Modes of entry of petroleum distilled spray-oils into insects: a review

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Abstract

Petroleum oils are some of the oldest and safest pesticides in use. In spite of the numerous improvements achieved in oil technology, the mode of entry and the insecticide action mechanism of these products have been the subject of considerable debate and conjecture over many years. The literature reviewed suggests that insecticide oils can penetrate the insect body through the integument as well as through the tracheal system. Suffocation by spiracle blockage was held as the most accepted theory on its mode of action. However, an in depth analysis of the interaction between oils and insects body surface from a physical perspective suggests that suffocation occurs only when insects are over-sprayed or dipped in oil. Based on this analysis, it is more likely that when petroleum oils contact the insect surface, capillary forces and complex physical interactions take place in the cuticular layer, which lead to differences in the melting point and permeability of cuticle waxes. This in turn, alters the waterproofing properties of the cuticle and also leads to penetration of spray oils that can be carried to different lipophilic tissues. The changes in the cuticle caused by oils, which range from changes in melting point of the cuticular wax layer to cuticle dewaxing, strongly suggest cuticular penetration as the foremost mode of entry of insecticide oils.

Key words: insecticide oils, petroleum distilled spray-oils, pesticides mode of entry.

Introduction

Petroleum oils are some of the oldest and safest pesticides in use and their history involves a remarkably broad list of developments in the framework of petroleum chemistry. Crude petroleum oils are mixtures of a large number of compounds that can be categorized as paraffin chains, unsaturated hydrocarbons, naphthene rings, aromatic rings, and asphaltic material (Chapman *et al*., 1952). Petroleum oils are commonly used in crop protection as diluents for formulated products in low-volume applications (Downer *et al*., 1993), as adjuvants for pesticides (Treacy *et al*., 1991) or as insecticides and ovicides themselves (Childers, 2002; Smith and Pearce, 1948), feeding deterrents and as oviposition repellents (Webb *et al*., 1994; Agnello, 2002).

Oils can improve insecticide's efficacy by enhancing plant coverage and penetration of active ingredients into the insect's body (de Licastro *et al*., 1983). Their synergistic effects on synthetic pesticides are related mainly to their carrier activity, which favors diffusion and transport of certain insecticide molecules into the insect body (de Licastro *et al*., 1983; Fontan and Zerba, 1987; Mulrooney *et al*., 1993).

Current specifications for synthesis, chemical purification and refining reflect more modern uses where advanced oil products are manufactured for specific treatments which are environmentally suitable, toxicologically safe and fit neatly into the integrated pest management concept. Pesticide efficacy of these highly refined "*narrow-range oils*" or petroleum-derived spray oils (PDSOs) increases with increasing distillation temperature (a parameter related to structure and molecular weight) of the oil until reaching an optimum point (Riehl, 1969). Median equivalent n-paraffin carbon number (nCy), based on ASTM D-2887 (ASTM, 1997),

is currently used to characterize the different oils. The physical and chemical characteristics of the PDSOs are defined by nCy, where "n" denotes a normal paraffin (alkane) molecule and "y" denotes the number of carbon atoms (Kuhlmann and Jacques, 2001). The customary carbon number values of spray oils are nC21, nC23, nC24 and nC25, range in which spray oils combine low phytotoxicity with insecticidal effectiveness.

In spite of the numerous improvements achieved in oil technology, the mode of entry and the insecticide action mechanism of PDSOs have been the subject of considerable debate and conjecture over many years. In turn, the impracticality of grouping them together with synthetic pesticides on the basis of their route of entry (fumigants, contact insecticides and by ingestion) and of their mode of action (mainly binding to specific receptors) constitutes the main hindrance for understanding how PDSOs perform as insecticides.

Suffocation by spiracle blockage has been mentioned as a key factor of oil toxicity (Davidson *et al*., 1991; de Ong *et al*., 1927) and is held as the most accepted theory on its mode of action (Najar-Rodriguez *et al*., 2008). However, blocked spiracles can only develop into a main mortality cause when a series of factors such as dose, insect taxa and developmental stage, and type of oil converge. In contrast to chlorinated pesticides, OP´s, carbamates and pyrethroids, oils have multiple targets and their biocidal activity depends on physical factors as surface phenomena, solubility, biochemical or metabolic traits and on the taxa and stage of the target organism (Stadler *et al*., 1996). Oils do not depend upon one property or means alone for their effectiveness, as synthetic pesticides do. The variety of toxicity signs observed in different insect species when treated with insecticide oils, range from negative effects on the feeding and oviposition behavior (Mensah *et al*., 1995;

Beattie *et al.*, 1995) to abdomen contractions, and dehydrated and darkened cuticle in dead insects (Najar-Rodriguez *et al.*, 2008). This has led to an array of assumptions from different authors on their mechanism of action.

In any case and independently from the type of insecticide used, the substance has to move from the external environment to its site of action in the target organism to be effective. Some authors have suggested that low polarity substances penetrate the insect cuticle through intersegmental membranes (Matsumura, 1975) or via wax and pore canals (Noble-Nesbitt, 1970). According to Gerolt (1983), the entry of insecticides by lateral diffusion through the integument and through the spiracle-tracheal system seem likely, fast and effective pathways. While a great deal of information about spray oil efficacy as well as their routes of entry and action mechanisms in insects has been provided by individuals working on each of these two pathways, gaps remain in our understanding of the functional role played by insect physiology and oil physics. Therefore, the primary purpose of this work is to review the main factors that govern the routes and modes of entry of oils in the insect body.

Lateral diffusion of spray-oils through the insect integument

Insect cuticle is a non living multilayered external matrix of carbohydrates, proteins and hydrocarbons that wraps all insect parts and forms the interface between the insect and its environment. It serves as the primary barrier to penetration of pathogens as well as of chemicals deposited on the insect's body surface (Gorb, 2001). The major components of epicuticular coating are often hydrocarbons, mainly methyl-branched alkanes, and several fatty acids, alcohols, aldehydes, ketones, wax esters, and esters of primary and secondary alcohols (Böröczky *et al.*, 2008). Apart from being a physical barrier, these compounds have a major role in cuticular waterproofing (Gibbs, 1995; 1998; 2002) and can act as semiochemicals in some taxa (Howard, 1993). Both functions may be affected by changes in the physical properties of epicuticular lipids and thus compromise water conservation as well as insect communication (Montooth and Gibbs, 2003).

