The physical action of three diatomaceous earths against the cuticle of the flour mite *Acarus siro* L. (Acari: Acaridae)

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Abstract: Experiments examined the accepted modes of action of the desiccant dust, diatomaceous earth (DE), against the flour mite, *Acarus siro* (L.) (Astigmata). Adult mites were exposed to three DE products for periods of 3, 18 and 72 h under conditions designed to allow partial desiccation of the mites without causing mortality. After exposure, the DE dust particles were washed off the mite bodies, and both the DE and the mites were examined for presence of cuticular hydrocarbons by gas chromatography–mass spectroscopy (GC-MS) analysis. GC-MS identified seven major cuticular lipids of chain length C_{13}–C_{26} that may have a role in the waterproofing of *A. siro*. After 18 h exposure, *n*-tridecane and several different long-chain fatty acid ethyl esters were detected on one of the DE products. After 72 h, *n*-tridecane was detected on all three DE products. Mite samples retained after removal of the DEs were examined by microscopy. Tentative evidence was observed by conventional low-power microscopy that might indicate uniform removal of the epicuticle. However, a detailed examination by scanning electron microscopy showed no signs of abrasion.

Keywords: astigmatid mites; *Acarus siro*; inert dust; diatomaceous earth; lipid; cuticle; mode of action

1 INTRODUCTION

Over the last decade there has been a resurgence of worldwide interest in using diatomaceous earth (DE) for management of arthropods associated with stored products. This naturally occurring material is mined from geological deposits of fossilised diatoms and is composed of over 90% silica. DE works against invertebrate pests as a desiccant and, owing to its inert nature, is seen as a benign alternative to chemical insecticides. Benefits include low mammalian toxicity, no harmful residues, persistence and stability at high and low temperatures.[1,2] DE products are registered for storage use in a number of countries including the USA, Canada, Australia, Japan, Indonesia, Germany, Saudi Arabia and Brazil. Since 2001, DE has received industry uptake in the UK for grain storage protection, with the launch of several products. Under UK national guidelines it is permissible to market DE as a plant protection product without the need for formal registration owing to its accepted mode of action, under an exemption as set out in the Control of Pesticides Regulations, UK, COPR 1986 regulation 3.[2] Nevertheless, the UK Pesticide Safety Directorate still requires notification of the intended use. However, this will change as the EU Plant Protection Products Directive (PPPD), 91/414/EEC, supersedes national regulations and harmonises registration across the EU. In the UK, industry guidelines for the use of DEs under damp maritime climate conditions are available through the Home Grown Cereals Authority's grain storage guide[3] and topic sheets,[4–6] which are based on field-validated research for use as a grain top-dressing when combined with aeration for cooling,[7] and for structural treatments.[8]

With regard to mode of action, it is widely accepted that DE causes desiccation by removing the waterproofing components of the epicuticle. Ebeling[9] stated 'Diatomaceous earths are both abrasive and slightly sorptive, and both types of action can result in desiccation when insects crawl among dust-coated kernels, thus rubbing all parts of their bodies against films of dust'. The original formulations of DE were believed to work primarily by abrasion, since the specific surface area of dust particles was small compared with the more sorptive silica aerogels. Modern formulations, based on better selection of particles and new sources of DE deposits, are believed to offer much greater adsorption of lipids, with workers considering oil-carrying capacity as a key predictor of dust efficacy.[10] Some formulations, referred to as enhanced DEs, are coated with silica aerogels, or have silica added, in order to increase this.[11] However, the present authors can find little work to demonstrate this absorptive action through direct measurement of lipids transferred directly from the cuticle onto DE particles, with virtually all previous studies concentrating on indirect indicators as aforementioned. There has been no full study at all on the mode of action of DE

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against stored product mites (order Astigmata), which are the primary target of these treatments in the UK. Preliminary mode of action work at this laboratory with the DE 'Dryacide' had not shown any abrasion to the cuticle, or ingestion of the dust, for the mites *Acarus siro* (L.) or *Lepidoglyphus destructor* (formerly *Glycyphagus destructor*) (Schrank) Cook and Armitage.[12] It was therefore proposed that DE probably works against these mites by cuticular lipid absorption.

Building upon this, in the present study, lipid analysis by gas chromatography–mass spectroscopy (GC-MS) and imaging by scanning electron microscopy (SEM) have been used further to elucidate the effects of DE on the cuticle of *A. siro*. In addition, a secondary aim of the study was to develop a better understanding of the mite cuticle which in turn can be exploited as a target for future generations of acaracides. The study comprised method development as well as the results reported here.

