The functional morphology of insect adhesive devices and its implications for ecology.

Being a Thesis submitted for the degree of Doctor of Philosophy

Department of Biological Sciences

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By

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Abstract

The aim of this thesis is to gain a better understanding of the constraints that affect insect adhesion with an emphasis on biological constraints such as plant defences against insects and the influence of abiotic factors on insect foraging. In chapter one of this theses, a literature review on the mechanisms of insect adhesion, the influence of attachment capabilities on foraging behaviour, plant-insect interactions, and synthetic insect barriers is presented, focusing on hymenoptera and coccinellids as representatives of the two basic insect pad types. In the following chapters we test the four leading hypothesis regarding insect adhesion (Contamination, Fluid absorption, Surface roughness and the effect of Surface Energy), before investigating the role of mechano-sensing via insect antenna on substrate choice and finally probing the link between surface properties and locomotion and adhesion. Throughout this thesis I use species of Hymenoptera and Coccinellids as representative species of the two basic adhesive pad types.

Publications arising from thesis

1) Orchard, M, J., Kohonen, M. and Humphries, S., (2012). The influence of surface energy on the self-cleaning of insect adhesive devices. *Journal of Experimental Biology*, **215**: 279 – 286.
2) Anyon*, M. J., Orchard*, M, J., Buzza, D, M, A., Humphries, S. and Kohonen, M, M., (2012). Effect of particulate contamination on adhesive ability and repellence in two species of ant (Hymenoptera; Formicidae), *Journal of Experimental Biology*, **215**: 605 – 616.

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~ ‘*Nature cannot be understood by pretending it is simple*’ ~ (Elton, 1965)

~ ‘*Si tibi voluntas eam, est nullum somnium*‘~ Walter Sobchak

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Chapter one

Insect Attachment:
Mechanisms and Ecological Implications

1.1 Introduction

The requirement for foraging animals to be able to adhere to surfaces with a range of inclinations and physical properties has led to the evolution of adhesive devices (adhesive pads, claws, prehensile tails) in many differing taxa, from mammals (including possums and tree gliders: (Gorb, 2007)), to amphibians such as tree frogs (Federle et al., 2006; Gorb, 2007; Smith et al., 2006), insect orders such as the Hymenoptera, Phasmatodea and Coleoptera, as well as arachnids. The ability to adhere to surfaces has evolved to such an extent that some insects (ants, flies, bees, beetles, locusts, aphids, grasshoppers) and lizards (geckos, skinks, and anoles) are able to climb microscopically smooth vertical surfaces and to adhere to surfaces even whilst hanging upside down**.** Aristotle noted as early as the 4th century BC the ability of geckos to run up and down tree trunks in any bodily orientation (Autumn & Peattie, 2002; Hansen & Autumn, 2005). To do this they have to be able to overcome both normal detachment forces (forces acting perpendicular to the surface) and shear forces (forces applied parallel to the surface) (Federle & Endlein, 2004; Federle, Baumgartner, & Hölldobler, 2004; Federle et al., 2002; Gorb, 2005; Pearson, 2008), whilst still maintaining the ability to move freely and with minimal energetic cost (Jander, 1963).

The ability to adhere to surfaces is crucial in all aspects of an insect's life history. Adhesion is important in mating (Bitar et al., 2009; Chapman, 2004; Frantsevich & Gorb, 2004; Gorb, Hosoda, & Gorb, 2009; Niederegger, Gorb, & Jiao, 2002), foraging (Chapman, 2004; Dejean et al., 2010; Eigenbrode et al., 2000; Federle et al., 1997; Holldobler & Wilson, 1990), nest building (Eigenbrode, 2004; Federle, Baumgartner, & Hölldobler, 2004; Federle, Rohrseitz, & Holldobler, 2000) and the ability to resist attack by predators (Attygalle et al., 2000; Eisner & Aneshansley, 2000; Federle, Baumgartner, & Hölldobler, 2004). In order to survive, insects must be capable of strong, yet quickly reversible attachment too many different substrates, from rough bark, wax covered leaves and glaucous (waxy) stems, to soil, sand, and rocks. So important is the ability to adhere to surfaces that many insects and arachnids have evolved the ability to adhere to surfaces whilst supporting more than 10–100 times their own body weight (Chapman, 2004; Dejean et al., 2010; Federle et al., 2001). Strong attachment allows insects to carry large food items, as well as providing protection against detachment by wind gusts, raindrops, and predators.

In the following sections a review of insect attachment mechanisms and their implications in ecology is presented.

1.2 Adhesion and friction: basic concepts

The purpose of this Section is to provide a brief description of adhesive and friction forces between surfaces, first for dry systems, and then for systems in which the surfaces are separated by a thin film of liquid. Comprehensive discussions of adhesion and friction forces may be found in standard texts, including those by Israelachvili, (2010), Bowden & Tabor, (2001), and Mate, (2008). Adhesion and friction are terms used to describe the forces acting between surfaces as a function of relative displacement (Figure 1.0). The adhesive force between two surfaces is defined as the magnitude of the force, which must be applied to separate the surfaces from contact. The frictional force between two surfaces is defined as the force required to slide one surface relative to another.

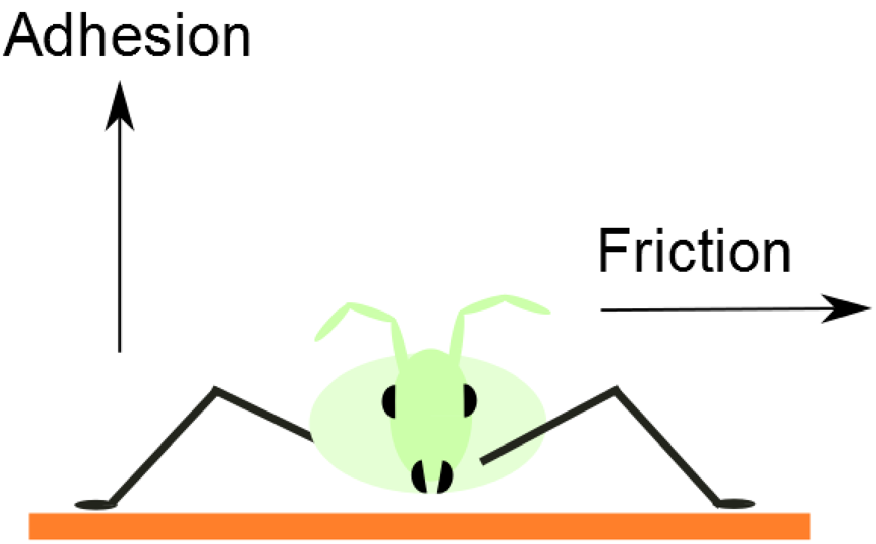

Figure 1.0: Schematic representation of how the principles of adhesion and friction affect insects (image shows stylised insect walking on flat surface in normal orientation).

Adhesive and frictional forces ultimately arise from the presence of intermolecular forces. The strongest such forces are electrostatic forces between charged molecules and those involved in chemical (covalent) bonds. However, electrostatic forces and covalent bonds are not relevant in the case of adhesion and friction in insect adhesive devices (Stork, 1980) and hence will not be considered further. In dry adhesion systems van der Waals forces are the most important contributor to adhesion (Israelachvii, 2010). The van der Waals force is a ubiquitous long-range attractive force, which arises from intermolecular dipole/dipole (‘Keesom’), dipole/induced-dipole (‘Debye’) and dispersion (‘London’) interactions. The magnitude of the attractive van der Waals force between two surfaces depends on the geometry and dielectric properties of the interacting surfaces and is a strong function of the separation between the surfaces. The van der Waals force F per unit area for two parallel flat surfaces separated by a distance D is given by the equation:

$$F = \frac{A}{6\pi D^2} \qquad [1]$$

Where A is the Hamaker constant. The magnitude of the Hamaker constant is determined by the dielectric properties of the interacting surfaces, but is typically in the range 10^{-20} – 10^{-19} J. All surfaces adhere to each other to some extent because of van der Waals forces. However, the magnitude of the van der Waals attraction between two surfaces decreases rapidly as the separation between the surfaces is increased. The reason that most surfaces do not strongly adhere is because they are rough, and hence close contact between the surfaces occurs only at a relatively small number of points (known as asperities).

There are two important approaches to increasing the magnitude of the adhesive force between rough surfaces, both of which are employed to some extent by insects (as discussed in detail in Section 1.2.1). The first is to increase the effective area of contact by introducing a liquid between the surfaces. This liquid (which, in the case of common adhesives, may later be allowed to solidify) fills in the gaps between the surfaces and therefore increases the area over which the attractive van der Waals forces act. The second approach to improving adhesion is to employ soft, often elastic, materials, which can deform to allow intimate contact even with rough surfaces. Indeed all biological adhesive pads characterised so far lizards (Geckos) (Autumn et al.,, 2006), Green Dock Beetle (*Gastrophysa viridula*) (Bullock & Federle, 2009), grasshoppers (Perez et al., 2006; Zhendong & Gorb, 2009) and tree frogs (Barnes et al., 2011) have a Young’s modulus of < 100 kPa. This low Young’s modulus means the surface is pliant and in this instance is referred to as making them ‘tacky’ allowing for adhesive contact between surfaces with little pressure (Alphonsus, 2002; Dirks & Federle, 2011). This has led to the prediction that all biological adhesive structures should be extremely soft (Dirks & Federle, 2011).

Friction refers to the force required to slide two contacting surfaces relative to each other and the static friction force is the force required to initiate sliding. The kinetic friction force is the force required to maintain sliding (at a given velocity). As with adhesion frictional forces arise from intermolecular interactions, and two limiting regimes may be identified. If two surfaces are in adhesive contact then in order to initiate sliding some critical shear stress must be applied in order to break the adhesive contacts between the surfaces. In the case that there is no adhesion between two surfaces, friction arises because in order to slide the surfaces relative to each other the asperities on opposing surfaces must be elastically or plastically deformed. Approximate values of friction forces can be predicted using the following equation:

$$F_r = \mu L \qquad [2]$$

Where F_r is the friction force, L is the load force (perpendicular to plane of contact, which acts to keep the surfaces in contact), and is μ the coefficient of friction. The magnitude of μ depends on the properties of the interacting solids (including the magnitude of adhesive forces, surface roughness, and elastic moduli) but typically falls in the range 0.04 – 1.00 (Huber et al., 2007).

1.2.1 ‘Wet’ adhesion and friction

As is discussed in Section 1.3.3, most insects rely on the presence of a liquid secretion between the adhesive devices and substrate to increase adhesive forces by maximising contact area (Figure 1.1).

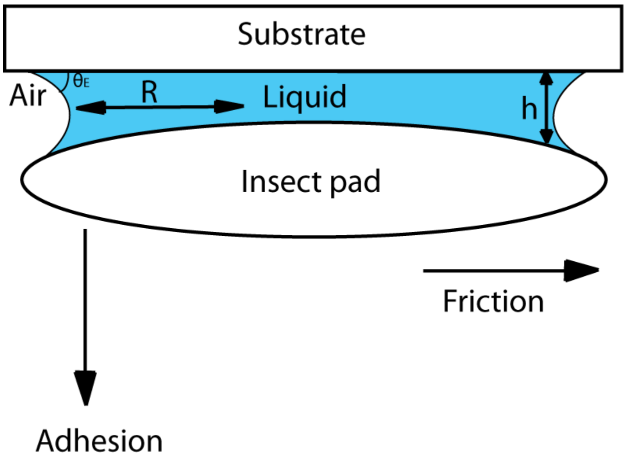

Figure 1.1: Illustration of the adhesive secretion in the contact region between the adhesive organ of an inverted insect and an idealised flat substrate, the extent to which the secretion wets/spreads across the substrate is related to the contact angle, R = the lateral radius of the liquid bridge, h = the thickness of the liquid bridge. The arrows show in which direction the secretion has to resist both adhesive and frictional forces, θ_E as defined by equation 3.

The presence of liquid in the zone of contact between two surfaces affects both adhesion and friction. In the case where the liquid at least partially wets the surfaces (i.e. where the contact angle θ of the liquid on the surfaces is less than 90°) an adhesive force, which is often significantly larger in magnitude than the van der Waals force, is generated. This adhesive force arises because the curvature of the liquid/vapour (air) interface results in the pressure in the liquid phase being lower than the pressure in the vapour phase (as dictated by the Laplace equation). In essence if a liquid droplet is trapped between two plates, then they will adhere strongly as long as the liquid wets them with an angle $\theta_E < \pi/2$. The internal angle θ_E is defined in Figure 1.1 above. As the two surfaces are bought together the separation distance between them can be defined as h. The droplet forms a capillary bridge between the two surfaces, characterised by radius R and a surface area of $A = \pi R^2$. The Laplace pressure within the droplet is derived as follows:

$$\Delta p = \gamma \cdot \left(\frac{1}{R} - \frac{\cos\theta_E}{\frac{h}{2}} \right) \approx \frac{2\gamma\cos\theta_E}{h} . \qquad [3]$$

Where P is the pressure within the droplet and γ is the surface tension of the liquid. The force within the droplet will be attractive as long as $\theta_E < \frac{\pi}{2}$. If $h \ll R$, it is equal to

$$F = \pi R^2 \frac{2\gamma \cos\theta_E}{h} \cdot \qquad [4]$$

The magnitude of this adhesive force depends on the separation between the surfaces, using the worked example from de Gennes et al., (2003, p6), if a droplet of water is trapped between two plates, using $R = 1\, cm$, $h = 5\, \mu m$, and $\theta_E = 0$ (best case with complete wetting of surfaces), using the Laplace equation gives a pressure drop of $\Delta p \sim \frac{1}{3} atm$ and an adhesive force $F \sim 10\, N$, which is enough to support one litre of water.

The presence of a thin film of liquid between two surfaces also gives rise to viscous force contributions to adhesion and friction as relative motion between the two surfaces requires that the liquid flow. The magnitude of viscous forces will depend on the viscosity of the fluid and the velocity of the flow, but will also depend on the geometry of the system. For simple geometries the forces can be calculated using the equations of fluid mechanics. For example, for two parallel circular disks of radius R which are bridged by liquid film of thickness h, (Figure 1.2) the force F which must be applied in order to separate the surfaces perpendicularly in a time t is given by:

$$F = \frac{3\pi \eta R^4}{4 t h^2} \qquad [5]$$

Where η is the dynamic viscosity of the liquid. For two parallel surfaces of area A which are separated by a liquid film of thickness h, the friction force is given by the equation:

$$F = \frac{AV\eta}{h} \qquad [6]$$

Where V is the relative sliding velocity of the surface. Finally, it should also be noted that the adhesive force described by Equation 7 might lead to dewetting of the film of liquid between two surfaces. Dewetting occurs when liquid is pinned by a contact point allowing liquid to be drawn out of the contact area (Figure 1.2). Such dewetting would give rise to dry adhesive contacts between the surfaces, which would further contribute to friction in general as discussed by Israelachvii (2010) or more specifically by increasing the rubberised 'dry' friction as discussed by Clemente and Federle (2012).

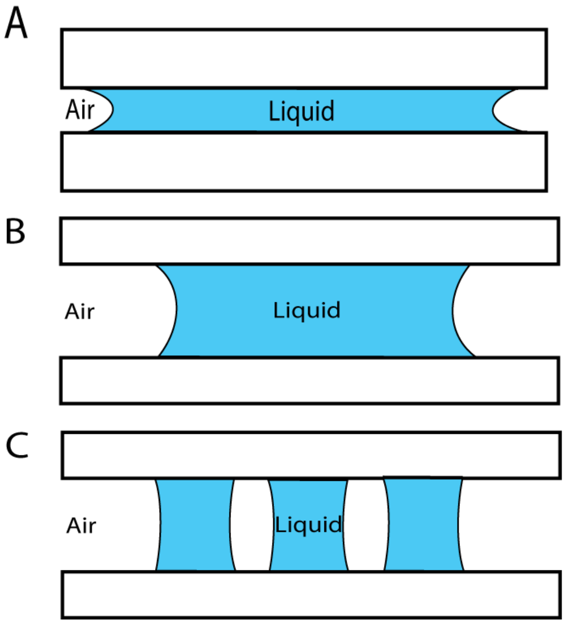

Figure 1.2: Simple model of adhesion between two solid plates separated by a liquid bridge, this model represents adhesion by surface tension (A), pulling apart the two surfaces (B) increases the air/liquid interface which requires work/effort, (C) localised dewetting as plates are pulled apart.

1.3 Insect attachment mechanisms

Insects use two forms of adhesive device; sickle-shaped claws located on the terminal segment of the legs (tarsi) and an adhesive pad, normally found on the terminal segments of the tarsi, but which can also be found on other sections of the leg. Individuals are able to switch between adhesive devices depending on the roughness of the substrate (Dai, Gorb, & Schwarz, 2002a). On rough substrates the claws are used to grip the surface asperities (> 30µm), whilst on smooth surfaces, where the claws would have no purchase, the adhesive pad is used.

1.3.1 Adhesive pads

There are two types of adhesive pad typically used by insects, commonly referred to as ‘hairy’ and ‘smooth’ (Figure 1.3-1.9). Some taxa such as Dermaptera (earwigs) and Heteroptera (true bugs) use both pad forms. It has been suggested that in Zoraptera (primitive flies), pad type may switch between flying and walking phenotypes (between generations), depending on environmental pressures (Beutel & Gorb, 2006). Hairy and smooth pad structures have evolved independently at least three times, although the exact number of times remains the subject of debate (Gorb, 2005; Gorb, 2001; Gorb & Beutel, 2001; Gorb et al, 2007).

The pad itself is typically made of a soft and deformable tissue, which can adapt to a rough surface, providing for intimate contact (Frantsevich et al., 2008; Scholz, Baumgartner, & Federle, 2008), as illustrated schematically in Figure 1.3.

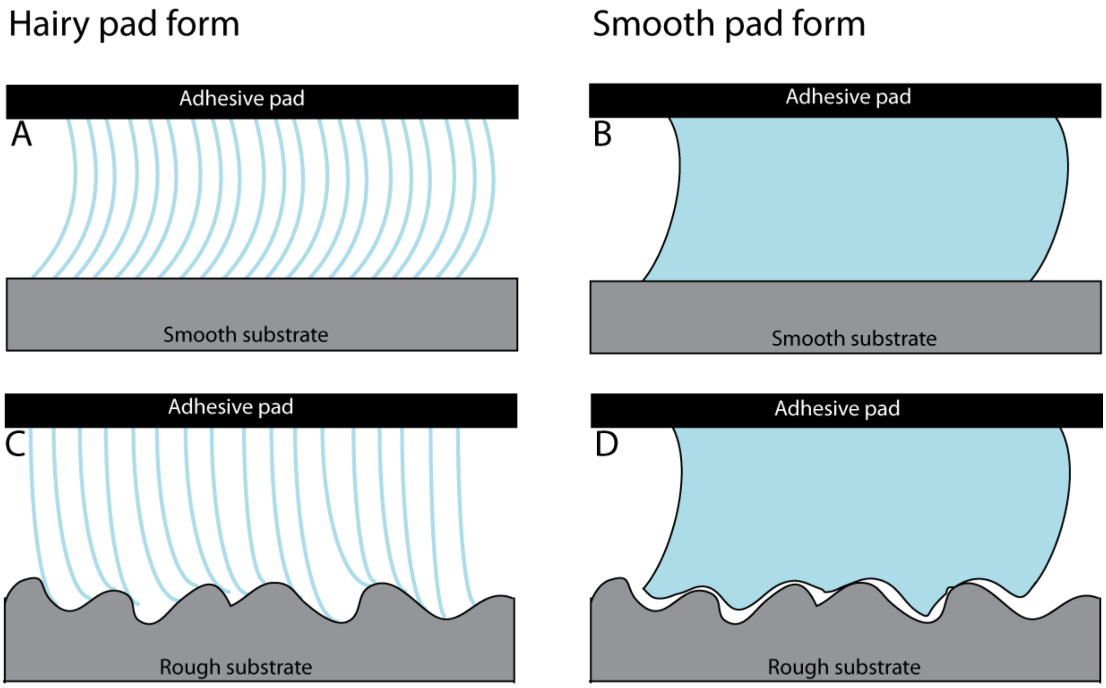

Figure 1.3: A schematic illustration of hairy (A and C) and smooth (B and D) adhesive pads. Both types of pad allow for intimate contact with both smooth (A and B) and rough (C and D) substrates. Adapted from Gorb 2005.

Hairy pad morphology

There is striking similarity between the hairs used for adhesion in insects, spiders and lizards (Stork, 1983) (Federle, 2006; Gorb, 1998) (Figure 1.4-1.7).

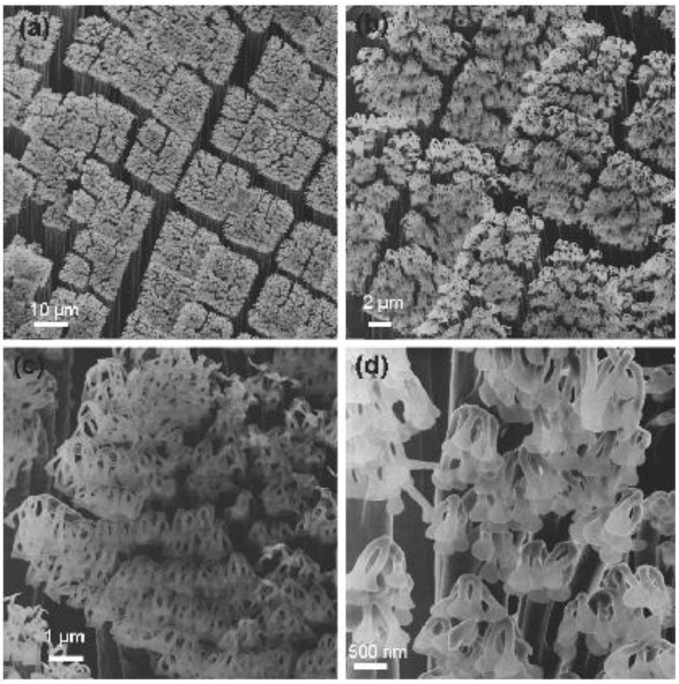

Figure 1.4: Helium Ion Microscopy (HIM) images of Gecko feet at differing magnification, showing organisation of setal types on adhesive pad. (Yang et al., 2011)

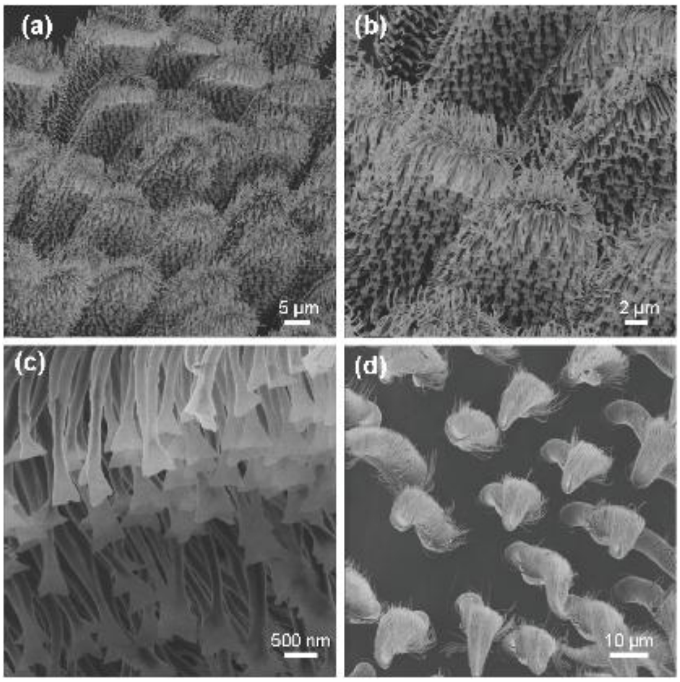

Figure 1.5: Helium Ion Microscopy (HIM) images of Spider feet at differing magnification, showing organisation of setal types on adhesive pad (Yang, et al. 2011).

Hairy adhesive pads in insects are covered with fine hairs (setae) suspended from a soft deformable cuticle normally found at the ends of the insect tarsi (Figure 1.3, A, C;

Figure 1.6). The setae can be fixed to the cuticle by either a socket, as in beetles, or directly into the cuticle itself as in flies. Setae in many insects are hollow to allow for the delivery of an adhesive secretion (Betz, 2003a) and are comprised of keratin like fibres. Adhesive setae are elongated structures ranging from 10 to 80 µm in length (Gorb et al., 2005; Gorb, 2001; Haas & Gorb, 2004; Willaims & Peterson, 1982). The terminal elements of the setae are typically very thin (0.04 – 50.00 µm) and often bifurcated to enhance contact area (Gorb et al., 2007a). Setal types and densities vary between species and sexes (Biata et al., 2009; Frantsevich & Gorb, 2004; Gorb, Hosoda, Miksch, & Gorb, 2010; Gorb, Hosoda, & Gorb, 2009; Niederegger, Gorb, & Jiao, 2002; Voigt et al., 2008). Representative images of hairy attachment pads are shown in Figure 1.6, while the differing morphology of setal tips can be seen in Figure 1.7.

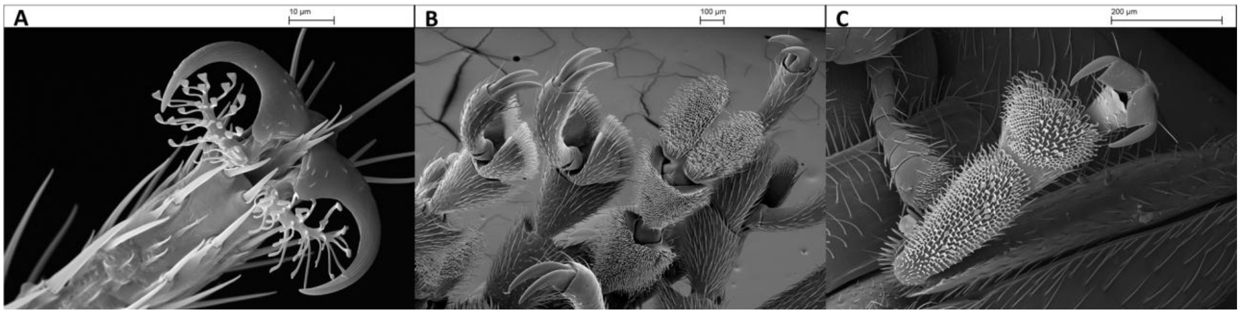

Figure 1.6: Representative SEM images of hairy adhesive pads, A, *Drosophila melanogaster*, B, *Pyrochroa serraticornis*, C, *Adalia bipunctata*.

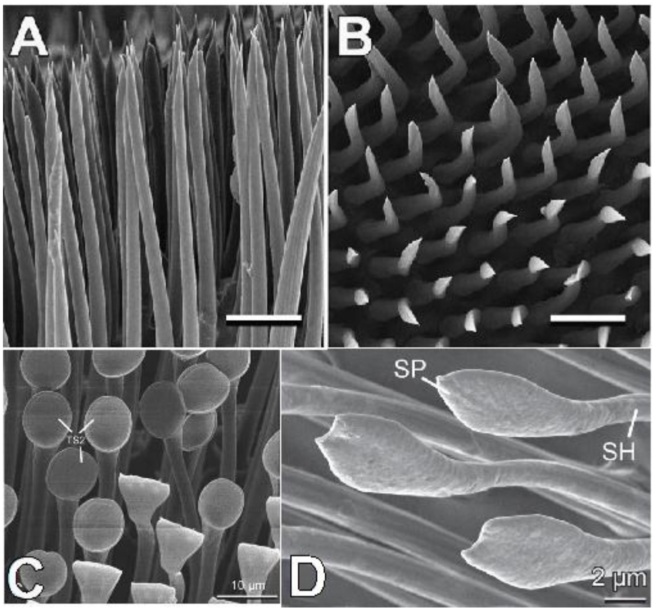

Figure 1.7: Differing setal tip morphology of hairy attachment pads, A & B, Pointed tips of the Colorado potato beetle *Leptinotarsa declineata* (Voigt et al., 2008), C, Discoid tips from dead lead beetle *Chrysolina fastuosa*(Gorb and Gorb, 2002) and D spatulae tips from the dock beetle *Gastrophysa viridula* (Hosanda and Gorb, 2010). Scale bars A & B 20 µm, C, 10 µm and D, 2 µm, sp, spatulae, sh, shaft, dt, discoid tip.

The maximum adhesive force available to an insect possessing hairy pads is directly correlated with the number of adhesive hairs it possesses (Stork, 1980a) and contact area (see equation 3) (Soto et al., 2010; Varenberg et al, 2006), with smaller setal tip area commonly compensated by increased setal density (Eimuller, Guttmann, & Gorb, 2008; Gorb, Gorb, & Kastner, 2001, Qian & Gao, 2006). The hairs employed by geckos and most spiders are appreciably thinner than those used by flies (Persson, 2008); this may be because the hairs used by flies are adapted to deliver the adhesive secretion and are hollow (Gorb, 1998), whilst the hairs used by geckos and spiders are not. Although recent work (Peattie, Dirks, Henriques, & Federle, 2011), suggests that an adhesive secretion may be used by arachnids as well. It has been suggested that hairy pads in larger animals, such as geckos, can adhere through Van der Waals forces alone (‘dry adhesion’). The jumping spider *Evracha arcuata* uses dry adhesion via tufts of hair found on the terminal segment of its legs (scopula), forcing the hairs into close contact with the substrate (Kesel, Martin, & Seidl, 2003; 2004). It is worth noting, however, that even the ‘dry’ adhesive systems used by geckos and some arachnids may be augmented by capillary condensation of water at the points of contact between the setae and the substrate (Barnes, 2007; Huber et al., 2005). In insects, however, hairy pads are always used in conjunction with an adhesive secretion.

Smooth pad morphology

The smooth pad form of adhesive device is found on the pretarsus and in most insects is comprised of a deformable membranous lobe that when combined with the claws allows for traction and locomotion on most surfaces (Chapman, 2004; Gorb, 2001). Smooth attachment devices can be both actively extended and retracted, as in Hymenoptera (where the pad is referred to as the arolium (Gorb, 2001)), or constantly deployed as in cockroaches (where it is called an epulanthae). The smooth extendible/inflatable pad is a folded cuticle sack that is held between the tarsal claws and is inflated by hydrostatic pressure controlled by the regulation of fluid reservoirs within the insect’s body (Endlein & Federle, 2008).

For example, while building nests weaver worker ants form a living latticework of ants that hold a series of leaves together while other ants from the colony pin the leaves together. The workers anchoring the structure are required to form strong adhesive contacts for many hours (Federle et al., 2001). Control of attachment forces is achieved by mediating the contact area of the adhesive pad, with the adhesive force being directly proportional to contact area (Holldobler & Wilson, 1983; Stork, 1980; Walker, Yulf, & Ratcliffe, 1985). Smooth pads may be found either protected between the tarsal claws (Figure 1.8 A) or on the terminal tarsal segments. However, as can be observed from Figure 1.8, although referred to as ‘smooth’ these adhesive devices show a range of topography and surface textures, including friction ridges and fribrillar appendages.

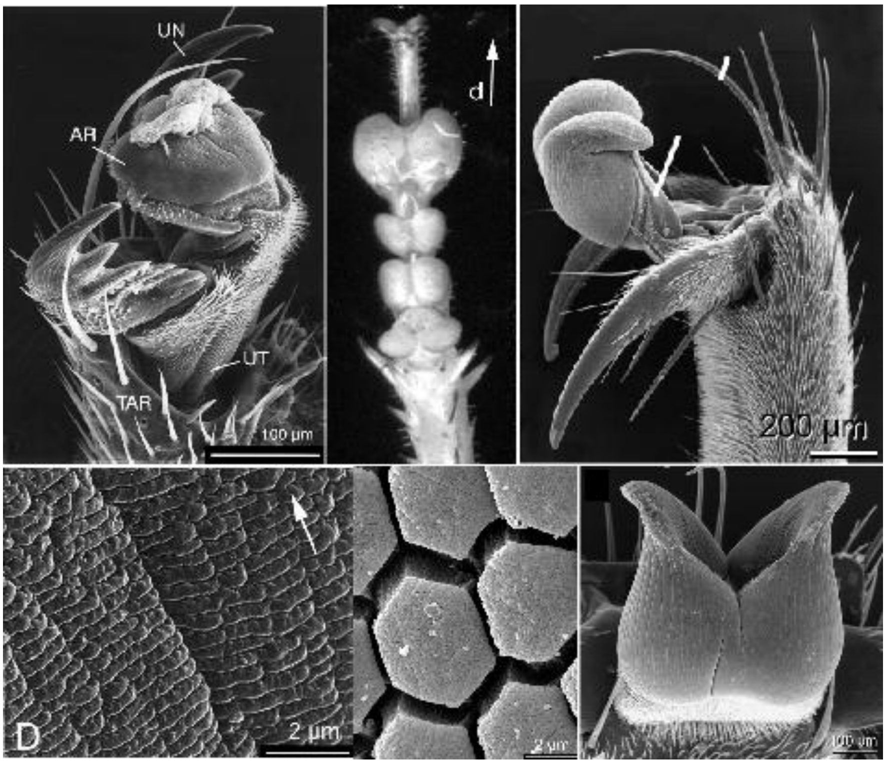

Figure 1.8: Comparison images of differing morphology of smooth attachment pads of insects, (A) scorpion fly (*Panorpa communis*) mid leg pretarsus, (B) great green bush cricket (*Tettigoniua viridissima*) with fixed euplantulae, (C) pre tarsus of the hornet *Vespas crabro* (D – F) high magnification images of (A-C) surface, respectively, (D) shows textured surface profile, €, shows hexagonal surface structure of euplantulae, (F) ventral SEM image of *V. crabro* arolium showing striations running the length of the axis. AR, arolium, CL, claws, TAR, tarsomer segment, SH sensing hairs, d, diostal direction. Images adapted from (A & D) Beutel and Gorb, (2001), (B & E) Gorb et al., (2002), (C & F) Frantsevich and Gorb (2002).

Although it is unknown how the adhesive secretion used by insects to maximise adhesion is delivered into the contact zone in smooth pads it is generally agreed that the arolium is in essence a fluid filled sack. Unlike setal hairs used by other attachment pads there are no pores through which the secretion can be delivered, as such Gorb and co-workers hypothesise that it is a sponge like structure holding the adhesive secretion in a fluid matrix (Beutel and Gorb, 2001; Gorb et al., 2002) (Figure 1.9), releasing it into the contact zone under the application of direct pressure and recovering fluid between steps through absorbance.

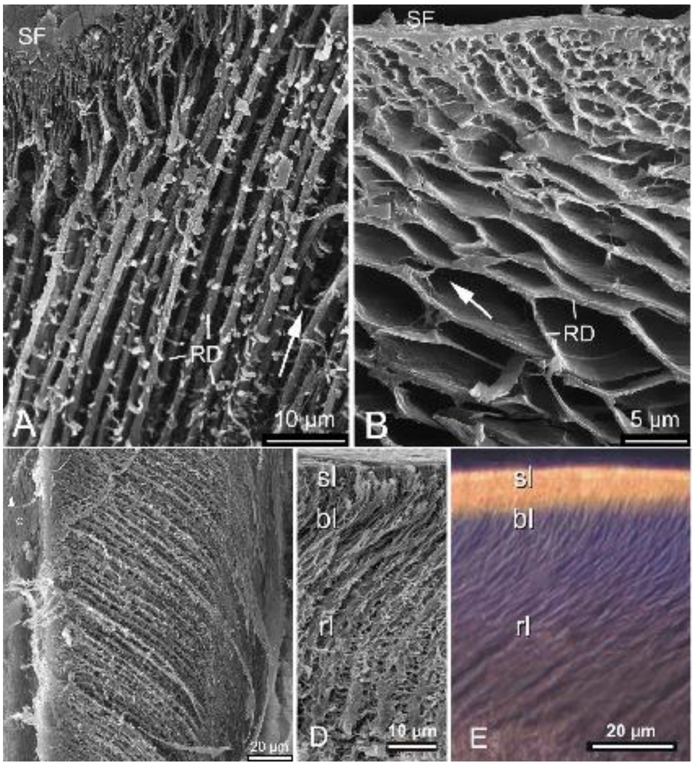

Figure 1.9: Internal structure of smooth attachment pads of (A) *Tettigonia viridissima* euplantulae, (B), Red Black Frog Hopper (*Cercopis vulnerata*) (Beutel and Gorb, 2001), C, internal rod like structures of *T. viridissima* at ~ 45° to the surface (Gorb et al., 2002), D, SEM image of internal structure of the locust *Locusta migratoria* showing crosslinking of internal rods, E, fluorescence microscopy of *L. migratoria* pad under UV (green and red band, superimposed) showing increased density of crosslinking towards outer surface (Goodwyn et al., 2006. SF surface, RD chitinous threads, Sl surface layer, bl branching rod layer.

Pad detachment

Leg movements mediate attachment and detachment of the pads, through a peeling action similar to that of pressure adhesive tape (Figure 1.10).

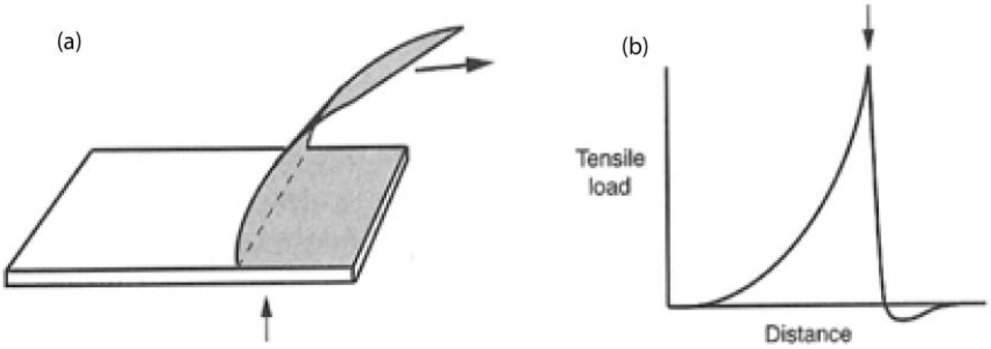

Figure 1.10: Simple schematic of peeling action of pressure sensitive tape from a flat surface, not the force applied to peel the tape from the surface gives rise to compressive forces behind the line of peeling (figure adapted from Vogel, 2003).

By pulling the pad in a proximal direction (towards the insect's body) attachment is enhanced; moving the pad in a distal direction (away from the insect's body), reduces attachment forces and allows the pad to be pulled off the substrate (Federle, 2006; Federle, Baumgartner, & Hölldobler, 2004; Federle et al., 2001). As discussed below, most insects also deploy an adhesive secretion between the pad and substrate in order to enhance the attachment forces.

The ability to vary the degree of contact between a surface and an insect's tarsal pad has evolved not only to deal with changes in substrate surface properties (Gorb, 2005), but also with differences in insect orientation and environmental influences. The rate of tarsal pad detachment, achieved through a peeling motion, is directly proportional to the total contact area used at any given time (Federle et al., 2001). Contact area can also be mediated by changes in gait patterns, in common house flies (*Musca domestica*) gait patterns changed in response to both physical orientation and the physical condition of individuals (Niederegger & Gorb, 2003; Niederegger, Gorb, & Jiao, 2002). Flies with removed tarsal claws took longer over some detachment actions depending upon both the direction and orientation of movement (Niederegger & Gorb, 2003).

Federle & Endlein (2004) have also shown that the Asian weaver ant *Oecophylla smaragdina* uses only a fraction of the total pad contact area available to them, even when walking upside down. However, contact area, increased when the ants were loaded with weights. On a smooth substrate with no load *O. smaragdina* used 14% of its pad area, whereas under loads of 30 mg the ant used 60% of its pad area. It has been hypothesised that the use of the full pad in the 'freezing-response' events (used in defence from predation or to increase attachment when subjected to increased wind conditions), results in an increase of adhesion through an increase in pad contact area at the cost of reduced locomotion (Endlein & Federle, 2009; Federle & Endlein, 2004).

Within the order Hymenoptera arolium extension is controlled through a combination of active and passive means triggered by leg movement (Federle et al., 2001; Gorb, 2007). Under normal conditions on rough substrates the arolium is not used, as the claws are able to interact with the realativly large surface asperities on the substrate (Endlein & Federle, 2008). Once the claws slip on a surface passive deployment of the arolium occurs, locking on surface irregularities to increase leverage and traction. While on smooth substrates once the tarsus moves past a *critical* angle the claw flexor muscle and downward force acting on the arolium causes the smooth adhesive pad to be passively deployed (Gorb, 2008). The extension of the arolium is always at the last moment indicating that it is not controlled by neuronal regulation but by the mechanical action of the claws slipping on the surface and rolling backwards around the foot. This action pulls on tendons that deploy the arolium (Drechsler & Federle, 2006; Eisner & Aneshansley, 2000; Endlein & Federle, 2008). The pad is removed from a substrate by a peeling motion, which is precipitated by a distal movement of the leg (pushing the leg away from the body).

Federle et al. (2001), suggest the arolium of bees is inflated through a joint mechanical-hydraulic mechanism, with hydraulic pressure applied from a gland in the upper leg: expanding the arolium. In the great green bush cricket (*Tettigonia viridissima*) the extension of the pad and the contact area (area of the pad substrate interaction) is actively controlled by the insect pushing down onto the substrate to directly influence contact area (Jiao, Gorb, & Schergie, 2000). This ability to remain attached to surfaces without a direct energetic cost can be modified to increase attachment forces through an increase in contact area from further extension of the arolium or behavioural changes such as lowering the centre of gravity or changes in gait. The biomechanical process involved in extending the arolium during locomotion is shown in Figure 1.10.

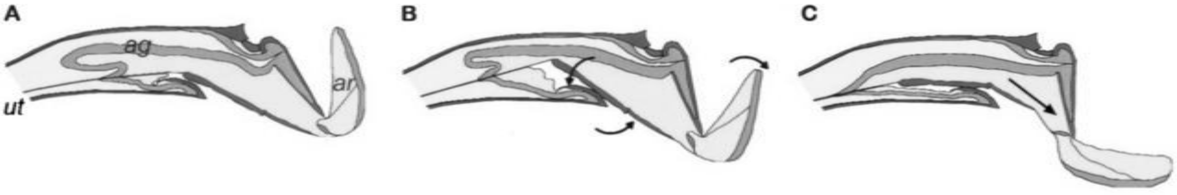

Figure 1.10: Model of the mechanics of arolium gland (ag), arolium (ar) active extension via pull on the unguitractor tendon (ut). Adapted from Federle *et al.*, 2001. A, claw flexor muscle is relaxed and the pad is in its resting position, B, the muscle has been tightened and the arolium is being extended, this occurs when the unguitractor tendon pulls the cuticle attached to the arolium up and backwards making it act like a hinge which in turn pulls the front of the arolium down, C, the arolium has been fully deployed the pull by the unguitractor has relaxed and the arolium has come to rest against the surface of the substrate.

1.3.3 Adhesive secretion.

Insects employ an adhesive secretion in order to increase the adhesive forces (Federle & Endlein, 2004; Gorb, 2005; Gorb, 2004; Persson, 1998; Persson, 2003; Qian & Gao, 2006). The secretion deposited in the footprints of insects can be seen at low magnification using light microscopy (Betz, 2003; Dirks et al., 2009; Federle, Riehle, Curtis, & Full, 2002; Geiselhardt et al., 2010; Knight, 2009; Votsch et al., 2002), and typical footprints for hairy and smooth pad types are shown in Figure 1.11. As discussed in Section 1.2.3, the effectiveness of the secretion in increasing attachment forces will depend on properties such as its spreading behaviour on the pad and substrate, surface tension, viscosity, and volume. However, any two surfaces that are joined by a thin layer of liquid which ‘wets’ both well (i.e. the liquid has a low or 0 contact angle), will demonstrate considerable adhesion.