Cuticular hydrocarbons are relevant to insect physiology from the qualitative and quantitative point of view. Insect cuticle constitutive lipids are highly diverse and can involve over 100 different compounds on a single individual, which may be present in very complex mixtures of different waxes and their isomers (Wigglesworth, 1945; de Renobales *et al.*, 1991; Dekker *et al.*, 2000). From a quantitative viewpoint, the increase in the amount of cuticular lipids confers enhanced desiccation resistance in insects. For example, in the goldenrod gall fly, *Eurosta solidaginis* (Fitch) (Diptera Tephritidae) the amount of cuticular hydrocarbons increases more than 40-fold in the overwintering larvae, which increases its desiccation resistance more than six-fold (Nelson and Lee, 2004). Moreover, parasitic insect species as the European beewolf, *Philanthus triangulum* F. (Hymenoptera Crabronidae) make deliberate use of this trait by applying large amounts of hydrocarbons on the body surface of its host (honeybee worker of *A. mellifera*) to lower water loss of the larval provisions (Herzner and Strohm, 2008).

The prompt penetration of oils through the insect integument has been shown through conclusive experiments by different authors. Using mixtures of parathion with paraffinic oils of different relative viscosity and identical polarity, de Licastro *et al.* (1983) and Fontan and Zerba (1987) found a linear correlation between the relative viscosity of the vehicles used and the percentage of cuticle penetration of the pesticide in the cone-nose bug, *Triatoma infestans* Klug (Heteroptera Reduvidae). Once in the insect's haemolymph, hydrocarbons are actively transported by lipophorins (Schal *et al.*, 2001). These specific lipoproteic complexes are capable of sequestering hydrophobic core lipids from the hydrophilic environment of the haemolymph (Blacklock and Ryan, 1993) and of transporting lipids and hydrocarbons within the insect body (Schal *et al.*, 2001), which finally accumulate in lipid-containing tissues (Taverner *et al.*, 2001).

Waterproof effectiveness of the insect cuticular hydrocarbon layer depends on its quali- and quantitative composition as surface lipids provide a better waterproofing barrier when they are in a solid than fluid state (Gibbs, 1998; Rourke and Gibbs, 1999; Rourke, 2000). Thus, straight- and long-chain saturated hydrocarbons provide the best protection against desiccation because these have higher melting points (Gibbs and Pomonis, 1995). However, cuticular waxes can be selectively affected by different organic solvents, ranging from a slight disturbance of the structural integrity of the wax layer (Wigglesworth, 1957) to complete dewaxing (Lockey, 1988). Likewise, apolar substances of low dielectric constant as PDSOs may interact with the overall lipid mixture on the insect cuticle depressing its melting point and increasing its permeability (Hurst, 1940; Rourke and Gibbs, 1999). This leads to an observed melting point range that is lower and broader than the melting point of either component or a sharp melting point at the eutectic temperature (Hägg, 1969). This behaviour can be explained in terms of interaction energy. The interaction energy of a pair of molecules is large when both are of similar polarity and it depends strongly on their mutual orientation. At low temperatures a sufficiently high proportion of neighboring molecules are able to adopt the orientations of low energy for the liquids to be miscible. As the temperature augments, considerations of energy become less important, and those of entropy become more important. Thus, the miscibility of PDSOs and cuticle waxes (e.g.) will most likely increase with temperature (Rowlinson and Freeman, 1961).

It is convenient at this point to briefly mention that the melting point depression is the physical driving force behind the liquid-like behavior of a mixture of solid compounds, which is primarily an entropic effect and can be estimated for each compound (Marcolli *et al.*, 2004). However, low melting point values of cuticular hydrocarbon mixtures do not always lead to higher water loss through the insect cuticle because the complex

interactions between saturated and unsaturated hydrocarbons in hydrocarbon mixtures are an added relevant factor involved in insect cuticular waterproofing (Gibbs, 2002; Gibbs *et al.*, 2003; Montooth and Gibbs, 2003; Herzner and Strohm, 2008). Hence, when applied on the insect's body surface, PDSOs come into a competing equilibrium with some of the components of the cuticle wax layer, breaking down its integrity by changing its melting point, which in turn increases cuticle permeability (Wigglesworth, 1945).

Dehydration is one of the main consequences of changes in cuticle permeability. Removal of cuticular waxes cause a "pin-hole effect" phenomenon, described for insects (Hurst, 1940; Wigglesworth, 1941; 1942) and millipedes (Cloudsley-Thompson, 1950), after submersion in organic solvents (ether, chloroform, etc.). Small water drops were shown to appear throughout the insect's body surface until the insect was completely coated with fine droplets, which evidenced dehydration. Moreover, the changes in the continuity of the insect cuticular wax layer may allow penetration of apolar compounds such as PDSOs, synthetic insecticides and non-electrolytes in general (Penniston *et al.*, 1969; de Licastro *et al.*,1983), and at the same time provide an escape for body water (Gibbs, 1998).

Inflow and diffusion of spray-oils through the spiracle-tracheal system

The general structure of the respiratory system in insects comprises spiracles, major respiratory openings in the exoskeleton, which lead into a cluster array of tracheae that branch into smaller tracheae and tracheoles.

Spiracles can be simple openings or complex associated structures that prevent the entry of particles and liquids into the tracheal system, thereby permitting respiratory gas exchange while minimizing water loss (Edney, 1977; Chapman, 1998).

The tracheal inflow as a mode of entry of non gaseous insecticides remained overlooked for decades. However, oil flowing into the air passages of insects was noted for the first time by Moore and Graham (1918), and addressed further by several authors (Roy *et al.*, 1943; Stadler *et al.*, 1996; Taverner *et al.*, 2001) with contradictory results (Najar-Rodriguez *et al.*, 2008). Recently Sugiura *et al.* (2008) provided conclusive evidence on this issue, showing that the flow of insecticide into the spiracles plays an important role in efficacy.