2 MATERIALS AND METHODS

2.1 Diatomaceous earths

Three DEs were tested:

1. Silico-Sec – >90% SiO_2, Biofa GmbH, Germany.
2. Protect-It – >90% SiO_2 'enhanced' by addition of silica gel (10% by weight), Hedley Technologies Inc., Canada.
3. Dryacide – 97% SiO_2 'enhanced' by coating with a silica aerogel (3% by weight) and 2% fluoride adjuvant, Dryacide Australia Pty Ltd.

All had been obtained from the manufacturers during earlier studies in 2001, 1998 and 1994 respectively, therefore allowing direct comparison with previous work. However, they do not necessarily represent current formulations (source of DE, particle size, etc.). Of these, only Silico-Sec is marketed in the UK. The precise oil-carrying capacity of these products was not available, although, in view of the added silica,[1] it is likely that they rank Protect-It > Dryacide > Silico-Sec.

2.2 Preparation of mites

Adults of an insecticide-susceptible strain of *A. siro* (strain 9528/2) were reared in large quantities using existing CSL methodologies (unpublished). Adult mites were 0–2 weeks old when used and were separated from the known aged cultures by sieving gently over a 500 μm mesh sieve. These mites were then tipped gently onto a 15 cm diameter Whatman No. 1 filter paper and left for sufficient time for the mites to cling to the paper. This paper was then inverted at a 45° angle above a second paper and gently tapped so that food and frass contaminants were removed from the mites. The mites were then examined under a low-powered binocular microscope and, if visibly clean, transferred into a holding flask with a sharp tap to the underside of the paper. This procedure was repeated on the food and frass fraction several times until all adult mites were removed. Visual examination immediately afterwards confirmed that the specimens were clean and undamaged.

2.3 DE treatment

For each experiment, 9 cm diameter glass petri dishes were treated by hand sieving either Dryacide, Protect-It or Silico-Sec DE at 10 g m^{-2} through a 250 μm wire mesh sieve onto the base of the dish. An additional dish was left untreated as a control. Adult *A. siro* that had been carefully removed from culture as described in Section 2.2 were added at 0.1 g per dish. An estimate of the number of mites contained in each 0.1 g aliquot was made by counting the number of adults in a 0.01 g sample under a low-powered binocular microscope. This gave an estimation of approximately 10 500 mites per replicate ($n = 3$ per run). The quantity of mites needed to allow detection of cuticular hydrocarbons had been determined from earlier experiments. The experiments were carried out under conditions designed to allow partial desiccation of the mites without causing mortality. Owing to the considerable time required to set up each experiment, a single replicate each of control and DE products for each exposure time was assessed on a separate occasion. This gave a total of nine experiments, consisting of three replicates each for either 3, 18 or 72 h exposure at constant conditions of 15 °C and 80% RH.

2.4 Separation of DE from mites

After exposure, the contents of each petri dish were rinsed onto a 100 μm stainless steel mesh placed in a Buchner funnel, using deionised water. The water was removed under vacuum. The mesh retained the mites while allowing the DE particles to pass through. The mesh was then removed from the funnel and placed into a clean petri dish. Immediately afterwards, the mites were checked using a low-powered binocular microscope to ensure that the fragile bodies had not been damaged during washing and that little, if any, of the DE remained. The mesh and retained mites were then subjected to solvent extractions. The DE/water suspension was collected during the mite washing and then filtered through a preweighed 9 cm diameter Whatman No. 6 filter paper (3 μm) to collect the DE. Solvent extractions were also carried out on the filter papers/retained DE. The mite replicates that had not been exposed to DEs were subjected to the same separation procedure for comparison.

2.5 Solvent extractions

Preliminary experiments had shown that extraction using chloroform gave larger amounts of the cuticular hydrocarbons than pentane, and chloroform was therefore used for the rest of this study. Typical chromatographic profiles with both extraction solvents are shown in Fig. 1. It was also found that all of the hydrocarbons could be extracted using a 15 min extraction period.

