Predation interactions among henhouse-dwelling arthropods, with a focus on the poultry red mite *Dermanyssus gallinae*

Running title: Predation interactions involving *Dermanyssus gallinae* in poultry farms

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**ABSTRACT**

**BACKGROUND**

Analysis of the poorly explored food webs of henhouse-dwelling arthropods would improve biological control against the poultry red mite (PRM) *Dermanyssus gallinae* (de Geer). This study aimed to identify trophic links among indigenous predatory arthropods, PRM, and alternative preys. *In-vitro* predation tests were carried out to assess (1) the ability of...
indigenous predators to feed on PRM juvenile and adult stages in two physiological statuses (unfed and freshly blood-fed) in the absence of any physical barrier, (2) predator preferences between PRM and astigmatic mites, and (3) predation interactions between PRM predators.

RESULTS

Ten arthropod taxa fed on PRM with predation rates ranging from 4 to 95% in our experimental conditions. They belonged to 1) Acari: *Androlaelaps casalis* (Berlese), *Cheyletus* spp., *Macrocheles muscaedomesticae* (Scopoli), *M. penicilliger* (Berlese), *Parasitus fimetorum* (Berlese), *Dendrolaelaps* spp. and *Uroobovella fimicola* (Berlese); 2) other Arachnida: *Lamprochernes nodosus* (Schrank) and a linyphiid spider; and 3) Insecta: *Lyctocoris campestris* (Fabricius). These predators varied in their preference for PRM stages and physiological statuses (unfed or freshly blood-fed). When given a choice, most predators preferred to feed on PRM than astigmatic mites. Bidirectional predation occurred within two pairs of PRM predators (*M. penicilliger–Lam. nodosus* and *A. casalis–Cheyletus* spp.), and *M. penicilliger* had a 100% predation rate on *A. casalis*.

CONCLUSION

Our study highlights the potential of various arthropod predators occurring naturally in poultry houses for conservation and augmentative biological control of PRM. Predation interactions between these predators should be accounted for before developing biocontrol agents against PRM.

Keywords
INTRODUCTION

MATERIALS AND METHODS

2.1 Arthropod sources

2.1.1 Arthropod predators and microbivore mites

2.1.2 PRM

2.2 Experimental arenas for predation tests

2.3 Predation test experiment

2.4 Predation test types

2.4.1 Prey-choice tests

2.4.2 Predator-to-predator interactions

2.4.3 Complementary tests

2.5 Data analysis

2.6 Ethical requirements

RESULTS

3.1 Predation on PRM

3.2 Preference of putative predators for PRM or astigmatic mites

3.3 Effect of stage and physiological status of PRM preys on predation preference
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>Predator-to-predator interactions</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>DISCUSSION</td>
<td>14</td>
</tr>
<tr>
<td>4.1</td>
<td>Potential food web involving PRM</td>
<td>14</td>
</tr>
<tr>
<td>4.2</td>
<td>Predators’ preference for different forms of PRM</td>
<td>15</td>
</tr>
<tr>
<td>4.3</td>
<td>Implications of interactions other than predator-PRM</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>CONCLUSION AND PERSPECTIVES</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>ACKNOWLEDGEMENTS</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>REFERENCES</td>
<td>18</td>
</tr>
<tr>
<td>8</td>
<td>TABLES</td>
<td>27</td>
</tr>
<tr>
<td>8.1</td>
<td>Table 1</td>
<td>27</td>
</tr>
<tr>
<td>8.2</td>
<td>Table 2</td>
<td>28</td>
</tr>
<tr>
<td>9</td>
<td>FIGURE LEGENDES</td>
<td>29</td>
</tr>
<tr>
<td>9.1</td>
<td>Figure 1</td>
<td>29</td>
</tr>
<tr>
<td>9.2</td>
<td>Figure 2</td>
<td>29</td>
</tr>
<tr>
<td>9.3</td>
<td>Figure 3</td>
<td>29</td>
</tr>
<tr>
<td>9.4</td>
<td>Figure 4</td>
<td>30</td>
</tr>
<tr>
<td>9.5</td>
<td>Figure 5</td>
<td>30</td>
</tr>
<tr>
<td>83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The poultry red mite (PRM) *Dermanyssus gallinae* (De Geer) is the most important pest of laying hens worldwide.\(^1\)\(^2\) PRM is a nidicolous ectoparasite attacking resting hens at night. After relatively rapid blood meal, it retires to hide in different microhabitats like cracks, crevices, and dry droppings in the farm building.\(^3\)\(^4\) PRM has significant effect on the health and welfare of hens, it can cause anemia, decreased egg production and increased hen mortality.\(^5\)\(^6\) The typical conventional control of PRM by means of synthetic acaricides is often not sufficient. In addition, the use of synthetic products has become increasingly reduced by stricter legislation regarding active ingredients.\(^7\)\(^8\) Therefore, different alternative methods of control have been developed such as plant-derived product,\(^9\) inert substances such as diatomaceous earth and silica,\(^10\) electronic perches, biological control by means of natural enemies like entomopathogenic fungi\(^11\)\(^12\) and predatory mites,\(^13\)\(^14\) and research on vaccines is making progress.\(^15\)\(^16\)