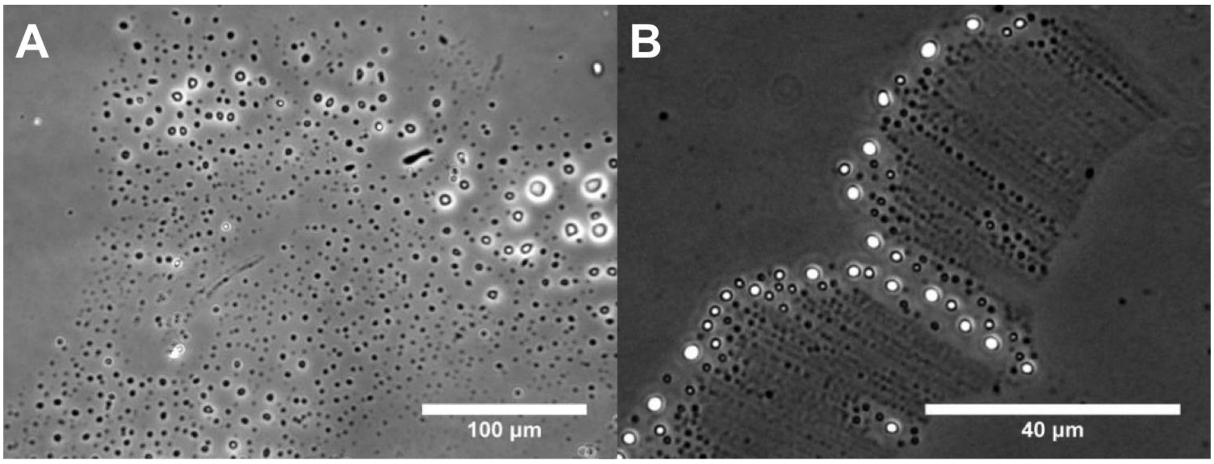

Figure 1.11: Optical micrographs (obtained using phase contrast mode) of (A) an *H. axyridis* footprint and (B) a *P. dives* footprint. The pattern of droplets observed in A is consistent with the arrangement and number density of setae in the hairy pads of *H. axyridis* (images supplied by Miss V. Knight).

Although subject to extensive research, the exact composition of the adhesive secretion is still unknown (Akiko & Ryohei, 1996; Attygalle et al., 2000; Gorb, 1998; Votsch et al., 2002). Although it is known that it is comprised of long chain hydrocarbons and waxes (Langer, Ruppersberg, & Gorb, 2004).

In recent years several authors have suggested that the adhesive secretion is actually an emulsion (a dispersion of water droplets in oil, or of oil droplets in water) (Casteren & Codd, 2010; Dirks et al., 2009; Drechsler & Federle, 2006; Federle, Riehle, Curtis, & Full, 2002; Gorb, 2005; Gorb, 2001; Votsch et al., 2002). It is possible however, that the "emulsion" seen in the literature is an artefact of the sample preparation, which involves freezing of the secretion. However the complex non-Newtonian behaviour of the secretion can also be explained by a model for single phase colloidal liquid of wax crystals (Betz, 2010, pp147). This colloidal suspension of solid alkane crystals suspended in a liquid alkene matrix might help explain the rate dependent viscosity changes observed in the adhesive secretion used by insects. However it is possible that the adhesive secretion changes between taxa or even species, with the bulk phase being relatively stable, differences in the adhesive performance could be due to unidentified chemical compounds used to optimise adhesive effort on plant surfaces commonly encountered by a specific species.

The contact angle (CA) is the angle at which a liquid makes contact with an interface (surface or liquid) (Figure 1.12) and is specific for a given system.

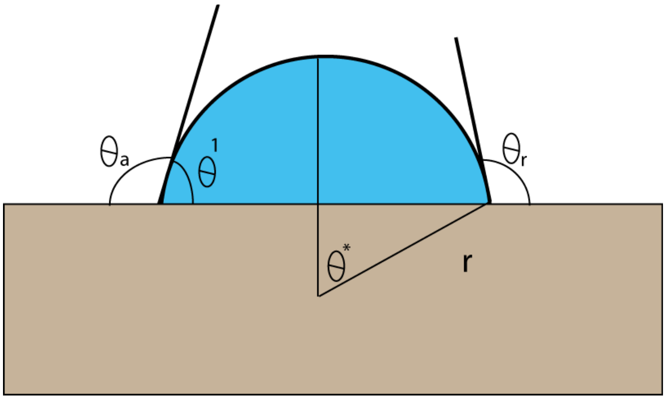

Figure 1.12: Side view of a liquid drop (volume v), on a smooth substrate. Θ^1 is the contact angle of the droplet on the substrate, θ^* is the internal angle of the droplet and r is the radius of curvature, θ_a and θ_r as defined by equation 6.

Contact angles show a liquids ability to wet a surface (Extrand, 2003; Extrand, 2006). The contact angle can be used to determine the composition of a secretion and its interaction with a substrate. If a liquid is attracted to a surface it will spread out and the angle of contact will be low whereas if the liquid is repelled by a surface it will bead up and the angle of contact will be high (Table 1) (Figure 1.13).

Table 1: contact angle measurements and ascribed surface wetting values for liquid solid interactions.

Contact angle	Degree of wetting	Adhesive force (liquid/solid)
$\theta = 0$	Liquid perfectly wets surface	Strong
$\theta < 0 < 90°$	Liquid wets high amount of surface	Strong
$90° \leq \theta < 180°$	Liquid wets low amount of surface area	Weak
$\theta = 180°$	Liquid does not wet surface	Weak/repulsive

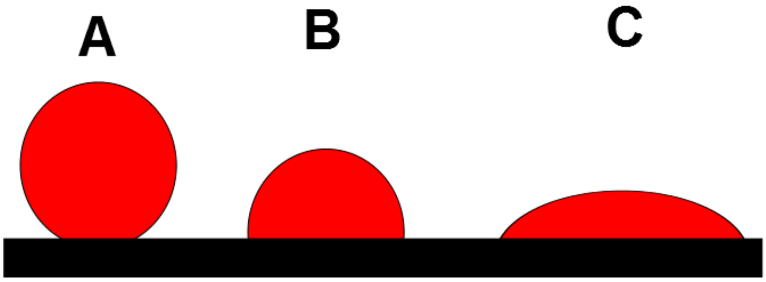

Figure 1.13: Typical droplet shape for liquid on solid surface with (A) very little wetting, (B) more wetting and (C) high level of wetting, droplet A has a high contact angle while droplet C has a lower contact angle.

The adhesive force between the liquid and the substrate causes the drop to spread over the surface whilst the cohesive forces within the liquid cause it to bead up limiting contact with the surface. In non-ideal systems (i.e. real substrates with non-smooth surfaces) the contact angle H is defined using the difference between the advancing (θ_a) and receding (θ_r) (shown in Figure 1.13 above), CA using the following equation.

$$H = \theta_a - \theta_r \qquad [6]$$

CA can also be reported as $CA = \theta_c$ where θ_c represents the contact angle equilibrium. The shape of a droplet is determined by the adhesive forces between the droplet and the surface and can be determined using the Laplace equation discussed earlier, while contact angles are determined by the cohesive forces within a liquid are derived using the Young equation,

$$\gamma_{SL} + \gamma_{LG} \cos\theta = \gamma_{SG} \qquad [7]$$

where γ_{SL}, γ_{LG} and γ_{SG} are the interfacial tensions between solid and liquid, liquid and gas and solid and gas respectively, interfacial tensions are described as forces per unit length, assuming a perfectly flat surface.

1.4 Attachment capabilities and ecology

1.4.1 Adhesion and copulation

As well as differences in attachment ability between species there are also differences between the sexes within a given species. In many species males constantly evolve new adhesive devices to hold the female during copulation (i.e. the changing elytra morph seen in water beetles discussed below) ("offensive traits"); forcing the female to evolve new defensive traits to reduce mating rates and ensure only the stongest males reporduce (Arnqvist & Rowe, 2002a; 2002b; Hardling & Bergsten, 2006). The dimorphism in attachment in ladybeetles allows males with the strongest attachment forces to successfully transfer genes, being unable to be pulled off the female during mating (Gorb, Hosoda, & Gorb, 2009b).

The effects of female driven attachment can be seen in the diving beetles, the great diving beetle (*Dytiscus lapponicus*) [Gyllenhal, 1808] and *Graphoderus zontaus verrucifer* [Sahlberg, 1824] (Hardling & Bergsten, 2006), where the female has two differing wing casing (elytra) morphs; smooth and granulated. The attachment pads of the male come in one of two morphs (one with small suction cups and one with larger suction cups) associated with the female elytra form to allow for attachment during mating. Changes in frequency of female elytra type results in a change in prevalence of each male pad form. Gorb *et al.* (2009) show a difference in the attachment abilities of the seven-spot ladybird (*Coccinella septempunctata*) with the males displaying a greater attachment force on smooth substrates and the females adhering better to rough surfaces. This difference in ability is thought to be related to the mating behaviour of this species, where the male has to cling onto the smooth elytra of the female for extended periods of time during mating and as such have a differing structure to the ‘hairy’ pads than the female, with a larger number of disc like spatula along with the setal forms commonly found in beetles. Adhesion also has an influence on oviposition behaviour post copulation (Biatar et al., 2009; 2010).

1.4.2 Foraging and adhesion

Southwood (1986) and Eigenbrode, (2004), suggests that the ability to attach to differing plant substrates was a pivotal point in the development of ant-plant symbiosis, opening up new resources, with the numbers of insect orders able to colonise terrestrial plants being limited by the basic morphology and abilities of their adhesive devices.

Adaptation of adhesion within a species allows for differing niches to be exploited (Orivel, Malherbe, & Dejean, 2001). By observing the adhesive abilities of 15 species of ant from the genus *Pachycondyla*, Orivel, Malherbe, & Dejean, (2001) found that seven species have small or absent adhesive pads which, when coupled with the narrow and straight design of the tarsal claws has led to the loss of ability to adhere to smooth surfaces. This was in direct contrast to the other eight species that possessed a large adhesive pad and wide placed curved tarsal claws. After comparing the life histories of each species and finding several species with large arolium that were ground dwelling they make two conclusions; firstly that the development of a large arolium should not be thought of as an adaptation for arboreal life but instead is a significant development towards the evolution of arboreal behaviour. Secondly that the ability to adhere to smooth substrates is directly related to the presence of a large arolium without which adhesion to smooth substrates would not be possible. Studies have shown, however, that environmental pressures can force arboreal ants to forage at ground level. By comparing behaviours between sister species, Gove & Majer, (2006) suggested that trees isolated through natural events or logging and agricultural practices could cause arboreal ants to forage more at ground level and found a correlation between activity levels of the generalist ant *Solenopsis geminate* (which is able to forage both at ground level and arboreally) and levels of agricultural sophistication, as previously hypothesised by Risch & Carroll (1982) and Nestel & Dickschen (1990). This change in behaviour may lead to loss of ecosystem function (Luck, Daily, & Ehrlich, 2003) and nutrient turn over as foraging ants move to novel environments.

It has been established experimentally that the magnitude of insect attachment forces depends on substrate properties such as roughness and surface energy (Dai, Gorb, & Schwarz, 2002; Federle, Rohrseitz, & Holldobler, 2000; Gorb, Hosoda, & Gorb, 2009; Hardling & Bergsten, 2006; Stork, 1980), with insects known to adapt adhesive ability to specific plant surfaces, driving host plant speciation. An extreme example, discussed in Section 1.4.3, is that found in the *Macaranga-Crematogaster* relationship, although other examples of such relationships can be found between the ant *Camponotus schmitzi* and the pitcher plant *Nepenthes bicalarata* (Bonhomme et al., 2011) and undescribed *Tetraponera* sp. (Formicidae, Pseuomyrmecinae) and its bamboo host (Klein, Maschwitz, & Kovac, 1993). Although the extent to which adhesive capabilities influence foraging behaviour and resource exploitation have not yet studied in detail, Detrain, Natan, & Deneubourg, (2001) suggest a link between substrate properties and foraging behaviour in *Lasius niger* (Linnaeus), suggesting that the physio-chemical differences between the two paper typesused as substrates in this trial might influence trail laying behaviours and subsequent recruitment. In a study by Bernadou & Fourcassie, (2008), a relationship between substrate coarseness and locomotory behaviour in the ant *Lasius niger* was found, with ants moving faster on fine sand substrates than on rough ones, suggesting that abiotic factors may influence adhesion and foraging behaviour. Although, as will be discussed in chapter two the relative sizes of the ant and particles used may have essentially represented a ‘solid’ surface, unlikely to foul the adhesive devices, with movement parameters most likely governed by the difficultly to traverse the differing surface profiles.

It is established that environmental factors such as relative humidity and temperature affect foraging strategies for insects (Holldobler & Taylor, 1983; Holldobler & Wilson, 1990; Whitford, Depree, & Johnson, 1980). It is known that some species show a distinct temperature regulated pattern to their foraging behaviour. For example, the ant *Caponotus vicinus* will start foraging at temperatures just above freezing but stops foraging at temperatures above 20 °C (Holldobler & Wilson, 1990). In a study by Holldobler & Taylor, (1983) it was found that the ant species *Nothomyrmecia macrops* (Clark 1951) hunts primarily after dark, with workers showing more activity at temperatures between 5 and 10 °C. It was suggested that this temperature trigger allows *N. macrops* to forage more efficiently by hampering the prey insects it finds in the canopy. This link between temperature, humidity and foraging activity might be explainable in terms of metabolic rates, but may also be explainable in terms of the effect of such environmental factors on adhesion. Temperature, for example, will obviously have an effect on the viscosity of the adhesive secretion used by insects, and hence would be expected to influence attachment forces. Humidity will also affect the surface energy of substrates such as mineral grains and plant stems, with water droplets forming on surfaces as well as potentially softening the material properties of the adhesive pad itself as seen in gecko's (Puthoff et al., 2010).

Indeed, recent studies have established a dependence of insect adhesive forces on temperature. The adhesive secretion of the ant *Oecophylla smaragdina* [Fabricius 1775] was found to have a static, temperature independent component and a dynamic component sensitive to temperature (Federle, Baumgartner, & Hölldobler, 2004a), similar to the temperature dependence of adhesion also observed in geckos (Losos, 1990). In a study on dynamic friction forces of *O. smaragdina* (Federle et al., 2004b) found that the relative forces were larger at 15°C than at 30°C, presumably due to a decrease in the viscosity of the adhesive secretion with temperature. The dependence of adhesive forces on temperature and other environmental factors, suggests that animals forage at times that would allow them to maximise their adhesive abilities and forage efficiently, an idea supported in part by studies showing changes in foraging activity in relation to temperature and humidity in tropical ants (Holldobler & Wilson, 1990; Levings, 1983; Levings & Windsor, 1984; Torres, 1984).

Foraging decisions of bumblebees was studied in a recent paper (Whitney, Chittka, Bruce, & Glover, 2009); where it was found that bumblebees preferentially forage on snapdragon flowers with a rough petal phenotype, landing on rough petals more often than on smooth ones. Whitney *et al.* (2009) compared ‘handling’ times (length of time the bee would spend manipulating the flower to obtain nectar) for the two flower forms and found that bees would spend less time manipulating the flowers with the smooth petal phenotype. Whitney *et al.* (2009) suggested that the rough petal phenotype allowed for greater adhesion and reduced handling times; making manipulation of the flower easier and increased the amount of nectar an insect could collect. No explanation for how the bees might tell which petal form they are going to land on was suggested, although it may be possible that the differing shaped cones of the rough and smooth phenotype may reflect light differently within the UV spectrum and allow the bees to choose.

1.4.3 Insect-plant interactions

Interactions between plants and foraging insects have been occurring since the cretaceous period (Holldobler & Wilson, 1990) with some insects subsisting on nectar and pollen from angiosperms and others eating the tissues of the plant themselves. This arms race between insects and plants has resulted in plants developing thicker cuticles, denser wax coverings, and dense coverings of spines and hairs on the outer surfaces to impede insect attachment and locomotion (Duetting et al., 2003; Eigenbrode, et al., 1996; Eigenbrode, Kabalo, & Stoner, 1999; Eigenbrode et al., 2000; Holldobler & Wilson, 1990; Schoonhoven, Loon, & Dicke, 2005; White & Eigenbrode, 2000), as well as developing unpalatable compounds such as *Coniine* (Mody et al, 1976), and highly movable surfaces which compromise adhesive ability and reduce predation by herbivores (Yamazaki, 2011). Over time the relationships between ants and plants have developed to be parasitic (Holldobler & Wilson, 1990; Schoonhoven, Loon, & Dicke, 2005), mutualistic (Buckley, 1982; Davidson & Mckey, 1993; Linsenmair et al., 2001) or symbiotic, with some plant species relying on an insect partner for protection from foraging herbivores (Linsenmair et al., 2001).

1.4.3.1 Anti-adhesive plant surfaces

The outer surfaces of most vascular terrestrial plants are covered in a range of differing structures derived from a mixture of lipids (epicuticular waxes), ranging from simple mono layers of wax to thick wax coverings and glandular trichomes (Guhling et al., 2005; Koch & Ensikat, 2008; Koch et al., 2004). Epicuticular waxes are often complex mixtures of alcohols, ketones, aliphatic hydrocarbons and fatty acids (Eigenbrode, 2004; Walton, 1990). These outer defences are used both to avoid desiccation through evaporation and as a barrier to insect pests. This reciprocal adaptation between insects and plants has led to the development of both behavioural and physiological adaptations within the insect world to negate the effects of the plant defences.

Holloway, (1969) suggests that smooth films of epicuticular waxes differ in surface energy, which could alter the effectiveness of the insect secretion to wet the surface and as such affect the magnitude of attachment forces (Eigenbrode, 2004), the effect of Free Surface Energy (FSE), a measure of the coefficient of friction of a surface on adhesion will be discussed in chapter three. In a study by Eigenbrode & Jetter, (2002) it was shown that insect adhesion is greater on waxes containing primary alcohols than on fatty acids. In a paper by Stoner, (1990) it was shown that plants might use waxes as a means of slowing down insect herbivores so reducing predation on the plant, possibly due to the wax disoloving hypothesis

More important than surface energy however, is the presence of rough or fragile waxy coatings. The epicuticular waxes covering the stems of terrestrial plants fracture and buckle as new waxes are formed beneath the outer layer and push outwards towards the surface. This constant production of new wax causes broken sections of the outer wax layer to be replaced as the new wax comes through. Once fractured, the waxes covering the stems of terrestrial plants are able to regenerate through the continued propagation of new waxes, thus avoiding desiccation of the vascular tissues. Through this process of new waxes pushing through to the surface a plant will typically have several differing layers of waxes on the same section (Koch et al., 2004), this regeneration could also allow for fouling of adhesive devices through the propagation of small wax particles.

The effects of waxy substrates on attachment abilities have been the subject of a growing body of literature. Gorb & Gorb, (2002) showed that the beetle *Chrysolina fastuosa* displayed poor attachment to plants with rough waxy surfaces and rates of movement dependant on substrate properties. Although smooth waxy surfaces did not affect attachment abilities, the covering of *Acer negundo* (maple tree) stems with waxy crystalloids disabled beetle attachment not only on *A. negundo* but also on other substrates for a short period afterwards (Eigenbrode, 2004; Eigenbrode & Jetter, 2002;

Eigenbrode et al., 2000). Although, the ability of rough and/or fragile waxy coatings on plant surfaces to reduce locomotion and adhesion in insects has been extensively demonstrated (Duetting et al., 2003; Eigenbrode, 2004; Eigenbrode & Jetter, 2002; Eigenbrode et al., 1996; Federle, Maschwitz, & Fiala, 1998; Federle et al., 1997, 2002; Federle, Rohrseitz, & Holldobler, 2000), no one has yet performed systematic studies of adhesive abilities on differing surface types (smooth, rough, changes in surface chemistry etc.).

Gorb & Gorb, (2002; 2006) have proposed four hypotheses to explain the anti-adhesive properties of rough waxy coatings.

1) *The contamination hypothesis:*

   The insect's adhesive pad may become contaminated by easily detachable wax crystals, from the epicuticular layer of terrestrial plants. As the insect steps on to the plant surface the wax crystals fracture and stick to the adhesive pad, reducing functionality.

2) *The wax dissolving hypothesis:*

   As the adhesive secretion is deposited into the contact zone it causes the epicuticular wax crystals to dissolve, becoming a slippery lubricating layer. Subsequently reducing both tangential and normal frictional forces, effectively limiting adhesive potential.

3) *The roughness hypothesis:*

   That the epicuticular wax crystals are layered in such a manner to give a micro-roughness scale, below the threshold that the adhesive pad is able to deform to.

4) *The fluid absorbing hypothesis:*

> That the outer layer of a plants surface may cause the secretion to be absorbed into the surface, potentially reducing available contact area and consequently adhesive force.

Several studies have been performed to determine which are the main driving factors in relation to the four main hypothesis of adhesion in insects. In 2006 Gorb and Gorb found evidence for the contamination hypothesis by observing the contamination of adhesive setae in the dead nettle leaf beetle (*Chrysolina fastuosa*) after being exposed to plant waxes. However, despite a growing body of literature the effects of contamination have so far been only empirically tested in hairy adhesive pads (Federle et al., 1997, 2002; Gaume et al., 2004; Gorb & Gorb, 2002; Gorb, 1998; Ishi, 1987; Lees & Hardie, 1988; Markstadter et al., 2000; Walker, Yulf, & Ratcliffe, 1985). Where instances of reduced adhesion were observed without any corresponding evidence for contamination it was suggested that the reduction of the contact area coupled with the absorption of the adhesive secretion by the epicuticular wax is involved, although the adsorption hypothesis has yet to be empirically tested. The continued interaction between the adhesive pads of insects and the broken particulate wax barriers of terrestrial plants make it conceivable that insects would possess a self-cleaning mechanism as described for gecko setae (Hansen & Autumn, 2005).

1.4.3.2 Insect-plant relationships

Foraging insects need to be able to move freely over surfaces with differing hydrophobic and hydrophilic properties (Clemente and Federle, 2012) and must be able to overcome changes in relative surface energies to fully exploit the localised environment (Peattie, 2009). Some predatory insects hunt preferentially on plants with a dense covering of lipophilic waxes (Eigenbrode, 2004) which hamper or limit the movement of prey items, whilst some insects prefer to deposit eggs on plant surfaces with increased levels of waxy blooms and as such afford their developing embryos some measure of protection from predators (Brennan et al., 2001). It is these behaviours, which could lead to foraging in novel environments and the development of new adaptations to exploit novel resources in ant-plant (myrmecophytic) relationships.

There is an ecological and energetic trade-off between growth and defence in plants, but plants associated with an insect partner seem able to invest more in growth because the insect protects them from attack from herbivores and fungal pathogens (Schoonhoven, Loon, & Dicke, 2005). Ant species in Africa have been shown to reduce damage to their host plants from herbivores by removing caterpillars (Bequaert, 1922), and *Pseudomyrmex* ants protect their host plants from grazing herbivores (Janzen, 1972). While plants have been shown to provide refuges (domatia) for symbiotic insects (Janzen, 1966; Beattie, 1985). However, it has been suggested that increased specialisation in both host plant and attachment ability of an insect could lead to a reduction in effective generalist competiveness (Davidson & Mckey, 1993; Federle, Rohrseitz, & Holldobler, 2000). Indeed, relationships between insects and plants differ in their degree of specificity and absolute species specificity (total symbiosis wherein without one the other species cannot survive) is rare (Davidson & Mckey, 1993). Increased specialisation in attachment and insect-plant relationships may be a means by which weaker ant species could escape high predation pressures from more aggressive carnivorous insects (Davidson & Mckey, 1993; Federle, Rohrseitz, & Holldobler, 2000).

The *Crematogaster sp* - *Macaranga* is the most widely studied myrmecophytic relationship, where the *Macaranga* tree provides food and shelter for the ants, and the ants protect the tree from invading neighbouring plants and herbivorous insects. Within the *Macaranga* genus there are differences in the density of epicuticular waxes, and the presence of this barrier is of utmost importance to the symbiotic ant species. Prominent waxy blooms on the stems of some *Macaranga sp* present an effective barrier. Generalist ants are unable to traverse the barrier whilst ants that have a close relationship with the waxy tree are able to forage and thrive without suffering from predation from more aggressive species (Federle, Rohrseitz, & Holldobler, 2000; Gorb, 2001, 2007; Orivel, Malherbe, & Dejean, 2001). The Crematogaster morpho-species is capable of traversing the 'glaucous' (waxy) surfaces of the host *Macaranga* plant without any apparent difficulty, building nests within the tissues and hollow stems of the tree, providing it with protection from other ant species and predation by herbivorous insects (Federle et al., 1997, 2002; Gorb, 2008; Itino et al., 2001). The ability of the symbiotic ant partner *Crematogaster* (Decacrema) to run over the waxy stem and avoid predation from more aggressive predatory ants is shown in Figure 1.14; here the symbiotic ant is safe above the wax boundary with the generalist sister species *Crematogaster* cf. a*rtifex* unable to cross the wax barrier. It is not yet known how the wax-running ants are able to adhere to the waxy blooms on *Macaranga* stems.

Figure 1.14: Effects of wax barriers in relation to ability to escape predation pressures. Both ant species in image are from the *Crematogaster* genus but only *Crematogaster (*Decacrema) species can move with ease on the wax covering of the *Macaranga* plant and as such escapes predation from *Crematogaster* cf. a*rtifex* (image taken from Federle *et al.,* 2000). A, area of *Macaranga* tree with un-manipulated waxy covering, B, area manipulated to remove waxy covering.

Study species

During the course of this research the adhesive abilities of the two main pad types (hairy and smooth), will be investigated with respect to the four main hypothesis (outlined previously) using representative species. Species were chosen based on their ease of procurement so as to ensure a good supply of sepcies throughtout the study, ease of maintenance and adhesive traits, with only species known to actively climb biological surfaces chosen. To probe for differences in pad types we use the hymenopterans *Polyrhachis dives* and *Myrmica scabrinodis* as representatives of insects with smooth pads, and the coccinellids *Harmonia axyridis* and *Adalia bipunctata* as representative of hairy padded insects. All the species used have been observed to have the ability to walk on waxy plant surfaces and in the case of *P. dives* has been observed to use its adhesive abilities to build nests.

Hymenopterans

Hymenoptera is one of the largest orders of insects described by Linnaeus [1758], including wasp, bees, ants and saw flies with some 13000 species currently described; all Hymenopterans so far described have smooth adhesive pads. With few exceptions members of this order are typically known as the social insects (Hölldobler and Wilson, 1990).

Polyrhachis dives (Smith, 1857)

Although not a true weaver ant *P. dives* (Figure 1.15) is often referred to as such due to its ability to use silks to build its nests (Hölldobler and Wilson, 1990), it is found across Asia and Australia and possesses a smooth attachment pad with the tarsi terminating in a claw. It shows dimorphism within the species with the queen approximately 12 mm in length, with two worker castes, foragers being 8 mm in length and the soldiers approximately 10 mm in length with larger mandibles.

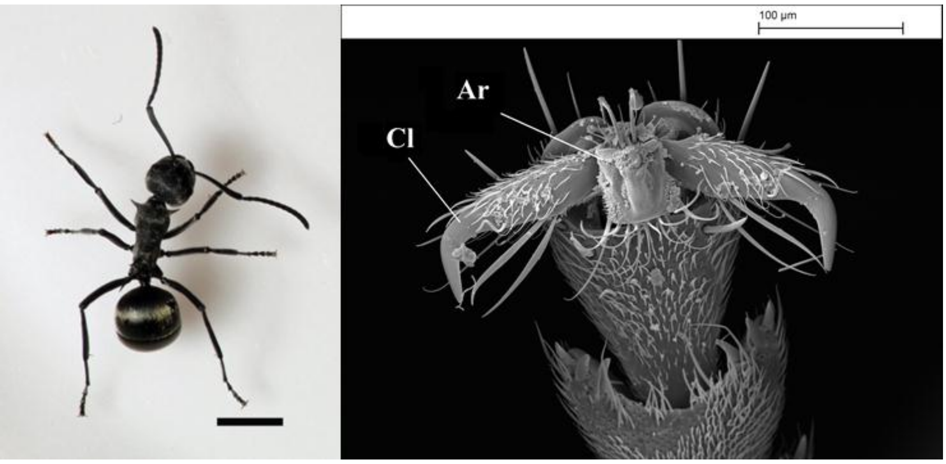

Figure 1.15: Asian weaver at *P. dives* on left and SEM image of its terminal tarsal segment showing adhesive devices on the right, Cl = tarsal claws, Ar = arolium, scale bar in ant image = 2mm.

Myrmica scabrinodis (Nylander, 1846)

M. Scabrinodis (Figure 1.16) is commonly referred to as the Spanish red wood ant; it belongs to the *Myrmica* family and is found living in grassland areas. There is little dimorphism between workers and queens within this species with queens being approximately 9 mm in length and workers being 6-7 mm in length. It belongs to the *Myrmica* genus and is found in grassland areas. It also possesses a smooth attachment pad with the tarsi terminating in a claw.

Figure 1.16: Spanish red-elbowed ant *Myrmica scabrinodis* on left and SEM image of its terminal tarsal segment showing adhesive devices on the right, Cl = tarsal claws, Ar = arolium, scale bar in ant image = 2 mm

Coccinellids

Coccinellids are a family of beetles known as ladybirds, ladybeetles or ladybugs, typically small insects ranging from ~1-10 mm in length. Described by Latreille [1807], they show a range of colour morphs and can be solitary or form large overwintering aggregations.

Harmonia axyridis (Pallas, 1773)

The Asian ladybird species *H. axyridis* (Figure 1.17) can now be found across the UK. It possesses a hairy attachment pad on the terminal tarsal segments with the tarsi terminating in a claw.

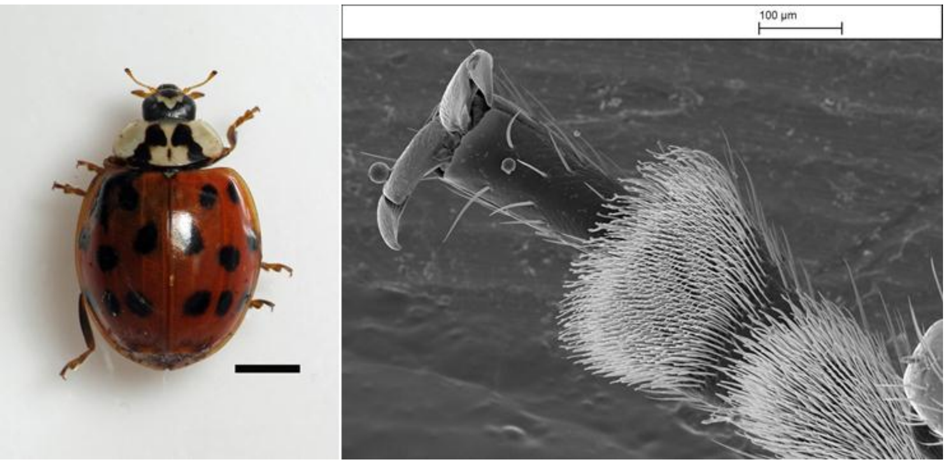

Figure 1.17: Asian Ladybeetle *Harmonia axyridis* on left and SEM image of its terminal tarsal segment showing adhesive devices on the right, Cl = tarsal claws, Ar = arolium, scale bar in ladybeetle image = 2mm.

Adalia bipunctata (Linnaeus, 1758)

The two spot ladybird *A. bipunctata* (Figure 1.18) is the second most common ladybird in the UK. It possesses a hairy attachment pad with the tarsi terminating in a claw similar to that possed by *H. axyridis* but on a smaller scale.

Figure 1.18: Two spot ladybeetle *Adalia bipunctata* on left and SEM image of its terminal tarsal segment showing adhesive devices on the right, Cl = tarsal claws, Ar = arolium, scale bar in ladybeetle image = 1mm.

Experimental chapters:

The aim of this thesis is to gain a better understanding of the constraints that affect insect adhesion.

Chapter two:

Aim: To extend the contamination hypothesis to cover smooth pads as well as hairy.

In this chapter I discuss the issues of particulate contamination of adhesive organs in ants and ladybirds. My findings support previous work regarding the contamination hypothesis in so much as the hairy pads became fouled, and add further support by showing for the first time that contamination of adhesive organs also occurs in insects with smooth pads.

Chapter three:

Aim: To determine if fouled adhesive devices can self-clean, and probe the effects of surface energy on self-cleaning abilities.

In this chapter I discuss the consequences of contamination of the adhesive devices, namely once they are fouled and adhesive contact is reduced and conversely adhesive force also reduced, can they self-clean or do insects need to actively preen. I find evidence for the passive self-cleaning of adhesive devices by friction between contaminating particles and substrate, with an interaction between the Free Surface Energy (FSE) of each.

Chapter four:

Aim: To determine the validity of the remaining hypotheses put forward by Gorb regarding insect adhesion, surface roughness, surface energy, and porosity.

As evidence for the contamination hypothesis was found in chapter one the work in this chapter deals with the three remaining hypothesis regarding insect adhesion. Using a novel surface (silica spray) I test for maximum angle of inclination within a beaker series, I test for angle of detachment on slide surfaces; I measure [normal] adhesive forces on a range of surface types and compared these forces to the maximum angle of inclination achieved in the beaker trials. I find that on the silica surface, FSE has no contributable effect on adhesion with insects failing to adhere to hydrophilic or hydrophobic surfaces, through the use of SEM images I find no evidence for contamination of the adhesive devices from degradation of the silica surface. Due to the nature of the surface type I was unable to control for porosity, but this has been shown to reduce adhesion in a recent publication E Gorb (2011). As such I conclude that it is surface roughness and more specifically roughness below a critical range <1µm that significantly reduces adhesion in insect. This result fits in with the critical adhesion range found for other insects and indeed is similar to the critical range found for geckos.

Chapter five:

Aim: To determine the ability of insects to assess the adhesive potential of a surface before traversing it. A recent paper by E Gorb (2011) suggests that they are able to walk on all surface types they come across but ‘choose’ to remain on surfaces that give good adhesion and I suggest also locomotive ability, this will addressed further in the final chapter.

In this study I find that the insect is able to determine between tacky, particulate and smooth surfaces, but unable to determine the FSE of smooth surfaces. I hypothesise that the tacky resins of plants surfaces and/or the broken wax coverings will reduce the mechano-sensing ability of insect antenna. That the ability to sense the adhesive potential of a surface gives the insect the ability to move through mosaic landscapes, that walking and flying insects will perform differently during this study, with flying insects poorer at the decision making regarding substrates, and furthermore that the difference is directly linked to the morphology of the antenna themselves.

Chapter six:

Aim: To link adhesive potential with foraging strategy.

I measured the [normal] adhesive forces on a series of experimental substrates, and also recorded movement parameters such as distance and velocity travelled on each substrate types. Although still preliminary, I find a link between substrate roughness and movement parameters, suggesting that adhesive potential directly influences speed and time spent on a substrate. In light of recent work by E Gorb (2011), the interesting fact here is that they stop at the interface between surface types and ‘choose’ to stay on the surface with the roughest substrate. This may be linked to changes in gait/posture/ centre of gravity needed between substrate types.

Chapter two

The effect of particulate contact fouling on adhesion in the hymenopteran species *Polyrhachis dives* and *Myrmica scabrinodis*

Evidence for the contamination hypothesis

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*Denotes joint authorship

2.1 Introduction

Insects have evolved numerous adaptations to enable them to move rapidly across natural surfaces within their ecological niches. Efficient adhesion is crucial for many different aspects of an insect's life, such as mating and oviposition (Bitar et al., 2009; Bitar et al., 2010), foraging and prey capture (Hölldobler and Wilson, 1990; Bauer et al., 2008), defence (Eisner and Aneshansley, 2000; Betz and Kolsch, 2004) and the selection and construction of nesting sites (Federle et al., 1997), especially for arboreal insects (Federle et al., 2002).

When surfaces are rough, insects can utilise their tarsal claws to attach to surface asperities (Federle et al., 2002). However, adhesion to smooth substrates is facilitated by special adhesive pads that have convergently evolved several times to conform to one of two main types: 'hairy' (arrays of microscopic setae) and 'smooth' (soft deformable pads) (Gorb and Beutel, 2001). It has been found that both pad types in insects deposit a liquid secretion to the contact zone during locomotion, with adhesion mediated by capillary and viscous attractive forces acting during static and dynamic situations, respectively (Nachtigall, 1974; Stork, 1980a; Walker et al., 1985; Ishii, 1987; Wigglesworth, 1987; Lees and Hardie, 1988; Dixon et al., 1990; Walker, 1993; Gorb, 1998; Federle et al., 2002). Adhesion has been found to be strongly related to the contact area of the attachment pads with the substrate, thus presence of the liquid aids adhesion by maximising the contact area between the pad and substrate by filling in micro-surface asperities (Vötsch et al., 2002; Drechsler and Federle, 2006; Dirks et al., 2009).

Many climbing insects (e.g. ants and beetles) spend much time walking on plant surfaces and require strong adhesion when walking vertically or upside down, sometimes carrying the equivalent of several times their own body weight (Hölldobler and Wilson, 1990). As such, it is necessary to continually ensure the effective functioning of their adhesive devices. However, it has been observed that many plants possessing fragile waxy layers or crystals are able to provide effective barriers against climbing insects (Stork, 1980b; Federle et al., 1997; Federle et al., 2000; Markstädter et al., 2000; Gorb and Gorb, 2002; Eigenbrode, 2004; Gaume et al., 2004; Gorb et al., 2008; Borodich et al., 2010). It has been proposed (Gorb and Gorb, 2002) that this anti-adhesive effect arises from the fact that the wax crystals are easily detached from the plant cuticle, breaking off when insects walk on them, contaminating the insects' attachment devices.

Contamination of attachment pads drastically reduces the contact area between the pad and the substrate, reducing overall adhesive forces. Substrate properties such as the surface energy and surface topography of these wax particles can influence the adhesive forces in insects, and a combination of these influences has been shown to drastically reduce the adhesive ability of beetles such as *Gastrophysa viridula* (Coleoptera; Chrysomelidae), which possess hairy adhesive pads (Gorb and Gorb, 2009). Similar effects have also been found for synthetic powder barriers, which have been found to form effective barriers against crawling insects (Briscoe, 1943; Alexander et al., 1944; Merton, 1956; Boiteau et al., 1994; Glenn et al., 1999); such barriers could potentially be used as an ecologically friendly method for the control of insect pest species (Boiteau et al., 1994; Hunt and Vernon, 2001). However, there have been few studies of how the anti-adhesive properties of natural or synthetic particle barriers depend on the physicochemical properties of the contaminating particles. In this chapter, we focus on the smooth adhesive pads of ants and study systematically how particulate contamination by synthetic powder barriers and the subsequent loss of adhesion depends on particle size, particle surface energy and humidity.

Insects are able to reduce the detrimental effects of attachment pad contamination by using a number of different strategies that can be categorised under (1) passive ‘self-cleaning’ mechanisms, which have been found in insects with both smooth and hairy pad types (Clemente et al., 2010; Orchard et al., 2012), as well as geckos (Hansen and Autumn, 2005; Lee and Fearing, 2008); and (2) active grooming behaviours (see Hosoda and Gorb, 2011). In particular, Clemente et al. have found that both smooth and hairy pads exhibit self-cleaning properties when contaminated with glass microspheres in a range of sizes (1–450 µm), finding that adhesion forces can return to normal after several steps (Clemente et al., 2010). Specifically for smooth adhesive pads, they found that self-cleaning was aided by shear movement of the tarsal pads in the proximal direction. Reduction of adhesive force has also been found to trigger grooming behaviour in beetles walking on manufactured nanostructured surfaces (Hosoda and Gorb, 2011), demonstrating that the reduction of adhesion or friction force between tarsal attachment pads and the substrate provides the insect with information on the amount of contamination of its adhesive pad, influencing their behaviour.

However, although grooming behaviours can remove particles from already contaminated attachment pads, to prevent contamination from initially occurring in the first place, it is reasonable to assume that insects may possess a system of detection and avoidance via their antennae. Specifically, it is possible that insects may also be able to use their antennae to ‘detect’ the material properties, such as surface morphology and roughness, of a substrate – in this case a powder barrier. Indeed, it is documented that insects use their antennae to detect numerous aspects of their surroundings (Kevan and Lane, 1985; Crook et al., 2008), with recent work demonstrating that the information relayed from tactile influences (Bernadou and Fourcassie, 2008; Bernadou et al., 2009) can be used in decision-making (Camhi and Johnson, 1999). However, this important question has yet to be addressed in a systematic way for loose powder barriers. Thus the second aim of this paper is to determine to what extent the ant species used are repelled by the powder barriers and how this behaviour may also be influenced by the physicochemical nature of the powder barrier. In order to study the effect of powder barriers on insect adhesion and repellence, worker ants from the species Polyrhachis dives Smith 1857 and *Myrmica scabrinodis* Nylander 1846 (Hymenoptera; Formicidae) were placed within the centre of circular barriers constructed of loose powders of synthetic particles, and their behaviour and adhesive ability after crossing the barrier was observed. These species are representative of the insect order Hymenoptera, both possessing smooth adhesive pads known as arolia (Gladun et al., 2009). These species were chosen in order to compare the behaviour and subsequent attachment ability of species of contrasting size and which are native to different ecological niches. Firstly, the insects' ability to climb vertical smooth surfaces after traversing the barriers was tracked. Secondly, the time spent investigating the barriers themselves with their antennae, a behaviour known as 'antennating' (Bernadou and Fourcassie, 2008), before the insect attempted to cross was recorded. During all experiments, the effects of the powder particles on attachment ability were investigated systematically by changing the particle material and size, and the relative humidity at which the experiments were performed, to elucidate the factors affecting insect adhesion and repellence.

2.2 Materials and methods

2.2.1 Insects

Worker ants were extracted from colonies of *P. dives* and *M.scabrinodis* purchased from a supplier (Anstore, Berlin, Germany). Colonies were held in glass formicaria in the laboratory and maintained at 20–25°C under a 14 h: 10 h light:dark cycle. Each species was fed an ant-feed mixture (Antstore), dried seeds and dried insects *ad libitum* several times a week. The length of the insects' claws and claw basal distance – defined here as the distance between the claws at the point at which they emerge from the tarsal cuticle – were measured by imaging the tarsi with a digital camera (Canon Powershot S31S, Canon UK Ltd, Reigate, Surrey, UK) connected to a Nikon SMZ800 stereo-optical microscope (Jencons-PLS, East Grinstead, West Sussex, UK) *via* an adaptor mount (MM99 S/N 3506, Martin Microscope Co., Easley, SC, USA). Digital images were analysed using the software package ImageJ 1.40, National Institutes of Health, Bethesda, MD, USA) (Rasband, 1997–2009). Visualisation of contamination of the insect tarsi and antennae was achieved using scanning electron microscopy (SEM). Insect samples were air-dried, coated with 2 nm of gold-palladium and imaged using a Zeiss EVO60 electron microscope in high-vacuum mode at 2 kV beam voltage and 100 pA probe current.

