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Population dynamics of a poultry hematophagous mite: characterization of the population growth and identification of factors of its slowdown using closed mesocosms

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Abstract

BACKGROUND: A thorough knowledge of the population dynamics of pests and of the main factors affecting population growth is an important prerequisite for the development of effective control strategies. Failures of various treatments aimed at regulating populations of *Dermanyssus gallinae* are regularly reported in poultry farms and pullulations occur very quickly after first detection. To finely characterize population dynamics of *D. gallinae*, and to identify the factors modulating population growth, we conducted two successive multi-generation experiments using closed mesocosms equipped with or without automatic counters and housing a host full- or part-time (three nights per week).

RESULTS: Population growth was very rapid and the adult to juvenile ratio very different from the prediction by a mathematical model. A male-biased sex ratio was observed in some mesocosms from 21 days and in most mesocosms from 35 days of population growth originating from an inoculum of adult females. A dramatic slowdown in growth was measured in mesocosms equipped with trackers, where the mites' path to the host was constrained. The slowdown in population growth induced by the intermittent presence of the host compared to its full-time presence was much less marked.

CONCLUSION: These findings suggest avenues of research for new management methods. They question the relevance of a critical threshold based on traditional trap monitoring to manage *D. gallinae*. Our results highlight a unique characteristic of *D. gallinae* that makes it a recalcitrant case to threshold-based practices recommended for integrated pest management (IPM) against other arthropod pests. The dramatic effect of a physical constraint for the mite to access the host (unnatural constrained path) confirms an observation made in 1917 and is a reason to design perches that are less conducive to parasite traffic.

Keywords: population dynamics; spatiotemporal constraints; sex ratio; mesostigmata; mites; experimental manipulations 2

1 INTRODUCTION

Understanding the factors that drive pest population dynamics is a major challenge for optimizing integrated pest management (IPM) in agroecosystems.[1,2] The main objective of IPM strategies is the regulation of pest populations, with the central principles including monitoring and treatment decisions based on critical thresholds.[3] Intervening at the right time in the pest population dynamics makes it possible to mitigate the environmental impact of pest control while maintaining a satisfactory yield level. To do so, monitoring of infestation levels is usually coupled with decision-making tools, which are essentially predictive models developed specifically for each pest.[4] The most time-consuming

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step in developing such models is the prior aggregation of biological knowledge of the species of interest and relevant meteorological input parameters.[5] Reproductive mode, developmental cycle parameters, density-dependent effects, and spatiotemporal dynamics at different scales are crucial information to achieve relevant IPM strategies. However, their study requires substantial work, so this information is often incomplete, especially in tiny arthropods such as mites. The small size of mites makes them very difficult to study, in particular with respect to spatio-temporal dynamics. For example, life tables and other information on reproduction generated by *in vitro* experiments on spider mites (Acari: Tetranychidae) have provided a necessary foundation for understanding the population dynamics of these plant-pest mites.[6,7] Subsequently, some particularly meticulous and time-consuming study has substantially refined the understanding of spatio-temporal dynamics by crossing different spatial scales and has allowed substantial progress in the definition of IPM strategies against plant pest mites.[8] As for meteorological parameters, the range of temperature requirements is an important element for deploying useful models in IPM against pest species in open fields or orchards,[9] although they are less likely to vary so much for greenhouse crop pests or pests infesting livestock buildings such as henhouses.

Livestock production lags far behind crop production in the implementation of IPM. *Dermanyssus gallinae* (De Geer, 1778), a blood-sucking mite responsible for significant economic losses in layer poultry farming worldwide, is particularly recalcitrant to many treatments, whether conventional or alternative.[10–13] *Dermanyssus gallinae* is an obligate hematophagous mite and its life cycle includes five stages: egg, larva, protonymph, deutonymph, adult. Only the last three need to feed. Each individual needs exactly two meals to reach the adult stage (transition from protonymph to deutonymph and from deutonymph to adult; the molt from larva to protonymph takes place spontaneously, without feeding). Then, the male stops feeding and the female has a blood meal before each oviposition, up to eight times in its life.[14] Like bed bugs or female mosquitoes, each individual only comes into contact with the host for rapid blood meals and spends its life at a distance in microhabitats. This lifestyle largely protects it from acaricides and other substances deployed by spraying. Furthermore, Maurer and Baumgärtner[15] and Mul *et al.*[16] highlighted the extreme heterogeneity of *D. gallinae* monitoring data obtained by trapping and/or *in situ* visual surveys between henhouses, but also within the henhouse from one period to the next, and the substantial amount of variation not explained by the variables considered.

Understanding the population dynamics of *D. gallinae* and its relationship to spatial configuration is necessary to make progress for environmentally friendly control and thus to improve the sustainability of the egg-producing industry. Yet we have uncovered a dramatic underestimation of this pest's population growth rate by the model built by Huber *et al.*.[17,18] This dramatic underestimation is likely due to imperfect estimates of reproductive and longevity variables collected in the literature for model parameterization.[18] The model of Huber *et al.*[17] describes by stochastic delayed differential equations for cohort individuals the demographic dynamics of *D. gallinae* and allows to simulate the population dynamics from a given number of mites at each life stage. To aid calculation efficiency, life stages are grouped into three groups (egg + larva, protonymphs + deutonymphs, adults). Unless otherwise stated, this model will be referred to throughout the text. In addition Dotson[19] detected *in vitro* a pattern of evolution of the sex ratio of successive clutches during the life of a female *D. gallinae*, with first clutches being 100% male and the last 100% female with gradual intermediate changes. *Dermanyssus gallinae* reproduces sexually, following a haplo-diploid system (males emerge from unfertilized oocytes and females from fertilized oocytes).[20] This is also the case with many other mites, such as *Tetranychus urticae* Koch, 1836 or, much more closely related, *Varroa destructor* Anderson & Trueman, 2000 or another poultry mite *Ornithonyssus sylviarum* (Canestrini & Fanzago, 1877), all of which can build up a population from a single virgin female through oedipal mating.[21–23] The evolution of the sex ratio in *D. gallinae* is consistent with the first egg always being male in *V. destructor*. However, unlike *V. destructor, T. urticae* and *O. sylviarum*, mating is required in *D. gallinae* to induce the laying of unfertilized eggs, thus male eggs[14] which rules out the possibility of population development from a virgin female. At the population level, one expects growth dynamics to slow down at the beginning by the limited production of females, i.e. individuals that produce progeny. Given the economic, health and ecotoxicological issues at stake, it is essential to better understand the factors that determine the mite's population dynamics.

The objective of this article is to characterize the temporal dynamics of populations of *D. gallinae* focusing on the evolution of the life stage and sex pyramid and to determine the main factors that affect population growth. To do so, we developed an approach based on successive prediction-experimentation-postdiction sequences, where the postdictions generated are translated into predictions to be tested by subsequent experimentation.[24] Our experimental systems consisted of closed mesocosms equipped with or without electronic trackers where the number of mites and their direction are automatically and continuously recorded and where a chick was accessible either from everywhere or via a constrained path for exhaustive tracking of foraging mites. Experiment A was motivated by the results and postdictions discussed earlier by Zriki *et al.*[18] and Dotson.[19] It was purely descriptive, with the objective to characterize the temporal population dynamics to evaluate how far observed population growth differs from population growth predicted by the Huber model.[17] In addition, we evaluated whether observed population growth and sex ratio supported the prediction of a peak in the proportion of males early in the population growth. Experiment A consisted in running both standard mesocosms that were destructively censused after various time intervals, and modified mesocosms equipped with electronic trackers allowing non-destructive temporal record of mite numbers in addition to the final destructive census. These tracker-equipped mesocosms differed from standard mesocosms by a constrained path from mite resting places to host and by holding chicks only three nights a week. As we recorded a much slower population growth in tracking mesocosms than in standard mesocosms, we conducted an additional experiment, experiment B, to test the hypothesis that the difference in population growth was explained by the difference in frequency of host availability better than by the difference in constraint on the path to the host. For this, we compared the growth of *D. gallinae* populations between standard mesocosms with intermittent presence of a chick and standard mesocosms with presence of a chick all the time. This second series of experiments is of major interest because it challenges an observation reported in 1917, which has never been exploited since then.[25]

2 METHODS

2.1 Biological materials

The birds used in the experiments were 3- to 60-day old chicks of *Gallus gallus domesticus* (Linnaeus, 1758) supplied by the INRAE infectiology platform (PFIE, Jouy en Josas, France). Chicks for each experiment were from a single clutch of the White Leghorn PA12 lineage. All experiments were conducted in compliance with relevant guidelines and regulations on animal experimentation. The mites were sampled a few days before the start of the experiments in a laying hen barn located at Pierrelattes (France), from the same farm as for the experiments conducted by Zriki *et al.*[18,26] (Farm DIN). Previous genotyping of several successive batches of dozens of mites randomly sampled at different locations and times in farm DIN showed that the poultry red mites that develop in this farm only belong to *D. gallinae* s.s. (more precisely, 100% of the genotyped individuals carried haplotypes of the haplogroup C, very common in French layer farms).[27] Selection of mites to be inoculated into the mesocosms was performed under a stereomicroscope, retaining the largest mites to form a 100% female inoculum as in Bartley *et al.*[28] Only live and active mites were collected, using a P1000 filter pipette tip connected to a vacuum pump. The tips were then sealed with parafilm. Inoculation was performed by introducing into the mesocosms each tip previously opened within hours of collection in order to avoid mortality at the beginning of the experiment.

