Research paper

Population fluctuation of predatory and sanitary importance mites (Acari) in commercial laying hens: Ecological interactions

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ABSTRACT

This study aimed to evaluate the mite fauna and their ecological interactions in commercial laying hen farms in the state of Rio Grande do Sul, Brazil. It was conducted from August 2013 through August 2014 with two sampling strategies (feathers and traps) in three different production systems: automated ($A_{1,2,3}$), semi-automated ($S_{1,2}$) and free-range (FR). A total of 38,383 mites were collected belonging to 23 families and 33 species, most of which were collected in feathers (74%) followed by traps (26%). There was higher abundance at S_1 (10,774-28.1%) and S_2 (11,023–28.7%) followed by FR (6972-18.2%), A_1 (1896-4.9%), A_2 (4775-12.4%), and A_3 (2943-7.7%). Higher richness was observed at S_1 (23 species), S_2 (18 species), and FR (19 species). *Megninia ginglymura* (Mégnin) (Analgidae) was the species with the highest health importance, eudominant on feathers, and its populations seems to be related with increased temperature. *Tuccioglyphus setosus* Horn et al. (Pyroglyphidae) seems to be influenced by relative air humidity and temperature. Predators with the highest populations were *Cheyletus malaccensis* (Oudemans) (Cheyletidae), *Typhlodromus transvaalensis* (Nesbitt) (Phytoseiidae), *Blattisocius keegani* (Fox), and *Blattisocius dentriticus* (Berlese) (Blattisocidae).

1. Introduction

Intensive egg production affects the welfare of laying hens and increases the risk of epidemics, and it can be affected by several complications, e.g. ectoparasitic mites (Guimarães and Leffer, 2009). Among the consequences of attacks by arthropods are decreased production due to increased eggshell fragility, as well as the fact that laying hens become anemic, restless, and aggressive towards each other (Sparagano, 2009).

Commercial laying hens are affected by mites of sanitary and economic importance worldwide. Since *Salmonella* vaccination is now widespread within the poultry industry, it seems that the new economic, welfare, and epidemiological problem is the poultry red mite, *Dermanyssus gallinae* (De Geer) (Dermanyssidae) (Sparagano, 2009). Aside from this hematophagous mite, *Ornithonyssus bursa* (Berlese) and *Ornithonyssus sylviarum* (Canestrini and Fanzago) (Macronyssidae) have been reported in poultry farms and *O. bursa* seems to have been replaced by *O. sylviarum*, which has recently been reported (Rezende et al., 2013), in the Brazilian poultry industry.

Among feathers mites, *Megninia ginglymura* (Mégnin) (Analgidae) is the most frequently reported in commercial laying hens in Brazil (Reis, 1939; Tucci et al., 2005; Rezende et al., 2015) and in the state of Rio

Grande do Sul (Silva et al., 2013; Rezende et al., 2013; Faleiro et al., 2015; Horn et al., 2016). This species spends its life cycle on its host and oviposits on feathers (Hernández et al., 2007). Apparently, it is not as abundant as *Dermanyssus* spp. and *Ornithonyssus* spp. and causes less significant damages as studies on its ecology and economic influence on poultry farms are scarce worldwide (Hernández et al., 2006). In Cuba, *M. ginglymura* was the most important sanitary species present in commercial laying hens in all provinces (Hernández et al., 2006); the population peak of this species there indicated a relationship with wet season (Hernández et al., 2007). In Mexico, the presence of two population peaks of *M. ginglymura*, one in July and another in November, suggests that seasonality affects population although the factors that influence population are yet unknown (Quintero et al., 2010). In the state of Rio Grande do Sul, higher population was related to the period ranging from February to April, with a peak in February, 16.3 mites/hen in free-range, and in April, with 22.3 mites/hen (Faleiro et al., 2015).

Tuccioglyphus setosus Horn and Klimov (Pyroglyphidae) seems to have a strong relationship with poultry farms, since it was observed in all laying hen management systems in Rio Grande do Sul. However, it was misidentified as *Pyroglyphus* sp. by Silva et al. (2013) and Horn et al. (2016) before it was described by Horn et al., 2017. This species

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was observed in laying hen facilities, laying hen feathers, and in nests of wild birds (*Columbina picui* (Temminck, 1813), *Columbina talpacoti* (Temminck, 1810), and *Zenaida auriculata* (Des Murs, 1847)). House dust mites of the Pyroglyphid family include free-living species that are mostly known as human associates living in house dust, upholstery, pillow and mattress stuffing, and causing allergies (Arlian, 1991; Lloyd, 2009; Tovey et al., 1981), and some species have economic importance as pests in stored food in warehouses and residential homes (Fain, 1990). There is no information on the ecology and food habits of *T. setosus*.

Alternative pest control using natural enemies allows the use of cleaner practices and is less environmentally harmful (Lesna et al., 2009). The traditional strategy in the control of pest species in poultry systems, *i.e.*, with synthetic chemical pesticides, tends to develop resistance in mites in the long term, and pesticides also have adverse effects on birds' nervous system and can be immunosuppressive and carcinogenic, as well (Nero et al., 2007; Marangi et al., 2009; Wright et al., 2009). *Androlaelaps casalis* (Berlese) (Laelapidae), *Gaeolaelaps aculeifer* (Canestrini) (Laelapidae) (Lesna et al., 2009), and *Cheyletus eruditus* (Schrank) (Cheyletidae) (Maurer and Hertzberg, 2001) are recognized as natural enemies of *D. gallinae*. The predators *Blattisocius dentriticus* (Berlese) (Blattisocidae) and *Cheyletus malaccensis* (Oudemans) (Cheyletidae) were observed feeding on *M. ginglymura* in the laboratory (Silva et al., 2016; Granich et al., 2017) and the highest potential for control was shown by *C. malaccensis*.