2.2.2 Powder particles

Polytetrafluoroethylene (PTFE; Sigma-Aldrich, Dorset, UK) and soda lime Ballotini glass (VWR-Jencons, Lutterworth, Leicestershire, UK) particles of various diameters, along with 1 µm diameter silica-glass (Angström Spheres, Fibre Optic Centre Inc., New Bedford, MA, USA), were used in this study. The PTFE and glass particles are representative of particles with low and high surface energy, respectively. Particles were separated into well-defined size fractions by manual agitation through a series of Endecott powder sieves (UKGE Ltd, Southwold, Suffolk, UK) of decreasing grating diameter between 500 and 10 µm. The geometry and morphology of the two materials differed, with glass particles shaped as regular spheres, in contrast to the PTFE particles, which were irregularly shaped and rough (Figure 2.0). Diameters reported for the PTFE particles were determined from the mean value of the major and minor length axes, which led to a small variation in the mean values of each fraction between materials, as reported in Table 2. Using light microscopy and SEM images, the physical size distributions of the particles within each fraction were determined using an in-built macro in ImageJ that counts and determines the size of objects within the image (Table 2).

Table 2: Measured diameters (± s.d.) of the polytetrafluoroethylene (PTFE) and glass particles after sieving into different sized fractions.

Material	**Mean diameter (µm)**
PTFE	476 ± 72
PTFE	123 ± 60
PTFE	105 ± 762
PTFE	21 ± 23
Glass	141 ± 259
Glass	111 ± 24
Glass	19 ± 8
Glass	1 ± 0.1*

Particle sizes were determined using optical and scanning electron micrograph images; typical sample size was ~150 particles. The 1 µm diameter glass particles had a standard deviation of <10% as defined by the supplier.

*Particles were used as supplied and not sieved

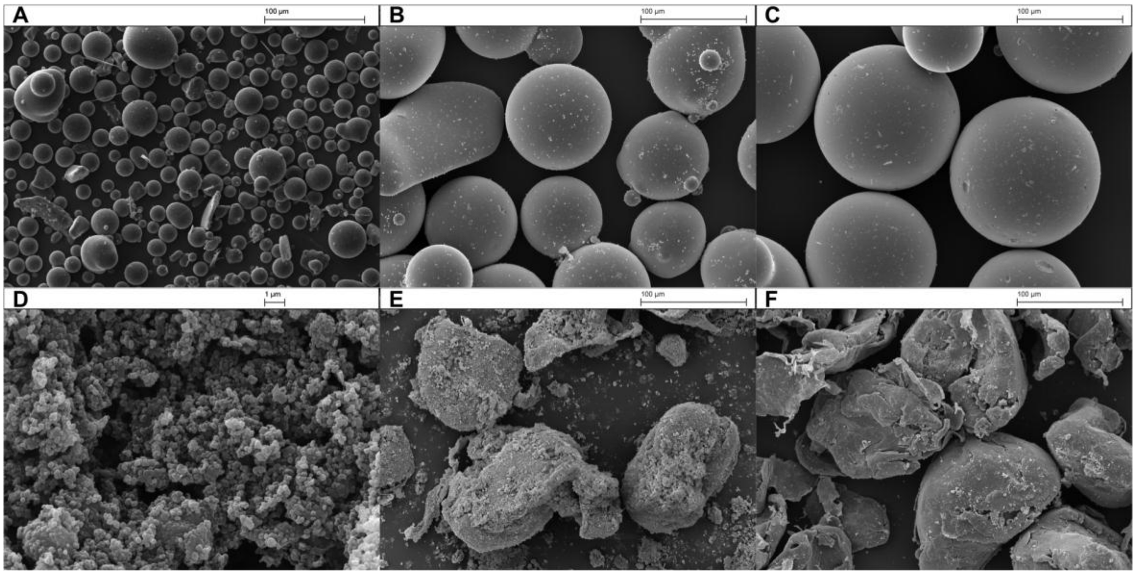

Figure 2.0: Scanning Electron Microscopy (SEM) images of some representative powder fractions of (A-C) glass and (D-F) Polytetrafluoroethylene (PTFE) particles used to construct the loose powder barriers. Glass particles are shaped as regular spheres, in contrast to the PTFE particles, which are irregularly shaped. The mean sizes of the particles are reported above in Table 2. Scale bars, 100 µm in all panels except D (1µm).

2.2.3 Barrier experiments

Circular powder barriers of ~1 cm width were constructed inside open glass Petri dishes of radius r = 6.4 cm and r = 3.3 cm for *P. dives* and *M. scabrinodis*, respectively (*h*dish>>*h*ant, where *h* is the height). Particles from each of the size fractions were gently poured manually along the inside wall of the dish using a small Teflon funnel. Prior to construction, Petri dishes were rinsed with HPLC grade iso-propanol (Fisher Scientific UK, Ltd, Loughborough, UK), wiped with a clean-room Spec-Wipe (VWR-Jencons) and dried with a filtered air supply. A fresh barrier was constructed for each replicate to reduce any effects of chemical signalling between workers from one experiment to the next. To neutralize any static charges, an ion gun (Zerostat 3, Milty, Bishops Stortford, UK) was used on each barrier before the experiments were begun. Petri dishes containing the barriers were placed upon an Ecotherm heat/cold stage (Torrey Pines Scientific Inc., Carlsbad, CA, USA) within a custom built Perspex chamber to allow for temperature control within the experimental arena (Figure 2.1). An air supply was passed through a series of moisture (R&D Separations MT200-4, Krackeler Scientific, Inc., Albany, NY, USA) and hydrocarbon traps (Agilent HT200-4, Agilent Technologies, Edinburgh, UK), which allowed control of the relative humidity (RH) of the airflow linked to the chamber.

RH was monitored using a HIH-4000-001 Integrated Circuitry Humidity Sensor (Honeywell Sensing and Control, Golden Valley, MN, USA) and logged with a Picoscope 3224 PC-based oscilloscope (Pico Technology Ltd, St Neots, UK). In order to study the effect of humidity on the number of ants to escape from a given fraction, the initial barrier experiments were carried out at 10, 50 and 70% RH (±5%) at a fixed temperature of 25±2°C. This range was chosen as it represented the natural range of RH each ant species was likely to encounter in their ecological niches or natural habitats when traversing dry surfaces (Hölldobler and Wilson, 1990). Finally, to avoid any moisture-induced improvement of adhesion between insect species and powder fractions during the experiments, all insects were held within closed dishes at the same RH for at least 30 min prior to use (Voigt et al., 2010). Control experiments were performed at each humidity level using clean dishes with no powders.

Workers were carefully extracted from their colonies and placed into the centre of the Petri dish, using soft metal tweezers, *via* a small access hole on the top surface of the chamber (Figure 2.1). Ants were observed for a maximum of 5 min, or until the ant had escaped, with each ant used only once and between 30 and 40 replicates performed for each parameter combination (*M. scabrinodis* *N*total 264, *P. dives* *N*total 277). Experiments were filmed from above using a digital camera (QuickCam Pro for Notebooks, Logitech UK Ltd, Slough, UK) controlled by HandyAvi (version 4.2;

Anderson's AZcendant Software) using the time-lapse capture mode, in a manner similar to that detailed by Loeffler (2009).

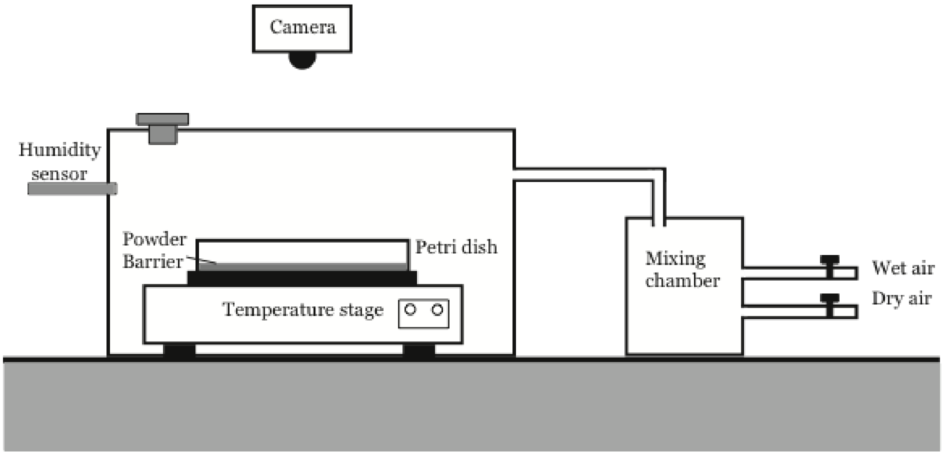

Figure 2.1: Schematic of the apparatus for the barrier experiments. The glass Petri dish sits on a heat/cold stage, which maintains the temperature within the arena for the duration of the experiment. The insects are carefully introduced to the centre of the Petri dish *via* an access hole at the top using soft tweezers and are filmed from above for 5min.

Two parameters were measured. Firstly, the effectiveness of a given type of powder in reducing adhesion was determined by recording the percentage of ants that were unable to escape having crossed the barrier. Specifically the results of each barrier experiment had three classifications; escape - the ant successfully escaped from the arena within 5 minutes, trapped - the insect attempted to, but failed to escape within 5 minutes, and no attempt - the insect made no attempt to cross the barrier and escape from the arena within 5 minutes. Denoting the number of ants that escaped, were trapped or made no attempt to cross the barriers as N_e, N_t and N_n respectively, the percentage of ants trapped for each parameter combination was defined as

$$\%Trapped = \frac{N_t}{N_t + N_e} \times 100 \qquad [8]$$

Although N_n needed to be taken into account, it was excluded from our analyses since these outcomes could not be attributed to any effects of contamination by the barriers. Secondly, the time spent investigating (antennating) the barriers themselves before either getting bodily onto or attempting to traverse the barrier itself was recorded. To determine to what extent the powders repelled the ants, the activity of each worker was recorded throughout the experiments and the length of time between the start of the experiment and the ant's first attempt to cross powder barrier threshold, *Tr*, was measured.

To investigate the effect barrier fragility has on the measured parameters, 19 µm diameter glass particles were also used to construct a series of solid, or 'caked', barriers for comparison. The caked barriers were prepared by constructing loose barriers, in the same manner as described above, which were then covered with a non-airtight plastic lid to protect them from any dust particles, and left exposed to the atmosphere for at least 24h (30–40% RH). Glass particles, such as those used in this study, form weak siloxane bonds at humidity's greater than 30% at the contact points of the particles because of the amount of water vapour present in the atmosphere, which leads to a slow solidification of the barrier (Bocquet et al., 1998; Fraysse et al., 1999; Bocquet et al., 2002). These barriers were sturdy enough to remain intact when the dish was inverted, but could be easily broken apart by manual pressure. This effect does not occur for PTFE particles, so this experiment could only be performed using high surface energy particles. All caked barrier replicates were performed under laboratory atmosphere (25±5°C, 35±5% RH) with the same procedure as above, and were filmed for a maximum of 10 min. During all experiments, no individual insect was used twice. Statistical analyses were performed using R v.2.8.1 (R Core Development Team, 2010). Escape data were analysed using a linear model with binomial distribution, and time repelled (*T*r) and time to escape (*T*e) were analysed with either an ANOVA for parametric data or a linear model for non-parametric data

2.3 Results

Individual workers were weighed and their claw length and basal distance were measured from optical and SEM images to allow for comparison of the two species (Table 3).

Table 3: Summary table of characteristic measurements made of the two ant species used in this study.

Species:	Mass (± s.e.m; mg)	Claw length (± s.d.; µm)	Base distance (± s.d.;µm)
Myrmica scabrinodis	4.83 ± 0.16	6.47 ± 7	34.7 ± 6.2
Polyrhachis dives	5.97 ± 0.21	110 ± 10	123.0 ± 26.2

Measurements were taken of multiple individuals of each species ($N = 10$)

Loose powder barriers

Trapping of ants

Control experiments with clean dishes trapped no ants of either species for all humidities investigated. Within the measured range when subject to Kaplan–Meir survival analysis, the effect of RH on the percentage of ants trapped by any loose barriers was not significant for either species ($\chi^2 = 3.52$, d.f. = 2, $P>0.05$), thus replicates from experiments across different RH values were subsequently pooled for further analyses.

The percentage of ants trapped, as defined by equation 9, was determined for each particle fraction (Figure 2.2). For both *P. dives* and *M. scabrinodis*, the percentage of ants trapped was found to be inversely related to the particle diameter for both materials (using a GLMM in accordance with Warton & Hui, (2011)), with smaller particles of both PTFE and glass trapping a significantly greater number of individuals (*P. dives*: glass, $F_{3,134} = 92.96$, $P<0.001$; PTFE, $F_{3,135} = 50.75$, $P<0.001$; *M. scabrinodis*: glass, $F_{3,135} = 41.037$, $P<0.001$; PTFE, $F_{3,121} = 15.04$, $P<0.001$). Within each species of ant, particle size was found to have a significant effect on the percentage of ants trapped, with smaller particles trapping a larger percentage of ants compared with larger particles, while Tr was also affected by particle size with ants taking longer to cross barriers made from smaller particles (GLMM: *P. dives*, $F_{7,223}$ = 48.70, P<0.001; *M. scabrinodis*, $F_{7,270}$ = 20.12, P<0.001). A similar result was found when comparing percentage of ants trapped and Tr between the two species, indicating that the relationship between contamination and particle size is similar for both species (ANOVA: $F_{7,501}$ = 10.11, P<0.001).

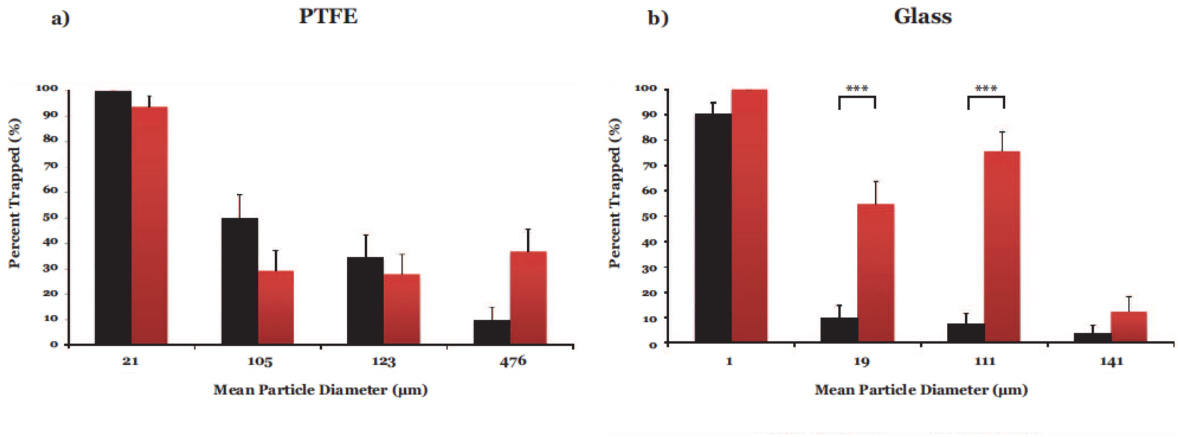

Figure 2.2: Percentage of ants trapped as a function of particle size for both (A) hydrophobic PTFE and (B) hydrophilic glass powder barriers. The percentage of *Polyrhachis dives* (black bars) and *Myrmica scabrinodis* (red bars) workers trapped is inversely correlated to particle size for both species (GLMM, F_7 = 43.982, P<0.001). ***P<0.001; all others not significantly different between species. Error bars show s.e.m.

Several *P. dives* workers that had traversed the different barriers, but were manually prevented from attempting to climb the vertical glass wall, were killed immediately after they had crossed the barrier and their tarsi were imaged *via* SEM (Figure 2.3 B-H); as a control, we also show the uncontaminated tarsi of *P. dives* (Figure 2.3 A). Contamination of the arolia by particles was observed for both PTFE and glass barriers made from small particles. Specifically, the arolia along with the tarsal claws and portions of the most distal tarsal segment were heavily contaminated by small particles (Figure 2.3 B–D) and the amount of particles observed to remain adhered to the tarsus and arolium increased with decreasing particle diameter for both materials. Indeed, the 1 µm glass particles almost completely coated the distal segment of the tarsi.

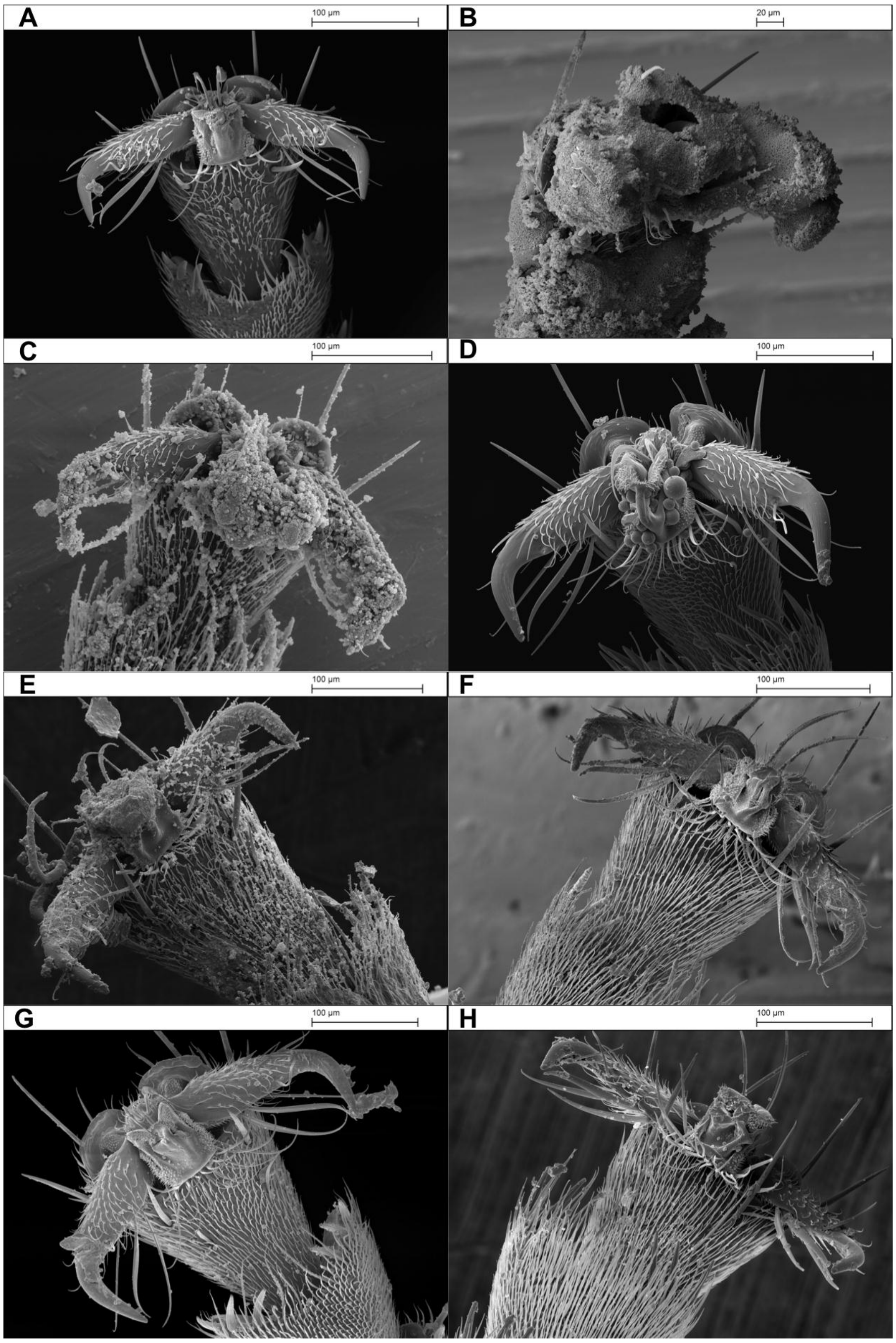

Figure 2.3: SEM micrographs of P. dives tarsi (A) uncontaminated, and after traversing powder barriers constructed of glass (B, D, F, H) and PTFE (C, E, G); (B) 1 µm glass, (C) 21 µm PTFE, (D) 19 µm glass, (E) 105 µm PTFE, (F) 111 µm glass, (G) 123 µm PTFE and (H) 141 µm glass. The level of contamination decreases with increasing particle size, and is not strongly affected by material type. Larger particles of glass and PTFE were not found to adhere to the arolium, as evidenced by particles in F, G and H. Ar, arolium, Cl, tarsal claws.

When comparing between *P. dives* and *M. scabrinodis*, the percentage of ants trapped by glass or PTFE powders did not differ significantly (GLMM: $F_{1,513} = 0.8802$, $P>0.05$); however, after closer examination it was found that when compared within a species there was a significant effect of particle type on the percentage of ants trapped (*P. dives*, $F_{1,277} = 8.88$, $P<0.05$; *M. scabrinodis*, $F_{1,278}$ 4.56, $P<0.05$). Specifically, we note that a significantly greater number of *P. dives* workers escaped from within the 19 µm glass barriers compared with the 21 µm PTFE barriers, even though the tarsi of the ants are clearly contaminated in both cases (Figure 2.3). This difference was found to be present and significant for both species (P. dives, $F_1 = 48.702$, $P<0.001$; M. scabrinodis, $F_1 = 20.122$, $P<0.01$; see Figure 2.2).

Repellent effects of barriers

Ants were observed to investigate several sections of the barriers with their antennae before crossing. Having touched the barriers, ants often spent time grooming their antennae and tarsi. In order to quantify the degree to which the ants were repelled by a powder barrier, the time taken before attempting to cross, *T*r, was measured for each species–material combination (Figure 2.4). It should be noted that although this measurement was used as an indication of the repellence of the barriers, it is only a qualitative estimate because, as mentioned above, a number of ants spent some of their time within the experimental arena grooming themselves. To attain a more accurate measure of actual time spent antennating before crossing the barriers, any time spent cleaning could be measured and subtracted from *T*r; this was the subject for a future study. A statistical difference was found when analysing *T*r as a function of particle diameter for both species (ANOVA: *P. dives*, $F_{7,225} = 14.41$, $P<0.001$; *M. scabrinodis*, $F_{7,272} = 20.21$, $P<0.001$). Time repelled data shown in Figure 2.4 indicate that for PTFE, *T*r is inversely related to particle size for both species of ant, with ants presented with larger particles taking a significantly shorter time to cross the barriers (Figure 2.4 A, C).

For glass particles, values of *T*r for 19 µm diameter particles were significantly greater than those for all other particle diameters (Figure 2.4 B, D). There was no significant difference between times measured for the particle types for *M. scabrinodis* (ANOVA: $F_{1,262}$ = 2.12, *P*>0.05); however, a significant difference was found for *P. dives* (ANOVA: $F_{1,275}$ =15.92, P = 0.03), indicating that, for this ant species, time taken to cross the barriers differed between glass and PTFE.

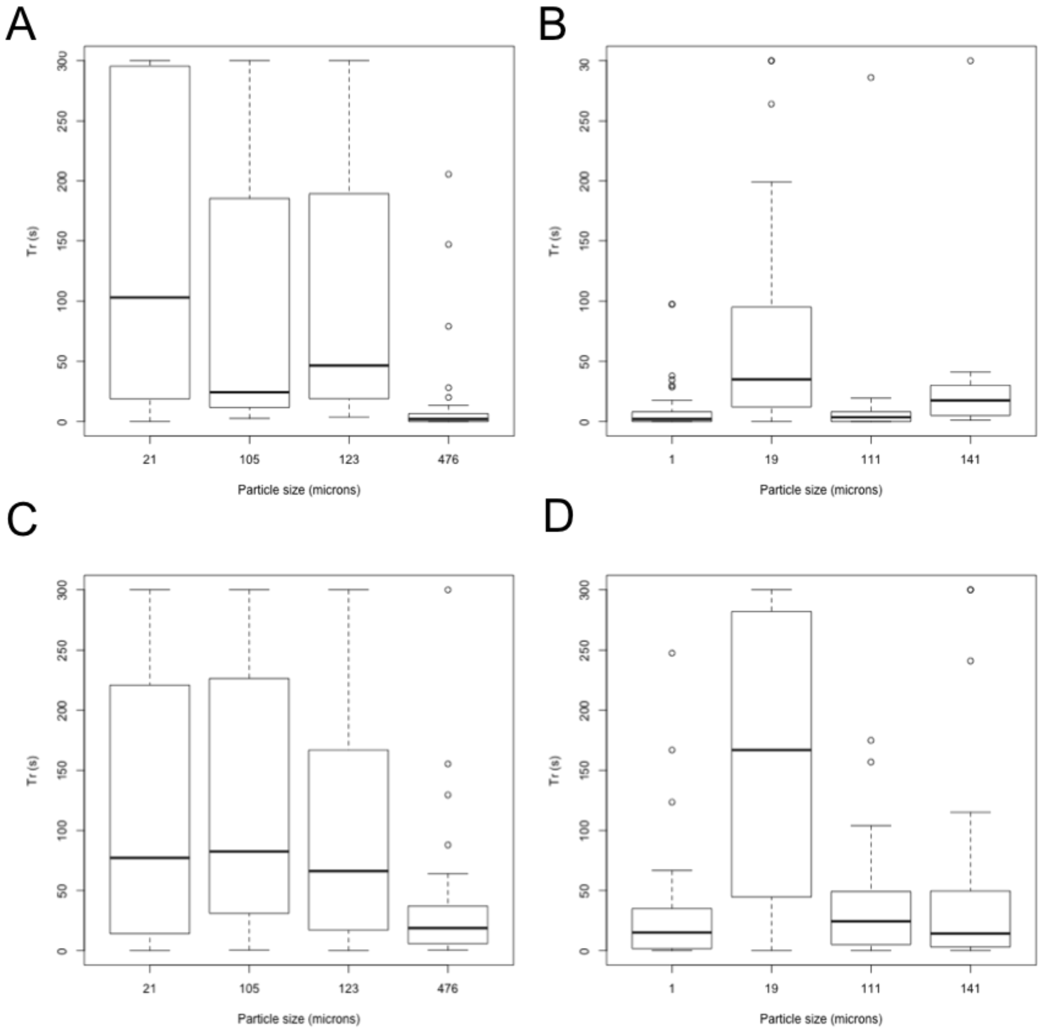

Figure 2.4: Time taken by (A, B) *P. dives* and (C, D) *Myrmica scabrinodis* to attempt to cross the threshold of the loose barriers, T_r *for* different mean particle diameters of PTFE (A, C) and glass (B, D). Experiments were capped at 300s (5 min). Plot shows medians (centre line), inter-quartile range (boxes) and the largest and smallest values (whiskers) that are not outliers (circles). Asterisks indicate median values that are significantly different from all other particle types: (A) ANOVA, F_3 = 7.47, 476 vs 21 µm $P<0.001$, 4776 vs 105 µm $P<0.05$, 476 vs 123 µm $P<0.01$; (B) ANOVA, F_3 =9.09, 19 vs 1 µm $P<0.001$, 19 vs 111 µm $P<0.001$, 19 vs 141 µm $P<0.05$; (C) ANOVA, F_3 = 5.94, 476 vs 21 µm $P<0.001$, 476 vs

105 µm P<0.001, 476 vs 123 µm P<0.001; (D) ANOVA, F_3 = 22.00, 19 vs 1 µm P<0.01, 19 vs 111 µm P<0.001, 19 vs 141 µm P<0.05; all others were not significantly different.

To ascertain the reason for the lack of antennating behaviour observed by ants for the 1 µm powder barriers, SEM images of the antennae of both ants were taken after workers had crossed barriers constructed of these particles. SEM micrographs of *P. dives* and *M. scabrinodis* antennae show hairs facing in the distal direction, the shafts of which are separated by approximately 5–10 µm. After crossing the 1 µm glass powders, the antennae of both species of ants show a coating of particles in between the hairs (Figure 2.5).

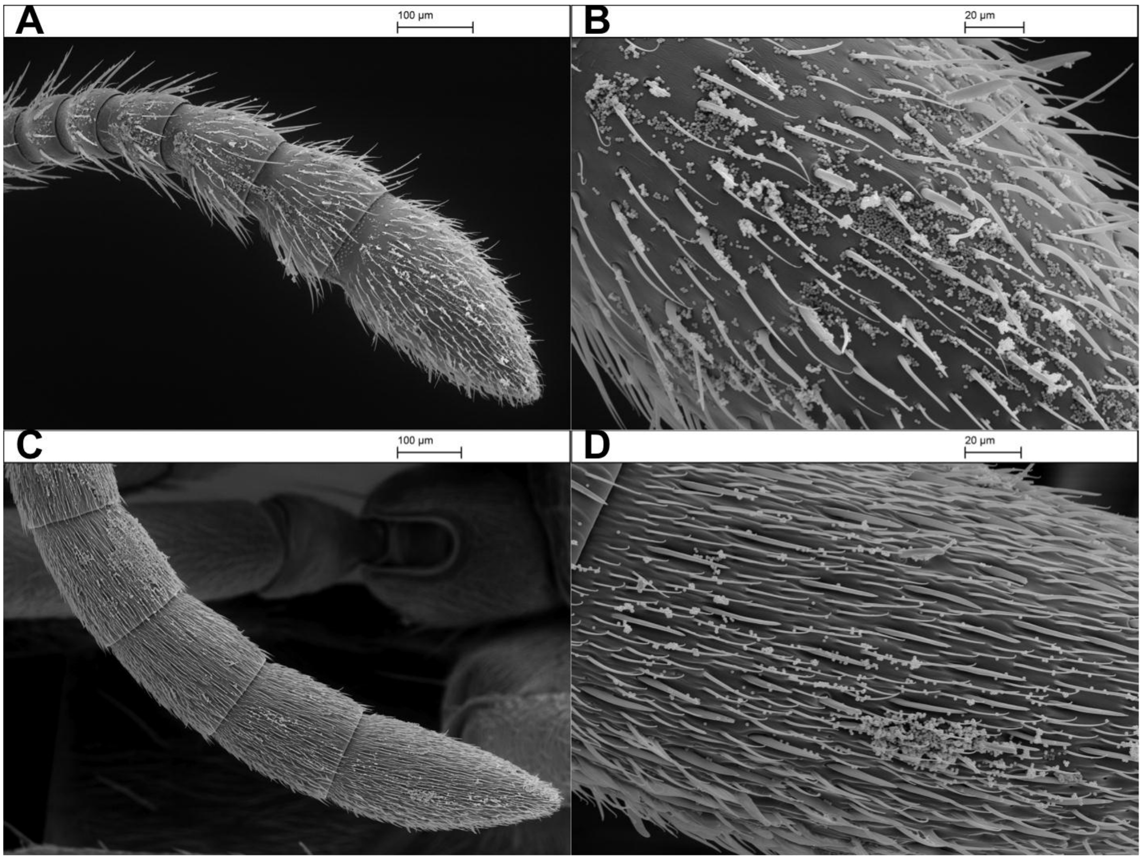

Figure 2.5: Images of Hymenopteran antenna fouled with 1 µm glass powder. A, B, *M. scabrinodis,* (A) view of antenna segments with clumps of powder seen on terminal sections, (B), image of terminal segment of antenna showing dusting covering of 1 µm glass particles, with fouling of mechano-sensing hairs. C, D, *P. dives*, (A), antenna fouled with 1 µm glass powder with clumps of powder seen on terminal sections, (D), close up image of antenna segment detailing particulate contamination around the base of the sensory hairs.

To determine the role antennae have on the time repelled, *T*r, a series of barrier experiments using 19 µm glass powders was carried out using ants with and without their antennae. A significant difference was found for both species when comparing *T*r between individuals with and without antennae: ants without their antennae spent a significantly shorter length of time investigating the barriers before crossing compared with ants with their antennae intact (ANOVA: *P. dives*, $F_{1,62} = 17.93$, $P<0.001$; *M. scabrinodis*, $F_{1,61} = 31.538$, $P<0.001$).

Rigid powder barriers

To determine whether the anti-adhesive effect and the observed repellence of the powder barriers is caused by their particulate nature and mechanical fragility, a series of caked powder barriers were constructed with 19 µm glass particles and escape experiments with both caked and fragile barriers were repeated. When comparing between caked and fragile barriers, the percentage of ants trapped as a function of barrier fragility for *M. scabrinodis* was found to be statistically significant ($F_{1,78}=102.6$, $P<0.001$). Specifically, only 7.5% of *M. scabrinodis* remained inside the arena at the end of the experiment with caked barriers compared with 82.5% for the loose barriers (Figure 2.6), whereas for *P. dives* there was no significant effect of barrier fragility on percentage of individuals trapped ($F_{1,64} =1.0$, $P=0.3$), with all individuals escaping within the time limit. When considering the time to escape (Figure 2.7), there was a significant difference found between the different barriers for both species of ant (ANOVA: *M. scabrinodis*, $F_{1,78} = 162.92$, $P<0.001$; *P. dives*, $F_{1,64} =13.076$, $P<0.001$).

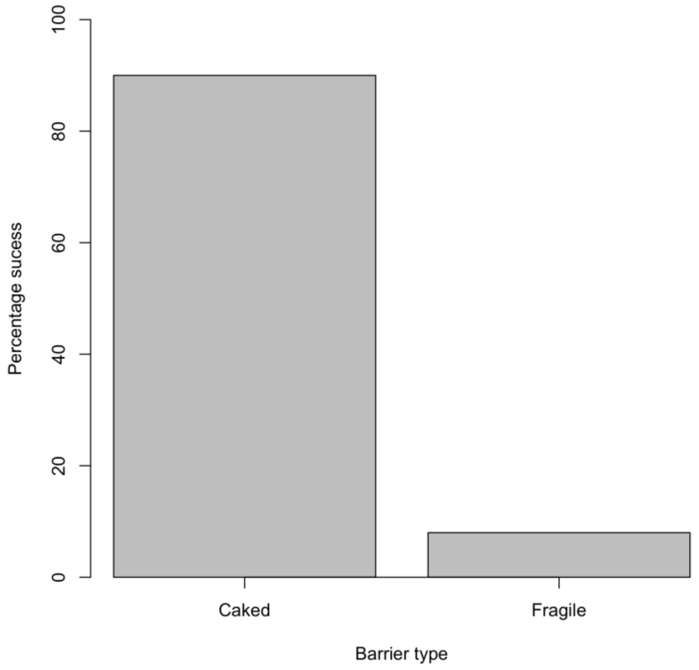

Figure 2.6: Percentage of *M. scabrinodis* workers trapped by loose (N=40) and caked (N=40) barriers constructed from the 19 µm glass particles. There was a significant decrease in the number of ants trapped by the caked barriers (F_1 = 102,6, P<0.001),

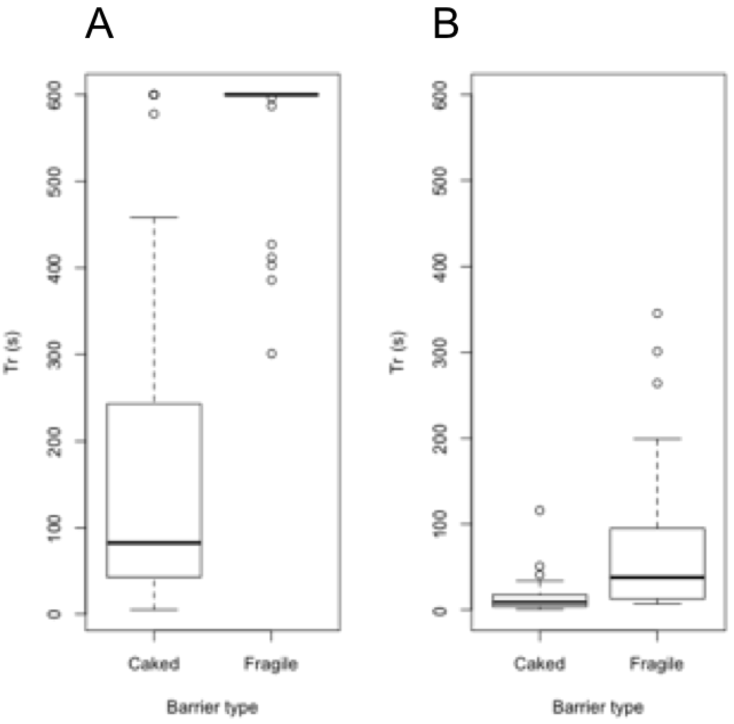

Figure 2.7: Time to cross the powder barrier and escape, Tr as a function of barrier type for (A) *M. scabrinodis* and (B) *P. dives*. *Te* was significantly different between caked and fragile barriers (*P. dives*, F_1 = 13.07, P<0.001; *M. scabrinodis*, F_1 = 162, P<0.001).

2.4 Discussion

Trapping of ants by loose powders

For loose powder experiments, after placing the ants within the centre of the dish, workers were observed to pause and briefly investigate the barriers with their antennae before moving to another section or attempting to cross. Barriers constructed of the largest particles did not present a problem for the ants to either cross or escape from the dish after crossing. However, after crossing the powder barriers constructed of smaller particles, a loss of adhesion on the vertical smooth walls of the Petri dish was observed for both species of ant, with these adhesion failure events becoming more frequent with decreasing particle diameter. Several ants were observed to fall from the vertical glass wall back into the powder barrier after temporarily achieving adhesion to the glass.

Smaller particles were found to trap a significantly greater percentage of ants for both species, suggesting that contamination becomes a greater problem for locomotion the smaller the particles the insect encounters. For example, powder barriers constructed of the 1µm diameter silica-glass particles and the 21 µm diameter PTFE particles each trapped over 90% of test insects for both species (Figure 2.2). The particle sizes found to heavily contaminate the arolium and tarsus of the ants corresponded well to those that also trapped greater than 50% of individual ants, with the exception of the 19 µm glass particles for *P. dives* (Figure 2.2). This is reasonable because heavy contamination reduces the available contact area between arolium and substrate, which dramatically reduces adhesion and friction forces (Gorb and Gorb, 2002; Hosoda and Gorb, 2011). Our results therefore give further confirmation that the ‘contamination

Hypothesis’ (Gorb and Gorb, 2002) proposed for hairy pad systems, also applies to insects with smooth adhesive pads. Ants with contaminated arolia, however, displayed no obvious change in behaviour whilst walking on a horizontal surface, suggesting that arolia are not deployed to a significant extent in this case.

From Figure 2.3 it can be seen that for both particle types when imaging with SEM, the arolia of ants that had traversed barriers made from the particles with diameters greater than approximately 100 µm were free from contamination or only lightly contaminated. One possible explanation for this observation is that when an ant crosses a powder barrier (consisting of multiple layers of particles), the relative magnitude of the competing forces between the pad and particles compared with inter-particle forces or particle weight may decrease with increasing particle size so that only particles below a certain threshold size will spontaneously adhere to the arolium.

These estimates predict that only particles with a diameter greater than 4 µm will not adhere to the arolia. This is more than one order of magnitude larger than the threshold size observed in Figure 2.3 and we therefore conclude that this is not the explanation for the observed threshold particle size.

We observed substantial contamination by large quantities of particles when particle diameters were smaller than the claw dimensions for both materials. For PTFE particles, heavy contamination was observed for particles with a mean diameter of 21 µm, light contamination was observed for 105 µm particles, and no contamination was observed for 123 µm particles. The light contamination by 105 µm diameter PTFE particles (Figure 2.3 E) appears to only consist of particulates of smaller size than the mean particle diameter. For glass, we observed heavy contamination by particles with mean diameters of 1 and 19 µm (Figure 2.3 B, D), and no contamination by particles with mean diameters of 111 and 141 µm (Figure 2.3 F, H). From Table 2 it can be seen that the standard deviation of the particle diameters for PTFE are relatively larger than those for glass, which suggests that only the smaller particles within a particular particle range adhere spontaneously – this may warrant further investigation.

We note that the transition from heavily contaminated arolia to non-contaminated arolia for *P. dives* (Figure 2.3) occurs at a particle size comparable to the claw dimensions (Table 3). We propose that the size dependence for contamination may be explained by the fact that individual particles with diameter comparable to or greater than the claw dimensions are prevented from adhering to the arolium by the presence of the claws themselves during locomotion, whereas particles much smaller than the claw dimensions are able to make contact with and contaminate the most distal tarsal segment of the ant, including the arolium, in large numbers (Figure 2.8). This leads to a reduction in real contact area with the substrate and a loss of adhesive force on subsequent steps, preventing the insect from scaling the vertical glass surface within the time limit. Thus we propose that, in ants, the claws may provide some protection from Contaminants that are large relative to the claw dimensions becoming affixed to the adhesive pad or interfering with efficient arolium deployment. Presumably, this would also work towards reducing the amount of active grooming the insect may need to perform to keep the arolium functioning efficiently (Hosoda and Gorb, 2011).

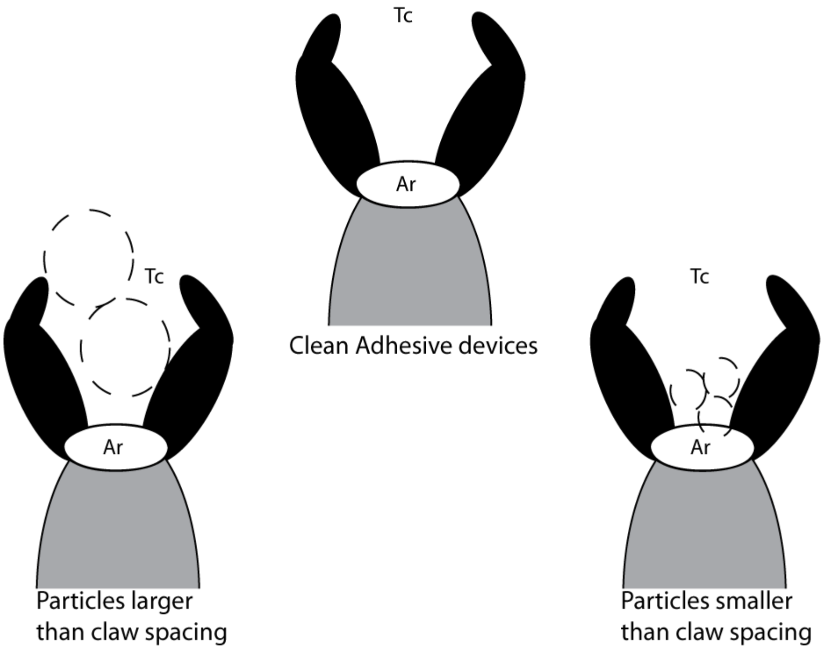

Figure 1.8: Schematic of ventral view of the distal tarsomere of an ant tarsus showing the arolium (Ar) and tarsal claws (Tc). A possible mechanism by which the tarsal claws may prevent large particles from attaching to the partially exposed arolium is shown. Particles with greater diameters than the claws dimensions are prevented from making contact with the soft adhesive pad.

It was found that a significantly lower percentage of ants were trapped by the 19 µm glass particle barriers than the 21 µm PTFE particles for both species of ant, even though the arolium and parts of the surrounding areas were contaminated in each case (Figure 2.3). In order to understand this difference, we consider the behaviour of the ants after they had crossed the barrier threshold. After crossing the powder and approaching the vertical glass wall, the forelegs of the ants were observed to slide in a downward direction on the walls of the petri dish in a scrambling, or shearing, motion as the ant attempted to gain adhesion to the surface. This behaviour was observed for both species, but *P. dives* were, in general, noticeably more active and would often spend a greater amount of time scrambling at the inner wall of the Petri dish attempting to escape.