2.2 Experiments

2.2.1 General principle

To study the population dynamics of *D. gallinae*, experiments were carried out in mesocosms mimicking portions of poultry farms which we specifically developed to allow development of mite populations over several generations under controlled conditions from a known inoculum of mites.[18,26] These mesocosms are miteproof experimental units, providing continuous housing for a chick, fed and watered *ad libitum*. Within each mesocosm, three artificial shelters provided mites with interstices comparable to those where mites typically accumulate in henhouses [see Fig. 1, later details and three-dimensional (3D) technical drawings on the MiteThru github (link in section 'Data Availability')]. Each mesocosm was provided with a chick and 200 live adult female mites at T0 and the mite populations were allowed to develop over several generations (Fig. 2). The size of the population after a given time in each unit was estimated by a destructive protocol (Fig. 3). The treatment of standard (STD) mesocosms was exactly as described in Zriki *et al.*[18, 26] and slightly adapted for the treatment of tracking (TR) mesocosms. This protocol allows the recovery of all mites present in each mesocosm at the time of the final treatment in two separate jars, one containing the mites present in the artificial shelters ('shelter' jar) and the other the mites present in the rest of the mesocosm ('content' jar), respectively. We exhaustively censused adult-like mites (salt-and-pepper colored deutonymphs and adults) in all portions of mesocosms, plus white life stages (protonymphs, larvae, eggs) in the 'shelter' portion. In addition, in order to explore the evolution of the sex ratio and get an estimate of the stage and sex pyramid, the precise stages of a random sample of mites from the 'shelter' jar of each mesocosm were identified, and sexes were determined for the adult stage (sex is only distinguishable in adults). This was carried out on the basis of morphological criteria, in two steps: first, distinguishing and counting using a stereomicroscope the white stages [eggs, larvae (six-legged), protonymphs] and large female adults, second, mounting the remaining mites between slide and coverslip and observing them under the light microscope. We then distinguished and counted deutonymphs, small adult females (very rare) and adult males. The distinction was made on the basis of five characters (i–v): (i) presence (male) or absence (deutonymph and adult female) of a mushroom-shaped spermatic orifice ventrally and centrally at the base of the gnathosoma; (ii) presence (male) or absence (deutonymph and adult female) of a small spur on tarsus IV; (iii) thickened, short, spermadactyl-bearing chelicerae (male) or long, tapered, hair-like and retractile chelicerae (deutonymph and adult female); (iv) presence (adult female) or absence (adult male and deutonymph) of a membranous flap covering the ovipore, located at the base of the gnathosoma (the longitudinal striations of the flap can be seen; the ovipore itself is invisible); (v) presence of a very thin sternal crescent-shaped plate with blurred contours just after the ovipore, at the base of the gnathosome (adult females) or ventral plate continuous from the opisthosoma to the base of the gnathosoma (adult male and deutonymph). Note that by far most of these individuals (the small adult-like mites) were either adult males or deutonymphs, small adult females were very rare (< 1%). We analyzed at least 200 individuals in jars containing > 400 mites, and at least half of the individuals in the others. Life stage and sex composition was assessed only in shelters.

Two types of mesocosms were used: standard (STD) mesocosms for which mite number was estimated only at the end of the experiment (destructive counting) and mesocosms equipped with a mite tracking device (TR mesocosms) measuring mite activity during the experiment (non-destructive counting; full description available on the Zenodo repository: see section 'Data Availability') besides the destructive counting at the end of the experiment. As STD mesocosms have already been the subject of two publications by our team,[18, 26] we will detail them at a minimum and further develop the description of the TR mesocosms derived from them. The structure of both types consisted of a polyvinyl chloride (PVC) cylinder 40 cm in diameter and 39 cm high for STD mesocosms or 59 cm for TR mesocosms (Fig. 1). The bottom was enclosed by a plastic plate and the top opening covered by a nylon membrane impermeable to mites and insects (mesh size 80 µm, 42 g/m^2, Diatex, Saint-Genis-Laval, France) sealed with silicone gasket (Sikaflex, Sika France SAS, Le Bourget, France). A side door of 20 cm diameter, sealed with a plastic lid and a silicone gasket, allowed the introduction of the chick. To provide suitable artificial shelters for the mites, three plastic smartcups (Smart Espresso, JMG, Basiglio, Italy) lined with pieces of Absopress cohesive containment tape (Laboratoire Marque Verte, Villers-lès-Nancy, France) and filter paper (Fisherbrand™ grade 600 cellulose general purpose filter paper; Fisher Scientific, Loughborough, UK) were attached against the wall of the PVC cylinder at equal distance from each other on the horizontal plane, at 34 cm from the bottom. In STD mesocosms, water and feed were supplied *ad libitum* to the chick by tanks that could be filled from the outside. Water was delivered by a Stilla nipple horizontal drinker (UFS, Guichainville, France) screwed through the mesocosm wall.

In STD mesocosms, a 38-cm-diameter circular metal plate with holes was attached 10 cm from the bottom, mimicking the slatted floors of free-range henhouses (with a 15-cm long plastic perch). In TR mesocosms, this was replaced by a 15 cm long and 3 cm wide rubber-coated aluminum perch fixed in a horizontal position on an L-shaped aluminum bar (vertical part: 42 cm long × 3 cm × 2 cm). This vertical bar was itself attached to a horizontal metal

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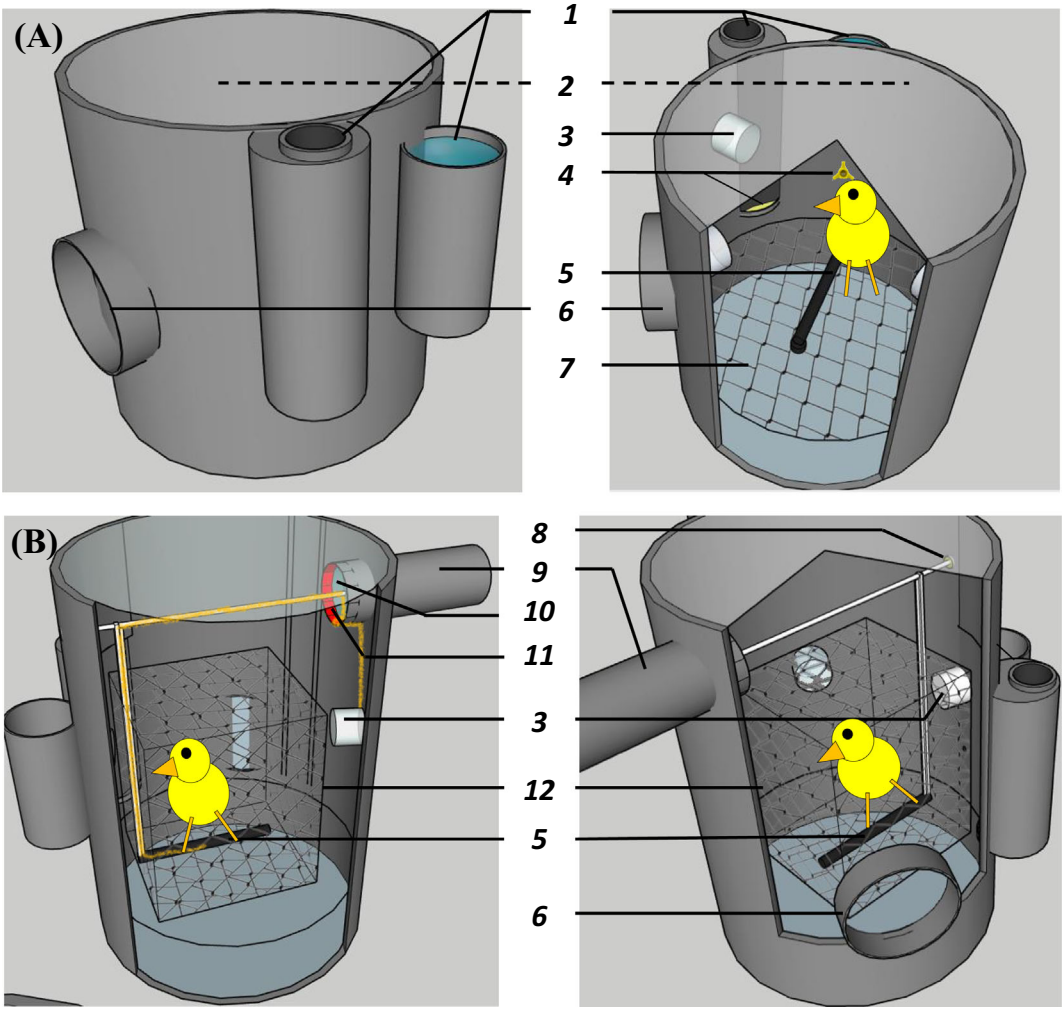


Figure 1. Design of the two types of mesocosm. (A). STD mesocosm. (B). TR mesocosm (waterer and feeder kept unused). At T0, two P1000 filter pipette tips containing each. 100 live mites (200 total), were opened and placed immediately on the mesocosm floor (under the 'slatted floor' metal plate in STD mesocosm). 1, water and feed tanks; 2, 80 µm nylon mesh membrane; 3, artificial shelter; 4, waterer and feeder; 5, perch; 6, opening; 7, perforated metal plate used as a floor for the chick ('slatted floor'); 8, lanolin barrier (hollow filled with lanolin around the stem); 9, location of the nanocomputer; 10, plexiglas disc on which mites are recorded by the camera of the tracking device; 11, LED strip surrounding the plexiglass disc; 12, cage containing the chick. Yellow line (B, left), way from artificial shelters (= mite 'homes') to the chick (a restricted way).

threaded bar covered with a thermoformable plastic sheath (total diameter 0.4 cm), one end of which passed through the center of the Plexiglas disk, forming the only access to the perch for the mites. The other end was attached to the opposite end in a cavity of the PVC wall filled with 10 mL of lanolin (in order to prohibit this access to the mites).

