Repellent or attractive effect of molecules in *Dermanyssus gallinae* and some predatory mites.

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Experiment report

Introduction

The poultry red mite (PRM), *Dermanyssus gallinae*, is the most important haematophagous ectoparasite of domestic poultry because of its cosmopolitan occurrence and abundance in poultry houses, great reduction in egg production, a major source of irritation and anaemia and in some cases its causal association with host death syndrome (Kirkwood, 1967; Kettle, 1993). *Dermanyssus gallinae*, belongs to the Order Mesostigmata which incorporates many mite species that vary considerably in morphology and behaviour. Many species are phytophagous, saprophagous or predatory free-living (Koehler, 1999; Gerson et al., 2008), whilst others, including PRM, have obligatory parasitic behaviour. The mite is also found to be a possible vector of a variety of poultry pathogens including avian spirochetes, Salmonella app., chicken pox virus and eastern equine encephalomyelitis (Valentine Moro et al., 2009; Lancaster and Meisch., 1986; Durden et al., 1993). *D. gallinae* poses a significant threat to egg laying hens in many parts of the world, including Europe (Sparagano O., et al 2009). In Europe infestation rates average more than 80%, with costs associated with both control and production losses estimated at €130 million per year for the EU egg industry (Van Emous R., 2005). Occasionally, *D. gallinae* causes dermatitis and a nuisance to people working at heavily infested poultry houses (Hoffman., 1987; Rosen et al., 2002). Control of this mite population primarily depends on further applications of various contact acaricides such as carbaryl, diazinon, dichlorvos and permethrin or fumigants such as sulfur dioxide. Plant essential oils may be an alternative source of materials for mite control because they constitute a rich source of bioactive chemicals and are commonly used as fragrances and as flavouring agents for food additives. Little work has been done, however, in relation to the acaricidal activity of plant essential oils against *D. gallinae*, although effectiveness of plant essential oils against insects has been well described by Isman (1999).

This report describes a laboratory study assessing the potential role of repellent or attractive effect of different molecules in the PRM. The study were hosted in the Laboratory of the Centre d'Ecologie Fonctionnelle et Evolutive in Montpellier with Professor Lise Roy's supervision for the time period between 15 and 28 November 2015. The study was under an STSM (Short Term Scientific Missions) funded by the COST Action FA1404 : Improving current understanding and research for sustainable control of the poultry red mite *Dermanyssus gallinae* (COREMI).

Materials and Methods

Mites

Poultry Red Mites (*Dermanyssus gallinae*) were obtained from hen farms located in municipalities of South France in the region of Avignon. The mites were preserved in the Laboratory of Centre d'Ecologie Fonctionnelle et Evolutive during the experiment inside transparent plastic containers qualified with suitable air flow, average temperature 20 ± 2°C. The mites were tested during the experiment in order to assure that they were vivid.

Bio-assay

Olfactory preferences of the mites were tested in a closed-system using glass Y-tube (Photo 1). Briefly, pressurised air was filtered through activated charcoal, humidified and split in two streams with the same flow. Air was then led through glass jars, where 50 ml vials for experiments that tested the response to volatiles, were placed for each experiment. Inside the plastic tubes 100μl of each of the tested compound were inserted and at the top of each of the vial a capillary tube with 0,53mm diameter were placed in order to obtain the regular release of volatiles. Afterwards the air motioned through plastic tubes finally arrived inside the glass Y-tube. The flow of streams was tested with a flowmeter and stabilised approximately to 117-119ml/min. The experiment's room temperature and humidity were recorded every day, as also the day time, with average 25 ± 5°C and 40-60% (±5). In order to minimise the influence of external factors, the room pressure was also stabilised as also the sun light was averted using window covers. Due to the well known attraction of PRM to the heat, heat power were placed in similar way under each component of the glass Y-tube and assure that there was the same heat inside the Y-tube. By the end of each assay, the glass Y-tube was washed with ethanol in order to weaken the odours, especially odours that the previous mite individual may have released, and new clean glass Y-tube were used for the next assay.

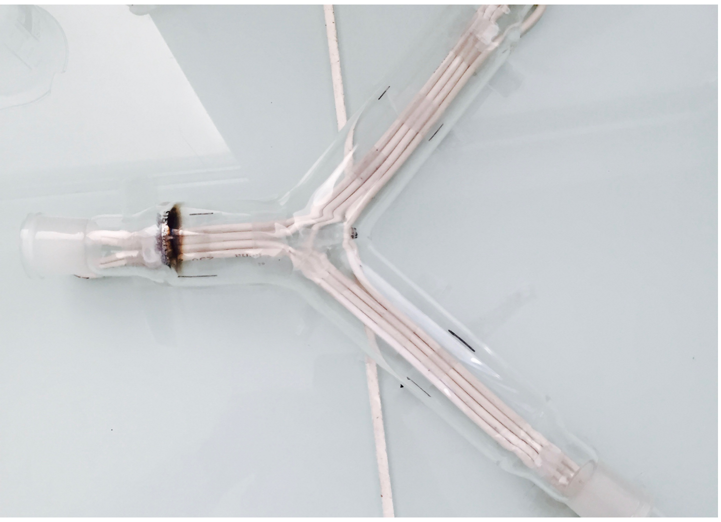

Photo 1: the glass Y-tube with the heat power where mites were inserted.