This behaviour occurred more frequently for smaller particles and often continued for some time, with the result that sufficient adhesion sometimes returned, and escape was achieved within the time limit for a number of ants. Additionally, after scrambling at the wall for some time, a number of ants would stop to groom their antennae and tarsi before continuing to attempt escape. This sequence of behaviours is similar to that found recently for the leaf beetle *Gastrophysa viridula* (Hosoda and Gorb, 2011), but included grooming of the antennae as well as the tarsus.

We suspect that contaminated tarsi of the ants could remove some adherent particles *via* the observed scrambling or shearing motion of the feet against the glass wall of the arena, in a behaviour akin to 'self-cleaning' in insects (Clemente et al., 2010), and geckos (Hansen and Autumn, 2005). However, this action will only be effective if (1) the downward pulling force exerted by the ant is large enough, and (2) the frictional force between the particle and the substrate is large enough to cause the particles attached to the pad to be dislodged during this shearing motion. We note that *P. dives* workers are on average stronger than *M. scabrinodis* owing to their larger size (Table 3). We also note that the friction coefficient of glass on glass is higher than for PTFE on glass (Lide, 2008). Thus, it is reasonable to assume that the observed scrambling motion should be most effective in removing the contaminating particles for *P. dives* contaminated by glass particles. This may explain why most of the *P. dives* workers (90.6%) were able to escape from the 19 µm glass powder barriers (Figure 2.2) even though the arolium was clearly contaminated by these particles (Figure 2.3), and would support the mechanism of self-cleaning in geckos proposed by Hansen and Autumn (Hansen and Autumn, 2005). It was found (Clemente et al., 2010) that with a shearing motion; smooth adhesive pads are able to remove adherent particles after several steps. Individuals of *P. dives* in the present study took longer than this to regain sufficient adhesion in order to escape. This could be due to several factors: (1) the deposition of particles, and subsequent recontamination of the arolium, from the glass surface as the ant attempted to escape from the same location of the dish; (2) a number of particles becoming embedded in the soft cuticle of the arolia within the contact zone; or (3) simply the sheer numbers of particles present in our case. As contamination and recovery time are strongly dependent upon contact area with the substrate, this continued presence of particles would slow the recovery process (Federle et al., 2002). This scrambling motion may work in a manner similar to that seen for hairy pads of insects (Clemente et al., 2010) and geckos (Hansen and Autumn, 2005); however, a detailed analysis of the mechanisms of the observed self-cleaning action in ants is beyond the scope of the current paper and is investigated in a separate publication (Orchard et al., 2012).

Repellent effects of barriers

As reported above, ants were observed to investigate the barriers with their antennae before attempting to cross. Ants probed several sections of the barrier with their antennae in a manner similar to that reported for stick insects assessing gap sizes (Blaseing and Cruhe, 2004) and for cockroaches performing orientation behaviours (Camhi and Johnson, 1999; Okada and Toh, 2006), before either crossing or moving to another section. This behaviour was observed for barriers constructed of all particle diameters and materials. Ant workers of both species were observed to be repelled by the powders to some extent, but particularly so with the smaller particles. Because the ants studied here are not repelled by smooth, flat surfaces of either PTFE or glass (M.J.A. and M.J.O., personal observations); this suggests that it is the particulate nature of the materials that causes the ants to be repelled. However, the 1 µm glass particles were an exception to this observation, with the majority of ants spending less time investigating these barriers compared with the others (Figure 2.4). Considering the low values of *T*r observed for ants crossing the 1 µm glass barriers (shown in Figure 2.4), this may also be explained to some extent by the ants' behaviour. In many cases, ants presented with 1 µm glass barriers did not stop to investigate the powder and simply ran across the threshold, moving up to the glass wall without hesitation. In the remaining cases, the ants only investigated for a relatively short time, as evidenced by the low values of *T*r in Figure 2.4. These observations suggest that the ants were either unable to detect the barriers or did not consider the barriers as something to be avoided.

Often it was observed that after having touched the barriers with their antennae ants would spend time cleaning, or grooming, their antennae in a way similar to that described by Wheeler (Wheeler, 1907) and others (e.g. Farish, 1972). It has been found previously that hairs present on the antennae are involved in detection of various aspects of an ants' environment, including airflow, chemical signalling, as well as tactile sensing (Hölldobler and Wilson, 1990; Bernadou and Fourcassie, 2008; Benton, 2008). In the present case, these hairs may also be used to gain some degree of direct tactile feedback on the physical properties of their environment, such as mechanical fragility, which subsequently influences the ants' behaviour. Contamination of the antenna's flagellomeres (sections) (shown in Figure 2.5) may inhibit the insects' ability to accurately detect tactile cues such as mechanical fragility and make the 1 µm diameter powder barriers essentially invisible to the ants used in this study, with a combination of dense contamination of the adhesive pads, tarsi and antennae, along with the apparent inability to detect the individual particles making this barrier particularly effective at preventing insect locomotion on smooth surfaces. To investigate this hypothesis, we performed a series of barrier experiments with 19 µm glass particles using ants with and without antennae. We found that ants without antennae spent significantly less time investigating the barriers before crossing than ants with antennae. The values for *T*r found in this case were similar to those found for ants crossing the 1 µm glass particles (Figure 2.4), providing evidence to support this hypothesis.

Rigid powder barriers

We note that for each species–material combination, the dependence of *T*r on particle diameter (Figure 2.4) demonstrates a trend similar to the relationship between particle diameter and the percentage trapped (Figure 2.2). This relationship suggests that repellence becomes more pronounced for particles that lead to a greater amount of contamination, which produces a significant reduction in adhesion *via* the reduction of the available contact area. The value *T*r measures the time taken by an insect to investigate the barriers with their antennae before crossing, and as such is not determined by arolia contamination. Instead, the correlation of *T*r with the percentage of ants trapped suggests that the ants are able to gather information about the barriers *via* the observed antennating action.

To determine whether the observed repellence was principally due to the barriers' particulate nature, escape experiments with both caked and fragile barriers were repeated. After placing the ants inside the circular barriers, it was obvious that the caked barriers were significantly easier to traverse and caused very little difficulty for the ants to subsequently climb the smooth glass wall of the dish and escape. A significantly lower percentage of *M. scabrinodis* were trapped by the caked barriers, and a significant drop in *T*e suggests that individuals of this species were not repelled by these rigid and rough surfaces. For *P. dives*, there was no significant difference found between the barrier types because all individuals of this species were able to escape. However, those *P. dives* workers that did escape took a significantly longer time to do so, as shown in Figure 2.7. Because the barriers differ only in their fragility, these results provide evidence to support the suggestion (see the previous section) that the fragile nature of the powder barriers is crucial to their effectiveness at trapping ants *via* contamination of the adhesive pads, in much the same way that plant epicuticular wax blooms function (Stork, 1980b; Gorb et al., 2008; Borodich et al., 2010), and that ants may assess the contamination risk of the powders by using their antennae to probe the mechanical fragility of the barriers.

Conclusions

We studied the escape of two different ant species (*P. dives* and *M.scabrinodis*) from circular powder barriers in order to determine the effect of barrier properties such as particle size, surface energy and mechanical fragility and environmental factors such as humidity on insect adhesion and repellence. Our results demonstrate that the anti-adhesive effect of barriers, constructed from loose synthetic powders, is due to contamination of the insects’ attachment devices causing a reduced contact area between the adhesive pad and the adherent surface, and was independent of RH within the range tested.

Adhesive loss is due principally to this loss of contact area between the substrate and the adhesive pad, preventing adhesion to smooth surfaces for some time after contamination. Our results therefore show that the ‘contamination hypothesis’, proposed previously (Gorb and Gorb, 2002) for hairy pad systems, also applies to insects with smooth adhesive pads. We found that contamination of the adhesive arolia, and the proportion of ants trapped by loose powder barriers, is strongly dependent on the size of the individual particles, but is less significantly dependent on particle surface energy and not dependent on environmental factors such as relative humidity. Specifically, particles larger than the tarsal claw base distance did not contaminate the arolia of either ant species, whereas particles smaller than the claw dimensions did, often-in great numbers. This suggests that the claws may offer the arolium some protection from being contaminated by particles that are large relative to the claw dimensions.

Workers of *P. dives* contaminated with high-energy particles regain adhesion after time spent scrambling at a high-energy smooth substrate in a shearing motion, similar to that seen in geckos and other insects in previous studies. This action may be a further example of ‘self-cleaning’ in smooth pads (Orchard et al., 2012). We also found evidence that ants used in this study were repelled by the loose powders, particularly by barriers made from the smaller particles, which lead to a greater amount of arolia contamination and loss of adhesion, with the exception of 1 µm particles. Repellence by a given powder barrier was significantly reduced when the mechanical rigidity of the barrier was increased. These results suggest that ants may be able to use their antennae to probe the mechanical fragility of the barriers and, furthermore, use this information to alter their behaviour in order to minimise the risk of contamination to their arolia. The ants’ ability to probe vital physical properties of its environment using its antennae will be the subject of a detailed investigation in the near future. Our results show that similar effects of contamination of adhesive pads in ants can occur for both natural (plant waxes) and synthetic particles. Results of this study show some agreement with data published for particulate control of insect pests (Briscoe, 1943; Alexander et al., 1944; Merton, 1956, Boiteau et al., 1994; Glenn et al., 1999; Puterka et al., 2000; Hunt and Vernon, 2001) and suggest that the results presented in these studies are likely a result of the small particle sizes used. Mimicking the effect of natural barriers could lead to the production of more efficient synthetic and nontoxic means of controlling pest species in agriculture, as well as for domestic purposes.

Chapter three

An investigation into the self-cleaning abilities of insect adhesive pads

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3.1 Introduction

Adhesive devices are often used to counteract the pull of gravity. Animals use several types of attachment mechanism from mechanical gripping using tarsal claws (Gladun et al., 2009) to specialised adhesive organs based on a variety of physical principles (Bullock et al., 2008; Drechsler & Federle, 2006). Insects use adhesive devices to enable foraging on a wide range of surfaces (Eigenbrode et al., 1999; Eigenbrode et al., 2000; Gorb et al., 2008) as well as for defence (Eisner & Aneshansley, 2000c) mating (Bitar et al., 2009; Voigt et al., 2008) and oviposition (Bitar et al., 2009). While insect adhesive devices include most known biological mechanisms, their adhesive organs are typically of two distinct types; smooth deformable organs (arolia) as found in Hymenoptera (ants, bees and wasps), or hairy adhesive pads as found in Coccinellids (ladybeetles) (Bullock et al., 2008; Frantsevich & Gorb, 2004; Niederegger et al., 2002). Both pad types deform locally to allow adhesion to rough surfaces, and increase adhesive contact area through secretion of small amounts of fluid delivered between the pad and surface (Betz, 2003; Dirks et al., 2009; Persson, 2003; Votsch et al., 2002).

Insect attachment must allow for strong yet releasable adhesion to be made between the tarsal pads and both horizontal and inverted surfaces (Federle, 2006). Any loss of adhesive ability is likely to be detrimental in terms of loss of foraging capability and an increased risk of predation. To enable adhesion insect feet are inherently ‘tacky’, employing an adhesive fluid secretion to maximise contact with a substrate (Gorb, 2005; Pohl & Beutel, 2004). The exact composition of the secretion is still unknown, although there is a general acknowledgement that it is an emulsion of lipophilic and hydrophilic components (Votsch et al., 2002), but no hydrophilic liquid has been identified (Geiselhardt et al., 2010). Indeed Dirks (et al., 2009), state that hydrophobic fluids stick well to both hydrophobic and hydrophilic surfaces and the inclusion of a hydrophilic component to the adhesive secretion used by insects has unclear benefits. Regardless of its composition, any secretion would only be of use on a rough surface where it would ‘fill-in’ the surface asperities to maximise contact area, however, due to the broken wax coverings of plant tissues and the fractal nature of weathered surfaces it can be expected that all surfaces are to some extent rough. In systems using fluid in this way contamination of the adhesive surfaces by loose material (contact contamination) can seriously compromise attachment ability (Clemente et al., 2010; Gorb & Gorb, 2006; Gorb et al., 2005). Contact contamination also reduces adhesive potential in biomimetic adhesive devices inspired by gecko feet (Lee & Fearing, 2008; Raibeck et al., 2008). The ability of insects to adhere to plant surfaces is also known to be dependent on surface properties (i.e. waxes, trichomes) (Eigenbrode, 2004; Eigenbrode et al., 1996; Eigenbrode et al., 1999; Eigenbrode et al., , 2000; Gorb & Gorb, 2002; Gorb & Gorb, 2006; Lee & Fearing, 2008; Raibeck et al., 2008). Plant waxes in particular are known to cause problems for foraging insects (Eigenbrode, 2004; Markstadter et al., 2000; Riedel et al., 2003) The differing frictional coefficients of the wax surfaces alter the ability of insect foot-secretions to wet plant surfaces (Gorb & Gorb, 2006; Wang et al., 2009). Frictional coefficients are used to describe the ratio of frictional force to normal load between two bodies, and are usually reported as static friction coefficients (μ_s) for stationary objects, and kinetic friction coefficients as (μ_k) for moving objects (Bowden and Tabor, 1950; Israelachvii, 2007). These coefficients of friction depend on the materials properties and the force(s) pressing them together. For example, dry glass on glass has a high static and kinetic coefficient of 0.94 and 0.4, respectively, while dry PTFE on PTFE has μ_s and μ_k of 0.04 and 0.04 respectively. Surfaces with high frictional coefficients generate greater frictional forces than those with low coefficients, i.e. glass on glass contact will generate more friction than PTFE on PTFE.

Some biological surfaces have been shown to exhibit self-cleaning properties, this self-cleaning of surfaces reduces or eliminates contact contamination. Given the prevalence and repercussions of contact contamination, self-cleaning adhesive devices would appear to be a highly advantageous trait. Self-cleaning with water droplets is known for both plant (Barthlott et al., 1997; Ma & Hill, 2006; Solga et al., 2007) and animal surfaces (Baum et al., 2001, 2002; Clemente et al., 2009; Clemente et al., 2010; Genzer & Marmur, 2008; Gravish et al., 2010; Hansen & Autumn, 2005). However, until recently no adhesive organs were known to have self–cleaning capabilities (Hansen & Autumn, 2005). Models of the mechanical attachment of gecko setae suggest that they are able to self-clean through energetic disequilibrium between the adhesive forces attracting the contaminating particle to the adhesive setae and those attracting it towards the substrate (Autumn & Gravish, 2008), similar to the theory for the influence of surface energies on friction and wear proposed by Rabinowicz (1961). Essentially, bringing the fouled contact organ into contact with a surface that has a greater attraction to contaminating particles than the device leads to the removal of the particles from the adhesive organ. This self-cleaning ability has been suggested to be unique to the setal pad form of geckos and by extension those possessed by some iguanian and scincid species, (Hansen & Autumn, 2005), which do not make use of a liquid secretion. It has also been suggested that the free surface energy (FSE, as defined by Israelachvii, 2007) of a substrate could alter the adhesive forces generated in the 'dry' adhesive system found in geckos (Autumn & Peattie, 2002). FSE also determines the spreading or 'wetting' of a liquid over a substrate, surfaces with a low FSE such as Fluon® for example, cause liquid to bead up. In general high frictional coefficients are found for surfaces with high FSE and vice versa (Israelachvii, 2007; Rabinowicz, 1961). Recent work has suggested that the 'dry' adhesive ability of gecko toe pads is influenced by relative humidity via forming capillary bridges between the spatulae and the substrate (Huber et al., 2005), softening of the ß-keratin of the spatulae allowing more intimate contact with the substrate (Puthoff et al., 2010), or enhanced by the use of an adhesive secretion (Hsu et al. 2011). Hui *et al.,* (2006) suggest that particle rolling where small movements of the adhesive setae push the contaminating particles to the outer edge of the adhesive pad may also contribute to the self-cleaning of the hairy pad structures in geckos. A similar model for self-cleaning of hairy pads in insects has been put forward by Clemente et al. (2010).

In the only study of insect adhesive pad self-cleaning to date (Clemente et al., 2010) suggest that the self-cleaning mechanisms proposed for the dry fribrillar adhesive pads of geckos could be logically extended to cover the setal pad forms of insects that use an adhesive secretion. Here we present a comparison of the self-cleaning abilities of the two main insect adhesive pad types. We compared the ability of insects with different types of adhesive pad fouled with particles differing in their FSE to escape over vertical walls also with differing FSE values. We used two differently sized species of Coccinellidae, the harlequin ladybeetle (*Harmonia axyridis* (Pallas 1773)) and the two spot ladybeetle (*Adalia bipunctata*) which have hairy pads, along with two differently sized species of ants, the Asian weaver ant (*Polyrhachis dives*) and the red elbowed Spanish ant (*Myrmica scabrinodis* (Nylander 1846)) which have smooth pads, to also determine what affect the physical size of an insect has on the self-cleaning ability of each pad type. We hypothesised that the physical size of the contaminating particle would influence the recovery of adhesion, and that such cleaning would depend on the FSE of both the contaminating particles and the substrate, with surfaces with high (glass) predicted to be better cleaning surfaces that those with low values of FSE, since it is reasonable that surfaces with high FSE values would generate sufficient shear forces to remove contaminating particles from the pad through friction, compared to those with low FSE.

For the purpose of this study, the time it takes an insect to recover adhesive ability by ‘scrabbling’ on the vertical walls, after its adhesive pads have been fouled, was defined as successful self-cleaning. Self-cleaning by shear forces would be facilitated by the higher frictional force between the contaminating particle and the substrate than the adhesive forces holding the contaminating particle to the pad. We show that there is an interaction between the physical properties of both the contaminating particle and the substrate that significantly influences the self-cleaning ability of insect adhesive pads.

3.2 Materials and methods:

3.2.1 Experimental design

Experiments were carried out using two species of ant: *Polyrhachis dives* (Smith 1857) and *Myrmica scabrinodis* (Nylander 1846) and two species of ladybeetle *Adalia bipunctata* (Linnaeus 1758*)* and *Harmonia axyridis* (Pallas 1773*)*. To test the effects of pad type on self-cleaning after contamination, two series of cylindrical vials (SiO_2 75 x 25 mm) were prepared; although the vial walls are curved, their radius of curvature is several orders of magnitude greater than that of the individual adhesive pads of the insects, and therefore can be considered a flat surface. All vials were cleaned by sonication in a hexane bath (Fisher Scientific HPLC Grade 95% n-hexane) for 1 minute then left to stand for 2 minutes and rinsed with isopropanol (99% pure Fisher Scientific HPLC Grade iso-propyl alcohol). After cleaning one series was left untreated ('clean glass' = high FSE, mean contact angle of 3° ± 2°), while the other was treated with DCDMS (dichlorodimethylsilane) (≥98.5% pure, Sigma Aldrich) in a vapour phase similar to that used by West *et al.*, (2007) ('treated glass' = low FSE, mean contact angle 105° ± 3°) to produce a low energy surface comparable to plant substrates (*Heliconia denisflora* 28.4° ± 4.3° and *Brassica oleracea* 160.3 °± 0.8°; (Barthlott, Neinhuis, & Schott, 1997). All vials were rinsed with hexane and blown dry with pure nitrogen before each replicate to ensure the surfaces were free from environmental contaminants such as dust. Contact angles of experimental surfaces were measured each day (*n* = 10), for the duration of the study by placing 5 µl of MilliQ purified water onto the surface and directly recording the contact angle using a Krüss DSA-10 drop shape analysis machine. This enabled us to verify that the surface treatments did not deteriorate over the course of the study.

Table 4: Mean mass, body length and adhesive pad contact area for each species. Adhesive pad contact area is average pad area across all legs, standard error of the mean is presented next to given values, (n = 20 individuals per species).

Species	Mass (mg)	Length (mm)	Pad type	Contact area (μm^2)
A. bipunctata	12 ± 0.6	4 ± 2	Hairy	$33.29 \pm 0.25 \times 10^3$
H. axyridis	35 ± 1.0	9 ± 2	Hairy	$114.60 \pm 1.17 \times 10^3$
M. scabrinodis	5 ± 0.2	5 ± 2	Smooth	$3.07 \pm 0.06 \times 10^3$
P. dives	15 ± 0.3	8 ± 1	Smooth	$40.65 \pm 0.39 \times 10^3$

To test the effects of pad type on self-cleaning after contamination, two series of vials (SiO_2 75 x 25 mm) were prepared; although the vial walls are curved, their radius of curvature is several orders of magnitude greater than that of the adhesive pads of the insects, and therefore we approximate them to be a flat surface for the purpose of this study. While we cannot rule out a minor bracing effect of paired legs we consider the additional frictional forces to be minimal given the vial curvature.

Contamination

Unlike previous mechanistic studies (Clemente et al., 2010) where the insects were restrained and artificially fouled with particles, in this study insects were allowed to free roam within an environment that would cause contamination in a more ‘natural’ manner. Insects were fouled with either glass or PTFE micro-spheres by being placed into a Teflon dish with a dusting of particles on its inner surfaces for two minutes. After contamination they were transferred to one of the two series of vials and left unperturbed under video observation. Two size classes of microspheres were used: ‘small’ (glass: 19 ± 8 µm SD (VWR-Jencons); PTFE: 21 ± 23 µm SD (Sigma-Aldrich)) and ‘large’ (glass: 111± 24 µm diameter µm SD; PTFE 123 ± 60 µm). For small insect species (*M. scabrinodis, A. bipunctata*) large particle sizes were not tested, as particle diameter was greater than the claw width, making them unlikely to foul (M. Orchard, pers. obs.).

Self-cleaning

All experiments including control tests were carried out in multiples of 20 replicates (total number of replicates in study; *P. dives* n = 200, *M. scabrinodis* n = 120, *H. axyridis* n = 200 and *A. bipunctata* n = 120) and were carried out at laboratory relative humidity (28-34 %) and ambient temperature (20-24 °C). Six vials were lined up in parallel and continuous video recordings were taken for 30 minutes (8 frames per second) using a webcam (Logitech Quickcam®Pro) controlled by HandyAvi (version 4.2; Anderson's AZcendant Software). Although the image resolution was not sufficient to view the adhesive devices in the large array, several analogous replicates were undertaken wherein the focal area of the camera was refined to show the adhesive devices more clearly.

The time taken to adhere (T_{adhere}) to the inner vertical wall of the glass vial was taken from the video footage. Insects were judged to have adhered when all six legs were on the vertical wall of the vial and the insect was able to move freely. The measured Tadhere was adjusted to exclude periods during which the insect was not moving under the assumption that there would be no effective shear or in-plane forces to shed contaminating particles. If an insect had not managed to adhere to the vial wall within 30 minutes the trial was awarded a censored value. This censored value was used to determine the numbers of insects that adhered to the inner vial walls within the 30-minute period (see Crawley, 2010 for further details on survival analysis). Additionally, time spent preening (T_{preen}) was recorded to allow for a comparison of active cleaning within each experimental group and between species, and compared using ANOVA and a *posthoc Tukey test*. It has been noted that reduced friction triggers grooming behaviour in beetles (Hosoda, & Gorb, 2010), so by comparing time spent preening allowed a defacto measure of the duration of contamination of the adhesive devices and as such an indication of the reduced friction between the adhesive device and the substrate.

All statistical analyses were carried out using R (ver. 2.10.1: R development core team, 2009). Survival analysis was used to investigate the relationship between 1) the physical size of the particle and the insect's ability to escape, 2) the physical properties of the substrate/particle interaction and the insect's ability to escape. Survival analysis was used as our data shows right censoring (Crawley, 2010). Survival analysis is used where the data shows censoring, that is if at the end of the study the event of interest has not occurred (in this case the ant had not managed to adhere). As it is impossible to say the individual would never adhere it is instead expected that the individual will escape at an unknown time in the future and the data is said to be censored. Survival analysis allows one to make statistical comparisons between censored data sets where standard statistics would suffer bias due to missing data (Crawley, 2010, Heagerty, 2005, and Heagerty et al., 2000). We used a linear model of the form $T_{adhere} = particle\ type \times particle\ size \times container\ type$ for large insects, while for small insects the particle size term was removed. Data were modelled using both an exponential distribution (which assumes constant hazard) and a Weibull distribution (which allows for non-constant hazard with time). ANOVA indicated that the Weibull distribution was the better fit to our data and those results are presented here. However, use of exponential distribution models does not qualitatively alter our conclusions. Non-significant terms were sequentially removed based on Akaike Information Criterion (AIC) values, leaving only parameters with a significant influence on the output of the model.

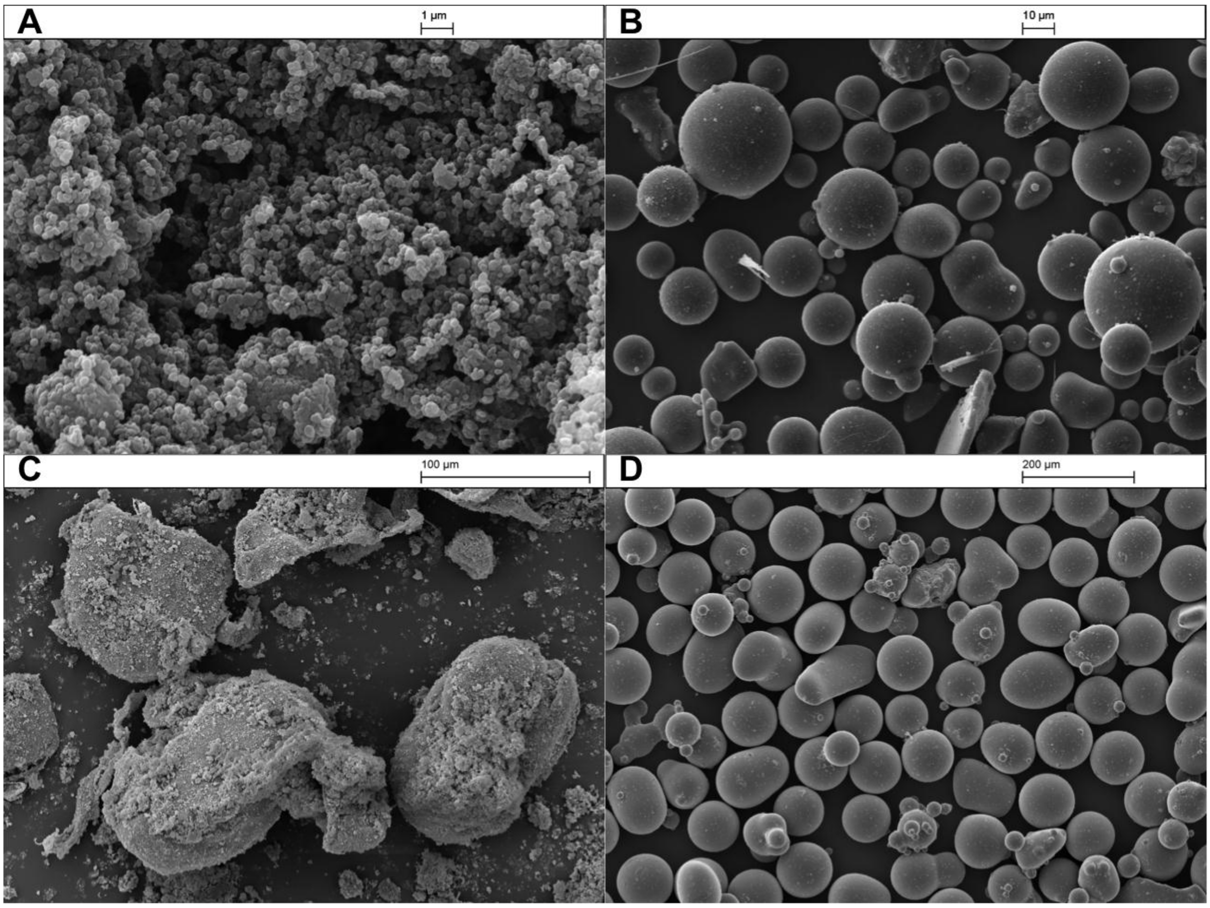


Figure 3.0: SEM micrograph of representative particles used to contaminate the adhesive devices of both ants and ladybirds in this study. A, 21 µm ± 23 µm PTFE particles, B, 19 µm ± 8 µm glass particles, C, 123 µm ±60 µm PTFE particles and D, 111 µm ± 24 µm glass particles.

3.3 Results

3.3.1 Effect of T_{preen} on self-cleaning

Several of the insects were observed to preen themselves during the experiments, as such insects that displayed high levels of preening behaviour were collected post experiment and imaged using SEM (Zeiss Evo 60 0.2 Scanning Electron Microscope), the resulting micrographs showed clean jaws for those insects contaminated with glass particles (Figure 3.1 A), while those contaminated with PTFE particles displayed contamination of the mouth parts (Figure 3.1 B), to emphasize the contamination by PTFE particles Figure 3.1 B is shown at a higher magnification than Figure 3.1 A. Duration of time spent preening (T_{preen}) was found to differ significantly between species (t = 2.8954, d.f. = 328, p = 0.004), although there was no significant difference between the large insects (t = -0.1433, d.f. = 195, p = 0.88) in time spent preening for either particle or substrate type both *A. bipunctata* and *M. scabrinodis* spent significantly more time preening than the larger insects (t = 5.065, d.f. = 129, p < 0.001). Mean duration in seconds (± s.e.m.) of preening behaviour is shown in Table 5 for the large insects while the data for small insects is presented in Table 6.

Table 5: Mean time spent preening (second's ± se) for large species, particle type, size and substrate combination. Column headings refer to particle substrate combination, Sg = small glass particles, SPTFE = small PTFE particles, Lg = large glass particles, LPTFE = large PTFE particles, Utv = untreated glass vial, Tv = treated glass vial.

Species	**Sg/Utv**	**SPTFE/Utv**	**Lg/Utv**	**LPTFE/Utv**
H. axyridis	709 ± 175	119 ± 108	28 ± 12	39 ± 31
P. dives	872 ± 8	278 ± 168	189 ± 0.17	428 ± 171
	Sg/Tv	**SPTFE/Tv**	**Lg/Tv**	**LPTFE/Tv**
H. axyridis	1030 ± 168	4 ± 3	84 ± 47	395 ± 4
P. dives	1702 ± 22	106 ± 95	146 ± 118	414 ± 167

Table 6: Mean time spent preening (second's ± se) for small species, particle type, size and substrate combination. Column headings refer to particle substrate combination, Sg = small glass particles, SPTFE = small PTFE particles, Lg = large glass particles, LPTFE = large PTFE particles, Utv = untreated glass vial, Tv = treated glass vial.

Species	**Sg/Utv**	**SPTFE/Utv**	**Sg/Tv**	**SPTFE/Tv**
A. bipunctata	1065 ± 135	982 ± 192	510 ± 212	456 ± 192
M. scabrinodis	1239 ± 203	473 ± 187	171 ± 133	1239 ± 118

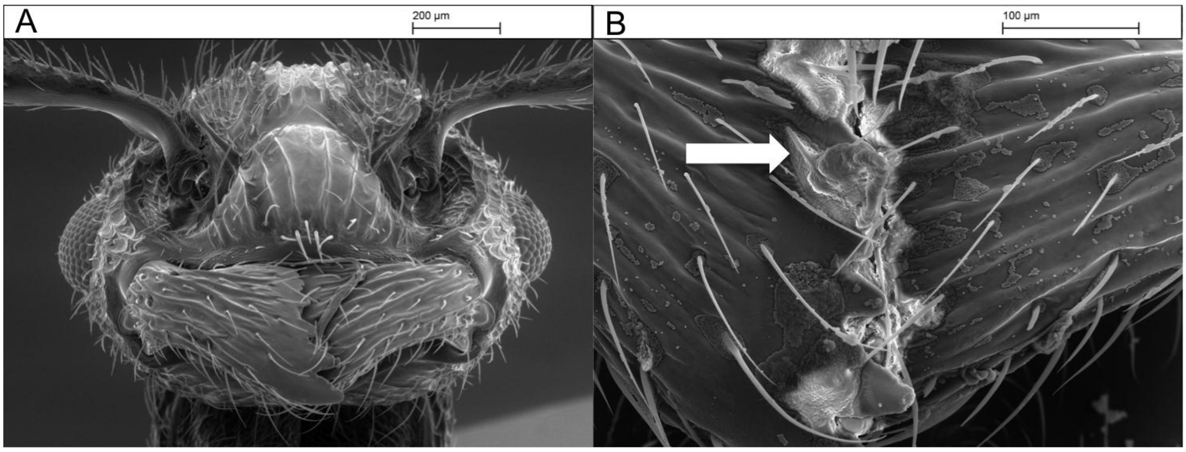


Figure 2.1: Scanning Electron Micrographs of A, *M. scabrinodis* and B, *P. dives* jaws post preening after contamination with A, glass and B, PTFE particles, post preening contamination of jaws was not found for ants fouled with glass spheres but can be observed in B for those insects fouled with PTFE particles.

3.3.2 Survivorship curves for controls of T_{adhere}

To provide a baseline of adhesive ability for each species controls were used, where an unfouled insect was placed in a representative vial of each type and *Tadhere* was recorded (Figure 3.2). To control for confounding factors such as pad size and behavioral differences we first analyzed the data by species. In a further analysis trials were grouped by the physical size of the insects (large (*P. dives* and *H. axyridis*) and small (*M. scabrinodis* and *A. bipunctata*) to probe the effects of physical factors such as the size of the attachment pad as a function of self-cleaning ability. The resulting survivorship curves for large species, particle and substrate combinations are presented in Figure 3.3, and those for the small species are presented in Figure 3.4.

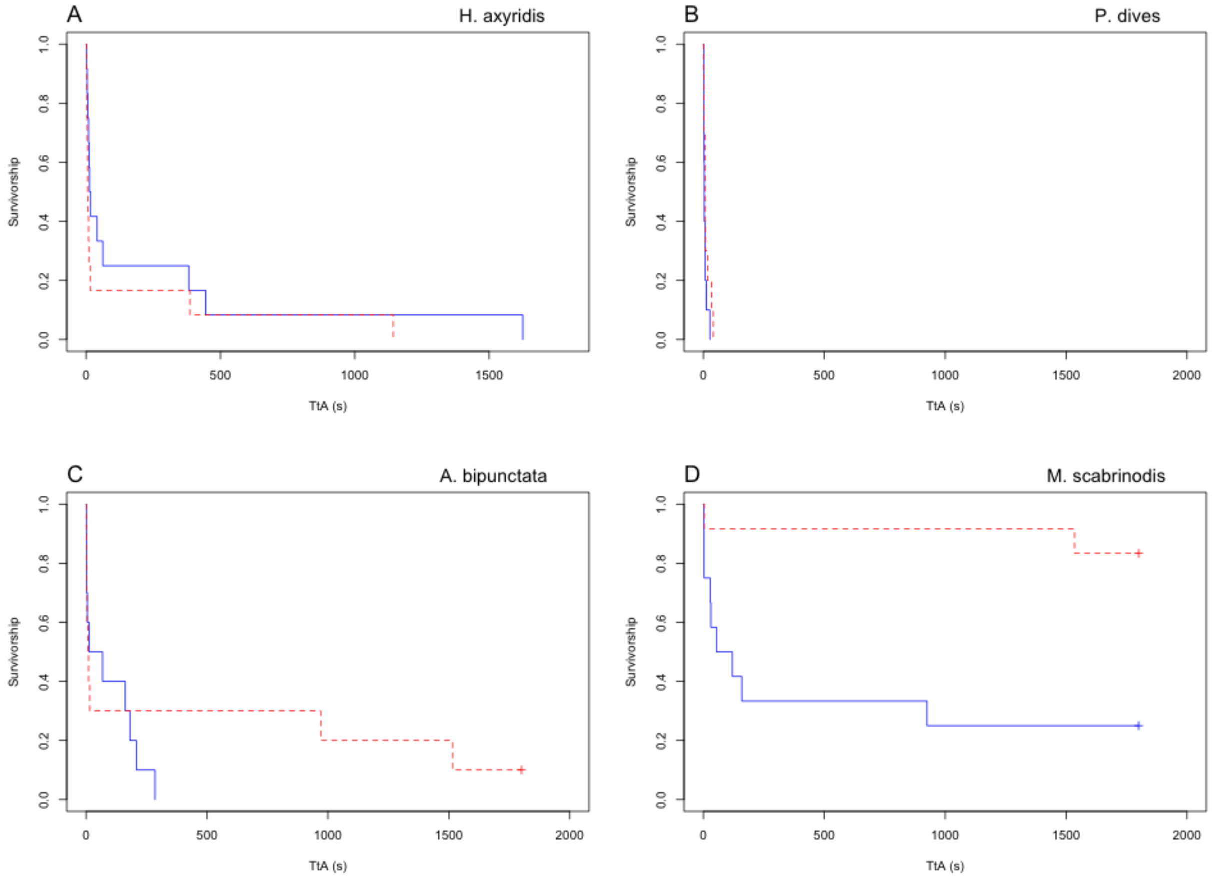


Figure 3.2: Survivorship curves of T_{adhere} controls for A, *H. axyridis*, B, *P. dives*, C, *A. bipunctata* and D, *M. scabrinodis*. For all plots solid blue lines = untreated vials, broken red lines = treated vials (n = 20 for each species vial combination). A step in the curve indicates an insect was successful in adhering to the inner vial wall, a + at the end of the line indicates there were still insects which had failed to adhere by the end of the 30-minute time period and when a line crosses the x axis it indicates all insects successfully adhered within the time period.

There was no significant difference between times taken to adhere to the control surfaces for large insects either within (harlequin ladybeetle (*H. axyridis*); d.f. = 1, F = 2.66, p = 0.10, Figure 3.2 A; weaver ant (*P. dives*); d.f. = 1, F = 1.60, p = 0.22, Figure 3.2 B) or between (d.f. = 1, F = 2.66, p = 0.109) a species. No significant difference between times taken to adhere to the control surfaces were found for the two spot ladybeetle (*A. bipunctata*); d.f. =1, F = 5.94, p = 0.23, Figure 3.2 C. However, there was a significant difference in $\boldsymbol{T_{adhere}}$ for the red elbowed Spanish ant (*M. scabrinodis*);

d.f. = 1, F = 5.94, p = 0.02, Figure 3.2 D, with a longer time taken to adhere to the treated vials. A difference in T_{adhere} was found between small species (d.f. = 1, F = 4.15, p = 0.04), with the *A. bipunctata* adhering in a faster time to both surface types when compared to *M. scabrinodis,* possibly due to the smaller contact area of the adhesive pad of *M. scabrinodis* (Table 4). Although no significant difference was found for time to adhere between large and small insects when compared between species (d.f. =3, F = 1.25, p = 0.29).

3.3.3 Effect of FSE and pad morphology on regaining adhesive ability

When the data were grouped according to pad type a significant effect of both particle (d.f. = 1, Z = 6.55, p < 0.001) and substrate (d.f. = 1, Z = 2.37, p <0.001) FSE was found for smooth pads. We find that insects contaminated with small PTFE particles taking longer to adhere to either surface type, but there was no interaction between the two terms (d.f. = 2, loglikelihood = -470.1, p > 0.001). For hairy pads only the FSE of the substrate had a significant influence on time taken to escape (d.f. =1, loglikelihood = -637.5, p < 0.001), irrespective of particle size or type more time was required to adhere to the treated vial surfaces.

3.3.4 Interactions of particle type, size and substrate on the regaining of adhesive ability

As can be observed from figure 3.3 and Figure 3.4 and Table 7 and Table 8, interactions between surfaces with a high frictional coefficient (glass on glass) offered better self-cleaning than glass on treated surfaces regardless of particle size, although it was found that PTFE was harder to remove on all surfaces for both pad types. Due to the numbers of insects that did not manage to adhere within the 30 minute time period the mean values are skewed.

Table 7: Mean ± standard error T_{adhere} values for large insects for all particle and substrate combinations, Due to the numbers of insects that did not manage to adhere within the 30 minute time period the mean values are skewed. Sg = small glass particles, SPTFE = small PTFE particles, Lg = large glass particles, LPTFE = large PTFE particles, Utv = untreated glass vial, Tv = treated glass vial.

Species	**Sg/Utv**	**SPTFE/Utv**	**Lg/Utv**	**LPTFE/Utv**
H. axyridis	653 ± 146	765 ± 171	782 ± 179	344 ± 77
P. dives	28 ± 6	1521 ± 340	1.41 ± 0.31	1371 ± 306
	Sg/Tv	**SPTFE/Tv**	**Lg/Tv**	**LPTFE/Tv**
H. axyridis	1590 ± 355	1338 ± 299	805 ± 180	1452 ± 324
P. dives	97.08 ± 21	1693 ± 378	21.9 ± 4	1203 ±378

Table 8: Mean ± standard error T_{adhere} values for small insects for all particle and substrate combinations, Due to the numbers of insects that did not manage to adhere within the 30 minute time period the mean values are skewed. Sg = small glass particles, SPTFE = small PTFE particles, Utv = untreated glass vial, Tv = treated glass vial.

Species	**Sg/Utv**	**SPTFE/Utv**	**Sg/Tv**	**SPTFE/Tv**
A. bipunctata	234 ± 52	817 ± 112	1432 ± 134	1344 ± 115
M. scabrinodis	560 ± 125	1326 ± 118	1628 ± 149	1521 ± 147

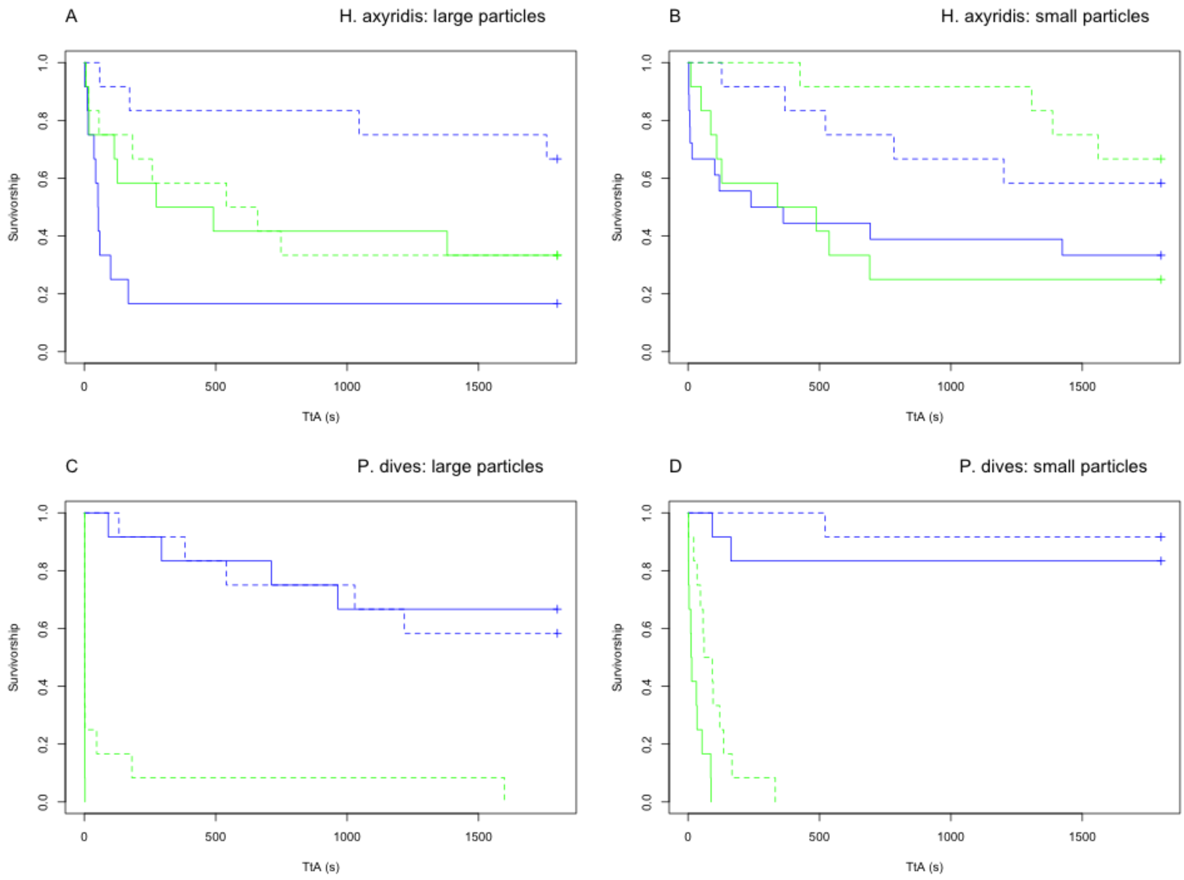


Figure 3.3 Survivorship curves for T_{adhere} in experimental treatments with large insects, (A) *H. axyridis,* (D) *P. dives* small particles, (B) *H. axyridis,* (C) *P. dives* large particles. Line colours; blue = PTFE particles, green = glass particles, continuous lines = untreated vials, dashed lines = treated vials (n = 20 for each combination for each species). A step in the curve indicates an insect was successful in adhering to the inner vial wall, '+' at the end of the line indicates there were still insects which had failed to adhere by the end of the 30-minute time period and a line crosses the x axis it indicates all insects successfully adhered within the time period.

