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Impeding movement of the Poultry Red Mite, *Dermanyssus gallinae*

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Highlights

- *Dermanyssus gallinae* is an economically important haematophagous ectoparasite of laying hens.
- Impeding mite movement could potentially prevent *D. gallinae* infestations or prevent mites feeding on birds.
- Thyme oil, sticky tape and insecticidal glue barriers contained 78-88% of mites within a specific area in *in vitro* experiments.
- Trials in an *in vivo* setting are needed to confirm the commercial applicability of these barriers.

Abstract

The poultry red mite, *Dermanyssus gallinae*, is an economically important hematophagous parasite of commercial egg laying hens, also affecting domesticated birds and companion animals. Conventional control of *D. gallinae* through acaricidal spraying is often ineffective, creating an urgent need to identify alternative management strategies for commercial and domestic infestations. Whilst integrated pest management is being considered for *D. gallinae*, the potential of impeding mite 'migration' routes, to either prevent initial infestation or manage established populations, has not been researched. Here we demonstrate that barriers of insecticidal glue, double sided sticky tape and thyme oil can contain *D. gallinae* within a specified area of a petri dish (78-88% of total mite population) and this level of containment was significantly greater than for negative controls (p values <0.05). Further studies in poultry houses are recommended to investigate the efficacy of these barriers in real world application and identity potential for barriers as a strategy for mite control.

Keywords: *Dermanyssus gallinae*, Insecticidal glue, Migration, Physical control. Poultry red mite, Thyme oil.

1 Introduction

The poultry red mite, *Dermanyssus gallinae*, is a blood-feeding ectoparasite of commercial egg-laying hens, as well as other bird species (Kristofík et al., 1996; Brannstrom et al., 2008). At their most severe, *D. gallinae* infestations in egg-laying units can result in death of hens due to substantial blood loss, with sub-lethal effects including irritation to birds, virus/disease transmission, reduced egg production and poor egg quality (Chauve, 1998; Cosoroaba, 2001). Infestations are prominent year-round in temperate climates, and average densities of 50,000 mites per bird are not uncommon (Kilpinen et al., 2005). It has been estimated that spraying and dusting to control mites as well as loss of eggs fit for consumption due to red mite pathology in layers costs the egg-production industry €130 million per annum within Europe alone (Van Emous, 2005). Spraying and dust controls cost €4.33/ 100 birds in caged systems and €3.83 / 100 birds in alternative systems (Lubac et al., 2003).

Spraying with synthetic acaricides and dusting with diatomaceous earth and silicas are currently the primary methods of *D. gallinae* control. Mite resistance to pyrethroid and carbamate sprays, however, is widely reported (Zeman and Zelezny, 1985; Beugnet et al., 1997; Marangi et al., 2009), and even where mites are susceptible to acaricide treatment, pest-product contact is hampered by the mites' reclusive lifestyle. *D. gallinae* spend the majority of their time secluded in the poultry house sub-structure, emerging only to feed for relatively short periods every few days.

Although non-conventional methods and integrated pest management are increasingly being considered for *D. gallinae* control, little work has been done to investigate the potential of repellent or physical barriers. This is despite the success of these barrier types against other ectoparasitic pests that display regular and repeatable 'migration' to and from the host (e.g. mosquito repellents and bed nets). Such barriers could be deployed in strategic areas to block short–range *D. gallinae* 'migration' routes in commercial settings, for example by isolating perches from nearby mite refugia, or by preventing initial pest establishment via longer range mite movement. For domesticated birds maintained in smaller 'hobby huts', for example,

new housing could theoretically be protected from founding *D. gallinae* populations in the wider environment by creating barriers to inward mite migration at house-environment interfaces (e.g. the house legs). Other forms of companion animal housing, such as small mammal hutches and bird cages, could be similarly protected from initial infestation, both from *D. gallinae* and other crawling pests (e.g. ticks). Diffuse barriers, as could be created through use of volatile repellents with a strong barrier effect, could potentially protect commercial poultry premises in much the same way.

The work described herein aimed to test existing barrier products to investigate their potential to restrict movement of *D. gallinae* in the laboratory as a preliminary study. Both potentially repellent, such as thyme oil (George et al., 2009), and physical barrier products, such as petroleum jelly (Kim et al., 2010), were selected for testing.

2. Materials and Methods

2.1 Mites

A population of mixed stage *D. gallinae* were collected from a commercial egg laying unit within the UK and stored at 4°C in a 75cm^3 conical flask (Corning, UK) with a vented cap as described by McDevitt et al. (2006) for up to 2 weeks before use in experiments.