The particular lifestyle of PRM as a nidicolous parasite living in a diversity of habitats in poultry farms makes this mite less likely to be reached by chemical treatment and more accessible for arthropod predators than ectoparasites living on the host. This suggests that PRM is an ideal target of biological control. Although biological control is well developed and has been successfully adopted to control pest arthropods in crop farming, this method has more recently begun to be developed against pests in livestock production and is still in its infancy.\(^1\)\(^7\) To date, five predatory
mites were experimentally shown to have potential in PRM biological control: *Cheyletus eruditus* (Schrank) and *C. malaccensis* (Oudemans), *Androlaelaps casalis* (Berlese), *Hypoaspis aculeifer* (Canestrini), and *Stratiolaelaps scimitus* (Womersley). The two predatory mites *A. casalis* and *C. eruditus* are currently mass-reared and used as biocontrol agents of PRM in laying poultry farms, but their efficiency in the field should be improved by complementary research. Arthropods associated to poultry production include several predatory taxa some of which are known to feed on various prey mite species. However, the ability of the majority of these predators to feed on PRM has not been investigated. Amongst the naturally-occurring arthropod predators in poultry farms, various taxa are known to dwell in poultry manure. Several are typically active hunters that have been recurrently observed into microhabitats other than this substrate, including in PRM traps. This makes them promising agents for PRM control in layer farm buildings. The effect of predator communities on their prey’s population may depend on direct and indirect interactions between these predators like competition and intraguild predation. Intraguild predation is very common among generalist predators that exploit common food resources which could significantly affect the dynamics of their shared preys. The effect of intraguild predation on prey regulation can be antagonistic, though many case reports do not confirm it, and it may even be synergistic. When focal and alternative preys share the same predatory species, the availability of alternative prey can also influence the behavior of the shared predator and can lead to either increased or...
decreased predation on focal prey. This depends on many factors including the relative size of prey populations and predator preferences. Astigmatic mites are microbivores/detritivores and they are the most frequent and abundant taxa in poultry manure. They may serve as main or alternative prey for many generalist predatory mites. Evaluating the preferences of potential predators of PRM between astigmatic mites (as possible alternative or competing prey) and PRM (as focal prey) is essential to predict the effect of these predators on pest regulation.

The physiological status of PRM in terms of duration since the last blood meal may have a substantial effect on predation. One can expect from the fresh blood meal either a facilitating effect on predation owing to the weakening of the highly extended cuticle (making it possibly easier to be penetrated by the predator’s chelicera) and the substantial slowing of PRMs’ movements (akinesis is observed quickly after feeding), or an antagonistic effect owing to the oxidative stress produced by the ingestion of fresh blood and/or the toxic products of its digestion (see adaptation mechanisms in hematophagous arthropods to dealing with feeding on fresh blood). The physiological status of prey in interaction with other factors (species, sex, and size of the proposed preys) was shown to be an important driver of prey selection by a spider predator that feeds on mosquitoes. Lastly, adult individuals of certain prey species have a greater ability to escape and better defense responses against predators than juveniles. Prey consumption by several phytoseiid mite predators was inversely related to prey size. Hence, predators may be more or
less prone to feed on freshly fed PRM and/or on different stages of PRM in
farms.