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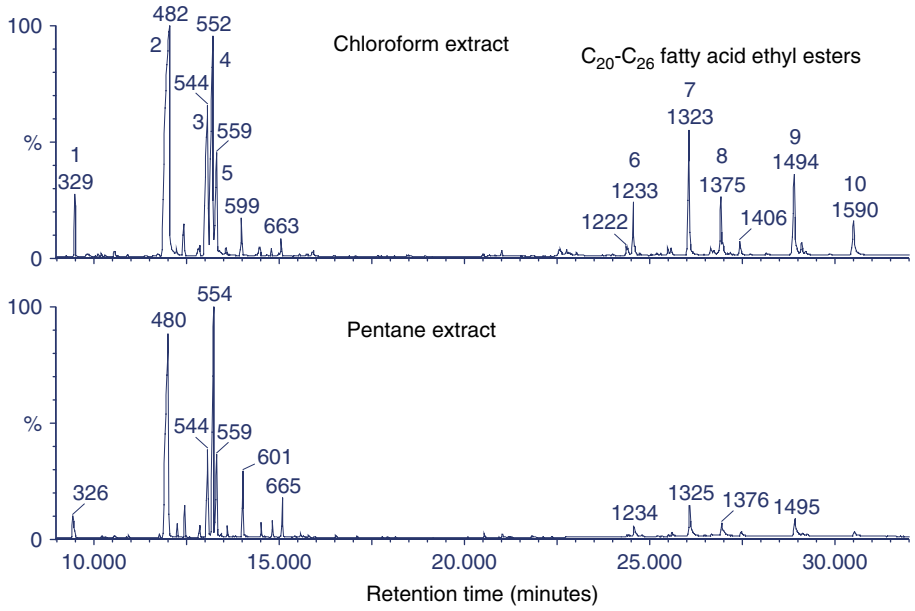

Figure 1. Comparisons of GC profiles for 0.1 g untreated *Acarus siro* extracts after 5 min soaking in either chloroform or pentane. Peak **1**, phenylacetaldehyde; peak **2**, 2-hydroxy-6-methylbenzaldehyde; peak **3**, unsaturated hydrocarbon; peak **4**, *n*-tridecane; peak **5**, unidentified aromatic aldehyde; peak **6**, C20:1 FAEE; peak **7**, C22:2 FAEE; peak **8**, C23:2 FAEE; peak **9**, C25:2 FAEE; peak **10**, C26:2 FAEE.

One day prior to extraction, the chloroform was purified by passing through a 20 cm column of neutral alumina (100–250 mesh, 30 mm internal diameter). Three-stage 15 min extractions were made for each sample by soaking samples in successive 2 mL aliquots of fresh solvent for 5 min at a time. For each sample the three solvent extracts were then combined and filtered through a Whatman No. 1 filter paper into a glass microreaction vessel (Supelco), to ensure no carryover of mite bodies or DE particles. This gave single 6 mL solvent extracts for each sample. The filter papers used to collect the DE were then air dried and reweighed to allow the amount of DE recovered to be calculated. A clean stainless steel mesh, clean filter papers, 0.001g of mite food, solvent and samples of each DE were also subjected to extraction, to act as controls. Each sample was then concentrated to 50 µL using a stream of dry argon prior to analysis.

The cuticular hydrocarbons were analysed by GC-MS on a Hewlett Packard 5890 series II GC coupled to a VG Trio-1 mass spectrometer. Injections (1 µL) were made using a Leap Combi-Pal autosampler in splitless mode (purge on after 1 min) at 280 °C onto a 50 m Chrompack CP-Sil 8CB capillary column (0.25 mm ID, 0.25 µm film thickness). The GC was operated in constant-flow mode at 15 psi at 50 °C. The oven temperature was initially 50 °C (1 min hold), then programmed to 310 °C at 10 °C min^{-1} and held at this temperature for 30 min. The mass spectrometer was operated in electron impact mode (EI+) at 70 eV and scanned from 33–550 amu once every second. The mass spectrometer source and interface temperatures were held at 200 °C and 310 °C respectively.

The treated mites, untreated mites and DE extractions were compared to show whether the lipids were absorbed from the cuticle.

The concentrations of the cuticular hydrocarbons present in the samples were calculated by comparison with injections of external standard solutions of *n*-docosane, $C_{22}H_{46}$ (100 ng µL), in chloroform.

Standards of tridecane, ethyl hexadecanoate, ethyl octadecanoate, ethyl (*Z*)-9-octadecenoate and ethyl eicosanoate (Aldrich Chemical Co.) were also analysed by GC-MS to confirm their presence in chloroform extract of *A. siro*.

Ethyl (*Z*)-11-eicosenoate (C20:1 FAEE) was synthesised from (*Z*)-11-eicosenoic acid using excess of ethanol and a trace of concentrated sulfuric acid. Ethyl (*Z*, *Z*)-13,16-docosadienoate was also synthesised from (*Z*, *Z*)-13,16-docosadienoic acid using the same method. Both esters were analysed by GC-MS to determine if they were present in *A. siro*.