In TR mesocosms, the tracking device was placed in a strategic area to count the mites moving towards the chick. In addition to comparing the size of the initial inoculum with the final size of the mite population, this made it possible to monitor the temporal evolution of the mite activity associated to mite nutrition throughout the experiment within each mesocosm. We hypothesized that activity measured by this device and the total population size are correlated with each other.

In order to record the totality of mite foraging activity using our tracking device, TR mesocosms were designed with an internal spatial arrangement that constrained the path of any foraging mite to pass over a single optical tracking area (Plexiglas disc). We therefore prohibited access to water and feed for the hen to prevent some mites from reaching their host from the water or feed trough and forced the bird to stay on a particular perch, connected only to the tracking area (Fig. 1(B)). The feed tank was kept empty and the nipple pipette was replaced by a silicone cap to condemn the water trough. Consequently, the TR mesocosms only housed hens during the night. Knowing that the mites need a minimum of 1 week to complete an egg-to-egg cycle[25,29] and because we observed that they do not feed before a minimum of 3–4 days of fasting in the laboratory, we decided to introduce the hens only during three nights per week (starting every Sunday, Tuesday and Thursday evening) in order to limit stress on chicks. The rest of the time, the chicks were housed individually in additional standard mesocosms called 'resting' mesocosms (no inoculation of mites). Each chick was associated with a single TR mesocosm and a single resting mesocosm. In order to compare the mite counts in TR mesocosms at the end of the experiment with the electronic counts, an exhaustive count of all individuals in both portions ('shelters' and 'content') was necessary. As the intermittent regime of the chick's presence strongly limited the accumulation of powdery material, we adapted the extraction protocol of the 'content' portion in order to retain and count all

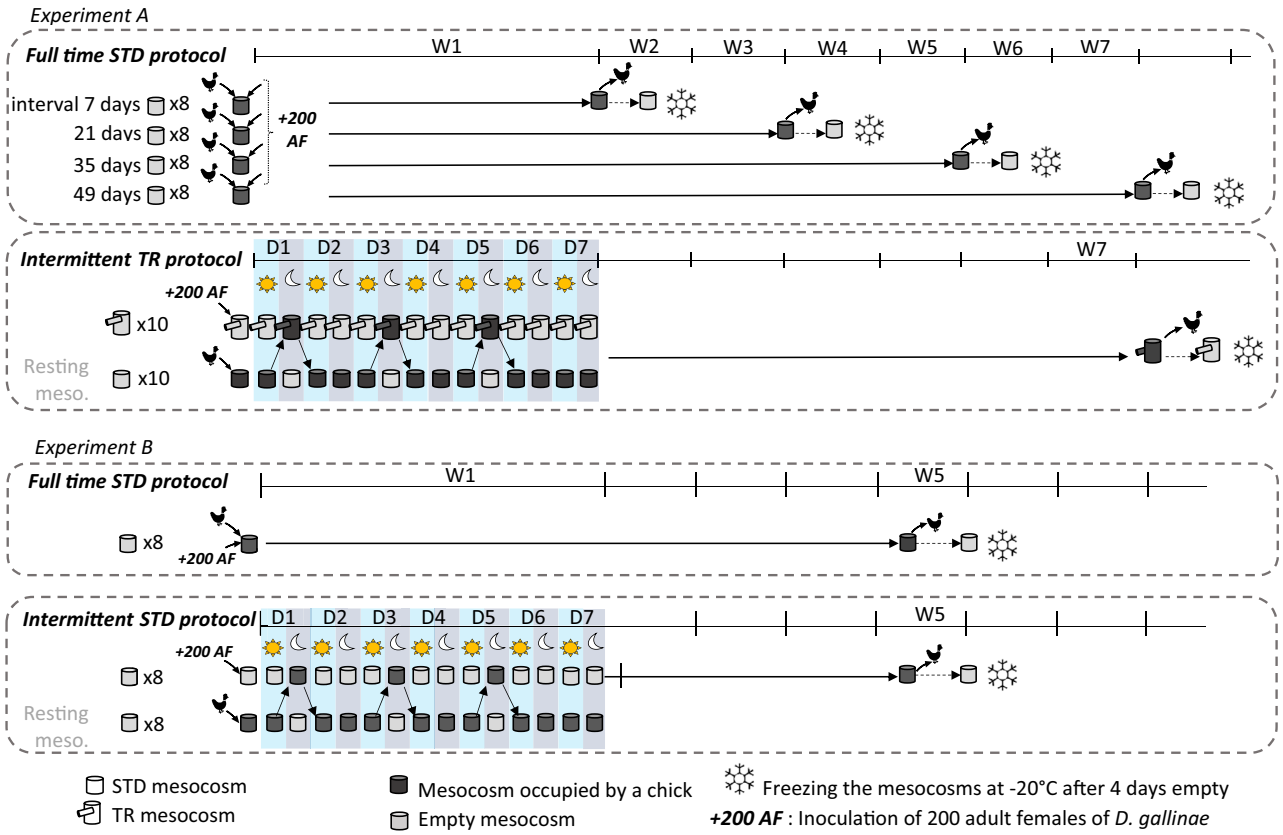


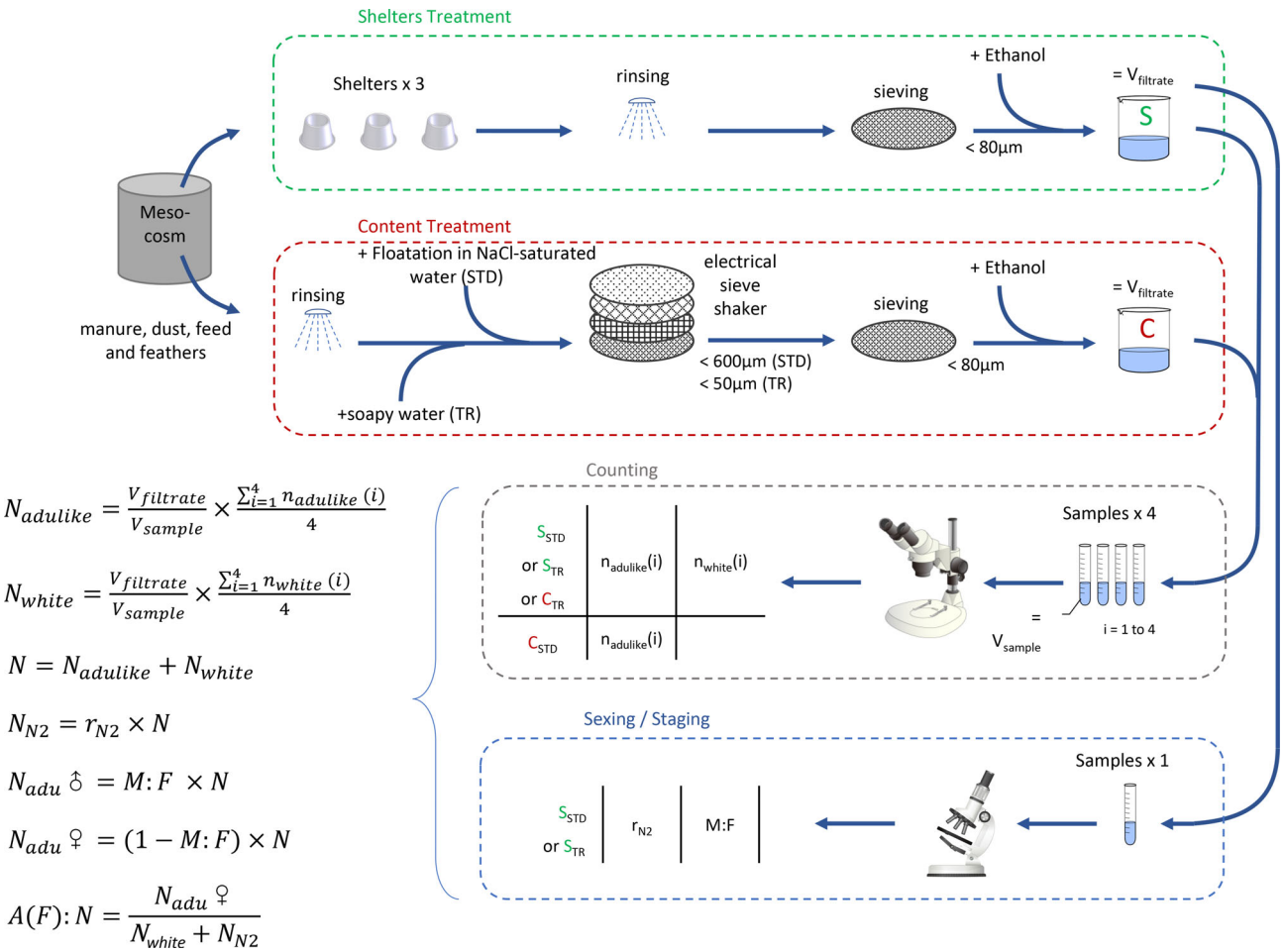
Figure 2. Experimental design. A chick and live adult females of *Dermanyssus gallinae* were introduced at T0 in each mesocosms and the chicks were removed 7 days before extraction and isolation of the mites. In experiment A, five series of mesocosms were implemented in parallel. Four series of STD mesocosms were implemented with four different time intervals spaced by 2 weeks each (1 week, i.e. 7 days; 3 weeks, i.e. 21 days; 5 weeks, i.e. 35 days; 7 weeks, i.e. 49 days) and one series of TR mesocosms, with the maximum time interval (7 weeks, i.e. 49 days). Chicks were present full-time in the STD mesocosms and part-time (nights starting on Sunday, Tuesday and Thursday evenings each week) in the TR mesocosms. In experiment B, two series of mesocosms were run in parallel, with a single time interval (4 weeks plus 2 days, i.e. 30 days) and two chick presence regimes (full-time and part-time exactly as in the STD and TR mesocosms of experiment A, respectively). In both experiments, each series of the STD mesocosms involved eight replicates, and the series of TR mesocosms involved ten replicates. W1, W2, …, week number 1, 2, …; D1, D2, …, day number 1, 2; AF, adult female of *D. gallinae*.

the life stages of mites in both portions, and not only adult-like mites in the 'content' portion like in STD mesocosms.