Bioassays on *Anticarsia gemmatalis* Hübner (Lepidoptera Noctuidae) larvae developed by Stadler *et al.* (1996), revealed that the inflow of oil into insect tracheae (figure 1) depends exclusively on capillary forces which act equally on living and dead insects, proving that tracheal blockage by PDSOs, when it happens, is a pure physical phenomenon. Oil "coats" the inner surface of the tracheae rather than blocking it and such "coating" is not continuous as small oil droplets appear aligned inside larger tracheae due to interfacial tension (figure 1a, 1b) (Stadler *et al.*, 1996). Oil can move deep into the tracheal system and reach tracheoles, tending to flow into larger tracheoles ($\Phi < 6 > 2\mu$; $\theta \leq 90°$) (figure 1c). In smaller tracheoles ($\Phi < 2.0\mu$) the capillary force pushes the liquid out of the tube ($\theta > 90°$) (figure 1d) (Stadler *et al.*, 1996). *A. gemmatalis* larvae possess one of the simplest spiracular closing mechanisms and are
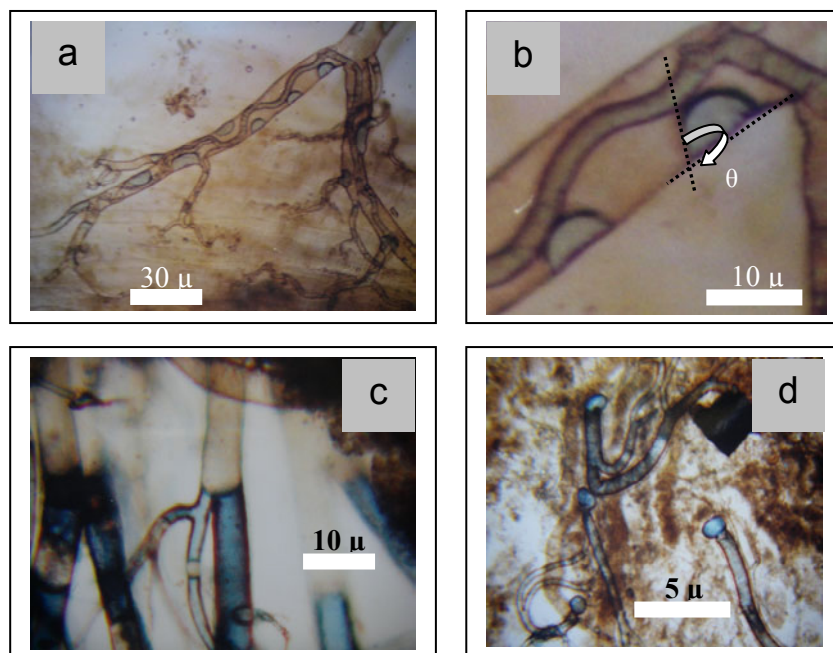
Figure 1. The inflow of PDSOs to the tracheae of lepidopteran larvae *A. gemmatalis* was confirmed experimentally by adding a liposoluble staining agent to the oil and observing it by means of a phase contrast microscope. a, b) Air-liquid interface inside the tracheal tubes, θ = contact angle $\approx 82.5°$; c, d) Capillary flow inside tracheoles (Stadler *et al.*, 1996).
(In colour at www.bulletinofinsectology.org)
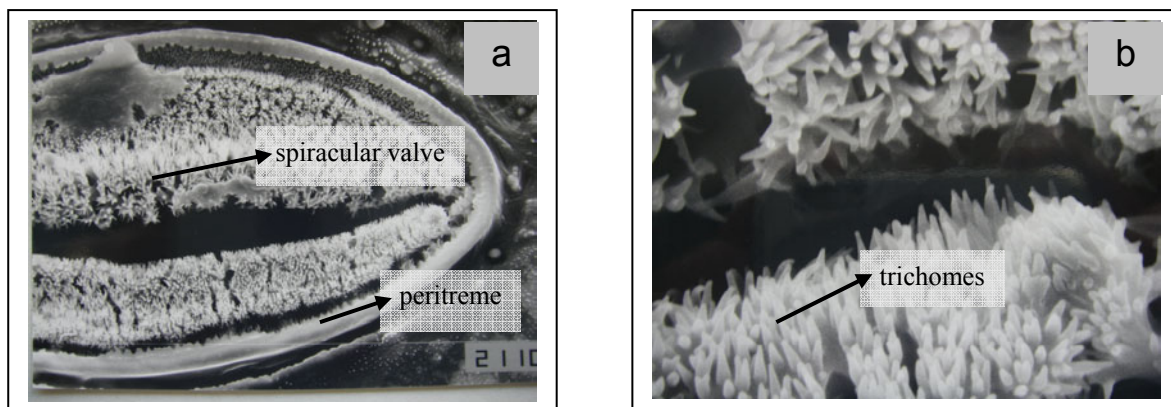
Figure 2. Scanning electronic micrography of the *A. gemmatalis* spiracle; a) 750x; b) 3500x.

therefore a good model for studying tracheal penetration by oils. The spiracles of *A. gemmatalis* larvae consist of an external valve system fixed directly at the peritreme, which can be pulled together over the spiracular opening (figure 2a). The spiracular valves of this species show intricate associated structures (trichomes) which form a filter apparatus (figure 1b). An atrium is present between the valve apparatus and the main tracheal trunk.

Similar results to those obtained on *A. gemmatalis* by Stadler *et al*. (1996) were obtained by Taverner *et al*. (2001) in dipping bioassays developed on *Epiphyas postvittana* (Walker) (Lepidoptera Tortricidae) larvae. These authors also found that oil flowed deep into the tracheal system reaching very small tracheoles (1–2 μm). A recent study by Sugiura *et al*. (2008) indicated that insecticide entry from the spiracles plays an important role in mediating a rapid knockdown effect in the German cockroach, *Blattella germanica* (L.) (Blattodea Blattellidae). In contrast, in bioassays on *Spodoptera litura* (F.) (Lepidoptera Noctuidae), Najar-Rodriguez *et al*. (2008) observed that PDSOs did not accumulate inside the main tracheal branches nor in small tracheoles at any rate or volume applied.

The tracheal inflow of insecticide oils depends on two main variables: the insect species and the oil type. The interaction between these two variables is what causes the discrepancies observed among different authors (Stadler *et al*., 1996; Taverner *et al*., 2001; Taverner, 2002; Sugiura *et al*., 2008; Najar-Rodriguez *et al*., 2008). Therefore, it is necessary to explore the tracheal inflow of hydrocarbons from a physics perspective to achieve a better understanding of the results obtained by different studies using different types of spray oils as well as different insect species.