Studies on population dynamics and efficient techniques for the control of sanitary mites are scarce in poultry farms in Brazil (Silva et al., 2013). Due to the importance of this activity to the economy of the state of Rio Grande do Sul, it is necessary to know the associated mites, their frequency, and the damages they cause. Considering that *M. ginglymura* seems to be affected by environmental conditions (Quintero et al., 2010) and predatory mites showed potential for the biological control of this ectoparasite in the laboratory (Silva et al., 2016; Granich et al., 2017), our hypotheses are that (1) environmental conditions (precipitation, relative air humidity and temperature) affect *M. ginglymura* and *T. setosus* populations in laying hen systems and (2) there is an association between the populations of *M. ginglymura* and *T. setosus* with predatory mites present in these systems. Therefore, the objectives of this study were to evaluate the mite fauna and their ecological interactions in commercial laying hen farms in Rio Grande do Sul, Brazil, and to support future studies using predatory mites as a biocontrol strategy applied in laying hen farms.

2. Material and methods

2.1. Study area

This study was conducted in different commercial laying hen systems from August 2013 through August 2014 in Lajeado municipality, Vale do Taquari, state of Rio Grande do Sul, Brazil.

Six poultry houses were sampled; in three of them the laying hen system consisted of automated vertical battery cages ($A_{1,2,3}$), two were semi-automated systems ($S_{1,2}$), and one was free range (*FR*) (Table 1). In the automated system, the laying hen were confined in metal cages on six floors with an area of approximately 450 cm²/hen (nine hens/cage), and cages were placed one on top of the other in stacks of four. Hen feed was provided in a metal structure, water was provided through nipple-type drinker, and eggs were collected by an automatic treadmill. In addition, feces were collected at least three times per week by treadmills at the bottom of the cage floors. In this laying hen system, there are screens throughout the laying hen house to prevent access from wild birds.

In the semi-automated laying hen system, the cages were arranged as stair steps with two stacks of cages in each poultry house. Feed and water were provided automatically and eggs were manually collected. The S_1 system was a wood structure in a "California house" style and S_2

was a "wide-span model" (Axtell, 1986). S_1 did not receive any type of pesticide application during the study period and was considered the semi-automated control.

In the other laying hen house evaluated, hen were raised free under a sawdust bed arranged on the ground, popularly known as free-range (*FR*). In Brazil, this system is popularly known as "caipira". Feed and water were provided automatically and eggs were collected manually. Nests were packed in a wooden structure with sawdust inside for egg maintenance. Laying hens were released during the day for sunbathing, ground pecking, and wing flapping.

Sampling efforts were different in the laying hen houses due to the absence of laying hens during some periods depending on the pause between the disposal of the old batch and entrance of the new batch of laying hens. Car access from other hen houses was denied throughout the study.

2.2. Mite samplings

To collect mites, we placed 16 traps made of 27-cm PVC pipe (50 mm diameter) with 13 0.8 mm holes and the ends closed by caps (PVC cap) and attached to the cages with a rubber band in each laying hen house (Tucci et al., 1988). Three slightly crushed paper towel sheets were placed inside the traps to provide shelter. Attracting substances were not used. Throughout the study period, traps were maintained at the same point and were replaced every 15 days. In A_1, A_2, A_3, S_1, and S_2, traps were arranged on the second floor of the cages, while they were placed on perches and inside the nests in *FR*. At each evaluation, the paper towel was collected, packed individually in plastic bags, labeled, and taken to the laboratory, where it was kept in a freezer (0 °C) for at least 24 h. For each evaluation, the paper towel collected was placed on Petri dishes and observed under a stereomicroscope.

To collect ectoparasites, ten laying hens were examined from each laying hen house, and chickens were selected along the length of the laying hen houses. A total of five feathers/hen were collected from each laying hen every 15 days. The feathers were placed in plastic containers with 70% alcohol for a minimum of 24 h prior to screening. Plastic containers were taken to the laboratory in paper boxes with Styrofoam inside. Screening was performed by filtering the alcohol in qualitative filter paper with 12.5 cm diameter and 80 g/m2 wt.

All mites were collected with a fine-tipped paintbrush and mounted in Hoyer's medium on microscope slides (Walter and Krantz, 2009). The slides were kept for up to 10 days at 50–60 °C to dry the medium, extension of legs, and for the diaphanization of specimens. Representative specimens of each species were deposited on the mites reference collection of the Museum of Natural Sciences at UNIVATES - University Center (ZAUMCN), Lajeado, Rio Grande do Sul, Brazil.

2.3. Data analysis

The data analyzed concerned the mites found in the laying hen houses evaluated sampled in traps and feathers. Several ecological indices were determined using the software DiVes 2.0 (Rodrigues, 2005):

i) Shannon-Wiener index (*H'*) expresses richness and uniformity. *H'* is determined by the formula $H' = -\Sigma\ pi\ Log\ pi$, where *pi* is the proportion of specimens of each species in relation to the total number of specimens found in the assessment performed (Shannon, 1948);

ii) Shannon's evenness index (*J*) expresses the equitability of abundances in a community and allows the assessment of species stability over time. *J* is determined by the formula $J = H'/Hmax'$, where the *H'* is the Shannon-Wiener index and *Hmax'* is given by the following expression: $Hmax' = Log\ s$, where *s* is the number of species sampled) (Brower and Zar, 1984).

iii) Berger-Parker dominance (*BPd*) considers the highest proportion of species with the highest number of individuals. *BPd* is determined by

Table 1
Characteristics of each laying hen house (A₁, A₂, A₃ – automated systems; S₁, S₂ – semiautomated systems; FR – free range) and management, between August 2013 and August 2014, in Vale do Taquari, Rio Grande do Sul, Brazil.

