



# Population-level impact of native arthropod predators on the poultry red mite *Dermanyssus gallinae*

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1 **Population-level impact of native arthropod predators on the Poultry Red Mite**

2 ***Dermanyssus gallinae***

3 Running title: **Impact of native predators on Poultry Red Mites**

4 Ghais Zriki<sup>1\*</sup>, Rumsais Blatrix<sup>1</sup>, Dominique J Bicout<sup>2</sup>, Olivier Gimenez<sup>1</sup>, Anne-Sophie

5 Soulié<sup>1</sup>, Liza Dadu<sup>1</sup>, David Degueudre<sup>1</sup>, Geoffrey Chiron<sup>3</sup>, Nathalie Sleenckx<sup>4</sup>, Lise Roy<sup>1</sup>

6

7 <sup>1</sup> CEFE, University of Montpellier, CNRS, EPHE, IRD, Université Paul-Valéry Montpellier

8 3, Montpellier, France

9 <sup>2</sup> Techniques de l'Ingénierie Médicale et de la Complexité Informatique, Mathématique et

10 Applications (TIMC, UMR CNRS 5525) Grenoble Alpes University, VetAgro Sup, Marcy

11 l'Etoile, France

12 <sup>3</sup> Institut Technique de l'AViculture (ITAVI) Lyon, Lyon Cedex 07, France

13 <sup>4</sup> Experimental Poultry Centre, Geel, Belgium

14

15 \*Corresponding author: G. Zriki - CEFE, 1919 route de Mende, 34000 Montpellier, France.

16 Tel.: 33 (0) 7 82 75 98 27. E-mail: [ghais.zriki@cefe.cnrs.fr](mailto:ghais.zriki@cefe.cnrs.fr)

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## 23   **Abstract**

24   Stimulating the regulation of pests by their natural enemies is a way to improve the  
25   sustainability of agriculture and respect for the environment. However, the presence of  
26   natural enemies does not guarantee the existence of a pest control service. To what extent  
27   are predatory mites commonly found in henhouses actually able to regulate a major egg  
28   industry pest mite, *Dermanyssus gallinae*? To answer this question, we have  
29   experimentally recreated portions of a poultry house ecosystem allowing the development  
30   of the pest over several generations in the presence of a chick and detritivorous mites  
31   (Astigmata) that are ubiquitous and abundant in layer farms. In these conditions, we  
32   compared the growth of *D. gallinae* populations in the presence and absence of native  
33   predatory arthropods. No effect of native predators on the growth of the *D. gallinae*  
34   population could be detected despite high initial predator-to-prey ratios and satisfactory  
35   growth of predator populations. Prey switching to the alternative prey Astigmata likely  
36   dilutes the effect of predation on the target prey. Further exploration is needed to see  
37   whether action could be taken to enhance the effect of top-down regulation.

38

39   Key-words:

40   prey-predator interactions, population dynamics, native assemblages, mites, bird  
41   ectoparasite

## 42 1. Introduction

43 Taking advantage of the top-down regulation of prey populations induced by  
44 predation or parasitism is the basis of biological pest control. Provided that such  
45 antagonistic interspecific interactions effectively regulate the pest population, the spread of  
46 this practice allows to replace pesticides and thus mitigate the environmental impact of  
47 agricultural activities (Bale et al., 2008; Tracy, 2015). However, empirical and even  
48 theoretical evidence (Abrams, 1992) suggests that this is not always the case. While it is  
49 relatively easy to demonstrate antagonistic interactions between individuals, it is far from  
50 easy to demonstrate the regulation of pest populations by natural enemies at appropriate  
51 scales in agrosystems (Furlong & Zalucki, 2010; Luck et al., 1988). Unlike parasitism rates  
52 that can be measured when dealing with parasitoids, predation events are notoriously  
53 difficult to identify (Furlong & Zalucki, 2010), especially with small arthropods such as  
54 mites (Koehler, 1999; Lindquist, 1975). This major hurdle makes it difficult to establish a  
55 link between the predation process and any reduction of the damage to plants or livestock  
56 (or any increase in production).

57 Taking advantage of biological pest control can be done either by locally releasing  
58 in agroecosystems exogenous natural enemies or by stimulating the development and  
59 activity of native natural enemies (Bale et al., 2008). In the latter case, it is expected that  
60 indigenous natural enemies will spread to farms from surrounding or nested uncultivated  
61 areas (e.g. grass strips, multi-species hedges) (Begg et al., 2017). In crop production, a  
62 long and rich experience of the different methods of biological control in different systems  
63 (Bale et al., 2008; Begg et al., 2017; Luck et al., 1988) makes it possible to implement  
64 integrative pest management strategies through the stacking of biodiversity service  
65 providers by cross-referencing data from various experiments (Hokkanen, 2017). In animal  
66 production, the development of biological control is still in its infancy, likely due to major  
67 interdisciplinary barriers between medicine on the one hand and environmental sciences

68 on the other hand (Destoumieux-Garzón et al., 2018). Biological control in livestock relies  
69 almost exclusively on a few cases of augmentative biological control (e.g. Knapp et al.,  
70 2018; Lesna et al., 2012; Skovgård & Nachman, 2004). Strategies related to conservation  
71 biological control are barely mentioned in the literature and have essentially been targeted  
72 against flies. Axtell (1986) suggested increasing the enemy density of flies on rare farms  
73 with low enemy densities without specifying how, except that he advised against the  
74 introduction of exogenous species. Hinton and Moon (2003) obtained promising results in  
75 poultry farms by acting on manure management to maximize the regulation of fly  
76 infestations by predatory mites, but have not led to operational implementation to our  
77 knowledge.