This study aimed to characterize the potential trophic interactions
involving PRM, alternative preys like microbivorous mites and arthropod
predators that usually share the same microhabitats with PRM or those
prone to hunt this mite in such microhabitats. Our specific objectives were
to (1) identify potential predators of PRM based on their ability to consume
various forms of PRM (different stages and physiological statuses) when no
physical barriers hinder the access of these predators to their preys, (2)
evaluate the effect of the presence of alternative preys like astigmatic
mites on the predation on PRM, and (3) assess whether predation
interactions can occur between PRM predators.

2 MATERIALS AND METHODS

2.1 Arthropod sources

2.1.1 Arthropod predators and microbivore mites

To maximize the diversity of arthropod predators to be tested, we sampled
arthropods from several barn layer farms located in the Drôme
department (Rhône-Alpes-Auvergne region, France). These farms were
selected based on previous in farm surveys. Barn layer farms have
slatted flooring under which manure is allowed to accumulate over long
periods (flock duration = ca. 1 year) which permits an important
development and establishment of manure-dwelling arthropods. We
focused on 13 taxa of arthropods including 12 manure-dwelling taxa and
one taxon of spiders. Certain mite taxa were multispecific and others were
monospecific (Table 1). These arthropods were not reared or maintained on any transitional diet in the lab. Manure samples were kept into plastic containers covered with nylon-filter lids (mesh size 80 µm) before the extraction of arthropods.

Manure-dwelling arthropod individuals were extracted by dry sieving of manure samples using a series of stacked sieves with decreasing mesh size (from 1000 µm to 180 µm). In order to identify and differentiate mite taxa, we used the definition of morphospecies in Roy et al. 2017. After performing predation tests on arthropods, the following taxa were identified at the species level: Pseudoscorpionida, Heteroptera (Insecta), *Macrocheles* spp. (Mesostigmata). For other morphoespecies, the taxonomic level was simply the level discernible under the stereomicroscope according to Roy et al. (species level for monospecific morphoespecies, higher levels for others; see Table 1).

2.1.2 PRM

PRM aggregates were collected from two farm buildings in sealable plastic bags and kept fasting for one to three weeks in an incubator at 15 °C before tests. To produce freshly fed PRMs, fasted individuals were introduced into a PVC cylindrical container (60 L, 40 cm in diameter) with a chick for 2 h at 25 ± 5 °C in complete darkness. The top of the container was sealed with mite-proof nylon mesh (100 × 100 µm, PE171.6, Diatex, France). Pieces of folded paper were put in the container to provide shelters for PRMs to aggregate after the blood meal and facilitate the collection of fed individuals.
2.2 Experimental arenas for predation tests

Predation tests were conducted following the protocol by El Adouzi et al. In short, each predator was tested in an experimental arena constituted by a well (flat-bottomed, 7 mm diameter) of a transparent polystyrene microplate (Nunc™ 167008 F96 MicroWell 96-well × 400 µL, external dimensions: 128 × 86 mm Cell Culture Microplate, Fyn, Denmark). We added 2–4 µl of 1.5 % agarose gel into each well to prevent dehydration of the arthropods, a technique validated by El Adouzi et al. Microplates were covered with stretched plastic paraffin film (Parafilm®, Bemis Co., USA).

2.3 Predation test experiment

A test consisted of confining one single predator and one or two preys (depending on the modality, see below) into a well of a 96-well microplate, wells of which were used as replicated experimental arenas. After a fixed contact duration, prey mortality was recorded in each arena. Controls consisted in the same single or paired prey(s) isolated into wells on the same microplate without any predator (same number of wells for controls as for tests with predators). Several different modalities of predator/prey(s) combination were tested on the same microplate and at the same time (= a series). To minimize the effect of random factors, (1) each predator modality was tested on different microplates successively, (2) two to four different modalities of predator/prey(s) combination were tested together in each series, (3) the set of modalities to be tested together was randomly rearranged for each series, so as to be free of dependencies between modalities. Each modality was replicated dozens of
times on a microplate (one predatory individual per taxon and per well on 2 to 4 columns, ie 16 to 32 individual tests, and a similar number of control wells), and this was repeated two to three time in different series. Predators were fasted for 24 h before being tested. Microplates were maintained in a climatic chamber at 25.0 ± 0.5 °C in complete darkness. Prey condition in each experimental arena was examined under a stereomicroscope after 24 hours of test duration. A prey was deemed to be dead if no movement was triggered by contact with a thin paintbrush. Arachnid predators do not swallow prey but only suck internal body liquids, making it difficult to differentiate predation events from prey natural mortality. Thus, in each series, prey mortality in predation tests was corrected by prey natural mortality recorded in the corresponding controls to obtain the predation rate.