2.6 Analysis of abrasion using SEM

Treated and untreated replicates were set up and the DE washed off the mites as described in Section 2.4. A minimum of ten mites from each replicate were examined for signs of abrasion using cryo-SEM at −180 °C on a Philips XL20 SEM.

3 RESULTS

3.1 Lipid absorption after 3 and 18 h exposure

Examination of the control samples showed no contaminating peaks in any of the areas of interest. The cuticular hydrocarbon *n*-tridecane, and both saturated and unsaturated fatty acid ethyl esters (FAEEs) with fatty acid chain lengths of C_{16}–C_{28}, were identified from extracts of the bodies of the treated and untreated mites (Table 1). None of these cuticular hydrocarbons was detected from DE removed from the mites after 3 h exposure. After 18 h, tridecane and several of the

Table 1. Comparison of cuticular hydrocarbons identified for *Acarus siro* compared with those found in *Acarus immobilis*

Acarus siro ($n = 6$)	*Acarus immobilis*[a]
C_{13}; *n*-tridecane[b]	C_{13}; *n*-tridecane
C_{13}; tridecene[b]	
C_{14}; *n*-tetradecane	C_{14}; *n*-tetradecane
C_{15}; *n*-pentadecane	C_{15}; *n*-pentadecane
C_{17}; *n*-heptadecane	C_{17}; *n*-heptadecane
	(*Z*)-8-C_{17}; (*Z*)-8-heptadecene
	(*Z*, *Z*)-6, 9-C_{17}; (*Z*, *Z*)-6,9-heptadecadiene
	C_{25}; *n*-pentacosane
	C_{26}; *n*-hexacosane
	C_{27}; *n*-heptacosane
	C_{28}; *n*-octacosane
	C_{29}; *n*-nonacosane
Ethyl hexadecanoate (C16:0 FAEE)	
Ethyl octadecanoate (C18:0 FAEE)	
Ethyl (*Z*)-9-octadecenoate (C18:1 FAEE)	
Ethyl icosanoate (C20:0 FAEE)	
C20:1;[b] C22:2;[b] C23:2;[b] C25:2;[b] C26:2;[b] C28:2[b] FAEEs	

[a] From reference 15.
[b] Major cuticular hydrocarbon components of *Acarus siro*.

main FAEEs from the mites were detected from two of the three Protect-It replicates. The amounts of tridecane from the various DE treatments were only approximately 0.2% w/w compared with that found on the control mites, and there was not enough of the other two FAEEs to allow quantification.

3.2 Lipid absorption after 72 h exposure

Lengthening of the exposure time to 72 h increased the amount of cuticular lipids extracted. Again, examination of the control samples revealed no contaminating peaks in the areas of interest, with the exception of the DE controls where small quantities of tridecane were found on each of the three DE products. Again, cuticular hydrocarbons of carbon chain length $C_{13}-C_{28}$ were identified from the bodies of the mites. However, amounts of tridecane along with three other compounds (two aromatic aldehydes and one aromatic alcohol) were found on all of the DE products after being washed off the treated mites (Table 2). Quantities of *n*-tridecane from these samples compared with the DE controls were ca 4 times greater, indicating that this was a genuine artefact of the experiment. After adjustment for the controls, these quantities varied between 0.2 and 0.3% w/w compared with that found on the untreated mites.

Comparison of filter-paper weights before and after collection of the DE showed a mean DE recovery from the washings of 93% (range 74–100%) for the three exposures ($n = 27$).

Ethyl (*Z*)-11-eicosenoate (C20:1 FAEE) was found to be present in mite extracts as a major component, peak 6 (Fig. 1), but ethyl (*Z*, *Z*)-13,16-docosadienoate was not found in mite extracts. Ethyl (*Z*, *Z*)-13,16-docosadienoate was considered to be the most likely candidate compound for peak 7.

Ethyl hexadecanoate, ethyl octadecanoate, ethyl *Z*-9-octadecenoate and ethyl eicosanoate (C16:0, C18:0, C18:1 and C20:0 FAEEs respectively) were also found to be present in the mite extracts, but only as minor components (Table 1).

3.3 Analysis of abrasion using SEM

SEM analysis of 200 images did not show the DEs causing any obvious signs of abrasion. However, when additional mites from the same samples were viewed using conventional low-powered microscopy, the DE appeared to have removed the shiny coating normally observed on untreated mites. This may have been indicative of uniform removal of the epicuticle containing the lipids.