78 In crops, several meta-analyses have revealed patterns that are sometimes  
79 counter-intuitive and which can be explained by the intrinsic complexity of ecosystems  
80 (e.g. Dainese et al., 2019; Karp et al., 2018). For instance, although conserving non-crop  
81 habitats almost always results in increased biodiversity, this does not come along with a  
82 spontaneous enhancement of pest control, or even sometimes triggers undesired effects  
83 (Karp et al., 2018). Higher-level trophic cascades and other biotic interactions interfering  
84 with the enemy-pest-crop cascade explain part of these counter-intuitive results (Abrams,  
85 1992; Chailleux et al., 2014). The effects of multiple predators on pest populations can be  
86 either increased or decreased compared to those of a single predator due to intraguild  
87 predation (predators feeding on each other) or to competition (Caballero-López et al.,  
88 2012; Colfer & Rosenheim, 2001; Finke & Denno, 2004, 2005; Janssen et al., 2006; Ong &  
89 Vandermeer, 2015). And their effects can be emergent (i.e., not simply additive) (Sih et al.,  
90 1998). Although generalist predators are proven to be effective biocontrol agents  
91 (Symondson et al., 2002), predation by generalist predators can be density-dependent  
92 (Abrams, 2004) and the relative abundance of a focal prey to other alternative prey may  
93 result in prey-switching behavior (Van Baalen et al., 2001). As a result, the co-occurrence

94 of pest and closely related non-pest species could also dilute the foraging effort of  
95 predators, resulting in a decreased control effect (e.g. Cardinale et al., 2003). Therefore, it  
96 is difficult, if not impossible, to predict the effect of a predator in an agroecosystem based  
97 solely on the effect of this single predator on a single prey.

98         Developing biological control in poultry farms is a major challenge for the  
99 sustainability of egg production to control the economically most important pest of laying  
100 hens worldwide, *Dermanyssus gallinae* (De Geer, 1778) (Mul, 2017; Sparagano et al.,  
101 2014). This haematophagous mite affects hen survival and egg-laying rate (Kilpinen et al.,  
102 2005; Wojcik et al., 2000) and is a reservoir and vector of several avian pathogens  
103 (Pugliese et al., 2019; Valiente Moro et al., 2009). In Europe, over 80% of layer farms are  
104 infested with *D. gallinae* and the annual cost of infestation control and production losses is  
105 estimated at 231 million euros (Van Emous, 2017). However, the implementation of  
106 efficient biological control is largely hampered by the general lack of knowledge about  
107 natural enemies and trophic cascades in poultry farming. Whilst native parasitoids are rare  
108 in European commercial henhouses (Roy et al., 2017), a high diversity of arthropod  
109 predators has been reported from these strongly anthropized systems (R. C. Axtell &  
110 Arends, 1990; Brady, 1970; Faleiro et al., 2015; Horn et al., 2016; Lesna et al., 2009; Roy  
111 et al., 2017; Silva et al., 2013; Young et al., 2019). The potential for predation on *D.*  
112 *gallinae* by several native predatory mites, a spider, a pseudoscorpion and three insects  
113 has been reported from *in-vitro* tests (Lesna et al., 2009; Stockton, 2004; Toldi et al., 2017;  
114 Zriki et al., 2020). Significant covariations between abundances of three taxa of predatory  
115 mites and of *D. gallinae* suggest that these predators are indeed associated to the pest in  
116 henhouses (Roy et al., 2020). Yet the link between predation processes and the regulating  
117 service of *D. gallinae* in farms could not be demonstrated (Roy et al., 2020), while it exists  
118 with one of the above mite taxa (*Androlaelaps casalis*) in the nests of wild birds (Lesna et  
119 al., 2009). The three predators of *D. gallinae* proved to be almost ubiquitous in the farms

120 studied, making it impossible to measure their specific effect in henhouses, unlike in the  
121 bird nests studied. It is therefore necessary to apply an experimental approach allowing to  
122 work on a population scale while controlling the presence or absence of predators. Lesna  
123 et al. (2012) conducted the only study of this type available today, but they only tested the  
124 individual effect of two non-native predator populations separately on *D. gallinae* as a  
125 single prey.

126 In order to make progress in developing biological control in an agroecosystem as  
127 poorly understood as henhouses, we need to better embrace what can happen in the  
128 henhouse ecosystem, accounting for potential emerging effects of native multiple  
129 predators and the impact of alternative prey. Besides the multiple predators reported from  
130 henhouses, ubiquitous detritivorous mites represent very abundant native alternative prey  
131 (Roy et al., 2017) and may dilute the impact of the native predators on *D. gallinae*. Our  
132 study aims at documenting the potential of native predators to regulate *D. gallinae*  
133 populations in a farm-like system. We experimentally measured the effect of the presence  
134 or absence of one or more native predators, collected directly from farms, on the growth of  
135 a *D. gallinae* population in the presence of alternative native prey. We addressed the  
136 following three questions, focusing on the two most promising candidates for biological  
137 control of *D. gallinae* according to Roy et al. (2020), namely *Androlaelaps casalis* and  
138 *Cheyletus* spp:

- 139 - Do native predators affect *D. gallinae* population growth in the presence of non-limiting  
140 quantities of alternative prey?
- 141 - Do increased complexity of predator assemblages maximize or minimize the effect of  
142 predation on *D. gallinae* population growth?
- 143 - How do initial biotic conditions determine the suppressive effect of predators on *D.*  
144 *gallinae*?

145

## 146 2. Materials and Methods

### 147 2.1. Study system

#### 148 2.1.1. Arthropod source

149 *Dermanyssus gallinae* individuals were collected in sealable plastic bags from  
150 aggregates found in hiding places in several barn layer farms located in the Drôme  
151 department (Rhône-Alpes-Auvergne region, France) and kept in the lab at 17°C. They  
152 were used in experiments after one to two weeks of fasting. This procedure resulted in  
153 synchronising the next gonotrophic cycle as females lay one clutch of eggs after each  
154 blood meal and need less than four days at 15-20°C for the whole process (Tucci et al.,  
155 2008). This was meant to reduce variation in population dynamics across replicates due to  
156 potential sampling bias regarding female physiological status.