2.4 Predation test types
2.4.1 Prey-choice tests
Prey choice tests were used to evaluate (1) the predation rate of each predator on each prey species and (2) the preference of predators between these offered preys. The predation rate (1) was estimated as the frequency of predation on a given kind of prey. Two prey individuals with (test) or without (control) one predator individual were introduced per well. The preference (2) was tested between two PRM developmental stages (unfed juveniles and unfed adult females), or two physiological statuses of PRM (freshly fed and unfed), or between unfed PRM and another prey mite, with a focus on Astigmatic mites. The preference according to the
physiological status and between PRM and Astigmatic mites was tested both with PRM juvenile and adult females. All in all, twelve putative predatory taxa were tested in 31 modalities of prey-choice test (Table 2).

2.4.2 Predator-to-predator interactions

We evaluated whether six native predators engaged in predator-prey interaction when no shared prey was offered, following five combinations: (1) *A. casalis* vs *Cheyletus* spp., (2) *A. casalis* vs *M. penicilliger*, (3) *A. casalis* vs *Lam. nodosus*, (4) *Lam. nodosus* vs *M. penicilliger*, and (5) *Dendrolaelaps* spp. vs *Pro. parascolyti*. The choice of these combinations was driven by the availability of predators in the successive sampling campaigns. For each predator pair, one individual of each of the two species (test) or a single individual (control) were introduced per well. Note that since each of the two predators confronted in the test wells is also a prey, two controls (one for each taxon) are associated with each test. Combinations one to five were tested 39, 24, 24, 22 and 24 times respectively.

2.4.3 Complementary tests

We performed a few complementary tests with the same protocol as prey-choice tests (2.4.1) to document trophic relationships between tested arthropods excluding PRM. Five predatory taxa and microbivore mites were subjected to predation tests according to different combinations described in Table 2. (Modalities 32 to 37).-
2.5 Data analysis

A predation test replicate was discarded from analyses if any of predator(s) or prey(s) had escaped from the experimental arena, or if the predator was dead at the moment of rating. In the case of predator-to-predator interaction tests, the replicate was discarded when the two predators were dead at the time of rating. In addition, a test modality was considered invalid in a series where natural mortality of prey(s) (or predators in the case of predator-to-predator interactions), i.e. in controls, exceeded 15% or differed significantly between the two prey species (or the two predators in predator-to-predator interactions; we used the Chi-squared test to check for independence of natural mortality rates between the two prey species).

To represent the potential trophic relationships between tested arthropod taxa, we considered the frequency of predation per putative predator on each prey species from all tests. The predation rate for each predator-prey pair in each test series was estimated by correcting prey mortality with natural mortality determined in the corresponding control (on the same series) using the Abbott formula\(^5\). The predation rate on each prey species and for each predator was calculated as the average corrected prey mortality over all test series. Predation rates on PRM were calculated by considering all PRM stages and physiological statuses tested indiscriminately.

To assess preferences of predators between prey species, Chi-squared tests were applied to test for dependence between prey mortality and
prey species in the presence of each predator. In prey-choice tests, to consider the three possible configurations (both prey dead, one prey dead, no prey dead), 2 x 2 contingency tables were constructed as follows: prey A dead or live x prey B dead or live. In tests of predator-to-predator interactions, only tests where one and only one predator had died were included in the analysis and 2 x 2 contingency tables were constructed as follows: predator A or B x live or dead. All chi-squared tests were conducted within the R environment,\textsuperscript{56} P values were calculated by Monte Carlo simulation with 2000 replicates.

2.6 Ethical requirements

All the experiments involving hens were conducted in compliance with regulations on animal experimentation (reference number of the Ethics committee: 036; project number: APAFIS#2339-2015101122029640 v4).