Note on the spatial setup of TR mesocosms: Given the marked negative geotropism in *D. gallinae* as in many parasitic and non-parasitic arthropods, including ticks,[30] we initially sought to position the camera at the level of the perch itself. This would have allowed us to count the mites truly 'climbing' onto the support and then onto a chick's leg, as they naturally do (LR, pers. obs.). For technical reasons and after numerous tests on different prototypes (image disturbed by the movements of the bird in the background, impossibility to impose a screen without substantially hindering the movements of the bird or the mites, chick droppings deposit on the tracking area), we designed the present system. Thus the access to the host for the mite is not only reduced, but also imposes a very unintuitive downward movement for the foraging mite.

2.2.2 *Tracking device in TR mesocosms*

All detailed technical, mechanical and computer information is available from the MiteThru github on the Zenodo repository (see section 'Data Availability'). The tracking device consisted of an electronic assembly involving an ARM processor single board nanocomputer (Raspberry pi 3 model B; Raspberry Pi Foundation, Cambridge, UK), a camera (Raspberry Pi PiNoir Camera Module V2; Raspberry Pi Foundation) and a 940 nm infrared LED strip (Solarex, Dessau-Roßlau, Germany). This type of equipment is increasingly used to develop specific systems for studying animal behaviors that are difficult to observe in the field (e.g. Hereward *et al.*[31]) or in the laboratory (e.g. Geissmann *et al.*[32]). We have developed a multi-target tracking system integrated into a mesocosm, typically at the interface between field and laboratory. The camera was placed behind the Plexiglas disc, in a central position (Fig. 1 and Fig. Z1 in the technical note on the Zenodo repository: see section 'Data Availability'). The bar providing sole access to the perch where the chick rested was fitted in the center of the Plexiglas disc, so that mites had to cross the Plexiglas disc from the perimeter to the center to reach the chick. The electronic assembly was carried out with pieces obtained through RS Components, Corby, UK and Farnell, Leeds, UK (assembly diagram and complete list of material on MiteThru github; see section 'Data Availability'). We wrote a program in Python 3 using openCV library (https://opencv.org/). The program took three photographs per second and based on their analysis deduced the position of the mites moving in the tracking area. It determined the path of the mites on a strip between two



$$N_{adulike} = \frac{V_{filtrate}}{V_{sample}} \times \frac{\sum_{i=1}^{4} n_{adulike}\,(i)}{4}$$

$$N_{white} = \frac{V_{filtrate}}{V_{sample}} \times \frac{\sum_{i=1}^{4} n_{white}\,(i)}{4}$$

$$N = N_{adulike} + N_{white}$$

$$N_{N2} = r_{N2} \times N$$

$$N_{adu}\,♂ = M{:}F \times N$$

$$N_{adu}\,♀ = (1 - M{:}F) \times N$$

$$A(F){:}N = \frac{N_{adu}\,♀}{N_{white} + N_{N2}}$$

Figure 3. Mesocosm treatment protocol after the end of the experiments A and B (including a minimum of 48 h freezing at −20 °C). The **S** or **C** filtrate were poured into a 1-L graduated beaker and 96 °C ethanol was added in sufficient quantity to reach a fixed volume ($V_{filtrate}$ = 300 mL for 'shelter' jars, $V_{filtrate}$ = 750 mL for 'content' jars). While the liquid was continuously homogenized in the beaker using a magnetic stirrer, a volume of V_{sample} = 10 mL was sampled by means of a syringe at mid-distance between the center and the edge of the beaker. To get $n_{adulike}(i)$ and $n_{white}(i)$ mites were counted with a stereomicroscope directly on a 80-µm nylon mesh after sampling, and distinguished based on color (salt-and-pepper and white individuals, respectively). The numbers $N_{adulike}$ and N_{white} of individuals were estimated as the product of the average of the counts in the four samples by the total volume $V_{filtrate}$ of the graduated beaker **S** or **C**, divided by the volume V_{sample} of the sample. *N* is the sum of $N_{adulike}$ and N_{white}. To establish r_{N2} and *M*:*F* stage and sex of a sample of mites from each 'shelter' jar of each mesocosm were identified by light microscopy (see text). N_{N2} and $N_{adu}♂$ were estimated as the product of *N*, the total number of mite in the mesocosm, by ratio r_{N2} or *M*:*F*, respectively, whereas $N_{adu}♀$ was estimated as *N* multiplied by the complement to one of *M*:*F*. Meaning of letters and signs: **S** or **C**, filtrate resulting from the treatment of the shelters and the content of a mesocosm, respectively; *i*, the number of the sample taken from the graduated beaker (i = 1–4); $n_{adulike}(i)$ and $n_{white}(i)$, number of adult-like mites (adults and deutonymphs) and number of white individuals (protonymphs, larvae, eggs), respectively, counted in the sample number *i*; $N_{adulike}$ and N_{white}, total number of adult-like mites (adults and deutonymphs) and of white life stages mites (protonymphs, larvae, eggs), respectively estimated in the mesocosm; *N*, total number of mites estimated in the mesocosm; r_{N2} and *M*:*F*, ratios of deutonymps and of males in adults in the mesocosm population, respectively. N_{N2}, $N_{adu}♀$, $N_{adu}♂$, estimated number of deutonymphs, adult females and adult males, respectively, in the mesocosm.

concentric circles whose center was that of the Plexiglas disc (diameters of 2.6 and 3.0 cm, respectively) and recorded the number of individuals that traveled through the area at time *t* according to their direction: either crossing the area towards the host ('IN') or the other way round ('OUT'). The data resulting from the analysis of the photographs were recorded continuously. In addition, in order to confirm the accuracy of the count without overloading the memory, the program recorded 15″ video captures at a minimum interval of 10 min if at least three mites were present in the tracking area (full program available on the MiteThru github).

2.2.3 *Conditions and course of experiment A and experiments B*

All the mesocosms were maintained throughout the experiments in an air-conditioned room (25.6 ± 0.8 °C, 79.2 ± 10.9% relative humidity; control from a probe placed in the center of the room at the height of the top of the mesocosms) and a 14 h:10 h light:dark light regime. Two series of experiments were conducted successively (Fig. 2).

Experiment A was dedicated to establishing the shape of the population growth curve and the evolution of the life stage and sex ratios. It involved in parallel STD mesocosms with four different intervals (7, 21, 35, 49 days, with eight replicates per interval) and TR mesocosms (ten replicates) with the maximum interval duration (49 days) (Fig. 3) and was conducted from January to March 2020. We extracted and counted mites at the end of each interval in all mesocosms. In addition, in the TR mesocosms, we continuously recorded the passage of mites on the Plexiglass disk during all the periods when chicks were present during the 49 days of the experiment. In STD mesocosms, the total duration