157 In barn layer farms, manure is allowed to accumulate over the flock period (ca. 12  
158 months) and hosts a high diversity of arthropods (Brady, 1970; Roy et al., 2017). Manure,  
159 as a source of arthropods, was collected from the same farm buildings as *D. gallinae* and  
160 stored at room temperature for one to two weeks before the start of the experiments. Live  
161 arthropods were extracted by dry sieving of manure immediately before being used in the  
162 experiments. Individuals were sorted by taxa and counted using a stereomicroscope into  
163 filter pipette tips connected by their base to a vacuum pump (aspiration is made possible  
164 by the porosity of the filter, which holds the arthropods). Each tip, containing a given  
165 number of individuals of a single taxon, was sealed with parafilm and stored separately in  
166 Eppendorf tubes for a few hours before starting the experiment. Mite taxa were classified  
167 following morphospecies defined in Roy et al. (2017). The source of astigmatic mites was  
168 either a farm population (collected with other mites from a farm in the present study, then  
169 sorted and multiplied in the absence of other taxa in the laboratory on yeast flakes), or a



170 pure lab population (sampled elsewhere and kept pure for years). As astigmatic mites are  
171 omnipresent and at high density in poultry manure (Brady, 1970; Roy et al., 2017), they  
172 were inoculated in large quantities in all experimental units to mimic conditions in farm  
173 buildings. In each mesocosm, approximately 3000 astigmatic mites were inoculated using  
174 a pipette tip as a gauge. For this purpose, a measurement was defined beforehand by  
175 grouping 3000 astigmatic mites (counted under the stereomicroscope) in a P1000 filter  
176 pipette tip and marking the filling level of the tip after settling by tapping. This manipulation  
177 was repeated 3 times to check for consistency in mite volume. Table 1 gives the list of  
178 arthropod taxa used in the experiments.

#### 179 2.1.2. Chicks materials

180 Specific-pathogen-free chicks belonging to the PA12 White Leghorn lineage were  
181 provided by the Plateforme d'Infectiologie Expérimentale (INRAE, Nouzilly, France). Each  
182 experiment was run with chicks from a single clutch.

#### 183 2.2. Experimental setup and general protocol

184 The regulating effect of various native arthropod assemblages on *D. gallinae*  
185 population development was tested through manipulative experiments using medium-sized  
186 experimental units, called hereafter mesocosms (details in Supplementary data 1).  
187 Mesocosms were mite-proof polyvinyl chloride cylinders, 40 cm in diameter, 39 cm high,  
188 designed to simulate the environment of a barn layer farm building and to host a chick for  
189 up to seven successive weeks with feed and drink *ad libitum* (1- to 7-week old chicks; two  
190 successive chicks needed to reach more than six weeks). A metal plate with holes fixed in  
191 a horizontal position 10 cm from the bottom (mimicking the farm slatted floor) allowed the  
192 chick to stand above the manure accumulation area. Chick water supply was regulated  
193 using a Stilla pipette screwed through the mesocosm wall (UFS, Guichainville, France).  
194 The top of mesocosms was fitted with a nylon-filter lid (mesh size 80  $\mu\text{m}$ ) allowing air flow  
195 while preventing arthropods exchange with the surrounding. Each mesocosm was

provided with three artificial shelters mimicking places sought by *D. gallinae* to aggregate and reproduce in poultry farm buildings (Supplementary data 1). Each of these shelters occupied 0.46 % of the internal volume of the mesocosm. Some details of the protocol were adjusted from one experiment to the next to take advantage of the knowledge acquired in the previous ones. These adjustments were meant to improve the capacity to detect any putative effect of native arthropods on *D. gallinae* population, and are explained for each experiment (see next section and Table 2).

In order to limit the risks of alteration of freshly blood-fed mites by the treatment necessary for their extraction and isolation from mesocosms (individuals of *D. gallinae* are more fragile after the blood meal), the chicks were removed from the mesocosms one week before the end of each experiment. At the end of the experiment, the development of arthropods was stopped by freezing at -20°C for 24 hours. To recover arthropods from mesocosms, a flotation method used to extract arthropods from soil samples (Edwards, 1991) combining washing and wet sieving was adapted. Arthropods from artificial shelters and from the coarse substrate (manure + dust + feed + feathers) of the remaining content were extracted separately and stored in 96 % ethanol in distinct jars. Arthropods other than mites were directly identified and counted on large-mesh sieves. For mites, abundance was estimated by extrapolating numbers of individuals from counts in four aliquots of 1/33<sup>rd</sup> and four of 1/75<sup>th</sup> volume for artificial shelters and coarse substrate respectively. Mites were counted in each aliquot following the method of Roy et al. (2017): they were filtered out through a nylon membrane, spread over the membrane, identified and counted under a stereomicroscope. As protonymphs cannot be unambiguously assigned to morphospecies, only adult-like individuals (deutonymphs and adult males and females) were counted. Astigmatic mites were introduced as a ubiquitous component of the poultry environment (role in degradation of manure and potential prey of introduced predators). Thus, their development was assessed through a qualitative check (we verified

222 that astigmatic mites were visible in the mesocosm by a rough observation with the naked  
223 eye), but abundance was not estimated. They reached a high level of development in all  
224 mesocosms.

### 225 2.3. Population-level impact of native arthropod assemblages on *D. gallinae* regulation

226 To test for an effect of native arthropods on the regulation of *D. gallinae* at the  
227 population level, three successive experiments were conducted in which the composition,  
228 the complexity and the richness of the assemblage of native arthropods was manipulated.  
229 Each experiment consisted in ten mesocosms for each of two to three modalities: one  
230 control and one to two test modalities. All mesocosms received one chick (replaced when  
231 dead), ca. 3000 astigmatic mites and a given number of *D. gallinae* individuals sampled  
232 from farm (Table 2). For test modalities, mesocosms were additionally inoculated with a  
233 specific assemblage of other arthropods (Table 2). Arthropod populations in the  
234 mesocosms were left untouched until the end of the experiment (for four to nine weeks  
235 according to the experiments).

#### 236 2.3.1. Experiment A. Impact of assemblage complexity: one versus two predatory species

237 The effect on *D. gallinae* regulation of potential interactions between two native  
238 predatory mites known for reciprocal predation (Zriki et al., 2020) was investigated using  
239 two test modalities (Table 2). They consisted in inoculating either *Cheyletus* spp. alone or  
240 *Cheyletus* spp. and *A. casalis* (Table 2). The inoculum of *D. gallinae* consisted in 400 adult  
241 females (their large size allowed discrimination from males and other stages using a  
242 stereomicroscope) and was given a one-week head start over predators.