	Laying hen stock and breed	Laying hen age	Sample effort	Pesticides
A_1	39,000 white Bovans	45 – 94 weeks 16 – 20 weeks (new batch from August 2014)	21 samples of feathers 10 laying hen/sample Total n$A_{1feathers}$ = 210 20 samples of traps 16 traps/sample Total nA_{1traps} = 320	Fipronil 1% (feed) in September 2013
A_2	60,000 50% white Bovans and 50% red Isa brown	68 – 98 weeks 17 – 37 weeks (new batch from April 2014)	20 samples of feathers 10 laying hen/sample Total n$A_{2feathers}$ = 200 18 samples of traps 16 traps/sample Total nA_{2traps} = 288	Fipronil 1% (feed) in September 2013
A_3	35,000 red Isa brown	99 – 109 weeks 19 – 54 weeks (new batch from December 2014)	17 samples of feathers 10 laying hen/sample Total n$A_{3\ feathers}$ = 170 16 samples of traps 16 traps/sample Total nA_{3traps} = 256	15% cypermethrin, 25% chlorpyrifos and 1% citronellal (pulverized) twice in April 2014
S_1	7,750 red Dekalb	45 – 88 weeks	18 samples of feathers 10 laying hen/sample Total n$S_{1\ feathers}$ = 180 18 samples of traps 16 traps/sample Total nS_{1traps} = 288	–
S_2	10,400 red Isa Brown	41 – 95	22 samples of feathers 10 laying hen/sample Total n$S_{2\ feathers}$ = 220 21 samples of traps 16 traps/sample Total nS_{2traps} = 336	Fipronil 1% (feed) in September 2013 and May 2014
FR	3,500 red Isa Brown	44 – 88 weeks	19 samples of feathers 10 laying hen/sample Total n$FR_{\ feathers}$ = 190 19 samples of traps 16 traps/sample Total nFR_{traps} = 304	Propoxur 1% (nests) in December 2013, January and August 2014

the formula $d = Nmax/N_T$, where *Nmax* is the number of specimens from the most abundant species and N_T is the total number of specimens from the sampling (Berger and Parker, 1970). Species constancy (*C*) was classified as constant when present in more than 50% of the samples (*C* > 50%), accessory when present in 25–50% of the samples (25% < *C* < 50%) and accidental when present in less than 25% of the samples (*C* < 25%) (Bodenheimer, 1955). The dominance (*D*) was defined by the formula $D\% = (i/t) \times 100$, where i = total number of individuals of a species and t = total individuals collected and clustered according to categories: eudominant (*D* > 10%), dominant (*D* > 5% < 10%), subdominant (*D* > 2% < 5%), eventual (*D* = 1% < 2%) and rare (*D* < 1%) (Friebe, 1983).

Climate parameters precipitation (mm), relative humidity of air (%) and temperature (ºC) for the study period were provided by UNIVATES University Center Meteorological Station, Lajeado, state of Rio Grande do Sul (Fig. 1).

A Principal Component Analysis (PCA) was performed with software used to investigate the correlation between abiotic end biotic parameters (PRIMER-E Clarke and Gorley, 2002) version 5.2.9, in traps and feathers. The main species with recognized predatory potential were considered for this analysis, besides the species *M. ginglymura* and *T. setosus*. In order to verify the highest inclinations and amplitudes of each species, rank-abundance curves were performed for traps and feathers.

3. Results

A total of 38,383 mites were collected from feathers and traps, belonging to 23 families and 33 species (Table S1). Most mites were

sampled from feathers (74%) and the others were collected from traps (26%). The highest abundance was observed in the semi-automated systems, S_1 (10,774) and S_2 (11,023), followed by *FR* (6972), while the lowest abundance was observed in automated systems (A_1: 1896; A_2: 4775; and A_3: 2943). Richness followed the same pattern of abundance, it was higher in S_1 (23) and S_2 (18), followed by *FR* (19), and lower in A_1, A_2 (13), and A_3 (12) (Fig. 2). Rank-abundance analysis showed that there was a drastic difference in the mite fauna present in feathers compared to traps, in terms of abundance and richness, with higher richness associated to traps (32 species) than to feathers (13 species) in the systems evaluated.

3.1. Ecological diversity indices

The automated systems had higher diversity and evenness indices (A_1 – *H'*: 0.7018, *J*: 0.6301; A_2 – *H'*: 0.5078, *J*: 0.4558; and A_3 – *H'*: 0.546, *J*: 0.5046) than semi-automated systems (S_1 – *H'*: 0.1977, *J*: 0.1452; S_2 – *H'*: 0.2764, *J*: 0.2202) or free range (*FR* – *H'*: 0.233, *J*: 0.1822) (Table 2). The lowest Berger-Parker dominance indices were observed in A_1 (*BPd*: 0.0538), S_1 (*BPd*: 0.0644), S_2 (*BPd*: 0.064), and *FR* (*BPd*: 0.0839) and the highest indices were observed in A_2 (*BPd*: 0.128) and A_3 (*BPd*: 0.1927).