3 RESULTS

By bringing together the results of all type of predation tests, we provide an insight into the potential trophic interactions among selected henhouse-dwelling arthropods, showing that PRM could potentially be part of this partial food web. (Fig. 1).

3.1 Predation on PRM

In our experiments, ten indigenous arthropod taxa fed on PRM, with predation rates (Abbott-corrected prey mortality percent) ranging from 100 to 4\%: M. penicilliger, Lam. nodosus, Cheyletus spp., M. muscaedomesticae, Lyc. campestris, linyphiid spider, Par. fimetorum, A. casalis, U. fmicola, and Dendrolaelaps spp. (in order of decreasing
predation rates, Fig. 1). The last two did so only occasionally (8 and 4% 
predation rates, respectively). In the presence of Uropodina spp. or of Pro.
parascolyti, PRM mortality did not exceed natural mortality in the 
corresponding predator-free controls.

3.2 Preference of putative predators for PRM or astigmatic mites

Of the seven arthropods tested as predators, four fed significantly more on 
PRM than on astigmatic mites in our conditions (Cheyletus spp.: $\chi^2 = 27, P$
$= 4.9 \times 10^{-4}$; A. casalis: $\chi^2 = 13, P = 9.9 \times 10^{-4}$; M. muscaedomesticae: $\chi^2 =$
$21, P = 4.9 \times 10^{-4}$; U. fimicola: $\chi^2 = 8.5, P = 7.9 \times 10^{-3}$; Fig. 2). Neither
Cheyletus spp. nor U. fimicola fed on astigmatic mites in the presence of
PRM juveniles.

3.3 Effect of stage and physiological status of PRM preys on predation preference

Cheyletus spp. and A. casalis fed significantly more on juveniles than on
adult females (Cheyletus spp.: $\chi^2 = 42, P = 4.9 \times 10^{-4}$; A. casalis: $\chi^2 = 12, P$
$= 4.9 \times 10^{-4}$; Fig. 3). Predation by M. muscaedomesticae and the linyphiid
spider did not differ significantly between the two stages.

The bug Lyc. campestris fed significantly more on freshly fed PRM
juveniles than on unfed ones ($\chi^2 = 8, P = 8.9 \times 10^{-3}$, Fig. 4A). Cheyletus
spp. fed significantly more on unfed adult PRM females than on freshly fed
ones ($\chi^2 = 17, P = 9.9 \times 10^{-4}$; Fig. 4B).

3.4 Predator-to-predator interactions

Predation occurred within all pairs of predators tested in our conditions
(Fig. 5). Predation was significantly asymmetric between the pairs M.
penicilliger–A. casalis ($\chi^2 = 48, P = 4.9 \times 10^{-4}$), and Lam. nodosus–A. casalis ($\chi^2 = 13.1, P = 9.9 \times 10^{-4}$). A. casalis did not kill any of the two other predators. It was more balanced and insignificant in the pair A. casalis–Cheyletus spp., but mortality was twice as high in Cheyletus spp. as in A. casalis. Predation interactions in the other pairs appear to be accidental.

4 DISCUSSION

4.1 Potential food web involving PRM

Although the predation interactions we observed occurred in the absence of any physical barrier between predator and prey, our results suggest that PRM in barn layer farms could be embedded in a food web containing at least ten arthropod taxa able to feed on PRM. Eight of these taxa are recorded to feed on PRM for the first time here: Lyc. campestris, Lam. nodosus, a linyphiid spider, M. penicilliger, M. muscaedomesticae, Par. fimetorum, U. fimicola, and Dendrolaelaps spp. The last two did so only occasionally. We also confirmed predation on PRM by natural populations of Cheyletus spp. and A. casalis which are actually commercially available for release in layer buildings as biocontrol agent of PRM, although in our experimental design predation by A. casalis can be considered moderate (19% predation rate).

