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First evidence of predation of the ant species *Lasius alienus* on the poultry red mite *Dermanyssus gallinae*

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Short note

ABSTRACT

The poultry red mite (PRM), *Dermanyssus gallinae* (De Geer, 1778) (Acari: Dermanyssidae), is a common and significant ectoparasite of the poultry industry worldwide. Although various biological, chemical, and physical methods have been attempted, an utterly successful control strategy has not been put forward yet. Our experimental investigations and observations revealed that the ant species *Lasius alienus* displays an effective predatory behavior on all biological stages of PRM. Our results also suggested that *L. alienus* is attracted by PRM-infested substrate at a distance. We concluded that predation by the ant on PRM is worth further investigation as it could possibly be an effective biological control strategy.

Keywords poultry red mite; *Dermanyssus gallinae*; *Lasius alienus*; predator; ants

Introduction

Dermanyssus gallinae (Poultry red mite, PRM) is a nidicolous, nocturnal and hematophagous ectoparasite that infests mainly chickens and other birds, and rarely other warm-blooded animals and humans (Roy and Chauve, 2010; George *et al.* 2015). PRM is the most common parasitic problem in commercial laying facilities worldwide (Tomley and Sparagano, 2018). Until today, many biological control methods have been attempted against PRM (Sparagano *et al.* 2014). In this context, some predator mites such as *Androlaelaps casalis*, *Gaeolaelaps aculeifer*, *Stratiolaelaps scimitus* and *Cheyletus eruditus* (Buffoni *et al.* 1997; Ali *et al.* 2012; Lesna *et al.* 2012) have been tested. However, an ideal mitigation goal has not been achieved yet (Mul *et al.* 2009; Sparagano *et al.* 2014; Knapp *et al.* 2018).

The ant species *Lasius alienus* (Förster, 1850) is one of the most abundant ant species in the Western Palearctic. It occurs mostly in natural open habitats, but is also found in light forest, forest edges and urban areas (wooded gardens, residential areas). It can reach nest densities up to 100 nests/100 m^2 (Seifert, 2018). Nests of this species are usually found in the soil, under stones, and occasionally in woods or some other materials. Workers (2-4 mm in length) establish foraging trails on the ground and in trees. The number of workers in a colony, which is active in cold and warm months, can be more than 10,000. Although the predation of many ant species on various arthropod groups has been well documented (Sanders and van Veen, 2011; Campolo *et al.* 2015; Milligan *et al.* 2016; Morris and Perfecto, 2016), there is no detailed data about *L. alienus*. However, this species was reported to consume both dead and small live insects, gather plant nectar, and feed on honeydew secreted by aphids. Workers can

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also forage in dwellings in search of food (Collingwood, 1979; Seifert, 1992; Robinson, 2005; Dussutour and Simpson, 2009).

This research was undertaken to look for evidence of possible predator activity of *L. alienus* on *D. gallinae*.

Materials and Methods

Background

In the study, a laboratory population of *D. gallinae* was used, which was established from the field samples collected from Turkish farms in 2015 and identified following Roy *et al.* (2009) and Di Palma *et al.* (2012). The mites were maintained in a parasite investigation unit (40º59′′N, 27º34′E; altitude: 17 m), of which parts were set up as a backyard coop complex in a woodland area. This coop complex contained a free-range area (4 x 5 m) and a roosting area (1x1.5 m) for chickens, with soil and concrete ground, respectively. The main component of the unit was enclosed by a wire mesh that allows the passage of ants.

The mites were placed on the solid top reservoir (25 x 50 cm size; 8 cm depth) of a four-legged (h: 20 cm) wooden roosting stand (Supplementary material 1). The reservoir was supplied with wood pieces and dry pine needles to provide hiding places for the mites. The stand was placed in the roosting area of the unit as an additional roosting site. Four to six adult hens were continuously present in the unit during the study period and were fed ad libitum.