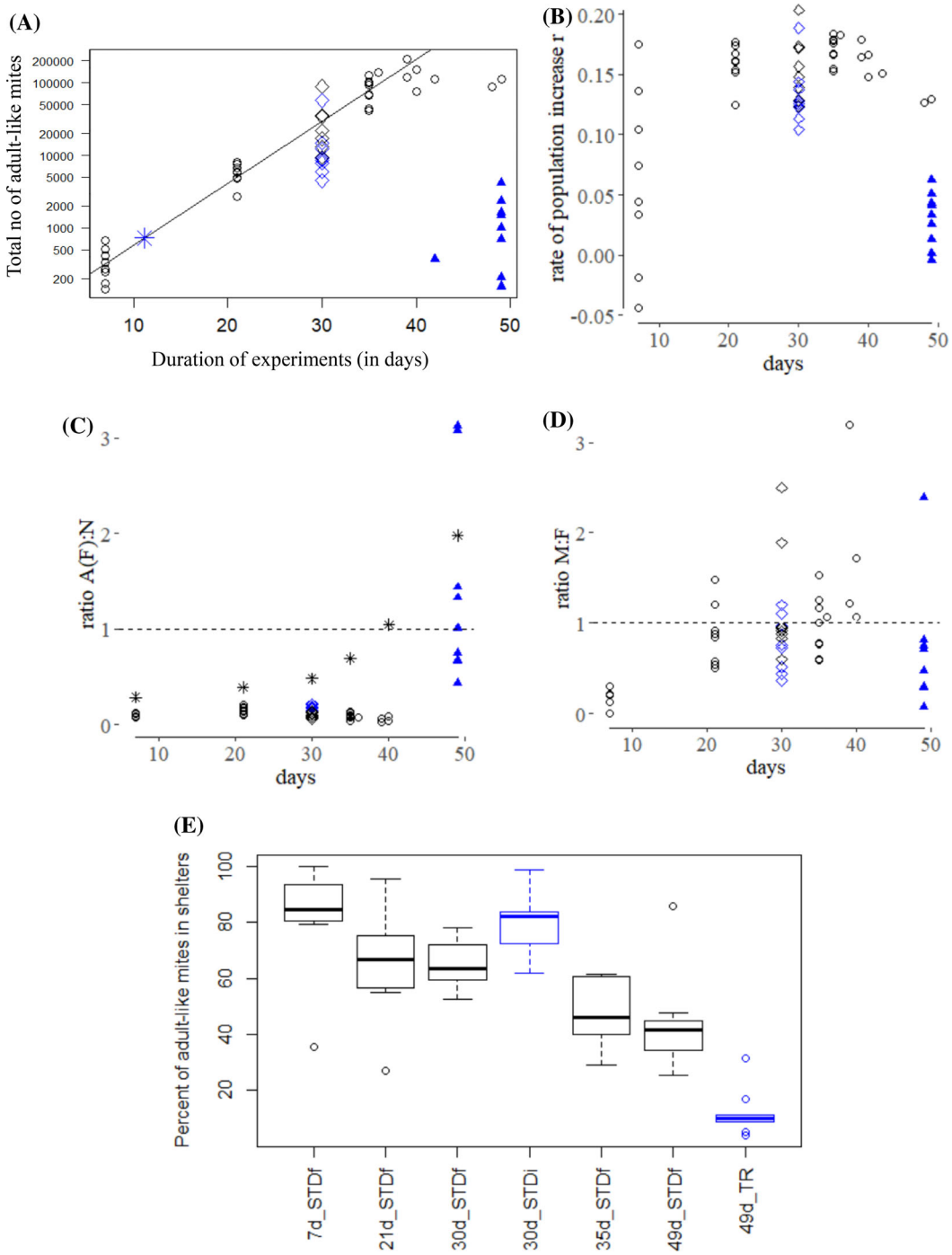


Figure 4. Demographic evolution of *Dermanyssus gallinae* in STD and TR mesocosms (experiments A and B) as measured from final mite counts. All figures represent raw data. In A-D a dot is a value directly from the final count of mites in a mesocosm (replicate) following the protocol described in Fig. 3, with the following meaning: open point shapes, STD mesocosms (circles for experiment A and diamonds for experiment B); solid triangles, TR mesocosms; black, full-time chicks, blue, intermittent chicks. (A) Number of adult-like mites (log scale) counted at the end of experiments as a function of the duration of the experiment. Note: it was not possible to calculate the number of adult-like mites estimated by the Huber model, because adult-like includes deutonymphs and excludes protonymphs (distinguishable with a binocular magnifying glass) while the Huber model treats all nymphs (proto- and deuto-) as a single group. Blue star, projection of the mean of the TR final counts. Note that chicks from the mesocosms with the longest interval (49 days) generally died before the expected term, which explains the spread of the highest duration values (no chick died in other mesocosms). (B) Evolution of the rate of mite population increase (*r*). (C) Ratio of adult females to juveniles as a function of the duration of the experiment and as predicted by the Huber model[17] (the model does not count adult males and only considers the sex ratio in adults). Black stars, predictions of the Huber model. (D) Male to female ratio in adults as a function of the duration of the experiment. Note that the Huber model considers a basic 1:1 sex ratio (dashed line on 1). Note: three of the mod1 STD mesocosms in experiment A ($T + 7$ days) only allowed to count females amongst adults. Therefore, three points are missing at $T + 7$ days corresponding to 0% of adult males (consistent with the overall low ratios at that interval). (E) Boxplots showing the temporal evolution of the percent of adult-like mites recorded from three artificial shelters relative to the whole mesocosm contents (intermittent STD or TR protocol in blue). Intervals 7, 21, 35 and 49 days in experiment A, interval 30 days in experiment B. Note that the 49-day interval was in fact in most cases lower due to chick mortality (see remark earlier, concerning A). STDf, full-time standard protocol; STDi, intermittent standard protocol; TR, intermittent TR protocol.

of the last interval was not reached in all mesocosms, due to chick mortality induced by the severity of the infestation. In these cases, the experimental duration was reduced to the period between T0 and chick death. In the STD mesocosms, each chick remained permanently until the end of the interval under test. In the TR mesocosms, each chick was introduced at night (between 7:30 p.m. and 8:00 a.m.) three times a week during the whole experiment and transferred to its own 'resting' mesocosm at 8:00 a.m. We were careful to check that we did not see any mites on the chick at the time of transfer to the resting mesocosm (although not seeing mites directly on the chick does not mean that no mites were there). We also carefully checked that the perch was free of mites at each chick transfer throughout the experiment. Acquisition of tracking data was made during all the nights with chicks present. The TR mesocosms were kept in darkness during the experiment to allow the tracking device to record the movements of the mites under stable infrared (940 nm) light conditions. They were exposed to white light only for about 20 min three times a week, before the transfer of the chick to the 'resting' mesocosm, in order to limit the risk of mite transfer. The resting mesocosms, however, were exposed to the same light conditions as the STD mesocosms. The wavelength 940 nm was chosen because it is outside the spectrum visible to the chicken (360–677 nm[33]) and well outside the range of thermal infrared emitted by the skin of homeothermic vertebrates (e.g. human skin emits between 5000 and 20 000 nm). The behavior of mites under infrared illumination by light emitting diodes (LEDs) at 940 nm was observed in preliminary tests and we did not detect any difference in behavior between infrared and white light (same type of trajectory, same apparent speed). At the end of each recording, a csv file was produced by the tracking software for each TR mesocosm, tracing the number of 'IN' and 'OUT' mites during the entire recording period. In order to evaluate the impact of the introduction and withdrawal of the chick on environmental conditions, we recorded temperature and humidity inside seven of the ten TR mesocosms every hour for 6 days, including two nights with chicks present (from Friday, February 28 at 5:00 p.m. to Wednesday, March 4, 2020 at 9:00 a.m.) using iButton Hygrochron Temperature/Humidity Loggers (Maxim Profile & IC Solutions, San Jose, CA, USA).

Experiment B was designed from postdictions drawn from experiment A and dedicated to test the effect of a temporal constraint of access to food resources on population growth in order to refine the interpretation of the results obtained. Here we compared the population growth when a chick was continuously present (as in the STD mesocosms of Experiment A) with that obtained with intermittent presence of chicks (as in the TR mesocosms), using STD mesocosms to control for the effect of the spatial configuration of host access. As in TR mesocosms of experiment A, the first night at T0 after inoculation of mites took place in the presence of chicks. A single 30-day interval was tested here, with eight replicates with full-time chicks and eight replicates with part-time chicks. This duration was chosen in order to obtain a substantial increase of the mite population, without reaching a problematic level of infestation for the chicks. This experiment was conducted in July 2020.

2.3 Statistical analyses of the data

All data were analyzed using the R software.[34] The data from the tracking program was compiled after each recording using the map_df() function in the purrr package into a table of the number of mites counted IN and mites counted OUT each day in each TR mesocosm. Another table was elaborated with the counts of adult-like mites at the end of experiments and a third one with the counts of life stages/sexes estimated from identified subsamples of mites (see earlier).

To identify the exponential growth among the tested intervals the rate of mite population increase (r) was calculated for each mesocosm as, $r = \ln[(Nf + 1)/200]/t$, where Nf is the final number of adult-like individuals, 200 is the initial number of adult females, and t the time period (in days) during which mites were allowed to feed on chicks. Hereafter, we will refer to the counts of mite corpses extracted from the mesocosms at the end of the experiment as 'eye counts' and to the counts of mite images that passed through the tracking area according to the rules defined above (either in the IN or OUT direction) as 'electronic counts'. To assess the consistency of the values obtained between STD mesocosms of experiment A and experiment B, we measured correlations between final eye counts of mites and the duration of the experiment in all mesocosms by computing Spearman's rank correlation coefficients. To evaluate the information provided by electronic counts on population dynamics, we also computed Spearman's coefficients between the final eye counts and the electronic counts accumulated over the whole experiment in TR mesocosms. Knowing that metamorphoses (protonymph and deutonymph) and oviposition events (adult females) each require a single blood meal and that adult females each lay one to eight eggs up to eight times in their life[14,25] it can reasonably be considered that each individual of a particular life stage passed in front of the camera at least the number of times corresponding to the number of meals it needed to reach its life stage, i.e. zero times for protonymphs (the egg hatched and the larva metamorphosed into a protonymph without a blood meal), one time for deutonymphs, two times for adult males and two to ten times for adult females. An iterative search of the number of image detections per life stage and sex was made in order to maximize the R^2 value of the correlation between the cumulated electronic count and eye count by assigning values between 0 and 23 to protonymphs, 1 and 24 to deutonymphs, and between 2 and 25 to adult males and females, while keeping the values of the image detection of each life stage lower than those of the next life stage.

In order to assess the extent to which our experimental results are consistent with the predictions of the Huber model,[17] we conducted simulations with the deSolve package[35] and compared the values obtained with ours, taking into account the particularities of the model (uncounted adult males, juvenile stages grouped in two groups). Note that we assume here that the corpses of all individuals (those that died at the end and those that died during the experiment) were counted as well, while the model only considers live mites at each time.

To get a rough idea of the spatial distribution of mites in the mesocosms, we visualized the percentage of adult-like mites found in the three shelters of the STD mesocosms with chicks present full-time.

3 RESULTS

The mean temperature in the TR mesocosms was 25.22 ± 0.67 °C in the absence of chicks and 26.50 ± 0.75 °C in the presence of chicks, with the mean in the presence of chicks +0.94 to +1.90 °C higher than the mean in the absence of chicks in each mesocosm. We did not find a marked effect of the presence of

the chick on the relative humidity inside the mesocosms, which remained close to the humidity measured in the center of the room (83.43 ± 3.80% in the absence of chick and 82.07 ± 3.85% in the presence of chick). The internal temperature and relative hygrometry of the TR mesocosms without chicks was very close to the temperature measured throughout the experiment centrally in the airconditioned room (25.6 ± 0.8 °C, 79.2 ± 10.9% relative humidity). This and the relatively small amount of variation induced by chick introduction and withdrawal suggest relatively efficient ventilation of the mesocosm interior, despite the thin membrane mesh covering the upper opening.

The raw data from Experiments A and B are available on the Zenodo repository (see section 'Data Availability').

