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Inert dusts and their effects on the poultry red mite (*Dermanyssus gallinae*)

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Abstract The haematophagous poultry red mite (*Dermanyssus gallinae*) is the most important pest of egg laying hens in many parts of the world. Control has often relied on chemical pesticides, but inert dusts, which are thought to kill target hosts primarily by desiccation, have become one of the most commonly applied alternative control methods for poultry red mite in Europe. This development has occurred despite a lack of knowledge of the efficacy of the different types of inert dusts and how this is affected by environmental parameters, e.g. the high relative humidity found in poultry houses. In this laboratory study the efficacy of different commercial inert dust products against *D. gallinae* is compared. All tested compounds killed mites, but there was a clear ranking of efficacy (measured as weight loss after 24 h and as time until 50% mortality), particularly at 75% relative humidity (RH). At 85% RH the efficacy was significantly lower for all tested compounds ($P < 0.001$). Weight changes over time followed an exponential evaporation model until the mites started dying whereafter the rate of evaporation increased again and followed a slightly different exponential evaporation model. A tarsal test showed that 24 h exposure to surfaces treated with doses much lower than those recommended by the producers is sufficient to kill mites as fast as when they were dusted with massive doses. These data emphasise the need for thorough treatment of all surfaces in a poultry house in order to combat *D. gallinae*.

Keywords Desiccation · Diatomaceous earth · Alternative control · Ectoparasite

Introduction

Dermanyssus gallinae (Acari: Dermanyssidae), the poultry red mite, is a blood-feeding mite of birds with a worldwide occurrence and is without any doubt the most important pest of egg laying hens in Europe (Chauve 1998). An estimated annual loss of 11 million Euros

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in the Dutch poultry sector alone emphasises the economic importance of this pest (van Emous et al. 2005). Infestation of a layer house with poultry red mites may cause significant irritation and anaemia in the birds and in severe cases may lead to death (Kilpinen et al. 2005). There have been reports of heavy mite infestations leading to reductions in egg production (Kirkwood 1967), and the mite is also known to be a possible vector of a number of avian pathogens, such as Newcastle Disease virus and avian spirochaetes and also of zoonotic bacteria such as Salmonella (Axtell and Arends 1990; Valiente-Moro et al. 2007). In addition, egg quality may be downgraded due to bloodspots from crushed mites on the eggshells (van Emous et al. 2005). Finally, mite infestation in a poultry house may be of nuisance to farm workers as it may cause dermatitis and severe pruritus (Auger et al. 1979; Höglund et al. 1995).

Dermanyssus gallinae generally hides in cracks and crevices in the near surroundings of the birds during most of the day and only spends brief periods of time on the birds for blood-feeding (Wood 1917). Control of poultry red mite has mainly relied on chemical pesticides, which has led to the development of resistance (Zeman 1987; Beugnet et al. 1997). In most countries these acaricides are only approved for usage in empty poultry houses in order to avoid chemical residues in eggs and poultry meat. This has severely restricted the possibilities for mite control and there is an urgent need for new and/or alternative control methods.

Inert dusts have been used for pest control for centuries, and so may provide just such an alternative. These dusts comprise a range of different minerals, diatomaceous earth (DE) products and synthetic silica products (Ebeling 1971; Subramanyam and Roesli 2000). Storage pests in particular have been treated with this type of agent, but a whole range of arthropod species have been targeted (Mewis and Ulrichs 2001; Faulde et al. 2006). In contrast, the treatment against poultry red mite using inert dust is a relatively new event. As the air humidity levels in poultry production facilities are often high this may hamper the efficacy of the inert dusts. Inert dusts mainly act as dessicants by absorbing the lipids of the cuticle surface (Ebeling 1971) leading to death of the arthropod as a result of water loss, so high humidity in general is less conducive to the action of inert dusts (Subramanyam and Roesli 2000; Nielsen 1998).