2.2 Physical barriers

Barrier treatments were applied to divide a 90mm petri dish (Thermo scientific, US) into two halves termed the 'pre-barrier' area and the 'post-barrier' area (Figure 1). A variety of adhesive and chemically repellent products were tested, based on a search of relevant scientific literature as well as consideration of pre-existing commercial barrier products (Table 1). These were applied to Petri-dishes as follows: Double-sided sticky tape (3M Ltd, US, width 2.5cm) was secured across the diameter of the plate ensuring the sides were fully covered. Two drops of 100% thyme oil (*Thymus vulgaris*) at 0.917g/ml (Calmer solutions Ltd, UK) were applied to an absorbent bandage tape 2 minutes before fixing the tape to the dish. Two drops

of Cade oil (*Juniperus oxycedrus*) at 0.870 g/ml (The Aromatherapy Shop Ltd, France) were placed in 5ml carrier oil, mixed together and two drops of the end mixture applied to a bandage tape as with the thyme oil. Petroleum jelly, detergent, and insect barrier glue (Agralan Ltd, UK) were each applied as continuous lines (~3mm wide) across the diameter of individual dishes. A negative control, with no barrier present, was included in all experimental replicates.

2.3 Experimental design

Each petri dish was placed in a 250ml square weighing boat (Starlabs, UK) filled with oil to prevent mites escaping (See Figure 1). Corrugated cardboard refuges (1cm^2) have been proven to attract and keep PRM (Nordenfors and Chirico, 2001) and so were placed in each of the post barrier areas to discourage return migration back across the barrier. At the start of each experiment, a group of mites (mean 93.5 s.d. 18.1) was placed in the pre-barrier area of each plate. Dishes were left at room temperature for one day (~24 hours) or three days (~72 hours) under natural lighting conditions (6-8 hours light / 16-18 hours dark) but away from direct sunlight. All barriers were tested using separate dishes/boats and each barrier treatment was replicated ten times. Another series of assays were conducted to evaluate selected barriers at 4°C and 37°C in order to evaluate the effect of temperature on the efficiency of barriers to contain mites. Each experimental replicate used new mites and newly constructed barrier/dish systems.

2.4 Counting mites

After the allotted time each dish/boat was placed at -20°C for three hours to immobilize all mites. The numbers of mites in each area were then counted. Those in the pre-barrier area, or stuck in the barrier, were deemed to have been contained by the barrier, whereas those in the post-barrier area and within the mite traps were deemed to have crossed the barrier.

2.5 Statistical testing

Following determination of a normal distribution, one-way ANOVA testing and post hoc analyses using Turkey's T test were calculated to determine significant variance in mites numbers contained by each barrier. Standard deviation between replicates of each barrier group was calculated to determine variance within each group of replicates.

3. Results

3.1 Experimental design validation

In all negative controls, a majority of mites migrated to the post barrier areas (24 hours = 66.1%, 72 hours = 62.9%) with ~34.5% of these found inside the cardboard traps. To gain temporal information on the speed of migration, counts of *D. gallinae* in the pre- and post- barrier areas were carried out at 30 minute intervals for the first two hours, then at 24 hours post-introduction (supplementary data). Mite numbers in the post barrier area increased gradually and continually over time for thyme oil, insect glue and sticky tape indicating that few, if any, mites traversed back to the pre-barrier area. For the petroleum jelly barrier, however, the counts showed variable numbers of mites in the pre- and post- barrier areas over time, similar to the negative controls, suggesting that return migration did occur.

3.2 Containment efficiency

Of the six barrier types that were tested, four were found to contain a significantly larger percentage of mites than the negative control (p values <0.05, see Table 2). The insecticidal glue showed the greatest containment efficiency preventing 97.5%

of mites from reaching the post barrier area after 24 hours (s.d. 3.66) and 87.7% of mites (s.d. 8.77) after 72 hours. These results were 63.7% higher at 24 hours and 50.6% higher at 72 hours (p values = 0.00) than negative controls. Other barriers that also contained significantly higher percentages of mites (p values <0.05) compared to the negative control were thyme oil (24 hours = 86.1%, 72 hours = 78.3%), sticky tape (24 hours = 86.0%, 72 hours = 82.6%) and petroleum jelly (24 hours = 63.5%, 72 hours = 64.8%). In contrast, barriers using cade oil (p value 0.99) or detergent (p value 0.14) did not show significant difference from the control after both 24 and 72 hours. No major differences were found in barrier efficiencies across all of the temperatures examined.