The analysis of the tracheal inflow phenomena using fluid dynamics shows that oil's inflow into the insect's tracheal system depends on oil's viscosity. The difference in diameter from tracheae to tracheole generates an overhead pressure on the fluid establishing a difference in infusion lengths by capillary forces and results in an enhanced inflowing speed. The time needed to achieve a given spatial difference between the liquid-filled tracheae is a function of pressure that will determine capil-

$$L^2 = \frac{\gamma\ r\ t\ \cos\theta}{2\eta}$$

Equation 1. The Washburn's equation describes capillary flow in porous systems (Adamson and Gast, 1997); where "t" is the time (min) for a liquid of dynamic viscosity "η" (N•s/m^2) and surface tension "γ" (N·m^{-1}), θ is the contact angle, and "L" the distance the fluid penetrates into a tube whose average radius is "r".

lary flow, ruled exclusively by surface phenomena. Capillary flow in fine pores is a spontaneous process, driven by an interfacial pressure gradient, ubiquitous in nature. Capillary rise is what brings water to the upper layers of soils (Smith, 2003) and allows wetting of natural fibers (Stadler *et al*., 2005), among other phenomena. The Washburn equation (equation 1) describes the flow by capillary effects and is widely used to model capillary flow (Adamson and Gast, 1997). This equation is the most common expression for capillary rise and assumes that the capillary pressure in a cylindrical tube in contact with an infinite liquid reservoir is compensated by viscous drag or wetting and gravity (Krotov and Rusanov, 1999).

Wetting can be defined as the tendency for one fluid to spread on or adhere to a solid surface (Bear, 1972). When two phases meet they try to minimize their interfacial area. The tension acting in the direction of the film surface is called the surface tension "γ" (N·m^{-1}). The liquid of a droplet assumes an equilibrium shape that is dictated by surface free energy considerations at the liquid-gas interface (γ_{lg}), the solid- gas interface (γ_{sg}), and the solid-liquid interface (γ_{sl}). The boundary between wetting and non-wetting conditions is generally taken at a contact angle "θ", which is defined under equilibrium conditions when three phases meet (solid, liquid, and gas) and described as the angle between solid sample's surface and the tangent of the droplet's ovate shape at the edge of the liquid droplet in contact with solids varies between θ = 0° (complete wetting, cos θ = 1) and θ = 180° (solid not wetted at all, cos θ = –1). A high contact angle is referred to as a low degree of wetting and indicates a low solid surface energy or low

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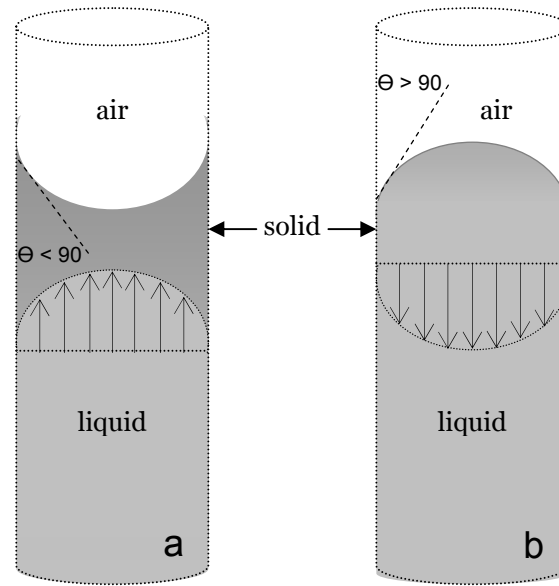
Figure 3. Schematic representation of a cross section of tracheoles with liquid inside. Contact angle (θ) of two different liquids in contact with a solid surface. θ at the solid/liquid interface is determined by the values of the work of adhesion of the liquid to the solid, and the work of cohesion of the liquid. a) When the adhesive force between liquid and solid is greater than the cohesive force within the liquid, the surface of the liquid will curve upward. The effect is of wetting and the capillary force (Fc) pulls the liquid into the tube. b) When the cohesive force within the liquid is greater than the adhesive force between the liquid and solid, the surface of the liquid will curve downward. The effect is of non-wetting and the Fc pushes the liquid out of the tube. The narrower the tube, the greater the rise of the liquid is. Increased ratio of adhesion to cohesion and greater surface tension also result in greater rise. The height the liquid rises will also decrease as a function of the density of the liquid.

chemical affinity between liquid and solid. A low contact angle is referred to as a high or sometimes complete degree of wetting and indicates a high chemical affinity.

The combined effects of wettability and interfacial tension cause the fluid to be imbibed into a capillary tube. Thus, on immersion of a capillary tube into a liquid, the liquid will contact the inside wall of the tube and penetrate forming a meniscus inside the capillary. The angle between the rim of the liquid and the capillary tube wall is named the wetting or contact angle "θ" (figure 3). Thus, wetting determines that the capillary force (*Fc*) (equation 2) will pull the liquid into the tube if θ is below 90° (figure 3a; figure 1c), and out of the tube if θ is larger than 90° (figure 3b; figure 1d).

On this basis, the fundamentals of tracheal blockage by PDSOs and whether they do so partially or totally can be understood. The oil inflow is driven by the sum of four forces acting on the liquid column (PDSOs) inside the insect tracheae. First, the capillary force; second, the friction force, which is a force that relates to the viscosity of the liquid; the third force arises from overhead pressure produced by breathing activity (in living insects) (Westneat *et al.*, 2003); the fourth force, the gravitational force can be neglected because of the small dimensions of the capillary tubes in diameter and in length.