In a first attempt to establish the mites in the facility, the population disappeared within the first week of introduction. Although ants' foraging trails leading to the top of the stand were observed, we did not realize that the ants may lead to the loss of the mite population. In the second attempt, we noticed that the mites were captured from their hiding sites by the ants during the daytime. Investigation of the area around the study units revealed natural occurrence of colonies of ants that we identified as *L. alienus* following Seifert (1992).

Demonstration of ant predation on PRM

Experiment 1

Foraging ants occurring naturally in the environment around the experimental unit in the summer period were attracted to the top of a white painted wooden table (75x100 cm, h: 80 cm) placed within the unit, using a piece of cotton impregnated with diluted commercial sugar and watermelon placed in a Petri dish (Supplementary material 2). The next day, two foraging trails originating from two distinct ant colonies led to the supplied food, and *ca.* 100 ants were observed on the Petri dish at any given time. Then, four new Petri dishes were placed (T0) around the initial dish (Figure 1A). The four new dishes contained respectively: (i) pieces of dry hen feces colonized by *ca.* 100 adults (fed female: 1-1.5 mm) and other stages (egg, larva, and nymph) of *D. gallinae*, (ii) mite-free dry hen feces, (iii) commercial sugar diluted with watermelon juice, and (iv) nothing (negative control). All the dishes were sealed with a thin cloth strainer to prevent the entry of ants and the exit of mites (Figure 1). Ant behavior was observed for one hour, starting immediately after setting the four Petri dishes (T0), and rechecked after 24 hours.

During the 1-hour observation period at T0, photographs were taken at the 5^{th}, 10^{th}, 20^{th}, 30^{th}, 40^{th}, 50^{th}, and 60^{th} minutes. The ants which climbed on the four new Petri dishes, were counted on the pictures. Video footage were used to record the time spent by each ant on the dishes.

Experiment 2

In order to observe the direct interest of the ants in mites, ants were attracted to the top of the white-painted wooden table as described in Experiment 1. Hen-bedding material containing