4. Discussion

The aim of this study was to identify pre-existing barrier products that could be of practical use in preventing *D. gallinae* migration onto hosts, or into host housing systems. Results suggest that insecticidal glue (Agralan Ltd, UK), double-sided sticky tape and thyme oil can all significantly (p values <0.05) contain mites within an area for at least 72 hours. Petroleum jelly appeared to act as an effective barrier to mite movement, though additional observation suggested repeat migration across this product was possible in *D. gallinae*.

Our results, in agreement with George et al. (2010), demonstrate that thyme oil, though not cade oil, is efficient at repelling *D. gallinae*. Negative controls of absorbent bandage only (i.e. no thyme oil) and bandage plus carrier oil only (no cade oil) barriers showed free movement of mites across the barrier (results not shown) indicating repellence was indeed due to thyme or cade oil. Though any barrier effect via repellence of volatile essential oils might be relatively short-lived, incorporation into a slow release carrier could overcome this issue, particularly for thyme oil which is known to maintain repellence to *D. gallinae* for extended periods vs other essential

oils (George et al. 2009b). In theory, thyme oil could be deployed as a diffuse barrier to manipulate existing mite populations or discourage inward movement of mites from the external areas. Use of physical barriers is likely to be more spatially explicit and confined to spot/strip application, though longer residual activities could be expected. This is supported by the successful use of non-drying insect glue to prevent experimentally isolated populations of *D. gallinae* from mixing with one-another in a single tiered cage system over a period of several months (George et al. 2010a).

Poultry houses can vary greatly in temperature over seasons, based upon housing construction and farm locality, with such variability having the potential to effect barrier efficacy (e.g. by altering product viscosity/volatility). Nevertheless, all of the effective barrier products tested here showed no obvious decline in containment efficacy at temperatures between 4°C and 37°C (supplementary data). As well, *in vitro* studies implemented seven day starved mites that may have reacted in a greater sense of urgency to mites found an *in vivo* setting. PRM *in vivo* instead feed every 2-3 days. Furthermore, our *in vitro* experiments presented PRM to exposed

environments, thus attraction to cardboard traps imitating refugia may have created a heightened attraction to mites in the confined space of a petri dish compared to the larger spaces of a standard poultry unit *in vivo*.

In summary, the work presented demonstrates that *D. gallinae* can be contained in vitro using a variety of commercially available barrier products. Further work is required to demonstrate the applicability of these barriers in real-world settings given the variation in poultry house environment compared to laboratory settings.

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Conflict of interest statement

All authors report there is no conflict of interest.

Table 1

Barrier material	Use of barrier in pest control	References
Double sided sticky tape	Used in collection techniques of several species of mites.	(Harvey and Martin, 1988; Tovey and Woolcock, 1994)
Bandage tape and thyme oil / cade oil	Proven to repel and even kill PRM.	(George et al., 2009)
Insect barrier glue	Used against adult vine weevil, winter moth, ants and earwigs.	Product description (Agarlan Ltd, UK)
Petroleum jelly	Proven deterrent against several species of phytophagic mites.	(Nicetic et al., 2001; Kim et al., 2010; Reddy and Bautista, 2012)
Detergent	Known to kill mites by break down of the exoskeleton cuticle.	(Edrees, 2013; Pritchard et al., 2015)
No barrier (negative control)	N/A	N/A

Table 1. Barrier materials selected to deter mite migration.

Table 2

Barrier type	After 24 hours			After 72 hours		
	Mean percentage of mites contained (and s.d.)	Percentage difference to negative control	P value	Mean percentage of mites contained (and s.d.)	Percentage difference to negative control	P value
Sticky tape	86.0 (±11.0)	52.1	0.00	82.6 (±8.5)	45.5	0.00
Thyme Oil	86.1 (±13.7)	52.2	0.00	78.3 (±16.3)	41.1	0.00
Insect glue	97.5 (±3.7)	63.7	0.00	87.7 (±8.8)	50.6	0.00
Petroleum jelly	63.5 (±23.3)	29.6	0.00	64.8 (±16.3)	27.6	0.00
Detergent	53.9 (±20.2)	20.0	0.14	66.7 (±22.3)	29.5	0.00
Cade oil	39.8 (±23.2)	6.0	0.99	58.1 (±8.9)	21.0	0.04
Negative control	33.9 (±16.3)	n/a	n/a	37.2 (±16.7)	n/a	n/a

Table 2: The mean numbers of mites contained by each barrier after a period of 24 hours and 72 hours. Each mean is derived from 10 repeats and is compared to the mean value of the negative control (no barrier). Significance testing using a post hoc Turkey's t test analysis derives a p value (significant <0.05) quantifying significance between each barrier type values and the negative control values.