Contact angle phenomena are further complicated when the surface of the solid is irregular (as may be the taenidia and the intima inside tracheae) and by contact angle dynamics and hysteresis (Walstra, 2003). There is a correlation between inflow resistance and surface roughness inside capillary tubes (Wasserthal, 1996) which is certainly a significant loss mechanism in PDSOs tracheal inflow (Tenan *et al.*, 1982). Furthermore, because of the small dimensions of tracheae, oils will flow inside the tubes according to *Reynold's* number (equation 3), which determines whether the flow in a pipe is laminar or turbulent (White, 1991). By this

$$Fc = 2\pi \; r \; \sigma \; \cos\theta$$

Equation 2. Capillary force (Fc) is the result of cohesion between like molecules of a particular liquid and adhesion between two unlike materials such as a liquid and a solid when an attractive force exists. Fc is calculated for circular capillary tubes here, "r" is the radius of the tube, "γ" is the surface tension (N·m⁻¹) and θ is the contact angle between the liquid and the wall of the tube (White, 1991).

$$Re = \frac{\delta \; V \; D}{\mu}$$

Equation 3. The Reynolds number (*Re*) is dimensionless and gives a measure of the ratio of viscous forces to inertial forces and quantifies the relative importance of them for given flow conditions (White, 1991). "δ" is the density of the fluid (kg/m³); "V" is the mean fluid velocity in (m/s); "D" is the diameter of the cylinder (m) and "μ" is the dynamic viscosity of the fluid (N·s/m²).
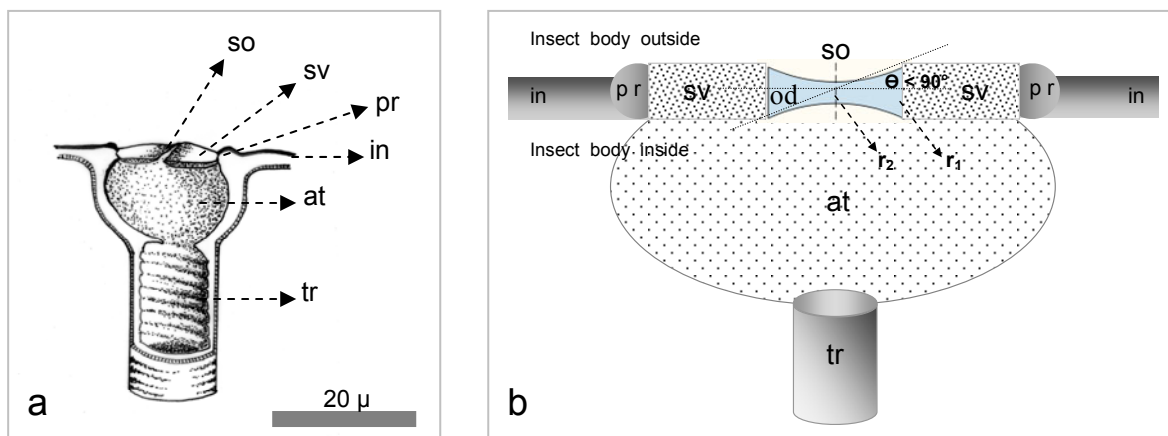
Figure 4. a) Sagital section of the *A. gemmatalis* spiracle. b) Schematic of a sagital section of the *A. gemmatalis* spiracle showing the oil drop forming the "pendular ring" (od) constrained to the slit-like spiracular valve lumen (so), bonded to the margins of the spiracular valve apparatus (sv) [heavy dotted area]; (pr) peritreme; (in) integument; (at) atrium [light dotted area]; (tr) tracheae; θ = contact angle of oil droplet and frontal border of the spiracular valves; (r) radius of curvature.

equation, the flow speed profile inside the tracheae will have a parabolic shape called *"Poiseuille flow"* and the velocity of the liquid will be highest in the centre of the tube, and will be zero at the wall of the tube (White, 1991).

Understanding capillary forces and looking at the anatomy of tracheal systems in lepidopteran larvae, it's easy to envisage how oil behaves under different scenarios when it enters in contact with the insect's body surface. In over-sprayed or PDSOs-dipped insects, oil will pour through the spiracular valves into the atrium and flow into the trachea following the principles of the Washburn equation (equation 1), as long as spiracles are open and an unlimited oil reservoir is present (Krotov and Rusanov, 1999). In contrast, when the mass of oil that reaches the insect body is limited (e.g. being dosed on the insect's body surface or when the caterpillar picks up a drop from the substrate), the fraction reaching the spiracular valve will not flow into the trachea because of the low surface energy between oil and inner tracheal surface. That means that in absence of any external pressure oil can reach the spiracular valve lumen (figures 4a; 4b, *so*), but the atrium (figure 4, *at*) will not fill spontaneously when it comes into contact with oil, because capillary forces at the valve lumen (lower radius) will resist the filling of the atrium (larger radius).

Then, the oil between valves will form a *"pendular ring"* (Finn, 1999) as a result of the contact of two rounded tinny surfaces as the tips of spiracular valves (figure 4b, *od*). There, the capillary pressure in general, can be expressed by the *Laplace* equation (equation 4). This is a more general expression of the pressure difference across a curved interface in a fluid, with the pressure on the concave side greater than that on the convex side, which can be expressed in equation 4.

As the wetting fluid saturation in the *"pendular ring"* is increased, the radii of curvature will be increased, and the capillary pressure will decrease. Vice versa, as the

$$Pc = \gamma\left(1/r_1 + 1/r_2\right)$$

Equation 4. The Laplace equation describes the capillary pressure difference (Pc) sustained across the interface between water and air, due to the phenomenon of surface tension. Where "r1" and "r2" are the two principal radiuses of curvatures of the interface in two perpendicular planes and "γ" is the surface tension (N·m^{-1}) (Finn, 1999).

wetting fluid saturation in the *"pendular ring"* is reduced, the radii of curvature will be reduced (figure 4b, r_1, r_2) and the capillary pressure will increase. Of course, when complex structures like trichomes are present on the spiracular valves (figure 2b) the fluid interface arrangements therein prevent using the above equation to calculate the capillary pressure.

Spiracle anatomy represents in some species one of the main hindrances to tracheal inflow of the oil (e.g. *A. gemmatalis*, figures 2a; 2b; 4a; 4b) and this factor, in conjunction with the lack of affinity between PDSOs and tracheal wall (low wettability, figure 1b; θ ≈ 82.5°), suggest that tracheal inflow of PDSOs is the less probable mode of entry of oils to the insect in field conditions. On the contrary, when insects are over-sprayed or dipped, oils enter promptly in tracheae and tracheoles due to capillary forces following the principles of fluid dynamics. However, due to the low chemical affinity between PDSOs and the tracheal wall, the diffusion of the oil through the trachea into the insect haemolymph is inefficient. To complicate matters further, gas exchange in inner tracheal walls is coupled with water vapor (Kestler, 1984). So, a water layer on the substrate (tracheae) introduces a new variable in the analysis, which definitively affects the value of θ.