Inert dusts are being marketed for control of *D. gallinae* in many countries in Europe, and are frequently relied upon as one of very few legal measures for mite control among poultry farmers. However, very little information is available on the efficacy of different products and on the effect of different levels of relative humidity on the efficacy of these products, although previous work has included application of inert dusts against poultry red mite (Melichar and Willowitzer 1967 cited in Ebeling 1971); Tarshis 1967; Kirkwood 1974; Chirico 2004; Maurer and Perler 2006). The aim of this study was to compare the efficacy of different commercial inert dust products representing pure DE, DE modified with synthetic amorphous silica, and pure synthetic amorphous silica products. Two inert dust products not marketed for pest control, but with a potential acaricidal effect, were also included in the study to investigate if they also acted as desiccants.

Materials and methods

Mites

All experiments were conducted using blood-fed adult female poultry red mites from a culture kept in the laboratory since 1997 fed on regular layer hens. The mites were allowed to feed the night before the experiments to obtain a homogenous group of mites with regard to their water balance.

Inert dusts

Several different types of commercial products containing inert dust were obtained from producers or retail companies. The inert dusts included natural DE products: Insecto (Natural Insecto Products, Costa Mesa, CA, USA), Diamol (O.W.A. Kemi, Skanderborg, Denmark), and SilicoSec (Miljøfluen, Gandrup, Denmark), DE's modified with synthetic amorphous silica: ProtectIt (Hedley Technologies, ON, Canada) and Fossil Shield 90.0 (FS 90.0, Bein, Eiterfeld, Germany), and pure synthetic amorphous silica products: RID (Rentokil, Glostrup, Denmark) and ID (confidential formulation under test). In some of the experiments kaolin and talc were included for comparison. Kaolin and talc are porous inert compounds widely used as carriers or diluents in dry formulations of chemical and microbial pesticides and were anticipated to have a limited effect on the mites, although other similar types of minerals have been used with success against stored product pests (Subramanyam and Roesli 2000).

Evaporation experiments

Groups of 100 mites were collected in Pasteur pipettes and exposed to a surplus (sufficient product to completely cover all mites in the pipette with further product to spare) of the different types of inert dust. After thorough mixing of mites and inert dust, the mites were poured out again and transferred to clean glass vials with a fine metal mesh lid. Groups of control mites were treated similarly (collected and transferred to glass vials). The initial weight of each group of treated mites was determined by weighing (Sartorius RC 210D balance, lower limit 10^{-5} g). All glass vials were placed in a closed box with a constant relative humidity in a temperature controlled room at 25°C. The humidity in the box was kept at 75 or 85% RH by means of saturated solutions of NaCl or KCl, respectively. At variable intervals (ranging from 2 h intervals in the beginning of the experiment to once a week in the end) the glass vials were weighed individually and the relative weight losses were calculated based on the initial weight of the mites. At the same time, the number of dead mites in each vial was estimated. This was done by visual inspection according to the scale shown in Table 1. For the data analysis the scaling was transformed back to the averages for each group.

All mites came from a laboratory culture where they were likely to have mated and produced eggs in the days following feeding. However, as each vial contained 100 adult female mites, each of which can produce 5–10 eggs, it was not possible to determine accurately the number of offspring produced. It was noted whether the egg production was clearly reduced compared to the untreated controls or completely prevented.

Table 1 Scale for estimation of *Dermanyssus gallinae* mortality and the average number of dead mites used in the data analysis

Scale	Dead mites	Average number of dead mites.
0	0–5	2
1	6–20	13
2	21–79	50
3	80–94	87
4	95–100	98

In each experiment three groups of 100 mites were used for the individual treatments and the experiment was repeated twice, i.e. a total of 900 mites per treatment. However, it was not practically possible to include all treatments in the same experiment (on the same day) and as control groups were included in all experiments the total number of control mites was higher than those treated with the products.