3.1 Results of experiment A

Despite expected variation, the shape of the growth curve over the tested intervals is quite consistent with typical population dynamics, consisting of a latency phase followed by exponential growth (smoothed by the log scale in Fig. 4(A)), followed by a plateau. Chick mortality was observed in STD mesocosms with the interval $T + 49$ days only (seven chicks) and near the end of the experiment, coinciding with the plateau. This is consistent with our previous results which showed a lethal effect of *D. gallinae* infestation in our mesocosms, with chicks tending to die past 35 days experiment starting from an inoculum of 400 adult females.[18] Adjusting the size of the inoculum (200 adult females) and the duration of the experiment to avoid chick mortality did not suppress it in the last interval. The *r* showed considerable

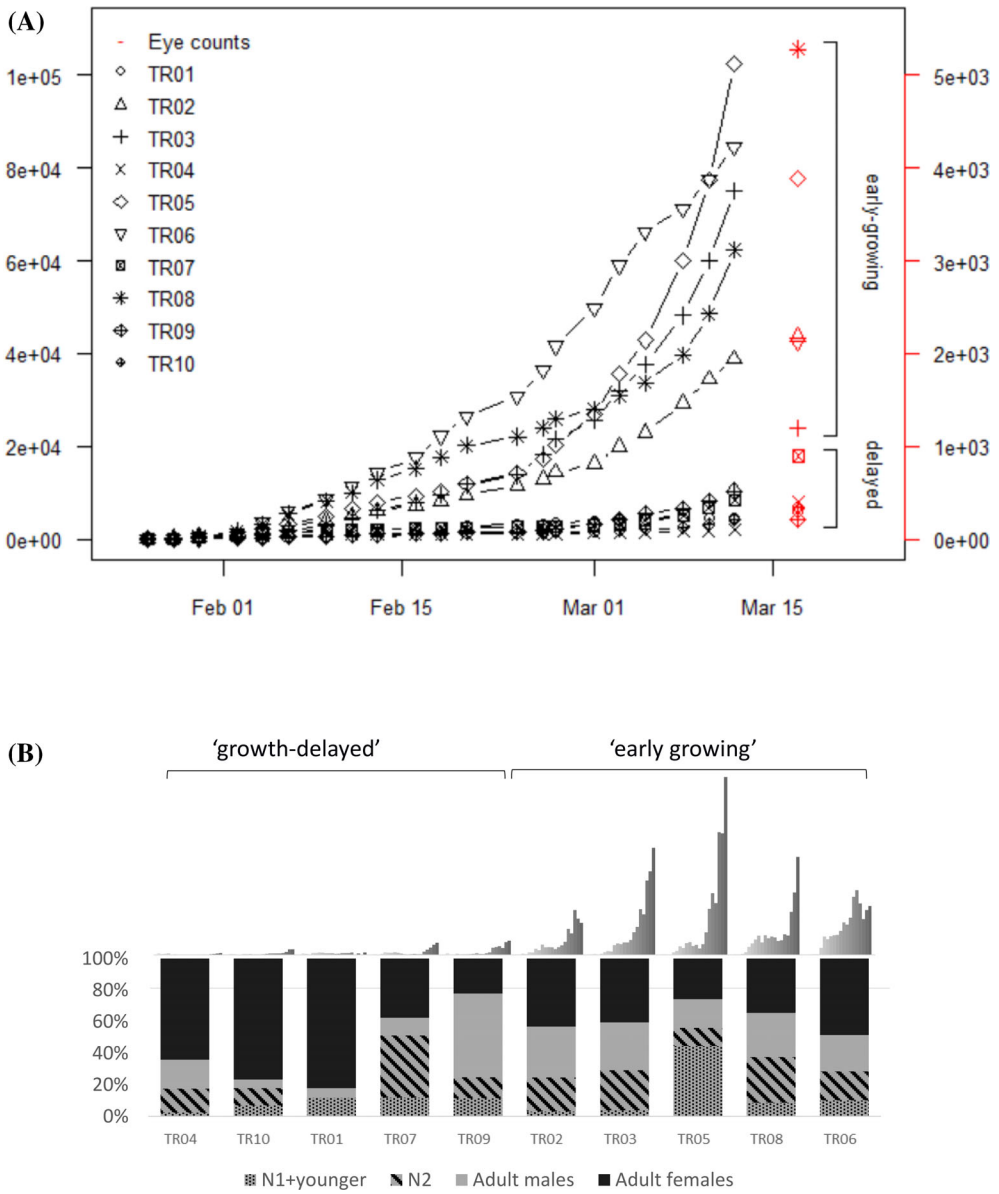


Figure 5. Results from TR mesocosms in experiment A. (A). Accumulation curves from electronic counts for all periods when chicks were present (three nights per week) in TR mesocosms (black) and final eye counts of adult-like mites from whole TR mesocosms (sum of artificial 'shelters' + remaining 'contents') (red). (B). Composition of populations in life stages and sexes in the different TR mesocosms ordered from the latest to the earliest growth (left to right). Top, daily mite counts (IN) across 49 days experiment. Bottom, proportion of life stage groups and sexes within adults. N1, protonymph; N2, deutonymph.

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variation at $T + 7$ days (range: −0.044–0.175, mean 0.063 ± 0.075) and was stabilized thereafter with fairly high values (range: 0.125–0.184, mean 0.161 ± 0.017) (Fig. 4(B)). Growth in TR mesocosms appeared to be severely slowed down compared to that observed in STD mesocosms (see Fig. 4(A) and 4(B)) and no chick mortality was observed there, contrary to STD mesocosm experiments of the same duration. Growth also appeared to be much more heterogeneous in TR than in STD mesocosms: among the ten TR mesocosms, five started very late and five showed an evolution of activity on the Plexiglass disc consistent with population growth dynamics (Fig. 5).

Linear regression of the eye count data with respect to the cumulative electronic counts shows a very low positive correlation (adjusted $R^2 = 0.466$; $F = 8.88$, df = 8, $P = 0.017$; Fig. 5(A)) and mean 24 ± 18 passes were electronically recorded per individual counted at the end of the experiment. According to the iterative maximization of the regression coefficient, the best combination of passes in front of the camera implies zero passes per protonymph, one per deutonymph, two per adult female, and 25 per adult male, for an R^2 adjusted to 0.548 ($P = 0.008$).

The comparison of the life stage and sex ratios from eye counts with the shape of the accumulation curve by mesocosm allowed the delineation of four demographic profiles as a function of adult sex ratio *M*:*F* (ratio of the number of males to the number of females) and stage ratio *A*(*F*):*N* (ratio of the number of adult females to the number of juveniles, i.e. deutonymphs + protonymphs + larvae + eggs) amongst the ten TR mesocosms (see Fig. 5(B)): profile 1, $M:F < 0.5$ & $A(F):N >> 1$ (TR04, TR10, TR01); profile 2, $M:F < 0.5$ & $A(F):N < 1$ (TR07); profile 3, $M:F > 1$ & $A(F):N < 1$ (TR09); profile 4, *M*:*F* close to 1 & variable *A*(*F*):*N* (*A*(*F*):*N* close to 1.5 in TR02, TR03, TR06, $A(F):N = 0.45$ and 0.86 in TR05 and TR08, respectively). We consider here the *A*(*F*):*N* ratio rather than the ratio of adults to juveniles because it corresponds to the ratio calculated by the model and facilitates comparison with the model outputs. The *A*(*F*):*N* ratios was always much higher in the TR mesocosms than in the STD mesocosms.

3.2 Results of experiment B

The final count values of *D. gallinae* in the full-time-chick modality in this experiment fit perfectly into the series of values obtained for different intervals during experiment A (Fig. 4(A)). This supports the idea that our system is robust and can generate reproducible results at intervals of several months. While it is true that population growth was slightly reduced with intermittent chick presence compared to the full-time-chick presence, the amount of difference was not comparable to that observed between STD mesocosms and TR mesocosms in experiment A (Figs 4(B) and 6).

3.3 Demographics, evolution of life stage and sex ratios in both experiments

A high positive covariation appeared between total adult-like counts and the experiment duration when considering the final adult-like counts of all STD mesocosms (from experiments A and B pooled; Spearman's $\rho = 0.927$, $S = 1343.4$, $P = 2.200.10^{-16}$; see Fig. 4(A) for data points distribution). This covariation was strongly decreased when adding the TR mesocosms (all mesocosms from both experiments; $\rho = 0.278$, $S = 23\,442$, *P*-value = 0.034; see Fig. 4(A) for data points distribution).

The number of protonymphs was higher than the number of adult-like mites (adult males + adult females + deutonymphs) in all mesocosms (STD + TR) except in five TR mesocosms (Fig. 4(C)). The ratio of adult females to juveniles (protonymphs + deutonymphs) observed in our STD mesocosms was always lower than predicted by the Huber model[17] and decreased with time (Fig. 4(C)). In STD mesocosms, the ratio of adult males to adult females (*M*:*F*) in the majority of mesocosms was less than one until $T + 30$ days in our STD mesocosms, with however a small number of mesocosms with *M*:*F* ratio > 1 at $T + 21$, $T + 30$ days. From $T + 35$ days to $T + 49$ days, the majority of STD mesocosms had *M*:*F* ratio > 1 (Fig. 4(D)). The TR mesocosms had quite different profiles, with much higher *A*(*F*):*N* ratios than the STD mesocosms as a whole and low *M*:*F* ratios as in the short duration STD mesocosms ($T + 7$ and $T + 21$ days). The total number of mites counted was positively correlated to the male-to-female ratio ($\rho = 0.688$, $S = 4424.6$, $P = 2.44 \times 10^{-7}$).