#### 243 2.3.3. Experiment B: Impact of assemblage complexity (continued): a nine-taxa 244 assemblage

245 The effect on *D. gallinae* regulation of a high-complexity assemblage of nine native  
246 arthropods (mites and insects), including five predators of *D. gallinae* was investigated

247 using a single test modality (Table 2). Experiment A revealed high levels of development  
248 of *D. gallinae*, suspected to blur any potential effect of predators and to lead to chick  
249 premature death. For ethical reasons, the size of the inoculum was reduced to 25 adult  
250 females of *D. gallinae* to delay the growth phase and thus limit the lethality of the  
251 infestation. At the same time, this allowed us to increase the predator-to-prey ratio. This  
252 number was fixed based on predictions using the population growth model of Huber et al.  
253 (2011) to lead to a final number of *D. gallinae* lower than the ones that induced chick  
254 mortality in experiment A. Simulations were conducted in R (R Core Team, 2020) with the  
255 deSolve package (Soetaert et al., 2010). The scripts are provided as a Supplementary  
256 data 2 and 3.

257 *2.3.4. Experiment C. Impact of a juvenile-feeding predator on a juvenile inoculum of D.*  
258 *gallinae.*

259 The effect of the predatory mite *A. casalis* when the inoculum of *D. gallinae* was  
260 composed of protonymphs was investigated using a single test modality (Table 2). Using  
261 protonymphs instead of adult females was meant to increase the probability to detect any  
262 effect of the predator on *D. gallinae* population, for two reasons. First, as this predator has  
263 a strong preference for protonymphs of *D. gallinae* over adult females and astigmatic mites  
264 (Zriki et al., 2020), it should be more efficient impacting population growth in experiment C  
265 than in the previous ones. Second, there would be less age variation within the first batch  
266 of adult females. As the number of eggs laid per clutch is correlated with age in *D. gallinae*  
267 females (Dotson, 1982), less age variation across inocula should translate into less  
268 heterogeneity in growth rates and final numbers of *D. gallinae*, and thus, more statistical  
269 power to detect a putative effect of predators on population growth. In addition, as the  
270 secondary sex ratio in *D. gallinae* seems balanced (Oliver Jr, 1966), an inoculum of 100  
271 protonymphs in experiment C should yield 50 adult females, reducing heterogeneity  
272 compared to the low initial number of females in experiment B. *Dermanyssus gallinae*

273 were not given a head start on predators in order to maximize the effect of the predator on  
274 the initial stage of population growth.

#### 275 2.4. Data analysis

276       Given that the demographic dynamics of a population is time dependent and  
277 typically includes a period of exponential increase after a latent period, that we are  
278 interested in the rate of increase and that we have conducted experiments of different  
279 durations, we have chosen to calculate the rates of increase of each mesocosm assuming  
280 an exponential growth of the population (Nordenfors et al., 1999), in order to compare the  
281 results of the three experiments. The rate of population increase ( $r$ ) was calculated for  
282 each arthropod taxon in each mesocosm as,  $r = \ln[(N_f+1)/N_i]/t$ , where  $N_i$  is the initial  
283 number of individuals,  $N_f$  the final number of individuals (for mites, only adult-like  
284 individuals were counted, see section 2.2), and  $t$  the time period (in days) during which the  
285 resource of the focal taxon was available (the chick for *D. gallinae*, the rest of the set up  
286 for native arthropods, see Table 2). In the formula of the rate, 1 was added to  $N_f$  to allow  
287 computation of the logarithm even when no mite individuals were found at the end of the  
288 experiment. Comparing  $r$  among control modalities tested differences in intrinsic rate of  
289 increase of *D. gallinae* population among experiments. The regulating effect of arthropod  
290 assemblages on *D. gallinae* population was tested for each experiment by comparing the  $r$   
291 of *D. gallinae* between control and test modalities. Comparisons were performed using  
292 Kruskal-Wallis and/or pairwise Mann-Whitney tests. P-values were adjusted with the  
293 Holm's method in case of multiple comparisons. To test for a subtler effect of predators on  
294 *D. gallinae* population in each experiment, we tested for a correlation between  $r$  of *D.*  
295 *gallinae* and the final number of native predators for each experiment (control and test  
296 modalities pooled). Statistical analyses were performed with R (R Core Team, 2020).

297       In order to determine whether the growth of *D. gallinae* in mesocosms ran  
298 successfully, the data obtained were compared with predictions from the mathematical

model by Huber et al.(2011). This was not trivial because the groups of individuals counted were not equivalent. The model considers and simulates the populations as a function of time for three groups of individuals: (1) eggs & larvae, (2) proto- & deutonymphs and (3) adult females; no number of adult males nor of dead individuals are provided. In our experiments, the number of individuals at the end of the experiment in each mesocosm was evaluated on the basis of the global count of adult-like individuals (deutonymphs + adult males and females). In addition, we did not distinguish between the dead and the living individuals. As a result, observed data consist in the summation of the whole model's group (3), part of model's group (2), adult males and dead individuals (all sexes and stages). To compare predicted and observed numbers of mites in different experiments, the predicted number of adult females (group 3) at  $t+n$  ( $n$ = duration of the experiment) was considered as the lower limit and the total sum of predicted individuals (all stages) as the upper limit of what could be expected as the number of adult-like individuals counted per mesocosm. In the latter, the absence of the model's group (1) from the experimental mite counts is likely to be only partially compensated for by the addition of the dead individuals. Indeed, in an experiment of comparable duration (6 weeks), the percentage of mortality estimated every week was  $< 3.5\%$  and eggs accounted for about 25% of the population at  $t+35$  days and  $t+42$  days in the study by Wang et al. (2018) conducted in individual cages. Therefore, eggs are likely to be more as are dead individuals at the end of experiment.

#### 2.5. *Ethics approval*

The experiments were approved by the Languedoc Roussillon ethics committee n°36 (project reference: APAFIS#1549-201805251343835v3). All experiments involving birds were conducted in compliance with relevant guidelines and regulations.