Tarsal exposure tests

Mites were exposed to different doses of inert dusts on treated filter paper discs. Discs were treated in Langs Bell, an apparatus for treating surfaces uniformly with dry substances in powder or dust form (Lang and Melte 1930). Initially, the Langs Bell was calibrated by blowing in different amounts of material and weighing the amount deposited on filter paper discs (6 cm diameter = 28.3 cm^2). The calibration test showed that there was an approximately linear relationship between the amount blown into the bell (x) and the amount deposited on the paper (y): $y(x) = 0.0398x - 0.000009$ ($R^2 = 0.93$). Whilst there may have been some variation using this method, it was nevertheless more precise as compared to other application methods such as sieving (Collins and Cook 2006) or using static electric plastic dishes (Fields et al. 2003). As some of the doses applied in the tests were too small to be weighed for each paper individually, the linearity of Langs Bell was used to extrapolate the calibration curve to these lower doses.

Filter paper discs (6 cm diameter) were treated with three different doses (and one untreated control) of three different types of inert dust: Diamol, SilicoSec and ID, representing two natural DE products and one synthetic silica product. These products also represented low, medium and high efficacies based on the results of the evaporation studies. The doses were 0.085, 0.34 and 0.85 $\mathrm{g/m}^2$.The treated paper discs were placed individually on a larger (7 × 7 cm) piece of fly glue (paper) trap (Cattle Shed, Silvanderson, Knäred, Sweden) and a group of 25 fed adult female mites were transferred to the filter paper disc. The bioassays were placed in plastic boxes with saturated solutions of NaCl or KCl to provide air humidities of 75 or 85% RH, respectively. After 24 h the remaining mites on the treated paper were sucked into Pasteur pipettes and kept in plastic boxes with the same relative humidity as before and observed daily for mortality over ~2 weeks or until all mites were dead. For each combination of inert dust product, dose and humidity, two groups of 25 mites were tested and the experiment was repeated once, i.e. a total of 100 mites per treatment.

Data analysis

In the evaporation experiment data were obtained on both weight change and mortality estimates. The weight loss after 24 h was noted and the data for the whole experimental period were fitted to a model for the relative weight change: $W(t) = W_{\mathrm{w0}}\ [1 - \exp(-kt)$ where W_{w0} is the water fraction of the weight at the start, and $-k$ is the rate of evaporation. This model is derived from the models for exponential water loss in Benoit et al. (2007). For the treatments with the slowest mortality (control, kaolin, and talc) the model was fitted only to those data before the mites started to die (the average time until the first mortality scores of 1). The remaining data were fitted to a slightly modified model: $W(t) = a(b - \exp(-kt))$ that does not necessarily intercept at 0% weight change.

The mortality scores were transformed back to estimated average mortality following Table 1. For day zero the mortality was by definition zero. The highest score of 4, corresponding to 95–100% mortality was recorded twice towards the end of the observation

Fig. 1 Examples of the weight changes of groups of *Dermanyssus gallinae* over time measured for **a** the ▶ untreated control mites at 75% RH, **b** kaolin at 85% RH, and **c** SilicoSec at 85% RH. The *curves* show the fitted exponential evaporation model $W(t) = W_{w0}\,(1 - \exp(-kt))$ for the entire experimental period (*dotted line*) and for the period until the mites start dying (*full line*). The *dashed line* shows the modified exponential model $W(t) = a(b - \exp(-kt))$ for the period from when the mites start dying

period where after the mortality was set as 100%. The estimated mortalities were fitted to a probit model (SAS Institute 2000) giving an estimate of the time elapsed following treatment until 50% of the mites were dead (LT_{50}).

Data on LT_{50} values and weight changes after 24 h for each of the two levels of relative humidity were log $(x + 1)$ transformed and tested for a significant effect of the treatment with a general linear model (SAS Institute 2000) followed by a multiple comparison analysis of the differences between the individual treatments (Tukey–Kramer, 0.05 significance level, SAS Institute 2000). The general linear model was also used to access differences between the two levels of humidity for each treatment.