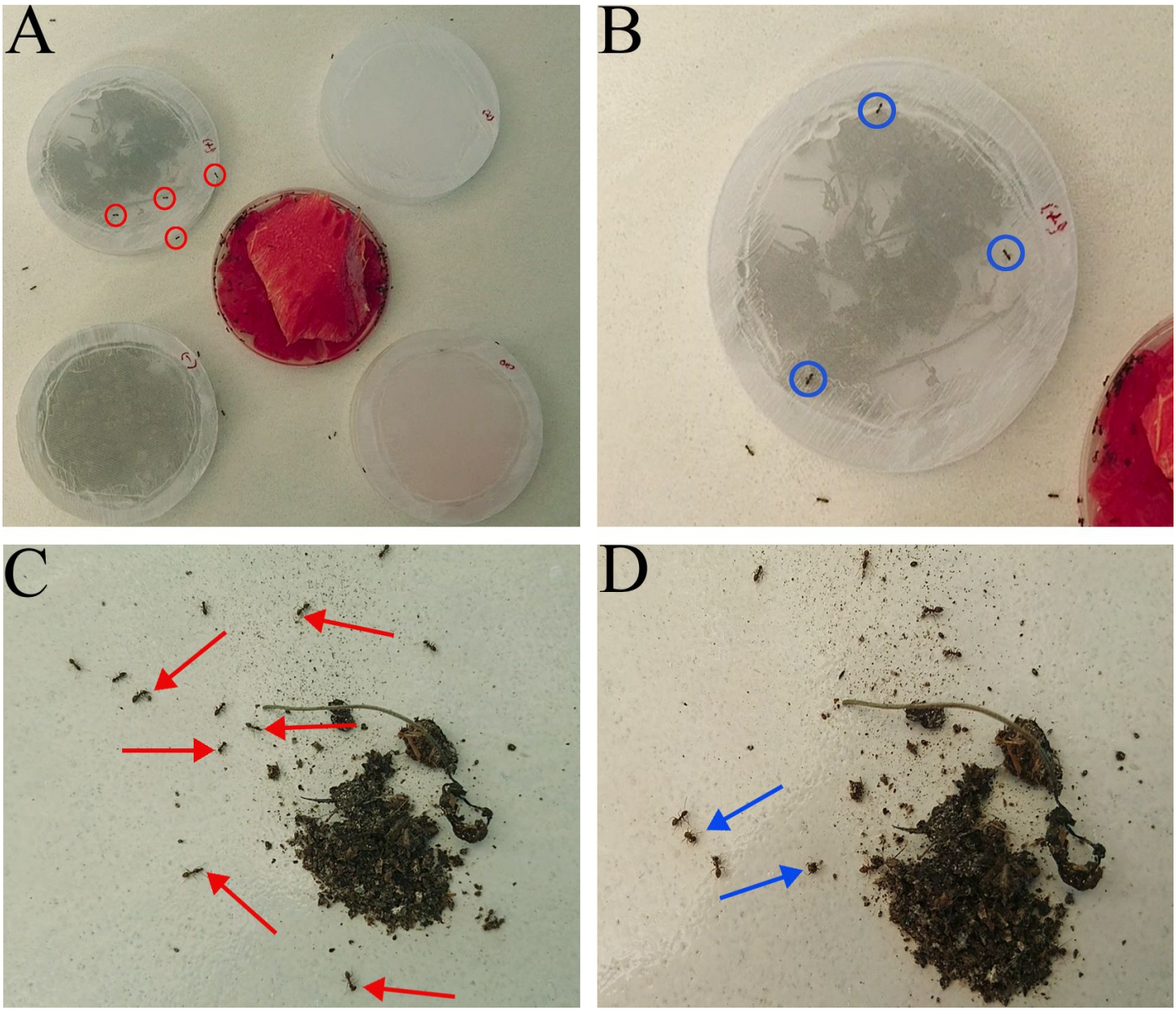


Figure 1 Illustration of the ability of the ant *Lasius alienus* to prey upon *Dermanyssus gallinae*. A. Among four petri dishes respectively with dry hen feces colonized by *D. gallinae* (top left), mite free hen feces (bottom left), diluted sugar (top right) and nothing (control, bottom right), ants (indicated by red circles) were more attracted by the first one. B. Ants trying to open the wire strainer on top of the Petri dish colonized by *D. gallinae*. C. Ants collecting mites and carrying them to their nests (red arrows). D. Ants bending their gaster to spray formic acid onto their mite preys (blue arrows).

live mites at various biological stages was placed next to the sugar-containing Petri dish and was readily invaded by foraging ants. Ant behavior was observed, and a video has been shot (Supplementary material 2). This experiment was repeated monthly during the warmer months to observe whether the interest of ants in mites was persistent across the season of ant activity.

Experiment 3

To observe the interest of the ants in different biological stages of PRM, the same setup as in Experiment 2 was established again. A pile of dry hen feces colonized by mites was divided into two equal pieces, in such a way as to include an approximately similar number of biological

forms of *D. gallinae* (white stages (eggs, larvae, unfed nymphs), and ~100 fed reddish nymphs and adults). One of the pieces was placed next to the sugar-containing Petri dish invaded by ants. The other piece, which was used as a negative control, was kept at -20 °C to hold the mites inactive. After 24 hours, the piece of the feces presented to the ants and the other piece kept in the freezer as a negative control were examined and compared under the stereomicroscope (Figure 2).

Results and discussion

Our first experiment suggested that the ants were most attracted by the dish containing dry hen feces colonized by *D. gallinae* (Table 1, Figure 1A, Supplementary material 2). Furthermore, during the 1-hour observation period at T0, it was observed that more than ten ants attempted to open the strainer on the top of the Petri dish containing the feces colonized by *D. gallinae* (Figure1B, Supplementary material 2). This type of effort was also observed in the dish containing mite-free hen feces on 3 ants during the 1-hour observation period at T0. As the strainer prevented direct contact between ants and PRM, this experiment suggests that ants are attracted by PRM and/or hen feces at a distance. At the 24th hour time point of the experiments; no particular interest of the ants was observed in any of the four Petri dishes.