4.2 Predators’ preference for different forms of PRM

Among the four tested arthropod taxa that showed substantial predation on PRM (predation rate > 10%), the largest-sized taxa (M. muscaedomesticae and the linyphiid spider) did not feed differentially on juveniles and adult PRM, whereas the smallest-sized (Cheyletus spp. and
A. casalis) preferred juveniles, consistent with previous findings that larger predators use a wider range of prey sizes.\textsuperscript{57,58} Interestingly, PRM consistently form multi-layered clusters with juveniles remaining in the center and adult females staying at the top.\textsuperscript{42} This arrangement was considered a protective behavior to preserve juveniles from predators. Furthermore, predators with different prey-stage preferences do not affect the population dynamics of prey and predator in the same manner.\textsuperscript{59} Mathematical modelling of prey-predator population dynamics suggested that only predators with no prey-stage preference allow Lotka-Volterra periodic prey-predator oscillations to occur and be maintained.\textsuperscript{59} Predators that prefer juvenile stages induce a rapid increase in the prey-predator oscillations' amplitude, which ultimately results in the extinction of both predator and prey.\textsuperscript{59} Selective predation focused on young preys is thus more likely to lead to extinction than non-selective predation.\textsuperscript{59,60-}

The predatory bug \textit{Lyc. campestris} was the only tested taxon that fed more on freshly fed juveniles than on unfed ones. This is perhaps because of the slower movement owing to blood ingestion, as preference for slow moving preys over faster ones was reported in other predatory bugs.\textsuperscript{41} An alternative explanation (though not exclusive) is that \textit{Lyc. campestris} could be prone to feed indirectly on vertebrate blood, as does an African jumping spider.\textsuperscript{45} The preference of \textit{Cheyletus} spp. for unfed females over freshly fed ones may be explained by the size and/or the motility of prey instead of deleterious effects of the blood meal as these predators did not show any preference for unfed/fed juveniles. In fact, individuals of predator taxa for which no preference for unfed/fed PRM (juveniles or...
adults) was detected frequently consumed both preys (fed and unfed PRM), showing no aversion to taking a potentially deleterious meal of vertebrate fresh blood. However, in these taxa, we may have missed preferences between unfed and fed PRM by hungry predators that may have recurrently chosen a particular status in the first act of predation.

4.3 Implications of interactions other than predator-PRM

The most abundant poultry mite taxon that can constitute an alternative prey to PRM predators is Astigmata. A significant preference for PRM over astigmatic mites was observed in *A. casalis*, *Cheyletus* spp., *Lam. nodosus*, and *M. muscaedomesticae*. The former two are known to feed and successfully develop on several species of astigmatic mites.\(^{38,39,61-63}\) *Cheyletus* spp. is a sit-and-wait predator ("ambush" foraging mode) and it rarely actively stalks its prey.\(^{64}\) As astigmatic mites are very slowly moving,\(^{38}\) these mites are less likely to encounter the cheyletid predator than the fast moving unfed PRM individual in the experimental arenas.

This could partially explain the complete absence of predation on astigmatic mites by this predator in our experiments. As our results show that intraguild predation is possible between several of the PRM predators, the natural regulation of PRM populations might be affected by the predator diversity in farms. Geden *et al.*\(^{21}\) reported the negative impact of intraguild predation between two naturally-occurring predatory mites, *Parasitus* sp. and *M. muscaedomesticae*, on the control of the house fly.

5 CONCLUSION AND PERSPECTIVES

Prior to the present study, information about native predators of PRM that
have been recorded from poultry farms to date was limited to three species $A$. casalis, $C$. eruditus and $C$. malaccensis.\textsuperscript{13,14,19} Our study revealed that six additional taxa native to poultry farms are substantial predators of PRM, all with predation rates on PRM higher than that of $A$. casalis. Among these, two (Par. fimetorum and $M$. penicilliger) are only found occasionally in poultry farms, and thus, do not seem to establish sustainably in this environment. The other four (the bug $Lyc$. campestris, the pseudoscorpionid $Lam$. nodosus, the mite $M$. muscaedomesticae, and the linyphiid spider) might be worth considering as additional candidates for augmentative biological control. They could also provide an unknown regulating ecosystem service that could be worth promoting via conservation biological control practices. Intricate interactions of varying degrees between arthropods in poultry ecosystems likely make actual food-webs different from the present reconstruction, as inferred from barrier-free tests. Ecosystem services provided by pest enemies are strongly dependent on spatial heterogeneity and layout coupled with the enemies’ dispersal capabilities.\textsuperscript{65,66} Special attention should be paid to spatial mapping of prey-predator meeting points in henhouses and identification of their determining factors to anticipate impediments to biological control owing to limited penetration by predatory mites into pest-infested areas.\textsuperscript{67} Further exploration of the potential of assemblages of native predators in controlling PRM through semi-field or field experiments would be most useful for future biocontrol applications in poultry houses.
6 ACKNOWLEDGEMENTS

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7 REFERENCES


George DR, Sparagano OAE, Port G, Okello E, Shiel RS, and Guy JH, Environmental interactions with the toxicity of plant essential oils to the poultry red mite *Dermanyssus gallinae*, *Medical and veterinary entomology* **24**:1–8 (2010).