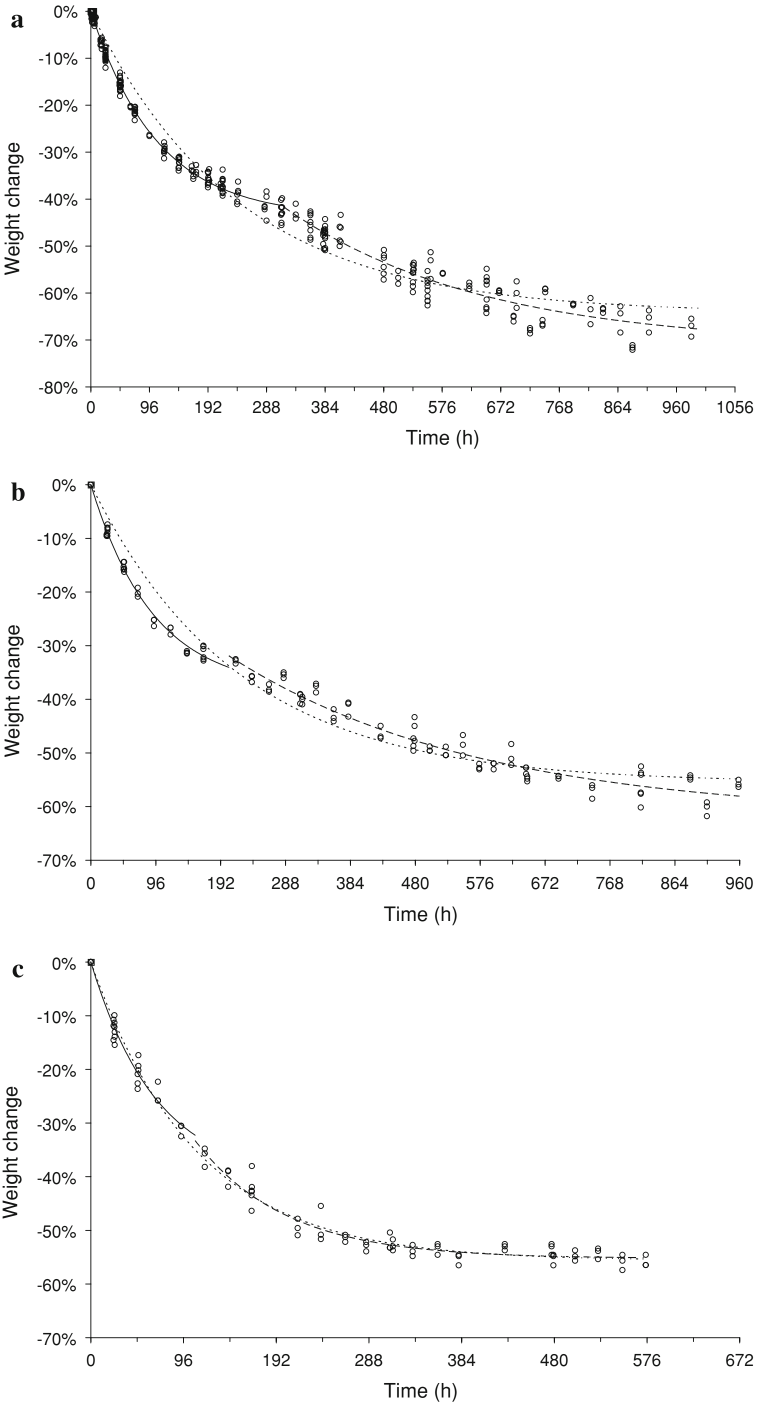
Data from the tarsal exposure tests were analysed by calculating the LT_{50} for each experiment with the probit procedure (SAS Institute 2000). In some cases, particularly with control treatments at high humidity, no mites died during the 2 week observation period. Thus a relevant LT_{50} value could not be calculated and a fixed value of 50 days was used based on the observations in the evaporation studies. There was also a lower limit to the measure of LT_{50} because the first observation was made after 24 h. If all mites were dead by then, the LT_{50} is ~0.6 day which is the lowest possible with this set-up. These data were also log $(x + 1)$ transformed and tested for significant differences between the three treatments at each dose and RH (Tukey–Kramer, 0.05 significance level, SAS Institute 2000).