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Concluding remarks

The literature available on PDOs has shown that the mode of entry of these substances into the insect body can be via integument as well as through the tracheal system, although the latter was mainly observed when insects were over-sprayed or dipped in oil.

The changes in the cuticle caused by oils, which range from changes in melting point of the cuticular wax layer up to cuticular dewaxing, and the appearance of a "pin hole" effect (Hurst, 1940; Wigglesworth, 1941; 1942; Cloudsley-Thompson, 1950), strongly suggest cuticular penetration of PDSOs occurs. Moreover, effective penetration of PDSOs was corroborated experimentally by Najar-Rodríguez et al. (2008).

It is well established that cuticular permeability depends primarily on the physical properties of cuticle surface lipids and that lipid melting results in greatly increased cuticular permeability in both intact insects and model membranes (Rourke and Gibbs, 1999). Qualitative and quantitative aspects of the dewaxing phenomenon and further penetration of PDSOs will depend mainly on the oil type, the dose administered as well as on the insect species and developmental stage tested. Finally, it´s possible that insect's haemolymph lipophorins (Blacklock and Ryan, 1993) are involved in the diffusion of PDSOs in the insect's body and aid in their transport to lipid-containing tissues, as shown by Taverner et al. (2001).

The insect tracheal system was experimentally confirmed as a route of entry of PDSOs by several authors (Moore and Graham, 1918; Roy et al., 1943; Stadler et al., 1996; Taverner et al., 2001; Sugiura et al., 2008). However, rather than blocking the tracheae, oil coats the inner surface as shown in figure 1a, where the air-liquid interface inside the main tracheal tubes can be clearly identified. This tracheal "coating" by the oil is not continuous as small droplets appear aligned inside larger tracheae due to interfacial tension (figure 1a, 1b) (Stadler et al., 1996). In contrast, tracheoles can become blocked by oil, depending on the tracheal radius and oil viscosity. Tracheal inflow by oils is a pure physical event which is independent of insect physiology and can be described by the Poiseuille equation (Tschapeck, 1961). Depending upon the contact angle defined by the interfacial surface energies of the solid-liquid-gas interface, fluids will either be drawn into or rejected from the tracheoles (figure 1c, 1d). For contact angles smaller than ninety degrees, the distance travelled by the fluid in the capillary tube is a function of the capillary dimension, and the properties of the fluid. For contact angles greater than ninety degrees, the force required for the fluid to move up the capillary is a function of the capillary dimension, and the properties of the fluid. Even in contact with larger doses of PDSOs, the formation of a "pendular ring" at the spiracular area (figure 4b) will prevent the inflow of the oil and spiracular blockage.

The literature reviewed suggests that the mode of entry of PDSOs to the insect body is through integument as well as through the tracheal system, although our in depth analysis of this from a physics perspective suggest the latter occurs only when insects are over-sprayed or dipped in oil. Tracheal blockage is not expected to be a phenomenon extensive to every oil class and insect species due to the relationship between physical properties of oils, and the dimensions of insect tracheae. The penetration of PDSOs through tracheal walls seems to be less relevant than the penetration through the integument due to its poor wettability by PDSOs. The large contact angle (figure 1b; $\theta \approx 82.5°$) is an unambiguous evidence for low affinity between these two media, denoting that oil does not diffuse easily through the tracheal wall into the insect body. Thus, the trachea is not the fastest and most efficient way of entry for PDSOs to the insect body. When oil comes in contact with the insect's body surface, capillary forces and complex physical interactions take place. Cuticular penetration of PDSOs, which causes physical changes in the permeability and melting point of the cuticle that lead to desiccation and penetration of the insecticide to other tissues, seems to be the most likely mode of entry of PDSO in the insect body.

References

ADAMSON A. W., GAST A. P., 1997.- *Physical chemistry of surfaces. 6th Ed.-* John Wiley & Sons Inc., New York, USA.

AGNELLO A. M., 2002.- Petroleum-derived spray oils: chemistry, history, refining and formulation, pp. 2-18. In: *Spray oils beyond 2000* (BEATTIE A., WATSON D., STEVENS M., RAE D., SPOONER-HART R., Eds).- University of Western Sydney, Hawkesbury, Australia.

ASTM, 1997: ASTM D 2887 - Standard Test Method for Boiling Range Distribution of Petroleum Fractions by Gas Chromatography.

BEAR J., 1972.- *Dynamics of fluids in porous media.-* Elsevier, New York, USA.

BEATTIE G., LIU Z., WATSON D., CLIFT A., JIANG L., 1995.- Evaluation of PSOs and polysaccharides for control of *Phylocnistis citrella* Stainton (Lepidoptera: Gracillaridae).- *Journal of Australian Entomological Society*, 34: 349-353.

BLACKLOCK B. J., RYAN R. O., 1993.- Structure and function of *Manduca sexta* haemolymph lipid transfer particle, pp. 25-43. In: *Insect lipids: chemistry, biochemistry and biology* (STANLEY D. W., STANLEY-SAMUELSON D. W., NELSON D. R., Eds).- University of Nebraska Press, Lincoln, USA.

BÖRÖCZKY K., PARK K. CH., MINARD R. D., JONES T. H., BAKER T. C., TUMLINSON J. H., 2008.- Differences in cuticular lipid composition of the antennae of *Helicoverpa zea*, *Heliothis virescens*, and *Manduca sexta*.- *Journal of Insect Physiology*, 54: 1385-1391.

CHAPMAN P. J, RIEHL L. A., PEARCE G. W., 1952.- Oil sprays for fruit trees.- *United States Department of Agriculture Yearbook of Agriculture*, 229-239.

CHAPMAN R. F., 1998.- *The insects structure and function, 4th ed.-* Cambridge University Press, UK.

CHILDERS C. C., 2002.- Practical use of horticultural mineral oils in integrated pest and disease management programs and their impact on natural enemies, pp. 332-348. In: *Spray oils beyond 2000* (BEATTIE A., WATSON D., STEVENS M., RAE D., SPOONER-HART R., Eds).- University of Western Sydney, Hawkesbury, Australia.