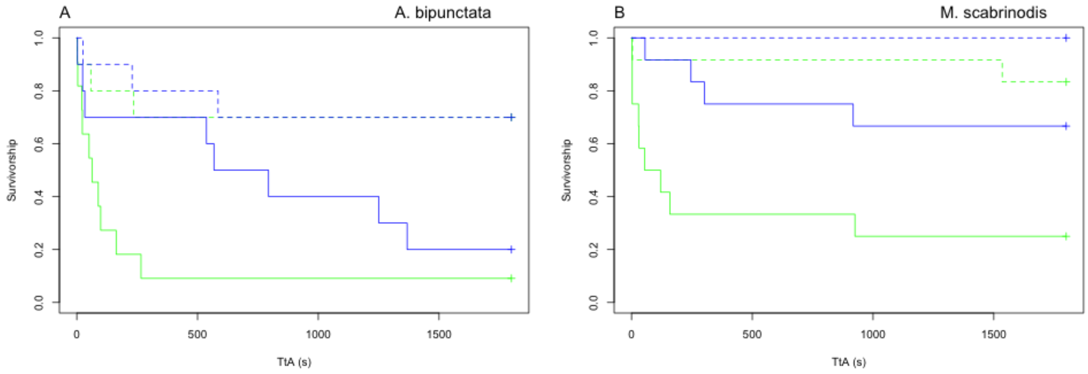


Figure 3.4: Survivorship curves for T_{adhere} in experimental treatments with small insects, (A) *A. bipunctata,* (B) *M. scabrinodis.* Line colours; Line colours; blue = PTFE particles, green = glass particles, continuous lines = untreated vials, dashed lines = treated vials (n = 20 for each combination for each species). A step in the curve indicates an insect was successful in adhering to the inner vial wall, '+' at the end of the line indicates there were still insects which had failed to adhere by the end of the 30-minute time period and a line crosses the x axis it indicates all insects successfully adhered within the time period.

We found a significant three-way interaction for T_{adhere} between particle size, particle FSE and substrate FSE for *H. axyridis* (d.f. = 7, loglikelihood = -458, $p < 0.001$). In *P. dives* time to adhere (T_{adhere}) was significantly longer for small particles when compared to large ones (D.F. = 1, Z = 3.758, $p < 0.001$), with a significant trend for individuals contaminated with particles of low frictional values (PTFE) taking longer to adhere than those contaminated with glass (d.f. = 1, Z = 0.57, $p < 0.001$) regardless of substrate properties. While an influence of substrate treatment was found to significantly affect T_{adhere}, with longer times taken to adhere to the treated vial walls regardless of contaminating particle size or type (d.f. 1, Z = 0.40, $p < 0.001$), there was also a significant interaction between the particle type and vial treatment, with those insects contaminated with PTFE particles attempting to adhere to treated vial walls taking significantly longer (d.f. = 4, loglikelihood = -311.5, $p < 0.001$) Figure 3.3 C and D.

The *A. bipunctata* results indicated a significant effect of the FSE of the contaminating particle (d.f. =2, loglikelihood = -172.7, $p < 0.001$), with a non-significant trend observed for substrate type ($p = 0.092$) although this was discarded using our AIC criteria. For *M. scabrinodis* time to adhere was influenced by both particle and substrate FSE but no interaction between the two (d.f. =2, loglikelihood = -113.2, $p > 0.001$) was found.

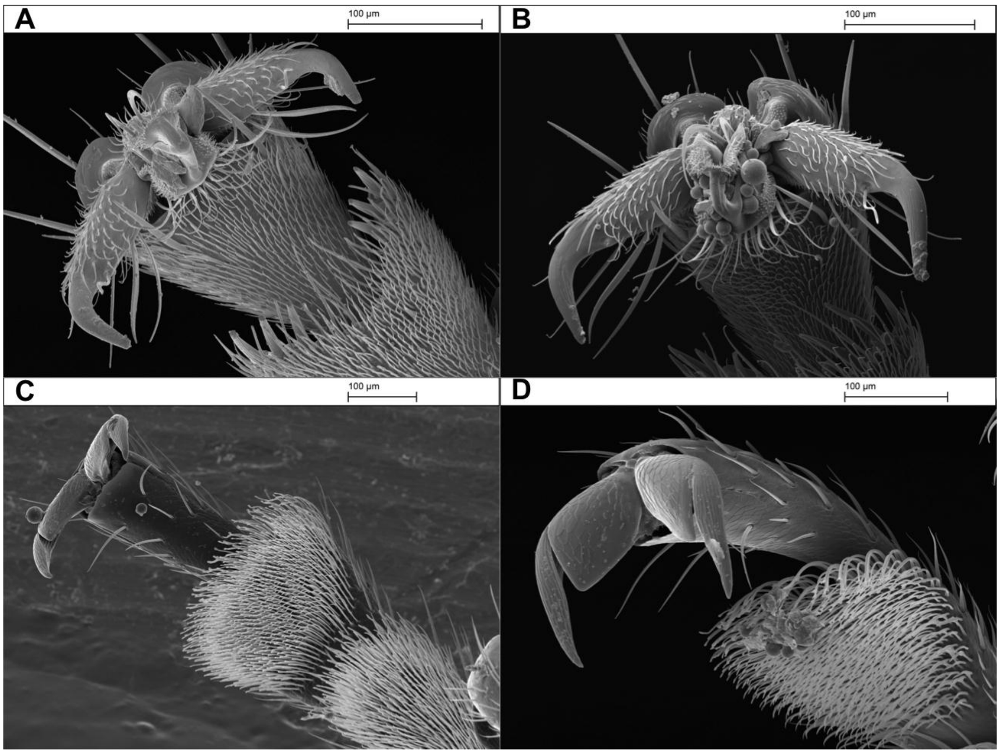


Figure 3.5: SEM micrographs of insect adhesive devices post fouling with 21 µm glass particles; A, *P. dives* untreated vial, B, *P. dives* treated vial, C, *H. axyridis* untreated vial, D, *H. axyridis* treated vial. Scale bar in all pictures is equal to100 µm.

3.4 Discussion

While a self-cleaning ability has been documented in a number of animal taxa (geckos: Hansen and Autumn, 2005, stick insects and dock beetles: Clemente *et al.,* 2010) and hypothesised more generally (Federle, 2006) the mechanics of self-cleaning in wet adhesive systems have been unclear. Here for the first time we were able to examine self-cleaning in freely walking, rather than tethered (Clemente et al., 2010), insects. We have been able to partially disentangle the interacting effects of FSE and pad type in the insect wet adhesion system. Results from the fouled insects show that although both hairy and smooth pad morphologies are capable of self-cleaning there are subtle differences between them. Adhesive pad types differed in both their self-cleaning ability (the number of contaminating particles removed) and the rate of recovery of adhesion once fouled (time taken to adhere to the vial walls), but these effects varied with the interplay of surface and particle FSE. Although while trapped in the vial the rear legs of the insect may have become re-contaminated with particles shed from the front limbs, this in itself is comparable to 'natural' events whereby the insect would have to 'clean' its feet while constantly being exposed to recontamination from plant waxes and environmental detritus, thus the level of recontamination is likely to be small. To aid in the analysis of self-cleaning, images of contaminated insect tarsi were obtained using a webcam connected via a stereo-microscope, each image was color inverted to produce a black and white image and then subject threashold analysis. This returned images wherein the contaminating particles were highlighted and number of contaminating particles removed in each image could be quantified. A series of images used to quantify the number of contaminating particles in each footstep are shown in Figure 3.6 (images shown are not colour invertend).

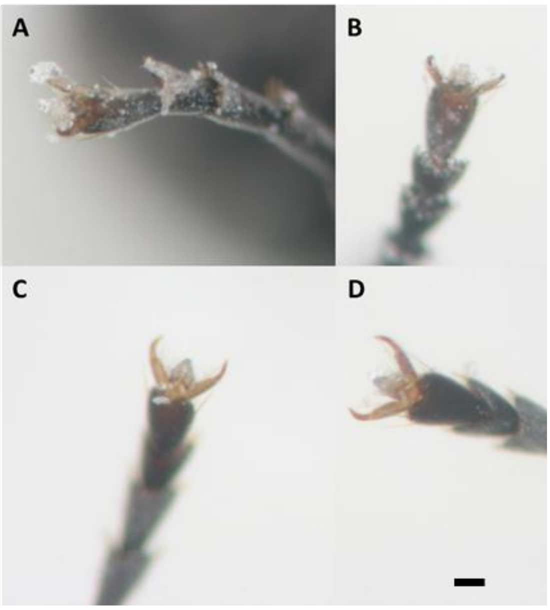


Figure 3.6: Sequence of non colour inverted images obtained to quantify the levels of contaminating particles left on the insect tarsi and adhesive orgnas between steps (time between images = 0.25 of a second). Image A – D show one step cycle, number of contaminating particles (white spheres on tarsi) can be observed to reduce when compared to previous frame, scale bar = 0.5 mm.

Effect of Preening time on self-cleaning

While time spent preening (T_{preen}), i.e. physically removing the contaminating particles from the insects body and adhesive organs did not differ between species of the same size, small insects were found to spend longer times preening than larger ones (Table 5 and Table 6) ($t = 5.065$, d.f. = 129.352, $p < 0.001$), possibly due to the relative size difference between small pads and particles compared to large pads and particles making it harder to scrabble at the inner vial walls. In order to assess the level of contamination of the jaws (a proxy for preening activity) a subset of insects used in the experiments were examined using SEM. Figure 3.1 shows examples of these images and visual confirmation of differences in the level of contamination between particle types can be seen.

Effect of FSE and pad morphology on regaining adhesive ability

While both pad types experienced loss of adhesive potential when fouled with microspheres, smaller particles were harder for both pad types to shed regardless of the physical size of the insect. The reported interaction between the FSE of the substrate and contaminating particles can be observed in Figures 3.3 and 3.4 where T_{adhere} was significantly longer for insects on treated glass surfaces post contamination with PTFE particles regardless of particle size, pad morphology or insect mass, resulting in a higher number of insects trapped at the end of the study. The ability to self-clean in ladybeetles (Coccinellids) was strongly influenced by the difficulty of adhering to surfaces with a low FSE, with, the difference in time taken to adhere to the surfaces depending upon particle sizes for *H. axyridis*, and for particle types for *A. bipunctata*. The ability to regain adhesion in both Hymenoptera (ants: smooth pads) was influenced by particle type and size, with an interaction between the frictional coefficient of the contaminating particle and substrate. The results of this study lend partial support to the results previously reported by Clemente et al (2010), where an effect of particle size was found for the regaining of adhesion in the dock beetle *Gastrophysa viridula,* with better recovery of adhesion for beetles compared to the stick insect *Carausius morosus*, when contaminated with large (45 µm) and small (1 µm) particles, but not for particles of 10 µm. We suggest that the difference found in the present study may be due to the differing morphologies of the pads, with smaller particles becoming trapped within the setal hairs of the hairy pads in a similar way to that reported by Clemente et al (2010).

The dense setae of the hairy pad type may simply allow for easier shedding of contaminating particles than for smooth pads through normal forces (acting perpendicular to the surface) as suggested by previous studies (Clementes et al., 2010; Hui et al., 2006; Hansen and Autumn, 2005), or through a rolling action (Hui et al.,

2006) where small lateral movements of the setae may facilitate cleaning by rolling the particle off the pad. Using this rolling argument (Clemente et al., 2010) suggested that the lack of setae on smooth pads leads to particles becoming embedded within the folds of the arolium. This at first seems counter intuitive since if the pads are better able to clean through shear forces then why would particles become trapped within the folds of the arolium. To explain this we put forward the following hypothesis, whist the arolium is fully deployed and at its maximum extension, particles would be rolled off the surface through contact with the substrate, however, once the arolium has started to reduce in size as the insect releases contact with the substrate (between steps) then any particles still on the surface of the arolium would become trapped due to the folding mechanism as the arolium is retained between the tarsal claws (Federle et al., 2001). We find support for this hypothesis in SEM images of the adhesive devices of ants that failed to self-clean within this study (Figure 3.7). It is also possible that although the arolium is less able to remove contaminants through application of normal forces it may be better able to self-clean through shear forces, which would be supported by the scrabbling and foot dragging behaviour insects were seen to perform during this study Figure 3.8 and Figure 3.9 (MO unpublished data). In the series of images presented in Figure 3.8 the concentration of particles deposited on the substrate can be observed to reduce over the course of a dragging action.

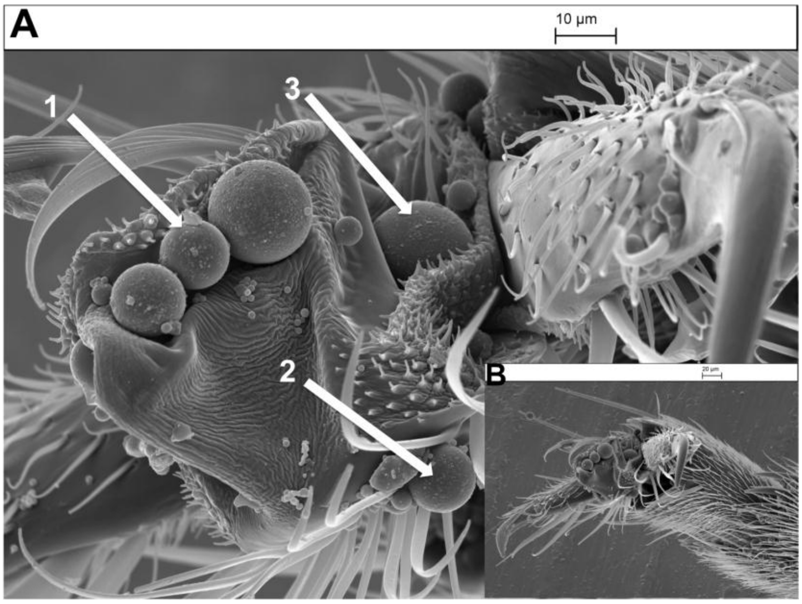


Figure 3.7: High magnification SEM micrograph of the (A) arolium of *P. dives* with 19 ± 8 µm SD um glass particles ensnared within the folds of the arolium. The outer edges of the arolium are comparatively clean while glass spheres can be seen held around the rolled distal edges (arrows 1 and 2) as well as within the folds adjacent to the tarsal claws (arrow 3). (B) Lower magnification micrograph showing placement of the arolium within the tarsal claws of *P. dives.*

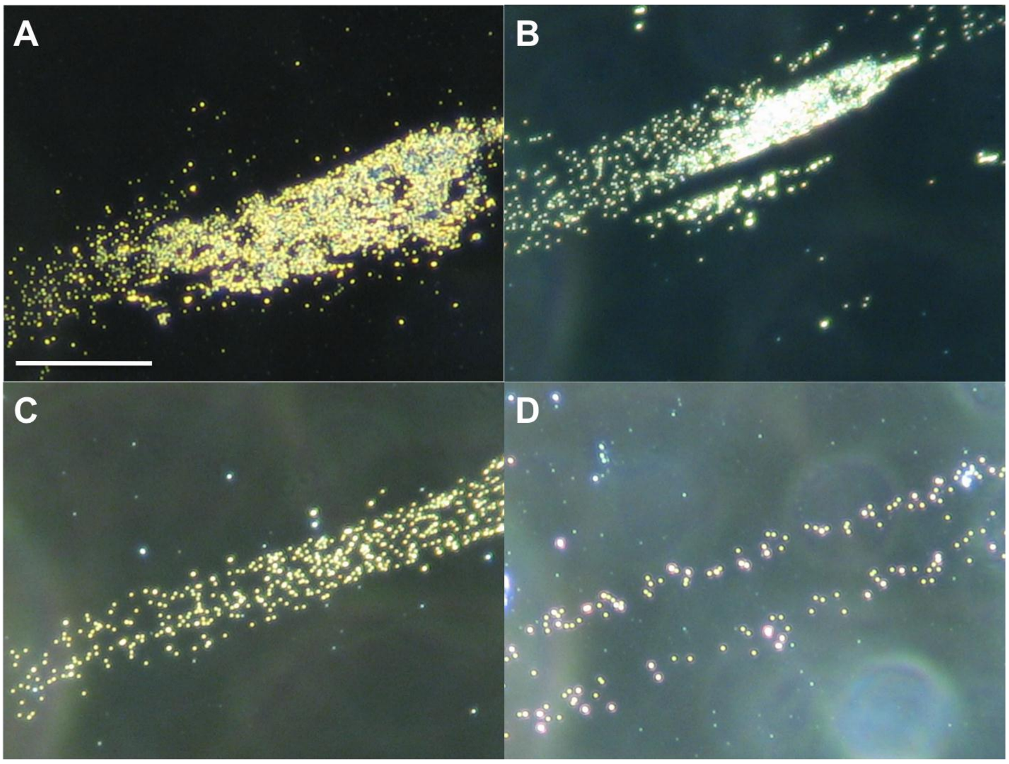


Figure 3.8: Optical phase contrast micrographs of large glass particles left behind in leg dragging behaviour observed in Hymenopterans during this study. Scale bar = 1 mm, all images taken at same scale, over a single microscope slide section.

Interactions of particle type, size and substrate on the regaining of adhesive ability

Although the glass particles used in this and previous studies are functionally spherical, the PTFE particles had a greater range of shapes and may have been better able to pack into gaps between setae (hairy pads) or in folds of the arolium (smooth pads). It seems likely that packing behaviours may have influenced the ability of the pads to remove PTFE particles compared to glass.

The weak ability of the hairy pads to adhere to the low energy substrate is interesting, as previous studies have suggested that the morphology of the hairy pads have been optimised for surface attachment (Federle, 2006). It is possible that on surfaces with a low FSE the adhesive secretion could bead up leaving it unable to wet the surface adequately, reducing capillary adhesion. Although this would be in contrast to current theory regarding the emulsion hypothesis and its benefits regarding attachment to both hydrophobic and hydrophilic surfaces.

We conclude that while the self-cleaning rate of both hairy and smooth pads in free roaming insects is similar, interactions between the relative FSE's of the contaminating particle and the substrate only had a significant effect on the hairy pad form suggesting that the mechanisms used by the two pad types do differ, and would suggest that this difference in ability has a morphological basis. Furthermore, when contaminated with large glass or PTFE particles individuals of *P. dives* were observed to drag their rear legs behind its body leaving behind a smear of fluid on the vial wall within which were trapped several contaminating particles. While this dragging behaviour was absent in the other species both the Coccinellids used in this study were occasionally observed to remain stationary for long periods of time, and upon moving again left behind yellow coloured liquid deposits ‘flushed’ from the adhesive pad on the substrate within which were trapped the contaminating particles (Figure 3.9 and Figure 3.10), although it should be noted that both the dragging and ‘flushing’ behaviours observed were only displayed infrequently and no insect exhibiting such behaviours were used in the subsequent analysis.

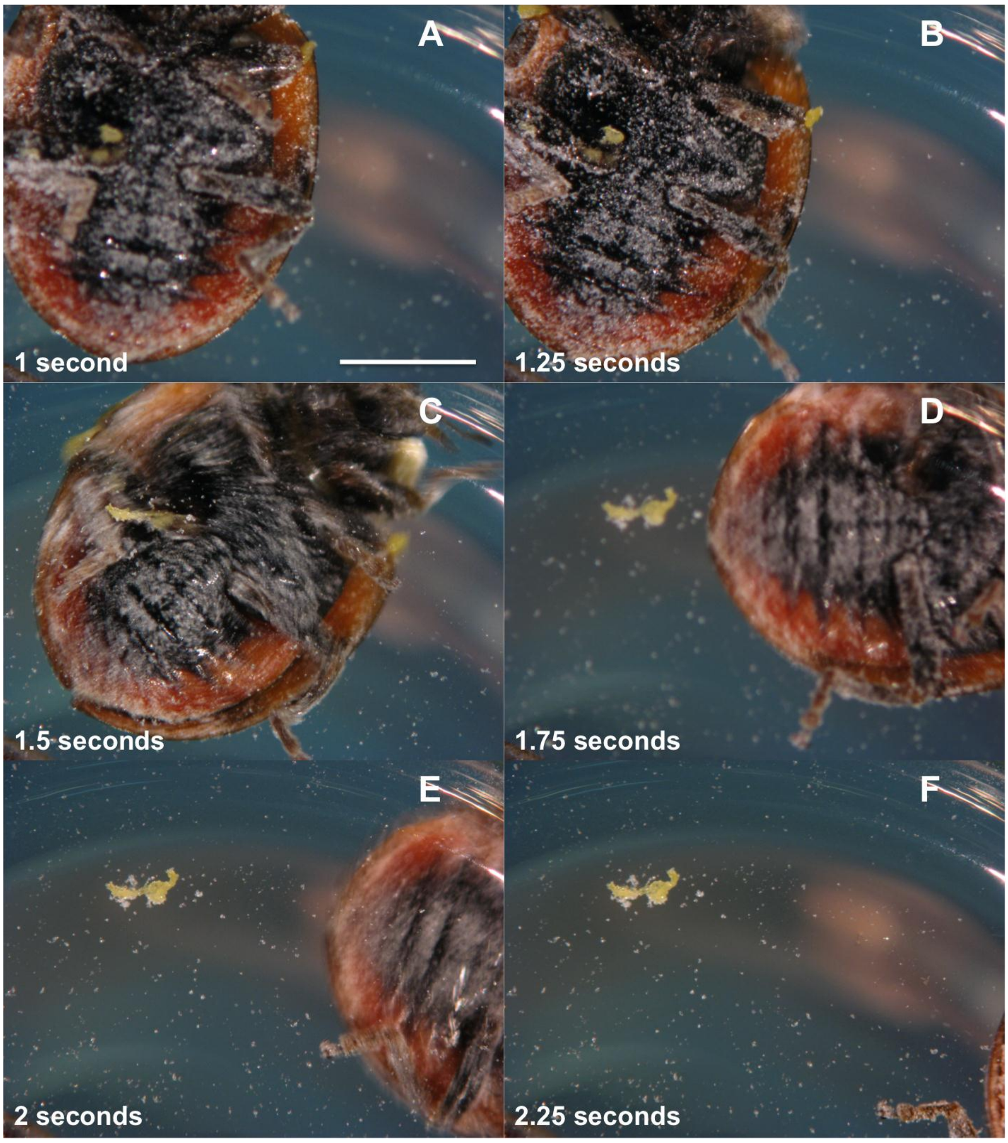


Figure 3.9: Optical micrograph images of flushing behaviour observed in *H. axyridis* Fouling particles can be observed in A and in subsequent images, as flushing behaviour continues the particles clump together in to a yellowish mass which is then left behind on the substrate as the insect walks off (D, E and F). Images shown at 0.25 second intervals, scale bar in A = 1 cm, all images to same scale.

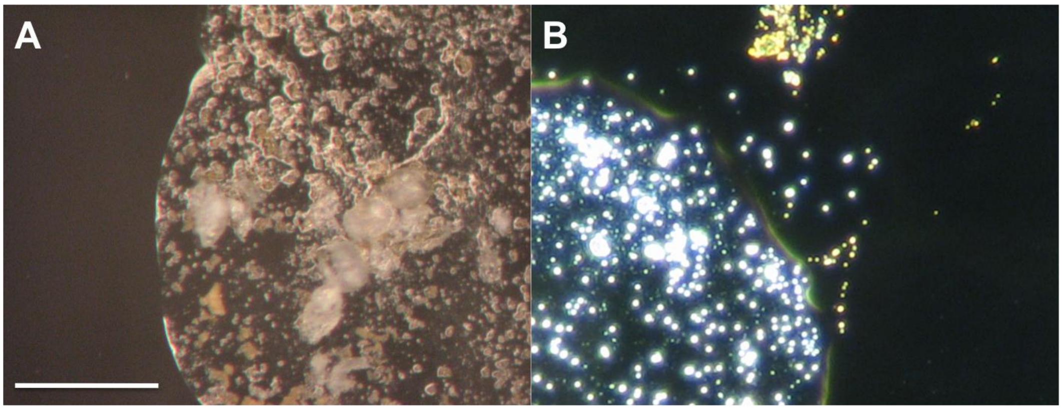


Figure 3.10: Optical images of particles flushed from the adhesive pad of H. axyridis, A, optical image of secretion droplet with contamination from large PTFE particles, B, phase contrast image of secretion droplet with contamination from large glass particles. Cuticular waxes can also be seen in image A, the presence of waxes and proteins in the secretion droplet may explain the complex rheological behaviour observed in previous studies. Scale bar = 1 mm, both images to same scale.

Although larger animals need not move faster by stepping more frequently but may simply have a longer stride, the larger insects in this study tended to move faster and step relatively more often than the smaller species (M. Orchard pers. obs.). This may have had an additive effect on the rate of adhesive recovery post contamination; a possible explanation for this is if both a large and small insect were trapped in adjacent vials but were constantly moving they would have a different moving degree, with the larger insect moving more frequent and an increased efficacy of the adhesive pads. This may explain why the larger insects preened less but regained adhesive ability faster. The extent to which these interactions influence the behaviour and life history traits of insects are open for further work and would suggest that it is impractical to generalise adhesive capabilities across species or even between differing size classes within a species.

Chapter four

Surface roughness determines adhesive potential in insects

4.1 Introduction

As described in section 1.4.3.1, four main hypotheses have been proposed to explain the adhesive abilities of insects: the contamination; roughness; wax dissolving and fluid absorbing hypotheses put forward by Gorb and Gorb (2002). The role of fragile particulate surfaces in contaminating the adhesive organs of insects has been demonstrated in chapters two and three and subsequent publications (Anyon et al., 2012; Orchard, Kohonen, & Humphries, 2012), and provides evidence for the contamination of both smooth and hairy adhesive devices. As such this chapter will address the remaining hypotheses regarding reduction of insect adhesion, namely the effect of surface roughness and porosity.

Factors such as surface contact area and porosity can affect the adhesive ability of insects and can be used to predict the adhesive potential of a species (Gorb et al., 2010). A surface profile of between 0.3-1.0 µm has been found to effectively reduce adhesion for both the green dock beetle (*Gastrophysa viridula*) (Peressadko and Gorb, 2004) and the common house fly (*Musca domestica*) (Gorb, 2001) and is hypothesised to be similar for other species with hairy pads, as supported by Scholz et al., (2010). The waxy epicuticular layer of terrestrial plants is made up of a dense covering of wax crystals with an aspect ratio (surface roughness) of between 0.3-1.0 µm (Peressadko & Gorb, 2004). It has been reported that a surface roughness order of 0.3-3.0 µm is the critical range for insect adhesion with adhesive abilities of both smooth and hairy pads failing within this range (Dirks & Federle, 2011; Gorb et al., 2005; Gorb, 2001; Peressadko & Gorb, 2004). Scholz et al., (2010), hypothesised that this 'critical' roughness range is related to the mechanical properties of the adhesive devices. By modelling the adhesive ability of the Indian stick insect (*Carausius morosus*) they found a critical roughness range for both claws (> 2µm asperities large enough to grasp with calws) and arolium (0.5-2.0 µm surface has smooth enough profile to enable arolium to make close contact). At roughness ranges < 2µm the arolium is thought to be unable to adhere to the substrate (due to mechanical stiffness) and any surface pores or structures are too small to allow purchase from the tarsal claws. Comparative studies of factors affecting adhesive potential in Tokay geckos (*Gecko gecko),* have found a relationship between the minimum root mean square roughness (RMS) profile of a substrate and adhesion similar to that reported for insects, with effective adhesion found on substrates exhibiting a RMS roughness of between 1.0-3.0 µm (Huber *et al.,* 2007). Any roughness scale smaller than this critical range results in a loss of adhesive ability due to insufficient contact between the adhesive organs and substrate, in a similar manner to that shown in Figure 4.0. While this may hold true for the dry adhesive mechanism of geckos, insects use an adhesive secretion that may allow them to increase the contact area thereby increasing adhesion (Pearson, 1998; 2003; Federle and Endlein, 2004; Gorb, 2004). As such this critical range is curious, and maybe affected not only by the pad being too mechanically stiff to allow for close enough contact, but may also be due to the physio-chemical properties of the surface altering the secretions abilities.

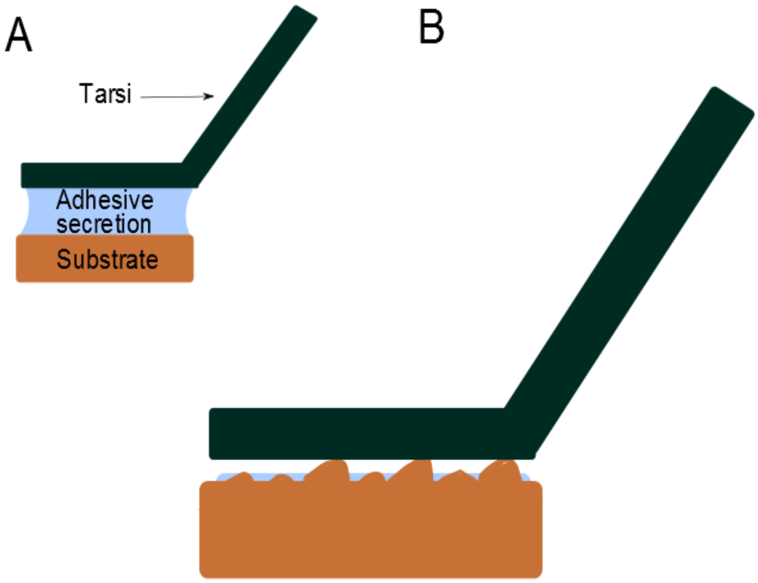


Figure 4.0: Simplified schematic of how surface roughness can limit adhesive contact, A, an ideal situation where the fluid wets both the pad and the substrate completely and adhesive contact is maintained, while in B, the fluid is insufficient to fill in the surface asperities and the pad is unable to deform on a localised scale to make dry friction with anything other than the asperity tips and an overall reduction in adhesive potential is observed.

Carnivorous plants employ a combination of fragile and low friction surfaces along with morphological adaptations to both trap and retain insects by impeding their adhesive abilities (Reidel *et al.*, 2003; Gorb and Gorb, 2006b). The structuring of the wax crystals of pitcher plants gives a micro-surface roughness exactly within this 'critical range', which inhibits the contact of an insects' adhesive organ and limits the adhesive contact area between the pad and the surface (Scholz et al., 2010b). An example of this is the epicuticular surface roughness of the lotus plant (*Nelumbo nucifera*), the surface of which has a dual scale roughness profile in effect having a nano-scale roughness profile over a micro-scale surface area, which reduces insect adhesion by limiting contact area, and helps the self-cleaning of the plant surface through extreme hydrophobicity (Hebert, 2009). It is this surface profile along with its covering of epicuticular waxes that makes it super-hydrophobic (Bhushan & Jung, 2006; Ming et al., 2005; Nosonovsky & Bhushan, 2005). The chemical properties of surface waxes on plants may also alter the effective adhesive ability via chemical changes in the adhesive secretion Geiselhardt *et al.* (2010).

Although current knowledge of insect adhesion allows for predictions using generalisations across species there are exceptions to the general rule. Symbiotic relationships between species such as the *Pameridea roridulae* and *Roridula gorgonias* relationship (Voigt and Gorb, 2008), the Crematogaster-Macaranga ant-plant symbiosis, and that between *Camponotus schmitzi* and *Nepenthes bicalata* (Schuitemaker and Staercke, 1933; Hölldobler and Wilson, 1990), allow the symbiont the ability to exploit and defend resources unavailable to other species due to specialised adaptations in both morphology and adhesive abilities. For example, although *R. gorgonias* is protected from herbivorous insects by a viscoelastic, adhesive secretion on its epidermis which traps a range of insects including ones of significant body mass (Voigt and Gorb, 2008), *P. roridulae* exhibits no difficulty adhering to or moving on the plants surfaces. A possible explanation for this ability to remain free where other insects become trapped is the dense epicuticular film that covers the body of *P. roridulae*. This layer is cohesively weak and is sloughed off, allowing it to remain free of the tacky surfaces of *R. gorgonias.* The *Crematogaster* morpho species that inhabit *Macaranga* trees show a marked morphological difference in mid leg size when compared to generalist sister species (Federle & Bruening, 2006). The increased leg length enables *Crematogaster* to have a lower centre of gravity whilst moving and as such reduce the likelihood of slipping on the waxy stems, allowing them to traverse the surfaces while their sister species cannot (Gorb and Gorb, 2006a). However, to date, no method has been put forward to explain the behaviour seen in the *C. schmitzi-N. bicalata* relationship. It seems plausible that the ant is able to adhere to the surface of the pitcher surface through a combination of morphological and behavioural adaptations including subtle changes in adhesive secretions and pad morphology, possibly due to a differing internal structure of the pad or differences in the physical properties of the pad material.

Several studies have measured the adhesive abilities of insects on both natural and artificial substrates (Brennan and Weinbaum, 2001; Eisner and Aneshansley, 2000; Federle *et al,* 2001). The techniques used range from centrifugal studies using a turntable to measure maximum detachment force (Federle *et al.*, 2000), to attaching an insect to a strain gauge and measuring maximum pulling force achieved across a range of surfaces (Eisner and Aneshansley, 2000). Merton (1965) found that insects had trouble adhering to surfaces coated with PTFE (Teflon). Fluon® which is a dispersion of polytetrafluoroethylene particles which can be sprayed or painted onto surfaces has been used to control the movement of entomological specimens by limiting their adhesive ability. To quantify the effect of Fluon® on the deployment of the adhesive organ in Hymenoptera, Endlein and Federle (2008), recorded the maximum arolium extension on three surface types (rough, smooth and Fluon® treated). Low frictional values of the Fluon® coated surfaces hindered the mechanical extension of the arolium, through a reduction in shear forces acting to extend the arolium. Arolium extension requires a proximal movement of the tarsi, which triggers the passive extension of the adhesive organ as the leg is pulled towards the insect's body (Endlein and Federle, 2008). This extension is facilitated by the sheer force generated between the arolium and the substrate, so Fluon®, by its inherent low free surface energy (FSE) inhibits extension of the arolium.

To the best of the author's knowledge no study has identified which factors gives surfaces anti-adhesive properties with respect to the wet adhesion used by insects. To this end the work carried out within this chapter will culminate in a novel anti adhesive surface that will allow us to probe the factors which effect adhesion in insects, by control of factors such as surface energy, porosity, surface roughness and the mechanical stability of all experimental substrates.

4.2 Materials and method

The Asian weaver ant *Polyrhachis dives* and the Harlequin ladybeetle *Harmonia axyridis* were used for this series of experiments (mean mass, and pad area presented in Table 9) as they are representative of each pad type (Smooth and Hairy) and observed to display comparable energetic levels (chapter three). Insects were maintained and cared for as described in chapter two. All experiments were performed at ambient laboratory temperature and relative humidity (25°C and 34% respectively).

Table 9: Mean mass, length and adhesive pad contact area given (for a single leg) for each species, standard error of the mean is presented next to given values, (n = 20).

Species	**Mass (mg)**	**Length (mm)**	**Pad type**	**Contact area (μm^2)**
H. axyridis	35 ± 1.0	9 ± 2	Hairy	114.60 ± 1.17$^{10-3}$
P. dives	15 ± 0.3	8 ± 1	Smooth	40.64 ± 0.39$^{10-3}$

4.2.1 Substrate adhesion trial

This series of experiments will be used to determine the effect of surface roughness, hydrophobicity and porosity on adhesive ability of insects using the substrates outlined in Table 10. Maximum angle of inclination achieved was compared between insects with intact tarsal claws and those with claws ablated.

Table 10: Physical properties of manipulated substrates used to determine adhesive potential on substrate types:

Substrate	**Surface roughness**	**Surface energy**	**Hypothesis tested**
Fluon® (F)	Micro scale	Low	1, 2
Silica (Si)	Nano scale	High	1, 2, 3
Irradiated silica (ISi)	Nano scale	Low	1, 2, 3
Clean glass (Cg)	N/A	High	1. 2
Large glass beads (Lb)	Micro scale	High	1, 3
Large glass beads treated with silica (LbSi)	Dual scale		1, 2, 3

The substrates outlined in table 10 above will be used to investigate the importance of the following hypothesis on insect adhesion, in both section 4.2.1 and 4.2.2:

<u>Hypothesis to be tested</u>

1) That surface roughness limits adhesive potential through mechanical stiffness of attachment materials.

2) That surface energy of a substrate limits adhesive potential in insects due to difference of the adhesive secretion in wetting such substrates.

3) That substrate porosity limits insect adhesive potential via wicking away of secretion.

First a twin series of clean glass beakers were constructed to give allow for manipulation of the surface energy of the substrates. These beakers were split into two series, one treated with a spray coating of silica particles (Si) suspended in ethanol (5% solution, 0.5 g in 10 ml of ethanol), with the other treated with Fluon® (F) (to give a micro textured low friction surface). A third series of clean beakers were left untreated and used as a control surface (Cg). All beakers were rinsed in hexane, and then blown dry with nitrogen before use. To determine if the maximum angle of inclination achieved while climbing the beaker sides was influenced by surface roughness the series of beaker trials was repeated with another series of beakers coated with a dispersion of 19 µm silica particles (Lb) prepared as the previous series, a subset of these beakers were then treated with the R202 silica spray (to give a dual scale roughness, suspected to limit insect adhesion) (LbSi), with maiximum angle of inclination compared for insects both with and without tarsal calws (claws removed as previously described). It should be noted that although the surface of these beakers was hydrophobic (CA of 5 µl water 105° ± 6°), they were not superhydrophobic due to the larger surface asperities. Maximum angle of inclination achieved was compared between substrates using an ANOVA and *pochoc Tukey test* (R, ver. 2.10.1: R development core team, 2009).

The beakers were then turned through 90° before insects were placed inside and their behaviour recorded (4 fps, for 1800 frames) for ten minutes using a webcam (QuickCam pro for notebooks) controlled by the freeware program HandyAvi (version 4.2; Andersons Azcendant software) (Figure 4.1). The adhesive ability of the insets was compared between the treated and clean series of beakers using a custom built plugin in ImageJ. The plugin allows calculation of the maximum angle of inclination (θ_i) for each insect on a frame-by-frame series.

To rule out possible contamination from particulates (as observed in chapter two and three) from degraded surfaces, the mechanical stability of the treated surfaces was also tested. Insects were first trapped within a treated beaker of each type for 30 minutes before sending for SEM imagining and percentage of contamination was quantified from micrographs, to rule out potential self-cleaning behaviours as observed in chapter three, another series of insects were again trapped within fresh treated beakers before being transferred to a PTFE beaker, limiting any possibility of self-cleaning due to frictional forces.

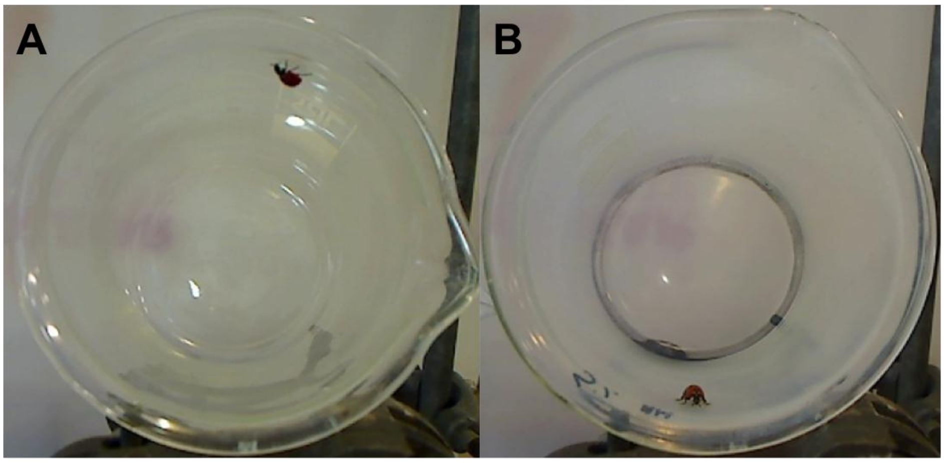


Figure 4.1: Digital images of *Harmonia axyridis* traversing A, a clean glass beaker, B, a beaker spray coated with a layer of 5% solution silica particles (super-hydrophobic surface).

4.2.2 The effect of surface energy on adhesive potential

To determine if the free surface energy (FSE) of a given substrate influenced the adhesive potential of an insect, a series of substrates were manufactured using a 5 % wt spray of nano sized particles using the same procedure as described above for the beaker trials, a subset of the treated slides were irradiated with UV light (UVP bulb) to give a twin series of slides with high and low surface energies with the same surface roughness profiles (Si and ISi slides). This irradiation removes the surface hydrocarbons to leave a super-hydrophilic surface (ISi). Other surface properties, such as length scale roughness and porosity, are unaffected, leaving two similar surface profiles (RMS 11.99 and 15.21 nm hydrophilic and hydrophobic surfaces ± 10 nm respectively) with differing FSE values (Table 10). Contact angles of 5 µl water on each surface were measured before use to quantify the FSE of each substrate before experimental use. To ensure complete surface coverage the slides were exposed in three differing positions for duration of 5 minutes each (Figure 4.3). Surfaces treated with the silica (Si) dispersions were originally super-hydrophobic (contact angles >150 °, Figure 4.2A), while irradiated surfaces (Isi) are super-hydrophilic (contact angle 6.3 °, Figure 4.2B). Silica surfaces were imaged by optical microscopy and Veco-Wyko white light interferometer techniques to determine if surfaces were physically different after irradiation.