Results

Evaporation experiment

For the slowest acting treatments (control, kaolin and talc) there were clear deviations from the exponential evaporation model when the data was fitted to the entire experimental period (*dotted line* in Fig. 1). These deviations gradually became smaller as the treatments killed the mites faster. For all the commercial inert dust products the deviations were so small that the exponential evaporation model provided a good approximation of the weight changes over the experimental period. For the slow-acting treatments the model fitted the data well until the mites began to die (*solid line* in Fig. 1). For the remaining data the modified model, that did not necessarily start at 0% weight loss, gave a good fit with the data (*dashed line* in Fig. 1).

For both weight loss after 24 h and LT_{50} values, data varied significantly for the different types of treatment at both levels of relative humidity (general linear model, $P < 0.0001$ for all combinations). Particularly at the lowest humidity there was a clear grouping of the products with regard to mite weight loss after 24 h (Fig. 2: treatments with similar letters are not significantly different). Kaolin and talc caused slightly, but significantly higher weight loss (11.9 and 12.1%, respectively) compared to the control group (9.5%). For three products: Insecto, RID and Diamol, weight losses of around 20% were recorded (18.2, 20.1 and 21.1%, respectively). Slightly higher values were found for ProtectIt (25.9%) and SilicoSec (27.9%). Finally, the two products resulting in the highest weight losses were FS 90.0 (37.3%) and ID (52.9%). The results for LT_{50} data followed almost the same groupings, with high weight losses resulting in low LT_{50} values and vice versa.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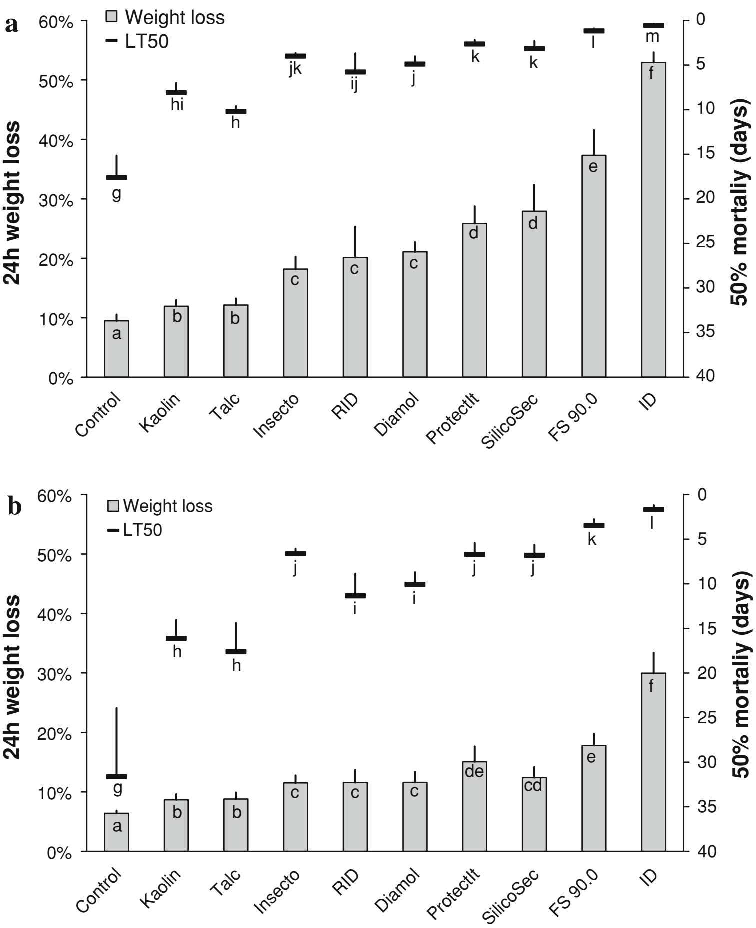

Fig. 2 Average weight loss of groups of 100 fed adult female *Dermanyssus gallinae*, relative to their initial weight before treatment and time until 50% mortality (for simplicity standard deviations are only shown in one direction). Results are provided from the two different levels of relative humidity used: **a** 75% and **b** 85%. *Bars* or *lines* with similar letters are not significantly different (0.05 level of significance, Tukey–Kramer multiple comparison, SAS Institute 2000). For all treatments there are significant differences for both weight change and LT_{50} at the two levels of humidity (GLM, SAS Institute 2000)

At 85% RH the weight losses were smaller and the LT_{50} values were higher, but the groupings remained almost the same as at 75% RH, although with more overlapping. One product, Insecto, showed a minor deviation from the general trend, in that the LT_{50} value was relatively lower than expected based on the weight change seen.