3.2. Biodiversity and mite fauna fluctuation

The families with the highest richness were Cheyletidae, with four species (*Chelacheles bipanus* Summers & Price, *Cheletomimus (Hemicheyletia) wellsi* (Baker), *C. eruditus*, and *C. malaccensis*, followed by Acaridae, with three (*Aleuroglyphus ovatus* (Troupeau), *Thyreophagus*
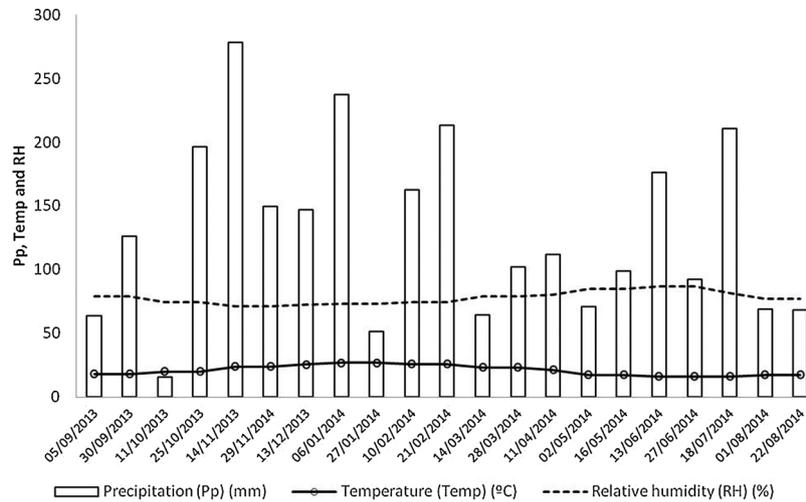
Fig. 1. Meteorological data: precipitation (Pp) (mm), temperature (Temp) (°C), and relative air humidity (RH) (%) in Lajeado, state of Rio Grande do Sul, between August 2013 and August 2014.

entomophagus (Laboulbéne), and *Tyrophagus putrescentiae* (Schrank)).

Megninia gynglymura was the species with sanitary importance and had high abundance, with 29,633 specimens (77.2%). It was present throughout the year in all systems evaluated, except for A_1, where its presence was first observed at the fifth sampling (Fig. 3 and 4 – the graph of A_2 is at a different scale). This species was considered constant in feathers and traps in all laying hen houses evaluated, except for A_1 where *M. ginglymura* was accessory in the feathers. In the feathers, it was eudominant in all systems. Additionally, it was eudominant in traps of S_1 and *FR*, dominant in A_1 and S_2, and subdominant in A_2 and A_3. In A_1 and A_2, the population peak of *M. ginglymura* occurred between January and March/2014, with 1.6 and 11.6 mites/feathers,

Table 2

Ecological indices of mite communities found in six laying hen houses (A_1, A_2, A_3 – automated systems; S_1, S_2 – semiautomated systems; FR – free range), between August 2013 and August 2014 in state of Rio Grande do Sul, Brazil.

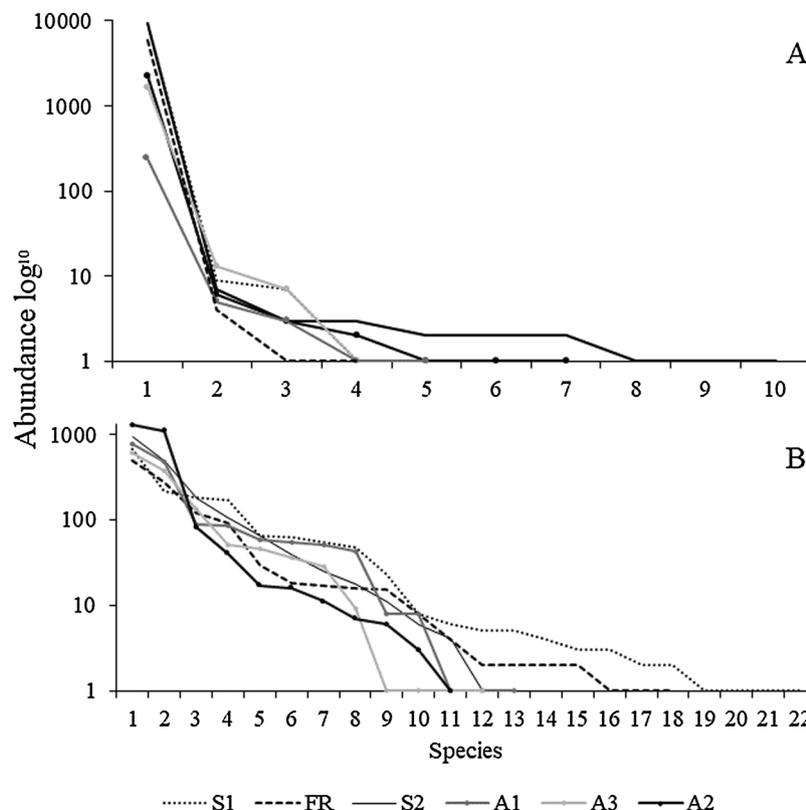| Index	A_1	A_2	A_3	S_1	S_2	FR
Number of species	13	13	12	23	18	19
Number of specimens	1896	4775	2943	10,774	11,023	6972
Shannon diversity (H') 2.0	0.7018	0.5078	0.5446	0.1977	0.2764	0.233
Shannon evenness (J)	0.6301	0.4558	0.5046	0.1452	0.2202	0.1822
Berger-Parker dominance (BPd)	0.0538	0.128	0.1927	0.0644	0.064	0.0839