CLOUDSLEY-THOMPSON J. L., 1950.- The water relations and cuticle of *Paradesmus gracilis* (Diplopoda, Strongylosomidae).- *Quarterly Journal of Microscopical Science*, 91: 453-464.

DE LICASTRO S. A., ZERBA E. N., CASABE N., 1983.- The relation between viscosity and penetration of some diethyl p-substituted phenyl phosphorothionates and oil carriers into the cuticle of *Triatoma infestans*.- *Pesticide Biochemistry and Physioogy*, 19: 53-59.

DE ONG E. R., KNIGHT H., CHAMBERLAIN J. C., 1927.- A preliminary study of petroleum oil as an insecticide for citrus pests.- *Hilgardia*, 2: 351-384.

DE RENOBALES M., NELSON D. R., BLOMQUIST G. J., 1991.- Cuticular lipids, pp. 242-253. In: *Physiology of the insect epidermis* (BINNINGTON K., RETNAKARAN A., Eds).- CSIRO Publications, East Melbourne, Australia.

DAVIDSON N., DIBBLE J., FLINT M., MARER P., GUYE A., 1991.- *Managing insects and mites with spray oils*.- University of California. Special Publication 3347.

DEKKER M. H. A., PIERSMA T., DAMSTÉ J. S. S., 2000.- Molecular analysis of intact preen waxes of *Calidris canutus* (Aves: Scolopacidae) by gas chromatography/mass spectrometry.- *Lipids*, 35: 533-541.

DOWNER R. A, HALL F. A., ESCALLON E. C., CHAPPLE A. C., 1993.- The effect of diluent oils on the electrostatic atomisation of some insecticides, pp. 203-215. In: *Pesticide formulations and application systems* (BERGER P. D., DIVERSETTI B. N., HALL F. R., Eds).- American Society for Testing and Materials, Philadelphia, USA.

EDNEY E. B., 1977.- *Water balance in land arthropods*.- Springer, Berlin-New York.

FINN R, 1999.- Capillary surface interfaces.- *Notices of the AMS*, 46 (7): 770-781.

FONTAN A., ZERBA E, 1987.- Mode of entry of insecticides in *Triatoma infestans*.- *Archives of Insect Biochemistry and Physiology*, 4: 313-323.

GEROLT P., 1983.- Insecticides – their route of entry, mechanism of transport and mode of action.- *Biological Review of the Cambridge Philosophical Society*, 58: 233-274.

GIBBS A. G., 1995.- Physical properties of insect cuticular hydrocarbons: model mixtures and lipid interactions.- *Comparative Biochemistry and Physiology*, 112: 667-672.

GIBBS A. G., POMONIS J. G., 1995.- Physical properties of insect cuticular hydrocarbons: the effects of chain length, methyl-branching and unsaturation.- *Comparative Biochemistry and Physiology*, 112: 243-249.

GIBBS A. G., 1998.- Water-proofing properties of cuticular lipids.- *American Zoologist*, 38: 471-482.

GIBBS A. G., 2002.- Lipid melting and cuticular permeability: new insights into an old problem.- *Journal of Insect Physiology*, 48: 391-400.

GIBBS A. G., FUKUZATO F., MATZKIN M., 2003.- Evolution of water conservation mechanisms in *Drosophila*.- *The Journal of Experimental Biology*, 206: 1183-1192.

GORB S. N., 2001.- *Attachment devices of insect cuticle*.- Kluwer Academic Publishers, Dordrecht, Boston, London.

HÄGG G., 1969.- *General and inorganic chemistry*.- John Wiley & Sons Inc., New York, USA.

HERZNER G., STROHM E., 2008.- Food wrapping by females of the European Beewolf, *Philanthus triangulum*, retards water loss of larval provisions.- *Physiological Entomology*, 33: 101-109.

HOWARD R. W., 1993.- Cuticular hydrocarbons and chemical communication, pp. 179-226. In: *Insect lipids: chemistry, biochemistry and biology* (STANLEY D. W., STANLEY-SAMUELSON D. W., NELSON D. R., Eds).- University of Nebraska Press, Lincoln, USA.

HURST H., 1940.- Permeability of Insect Cuticle.- *Nature*, 145: 462-463.

KESTLER P., 1984.- Respiration and respiratory water loss, pp. 137-183. In: *Environmental physiology and biochemistry of insects* (HOFFMAN K. H., Ed.).- Springer Verlag, New York, USA.

KROTOV V. V., RUSANOV A. I., 1999.- *Physicochemical hydrodynamics of capillary systems*.- Imperial College Press, London, UK.

KUHLMANN B., JACQUES D., 2002.- Classifications, standards and nomenclature-mineral oils, agricultural mineral oils and horticultural mineral oils, pp. 29-38. In: *Spray oils beyond 2000* (BEATTIE A., WATSON D., STEVENS M., RAE D., SPOONER-HART R., Eds).- University of Western Sydney, Hawkesbury, Australia..

LOCKEY K. H., 1988.- Review. Lipids of the insect cuticle: origin, composition and function.- *Comparative Biochemistry and Physiology*, 89B: 595-645.

MARCOLLI C., LUO B. P., PETER T., 2004.- Mixing of the organic aerosol fractions: Liquids as the thermodynamically stable phases.- *Journal of Physical Chemistry*, 108A: 2216-2224.

MATSUMURA F., 1975.- *Toxicology of insecticides*.- Plenum Press, New York, USA.

MENSAH R., HARRIS W., BEATTIE G., 1995.- Response of *Helicoverpa spp.* and its natural enemies to petroleum spray oils in cotton.- *Entomophaga*, 40: 263-272.

MONTOOTH K. L., GIBBS A. G., 2003.- Cuticular pheromones and water balance in the house fly, *Musca domestica*.- *Comparative Biochemistry and Physiology*, 135A: 457-465.

MOORE W., GRAHAM S. A., 1918.- Physical properties governing the efficacy of contact insecticides.- *Journal of Agricultural Research*, 13: 523-538.

MULROONEY J. E., WOMAC A. R., GREEVER J. C., 1993.- The influence of carrier oil viscosity on the transfer of bifenthrin from cotton to tobacco budworm larvae.- *Southwestern Entomology*, 18 (2): 91-100.