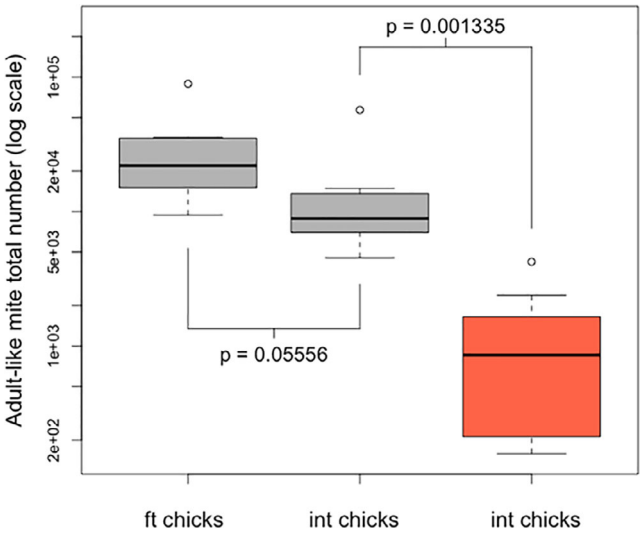

Figure 6. Final counts from STD mesocosms in experiment B (gray) with different chick regimes and from TR mesocosms in experiment A (red). ft chicks, presence of full-time chicks; int chicks, intermittent presence of chicks. *P*-values from Wilcoxon tests.

3.4 Overview of the spatial distribution of mites in the mesocosms

The three artificial shelters occupied 1.38% of the mesocosm volume and were located at a distance from the mite release point on the vertical wall of the mesocosm (34 cm above the floor on which the mites were released). The percentage of adult-like mites counted in the three artificial shelters of each STD mesocosm with chick present full-time shows that more than half of the mites were in the artificial shelters until the 30-day interval (Fig. 4(E)). Furthermore, this relative distribution evolved over time, with the proportion of mites in these small volumes being maximal at the beginning of the dynamics (more than 80% of mites counted at $T + 7$ days) and gradually decreasing thereafter. Moreover, visual observations made during the experiment, without touching the material in order to avoid disturbing the mites, did not generally allow to observe any aggregates before the 30-day interval. Then, the first visible aggregates were found inside the artificial shelters (made of transparent plastic), then others were added to the walls as time went by. Finally, in parallel experiments in two mesocosms, during which we were able to disturb the interior of the mesocosm, we found that while no mites were detectable by naked eye observation of the walls

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and objects in the mesocosm and while massive aggregations were visible in the artificial shelters, significant numbers of individuals were contained in the manure accumulated under the slatted floor (1 day after collecting handfuls of manure and placing them in tightly sealed ziplock bags, hundreds of mites were aggregating in the corners of the bag).

4 DISCUSSION

4.1 Characterization of temporal population growth of *D. gallinae*

The very good agreement between the duration of the experiment and the number of mites counted in the STD mesocosms, whatever the experiment, demonstrates the utility of this system for exploring the population dynamics of a hematophagous arthropod. The population dynamics of *D. gallinae* proved to be very aggressive (> 405-fold increase in adult-like number in 35 days). In line with our previous finding,[18,26] it was much faster than predicted by the Huber model[17] (< 64-fold increase in 35 days). The plateau reached after 35 days of experimentation shows a limit imposed by the tolerance of the chick to infestation.

In TR mesocosms, the evolution of activity measured on a passage necessary to take blood meals reflects well the demographic dynamics of *D. gallinae* despite disparities between the dynamics thus measured and the final eye counts. Indeed the time evolution of daily electronic mite counts over 49 days was consistent with the expected shape of the first two steps of a population dynamic logistic curve (latency and growth)[36] in at least the five mesocosms where population growth was initiated (Fig. 5). Even if the correlation between the physical and electronic counts was weak, the five early growing mesocosms (TR02, TR03, TR05, TR06, TR08) all showed higher eye count values than the five delayed-growing mesocosms. However, although the populations in early-growing TR mesocosms apparently started the second phase of the Verhulst logistic model (growth), they were probably still far from the third phase (equilibrium) at the end of experiment in view of the weak slope inflection (Fig. 5(A)). This is consistent with the very low census values from eye counts. Eye counts at $T + 49$ days correspond to values from STD mesocosms at the 7- and 21-day intervals (Fig. 4(A)), suggesting a delay of 28 to 42 days in TR mesocosms.

The difference in population growth between STD mesocosms with full-time and intermittent chick presence in experiment B was very small compared to the huge difference between STD and TR mesocosms in experiment A (Figs 4(B) and 6). Therefore, neither the effect of the time constraint for resource access (frequency of host availability) nor the effects of intermittent chick presence on abiotic conditions [variations in ambient temperature, probably also in carbon dioxide (CO_2) levels, strength of the temperature gradient, CO_2, kairomones] can alone explain the growth suppression observed in the TR mesocosms. A drastic slowdown in population growth occurred in TR mesocosms over the period, not a simple lag in the onset of growth since the *A* (*F*):*N* ratio was higher in all TR mesocosms than in STD mesocosms, including the 7-day STD mesocosms. This supports the observation reported by Wood in 1917[25]: 'The ease with which mites reach the host has a decided bearing on the rapidity of increase. Hungry mites, though placed quite near a fowl, have great difficulty in finding the host unless the means of access is direct. This fact would account for the mite preferring to hide on the roost. When mites are found all over the walls the infestation must be a heavy one.'

Four demographic profiles defined on the basis of life stage and sex ratios (see Results section) can be reconciled with the tracker accumulation curves as follows: profile 1, transition from latency phase to growth (adult females dominated and there were almost no adult males yet, as in $T + 7$-day STD mesocosms), profiles 2 and 3, beginning of growth phase, with a peak of adult males in profile 3 (first oviposition of newly emerged females) and increase in the number of juveniles in both; profile 4, growth phase in progress, with an almost balanced sex ratio and a highly variable number of juveniles relative to the number of adult females. The absence of density-dependent effects (population density still well below equilibrium, no chick mortality) may explain the balanced *M:F* ratios or in favor of females in profile 4 (females could complete all their gonotrophic cycles).

4.2 Time evolution of the stage and sex ratios

We recently found that the mite population growth as predicted by the Huber model[17] was much lower than empirically observed in controlled mesocosms.[18] Here we discover an additional discrepancy between model and experimental results regarding the life stage ratio with a strong and constant dominance of nymphs in STD mesocosms while the Huber model predicts an increasing dominance of adult females. The marked dominance of nymphs over adults reported here was also noted by Wang *et al.*[37] in cage experiments of similar durations and numbers of inoculated mites. Considering that the STD mesocosms with full-time presence of chicks best reflect the farm conditions, these discrepancies may indicate a poor knowledge of the duration and survival of the different life stages, essentially established by *in vitro* experiments.[15,25,29,38,39]

Furthermore, recurrent and transient peaks of adult males seem to have occurred at different times of the experiment since mesocosms with *M:F* ratios > 1 were regularly noted among mesocosms with inverse or balanced ratios from $T + 21$ days. Based on Dotson's[19] statements on the evolution of the sex ratio of a female's clutches, as we started with females of various ages, it was expected that the eggs laid would give birth to a mixture of males and females in the first cycle, consistent with the small *M:F* ratio in STD mesocosms during the first intervals. From the succeeding generation, an increased number of males was expected at least during the first two cycles, born from the eggs laid by the first-generation mesocosm females, consistent with the drastic increase of *M:F* ratio at larger intervals. This pattern could be prolonged in successive waves during the growth phase due to density-dependent variations in female longevity (competition) (if females die younger, the proportion of males should increase). This is consistent with the positive covariation between *M:F* ratio and mite number in our data.

4.3 Respective roles of temporal and spatial constraints to host access in population growth of *D. gallinae*

Three main elements distinguished STD mesocosms from TR mesocosms in experiment A: frequency of host availability (full-time *versus* intermittent), light regime (standard photoperiod in natural light *versus* quasi-continuous darkness), the presence of local infrared illumination at 940 nm (i.e. outside the visible spectrum of the chicken and still far from the thermal infrared range) and physical access to the host (unconstrained *versus* spatially restricted and unnatural). Experiment B showed that reducing temporal access to the host only slightly impacted population growth, and that this impact was not comparable to the difference between STD and TR mesocosms in experiment A. Darkness was

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shown to increase population growth in *D. gallinae* instead of slowering it.[37] Although we cannot completely eliminate a possible effect of the LEDs on the mite's behavior, the infrared light emitted around the Plexiglas plate does not constitute a barrier in any way. Indeed, consistent with the preliminary behavioral observations, the numerous videos recorded during the experiments show that the mites actively cross the tracking area in both directions, alone or in groups. Furthermore, the wavelengths detected by two mites closely related to *D. gallinae* are all much higher than 940 nm (3600–7000 nm in *Laelaps echidnina*, 5000–10 000 in *Varroa jacobsoni*[40,41]). Although we cannot definitively conclude that the infrared wavelength used does not interfere with the system, all of this evidence suggests an absence of effect of the LEDs on mite and chick behavior. As a result, the slower population growth in TR mesocosms cannot be explained by the first three elements and the spatial configuration of access to the food resource was the factor that most likely constrained population growth in our TR mesocosms.

The physical nature of the substrate and the spatial structure of resource distribution are already known in mites to affect predator's access to its prey and population development and dynamics.[42–44] For instance, predation of Astigmata by predatory mites was impaired in the presence of wheat flour compared to semolina or wheat grain.[42] Therefore, the accumulation of powdery or granular material (dust, skin scales, feed powder, *etc.*) in STD mesocosms with a chick present full-time could have hindered the ability of mites to find their host. This did not seem to noticeably affect the pathway of *D. gallinae* to its host since it grew at best in these conditions. Like in the poultry houses, powdery or granular material were not homogeneously distributed, which may explain the low limiting effect.