During Experiment 2, ant movements directed to the sugar-containing Petri dish changed immediately after placing the hen-bedding material containing mites. The ants started to move quickly and irregularly and began to collect mites, carrying them subsequently to their nests (Figure 1C, Supplementary material 2). Ant workers bent their gaster and likely sprayed formic acid when catching live adult mites, but rarely when catching larvae and nymphs (Figure 1D, Supplementary material 2). The repetitions of this experiment during the warmer months showed that the interest of ants in mites was persistent across the season of ant activity. Although a quantitative measurement could not be done, no qualitative change was observed in this interest during the warmer months.

In Experiment 3, the stereomicroscope examination performed at T+24th hour showed that the workers of *L. alienus* took away the eggs, larvae, nymphs, adults, and even eggshells of *D. gallinae* from the piece presented to them for twenty-four hours (Figure 2).

The localization of *D. gallinae* in the upper parts of the poultry housings has been considered a restrictive factor for the effectiveness of some predatory mite species on PRM, as those predatory mites are mostly located in manure, on the bottom of housings (Lesna *et al.* 2012). In our study, we observed that ant workers were able to find and eliminate all forms of PRM within a range of several meters from their colony, even at the top of the stand, including from dried chicken feces where PRM frequently hide. This suggests that *L. alienus* workers would be efficient at finding mites in the different parts of the hen house. Furthermore, no ant was seen on the chickens or their food, and no chicken was noticed to eat the ants or interfere with them. So, there is little risk that this ant species would be a vector of poultry pathogen

Table 1 Results from experiment 1. Mean ± sd number of ants counted over the seven successive pictures during the 1-hour observation period at T0 and mean ± sd duration of visit time per ant on the dish. Hen feces + PRM, dish containing the hen feces colonized by *D. gallinae*; hen feces, mite-free hen –feces; Sugar, dish containing diluted sugar; Control: empty dish.

Materials	Number of ants	Time spent (seconds)
Hen feces + PRM	132 ± 15.9	46 ± 48.5
Hen feces	121 ± 20.3	19 ±18.7
Sugar	73 ± 17.2	10 ± 5.7
Control	18 ± 7.5	11 ± 5.6