Fig. 2. Rank-abundance curves of fern species, comparing the edge and inner part of the study sites. A_1, A_2, A_3 – automated systems; S_1, S_2 – semi-automated systems; FR – free range.
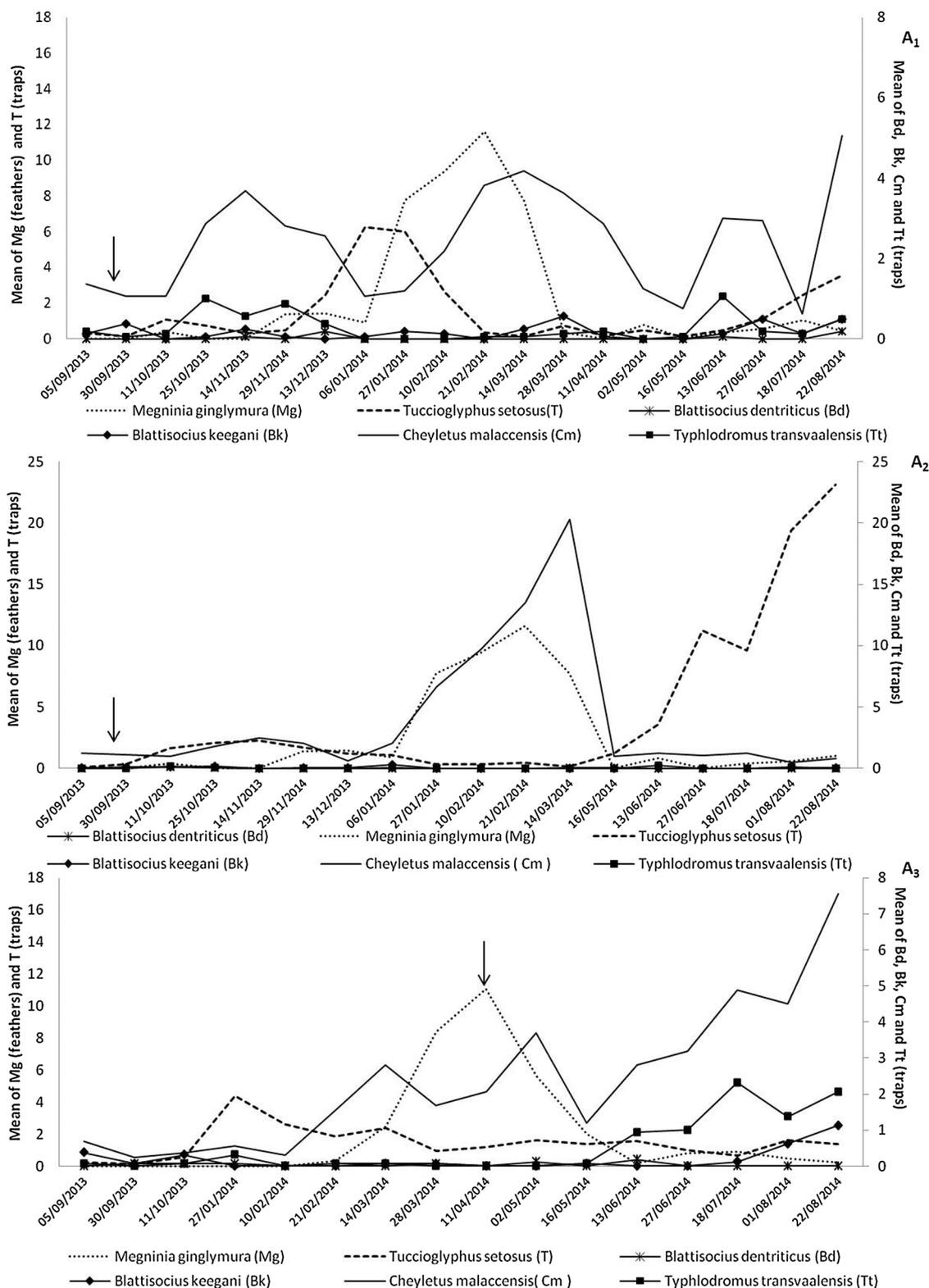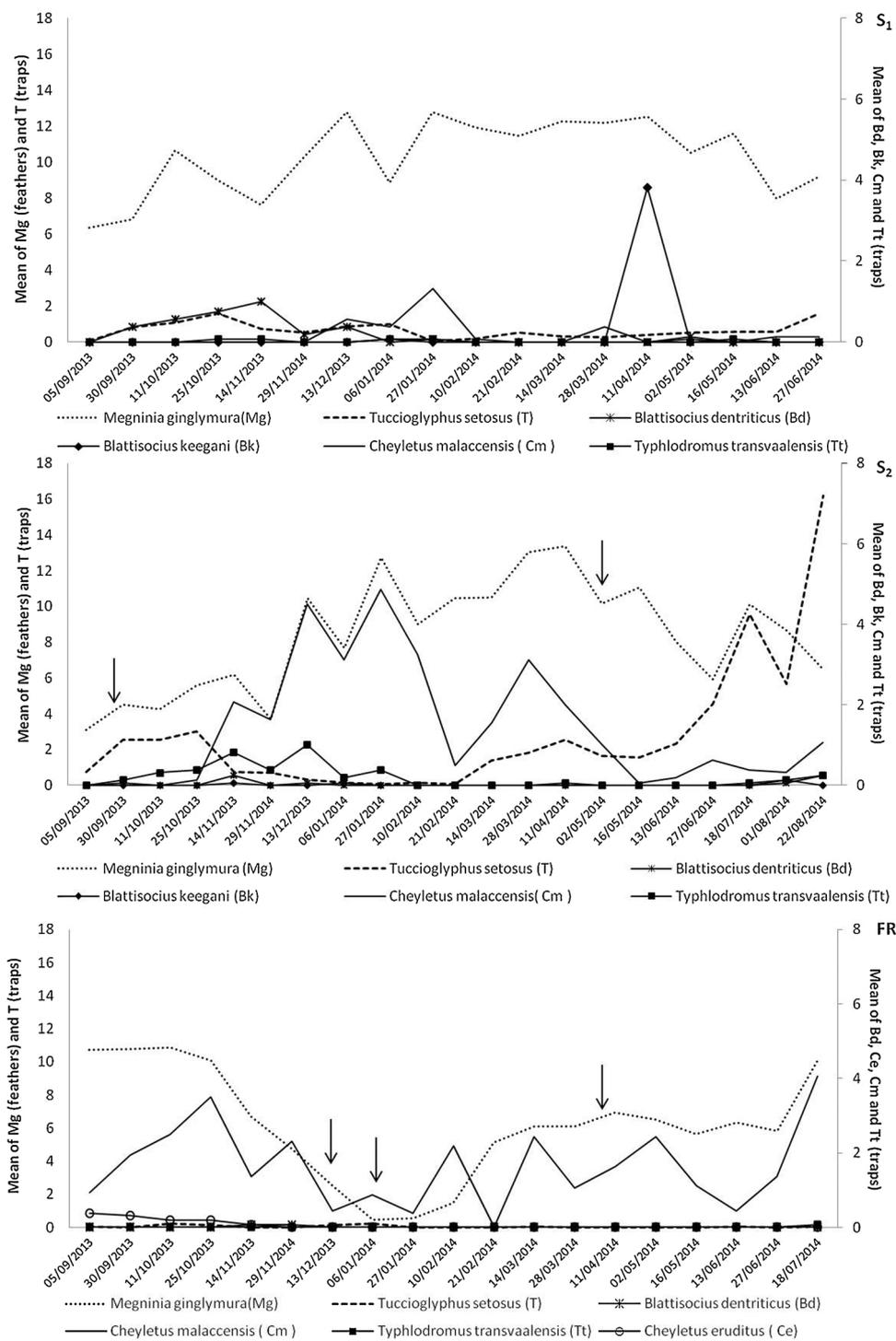
Fig. 3. Mite population fluctuation in automated (A_1, A_2[*] and A_3) laying hen houses between August 2013 and August 2014 in Lajeado municipality, state of Rio Grande do Sul, Brazil. * A_2 graph scale is different from the others due to a high population peak of *Tuccioglyphus setosus*.