The 3D organization of access to the chick could strongly affect the mite's ability to find its host: the horizontal bar connecting the inner wall of the mesocosm to the perch, via a third vertical piece, was located above the chick and required a downward movement of the foraging mite for 42 cm. Therefore, the path imposed in the TR mesocosms could exhibit a highly disruptive paradox for the mite because it counters the negative geotropism of hematophagous foraging mites. Early colonization of artificial shelters attached on the wall 34 cm above the floor by mites released on the floor (about 20 cm from the vertical wall) in STD mesocosms does confirm the negative geotropism of *D. gallinae* for shelter selection. However, it also demonstrates the propensity of this very lively and active mite to travel > 20 cm downward to reach the support on which the host stands (24 cm below the artificial shelters). Thus, the constraint of a downward path towards the chick does not well explain the delay in population growth in TR mesocosms. However, the path conformation in TR mesocosms necessarily implies contradictions in the mite's response to stimuli produced by the chick, i.e. heat, CO_2, body odor.[45–47] It is very likely that during foraging, many mites tended to descend along the wall of the TR mesocosom, as they did in the STD mesocosms, in order to access the chick from below and ended up on the floor, with no way to reach the source of the stimuli. Moreover, if we consider that the stimuli are emitted more or less in a star shape around the chick perched in the middle of the perch, once the access through the plexiglass plate is reached, the mite must advance in a positive gradient along the upper horizontal bar (up to its middle) and then in a negative gradient (until it reaches the vertical bar). Thus the high number of electronic counts per individual counted by eye count at the end of the experiment could partly result from many mites turning back several times without reaching the vertical bar.

Although we did not investigate the spatial distribution of mites in the mesocosms in detail, it was clearly very different between the two types of mesocosms given the very low percentage of mites counted in the artificial shelters of the TR mesocosms. Perhaps the elevation of the TR mesocosm body (20 cm higher than the STD mesocosms) affected the spatial distribution by widening the gap between the artificial shelters (located at the same height in both types of mesocosms) and the top membrane: mites turning back after hitting the top membrane were less likely to encounter an artificial shelter in TR mesocosms. Alternatively, it may be that the continuous darkness encouraged the mites to position themselves elsewhere and/or that the lack of poultry feed encouraged them to settle in the feeder tank. In any case, the main route taken by the mites to reach the Plexiglass disk probably started from sites other than artificial shelters. The position of these sites remains undetermined but outside the perch itself (verified throughout the experiment).

Regardless of the aggregation sites of the mites, once the unnatural constrained path to the host is discovered, it is possible that individuals learn from stage to stage to use the space, as has been shown in *V. jacobsoni*.[48] By allowing optimization of energy expenditure in females *D. gallinae*, which can complete up to eight gonotrophic cycles, including each a blood meal,[14,25] the reduction of foraging time through transstadial learning could facilitate the sequencing of ovipositions. Thus, the learning time per individual may explain part of the observed lag. In addition, the staggering of growth phase onsets between TR mesocosms over the duration of the experiment suggests that a key event occurred at different times. Pioneer mites may chemically mark the path to the host as ants do, for example, helping others find this unnatural path without experience. Depending on the proportion of 'rover' and 'sitter' phenotypes or equivalent (as described in insects and nematodes[49,50] and probably present in many other animals[51]) the probability of the pathfinding event occurring within a given time period could explain the heterogeneity in growth phase onset dates. If a small number of individuals are likely to explore the environment, the probability of finding the path may be low enough that the self-organization resulting from the concomitance of learning and/or trail marking and the frequency of exploratory traits takes time to emerge.

Lastly, the discrepancies observed between electronic and eye counts could be partly explained by a sex-seeking behavior of adult males resulting in more counts than expected. Although males do not appear to feed,[25] maximization of the regression coefficient between eye counts and electronic counts suggested they contributed substantially to the latter. It is very possible that adult males come and go on the way to the host, looking for females to fertilize. In any case, it is clear that the functional and evolutionary study of foraging, sheltering and sex-seeking behavior in *D. gallinae* is an important issue for advancing its management.

5 CONCLUSION AND PERSPECTIVES

We characterized precisely the temporal dynamics of population growth of *D. gallinae* from a medium-sized inoculum, including the temporal evolution of the stage and sex ratios. This has advanced the fundamental knowledge of the population dynamics of *D. gallinae* and opens new avenues for experimental investigation of population dynamics of this poultry pest to progress its

management. The rapid transitions from medium to high infestation (about 11 times more mites in 2 weeks, between $T + 21$ and $T + 35$ days) makes it difficult to define a critical threshold relevant for treatment decisions based on the evolution of mite trapping over time, as we had imagined to recommend.[13] Traditional trap-based monitoring means are generally not very sensitive and curative treatments are generally not very effective when infestation is high.[52] A means of very early detection of accelerating mite population growth at any point in the henhouse would be required for such a threshold to be useful, maybe by deploying throughout the building a set of very sensitive automatic monitoring systems (e.g. the system whose prototype is presented by Mul et al.[53,54]) or recording changes in poultry behavior due to infestation using audio video technologies, as in other livestock systems.[55,56] And maybe treating as soon as *D. gallinae* is detected could be a better solution than defining a critical threshold, contrary to what is generally advocated in IPM against arthropod pests.

Our study also confirmed the occurrence of male peaks during the growth phase from inocula of adult females. This information is a valuable addition to the basic knowledge available on *D. gallinae*. Although it is not immediately applicable for operational purposes, it opens up avenues of exploration for a better understanding of the problem on farms. The long latency phase observed in farms after the empty period[10] could be explained by this particularity: if the residual populations (persisting after the empty period or arriving in the building with the pullets) are dominated by one stage, the arrival of the first clutches of eggs could be synchronized and give rise to cohorts almost entirely made up of males. It would take several laying cycles to reach a marked increase in the number of females to see an exponential increase.

Finally, our results highlight the major role of spatial configuration in relation to host access for the mite as a determinant of population growth. In line with Wood's observations,[25] the spatial configuration seems to be a factor determining the temporal dynamics of *D. gallinae* populations. Finding a physical way to complicate access to resting hens on farms may be a promising avenue, as recommended in 1917 by Wood: '[…] to aid in control work, the roosts should not be connected up with the walls of the chicken house unless some method of preventing the access of the mite to its host is used. The same may be said of the nests. The simpler and more isolated the roosts and nests, the easier it will be to eradicate the mite'.[25] Combined with the direct action of silica on *D. gallinae*,[57] the specific application of this powder on perch access points could enhance its effectiveness. Intercalating materials that are not conducive to mite travel at the connections between the perches and the rest of the structures could also help limit infestations, in combination or not with electrified perches such as 'Q-Perch'. In a more innovative direction, the slowing of growth in the presence of top access to the chick suggests that perches suspended by links alternating horizontal and vertical segments could strongly reduce infestation. However, we did not explore with precision the rules of the spatio-temporal dynamics of this mite. Studies on the local spatio-temporal distribution and dynamics of the mite populations as was done on spider mites (e.g. Azandémè-Hounmalon *et al.*[8]) are now an important challenge to progress in the management of this poultry pest. To make relevant recommendations for anti-mite facilities in poultry houses, it would also be useful to explore the ability of mites to learn the path and to test the hypothesis of a marking pheromone allowing conspecifics to benefit from the experience of a small number. Furthermore, in order to evaluate the sustainability of management methods based on spatial configuration, it would be wise to study the genetic structure related to foraging and to evaluate the frequencies of 'rover'/'sitter' alleles and equivalents.[49] Indeed, depending on the potential of individuals to explore the environment to a greater or lesser extent, imposing constraints on the path to the chick could be met with resistance more or less quickly, all the more that a negative frequency-dependent selection may occur on these alleles.[58]

More generally, our results highlight that our knowledge of the mite's life cycle timing needs to be refined. We argue that fundamental knowledge of the biology and population dynamics of *D. gallinae* is still lacking and that this substantially hinders progress in prevention and control strategies for this mite. The extent of the gaps in knowledge of the biology of *D. gallinae* is probably even greater than reported in the DISCONTOOLS database (https://www.discontools.eu/database/112-poultry-red-mite.html).

ETHICS APPROVAL

The experiments were approved by the Languedoc Roussillon ethics committee n°36 (project reference: APAFIS#30980-2018052513543835v6). All experiments involving birds were conducted in compliance with relevant guidelines and regulations.

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CONFLICT OF INTEREST

The authors declare they do not have any conflict of interest.

AUTHORS CONTRIBUTION

Lise R, DJB, RB, SD and NS conceived the ideas, Laurent R (electronic tracking system) and DD (configuration of mesocosms) conceived and designed methodology; Lise R, SD, A-SS, LD, RB collected the experimental data; DJB and Lise R conducted the model simulations, SD and Lise R analyzed the experimental data; Lise R and RB led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY

The three following resources are hosted on the Zenodo Open Science repository:

Zenodo1 (MiteThru github): Technical information, Python script and technical drawings about the electronic tracker.

DOI: 10.5281/zenodo.5145391

URL: https://zenodo.org/record/5145391#.YcBdkbrjI2w

Zenodo2 (Electronic monitoring of small arthropods in closed mesocosms): technical note gathering the bases of the approach adopted and the details of the preliminary and validation experiments.

DOI: 10.5281/zenodo.6498957

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Zenodo3 (Raw data): Here are provided two tables: 'eye counts' displays the number of mites counted in the mesocosms of experiments A and B at the end of the experiments, with indication of the stages and sexes counted; 'electronic counts' displays number of mites counted IN and OUT in the TR mesocosms per night with chick present during experiment A.

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