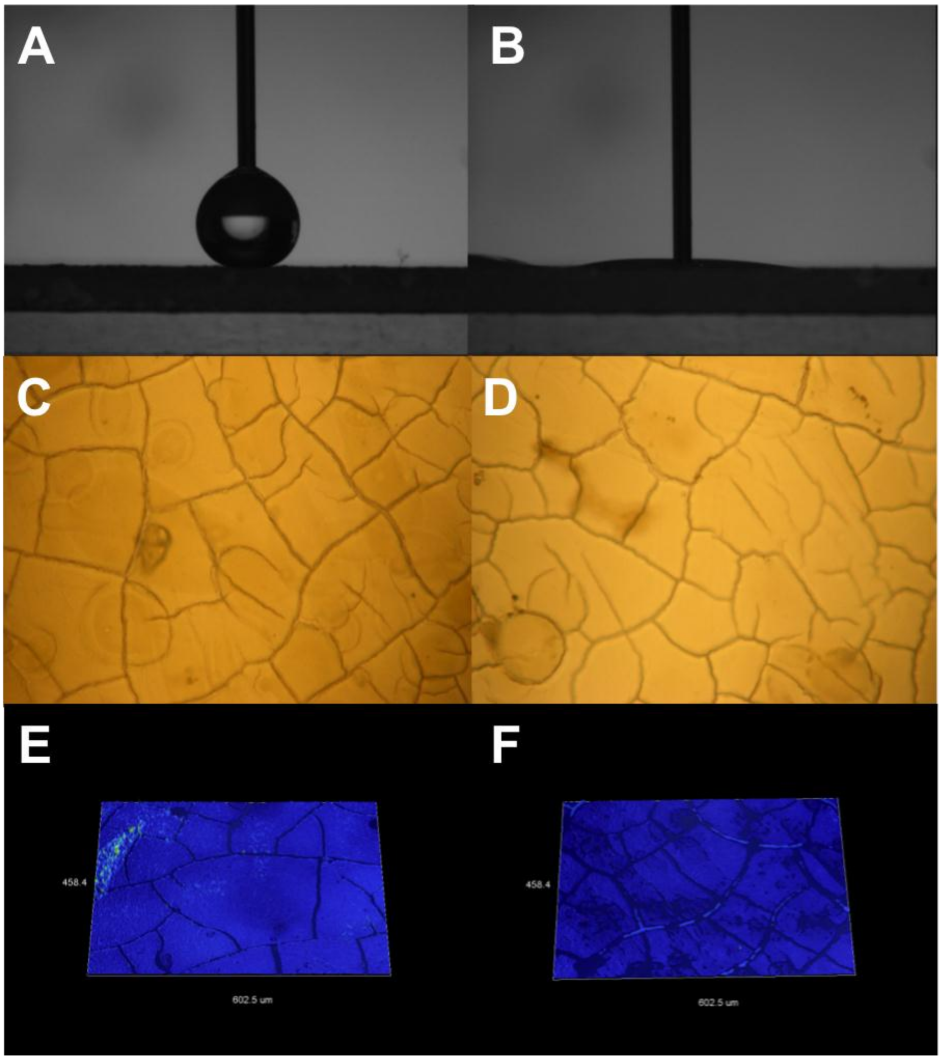


Figure 4.2: Comparable surface array, A and B 5 µl water droplets on superhydrophobic substrates before (A, Si) and after (B, ISi) irradiation with UVDB bulb, C and D, optical images of superhydrophobic substrate (5% wt silica suspension), pre (C) and post (D) irradiation, E and F digital images of superhydrophobic substrate pre (E) and post (F) irradiation, images captured using a Veco-Wyko white light interferometer.

Insects were placed on a slide surface and slowly rotated up to 180° or until it dropped off the slide. This was repeated for insects both with and without intact tarsal claws (tarsal claws removed using microscissors). The maximum angle of inversion of each insect was recorded using a simple tilt bed (Figure 4.4). Angle of detachment for each species on all substrates was compared using a nested ANOVA (R, ver. 2.10.1: R development core team, 2009).

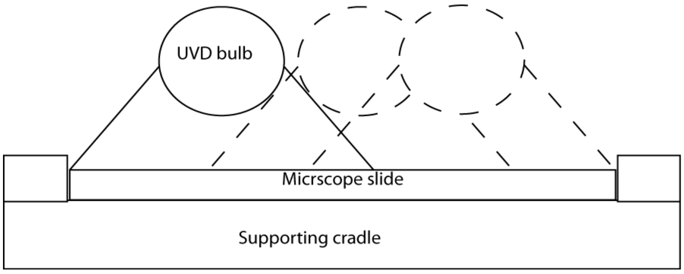


Figure 4.3: UVD bulb irradiating the surface of a super-hydrophobic slide (ISi), slide was moved to allow bulb to burn in the three positions, last two positions shown as dotted lines.

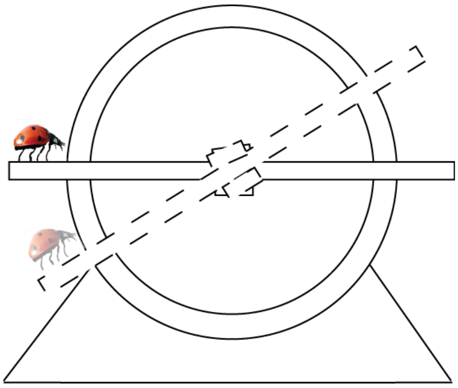


Figure 4.4: Schematic of tilt angle apparatus, insect is placed on level platform and it is slowly rotated around its axis, *H. axyridis* shown larger than actual size to facilitate demonstration.

4.3 Results

4.3.1 Substrate adhesion trial

In the following boxplots we plot the maximum angle of inclination achieved by both *P. dives* and *H. axyridis* within each of the beaker series (Figure 4.5 and Figure 4.6 respectively). Maximum angle of inclination was then compared using ANOVA and *posthoc Tukey test,* to determine differences both within and between species.

A significant difference was found for maximum angle of inclination between control (Cg) and experimental surfaces for both species (*P. dives;* $F_{4, 230} = 178.12$, $p < 0.01$; *H. axyridis;* $F_{4, 230} = 194.83$, $p < 0.01$), as well as for those insects with intact tarsal calws compared to those with ablated ones (*P. dives;* $F_{1, 230} = 17.08$, $p < 0.01$; *H. axyridis;* $F_{1, 230} = 48.43$, $p < 0.01$), with a larger angle of inclination achieved on all surfaces by insects with intact tarsal calws. However, no significant difference was observed between experimental surface types for either species (*P. dives;* $F_{4, 230} = 139.53$, $p = 0.46$; *H. axyridis;* $F_{4, 230} = 99.35$, $p = 0.35$), although a significant difference was found between species ($F_{1, 460} = 25.48$, $p < 0.01$), with *P. dives* consistently able to achieve a higher angle of inclination than *H. axyridis* on the experimental surfaces, with and without ablated claws.

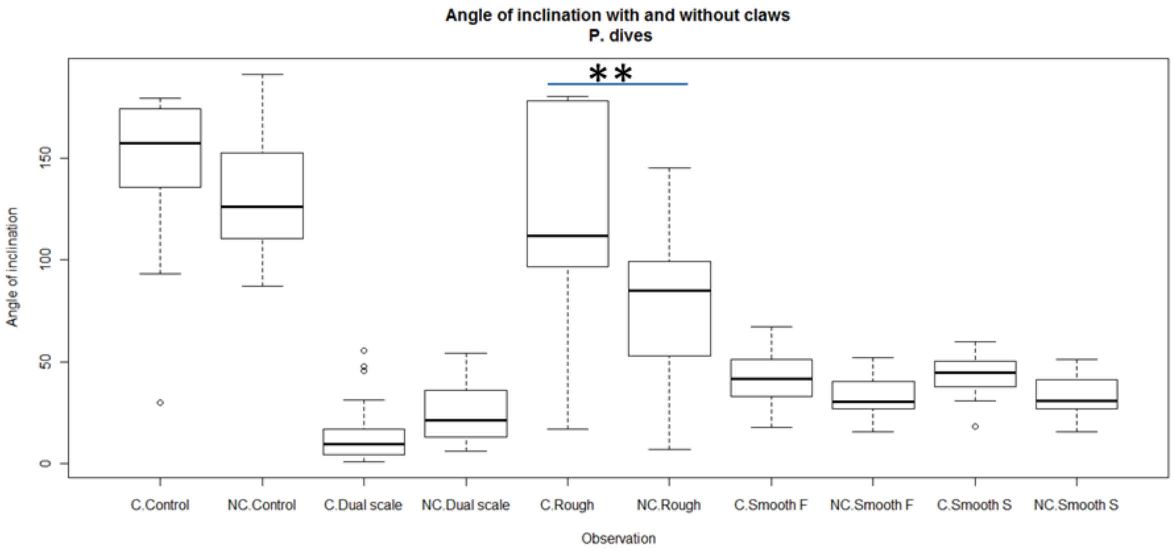


Figure 4.5: Maximum angle of inclination achieved on Control (Cg), Dual scale (LbSi), Fluon (F) and silica (Si & ISi) treated experimental beakers for *P. dives*, C = ant with claws, NC = ant without claws, Control = clean glass beakers, Dual scale = treated with 19 µm beads then sprayed with silica treatment, Smooth F = Fluon, Smooth S = silica treated, n = 22 for each surface, * = significant differences between means at the 99 %.

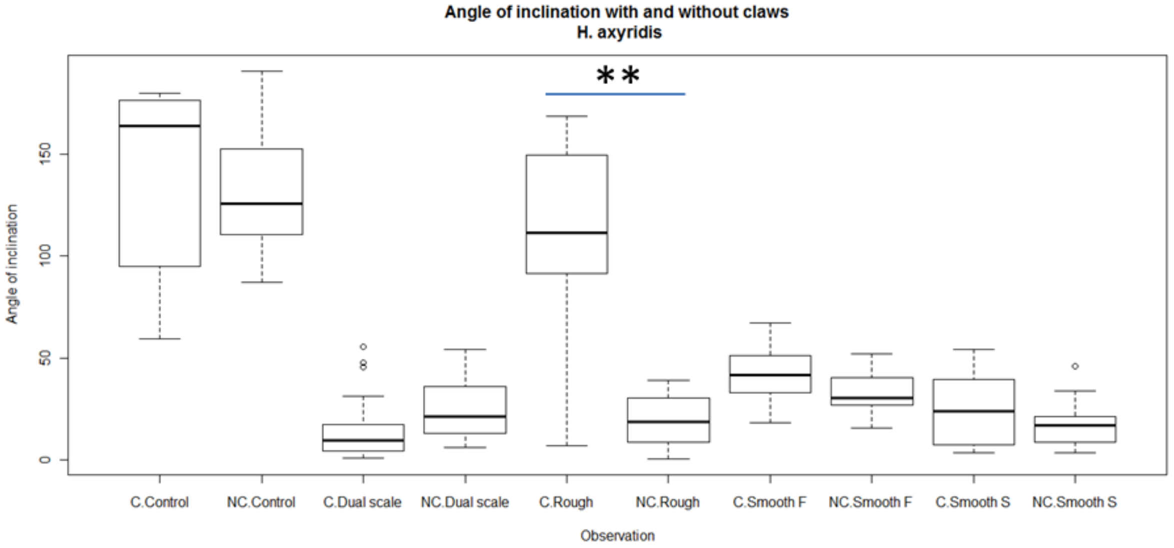


Figure 4.6: Maximum angle of inclination achieved on Control (Cg), Dual scale (Lb), Fluon (F) and silica (Si & ISi) treated experimental beakers for *H. axyridis*, C = ant with claws, NC = ant without claws, Control = clean glass beakers, Dual scale = treated with 19 µm beads then sprayed with silica treatment, Smooth F = Fluon, Smooth S = silica treated, n = 22 for each surface, * = significant differences between means at the 99 %.

There was a significant difference in angle of inclination achieved for species with and without intact tarsal claws on surfaces constructed using 19 µm glass beads (Lb) and those surfaces with a dual scale roughness (LbSi) (*H. axyridis*; $F_{1, 46} = 133.11$, $p < 0.01$; *P. dives;* $F_{1, 46} = 105.39$, $p < 0.01$ (figure 4.5, 4.6 respectivly)). No significant difference in maximum angle of inclination achieved was found between species for insects with or without tarsal calws ($F_{1, 460} = 2.51$, $p = 0.11$), suggesting that both adhesive devices function well on surfaces with a RMS sufficient to allow for purchase regardless of hydrophobicity. However there was a significant interaction between maximum angle of inclination achieved and substrate properties, both within (*P. dives*, $F_{4, 230} = 7.39$, $p < 0.01$; *H. axyridis*, $F_{4, 230} = 37.34$, $p < 0.001$) and between each species ($F_{4, 460} = 36.53$, $p < 0.001$), for insects with and without tarsal claws.

4.3.2 Adhesion measurement

To determine if movement parameters such as maximum angle of inclination were related to adhesive potential on each of the substrate types a custom movement stage was constructed Figure 4.7, with [normal] adhesive forces then measured experimentally on each experimental surface. This stage allowed controlled manipulation of live insects; cotton thread was tied around the thorax of specimens of *P. dives* and *H. axyridis* (with care taken to avoid trapping any legs (insert Figure 4.7), this thread was then attached to the underside of an electronic balance, supported directly above the movement stage. The movement of the stage was controlled by an electronic motor (MFA/combo drills RE280/1) connected to a regulated constant 12 V supply, operated by a double pole double throw switch; the motor was then attached via a drive shaft to a micromanipulator stage. By changing the polarity of the circuit it was possible to lower or raise the stage at a set velocity (4.41 $mm^{s\ -1}$). A specimen was placed on the substrate, and then the stage was moved downwards away from the insect (Figure 4.7 A and B). As the insect moved away from the balance it exerted a pull on the attachment thread that is registered by the balance. These recorded forces were compared against a control surface (clean glass) using an ANOVA with a *Dunnetts posthoc test* used to compare results for each surface independently against a control (Mathmatica version 8, Wolfram mathematics).

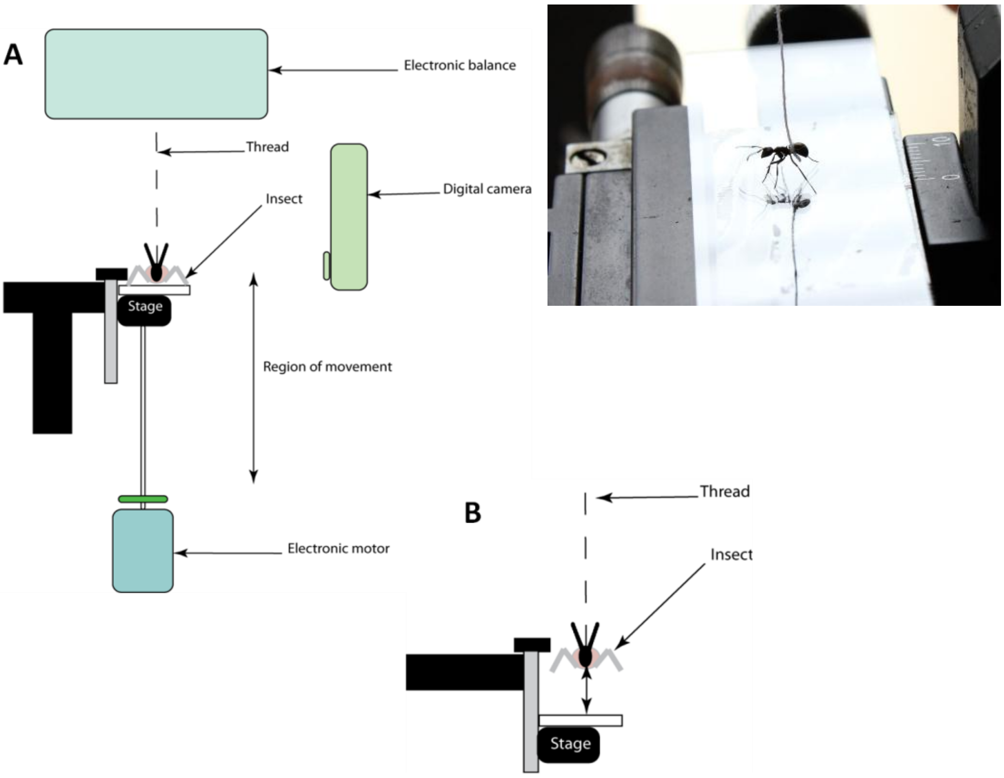


Figure 4.7: Schematic of movement stage used to record adhesive forces, A, whole set up viewed front on, B, close of stage, free space underneeth insect is equal to adhesive strain, insert shows *P. dives* specimen with thread tied around thorax.

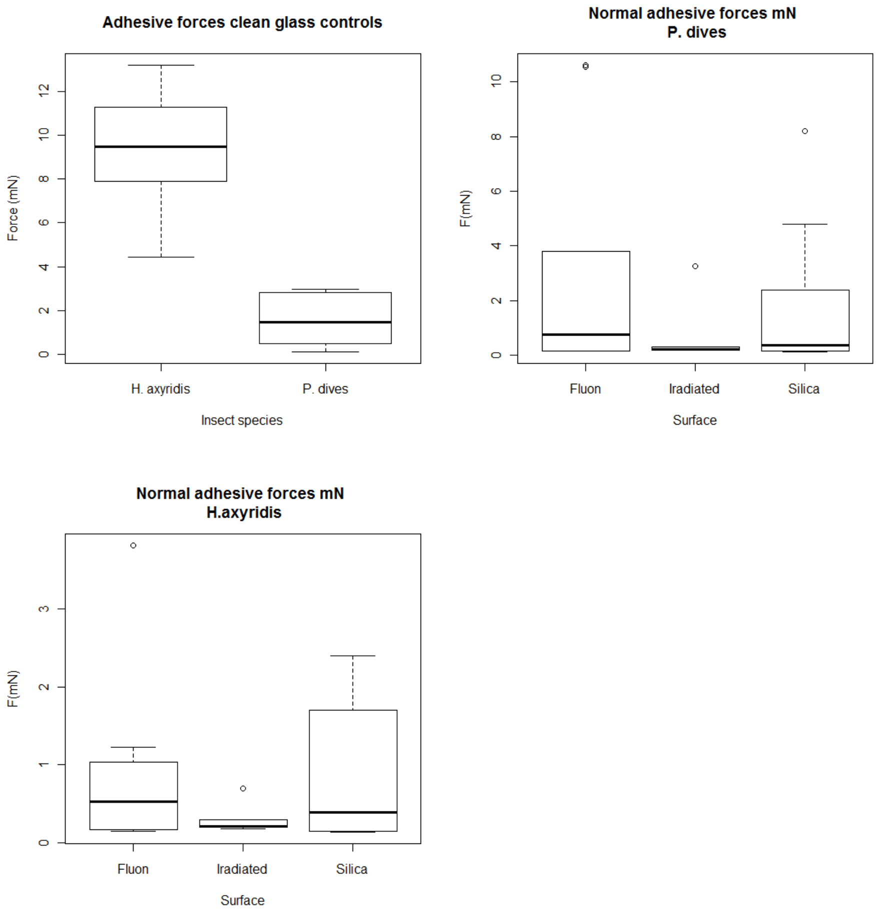


Figure 4.8: (A) Box and whisker plot of adhesive forces for *P. dives* and *H. axyridis* on clean glass control surfaces, thick black line represents mean value with outliers are shown as hollow circles (n = 20 for each species), (B) adhesive forces recorded for *P. dives* on each of the treated surfaces, Fluon (F), Silica (Si) and irradiated silica (ISi), thick black line represents mean value with outliers are shown as hollow circles (n = 22 for each substrate), (C) adhesive forces recorded for *H. axyridis* on each of the treated surfaces, thick black line represents mean value with outliers are shown as hollow circles (n = 22 for each substrate).

When comparing [normal] adhesive forces on the control surfaces between species (Figure 4.8), a significant difference in maximum forces generated were found, with larger forces being generated by the hairy pad of *H. axyridis* ($F_{1, 18} = 79.99$, $p < 0.01$). No significant difference was found when comparing [normal] adhesive forces between experimental surfaces for *P. dives* ($F_{2, 27} = 1.54$, $p = 0.2$) or *H. axyridis* ($F_{2, 27} = 1.86$, $p = 0.17$) or between species ($F_{1, 54} = 3.52$, $p = 0.06$), suggesting that adhesive forces are not dependent on the surface energy of a substrate (Si & ISi).

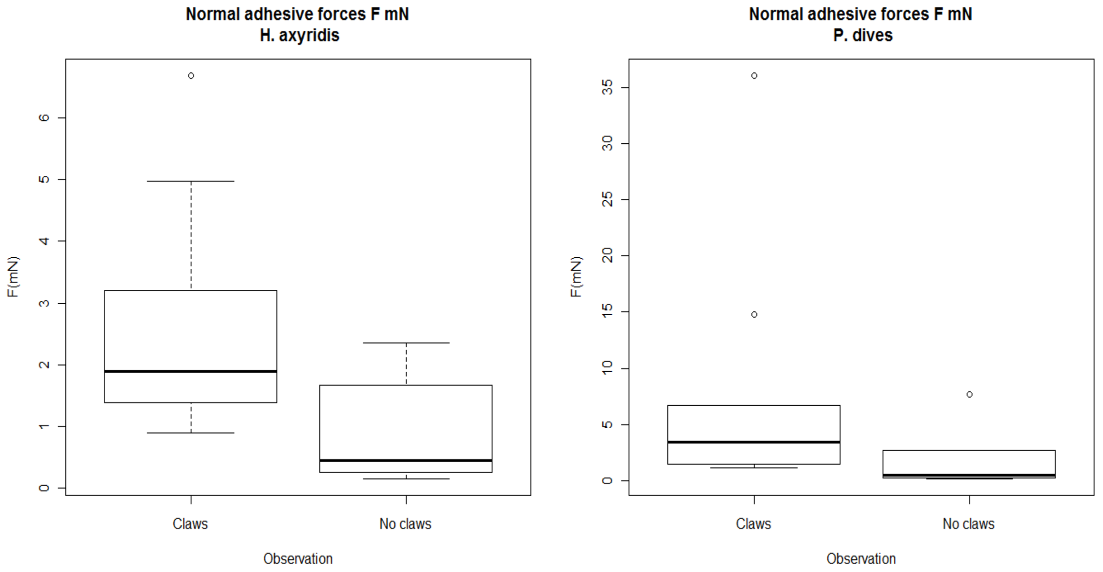


Figure 4.9: Box and whisker plot of normal adhesive forces on hydrophobic surfaces with a 19 µm ± 3 µm surface roughness, for both *H. axyridis* and *P. dives*, thick black line represents mean value with outliers are shown as hollow circles (n = 20 for each substrate).

When comparing absolute adhesive forces (FmN) for insects with intact and removed tarsal claws (Figure 4.9), a significant difference in adhesive ability was found for *H. axyridis* ($F_{1, 18}$ = 7.65, p < 0.01) but no such difference was observed for *P. dives* ($F_{1, 18}$, p = 0.15). This suggests that the primary adhesive device used by *P. dives* is the arolium and removal of or damage to the tarsal claw system may be of little consequence.

4.3.3 Tilt angle study:

The maximum angle of inclination achieved on inverted slide surfaces for both species are given in Table 11 and Figure 4.10.

Table 11: Maximum angle of inversion achieved for both species on each substrate type using tilt bed.

Insect	Substrate	Maximum angle of inclination °
P. dives	Control	180
	Super-hydrophobic	32.4
	UV treated super-hydrophobic	33.8
	Fluon®	46
H. axyridis	Control	180
	Super-hydrophobic	32.6
	UV treated super-hydrophobic	33
	Fluon®	47

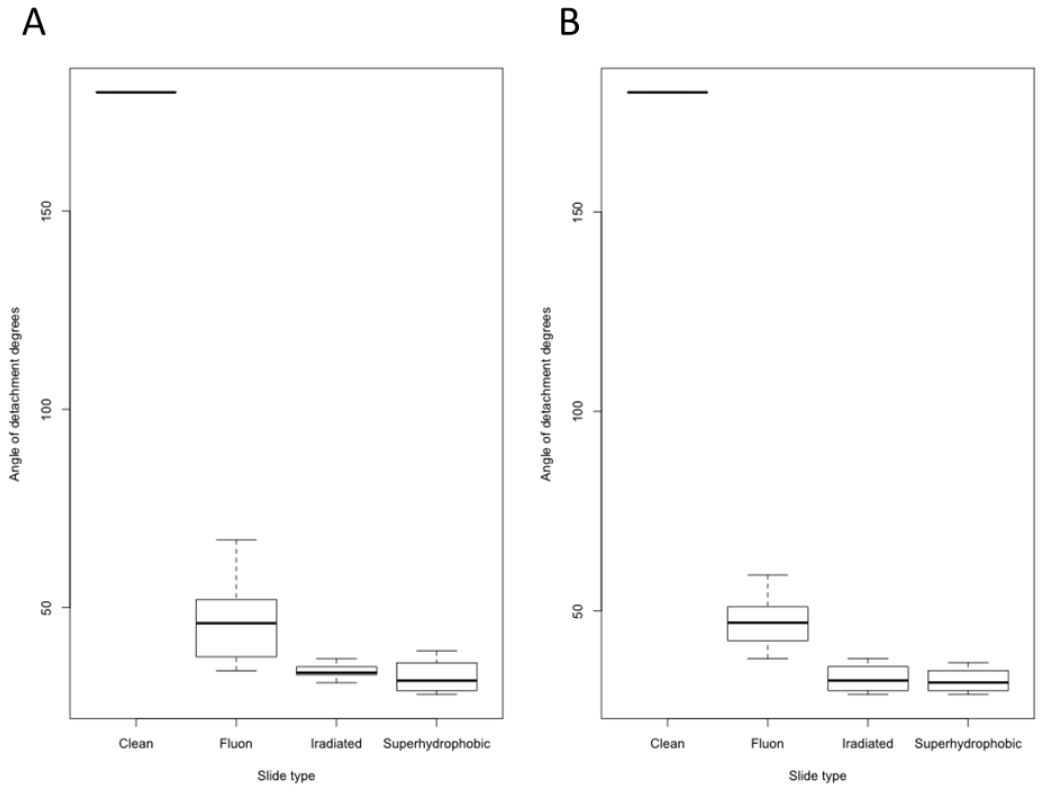


Figure 4.10: Box and Whisker plot of maximum angle of inclination achieved for both (A), *P. dives* and (B) *H. axyridis* on a range of slide surfaces, Clean (Cg), Fluon (F), Silica (Si) and irradiated silica (ISi), , note that on clean glass surface the insects were able to adhere to the surface even when inverted 180°.

There was a significant difference between the high and low energy surfaces in angle of detachment for both *P. dives* ($F_{3, 38} = 15.74$, $p < 0.001$) and *H. axyridis* ($F_{3, 38} = 7.98$, $p < 0.001$), while there was no significant difference in detachment angle for either species with respect to low energy substrate types (Si & ISi) (*P. dives;* $F_{3, 38} = 22.1$, $p > 0.05$; *H.* axyridis, $F_{3, 38} = 17.33$, $p > 0.05$).

4.3.4 Contamination

To determine if the insects used in this study showed poor adhesion (reduced maximum angle of inclination) on the experimental surfaces due to contamination of the adhesive devices, SEM images of insect tarsi were taken. These revealed little contamination from either the silica or irradiated silica surfaces (Figure 4.11).

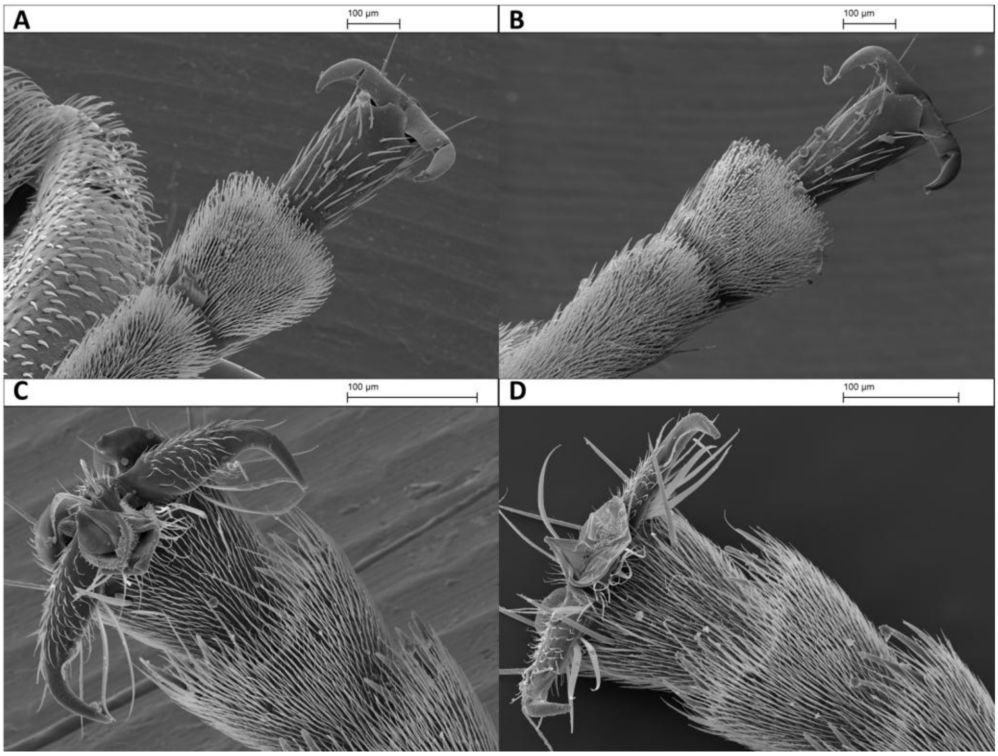


Figure 4.11: SEM images of insect adhesive devices used to determine levels of contamination from treated surfaces, A and B *H. axyridis* silica and irradiated silica treated surfaces respectively, C, D *P. dives* silica and irradiated silica (Si & ISi) treated surfaces respectively. SEM images of fouled tarsi *P. dives* Figure 2.3.

4.4 Discussion

During the course of this study it was observed that both pad types show the same trend for decrease in adhesive potential related to an increase in surface roughness supporting hypothesis 1. Although there are subtle differences in rate of self-cleaning between the two morphologies with interactions between the FSE of contaminating particles and substrate.

Substrate adhesion trial

On surfaces with an aspect size of < 20 nm (Silica substrates, nano scale low friction surfaces, Si & ISi), insects were not able to adhere sufficiently and were seen to slide backwards once they stopped actively trying to climb the walls (in the beaker trials) similarly in the tilt table study the RMS surface profile influenced the maximum angle of inversion obtained by the specimens, again with insects having difficulty adhering to the silica surfaces (nano textured low friction surfaces, Si & ISi). This effect of surface roughness can be seen more clearly when it is compared to the results from the other series, when placed on rougher substrates with surface asperities size 19 µm ± 3 µm (Lb & LbSi). On these rougher substrates (Lb) insects were able to move freely/gain a higher angle of inclination, represented by the increased angle of inclination when compared with those achieved on the other experimental surface types (LbSi, Si, ISi, F). The super-hydrophobic surfaces used in this study show the same surface profile and properties analogous to that reported by Ming *et al.,* (2005). Unlike Ming's study where surfaces were shown to need dual scale roughness on both the micro (F) and nano scale (Si & ISi), wyko imaging of our surfaces show them to be relatively featureless (Figure 4.2).

In a study carried out by Huber *et al.*, (2007), surface roughness was found to influence adhesive force generated by the seta of the Tokay gecko (Gecko gecko). It was shown that surfaces with a RMS roughness of between 100-300 nanometres (1.0-3.0 µm) were sufficient to reduce adhesive potential through reduced contact between the setal tip and the surface asperities, due to mechanical stiffness of adhesive organs. In our study adhesion was found to be significantly reduced on surfaces with a RMS profile of approx < 20 nm, a probable reason for this is again reduced contact between the ladybirds setal tip and the surface profile. *H. axyridis* has a mean setal tip diameter of 50 nm ± 10 nm (measured using ImageJ on 50 differing setal tips imaged using SEM).

It is plausible that mechanical failure of the treated surfaces (silica, Fluon®) could lead to contamination of the adhesive organs in a manner similar to that already observed in previous chapters. However, SEM images of insect tarsi were taken in an attempt to rule out contamination as a factor in this study. The images qualitatively revealed little contamination from either the Fluon® (F) (micro textured low friction surface) or super-hydrophobic treated (nano textured low friction surface, Si & ISi) substrates Figure 4.12, so failure to adhere to the experimental surfaces must be due to other factors, such as surface energy and roughness profile.

Tilt angle study

The results suggest that there is no quantifiable difference between the angel of detachment for either *P. dives* or *H. axyridis.* There is a significant difference between the high and low energy surfaces (Cg, F, Si & ISi) in angle of detachment, while there is no significant difference in detachment angle for either species with respect to low energy substrate types.

A previous study (Gorb and Gorb, 2002) found that insects were capable of changing bodily orientations and centre of gravity on friction poor substrates to increase adhesive potential it is unlikely that orientation alone can compensate for the low friction coefficient of the treated silde surfaces.

On other substrates such as smooth clean glass the insects could be inverted totally, while on experimental surfaces they slipped off the slide at angles < 50°. It was observed that regardless of surface energy (hydrophobic or hydrophilic, Si & ISi), the insect would lose grip on the slide and fall backwards, this again suggests that the FSE of a surface is not crucial in determining adhesive potential, and that the effect of FSE is similar between pad types. It should be noted that although the adhesive secretion used by insects has been hypothesised to enable adhesion between a range of surface types irrespective of FSE, surface profiles (within known mechanical limits) etc. It has been shown that FSE of a substrate can significantly alter the adhesive potential of an insect by altering its ability to passively self-clean (Orchard, Kohonen, & Humphries, 2012).

Discussion regarding the effects of pad types:

Although the roughness scale is approximately the same as that shown to reduce adhesion in geckos (Huber *et al.*, 2007), it is somewhat counter intuitive to assume that this would result in a sufficient loss of adhesive potential in insects it is well documented that insects use an adhesive secretion to minimise the effects of surface profiles and as such increase both contact area and subsequently adhesive force. As such the loss of adhesive ability observed on these substrates may be a result of a combination of factors such as RMS roughness and porosity, if the secretion is indeed an oil in water or water in oil emulsion as suggested by Gorb (2008), it could plausibly be either wicked away through the surface pores or bead up in front of the adhesive organs and create a surface upon which the insects would effectively aquaplane as seen in pitcher plants (Bauer & Federle, 2009; Bauer, Willmes, & Federle, 2009; Bohn & Federle, 2004; Gorb, 2008).

However, if the adhesive secretion used by insects is indeed an emulsion then the extreme hydrophobicity of the epicuticular waxes could cause beading of the secretion resulting in subsequent loss of contact area and adhesive potential through localised dewetting. This coupled with the range of surface roughness could theoretically result in lost contact through mechanical stiffness of the arolium itself, or even through an increase in the liquid thickness resulting in it becoming a lubricating instead of adhesive layer, thus allowing slippage of the limb during locomotion. The loss of adhesive ability in *H. axyridis* may be in part due to the stiffness of the setae themselves; this influence of setal flexibility has been shown to be an important factor in studies on geckos (Autumn and Gravish, 2008). Due to the size of the measured setal tip area and the corresponding RMS of the surface profiles it is likely that the tips of setae are unable to make any real contact with the substrate that would be sufficient to maintain significant adhesive forces. However, no study has addressed the stiffness or flexibility of the cuticular sac that makes up the arolium used by Hymenoptera; it is possible that the hydraulic pressure that inflates the sac is unable to force it into sufficiently close contact with the substrate once the surface roughness decreases below a critical point.

It is important to remember that the RMS roughness does not describe all aspects of a surface, for example no quantification of the porosity is given. The data from this study supports previous work carried out by Barnes et al., (2011) and others (Betz, 2002; Chen & Gao, 2007; Gorb et al., 2010; Kwak & Kim, 2010; Nimittrakoolchai & Supothina, 2008; Persson, 2007) and shows that both RMS and dual size roughness play an important role in wet adhesive systems and that its influence on adhesion is constant regardless of substrate FSE or porosity.

It is important to note that clipping of tarsal claws has been shown to reduce adhesion in lizards (*Anolis carolinensis*), by 40% when two claws were clipped but by 60% when four were clipped. This reduction in adhesive effort is thought to be due to damage to the tendons that reduced toe pad function (Bloch & Irschick, 2010). This reduction was observed even though the adhesive performance was measured on smooth substrates and the claws have no direct effect on toe pad function. It is plausible that the reduction in adhesive effort seen in our results was due to damage to the soft tissues surrounding the toe pad, or due to increased pain in the injured digits that altered behaviour and posture. However, care was taken in this study to avoid clipping the claws to short and causing possible damage.

The work presented here coupled with that carried out in the previous experimental chapters will be used to build a firm basis for the theory affecting the final experimental chapter, linking locomotion and adhesive ability. This work is already being tentatively undertaken for some species of Gecko (Autumn, Dittmore, Santos, Spenko, & Cutkosky, 2006; Kwak & Kim, 2010; Nam et al., 2009), but similar studies in insects are lacking. Finally it must be noted that due to the nature of fractal mathematics any surface area measured depends in part on the size of the measuring probe and as such all readings are to some extent arbitrary (Packman, 2003).

Further work:

Further to the work carried out in this series of experiments future directions could include determining the adhesive potential of a single setal hair or group of hairs removed from an adhesive pad. This is inherently tricky as the adhesion system evolved in insects uses an adhesive secretion, to obtain significant and meaningful results a manner by which the delivery of the secretion into the contact area of a seta would have to be devised. In an attempt to do just such a thing recent work has involved detailing the pull off force of live insects on several surfaces, however, the manner by which these studies have been carried out would seem to bear non consistency with each other, with differing insects and substrates being used for most studies, as such the results obtained are only analogues to each other and not directly comparable. One of the major concerns with using removed seta is the drying out of the adhesive secretion or the loss of any possible passive/active control of the secretion composition derived from the insect's behaviour.

In conclusion we find that in this study FSE had little effect on adhesive ability, with insects failing to adhere to hydrophilic and hydrophobic substrates alike, that the reduction in adhesive strength and ability to walk on the surfaces was irrespective of contamination of the adhesive organs as proven by SEM imaging of the tarsi of insects post exposure to experimental surfaces. We conclude therefore that adhesion was predominantly affected by surface roughness, and that both species had similar results with respect to the surface profiles. However, due to the nature of the silica treatment it was not possible to control for surface porosity in this study, and as such a reduction in adhesive forces may also be due in part to the porosity of the surface. The novel silica treatment devised in this study has potential for commercial usage and consistently outperforms the Fluon® treated surfaces, is easy to apply, dries quickly and is mechanically stable, and could be developed further to protect from ingress of pest species into hospital or food stores. Furthermore, the silica treatment may provide pest management of agricultural species, as through characterisation of specific insects adhesive potential it would be possible to tailor the treatment to take advantage of differences in specific adhesive abilities.

Chapter five

Mechano-sensing properties of insect antenna and their influence on foraging behaviours

5.1 Introduction

The work carried out in this chapter deals with the questions raised in previous chapters. Namely, if an insect can become fouled by the environment (plant waxes, detritus, industrial pollutants), and is repelled by particulate barriers, then can mechano-sensory feedback from the antennae be used to determine where to forage by assessing a 'risk' associated with loss of adhesion and substrate properties? The mechano-sensing abilities of insect antennae have not been well explored in the literature. Although Wasmann (1899), determined antennal movements could be a described as a complex language, little since has been done to investigate the behaviours concerned with movements of the antenna and communication. Lenoir & Jaisson (1982) suggest that in ants the touching of conspecifics with both antenna and forelegs is a manner of communication whereby individuals recruit other nest mates in foraging and tandem running behaviours (Holldobler & Wilson, 1990). Tandem running is instigated by the lead ant which runs up to a nest mate as described, beating the other individual's body rapidly with its antennae before moving away, the nest mate then follows the instigator towards the resource (i.e. food source or new nest site). It has been shown that the lead ant will not move away unless it feels the recruited ant's antennae touching it (Holldobler, 1984). Although, the act of recruitment itself its generally well understood, it is relatively poorly defined in the literature and cannot be clearly distinguished from other behaviours such as alarm responses (Holldobler & Wilson, 1990). Due to our poor understanding of antenna movements and related behaviour it is possible that other information is passed on through antennal gestures that we are currently unable to interpret.

The complex behaviours such as brood care and construction of nests exhibited by social insects are usually carried out in subterranean conditions, and as such must depend on the tactile senses of the insect to a large degree. Indeed, it has been suggested that the tactile sense may compensate for poor vision (Bernadou & Fourcassie, 2008; Eltrinham, 1933; Kevan & Lanet, 1985). It is also well established that Hymenopterans use antennae to assess returning foragers and other individuals when determining nest mate recognition (Chapuisat et al., 2005; Helanterä & Sundström, 2007; Martin, Helanterä, & Drijfhout, 2008; Ozaki et al., 2008; Pfennig et al., 1983).

Insects can use their antennae to assess gap width (Blaesing & Cruse, 2004), enable wall following behaviour and location of obstacles (Okada & Toh, 2004; 2006), assess the thickness of wax comb walls in bee hives (Martin & Lindauer, 1966), to enable finding of nectar rewards by following roughness gradients (Kevan & Lanet, 1985), as well as for receiving chemical signals from conspecifics and following pheromone trails (Hansson et al., 1992; Nakanishi et al., 2010; Ozaki et al., 2005; Webb, Harrison, & Willis, 2004). However, there is a significant gap in the literature regarding what information an insect receives about its physical environment from its antennae and how this influences its behaviour. The majority of the work carried out into the sensing abilities of antennae has been towards developing more sensitive tactile abilities for robotic development (Lewinger et al., 2005).

While studying the tactile senses of cockroaches, (Okada & Toh, 2004; 2006) it has been demonstrated that even blinded, cockroaches have the ability to find and move towards objects based on tactile sensing alone. Kevan & Lanet, (1985) demonstrated that Honeybees are able to determine the microtexture of petal surfaces in *Helianthus* *annuus* which has a difference in surface texture at either end of the petal, moving along a roughness gradient until they find the nectar reward. Kevan and Lane (1985) also suggest that the ability to determine between surface profiles of plants may lead to specialised relationships between plants and pollinators.

An insect's body is covered with a hard cuticular shell effectively isolating it from the outside world (Wigglesworth, 1964), so any feedback from spatial environmental stimuli comes from visual, chemical or tactile feedback (Kaneko, Kanayma, & Tsuji, 1998; Pearson & Franklin, 1984; Pelletier & McLeod, 1994). To enable tactile feedback, several sections of the outer cuticle are modified to become sense organs (Okada & Toh, 2006; Wigglesworth, 1964) Figure 5.0. This modification comes from the adaptation of a single epidermal cell. This cell splits to form a cluster of cells, from which, a single thread like structure called an axon grows. This axon then connects to the nerve fibre allowing any stimulus from the cell group to be relayed to the central nervous system. Although the concentration of sense cells is higher on antennae they are also found on other regions of the insect body (Eltrinham, 1933).

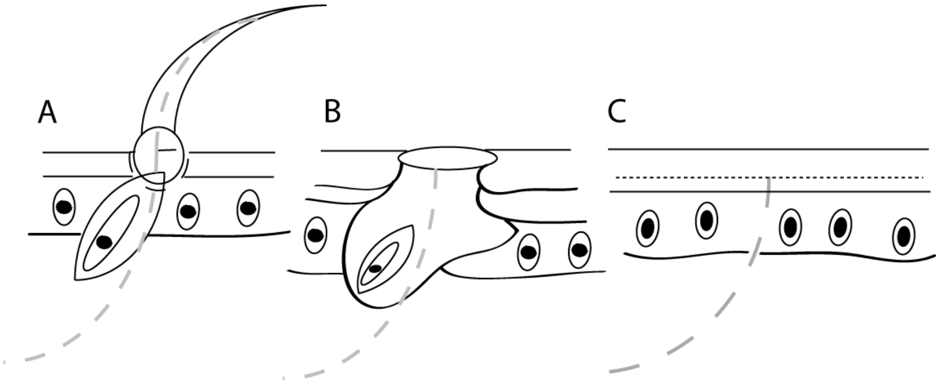


Figure 5.0: Three types of mechanical sensing organs found on insect antenna. A tactile hair, B, Campaniform organ, C, Chorodontonal organ. Schematic shows first layers of epidermal cells with primitive nerve fibre (axon) shown as dotted grey line.

