of Bionic Engineering*, *supplement*, 164-171.

LIST OF FIGURE CAPTIONS

Fig. 1 Attachment force measurement system (a) and a typical attachment force-time curve (b) used for exhibiting the changes of the force in real-time.

Fig. 2 SEM images of the slippery zone in *N. alata* pitcher. (a) Overall image of the slippery zone. (b) Specific image of lunate cell. (c) Specific image of epicuticular wax crystals.

Fig. 3 SWLI images of slippery zone in *N. alata* pitchers. (a) 3D image surrounding the lunate cell, the scanning range is 160×120 *um*. (b, c) 2D image and its profiliograms of slippery zone surrounding lunate cell. The white line in (b) shows the axes for profiles.

Fig. 4 Maximal attachment force generated by locusts on slippery zone and stainless steel plate with different slanting angles. (a) Presenting the maximal values. (b) Exhibiting the decreasing trend.