Figure 1

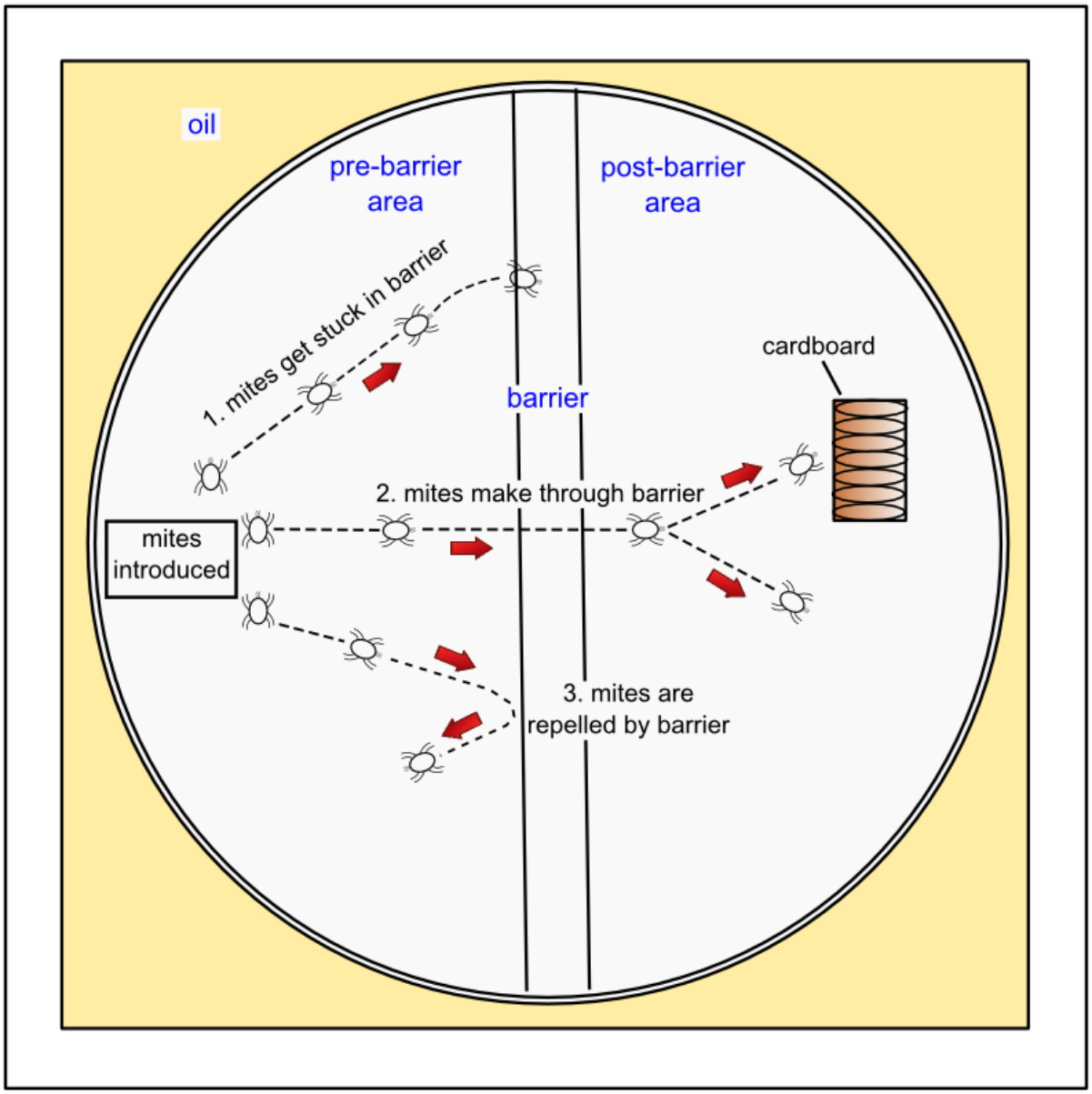

Figure 1: Testing the efficiency of barriers to contain mite migration. A barrier is created across the diameter of a 90mm petri dish separating it into three areas: the pre-barrier area, the barrier and the post-barrier area. The dish is placed in a weighing boat filled with oil to prevent mite migration outside the dish. A 1cm^2 piece of corrugated cardboard is placed in the post-barrier area to stimulate mite migration.

Mites are introduced in the pre barrier area and are either trapped by the barrier (option 1), mites traverse the barrier (option 2), or contained within the pre-barrier area (option 3). After 24 or 72 hours mite numbers in each area are recorded. Mites are a mixed stage population measuring 0.2-1.0mm in length.