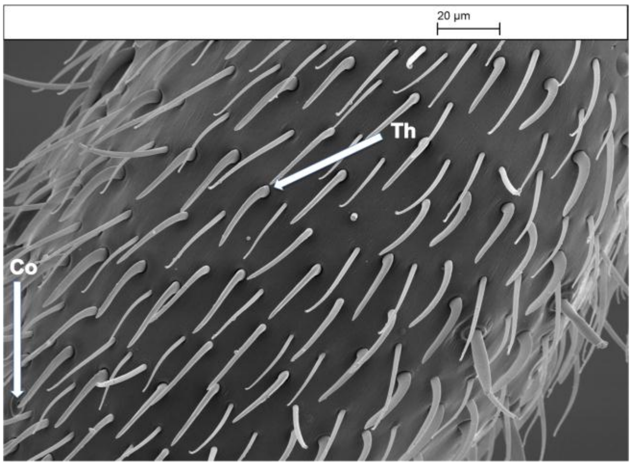


Figure 5.1: SEM image of P. dives antenna, showing Campaniform organ (Co) and numerous tactile hairs (Th).

Tactile sensing hairs have been identified in several texts including Snodgrass (1926), where the main mechano-sensing organs are identified as Trichoid (Figure 5.0 A) (long thin hairs with a terminal nerve fibre), Basiconic (short peg like hairs, thick walled and connected to a single nerve fibre) and campaniform sensillae (Figure 5.0 B). Basiconic sensillae are generally regarded as trichoid sensillae with reduced hair length. They are both typically found all over the body and may stand proud of the surface as on the antenna or are recessed in cavities of the cuticle. Campaniform sensillae have varied shapes but are invariably of simple structure (Figure 5.1), typically a thin walled dome like structure connected to an internal nerve fibre. Again these can be found over most of the insect body. The Chorodontal organ (Figure 5.0 C) is of unknown function although it is speculatively linked to tactile sensing (Snodgrass, 1926).

Insect antennae are covered in sensory hairs, which have chemo-, mechano- and thermo- receptive capabilities (Holldobler & Wilson, 1990; Krause & Durr, 2004; Okada & Toh, 2004; 2006), enabling an insect to build up an image of the localised environment. Previous studies have focused on the function of mechano-sensory feedback from antenna and forelimbs (both ipsilateral and contralateral), in decision making when avoiding obstacles or gaps in the insects path (Blaesing & Cruse, 2004;

Kaneko, Kanayma, & Tsuji, 1998; Lewinger et al., 2005; Okada & Toh, 2004; 2006; Pearson & Franklin, 1984; Pelletier & McLeod, 1994). Kaneko et al. (1998), suggests visual recognition in insects in general is poor, with an inability to judge the shape of or distance to an object correctly. In contrast, the desert locust (*Schistocerca gregaria*) utilises both visual and mechano-sensory feedback mechanisms to determine leg placement and locomotion in novel environments (Niven et al., 2010).

As previously stated (section 1.4.3), many differing plant surfaces have evolved, which inhibit insect adhesion and slow locomotion (i.e. waxes and trichomes) (Eigenbrode & Jetter 2002; Eigenbrode et al. 1996; Eigenbrode 2004). These surfaces can be either tacky (e.g. sundew plants), non-stick (e.g. the peristome rim of pitcher plants), fragile (e.g. outer wax layers of glaucous plants) or any combination of the above. Due to the changing physical properties of both biotic and abiotic surfaces with which an insect would come into contact while foraging, it is probable that the antenna facilitate locomotive decisions. This ability to discriminate between surfaces would support the hypothesis put forward by Geiselhardt et al. (2010), that the chemical nature of a surface can reduce adhesion through mediation of the tarsal secretions. It is therefore likely that insects probe the surface properties of a given substrate for adhesive potential before crossing. This raises questions regarding the differences in antenna morphology between flying and walking insects, with walking insects typically having longer antennae with an increased number of trichoid hairs and more antennal segments than flying insects (Figure 5.2). This difference in morphology may be due to antenna in some flying insects acting primarily as a gyroscopic balance for orientation during flight (Sane et al., 2007).

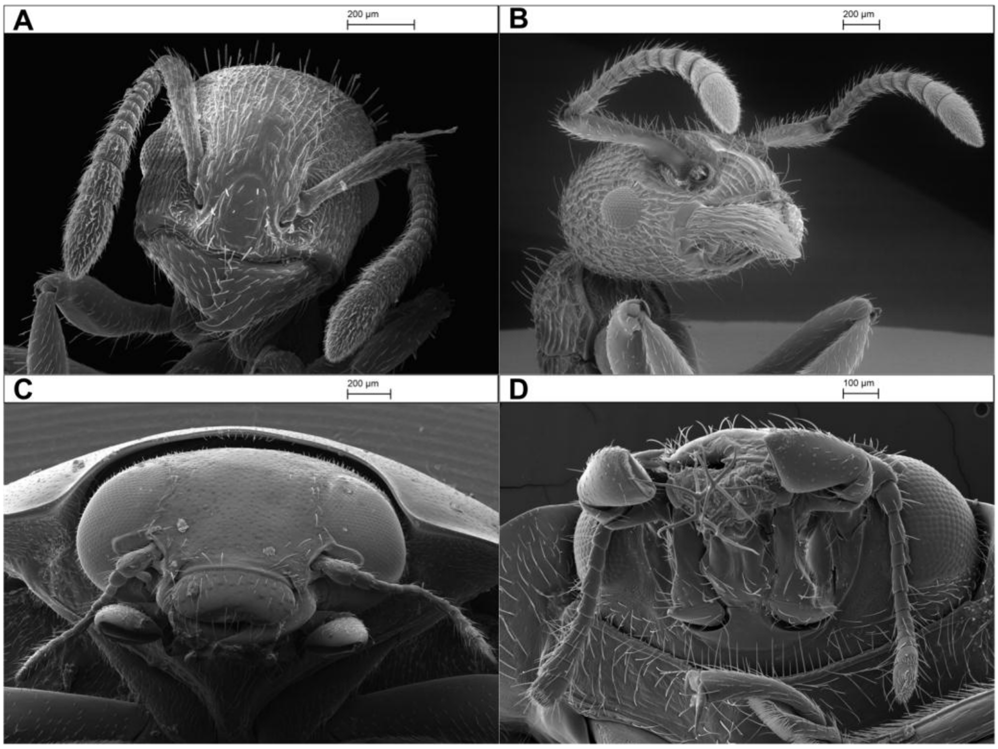


Figure 5.2: SEM images of insect antenna, A, *Leptothorax acevorum,* B, *Myrmica scabrinodis (Nylander 1846),* C, *Harmonia axyridis (Pallas 1773),* D, Cocinella septempunctata. Images of Hymenopteran (A and B) and Coccinellids (C and D) insect antenna are taken at same magnification. As can be observed the two Hymenopterans species have considerably longer antenna with more antennal hairs than those of the Cochinellid species.

Detailed models determining the tactile working range and sensory capabilities of insect antennae are lacking, and the tactile abilities of insect antenna are poorly understood at best, as previously mentioned (Krause & Durr, 2004). Although the structure of an insect’s antenna may differ with locusts, crickets and stick insects having a hinge joint to join the antenna to the head, while ants, bees and cockroaches have a ball and socket joint giving higher dexterity (Krause & Durr, 2004; Sane & McHenry, 2009), they function in similar ways. Antenna function studies tend to incorporate models based on fluid dynamics, e.g. Krause and Dürr (2004), as the underlying physics of antenna movement is largely in the domain of fluid mechanics (Barth, 2004). An understanding of the mechanics and kinematics of the antenna could help elucidate both the evolutionary trends and functional properties underlying the development of antenna in general (Krause & Durr, 2004; Sane & McHenry, 2009).

Due to the large number of mechano sensitive hairs on the antenna we hypothesise that insects will be able to determine between particulate and tacky substrates, but will prove ineffective at discerning the adhesive qualities of substrates with manipulated surface energies. We also hypothesise that the flying insects used in this study will show poorer abilities at determining between substrate and barrier properties than walking insects. Insects in this study were exposed to a series of barriers to replicate different 'natural' surfaces, particulate barriers (mimicking broken epicuticular wax coverings), Petroleum jelly (mimicking resinous secretions found on plant limbs) and barriers of plant material made from the removed tips of the sundew plant (*Drosera sp.)* (Mimicking a natural tacky barrier). This is the first attempt to systematically probe the biomechanical feedback properties of insect antennae with regards to environmental stimuli and decision-making.

5.2 Materials and method

Insects were maintained as lab stocks and fed a mix of chopped mealworms and honey for the ants (*Polyrhachis dives*), while ladybirds (*Harmonia axyridis (Pallas 1773)*) were fed a prepared artificial food source (Majerus & Kearns, 1989) *ad libitum* three times a week. Experiments were split between two groups. Group one were exposed to clean glass slides of differing surface energies to determine if insects can discriminate between substrate energy levels before attempting to climb, while group two were exposed to barriers of differing types (glass beads, plant material, Petroleum jelly) with a standardised gap (1.5 cm) randomly assigned along the slide, to determine if they could ascertain the likelihood of contact fouling or entrapment before crossing a surface. The substrates outlined in table 12 will be used to investigate the importance of the following hypothesis on insect adhesion, in section 5.2.1.

Table 12: Physical properties of manipulated substrates used to determine adhesive potential on substrate types:

Substrate	**Surface roughness**	**Surface energy**
DCDMS	N/A	Low
Silica (Ts)	Nano scale	High
Irradiated silica (Is)	Nano scale	Low
Clean glass (Cg)	N/A	High

Hypothesis to be tested

1) That surface roughness provides tactile feedback informing foraging decisions.

2) That Foraging insects are unable to determine the FSE of substrates whilst foraging.

5.2.1 Substrate choice test

All slides were sonicated in hexane (Fisher scientific UK, 99% pure) for two minutes then blown dry with nitrogen. A sub-section of the slides were then treated differently to give a range of free surface energy (FSE) values and surface profiles, physical properties of each slide type outlined in Table 12, this was performed to determine if insects can choose between substrate energy types which may limit adhesive abilities while foraging.

Glass slides destined to have a low FSE were either treated with silica spray or placed inside a clean glass jar where they were exposed to DCDMS (dichlorodimethylsilane) in a saturation phase. After two days the chemical changes to the surface of the slides were complete, and then the slides were rinsed in hexane to remove any residual chemicals and remove the build-up of PDMS (polydimethylsiloxane), which releases hydrochloric acid as a by-product and is highly toxic to insects. A further series of slides were treated with a spray coating of silica particles (nano sized particles which form 1 µm aggregations) (5% solution, 0.5 g in 10 ml of ethanol), which once dried gave a super-hydrophobic surface. Finally, a second series of the spray-coated silica slides were exposed to a UVB bulb emitting ozone gioving a nano textured low friction surface. This stripped away the surface hydrocarbons of the super-hydrophobic surfaces to give a super-hydrophilic surface. Once slide treatments were complete, contact angles of 5 µl of water were recorded on all surfaces to ensure a range of surface energies had been achieved. Silica sprayed slide surface had mean contact angles > 150°, irradiated silica surfaces had mean contact angle < 6°, DCDMS treated slides had mean contact angle of 98°, with a mean contact angle on clean glass of 3° (Figure 5.3 A, B, C and D respectively).

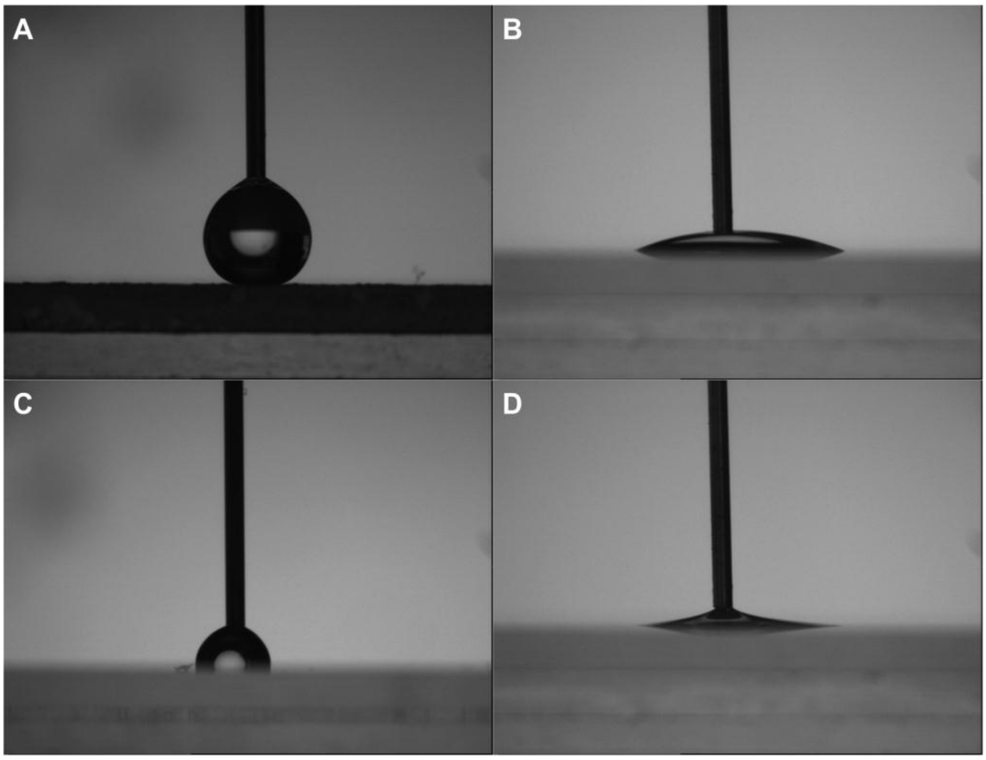


Figure 5.3: Contact angle images of 5 µl of water on (A) silica treated surface (Ts), (B) irradiated silica surface (Is), (C) DCDMS treated and (D) clean glass slide (Cg) surfaces used in this study.

Insects were placed inside an experimental arena (two glass chambers 14 x 14 cm joined along one edge with silica sealant) while an attractant (1 ml of sugar water solution, Antstore.net, sugar-honey-fluid 100 ml) was placed into the other side of the chamber. To reach the attractant, insects would have to traverse a choice of artificial substrates (Figure 5.4), presented at both horizontal and inclined (5º) positions with numbers of failed and successful attempts to traverse the substrates being compared with a linear model with binomial error distribution (R version 2.10.1: R development core team, 2009).

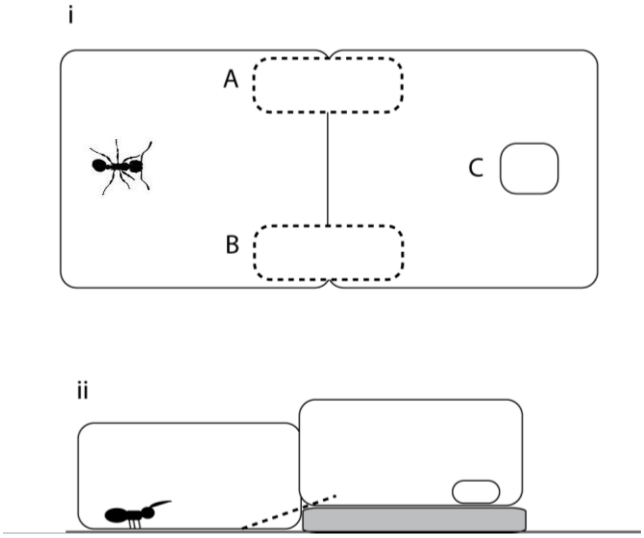


Figure 5.4: Schematic of substrate choice arena (I), top down view of arena A and B are horizontal microscope slides that can be changed to alter substrate energies allowing a choice of substrates for the insect to traverse, C is the attractant. (ii), side view of elevated arena, with slide surfaces presented at 5° inclination. Ant and angles shown not to scale.

5.2.3 Barrier navigation test

Trials were carried out by placing three slides of the same slide/barrier combination inside a Perspex container (Figure 5.5) with an ant placed one slide of the barrier, and an attractant (food reward) placed at the opposite end. To determine an insect's ability to discriminate between barrier properties four series of clean glass slides were constructed. A control series (Ct) was left untreated with no barrier in place while the other series were prepared as follows: One series with a covering of 19 µm glass powder (Gp), another with a thin covering of petroleum jelly (Pj) and a third series with sections of the tacky resinous tips of the sundew plant (Pt) glued in place to form a barrier. A small clear gap (1.5 cm) was left on all slides. The slide types used for the substrate were rinsed in Hexane and blown dry with Nitrogen before use. A webcam controlled by HandyAvi (version 4.2; Anderson's AZcendant Software) was used to record insect movement and behaviour as it probed the barrier and time taken to find the gap and cross the slides was extracted from these videos. All trials were run for a maximum of 10 minutes, if the insect had not crossed the barrier or navigated the gap within this time frame, the experiment was halted. To assess levels of contamination/repellence between barrier types, time taken to antenate each barrier before finally crossing the barrier to the food attractant were compared using. Differences between these time sets were analysed using ANOVA (R version 2.10.1: R development core team, 2009).

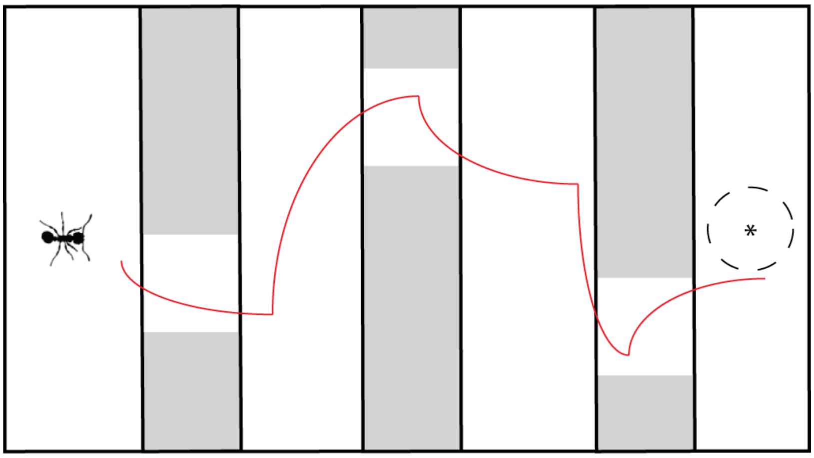


Figure 5.5: Schematic of experimental arena for substrate navigation study, ant placed in the left hand side with a series of three barriers between it and the food reward at the other end. Typical searching path observed in this study shown in red, food attractant marked with *. Ant not shown to scale.

5.3 Results

5.3.1 Substrate choice test

In Figure 5.6 and Figure 5.7 we plot the percentage of successful attempts to cross the differing substrates for both *P. dives* and *H. axyridis* for both flat and inclined surfaces. Number of insects successfully crossing the substrates differs significantly between inclined surfaces (*P*. dives; $F_{5, 117} = 9.709$, $p = < 0.001$, *H. axyridis*; $F_{5, 126} = 39.43$, $p = < 0.001$), but not for surfaces presented horizontally (*P*. dives; $F_{5, 131} = 26.18$, $p = > 0.05$, *H. axyridis*; $F_{5, 120} = 23.65$, $p = > 0.05$) and not between species when presented with inclined ($F_{5, 243} = 19.27$, $p > 0.05$) or horizontal surfaces ($F_{5, 251} = 22.83$, $p > 0.05$).

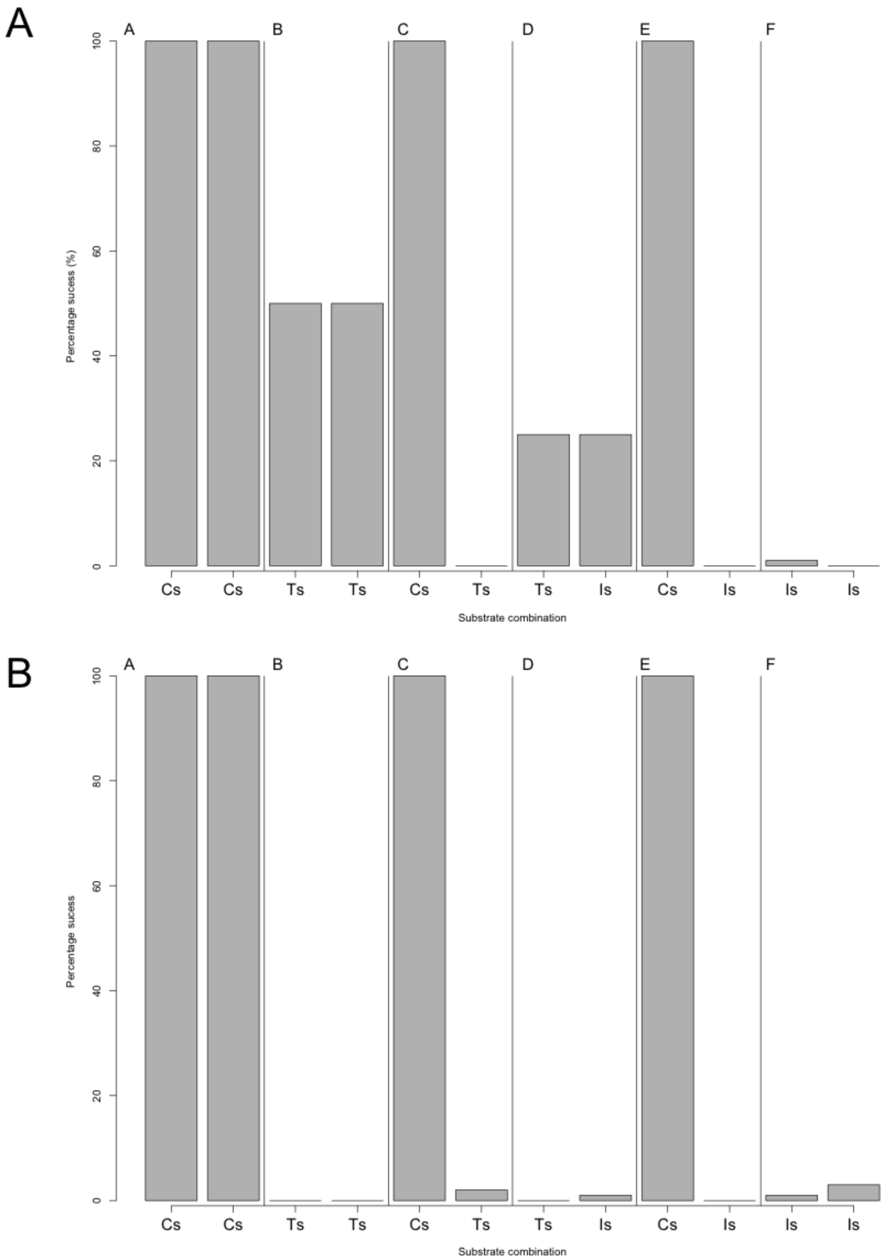


Figure 5.6: Percentage of successful attempts by *H. axyridis* to cross (A) flat substrates and (B) inclined substrates (surfaces presented at a 5° inclination) with differing combinations of FSE (A-F, experiment results separated by horizontal black line), Cs = clean slide, Ts = treated slide, Is = irradiated slide.

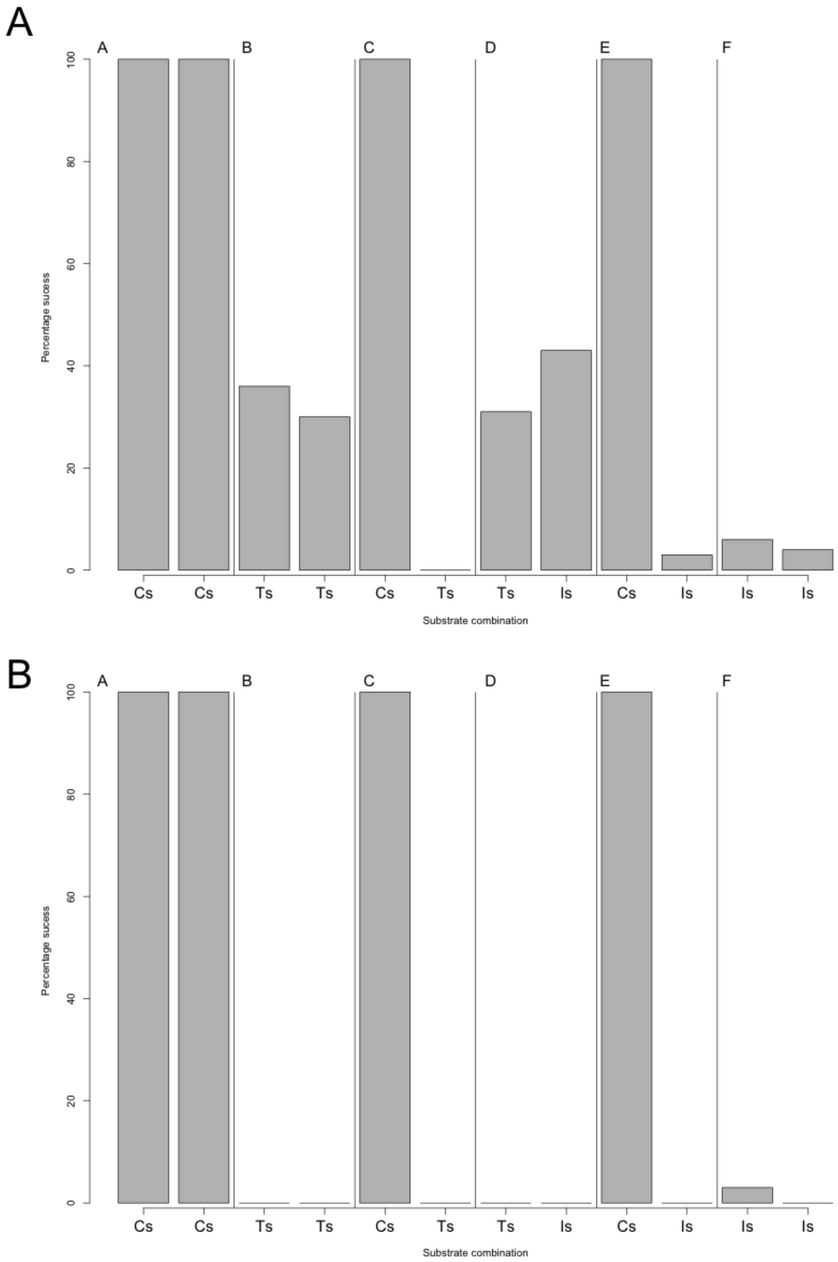


Figure 5.7: Percentage of successful attempts by *P. dives* to cross (A) flat substrates and (B) inclined substrates (surfaces presented at a 5° inclination) with differing combinations of FSE (A-F, experiment results separated by horizontal black line), Cs = clean slide, Ts = treated slide, Is = irradiated slide.

5.3.2 Barrier navigation test

In Figure 5.8 and Figure 5.9 we plot time to approach and investigate and traverse the barriers for both *P. dives* and *H. axyridis* respectively. Time taken investigating barriers differed for all experimental barrier types, with longer taken to investigate the initial barrier than either of the subsequent barriers for *P. dives* (Ct, $F_{2, 81} = 11.54$, $p < 0.05$; Gp,

$F_{2, 81}$ = 79.55, p < 0.001; Pt, $F_{2, 81}$ = 140. 86, p < 0.001; Pj, $F_{2, 81}$ = 106. 93, p < 0.001) and *H. axyridis* (Ct, $F_{2, 81}$ = 4.36, p <0.05; Gp, $F_{2, 81}$ = 6.25, p < 0.001; Pt, $F_{2, 81}$ = 7.65, p < 0.001; Pj, $F_{2, 81}$ = 61.92, p < 0.001). Time taken to investigate each subsequent barrier differed significantly from time taken to investigate the initial barrier for all barriers; the p values for each barrier combination are presented in tables 13 and 14.

Table 13: P values for time taken to probe the initial and subsequent barriers in navigation test for *P. dives.*

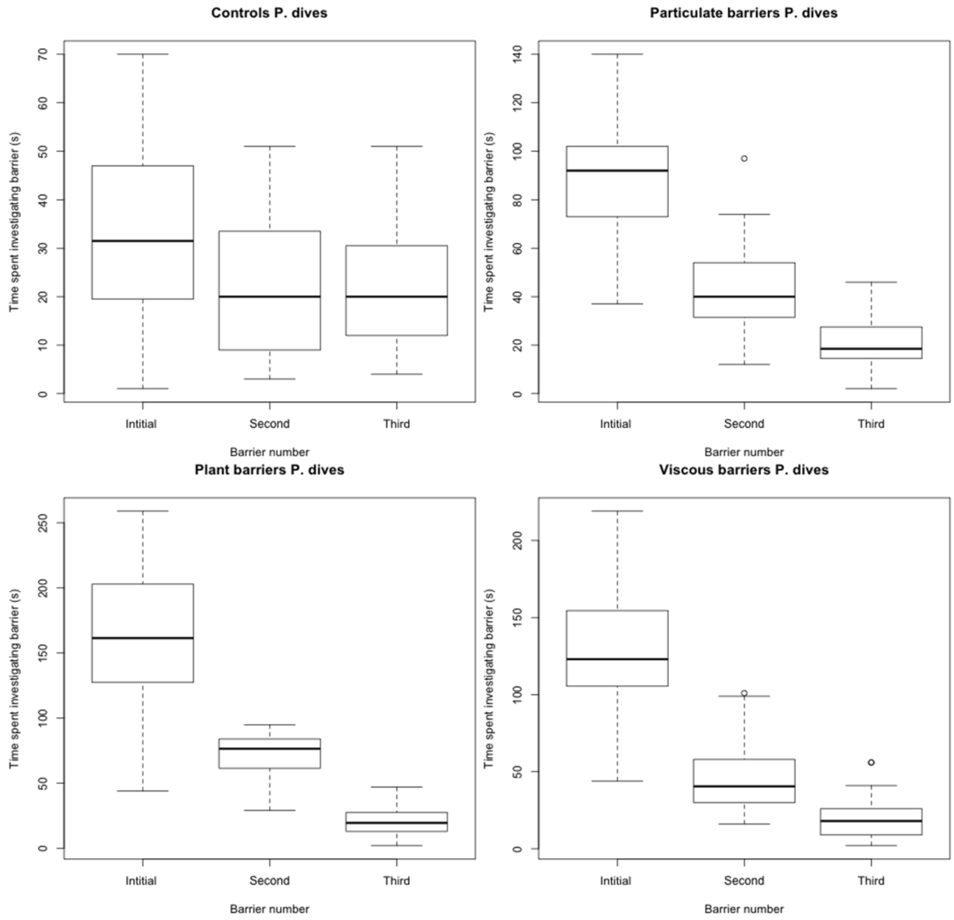
Barrier type	P value barrier combination
Controls (Ct)	Initial – second p < 0.05
	Initial – third p < 0.05
	Second – third p = 0.998
Glass powders (Gp)e	Initial – second p = 0.05
	Initial – third p < 0.001
	Second – third p < 0.001
Plant (Pt)	Initial – second p < 0.01
	Initial – third p < 0.001
	Second – third p = 0.001
Viscous (Pj)	Initial – second p < 0.001
	Initial – third p < 0.001
	Second – third p < 0.001

Table 14: P values for time taken to probe the initial and subsequent barriers in navigation test for *H. axyridis.*

Barrier type	P value barrier combination
Controls (Ct)	Initial – second p < 0.05
	Initial – third p < 0.05
	Second – third p = 0.998
Glass powders (Gp)	Initial – second p = 0.05
	Initial – third p < 0.001
	Second – third p < 0.05
Plant (Pt)	Initial – second p = 0.4
	Initial – third p < 0.001
	Second – third p = 0.07
Viscous (Pj)	Initial – second p < 0.001
	Initial – third p < 0.001
	Second – third p < 0.001

The physical properties of a barrier was found to have a significant effect on all interaction times when compared between species ($F_{4, 640}$= 11.28, p = 0.002). With *P.*

dives taking significantly longer to traverse viscous barriers compared to particulate ones ($F_{2,\ 160} = 7.56$, $p = < 0.05$), no significant difference was found for *H. axyridis* when comparing times to interact with either of the barrier types (viscous or particulate) ($F_{2,\ 160}$, $= 3.54$, $p = > 0.06$).



Controls P. dives

Particulate barriers P. dives

Plant barriers P. dives

Viscous barriers P. dives

Figure 5.8: Plots of time taken by *P. dives* to investigate each barrier in the series. Data presented for all barrier combinations.

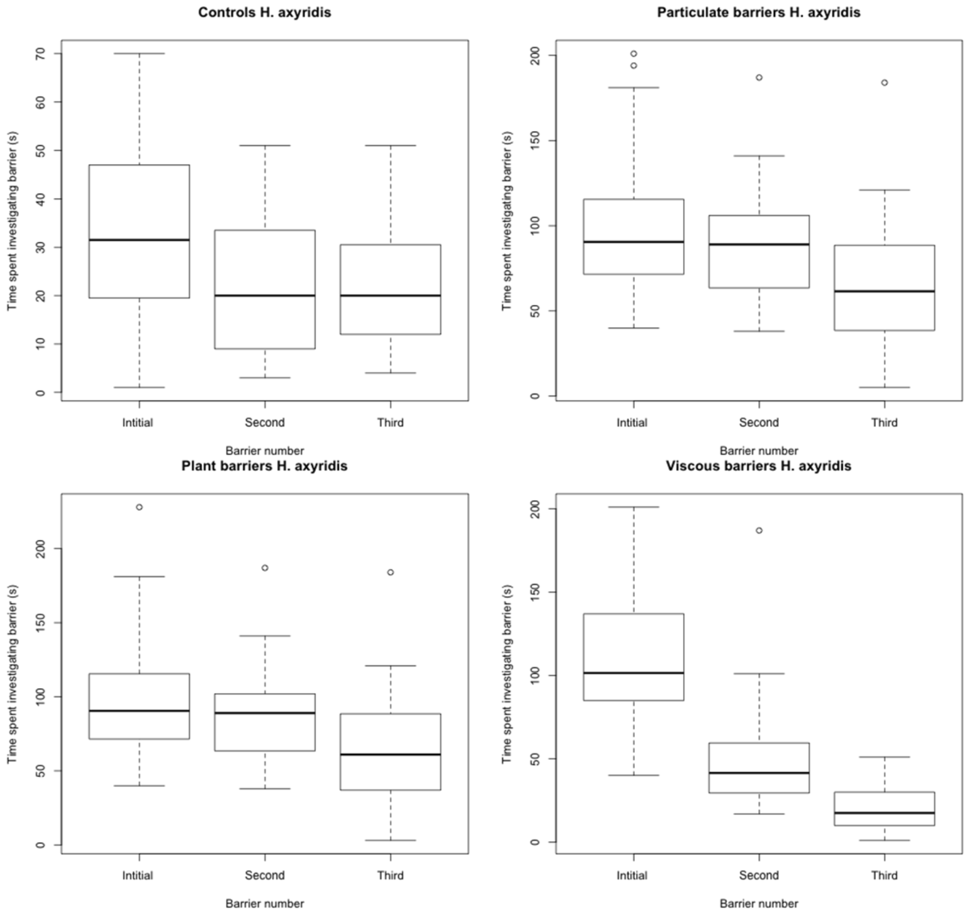


Figure 5.9: Plots of time taken by *H. axyridis* to investigate each barrier in the series. Data presented for all barrier combinations.

5.4 Discussion

The results from this study support our initial hypotheses, firstly that there is a difference between flying and walking insects in ability to determine between particulate and tacky barriers, and that both flying and walking insects would be unable to discriminate between the surface energies of a smooth substrate. Secondly that flying insects would be poorer at determining the potential risk associated with differing barrier types. Within the substrate choice study neither *P. dives* nor *H. axyridis* were observed to discern between substrates when presented in either combination, with numbers of attempts to cross being equal among all replicates. However, the number of successful crossings of each substrate differed between combinations presented horizontally and inclined (Figure 5.6 and Figure 5.7). This inability to discern energy levels supports previous findings (Gorb *et al.*, 2007) where it is suggested that insects can adhere to both hydrophilic and hydrophobic surfaces while foraging. However, the inability to adhere to the differing substrates when presented at an inclined angle would seem to contradict this generalisation.

5.4.1 Barrier navigation

Within the barrier navigation study, barrier properties were found to significantly alter time spent investigating barriers before crossing for *P. dives* but not for *H. axyridis*. Although time spent investigating each subsequent barrier significantly decreased for all but control surfaces for *P. dives* and *H. axyridi*s (Table 13, Table and Figure 5.8 and Figure 5.9). *P. dives* and *H. axyridis* showed comparable times investigating each barrier initially, a significant difference in time to probe the barriers a second time and finally cross the barrier. Further analysis suggests barriers made from plant material affect time to find the gap within the barrier more than those made of Petroleum jelly with a significant difference for *P. dives* (F = 6.07, d.f. = 1, $p < 0.01$) but not for *H. axyridis* (F = 0.032, d.f. = 1, p = 0.84). This suggests that the extra time taken to traverse the barrier is a function of barrier properties and the loss of sensing ability due to contamination of the antenna's tactile hairs. Indeed this is supported by observations of an increase of preening time between the barrier types. This difference in both time spent preening and time associated with probing barriers suggests that although the antenna of *H. axyridis* became fouled in a similar manner as those of *P. dives* they are less sensitive to subtle differences in barrier properties. Comparisons between *P. dives* and *H. axyridis* suggest that *P. dives* are significantly more affected by viscous barriers than *H. axyridis*. As such the following working hypothesis is presented to explain the differences observed between *P. dives* and *H. axyridis.* We propose that the deadening of mechano-sensing ability of insect antennae is related to the loss of movement of the tactile hairs when exposed to particulate and viscous barriers (Figure 5.10) supported up by previous observations from the powder barrier work with 1 µm glass particles (Figure 5.11).

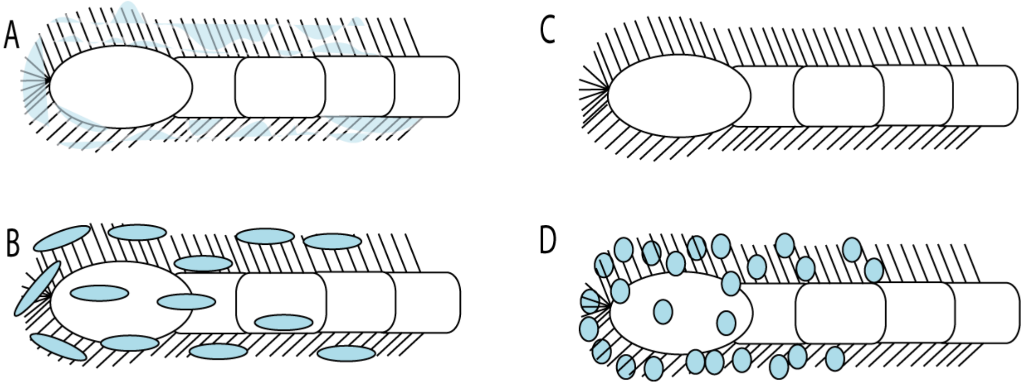


Figure 5.10: Loss of antenna mechano-sensing function from; A, antenna covered with a tacky residue, seeped not only between the hairs but also the base of the hairs themselves reducing movement, B, antenna after touching a tacky barrier, the hairs are clumped together and have a reduced sensing ability, C, represents a clean un-fouled antenna, D, displays the effect of particulate fouling on insect antenna, filled circles on antenna represent fouling wax particles

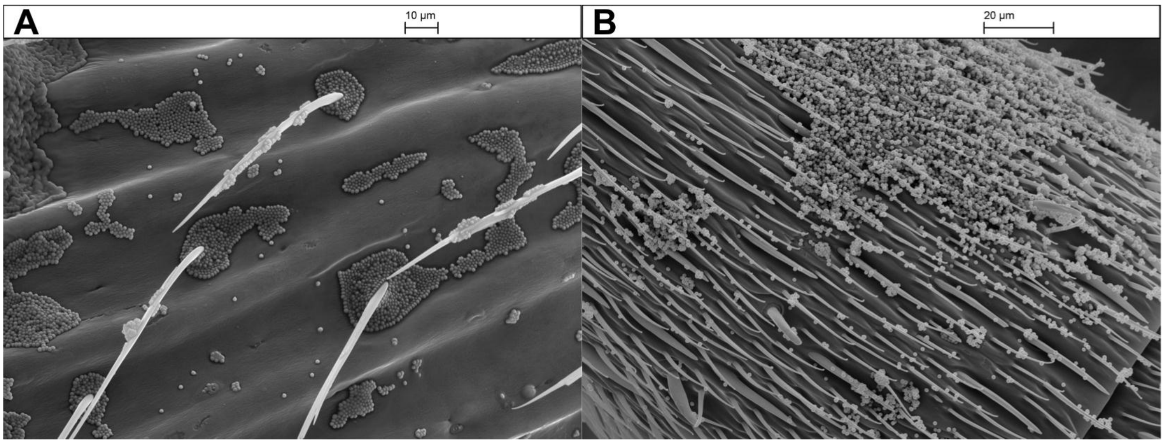


Figure 5.11: SEM image of tactile sensing hair of *P. dives* antenna jammed by 1 um glass particles, effectively turning off the sensory capability of the antenna section.

Although the adhesive organs of Lepidopterans such as the European corn borer (*Ostrinia nubilalis*) (Catatayud et al., 2006; Marion-Poll, Guillaumin, & Masson, 1992), the flour moth (*Ephestia kuhniella*) (Anderson & Hallberg, 1990), the European sunflower moth (*Homoeosoma nubulella*) (Faucheux, 1991), and the Spruce bud worm (*Choristoneura fumiferana*) (Mitchell & Seabrook, 1974), are known to have mechano- and chemo- sensitive capabilities, and as such may be able to discern the physio-chemical nature of a substrate; with those of the Diptera *Chrysomya nigripes* also having been suggested to have the same capabilities (Ngern-klun et al., 2007). No such abilities have been described for the smooth adhesive organs (arolium) employed by *P. dives*. The results would also seem to concur with previous work on the sensing ability of the arolium reported by Eltringham (1933), where he suggests the arolium of hymenoptera can be considered to be essentially deaf due to the apparent lack of sensing receptors. The inability to sense the substrate through the arolium may have led to evolution of the extensive antennating behaviour exhibited by social Hymenopterans (in this case *P. dives*).

The ability to discern between particulate or tacky substrates would be a useful trait for walking insects, which are limited in their ability to move through three-dimensional space. Flying insects seem less able to determine between particulate and tacky substrates, and it is likely that this is a function of their antennae morphology, which in some species for example the Hawk moth (*Maduca sexta*) has evolved to mediate flight stability and gyroscopic control (Sane et al., 2007). As such, the antennae of *H. axyridis* do not reach to the substrate and they are seldom seen to attempt to antenate, unlike *P. dives,* which probes the substrate and immediate surroundings before placing each foot, as recorded in other species (Durr, Konig, & Kittmann, 2001; Durr, Krause, & Schmitz, 2003; Horseman, Gebhardt, & Honegger, 1997). This result is surprising, as it could have been expected for the flying insect to possess a more developed tactile sense, due to the requirement to walk on some plant surfaces while foraging. However, *H. axyridis* was consistently worse at determining the associated risk of each barrier or substrate and it would seem that the antennae function as a gyroscope aid and pheromone detector first, and a tactile sensor second. This suggestion would seem to contradict the results reported for Honeybees (*Apis sp.*) by Martin and Lindauer (1966). However, it is likely that bees possess a more discerning tactile sense to enable them to determine between petal surfaces during pollination events, while *H. axyridis* has reduced tactile senses and forages from a combination of tactile and visual cues (Obata, 1986). It is also likely that the results discussed by Whitney et al. (2009), wherein bees displayed a clear preference for a rough petal phenotype, are influenced by tactile sensing of the bees; with bees able to discern from tactile senses the petal surfaces they are best able to manipulate to increase foraging efficiency.