Maurer V and Perler E, Silicas for control of the poultry red mite *Dermanyssus gallinae* (2006).


18 Lesna I, Sabelis MW, van Niekerk TGCM, and Komdeur J, Laboratory tests for controlling poultry red mites (*Dermanyssus gallinae*) with


49 Xiao Y and Fadamiro HY, Functional responses and prey-stage preferences of three species of predacious mites (Acari:...


### Table 1. List of arthropods species/morphospecies collected from poultry farms and used in predation tests.

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<tr>
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<th>Suborder</th>
<th>Family</th>
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<th>Guild</th>
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<td>Dermanyssidae</td>
<td><em>Dermanyssus gallinae</em></td>
<td>hematophagous</td>
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<td></td>
<td>Gamasina</td>
<td>Laelapidae</td>
<td><em>Androlaelaps casalis</em></td>
<td>predator</td>
</tr>
<tr>
<td></td>
<td>Gamasina</td>
<td>Digamasellida</td>
<td><em>Dendrolaelaps presepeum</em> (dominant</td>
<td>predator</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>species), <em>Dendrolaelaps</em> spp. and</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>unidentified Digamasellidae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gamasina</td>
<td>Melicharidae</td>
<td><em>Proctolaelaps parascolecti</em></td>
<td>fungivorous/omnivorous</td>
</tr>
<tr>
<td></td>
<td>Gamasina</td>
<td>Parasitidae</td>
<td><em>Parasitus fimetorum</em></td>
<td>predator</td>
</tr>
<tr>
<td></td>
<td>Gamasina</td>
<td>Macrochelidae</td>
<td><em>Macrochelidae muscaedomesticae</em></td>
<td>predator</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>M. penicilliger</em></td>
<td>predator</td>
</tr>
<tr>
<td></td>
<td>Uropodina</td>
<td>Urodinychidae</td>
<td><em>Urobovella fomicola</em></td>
<td>predator and detritivorous</td>
</tr>
<tr>
<td></td>
<td>Uropodina</td>
<td>other families</td>
<td>Uropodina spp.</td>
<td>predator and detritivorous</td>
</tr>
<tr>
<td>Trombidiformes</td>
<td>Eleutherengoni des</td>
<td>Cheyletidae</td>
<td><em>Cheyletus spp.</em></td>
<td>predator</td>
</tr>
<tr>
<td>Sarcoptiformes</td>
<td>Astigmata</td>
<td>various families</td>
<td>-</td>
<td>microbivore and detritivorous</td>
</tr>
<tr>
<td>Pseudoscorpionida</td>
<td>Locheirata</td>
<td>Chernetidae</td>
<td><em>Lamprochernes nodosus</em></td>
<td>predator</td>
</tr>
<tr>
<td>Aranea</td>
<td>Araneomorpha</td>
<td>Linyphiidae</td>
<td><em>Linyphiidae</em> sp.</td>
<td>predator</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Heteroptera</td>
<td>Anthocoridae</td>
<td><em>Lyctocoris campestris</em></td>
<td>predator</td>
</tr>
</tbody>
</table>
Table 2. Table 2. Modalities of predation tests performed between putative predators and preys. Each line corresponds to a predator taxon and each column to a prey combinations it was confronted with. M1-M37: Modality ID, n= number of test replicates for each modality; (n) represent only the number of retained replicates of validated test in all test series. (A replicate= one predator individual with two prey individuals into one well of a microplate), N1: protonymph of PRM, AF: Adult female of PRM.