### 3. Results

### 324 3.1. *Impact of native arthropod assemblage on D. gallinae population*

325        Estimated final numbers of *D. gallinae* in control modalities showed high variance  
326 among replicates within each experiment (Table 3). In none of the experiments did the *r* of  
327 *D. gallinae* vary significantly between control and test modalities (Fig. 1; experiment A:  
328 Kruskal-Wallis statistic = 5,  $P = 0.09$ ; experiment B:  $U = 37$ ,  $P = 0.55$ ; experiment C:  $U =$   
329 53,  $P = 0.88$ ). In addition, *r* of *D. gallinae* and final estimated numbers of native predators  
330 were not correlated (Supplementary data 4). Experiments A and B showed successful  
331 population growth whereas in experiment C *r* was close to zero (failed population growth)  
332 (Fig. 1). Based on the model predictions, experiments A and B generated values much  
333 higher than predicted (9.5 times on average (max. 14.7 times) and 7.5 times on average  
334 (max. 9.8 times) respectively). In experiment C, observations were consistent with the  
335 prediction.

### 336 3.2. *Population increase of other native arthropods*

337        *Androlaelaps casalis* and *Cheyletus* spp. showed high rates of population increase  
338 in all experiments (Fig. 2). Most other native arthropods (experiment B) failed to develop (*r*  
339 close to or below 0).

### 340 3.3. *Spatial co-occurrence of D. gallinae and predators*

341        In terms of spatial organization, the different arthropods can move and stay in  
342 different places in our mesocosms: in the artificial shelters that we have designed to be  
343 suitable for *D. gallinae* (high stratum), in the manure that accumulates under the holed  
344 metal plate (low stratum), in the interstices formed by the junction points between the  
345 different elements and under the feeder (various strata). In other experiments carried out  
346 in the same mesocosms, the distribution of *D. gallinae* evolved from about 80% in artificial  
347 shelters (20% elsewhere) at the beginning of the population dynamics to 50% in artificial  
348 shelters in the growth phase (LR, RB, unpublished data). As expected, artificial shelters

349 were colonized by *D. gallinae* in all mesocosms in the present study. In Experiment C,  
350 where there was no development of *D. gallinae*, the mean percentage of individuals  
351 counted in these shelters relative to the whole mesocosm was  $76.55 \% \pm 31.65$ .

352 We do not know a priori how predatory arthropods are distributed in our  
353 mesocosms. While the genus *Cheyletus* encompasses typically sit-and-wait predators,  
354 *Androlaelaps casalis* is a vagile and active hunter which can move quickly over several  
355 meters to search for prey (Barker, 1968). *Cheyletus* spp. co-occurred with *D. gallinae* in  
356 shelters of 18 and 10 mesocosms out of 20 and 10 in experiments A and B respectively.  
357 *Androlaelaps casalis* co-occurred with *D. gallinae* in shelters in 2, 10 and 9 mesocosms  
358 out of 10, 10 and 10 in experiments A, B and C respectively. No other arthropod taxon was  
359 found in shelters.

#### 360 3.4. Chick death

361 The protocol involved the systematic and immediate replacement of any dead chick.  
362 At the beginning of each experiment, a small number of chicks died, a mortality which was  
363 considered normal ( $< 3\%$  of the flock). In experiment A, recurrent chick death was  
364 recorded from 35 days of contact with *D. gallinae* when mortality was almost nil before and  
365 for no apparent reason other than mite infestation. Because most replaced chicks died at  
366 that time within one or two days, we considered, for ethical reasons, this peak mortality as  
367 a cut-off point and made the decision to remove chicks from the experiment early (at T+39  
368 days, instead of the 60 days of presence of chicks initially foreseen) and mesocosms were  
369 kept running without chick for an additional month in order to temporarily block the growth  
370 of the *D. gallinae* population (see Table 2). Knowing that we observe massive mortality  
371 after 3 weeks in *D. gallinae* populations kept in the laboratory in the absence of a host, we  
372 expected that the *D. gallinae* population would have become small enough to allow new  
373 chicks to remain healthy. We further expected that this might maximize the impact of  
374 predators on the *D. gallinae* population in the test modalities. This sanitary action was not



375 enough to make the *D. gallinae* population tolerable for the new chicks, whose  
376 immediately high mortality was a definitive cut-off point, whatever the modality. In  
377 experiment B, recurrent chick death was also recorded before the planned end of the  
378 experiment, after we replaced old chicks (7 weeks old) by younger ones (two weeks old)  
379 despite the substantial reduction in the size of the inoculum. We again removed the chicks  
380 at the time of peak mortality, and we then terminated the experiment. In these two  
381 experiments considered together, the relative number of mesocosms with chick death did  
382 not differ significantly between control (6 out of 19) and test (13 out of 30) modalities (Chi-  
383 squared = 0.27,  $p = 0.60$ ). Estimated final number of *D. gallinae* was significantly higher in  
384 mesocosms with chick death (median [min-max] = 98,919 [33,709-153,091]) than in  
385 mesocosms without chick death (median [min-max] = 52,598 [1,196-142,931]) (Mann-  
386 Whitney U-test,  $U = 461$ ,  $p = 0.00018$ ).

387

#### 388 4. Discussion

389 Our innovative experimental system makes it possible for the first time to test at a  
390 population level the effects of the predation process between mites growing on coarse  
391 substrates, as has been done on mites growing on plant substrates (e.g. Huffaker, 1958).  
392 The mite-proof system allows control of inoculum size and freedom from unwanted  
393 introductions during experiments in cages reported by Lesna et al. (2012). Dozens of  
394 replicates can be carried out in parallel in the same room thanks to the moderate size of  
395 each unit. The development of the target prey *D. gallinae* from inocula consisting of adult  
396 females and the development of the alternative prey (Astigmata) was very satisfactory.  
397 The development of predators was consistent with our expectations of success, despite  
398 disparities between taxa. The two most promising predator taxa, *Androlaelaps casalis* and  
399 *Cheyletus* spp. have developed well in all the experiments. Among the arthropods that  
400 failed to develop, this was expectable for some because the duration of the experiment

401 was less than or very close to the life cycle time (e.g. *C. pumilio*, Uropodina). For others,  
402 growth failure may be explained by a resource defect. For example, mites of the genus  
403 *Dendrolaelaps* typically feed on nematodes, a group we have not introduced into the  
404 system. Finally, the artificial shelters were largely occupied by *D. gallinae* and the  
405 recurrent presence of *A. casalis* and *Cheyletus* spp. in these prey-occupied shelters is  
406 consistent with the observations of Maurer et al. (1993) for the same taxa in traps placed  
407 in henhouses. These artificial shelters thus perform well as microhabitats for the various  
408 interactors.