For all treatments there were significant effects of the humidity ($P < 0.001$ for all treatments) on both the LT_{50} values and weight change data; the LT_{50} being lower and the weight change being higher at 75% RH than at 85% RH.

At 85% RH, only the two most efficient products FS 90.0 and ID resulted in reduced egg production. At 75% RH, both of these products resulted in zero or very low egg production, and the five inert dusts (Insecto, RID, Diamol, ProtectIt, and SilicoSec) caused reduced egg production also. The kaolin and talc treatments had no observable effect on egg production.

In all experiments, the eggs that were produced hatched and the nymphs were not affected by the treatments.

Tarsal exposure test

There was a clear order of efficacy among the three inert dust products with ID being the most efficient and Diamol the least efficient (Fig. 3). However, with the lowest doses at 85% RH the differences between Diamol and SilicoSec are not significant. For all three products, increasing the dose or lowering the humidity results in lower LT_{50} values. It should be noted that the levelling out of the dose-effect at the highest doses of ID (particularly at 75% RH) was affected by the length of the observation interval (first observation after 24 h).

Comparing the LT_{50} values in this test with those obtained in the evaporation experiments (*arrows* in Fig. 3) showed that 24 h exposure on a treated surface (with the highest doses) was equally as efficient, if not more so, than the maximum exposure level achieved when mixing the mites with a surplus of dust.

Discussion

The potential of inert dusts for controlling insect pests, particularly stored product pests, has been known for centuries. The available literature on the effect of inert dusts on stored product pest covers a wide range topics, from efficacy (Mewis and Ulrichs 2001) to how the dust directly affects the insects (Ulrichs et al. 2006). To some extent this body of research covers the effect of these products on the Acari, particularly storage mites (Collins and Cook 2006) but little is known about how the inert dusts affect blood-sucking mites. Blood sucking ectoparasites such as the poultry red mite differ from the more commonly studied stored product pests because they consume relatively large amounts of avian blood which contains almost 90% water (Lehane 2005). An adult female poultry red mite ingests ~0.2 mg of blood per meal (Sikes and Chamberlain 1954). Despite the large amount of water ingested, the most efficient inert dusts in this study cause almost complete loss of water within 24 h at 75% RH (and 25°C) with the maximum dose applied in the evaporation studies. This indicates that the mites would not be able to compensate for this water loss by taking in a new blood-meal, at least not with the fastest acting products tested here. It is possible that with the more slowly acting products, additional blood-meals could replenish the mites' water loss, but further study would be needed to confirm this.

The initial weight loss of the untreated control mites can be attributed mostly to the defecation taking place in the first couple of days after feeding. The faeces remained in the glass tube but would have dried out with the water component passing through the mesh lid. This would have been the same for the treated mites but with additional water loss coming from evaporation across the mites' cuticle (Ebeling 1971). The present study has shown that the weight changes follow the exponential evaporation model of Benoit et al. (2007) with some modifications. For the untreated control mites the model fitted the weight changes until the mites started dying, then the rate of evaporation increased again indicating that the living mites had an active protection mechanism against evaporation. The weight changes from this point followed a new exponential evaporation model. With the faster acting treatments the transition point where the mites started dying was no longer detectable in the present experiment.