<table>
<thead>
<tr>
<th>Predator species/ morphospecies</th>
<th>Prey combination (one individual of each type)</th>
<th>Prey-choice tests</th>
<th>Complementary tests</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfed N1 &amp; unfed AF</td>
<td>Unfed N1 &amp; unfed AF</td>
<td>Unfed N1 &amp; fed N1</td>
<td>Unfed AF &amp; fed AF</td>
</tr>
<tr>
<td><strong>A. casalis</strong></td>
<td>M1 (n=37)</td>
<td>M8 (n=76)</td>
<td>M15 (n=42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dendrolaelaps spp.</strong></td>
<td>M2 (n=43)</td>
<td></td>
<td>M16 (n=21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pro. parascolyti</strong></td>
<td>M3 (n=22)</td>
<td>-</td>
<td>M9 (n=29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P. fimetorum</strong></td>
<td></td>
<td>-</td>
<td>M17 (n=23)</td>
</tr>
<tr>
<td><strong>M. muscaedomesticae</strong></td>
<td>M4 (n=35)</td>
<td>M10 (n=28)</td>
<td>M18 (n=22)</td>
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<tr>
<td><strong>M. penicilliger</strong></td>
<td></td>
<td></td>
<td>M24 (N=23)</td>
</tr>
<tr>
<td><strong>U. fimicola</strong></td>
<td>M5 (n=75)</td>
<td>M11 (n=31)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Uropodina spp.</strong></td>
<td></td>
<td></td>
<td>M12 (n=15)</td>
</tr>
<tr>
<td><strong>Cheyletus spp.</strong></td>
<td>M6 (n=37)</td>
<td>M13 (n=67)</td>
<td>M19 (n=56)</td>
</tr>
</tbody>
</table>
### Table: Prey Items

<table>
<thead>
<tr>
<th>Species</th>
<th>M7 (n=15)</th>
<th>M20 (n=23)</th>
<th>M26 (N=44)</th>
<th>M37: Astigmatic mites (22)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lam. nodosus</em></td>
<td></td>
<td>M14</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Linyphiidae sp.</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>L. campestris</em></td>
<td>-</td>
<td>-</td>
<td>M21 (n=15)</td>
<td>M27 (N=30)</td>
</tr>
</tbody>
</table>

(†): Two individuals of the same prey species were offered.
9 FIGURE LEGENDS

9.1 Figure 1.
Primary reconstruction of potential food-webs between arthropods in layer farms centered on *D. gallinae* (PRM) as a prey according to our in-vitro tests. Arrows are orientated from prey to predator. Values at the start of arrow indicate percentage of predation (with Abbott correction), thickness of arrows proportional to this percentage, dashed line = absence of interaction, no line = untested interaction.

9.2 Figure 2.
Mortality (%) in *D. gallinae* and astigmatic mites in the presence of single predatory individuals. *n* = number of tested replicates. Black: mortality of *D. gallinae*, white: mortality of astigmatic mite, grey: mortality of both preys. Bars are labeled with * when mortality rates differ significantly between preys within the corresponding modality. Significant difference means predator preference for the prey with the highest mortality rate.
(black + grey for *D. gallinae*, white + grey for astigmatic mite). (\(P < 0.05\), Chi-squared tests).

**Figure 3.** Mortality (%) of *D. gallinae* females and juveniles in the presence of single predatory individuals over a 24-h contact period. \(n\) = number of tested predators. Black: mortality of unfed females, white: mortality of unfed juveniles, grey: mortality of both preys. Bars are labeled with * when mortality rates differ significantly between preys within the corresponding modality. Significant difference means predator preference for the prey with the highest mortality rate (black + grey for females, white + grey for juveniles). (\(P < 0.05\), Chi-squared tests).

**9.3 Figure 3.**
9.4 Figure 4.

Mortality (%) in *D. gallinae* with two physiological statuses. A: unfed and freshly fed juveniles; B: unfed and freshly fed females. 24-h contact period. n = number of tested predators. Black: predation on freshly fed prey, white: predation on unfed prey, grey: predation on both preys. Bars are labeled with * when mortality rates differ significantly between preys within the corresponding modality. Significant difference means predator preference for the prey with the highest mortality rate (black + grey for
freshly fed preys, white + grey for unfed preys). \(P < 0.05\), Chi-squared tests).

9.5 Figure 5.

Proportion of killed individuals in different combination of native henhouse-dwelling arthropod predators. No extraguild prey was offered, No: number of test replicates for each combination. (Chi-squared tests).