Prior to the entry of each mite inside the glass Y-tube, mites were tested for being vivid and making vigorous movements. For each assay one female, vigorous mite was chosen and released individually at the down-wind end of the stem of the Y-tube. They were given the opportunity to walk upwind and choose for one of the odour sources for a maximum period of 15 min. 'Odour Choice' was recorded as soon as the mite entered either the left or right arm. If a mite remained in one arm for a consecutive period of 5 min, this choice was recorded as 'Final Choice' and the mite was then discarded. All other situations were recorded as 'No Choice'. Finally the movements of the mites inside the glass tube were recorded using a layout in order to detect and describe the overall movement of mites through the tubes and at the end of each day all the data were recorded using a table.

Experiments

The repellent or attractive effect of different molecules to mites were tested using this behavioural experiment. Especially, in the begging of the assays, one substance was included and afterwards tow substances in order to compare the preference of mites to one of them. Furthermore during the experiment five different odours were tested namely, Ammonia, Lactic Acid, Eugenol, Feathers and Droppings. Overall during the experiment, the response of 100 mites was assessed. Stimuli were alternated between left and right arms after each assay. Before the entrance of each mite inside the glass Y tube, there was a minimum time period of 2 minutes of air flow through the tubes.

Response to odours and results

We investigated the response of mites to ammonia (A), eugenol (E) and lactic acid (LA) as also the response to host natural odours such as feathers (F) and droppings (D). To begin with, 30 mites were tested using ammonia as the only odour for the assays, 13/30 (43,4%) choose 'ammonia', 7/30 (23,4%) 'no ammonia' and 10/30 (33,4%) 'no choice'. Moreover 10 mites were assessed using lactic acid and 4/10 (40%) selected 'lactic acid', 2/10 (20%) selected 'no lactic acid' and 4/10 (40%) 'no choice'. For the assays testing eugenol, 10 mites were tested and the the majority of them preferred 'no eugenol' 8/10 (80%) and only 1/10 (10%) selected 'eugenol' and 1/10 (10%) 'no choice' (Table 1).

mite response to eugenol on 23/11/2015

	day time	duration	capillary	left	right	movement	result
1	17:45	5m	0.53mm	-	E	directly	No Eugenol
2	17:55	5m	0.53mm	E	-	directly	No Eugenol
3	18:05	3m	0.53mm	-	E	directly	No Eugenol
4	18:10	7m	0.53mm	E	-	around	No Eugenol
5	18:22	5m	0.53mm	-	E	around	No Eugenol
6	18:30	5m	0.53mm	E	-	around	No Eugenol
7	10:18	10m	0.53mm	-	E	around+	No Choice
8	10:32	5m	0.53mm	E	-	around	No Eugenol
9	10:42	5m	0.53mm	-	E	directly	Eugenol
10	11:00	5m	0.53mm	E	-	around	No Eugenol

Table 1: the data from the assays testing the repellent effect of eugenol to mites.

As for the assays using the host odours, feathers were obtained by cutting feathers located in the neck area and the area of amara from hens living in farms in South France and droppings from the same farms. Thus, 10 mites were tested using feathers and 6/10 (60%) selected 'feathers', 2/10 (20%) selected 'no choice' and 2/10 (20%) 'no feathers'. Additionally 10 mites were used for testing the response to droppings and only 2/10 (20%) preferred 'droppings', 4/10 (40%) 'no choice' and 4/10 (40%) selected 'no droppings'.

Eventually we conducted 3 tests in order to compare the response of mites to 2 different molecules simultaneously in the same assay. In detail, we compared ammonia versus eugenol, ammonia versus feathers and feathers versus droppings. Especially in the experiment ammonia versus eugenol, 10 mites were used and 4/10 (40%) choose 'ammonia', 3/10 (30%) choose 'eugenol' and 3/10 (30%) 'no choice'. Moreover in the assays assessing ammonia versus feathers, 5/10 (50%) preferred 'feathers', 2/10 (20%) preferred 'ammonia' and 3/10 (30%) 'no choice'. Finally for the assays testing both natural host odours, we used 10 mites and 9/10 (90%) selected 'no choice' and only 1/10 (10%) selected 'droppings'.

Discussion

This experiment was an ideal start for setting up this particular experiment, stabilise the parameters and improving the functionality of the system. Primarily, this STSM was the first and vital part for this experiment and the first step in order to build scientific bridges and for future collaborations between France and Greece about this project or relevant researches.

It is well known that different kind of molecules have a notable effect on *D. gallinae* mites, some of them attractive and some other repellent. Odours sources like the host feathers have been shown to have an attractive effect to mites (Koenraadt et al., 2010). A variety of chemical compounds may be responsible for the attraction of *D. gallinae* towards feathers. Many essential oils are known to posses various bioefficacies such as ovicidal, repellent, antifeeding and biocidal activities against various arthropod pests (Isman,1999). Molecules such as eugenol are very likely to have a strong repellent effect to mites and conversely host odours like hen features seem to have an attractive effect. Additionally other molecules, for instance the ammonia, is not clear if it is a repellent or an attractive substance to those mites. The results reported here provide evidence for the potential repellent and attractive effect of different molecules to engored female *D. gallinae*. Herein, the study indicated that *D. gallinae* mites were attracted by host odours like feathers and were not by odours such as eugenol. The data gained from the assays clearly show and stress the repellent effect of eugenol to those mites, conversely the data are not clear about the effect of lactic acid, droppings or ammonia. Overall in this report 100 mites were tested during the tow week time period, and it is necessary to continue the experiment conducting more assays in order to have a complete and objective result about the effects of those odours to *D. gallinae* mites.

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