NAJAR-RODRÍGUEZ A. J., LAVIDIS N. A., MENSAH R. K., CHOY P. T., WALTER G. H., 2008.- The toxicological effects of petroleum spray oils on insects – Evidence for an alternative mode of action and possible new control options.- *Food and Chemical Toxicology*, 46: 3003-3014.

NELSON D. R., LEE R. E., 2004.- Cuticular lipids and desiccation resistance in overwintering larvae of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae).- *Comparative Biochemistry and Physiology. B, Biochemistry & Molecular Biology*, 138: 313-320.

NOBLE-NESBITT J, 1970.- Structural aspects of penetration through insect cuticles.- *Pesticide Scence*, 1 (5): 204-208.

PENNISTON J. T., BECKETT L., BENTLEY D. L., HANSCH C., 1969.- Passive penetration of organic compounds through biological tissue: Non-steady state theory.- *Molecular Pharmacology*, 5: 333-341.

RIEHL L. A., 1969.- Advances relevant to narrow-range spray oils on citrus pest control, pp. 897-907. In: *Proceedings of the First International Citrus Symposium*, Vol. 2.- Riverside, California, USA.

ROURKE B. C, GIBBS A. G., 1999.- Effects of lipid phase transitions on cuticular permeability: model membrane and in situ studies.- *Journal of experimental Biology*, 202: 3255-3262.

ROURKE B. C., 2000.- Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*.- *Journal of experimental Biology*, 203: 2699-2712.

ROWLINSON J. S., FREEMAN P. I., 1961.- Lower critical solution points in hydrocarbon mixtures.- *Pure and Applied Chemisry*, 2 (1-2): 329-334.

ROY D. N., GHOSH S. M., CHOPRA R. N., 1943.- The mode of action of pyrethrins in the cockroach, *Periplaneta americana* L.- *Annals of Applied Biology*, 30: 42-47.

SCHAL C., SEVALA V., CAPURRO M., SNYDER T. E., BLOMQUIST G. J., BAGNÈRES A. G., 2001.- Tissue distribution and lipophorin transport of hydrocarbons and sex pheromones in the house fly, *Musca domestica*.- *Journal of Insect Science*, 1 (12): 1-11. [online] URL: http://insectscience.org/1.12/.

SMITH E. H., PEARCE G. W., 1948.- The mode of action of petroleum oils as ovicides.- *Journal of Economic Entomology*, 41: 173-180.

SMITH J. E., 2003.- Capillary rise of soil water, pp. 861-864. In: *Encyclopedia of water science* (STEWART B. A., HOWELL T. A., Eds).- Marcel Dekker, New York, USA.

STADLER T., FORNÉS A., BUTELER M., 2005.- Interfacial forces and permeation of the codling moth cocoon silk.- *Bulletin of Insectology*, 58 (1): 57-64.

STADLER T., SCHANG M. M., ZERBA E., 1996.- Caracterización fisicoquímica y toxicológica de algunos aceites minerales de uso fitosanitario.- *Revista RIA*, 27 (1): 67-80.

SUGIURA M., HORIBE Y., KAWADA H., TAKAGI M., 2008.- Insect spiracle as the main penetration route of pyrethroids.- *Pesticide Biochemistry and Physiology*, 91 (3): 135-140.

TAVERNER P. D., GUNNING R. V., KOLESIK P., BAILEY P. T., INCEOGLU A. B., HAMMOCK B., ROUSH T., 2001.- Evidence for direct neural toxicity of a "light" oil on the peripheral nerves of lightbrown apple moth.- *Pesticide Biochemistry and Physiology*, 69: 153-165.

TAVERNER P. D., 2002.- Drowning or just waving? A perspective on the ways petroleum-based oils kill arthropod pests of plants, pp. 78-87. In: *Spray oils beyond 2000* (BEATTIE A., WATSON D., STEVENS M., RAE D., SPOONER-HART R., Eds).- University of Western Sydney, Hawkesbury, Australia.

TENAN M. A., HACKWOOD S., BENI G., 1982.- Friction in capillary systems.- *Journal of Applied Physics*, 53: 6687-6692.

TREACY M. F., BENEDICT J. H., SCHMIDT K. M., ANDERSON R. M., 1991.- Mineral Oil: enhancement of field efficacy of a pyrethroid insecticide against the boll weevil (Coleoptera: Curculionidae).- *Journal Economic Entomology*, 84 (2): 659-663.

TSCHAPEK M. W., 1961.- *Water and its condition in soil*.- Research Bulletin Special Report. Manuales de Ciencia Actual. Consejo Superior de Investigaciones Científicas Instituto José María Albareda de Edafología y Biología Vegetal. Madrid, Spain.

WALSTRA P., 2003.- *Physical chemistry of foods*.- Marcel Dekker, New York, USA.

WASSERTHAL L. T., 1996.- Interaction of circulation and tracheal ventilation in holometabolous insects.- *Advances Insect Physiology*, 26: 297-351.

WEBB R. E., MCALLEN W. H., FINNEY J. A., VENABLES L., WHITE G. B., WIEBER A. M., COHEN D. L., 1994.- Destruction of gypsy moth egg masses (using surfactants, detergents, oils or conventional insecticides) for quarantine and community action programs.- *Journal of Entomological Science*, 29 (3): 305-317.

WESTNEAT M. W., BETZ O., BLOB R. W., FEZZAA K., COOPER W. J., WAH-KEAT L., 2003.- Tracheal respiration in insects visualized with synchrotron X-ray imaging.- *Science*, 299 (5606): 558-560.

WHITE F. M., 1991.- *Viscous fluid flow, 2nd edition*.- McGraw Hill, New York, USA.

WIGGLESWORTH V. B., 1941.- Oils aiding loss of water from the cuticle.- *Nature*, 147: 116.

WIGGLESWORTH V. B., 1942.- Some notes of the integument of insects in relation to the entry of contact insecticides.- *Bulletin of Entomological Research*, 5: 61-65.

WIGGLESWORTH V. B., 1945.- Transpiration through the cuticles of insects.- *Journal of Experimental Biology*, 21: 97-114.

WIGGLESWORTH V. B., 1957.- The physiology of insect cuticle.- *Annual Review of Entomology*, 2: 37-54.

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