409       Regarding the growth of focal prey, in the two experiments with inoculation of adult  
410 females, the growth of the focal prey population evolved consistently with the duration of  
411 the experiment and the size of inocula since the final numbers varied substantially  
412 between experiments A and B but maintained similar  $r$  values (Fig. 1). In experiments A  
413 and B, the intrinsic  $r$  (mean 0.14 and 0.13 resp.) was slightly lower than the  $r$  estimated by  
414 Lesna et al. (2012) (mean 0.15 females/female/day) under temperature conditions very  
415 close (26°C) to ours (26°/23°C) and close to that estimated by Maurer and Baumgärtner  
416 (1992) (0.12 females/female/day) at 25°C. However, our  $r$  was underestimated compared  
417 to these two studies, as it does not consider eggs, larvae and protonymphs (we only count  
418 adult-like individuals). The heterogeneity of development success among replicates within  
419 modalities in each experiment was consistent with studies that have carried out  
420 experiments involving hens in isolated cages, artificially infested with *D. gallinae*: Lesna et  
421 al. (2012) and Mul et al. (2017) reported developmental failures in >15% of replicates.

422       Despite the massive growth of predators prone to feed on *D. gallinae*, no negative  
423 effect was detected on the growth of the pest population after several generations,  
424 whether with single or multiple predators. Moreover, the mortality of the chicks was not  
425 diminished by the presence of predators. Would confinement alter prey-predator  
426 interactions in the mesocosms, an exaggerated negative predator effect would be

427 expected due to the forcing of the prey-predator encounter, rather than a decrease in the  
428 effect (Luck et al., 1988). Given that both pest-feeder predators were actually encountered  
429 in the presence of prey in artificial microhabitats, it is unlikely that the lack of effect is due  
430 to a lack of prey-predator encounter.

431         Astigmatic mites were very abundant in our mesocosms and may have diluted the  
432 effect of predators on *D. gallinae*. Predation rate by mite predators on plant pests was  
433 shown to decrease, increase or be unchanged in the presence of Astigmatic mites  
434 (Grosman et al., 2011; Rueda-Ramírez et al., 2018). The effectiveness of biological control  
435 by mite predators in the presence of an alternative prey can also vary depending on the  
436 stage composition of the primary prey population (Azevedo et al., 2019). Thus, the effect  
437 of an alternative prey on biological control by mite predators is complex. On the one hand,  
438 alternative prey may boost the population of predators and enhance pest control. On the  
439 other hand, alternative prey may divert predators from the pest and reduce the  
440 effectiveness of the pest control service. In the case of our study, we suggest that prey  
441 switching to the very abundant Astigmata may well explain the lack of effect of predators  
442 on *D. gallinae*. Interestingly, *A. casalis* and *Cheyletus* spp. had been shown *in vitro* to  
443 prefer *D. gallinae* to the alternative prey Astigmata (Roy et al., 2020; Zriki et al., 2020).  
444 This apparently contradictory result may be due to behavioural differences between the  
445 two species: astigmatic mites are very slow-moving mites, whereas *D. gallinae* is swift and  
446 often active. Predators may have preferred *D. gallinae in vitro* because of the increased  
447 likelihood of encountering them in small *in-vitro* arenas. The reverse situation may occur in  
448 mesocosms where confinement is much lesser. Prey switching from *D. gallinae* to  
449 Astigmata may even be more common in mesocosms than in henhouses because the  
450 distances between feed and manure, where Astigmatic mites tend to grow more, and the  
451 microhabitats in the upper stratum, where *D. gallinae* preferentially accumulates, are  
452 substantially reduced compared to henhouses (dozens centimeters vs. several meters).

453 This may lead predators in henhouses to encounter *D. gallinae* more frequently away (e.g.  
454 on perches) from areas where astigmatic mites are found in high densities (e.g. in manure)  
455 than in mesocosms.

456 No emerging effects (positive or negative) from multiple predators were detected  
457 either. Of course, we could not consider the effect of multiple predators in their entirety  
458 (some native mite species could not be integrated into the multitaxa modalities), nor the  
459 various possible interactions with many uncontrolled organisms (nematodes, fungi,  
460 protozoa, bacteria...). We have achieved the development of communities involving a  
461 variety of native predators, including the two most promising ubiquitous taxa from previous  
462 studies. Nevertheless, we cannot guarantee that the absence of effect really reflects what  
463 happens in a real farm.

464 The lack of effect of predators of *D. gallinae* does not even seem to be affected by  
465 the initial biotic conditions. Considering the two focus predators *A. casalis* and *Cheyletus*  
466 spp., the initial predator to prey ratios (1:10, 1:0.6 and 1:5 respectively in experiments A, B  
467 and C) were in the range or higher than ratios that induces significant reduction in the mite  
468 prey population in a greenhouse prey-predator system (e.g. 1:20, and in some cases up to  
469 1:60; Opit et al., 2004). Certainly, the 1-week time lag between the introduction of *D.*  
470 *gallinae* and that of the predators in experiments A and B decreased these ratios. But in  
471 experiment C, prey and predators were introduced at the same time as the predator, so  
472 that the initial ratio was strictly 5 prey individuals per predator. With such a high predator to  
473 prey ratio, a strong reduction of the *D. gallinae* population was expected in the test  
474 modalities, all the more that the prey was the preferred stage (protonymphs) of the  
475 predator tested (*A. casalis*, Zriki et al., 2020). The lack of a difference between control and  
476 test modalities could simply have been the result of a sudden death of the protonymphs at  
477 the beginning of the experiment, since we count dead mites along with living ones. But  
478 most of the mites remained alive since the majority of the individuals were recovered from

479 artificial shelters (the only part where both protonymphs and adult-like were counted;  
480 protonymphs actively sought and found suitable microhabitats after inoculation) and very  
481 few had the appearance of individuals dead before final treatment (the bodies were for the  
482 most part intact and flexible, instead of dry and broken by sieving). Since these prey did  
483 not complete a full cycle in spite of remaining alive and the predators developed properly,  
484 the lack of difference between control and test modality suggests that the predator to prey  
485 ratio even increased throughout the experiment.