respectively, and the highest mean value in February/2014. Synthetic chemical pesticide was applied in September/2013, which is the period of low population. In A_3, there was a late population peak, in April/2014, with a mean value of 11.1 mites/feathers. In the semi-automated systems, the populations remained with high numbers during most of the period. In S_1, high population extended from November/2013 to

April/2014, averaging 12.8 mites/feathers in December/2013 and January/2014; in S_2, mean values were high between December/2013 and June/2014, with the highest mean value in April/2014, 13.4 mites/feather. In *FR*, populations with high number were observed from September to November/2013, with a high population peak in October/2013, 10.9 mites/feathers. Mite populations increased

Fig. 4. Population fluctuation of mites in semi-automated (S_1 and S_2) and free range (*FR*) laying hen houses between August 2013 and August 2014 in Lajeado municipality, state of Rio Grande do Sul, Brazil.

immediately after the application of synthetic chemical pesticide, with a new population peak between February and June/2014.

Among predators, *C. malaccensis* was present in the systems throughout the sampling period, with 3511 individuals (9.1%). This species was the most constant and eudominant in traps of all systems, except for S_1, where it was accessory and subdominant. In the feathers, this predator was observed in A_1 and it was accidental and rare in the other laying hen houses. In A_1, *C. malaccensis* populations increased after the population peak of *M. ginglymura* started in January/2014, but there was no significant correlation between these populations. In A_2,

laying hen house with high abundance of *C. malaccensis*, the population peak occurred between January and March/2014, coinciding with the population peak of *M. ginglymura*. In A_3, the population peak of *C. malaccensis* occurred between February and August/2014. In S_2, population peak was between December/2013 and February/2014 (4.9 mites/traps in January/2014). In *FR*, the population of this predator remained high, with several population peaks between September and November/2013, and from February and March to May/2014.

The other mite collected, *C. eruditus*, was found only in lower population in *FR* (18 specimens – 0.05%).

The second most abundant predatory mite present in all systems was *Typhlodromus transvaalensis* (Nesbitt), with 309 specimens (0.8%), considered constant in traps of A_1, A_2 and S_2, where they were dominant, eudominant, and subdominant, respectively. This species was observed in feathers in S_1 and S_2, and was considered accidental and rare. In A_1, there was a period with slightly higher population between October and December/2013 and in June/2014. In A_3, population peak occurred between June and August/2014 with a mean value of 2.3 mites/traps in July/2014. In S_2, the highest populations were registered between October/2013 and January/2014, with 1 mite/trap. In A_2, S_1 and *FR,* populations were very low.

Blattisocius dentriticus and *Blattisocius keegani* (Fox) (Blattisocidae) were predatory mites with low abundance evaluated for potential biological control. Populations of both predators remained low during the study period. *Blattisocius keegani* was the third most abundant predator in at least five laying hen houses (A_1, A_2, A_3, S_1, and S_2), totaling 187 specimens (0.5%). This predator was constant and subdominant in the traps of A_1 and A_3; accessory and rare in A_2; accidental in S_1 and S_2, where it was subdominant and rare, respectively. In the feathers, low numbers of *B. keegani* were also observed and it was considered accidental and rare, except for A_1, where it was absent. This species was absent in the *FR* system. There were only 93 specimens of *Blattisocius dentriticus* (0.2%), it was thus considered accessory and subdominant in the traps of S_1 and accessory and rare in A_2 and A_3. In the traps of other laying hen houses it was considered accidental and rare. In the feathers, this species was present in A_1 where it was accidental and rare; in S_1, it had a population peak between September and November/2013.