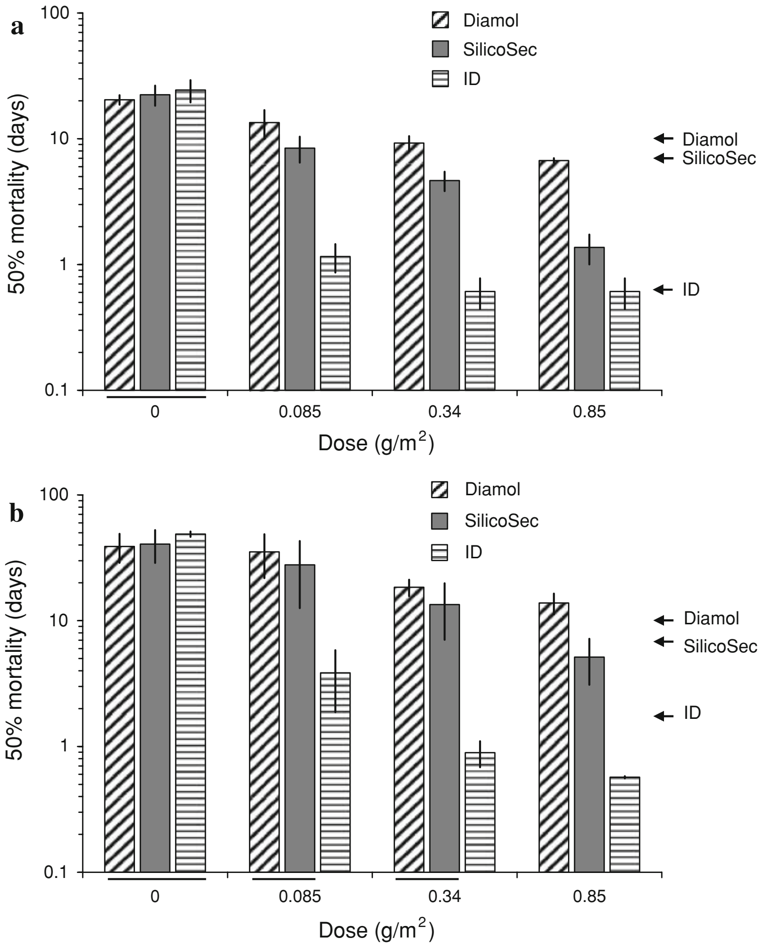

Fig. 3 Results from the tarsal exposure trials with *Dermanyssus gallinae*. Average LT_{50} values (with standard deviations) for the four tests at each dose (and untreated control) at 75% RH (**a**) and 85% RH (**b**). *Lines* below the *x*-axis indicate results that are not significantly different (0.05 level of significance, Tukey–Kramer multiple comparison on log(x + 1) transformed data, SAS Institute 2000). *Arrows* to the *right* indicate LT_{50} values from the evaporation studies. There is a lower limit to the LT_{50} determination at around 0.6 days due to the 24 h observation interval

There were clear and significant differences between the different types of inert dust, both with regard to weight loss and speed of mite knock-down. The most efficient product was the synthetic silica product ID that resulted in 50% mite mortality after 0.6 days at 75% RH and 1.7 days at 85%. These values may even be underestimating the efficacy of this product because knock-down was so quick that the observation intervals were too long to determine the LT_{50} precisely. The other types of inert dust fall in between the efficacy of ID and that of kaolin/talc as the least efficient compounds. The general rule concerning the efficacy of the different groups of inert dusts was as follows (least—most efficacious): clay and other minerals < pure DE's < modified DE's < pure synthetic amorphous silicas. This hierarchy is based mainly on the oil absorption capacity of these dust-based products which is dependent on their particle size distribution (Subramanyam and Roesli 2000). ProtectIt is a DE modified by the addition of a silica aerogel. However, in this experiment the pure DE, SilicoSec, was as efficacious as ProtectIt when comparing LT_{50} values, and SilicoSec killed mites significantly faster compared to another pure DE, Diamol. Similar results were found by Faulde et al. (2006) in a study on the German cockroach, *Blattella germanica*. FS 90.0 is also a modified DE, but has been modified by coating the DE particles by adding silica aerogel treated with dichlorodimethyl-silane. The results confirm that this produced a higher efficacy against poultry red mite. RID is an inert dust containing pure synthetic silicon dioxide like the product ID. However, while ID proved to be the fastest acting inert dust in the current study, RID performed relatively poorly. Kaolin and talc both proved to have a slight, but statistically significant acaricidal effect.