If the results for *H. axyridis* are generally applicable then an inability in flying insects to assess substrates for adhesive potential as suggested by their increased length of time to navigate the barrier set up when compared to *P. dives,* is likely to be offset by the fact they can simply fly over or away from any substrates that may cause problems for adhesion. It is also likely that walking insects will show a greater ability to discriminate between risky substrates. However, this still leaves the question of how surfaces such as the peristome rim of pitcher plants are able to trap such a wide range of insects and most of them unable to fly. This study suggests that the ability of species such as pitcher plants to trap insects is in part due to the structure of its surface. Due to the nature of antennae sensing it is probable that the insects cannot tell that the change in roughness scales between the peristome rim and the outer pitcher surface, and because the surface is non-particulate or tacky they sense no associated risk with walking onto the rim. The rim surface has a low surface energy that would impede adhesion and coupled, with the nanoscale capillary tubes present ‘wicking’ the adhesive secretion the contact zone of the adhesive pad reducing adhesion on an already slippery surface.

Chapter six

The influence of surface properties on the foraging and movement patterns of Hymenopterans (Formicidae; *Polyrhachis dives*)

6.1 Introduction

How an organism moves through an environment directly influences the likelihood of encountering mates, prey and predators (Wiens, et al., 1995), while at the same time influencing spatial dynamics of populations and nutrient turnover in an environment (Bunce and Howard, 1990; Weins, *et al.*, 1995). The movement characteristics of organisms have been studied extensively (Wiens et al., 1995), and have been categorised as searching (Bell, 1991) and dispersal behaviours (Bunce & Howard, 1990; Stenseth & Lidicker, 1992), with the behaviours split between resource usage and simple models of invasion routes (Fagan et al., 2002). Hanski, (1980) pondered what exactly the driving forces behind the structure of spatial moment within a species are and how they drive the structuring of communities. However, almost all models of animal movement focus on either short or long distance movement with little overlap between the two, which is potentially problematic as there is no certainty that the two are separate (Hanski, 1980; Taylor, 1978). The study of movement on both large and small spatial scales can help inform models of resource use by an individual over its lifetime and help highlight areas used by migrating species (Arellano et al., 2008).

The study of animal movement uses both theoretical and empirical approaches to understand resource and landscape usage. Studies have included the use of fractal analysis to determine landscape use from field observations, as well as random walk and diffusion models to predict spatial distributions (Berg, 1993; Byers, 2001; Jeanson et al., 2003; Okubo, 1980), in terms of insect studies have been performed on ants and butterflies (Jeanson et al., 2003), Elodes beetles (*E. obsoleta, E. extricata, E. hispilabris*) and Grasshoppers (*Opeia. obscura*, *Psoloessa delicatula* and *Xanthippus* *corallipes*). Random diffusion models have been used to predict range expansion (Fournier, Deneubourg, & Fourcassi, 2003; Patterson et al., 2008) as well as to gain deeper insights into how movement of individuals can influence spatial population dynamics, resource usage and to help control pest species (Marsula & Wissel, 1994), both Brownian motion and Levy walk models have been incorporated to predict temporal and spatial movements in animals (Gautestad, 2012).

Small scale differences in the topography of local landscapes are known to be important for walking insects (Hanski, 1980), which use structural correlates such as roughness (Bernadou & Fourcassie, 2008; Camhi & Johnson, 1999; Erber, Kierzek, & Sander, 1998; Kevan & Lanet, 1985; Okada & Toh, 2004; 2006), to physically orientate themselves within an environment. Wall following behaviour in ants and cockroaches has also been well documented (Camhi & Johnson, 1999; Dussutour, Deneubourg, & Fourcassie, 2005), wherin the indivdual insect moves along a wall edge maintaing contact with its antenna. It is suggested that instead of being attracted by long-range stimulus it is instead the physical contact with an obstacle that guides insect movement (Creed Jr & Miller, 1990; Hanski, 1980).

In a study on the small scale movement patterns of insects, Weins et al., (1995) followed the movements of two differing insect species across a micro landscape (25 m^2). Fractal dimensions of movement pathways were calculated in accordance with Mandelbrot, (1983), using the following equation:

$$L(\delta) = K\delta^{1-D} \qquad [9]$$

Where D is the fractal dimension, calculated by regressing L (where L is the measured length of the pathway) on the natural log of δ, with δ representing a known measurement scale (e.g. km, m, cm). Fractal dimensions of pathways range between 1 and 2 with values between closer to 1 straighter (more linear) and ones closer to 2 covering more of the substrate (being more tortuous). The intercept of the slope of the regressed measurement scale K is rarely used in analysis although it may hold hidden information (Weins *et al.,* 1995). Weins (*et al.,* 1995); found that the movement pathways of the insects differed, with ants taking a more tortuous pathway compared to the more linear movement observed in grasshoppers. This difference in pathways possibly reflects the way in which each insect species perceives its environment, with ants searching for patchy localised distribution of seeds and grasshoppers moving between abundant patches of resources. Although D is scale dependent, in so much that if you measure it at differing length scales the value of D will change, if the measurement scale is kept the same as in Weins (*et al.*, 1995), then difference in D can be related to how the insect experiences its environment.

Furthermore, Weins (*et al.*, 1995), went on to propose a model to explain the movement of an individual: If an organism is moving in a linear fashion through an area with an evenly distributed resource (i.e. grass), then its encounter rate for the resource can be described $q = ct^{D_r}$ where the fractal dimension $D_r = 1$ and c = resource density. If the reward for searching is constant with time (i.e. not limited by effort), the only way to increase q is to move to another patch with a higher abundance of resources, and the quickest way to do this is to move in a straight line until a new patch of resources is found. If however, the resource is not evenly distributed it predicts the movement pathway of the individual to be more tortuous, as the benefits of searching sub regions of an environment increase.

However, in this chapter fractal dimensions of movement were calculated using the more recent model proposed by (Abaigar et al., 2012):

$$FD = \frac{logN}{\log N + log\, d_{max/L}} \qquad [10]$$

Where FD = fractal dimension, N = total number of steps in the track, L = total displacement (track length).

Here I use ant movements to show that parameters such as velocity and distance travelled are not constant throughout an environment but differed with landscape types. I compare velocity and distance between track points on substrata with known surface roughness regimes, and use a custom mechanical stage to measure the [normal] adhesive force of tethered insects on each of the experimental substrate types. I hypothesise that movement parameters such as velocity and distance are not constant throughout an environment but in fact differ between homogenous and mosaic landscape types; furthermore we propose a hitherto unreported link between adhesive ability of insects and movement parameters, such as dispersal and migration events.

6.2 Materials and method

To determine how substrate properties affect foraging insects we first measured mean distance and velocity travelled between track points on a range of known surface types reporting mean values for both controls and experimental surface combinations. Secondly we compared the adhesive ability of the insects on each surface type with a moving stage, reporting [normal] adhesive force on each substrate.

6.2.1 Foraging study

In order to record speed and velocity of free moving ants on a range of surfaces an experimental arena was constructed with a nest site and food source placed randomly on either side (Figure 6.0). Nest and food position were randomised using a number generator (1-6) with three positions on each side of the arena equidistant from each other, each nest held 30 ants and movement parameters were measured once ants were two body lengths away from next site to minimise pathway disturbance from foraging ants returning to the nest. Pathway measurements were taken for 20 ants for each set up (10 ants for each side), with pathway length kept to 1000 track points for each ant. The arena was constructed to allow the substrates to be easily changed between replicates, allowing manipulation of local landscape parameters (in this case surface roughness). To determine if distance and speed were correlated with adhesive ability the substrates were constructed using sandpaper of known grit sizes (**Error! Reference source not found.**). First a series of control replicates were performed where both sides of the arena had the same substrate grit size, secondly the substrates were randomised using the same program as for nest position so that each side had a differing substrate grit size.

To record the ant behaviour a digital camera (Panasonic SDR – S26), controlled by HandyAvi (version 4.2; Anderson's AZcendant Software) was set up above the arena and continuous video recordings were taken for 4 hours. These videos were then imported into ImageJ (http://rsbweb.nih.gov/ij/) for analysis using the manual tracker (MtrackJ) plugin. This allows for the movement of the ants to be monitored frame by frame (creating a 'track' for each insect), to calculate speed, direction and distance for each insect. Movement pathways were subject to fractal analysis to determine if behaviours differ between substrata

Table 14: Mean grit sizes of substrates used in foraging arena, all sizes reported are from manufactures (B&Q) published tolerances.

Sandpaper grit size (wt)	Average particle diameter (µm) ± 10%
60	269
80	201
100	162
150	100

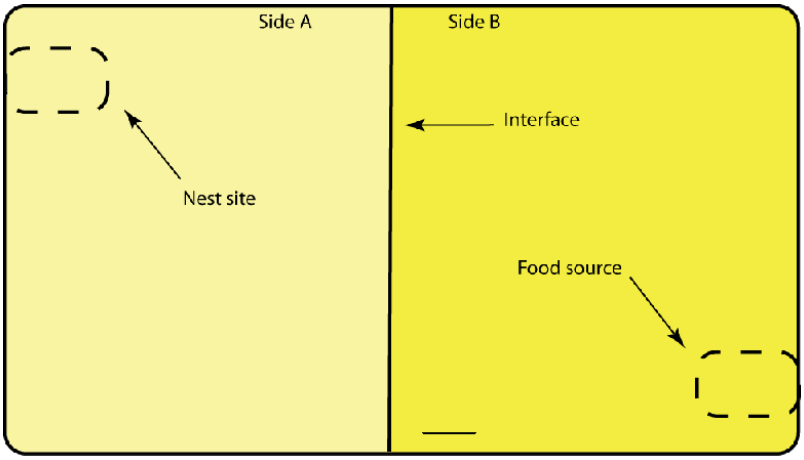


Figure 6.0: Foraging arena used to observe ant trails on alternate substrate types; nest and food source positions were randomly generated between each trial, as were substrate type, scale bar = 4cm.

6.2.2 Adhesion measurement

To determine if movement parameters such as speed and distance were related to adhesive potential on each of the substrate types, F (mN) compared to a clean glass control surface, a custom movement stage was constructed Figure 6.1. This stage allowed controlled manipulation of the insects; cotton thread was tied around the thorax of cooled specimens of *P. dives* (with care taken to avoid trapping any legs), this thread was then attached to the underside of an electronic balance, supported directly above the movement stage. The movement of the stage was controlled by an electronic motor (MFA/combo drills RE280/1) connected to a regulated constant 12 V supply, operated by a double pole double throw switch; the motor was then attached via a drive shaft to a micromanipulator stage. By changing the polarity of the circuit it was possible to lower or raise the stage at a set velocity (4.41 $mm^{s\ -1}$). For the trials an ant was placed on the substrate, then the stage was moved downwards away from the insect (Figure 6.1 A and B). As the insect moved away from the balance it exerts a force on the attachment thread that was registered by the balance. These recorded forces were compared using an ANOVA with a *Dunnetts posthoc test* used to compare results for each surface independently against a control (Mathmatica version 8, Wolfram mathematics).

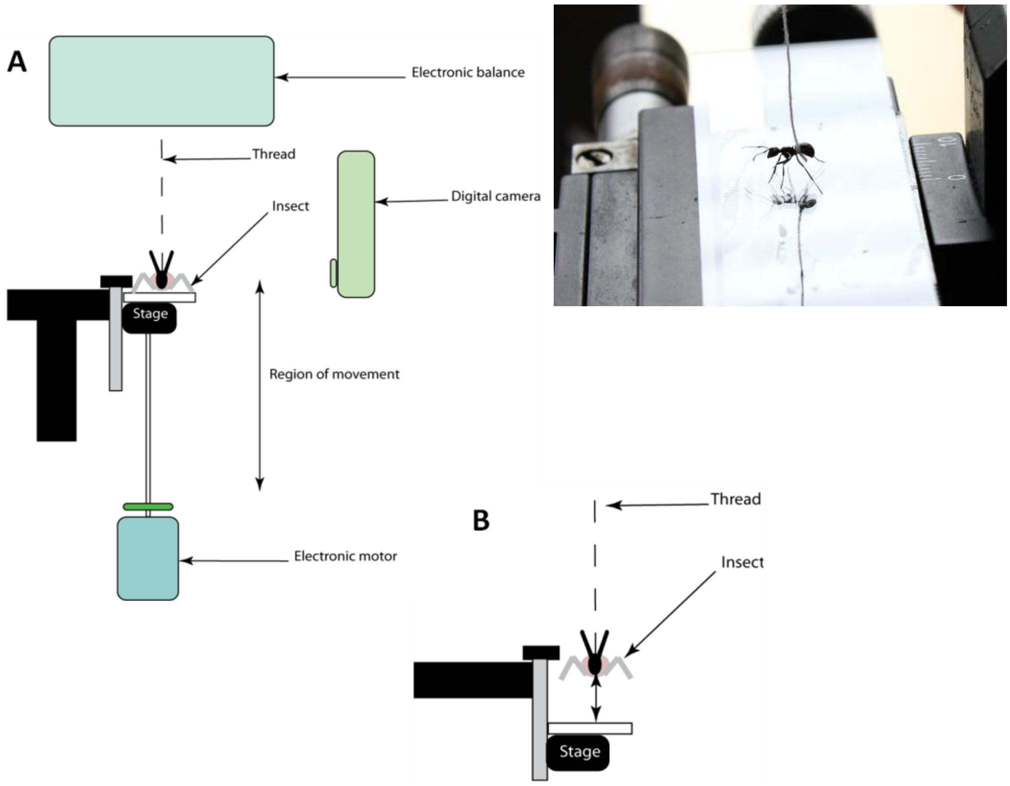


Figure 6.1: Schematic of movement stage used to record adhesive forces, A, whole set up viewed front on, B, close of stage, free space underneeth insect is equal to adhesive strain. Insert is digital image from webcam showing *P. dives* whith thread tied around thorax.

6.3 Results

6.3.1 Foraging studies

There was no significant difference between mean velocity or distance moved on control substrates ($F_{3,\ 76}$ = 5.40, p = 0.4, $F_{3,\ 76}$ = 2.45, p = 0.100, respectively). However, a significant effect of substrate was found when analysing the experimental data, with ants typically moving further and faster between track points on coarser substrates (Figure 6.2 B) (particle size; 100 µm – 269 µm*;* $F_{5,\ 114}$ = 53.78, p < 0.001; 162 µm –269 µm, $F_{5,\ 114}$ = 68.1, p < 0.001), although this trend disappears at the threshold for arolium extension (201 µm – 269 µm, $F_{5,\ 114}$ = 2.24, p = 0.13). At this threshold (approx. 150 µm, from chapter two), time taken between steps may be influenced by the passive action of arolium extension on substrates with a range of particle sizes both above and below this critical threshold.

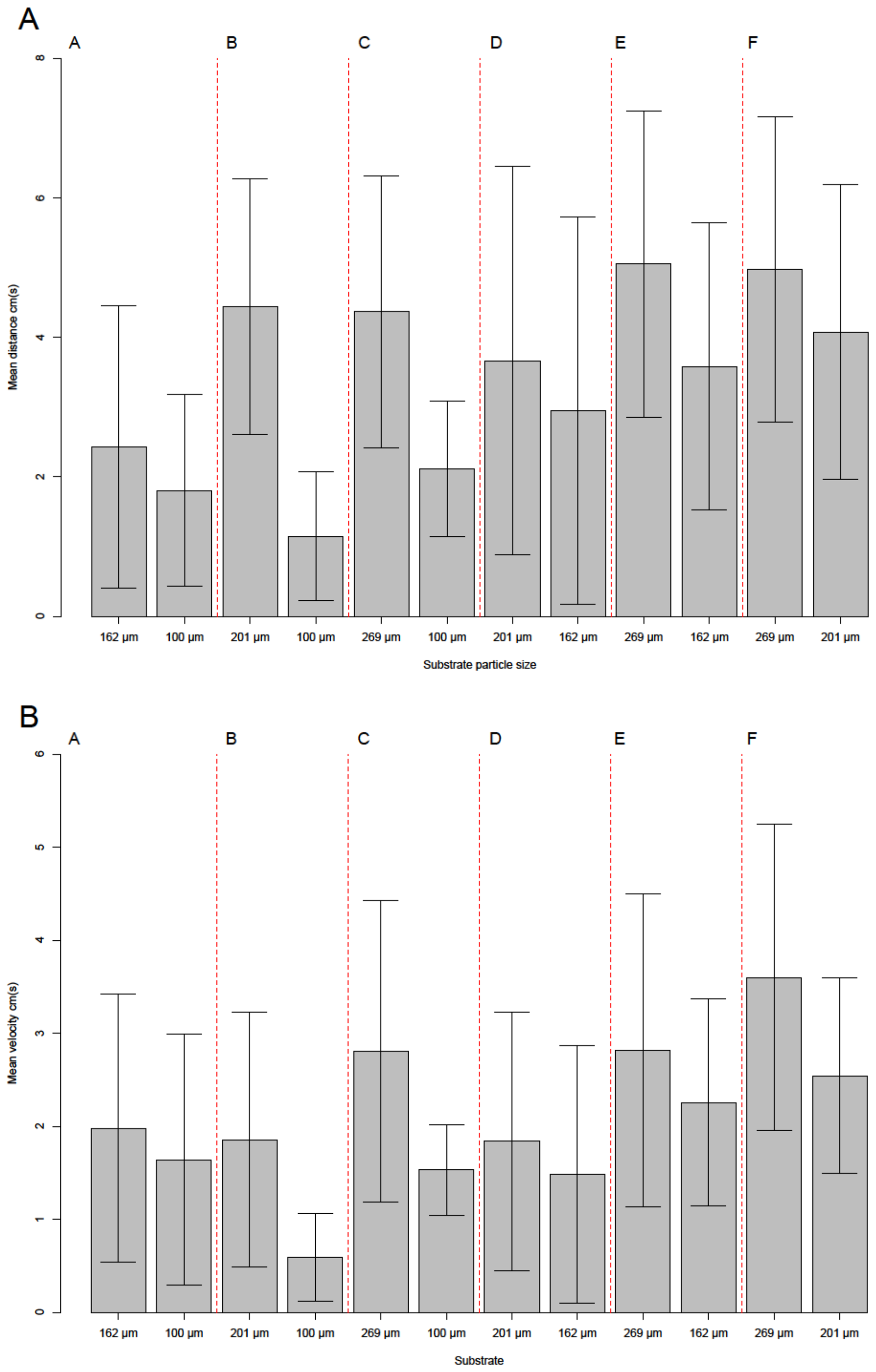


Figure 6.2: Mean distance (A) and velocity (B) travelled between track points for each substrate type, (A-F, experiment results separated by red horizontal dotted line), tracks comprised of 1000 steps, and error bars show sem.

Representative movement pathways of ants on each substrate are presented in Figure 6.3 while the resultant *D* of each pathway is presented in Table 15. Ants typically exhibit a more tortuous movement path on coarser substrates, with *D* increasing and movement pathways becoming more linear as substrate coarseness is reduced.

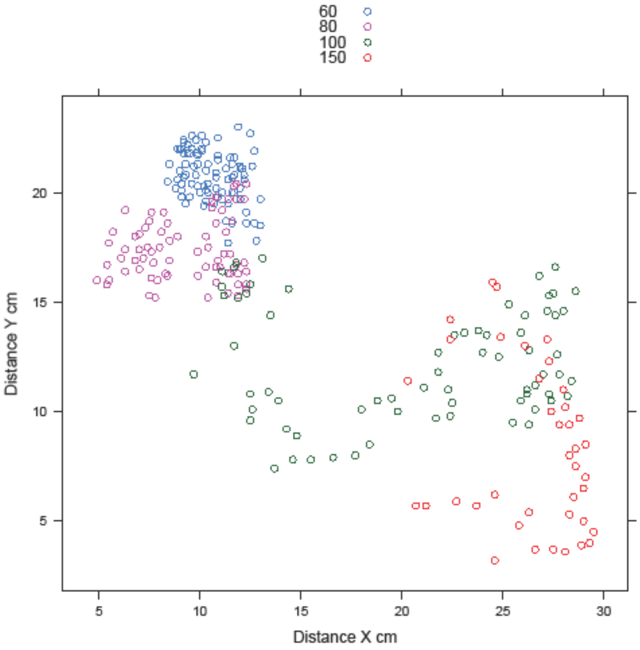


Figure 6.3: Example movement pathway (100 step lengths) for *P. dives* on each substrate type; key at top of plot indicates which colour represents movement pathways on each substrate type.

Table 15: Fractal dimension (*D*) of movement *P. dives* movement pathway (1000 step lengths) on each substrate type.

Substrate	*D*	*Nominal substrate particle size µm*
60 wt	1.87	269
80 wt	1.793	201
100 wt	1.73	162
150 wt	1.593	100

Ants displayed a lower value for *D* on coarse substrates, moving both further and faster between track points, covering the area more thoroughly (Figure 6.3), with an increase of searching effort within a confined space.

6.3.3 Adhesion results

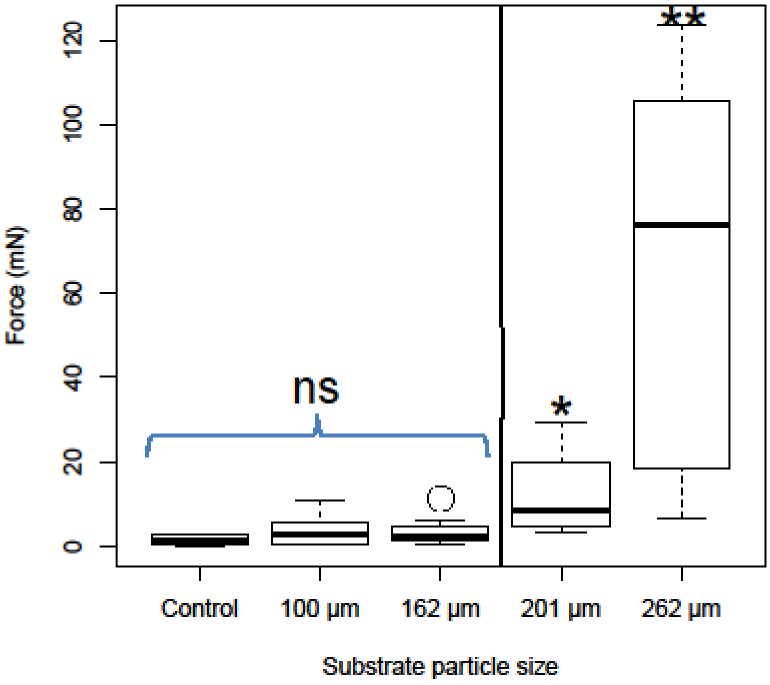


Figure 6.4: Measured adhesive forces in the normal plane for *P. dives* on control and experimental surfaces (n = 20 for each surface), mean value is marked with a black line, box upper and lower quartiles with outliers depicted by whiskers, outliers above 2.5 times the mean are shown as hollow circles. Adhesive force (mN) is depicted on y-axis; surface type (particle size) is shown on the x-axis. Ns = not significantly different, * = significant at the 95% level, ** = significant at the 99% level.

Adhesive force (mN) differed significantly between surface types, with a positive correlation between adhesive force (mN) and substrate particle size (Spearman rank correlation r = 0.611). Forces recorded on clean glass, 100 µm and 162 µm sandpaper were not significantly different from each other (ANOVA, posthoc Dunnetts test; $F_{2, 18} = 2.79$, $p > 0.05$; $F_{2, 18} = 2.64$, $p > 0.05$; $F_{2, 18} = 3.47$, $p > 0.05$, respectively), while adhesive forces differed significantly for surfaces with a grit size > 200 µm (ANOVA, posthoc Dunnetts test; 201 µm; $F_{2, 18} = 13.4$, $p < 0.001$; 269 µm; $F_{2, 18} = 22.98$, $p < 0.001$), with maximal adhesive forces recorded on the 269 µm particle size sandpaper.

6.1 Discussion

6.4.1 Foraging analysis

Distances and velocity between track points were not significantly affected by substrate coarseness in homogenous environments, while those recorded on mosaic environments did. This change in movement rates in relation to substrate roughness may be due to the time taken to extend and retract the arolium between steps. Although this reflexive action is by its very nature a quick movement it must take up a finite period of time with which to perform each action; we suggest that on substrates with a coarse grit size adhesion and locomotion are linked by time taken from mechanical interlocking of the tarsal claws on surface asperities. As such there would be no need for extension and retraction of the arolium and each stepping action would be relatively faster. Although it was not possible to measure time taken to extend and retract the arolium between steps in this study it is theoretically possible to measure this in future studies using high-speed cameras and microscopy techniques.

Movement patterns were observed to differ between homogenous and mosaic surface types (Figure 6.2 A and B), both in terms of distance and velocity between track points but also in the intensity of searching behaviour with ants displaying a more Brownian movement pathway on coarser substrates with an increase in *D* as substrate coarseness decreased (Figure 6.3 and Table 15), matching the adhesive force results recorded on each substrate type (Figure 6.4). When insects were presented with a mixture of substrate types they were observed to approach the interface between surface types but 'choose' to remain on the side of best adhesive ability (Figure 6.5 B). Although this ability to choose substrates based on adhesive potential has been speculated upon in previous chapters, it would seem to offer new insights to previous work as well, helping to explain why the fractal dimension of pathways (D) of insects differ between larval and adult forms (as found by Weins *et al.*, 1995).

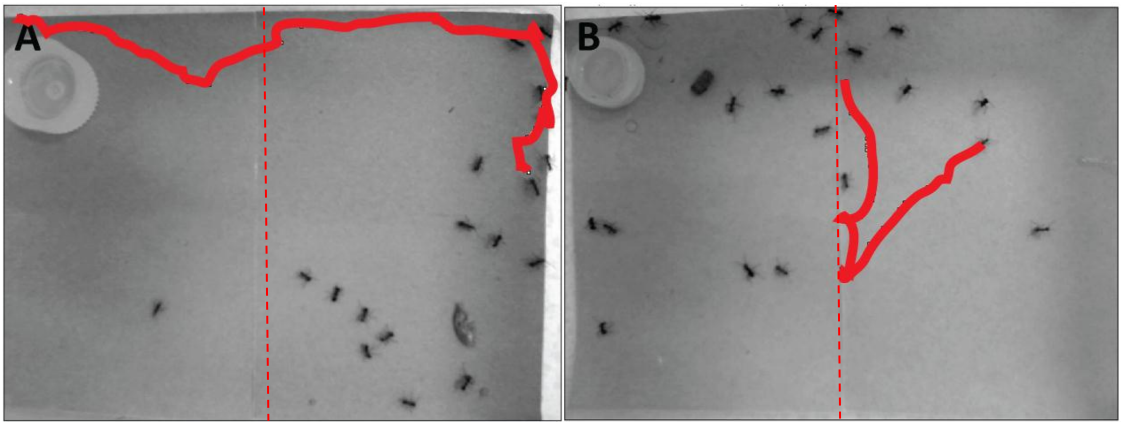


Figure 3: Digital trail of ant movement (red line) between, A, homogenous landscapes and B, mosaic landscape types (in the case of B, the right hand substrate has the larger grit size), only one ant trail shown per surface to aid clarity. Dotted red line represents the interface between substrates.

Mechanical stage

The results from the adhesive trial would suggest there is a threshold at which the tarsal claws provide the majority of adhesive force through interlocking with relatively large surface asperities (Figure 6.4), with a similar trend as that observed by Dai *et al.*, (2002), for roughness dependent friction forces generated by the tarsal claws of the beetle *Pachnoda marginata*. This is further supported by the behaviour of the ants on surfaces with a grit size > 200 µm, where specimens were observed to orientate themselves in a head downwards position to allow for maximum adhesive force to be generated by the pads. The change of orientation would maximise the adhesive area available and as such increase maximum adhesive forces on substrates with a roughness greater than the claw tip diameter, allowing for closer interlocking of claws with asperities. Although the interlocking of claws with surface asperities helps aid adhesive performance there must be a limit at which larger surface asperities become a hindrance to adhesion and locomotive abilities, and such a result may have been recorded in Bernadou and Fourcassie (2008). In their study Beradou and Fourcassie observed that individuals of *Lasius niger* exhibited more sinuous pathways on finer substrates, while this may seem to contradict the results from this study, the difference can be easily explained if one considers the relative sizes of the ants and substrate types used. While the substrates were presented loose they were unlikely to have influenced locomotive choices by causing fouling of the attachment devices as the smallest particle diameter was 400 µm (much larger than the claw base dimension) and due to the oval shape of the particles they may have been subject to packing behaviours making them much more stable. It is likely the more sinuous trajectories were observed due to the relative sizes of the ant and particles, with ants having to scrabble over the larger particles to make progress and as such ‘choose’ to stay on the substrate that allows for better locomotion.

However, it must be noted that while the use of clean glass surfaces or arbitrary roughness scales may lend itself to understanding of adhesion in laboratory conditions, the insects themselves are evolved to maximise their adhesive potential on ‘natural’ surfaces. An example of this would be the wax running behaviour seen in the *Crematogaster sp* complex studied by Federle and co-workers (Federle & Endlein, 2004; Federle & Rheindt, 2005; Federle, Jetter, Riederer, & Ho, 2000; Federle, Riehle, Curtis, & Full, 2002; Federle, Rohrseitz, & Holldobler, 2000), where it was found that generalist ants would not walk on waxy surfaces (Federle, Rohrseitz, & Holldobler, 2000). This led onto the hypothesis that there was a ‘trade off’ between the ability to adhere to waxy and general substrates. With further studies (Gorb & Gorb, 2006d) finding that ‘wax runners’ have longer middle legs than sister species which avoid walking on waxy surfaces, and as such attributed the ability to move freely on waxy surfaces to this physiology allowing a lower centre of gravity and a wider spread of weight over a substrate.

However, recent work (Gorb & Gorb, 2011), suggests that is simply not the case. By removing the waxy coating from a stem of three differing plant species and recording the distance traversed on each surface type they conclude that ants avoid walking on waxy surfaces not because they are incapable of doing so but because they have a choice of other substrates that do not require additional locomotory efforts or time to adapt body posture to before traversing. This would in turn suggest that the wax running ability seen in the *Crematogaster* complex is not governed by the evolution of a symbiotic relationship between the ant and plant but rather a more aggressive ant simply outcompeting other species for a resource. This in turn would bear out some of the observations from the study, with ants typically moving at a matched rate of both distance and velocity between track points in homogenous environments but moving in differing manners in heterogeneous environments. This linked with the ability to 'probe' substrates immediately in front of the ant (as observed in previous chapters), would allow the insect to move through an environment locating food with the minimum energetic cost to the individual.

It is likely that the differing movement patterns observed during this study are representative of small scale movements of insects in relation to surface roughness, and that this roughness driven movement and adhesion may limit both weight (g) and abundance of prey items brought back to a nest site by ant species. We suggest future work should include the modelling of insect movement to include not only obstacle avoiding behaviours but also adhesive potential; orientation, mechanical stimulus [from antenna], the physiochemical properties of substrates and incorporate factors such as foraging, mating and anti-predator behaviours, along with fractal analysis of movements (Crist et al., 1992; Crist, & Wiens, 2011; Wiens et al., 2012), optimal foraging theory

(OF) (Krebs, 1978), and ideal free distribution theory (IFD) (Fretwell & Lucas, 1970). If these parameters are included in a model it may be possible to map the spread of invasive species by predicting which direction movement is likely to occurr in relation to environmental, biotic and abiotic pressures at both large (across landscapes at the km scale) and smaller spatial scales, helping the management of invasive species by identifying which plant species they are likely to be foraging or ovipositioning allowing for a higher percentage to be captured in field work.

General discussion:

Summary

During the course of this thesis we have examined the influence of the four main hypothesis proposed by Gorb and Gorb (2002) on the wet adhesive system used by insects and how they influence the behaviour of foraging insects. In chapter two we confirm that contamination occurs not only for hairy pads as previously shown (Clemente et al., 2010; Gorb & Gorb, 2006; Gorb, 2001; Gorb, 2007), but also for smooth pads associated with Hymenoptera (Anyon et al., 2012), concluding there is a critical size range for particles capable of contaminating the adhesive devices. Evidence from chapter three suggests that although the pad types are both able to self-clean there are subtle differences between the two morphologies in rate of self-cleaning, with a significant interaction between the free surface energy (FSE) of both the fouling particle and substrate (Orchard, Kohonen, & Humphries, 2012). In chapter four it was determined that out of porosity, surface energy and surface roughness, it is surface roughness that is the most important factor in limiting insect adhesion, with the potential critical roughness regime being reported in the literature being associated with the material properties of the adhesive organs themselves, having implications for agricultural practices relating to pest species. In chapter five we investigated the role of mechano-sensing hairs on antenna in substrate choice trials, showing that insects are able to use the antenna to probe substrates for ‘risk’, choosing to avoid tacky and particulate barriers as they pose a higher risk of entrapment and loss of adhesive ability. However, it was also shown that insects are unable to asses the FSE of substrates, which has implications for pest management schemes and medical applications. While in chapter six we show how the link between substrate coarseness and adhesion can feedback into foraging and substrate choice decisions in Hymenoptera.

Implications for the field of insect adhesion

Material science

Currently the study of insect adhesion has implications for many fields of research including material science and the manufacture of new pressure sensitive adhesives (Chen & Gao, 2007; Davies et al., 2009; Federle, 2006; Gorb, et al., 2007), to self-cleaning adhesive tapes (Lee & Fearing, 2008), capable of maintaining adhesive properties after multiple applications. Using the study of insect adhesion it is possible to tailor a tapes adhesive potential for a particular purpose, changing the shape of the contact points to maximise adhesion on a range of surface types (Greiner & Arzt, 2007). Studies of the structure of biological adhesive pads found that there are marked changes in relation to the mass of the organism and adhesive potential between dry and wet adhesive systems (Figure 7.0) (Federle, 2006), suggesting a trade off between body mass and adhesive potential.

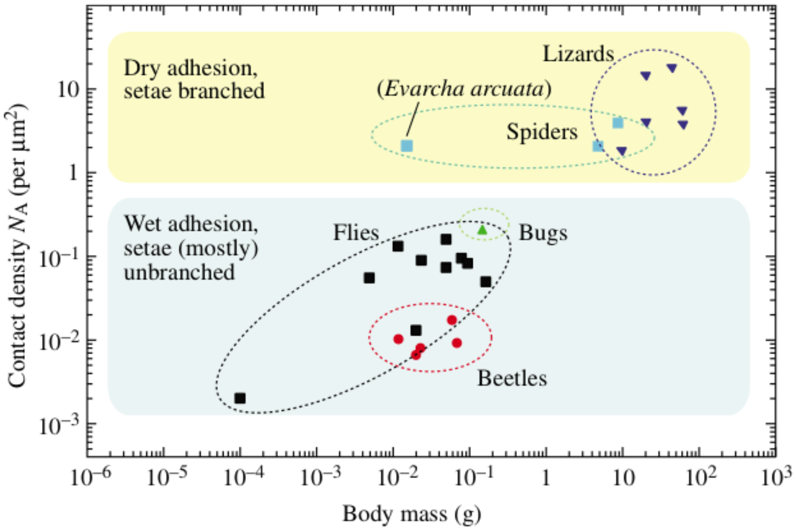


Figure 7.0: Relationship between contact setal density (Na) of hairy pads and body mass for a variety of taxa (image taken from Federle, 2006).

Studies on the mechanical properties of biological adhesive pads (Bullock & Federle, 2011; Gorb et al., 2007; Kwak & Kim, 2010) found that insects use predominantly thicker and less flexible hairs than those employed by lizards such as geckos. This allows for a more compliant outer surface and as such relatively larger area of real contact between the tips of the hairs and the substrate for geckos than for insects, a notable morphological change in the overall design of the hairs can be observed as well, with hairs in insects typically un-branched and relatively stiff and those in lizards being bifurcated. The branching of the hairs may also be an evolutionary design to avoid matting of the adhesive devices during locomotion, although this may also be facilitated by the thin layer of phospholipids covering of the setal hairs in geckos (Hsu et al., 2011; Puthoff et al., 2010). While, when the setae on beetle adhesive pads are examined closely corrugations can be observed on the dorsal surface that would prevent the hairs from sticking together.

It should be noted also that while classically referred to as 'dry' the adhesive system of geckos and spiders have been found to be influenced by relative humidity (RH) (Huber et al., 2005; Niewiarowski et al., 2008; Puthoff et al., 2010b) and surface roughness (Kesel, Martin, & Seidl, 2004; Niederegger & Gorb, 2006; Wolff & Gorb, 2012), with some studies inferring that geckos and spiders use a phospolipid secretion to enhance adhesive contact (Niewiarowski et al., 2008; Peattie et al., 2011), determining that the formation of capillary bridges at the terminal tips of the seta aid in generating adhesion and that as RH rises the material stiffness of the adhesive pads reduces with the seta themselves becoming more pliant and able to form more intimate contact with the substrate (Huber et al., 2005; Puthoff et al., 2010).

Implications for agricultural practices

While the study of insect adhesion can help shed light on past agricultural studies on pest insect species that cause damage in agriculture (Hsu et al., 2011) and can upset ecosystems (Adriaens, Gomez, & Maes, 2008). A number of methods exist to deal with pest species, from spraying with pesticides, sterile insect techniques (SIT), and treating surfaces with abrasive materials (Cooper et al., 1995; Glenn et al., 1999; Marsula & Wissel, 1994; Tingle, 1996). Spraying with pesticides can cause a reduction in pest species numbers and in a trial by Cooper et al. (1995) found that sprays of Insect Growth Regulators (IGR) can reduce abundance of *Locusta migratoria capito.* Whilst the field trials of Cooper (*et al,*. 1995) found negligible impact on non target species, a follow up study by Tingle (1996), found that numbers of non target species (Lepidoptera; Arididaae) were reduced within areas treated with diflubenzuron (which reduces chitin synthesis in insects). It is likely the reduction in pest species found in the study by Glen (et al., 1995), is due to the contact fouling of the adhesive devices of arthropods, however, due to the nature of their study it is possible that the spraying of plant surfaces also affected pollinator behaviour. If a systematic study of the adhesive properties of pest species were carried out it might be possible to develop a treatment that reduced the adhesive ability of pest species but left pollinating insects unaffected; either by covering plant surfaces with a critical roughness range that affects the pest species only or perhaps treating the stems/trunks of plants to make them ‘slippery’ thus limiting the damage done by walking herbivorous insects.

Biomimetic studies into insect adhesion (and adhesion in general) and antenna sensing have increased in recent years with Lewinger *et al.,* (2005) suggesting that by incorporating feedback from sensitive antennae arrays, sophisticated robots would be able to locate, identify and avoid obstacles in their path. New research being undertaken in Australia aims to do just this by developing novel ‘robotic scouts’ capable of searching inside collapsed buildings to discover potential casualties and are heavily influenced by studies of adhesion and sensory feedback from antenna, with the robot itself modelled on the locomotive abilities of geckos (Clemente pers comms). However, to fully develop these novel robots requires significantly more research into mechano-sensing of antennae in general and a move away from the historical focus of behavioural studies of chemical communication (Stanczyk et al., 2010). Although recent research has been undertaken into the combination of more sophisticated antennal sensors and locomotive models in an effort to increase the efficacy of remote survey robots used to locate victims of natural disasters (Gravish et al., 2010; Schargott, Popov, & Gorb, 2006; Tamelier et al., 2011; Varenberg et al. 2011; Zhao et al., 2009).

Implications for ecology

Sexual dimorphism in attachment has been reported in species such as the Colorado potato beetle (*Leptinotarsa decemlineata* [Say 1824]) (Voigt et al., 2008), millipedes *Orthomorphella pekuensis* (Chung & Moon, 2008), the blow fly (*Calliphora vicina*) (Niederegger, Gorb, & Jiao, 2002), water striders (Arnqvist & Rowe, 2002a; 2002b) and two species of water beetle *Dytiscus lapponicus* and *Graphoderus zonatus verrucifer* (Hardling & Bergsten, 2006). These differences in adhesive forces between sexes have been suggested to relate to differences in setae types found on the pads as well as changes in the composition of the cuticular lipids themselves can differ between males and females; e.g. female Northern Walking Stick Insect (*Diapheromera femorata*) (Warthen, Uebel, Lusby, & Adler, 1981) have higher concentrations of wax esters including long chain hydrocarbons (C_{24} and C_{28}) than males of the same species. Although not empirically proven it is possible that the differences in adhesion between male and female insects could lead to the differing sexes optimally foraging on differing plants (with differences in surface chemistry and topography (waxy and smooth surfaces)). Recently a series of studies using Atomic Force Microscopy (AFM) have begun identifying the material properties of the adhesive pads and secretions employed by insects (Piesker & Gorb, 2012), determining that the adhesive secretion of ladybeetles and flies have differing evaporation rates. This difference in evaporation rates suggests that the bulk of the adhesive fluid changes between species, possibly being specific for substrates found within a given niche. As such it is possible to probe the adhesive differences between insects, and between sexes in relation to the material stiffness of the adhesive devices themselves as well as the stiffness of preferred substrates (Federle pers comms).

The future of insect adhesion

In conclusion we find that the understanding of a specific insect's attachment capability could help explain the evolution of host-plant selection and the beginning of insect-plant symbiosis. By characterising attachment capabilities of invasive insects may also help the establishment of novel integrated/ecologically based management programs for invasive species. However, there are many unanswered questions within the field, for example how does the mass of an insect limit the surfaces to which it can adhere, how do adhesive abilities and decision making influence foraging decisions in social insects, histological studies are needed to determine the delivery method of the adhesive secretion in smooth pad system, is it a fluid filled matrix as suggested by Beutel and Gorb, (2001) and Gorb (et al., 2002) or is there a previously unreported method of fluid delivery. Finally an important question is what factors influence host plant switching and how are localised changes in habitat affecting population dynamics through loss of preferred plants to forage or oviposition.

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Appendix

Statistical analysis

Survival analysis example

Survival analysis starts with a survivorship value of 1 and stays at 1 until the first individual escapes. For example if you had five observations of an event occurring at known weeks (12, 17, 29, 35 and 42), then the survivorship value of 1 would continue until week 12. After week 12 the survivorship value would drop to 4/5 = 0.8 where it would stay until week 17 where it would drop to 0.8 x 3/4 = 0.06. At week 29 it would drop again and so on with the probability of survival continuing to drop until the end of the events.

In the above example this would give two groups those that “died” (in this case escaped) $d(t_i)$ and “those at risk” (in this case still trapped within the experimental arena) $r(t_i)$. By assuming an exponential distribution and a “constant hazard” (risk of escape), according individuals a score of either 1 (time of death or in this case event happened and they escaped) or 0 (still alive when last seen or in this case trapped within the experimental arena) it is possible to analysis for statistical differences between treatments (in this case particle type, particle size and relative humidity).