4 DISCUSSION

A comprehensive summary of the cuticular structure for *Acari* can be found in Evans,[13] although there is little detail available regarding the cuticle of astigmatid mites. For astigmatid mites the cuticle is the main respiratory surface and plays a major role in osmoregulation, since these mites lack an organised respiratory system, and transpiration occurs by diffusion directly through this surface. Hughes[14] states that the cuticle is composed of a thin exocuticle, covered by an epicuticle, lying directly above the epidermis. A distinct chitinous endocuticle is missing, except where the exocuticle is thickened to form the ring-shaped sockets of sensory setae. Previously, only *n*-tridecane had been identified as a cuticular

Table 2. Concentrations (ng μL^{-1}) of compounds identified on DE washed off treated mites after 72 h exposure

	(a) Tridecane from washings	(b) Tridecane on DE controls[a]	Tridecane (a − b)	2-Hydroxy-6-methyl-benzaldehyde	Phenyl-acetaldehyde	2-Phenyl-ethanol
Control mites[b]	430.2	n/a	430.2	587.4	33.4	18.4
Dryacide	2	0.57	1.43	0.13	0.06	0.012
Silico-Sec	1.57	0.25	1.32	0.02	0.035	0.001
Protect-It	1.22	0.58	0.64	0.012	0.017	0.037

[a] Solvent extractions on DE with no exposure to mites.
[b] Solvent extractions from untreated mites.

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hydrocarbon of *A. siro*.[15] Although the function of this compound is unknown, it has been suggested that it may serve as a solvent for the semiochemicals such as neral and neryl formate that are produced by some mite species.[16]

In the present study, three major volatile, non-lipid-like components were identified: phenylacetaldehyde, 2-hydroxy-6-methylbenzaldehyde and an unidentified aromatic aldehyde. Five of the seven major cuticular hydrocarbon components were also putatively identified and two positively identified: tridecane and ethyl *Z*-11-eicosenoate, peaks 4 and 6 (Fig. 1). Two of these identified major components (*n*-tridecane and an unsaturated hydrocarbon of the same chain length) are of identical chain length to one found in *Acarus immobilis* Griffiths.[15] The other five were esters of long-chain fatty acids $>C_{20}$. 'Conventional wisdom' dictates that these longer-chain lipids ($>C_{20}$) are the most likely to be responsible for waterproofing of the cuticle, as longer-chain hydrocarbons give more protection against water loss and are non-volatile, so are not constantly lost through evaporation, as is *n*-tridecane. However, all the major cuticular components detected are liquids at room temperature.

The results reported here are important additions to current knowledge. The cuticle has been identified as one of several potential physiological targets for the development of new acaracides. Not only is the cuticle an important site for osmoregulation/respiration but it is also an anchorage for muscles and protects against physical damage and penetration of pathogens.[17] For example, a current study at this laboratory is looking to develop an entopathogenic fungal-based biocontrol agent, and such information is useful when deciding upon formulation.

The only cuticular lipid detected in significant amounts on all of the DE products that had been rinsed off the mites was tridecane (Table 2). This was perhaps not surprising, as this is the most abundant lipid available for uptake (Fig. 1). Only in one case did DE appear to absorb a wider range of *A. siro* lipids. It is interesting that these were only found on Protect-It, which was marginally the most effective DE product against mites in an earlier study.[18] It was surprising that these two longer-chain lipids (C23:2 and C25:2 FAEEs) were not subsequently redetected at the longest exposure, but this may indicate that recovery of these was near the limit of detection of the method. In contrast, medium chain-free fatty acids – palmitic acid, stearic acid, oleic acid and linoleic acid – have been detected by other workers on DE removed from the bruchids *Callosobruchus maculatus* (F.) and *Acanthoscelides obtectus* (Say) after only 6.5 h exposure at 25 °C, extracted in hexane and analysed by gas chromatography.[19] However, the amount of lipids available for extraction from the bruchids would have been very much higher, and it may have been that the higher temperature in this study also had an effect, increasing the rate at which the lipids were removed.

As in a previous study,[12] there were no obvious signs of puncture marks, gouges or other signs of overt damage caused by the DE. However, it is possible that the thin epicuticle may have been uniformly worn down or removed, and this would not have been obvious unless viewed as a section. Further work is needed to confirm any abrasive action of the DE, using thin-layer electron microscopy (TEM).

However, this study does raise questions about the role of tridecane. With the lack of other evidence to the contrary, and regardless of its volatile nature, it may play a role as a waterproofing agent. This is further supported by the fact that the pheromone 2-hydroxy-6-methylbenzaldehyde,[16,20] which was present in greater quantities in the mites, was only absorbed at 1.5–10% as much as tridecane. If tridecane does have this role, then it could be concluded that DE has a similar mode of action against mites as hypothesised for insects. More in-depth studies are needed to support these results.

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