The general theory for the mode of action of inert dusts on arthropods is that they destroy the protective wax layer of the cuticle (Ebeling 1971), and it is possible that even such relatively harmless compounds as kaolin and talc absorbed some of the wax layer thus increasing the permeability of the cuticle. A study on the control of rice pests using entomopathogenic fungi showed that treatments in which the biocontrol agent was formulated in kaolin or talc provided significantly better as pest control products compared to untreated conidia (Samodra and Bin Ibrahim 2006). This might be an example of the inert dusts acting as synergists, as suggested by the authors. Perhaps more likely was that the compounds caused additional mortality to that caused by the fungus. According to Ebeling (1995), kaolin is worthless as a desiccant dust unless its pores are enlarged by acid- and heat-activation, and such derivates have been successfully applied against stored product pests (Permual and Le Patourel 1992). However, unmodified clays and other minerals have often been used as grain protectants against arthropod damage (Subramanyam and Roesli 2000). This suggests that dusts other than those containing high proportions of silicon dioxides may have pesticidal effects, although these are likely to be much reduced compared to synthetic silicon dioxides.

Exposing the mites for 24 h on treated surfaces confirmed that they were able to pick up enough product to be killed within less than a day for the most efficient product tested, ID, and 4.5 days (at 75% RH) with the less efficient Diamol. The doses applied in the current study were generally much lower than those recommended by companies marketing these inert dusts for use against poultry red mite or other pests. The recommended application rate of Fossil Shield is 1–3 g per layer hen in cage systems, which is notably higher than even the highest dose applied in the tarsal test assay. However, even at the relatively low doses used in this experiment, the efficacy of the products tested was comparable to that achieved in the evaporation experiment where massive doses of product where used in a maximum exposure procedure (Fig. 3). This suggests that the mites were susceptible to low doses of inert dusts provided they picked up the particles from a treated surface for a continuous period of 24 h. This emphasises the need for thorough treatment of all surfaces in a poultry house and also the need for reapplication where the treated surfaces become covered by environmental dust and debris.

Pest knock-down, or speed of kill, is an important factor when choosing between inert dust products, particularly if a product acts fast enough to prevent production of offspring. Another important factor is to what extent environmental variables such as humidity affects the efficacy of a product. A poultry house is a relatively humid environment and the humidity can rarely be controlled in layer houses other than by ventilation and temperature control. Nordenfors and Höglund (2000) recorded temperature fluctuations over 1 year in an aviary system. During summer the humidity fluctuated in the range 40–80%RH and in the winter the range was 60–85% RH. The results of the present study showed that the efficacy of all of the inert dusts tested was significantly lower at 85% RH compared to 75%

RH. In theory, synthetic silicon dioxide and modified DE's should be less dependent on lower humidity due to the hydrophobicity of these products. In the current study, however, all inert dusts showed decreased efficacy at the highest humidity level, so even the two tested pure synthetic silicas and the two modified DE's were susceptible to an increase from 75% RH to 85% RH. Mewis and Ulrichs (2001) observed a similar result for Fossil Shield in a study on stored product pests, and Faulde et al. (2006) found that SilicoSec, Diamol and Fossil Shield 90.0 were less effective than other modifications of Fossil Shield at 85% RH. Although wax sorption can take place in a water saturated atmosphere, provided the insect cuticle is in continuous contact with the dust (most notably for the silica aerogels), early reports also showed that knock-down will be prolonged compared to that achieved under ambient humidity conditions (Ebeling 1995).

In conclusion, based on the results of the current laboratory study, higher application rates of all the inert dusts, including the highly efficacious ones, must be applied if the humidity of the poultry house reaches 85% in order for these products to retain the efficacy shown at lower humidity levels. There are significant differences in the efficacy of the different products tested, suggesting that some may be more effective than others in poultry red mite control. Nevertheless, other factors including the price of any product and the ease of application would also need to be considered before selecting one product above another for application in poultry houses.

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