Among generalist species, *T. setosus,* with 3294 (8.6%), was the most abundant species present throughout the sampling period in all laying hen houses, except for *FR* where its population was low. This species was considered constant and eudominant in the traps, except for *FR* where it was accessory and eventual. In the feathers, it was accessory and rare in A_3; accidental and rare in A_2 and S_2; and accidental and subdominant in A_1. This species was absent in the feathers of S_1 and *FR*. In A_1, synthetic chemical pesticide application in September/2013 did not prevent high populations between December/2013 and February/2014, with higher mean value in January/2014 (6.3 mites/feathers). In A_2, high mean values of its population occurred between June and August/2014, with a population peak in August/2014 (23.1 mites/traps). Due to this high mean population in this system, its graph is shown at a different scale from the others (Fig. 3). In A_3, the population remained low and the mean value oscillated between 0.7 and 4.4 mites/traps with a peak in January/2014. In S_1, the population remained low with no significant population peak. In S_2, there were two population peaks between September and October/2013 and another peak from March to August/2014 with high mean values in August/2014 (16.2 mites/traps). In *FR*, the population remained low with high mean value in December/2013, 0.13 mites/traps.

3.3. Correlation between abiotic end biotic parameters

The two first two components of PCA for feathers explained ca. 55% of the variation between the different laying hens (Fig. 5). Component 1 was most correlated with total mite abundance (0.54), abundance of *M. ginglymura* (0.53), and temperature (0.45). Component 2 was most correlated with relative humidity (0.51), followed by abundance of *M. ginglymura* (0.44) and temperature (-0.44). In general, the relationship between *T. setosus*, relative humidity and richness is indicated. The PCA for traps explained 55% of the variation between the different laying hens (Fig. 6). Component 1 was more correlated with the abundance of *T. setosus* (0.48), followed by total mite abundance (0.53) and temperature (-0.41). Component 2 was most correlated with relative air humidity (-0.59), followed by temperature (0.44) and total mite richness (-0.44). In traps, *M. ginglymura* was more related to free range and automated systems with temperature as determining factor while increase in temperature is related to higher abundance of this species. In

traps, the abundance was not related to *M. ginglymura*.

To investigate the possible relationships between predator-prey in a second PCA analysis, we found the following: the first two components of the PCA for feathers explained ca. 62% (Fig. 7); component 1 was more correlated with *M. ginglymura* abundance (0.580) followed by *B. keegani* (0.526); component 2 was more correlated with *T. transvaalensis* (0.562) and *T. setosus* (-0.536). The PCA for traps explained ca. 65% of the variation between the different laying hens based on abundance (Fig. 8). Component 1 was more correlated with *M. ginglymura* abundance (-0.545) and *T. transvaalensis* abundance (0.510). Component 2 was more correlated with abundance of *T. setosus* (-0.762) and *C. malaccensis* (0.367).

4. Discussion

Data found in this study are important as preliminary information for the identification of associated species and evaluation of their population dynamics in the management of different laying hen houses. Management affects mite abundance, richness, and diversity in laying hen systems (Horn et al., 2016). Furthermore, *M. ginglymura* stood out as the mites with the highest sanitary importance associated to laying hens in the study region. This species is strongly associated to feathers, but in laying hen systems with high population densities it seems to leave the hen and look for a new host, and could thus be captured by traps designed to catch predatory mites and other ectoparasites such as *D. gallinae*, which crawls up the hen only for blood feeding. No hematophagous mites were found. *Megninia ginglymura* feed on skin, fat, and feather parts, and mite saliva can cause lesions, allergic reactions, serious scabs, stress, crust formation, and secondary bacterial infections (Tucci et al., 2005). Few studies confirm the percentage of economic loss. Therefore, it is of utmost importance to further the knowledge of the management of this species in commercial laying hens. The laying hens in the *FR* system had improved visual appearance, with no skin injuries and practically intact feathers until the discard phase due to senescence and reduced egg productivity while the opposite was observed in all other systems (Horn et al., 2016).

4.1. Mite fauna

The highest richness was found in traps, while the highest abundance was associated to feathers. Automated systems seem to induce a lower abundance of mites of sanitary importance compared to the other systems. Even with higher confinement of hens, abundance in automated systems was lower than or similar to other laying hen systems (Horn et al., 2016), while higher mite richness was observed in semi-automated systems (S_1; S_2) and *FR*. The highest richness was associated to laying hens without synthetic chemical pesticides (S_1), and total mite abundance was not significantly high compared to the other laying hen houses (Horn et al., 2016).

These data show a typical mite stability pattern in the community and ecological balance with the highest richness and more rare species found in traps. This pattern also appears in the *FR* system. In the other systems, there seems to be decreased richness. The higher number of exclusive species and the higher community heterogeneity among traps compared to feathers show that mite fauna community has a more equitable distribution with a larger number of rare and less abundant species (Magurran, 1988).

Automated laying hen houses had higher diversity (H') and evenness (J) than the other systems. The lowest dominance was observed in semi-automated laying hen houses, *FR*, and A_1.

Regarding mite fauna associated with each kind of mite sampling, traps had higher mite stability in the community while feathers had an extremely high abundance of only one species (*M. ginglymura*). It is worth of note that predator abundance was very low and these data could influence the PCA evaluations on feathers (Figs. 7 and 8).