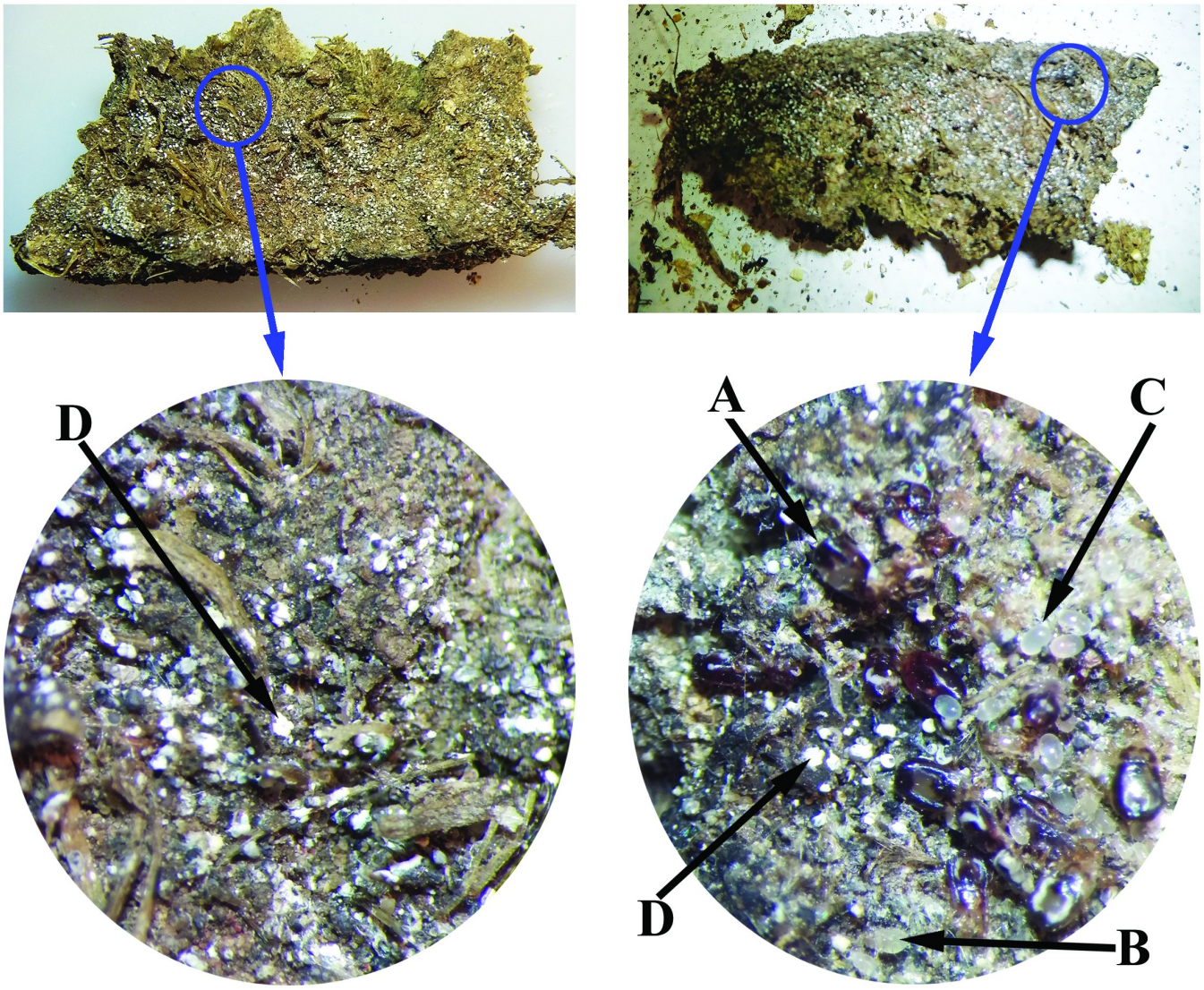


Figure 2 A pile of dry hen feces colonized by *D. gallinae* was divided into two pieces in Experiment 3. The left one was exposed to ants for 24 hours. The right one was not exposed to ants and was used as a negative control after being kept at -20 °C to hold the mites inactive. A: adult mite, B: early biological forms, C: egg, D: white residues of PRM aggregate.

or parasite. In fact, some ant species are known to be the intermediate host of some poultry parasites (Taylor *et al.* 2016), but there is no report on the vector potential of *L. alienus* for any vertebrates. It was reported that the workers of some ant species (e.g., the fire ant *Solenopsis invicta*) could sting and bite chickens (Tomberlin and Drees, 2007). However, *L. alienus* does not have a sting, and, unlike its close relative species *L. niger*, it is known as a non-aggressive species (Collingwood, 1979; Robinson, 2005).

We showed that *L. alienus* is a potential predator on all biological stages of PRM. This prey-predator interaction is worth investigating more thoroughly as it might lead to an effective biological control strategy.

Supplementary data

Supplementary material 1

General view of the wooden roosting stand used for the propagation of *D. gallinae*.

Supplementary material 2

Video showing *L. alienus* workers trying to open strainer to get inside the Petri dish that contained hen feces colonized by the poultry red mite *Dermanyssus gallinae* (PRM) in experiment 1 (Part I) and hunting PRM in experiment 2 (Part II).

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