486         Prey switching aside, the population dynamics of the prey, relative to that of the  
487 predators likely partly explains the general lack of effect of predation on populations of *D.*  
488 *gallinae*. The final numbers of *D. gallinae* in experiments that produced satisfactory growth  
489 were well above the upper limits of the model predictions (Table 3). This suggests that  
490 some parameters of the model (stage duration, survival rate, severity...) are far from  
491 reality. The fundamental knowledge of many biological parameters is based on a few life  
492 tables from *in-vitro* studies (especially Maurer & Baumgärtner, 1994; Nordenfors et al.,  
493 1999; Tucci et al., 2008) which provide valuable but patchy information. Recent *in-vivo*  
494 studies add valuable information such as feeding rate and life cycle duration with the  
495 permanent presence of hens (Wang et al., 2018; Wang et al., 2019), however, information  
496 is still lacking. The stage that makes up the starting inoculum strongly affects the  
497 subsequent population growth since no development could be observed with inocula  
498 composed of protonymphs (experiment C, Fig. 1). The model predicted almost perfectly  
499 the number of individuals in the different stage groups in experiment C, although we  
500 cannot clearly explain this result. Either the match between the model and the result of the  
501 experiment is fortuitous, involving some computation artefact that mimic a biological fact,  
502 or model parameters involving nymphs are more reliable than those involving adult  
503 females (as experiments A and B showed a weak match between model predictions and  
504 experiment results when starting with adult females). In the first case, the biological fact

505 might be the inability of protonymph to get suitable blood meals in the absence of adults.  
506 Indeed, only part of the mites had obviously fed during the 4-week experiment (molted into  
507 adult-like). The protonymphs constituting the inoculum thus appear to have performed little  
508 feeding activity, although alive.

509         In conclusion, no effect of predation on the population growth of *D. gallinae* could be  
510 demonstrated in the present study, despite an innovative and successful experimental  
511 system, working at a population level, in native communities of mites associated with  
512 poultry. The presence of alternative native prey and the peculiar population dynamics of *D.*  
513 *gallinae* may explain this result. A huge gap in knowledge of *D. gallinae* population  
514 dynamics was uncovered by chance in the course of our study. It may not only explain the  
515 lack of effect of predators on the development of the target prey, but may also explain  
516 many other failures to control this pest in field. Indeed, our results suggest that killing  
517 individuals does not significantly affect *D. gallinae* population and/or the rate of removal by  
518 predators is too small to affect the population in farm-type contexts. In order to refine the  
519 comparison between model predictions and experimental results, it would be wise to  
520 consider dead mites in the Huber et al. (2011)'s model. This will require not only the  
521 rewriting of parts of the model, but also the evaluation of the degradation kinetics of mite  
522 corpses.

523         The present study based on non-limiting quantities of astigmatic mites is a key step  
524 in the implementation of biological control of *D. gallinae* since it mimics a typical farm  
525 environment. However, prey-switching is density-dependent par excellence, it could vary  
526 substantially according to local densities of astigmatic alternative prey, thus among  
527 henhouses, but also among microhabitats within henhouses. Therefore, a thorough  
528 measure of the effect of astigmatic mite density on predation on *D. gallinae* would be  
529 needed to fully assess its local effect. Mapping prey-predator meeting points in henhouses  
530 and comparing this map with astigmatic mites' densities would help to determine the

531 extent to which local top-down regulation could be reinforced by targeted practices. Lastly,  
532 we have been able to observe the result of population growth of *D. gallinae* in our farm-like  
533 units, but the kinetics are unknown. It would be useful to dissect the temporal dynamics as  
534 the experiment progresses, which will require consequent adjustments to the system (e.g.  
535 insertion of an electronic mite tracking system in the mesocosms).

536

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552

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556

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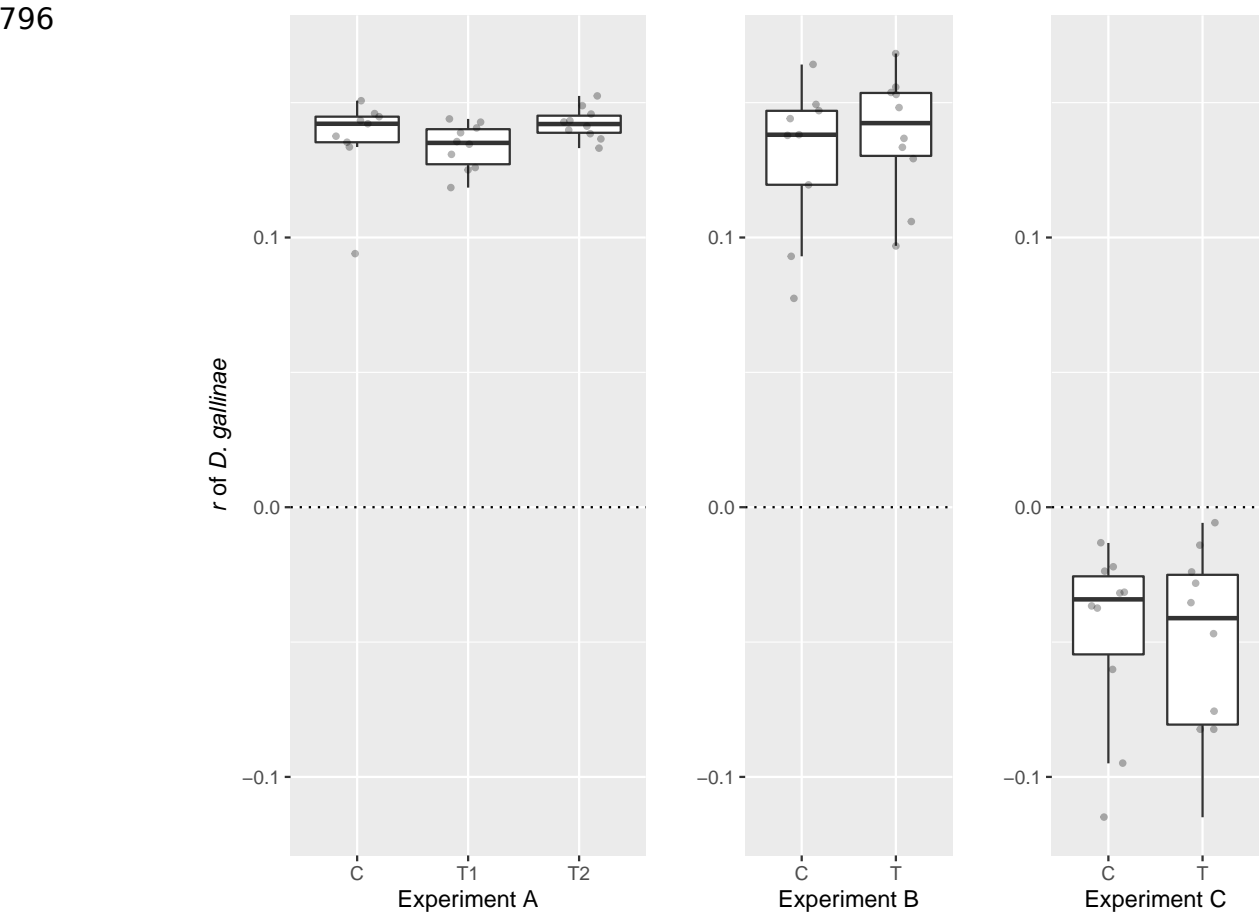
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790 **Figures**

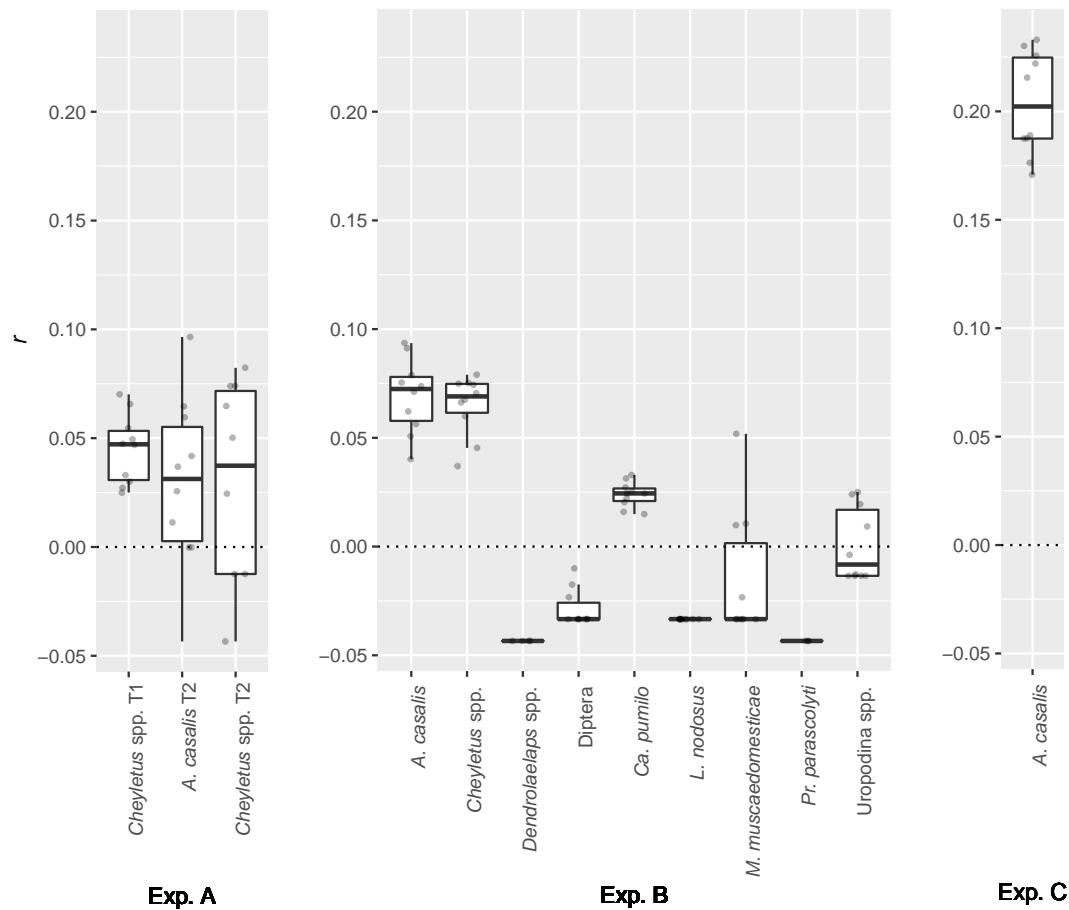
791

792 **Figure 1.** Rate of *D. gallinae* population increase ( $r$ , in number of individuals per day) in  
793 control (C) and treatment (T) mesocosms of three experiments. Treatments consisted in  
794 adding predators of *D. gallinae*. Horizontal bold lines represent median, boxes represent  
795 inter-quartile range, and whiskers extend to 1.5 times inter-quartile range.



797 **Figure 2.** Rate of population increase ( $r$ ) of native arthropods in three experiments in  
798 mesocosms. Horizontal bold lines represent median, boxes represent inter-quartile range,  
799 and whiskers extend to 1.5 times inter-quartile range.

800



801 **Supplementary data**

802

803 **Supplementary data 1.** Description of mesocosm design.

804 **Supplementary data 2.** Script used for the simulation of population dynamics of

805 *Dermanyssus gallinae* using the model of Huber et al. (2011) in R. This script allows to set

806 the parameters used by the model (Table 1 of Huber et al. 2011), to calculate the basic

807 reproductive number and the carrying capacity of the adult population and to perform

808 model simulations using the dede function (to solve the delayed differential equations

809 numerically).

810 **Supplementary data 3.** Script used for the simulation of population dynamics of

811 *Dermanyssus gallinae* using the model of Huber et al. (2011) in R. This script contains the

812 model equations (a system of delayed differential equations).

813 **Supplementary data 4.** Rate of *D. gallinae* population increase ( $r$ ) as a function of the

814 estimated total number of predators at the end of three experiments in mesocosms.

815 Control modality (initial inoculum with *D. gallinae* alone): circles, test modalities (initial

816 inoculum including an assemblage of native arthropods): crosses and triangles. rho:

817 Spearman's correlation coefficient.