Overall, mite fauna had obvious population peaks, and some species
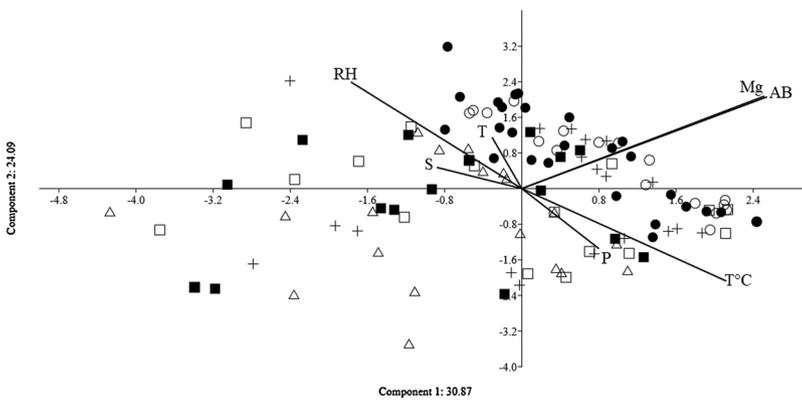
seemed to show resistance to synthetic chemical pesticides used by the farmers to control *M. ginglymura.*

4.2. Population dynamics of M. ginglymura and T. setosus

Quintero et al. (2010) reported the presence of two population peaks of *M. ginglymura* in Mexico, one in July and one in November, suggesting that seasonality affects population. However, the factors that affect population are yet unknown. Population peaks of *M. ginglymura* occurred between September/13 and April/2014 in the laying hen systems, coinciding with periods of high mean temperatures. The period of high population occurred from February to April, reaching a peak in February, with 16.3 mites/hen in the free-range system, and in April with 22.3 mites/hen (Faleiro et al., 2015). These authors associated these differences to seasonal temperature, with population peaks occurring in free-range systems in warmer months and in battery cages in colder months.

The application of synthetic chemical pesticide in A_1 e A_2 did not seem to have been effective in the control of *M. ginglymura* population, because there was a population increase shortly after the application. In A_3, there was a late population peak probably due to the introduction of a new batch of laying hens in January/2014. There was decreased *M. ginglymura* populations with the use of pesticides. However, due to the late peak, the actual capacity of pesticides to refrain population growth remains unclear, since this decrease could be related to environmental conditions. In *FR*, populations were affected by pesticides in December/2013 and January/2014. However, it is noteworthy that immediately after the application there was a fast increase in population, indicating that there is a tendency towards resistance to pesticides. Laying hen houses without use of pesticides (S_1) act as a control, helping to elucidate the ecology of this species, which seemed to have population peaks during periods of higher mean temperatures. Our data showed a higher relationship of *M. ginglymura* population with temperature. The population increases of this species in Cuba occurred between May and

June and extended until December, indicating that population is related to wet season (Hernández et al., 2007).

Regarding *T. setosus,* although there was no apparent sanitary risk for laying hens due probably to the fact that this is a dust mite, as are most the mites of Pyroglyphidae family, some information about it has been elucidated as nothing is known about this species, which has been described in this environment (Horn et al., 2017). *T. setosus* has a strong relationship with poultry farms, having been observed in all laying hen management systems throughout the year and in high populations (second most abundant species in feathers and traps).

Tuccioglyphus setosus was collected in feathers and traps. Population peaks were not concomitant in the laying hen systems, but oscillated between September/2013 and February/2014 (A_1, A_2, A_3 and S_2), and an additional peak in A_2 was observed between June and August/2014. A multivariate analyses showed a direct relationship with relative air humidity and *T. setosus* abundance.

4.3. Promising biological control agents

Cheyletus malaccensis seems to be the most important predatory mite observed. This corroborates the findings by Faleiro et al. (2015) who suggested that *D. gallinae* is a suitable prey for this predator. This predator was evaluated with the prey items *Acarus siro* L. (Acaridae), *A. ovatus*, *Caloglyphus redickorzevi* Zachvatkin (Acaridae), *Caloglyphus rodriguezi* Samšiňák (Acaridae), *T. putrescentiae*, and *M. ginglymura* (Pekár and Hubert, 2008; Palyvos and Emmanouel, 2009, 2011; Cebolla et al., 2009; Al-Shammery, 2014; Granich et al., 2017). When the results obtained in the life table of *C. malaccensis* feeding on *M. ginglymura* are compared with studies that evaluate other preys, the best results so far have been obtained when feeding on *M. ginglymura* (Granich et al., 2017). The population peaks of this predator seem to coincide with population peaks of *M. ginglymura* (Figs. 3 and 4). *Cheyletus malaccensis* seems to be the most common predator when there is a decrease in populations of *M. ginglymura*. This species showed higher population
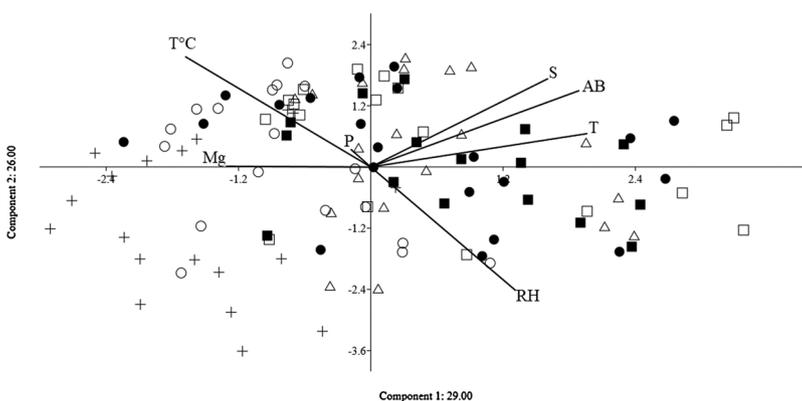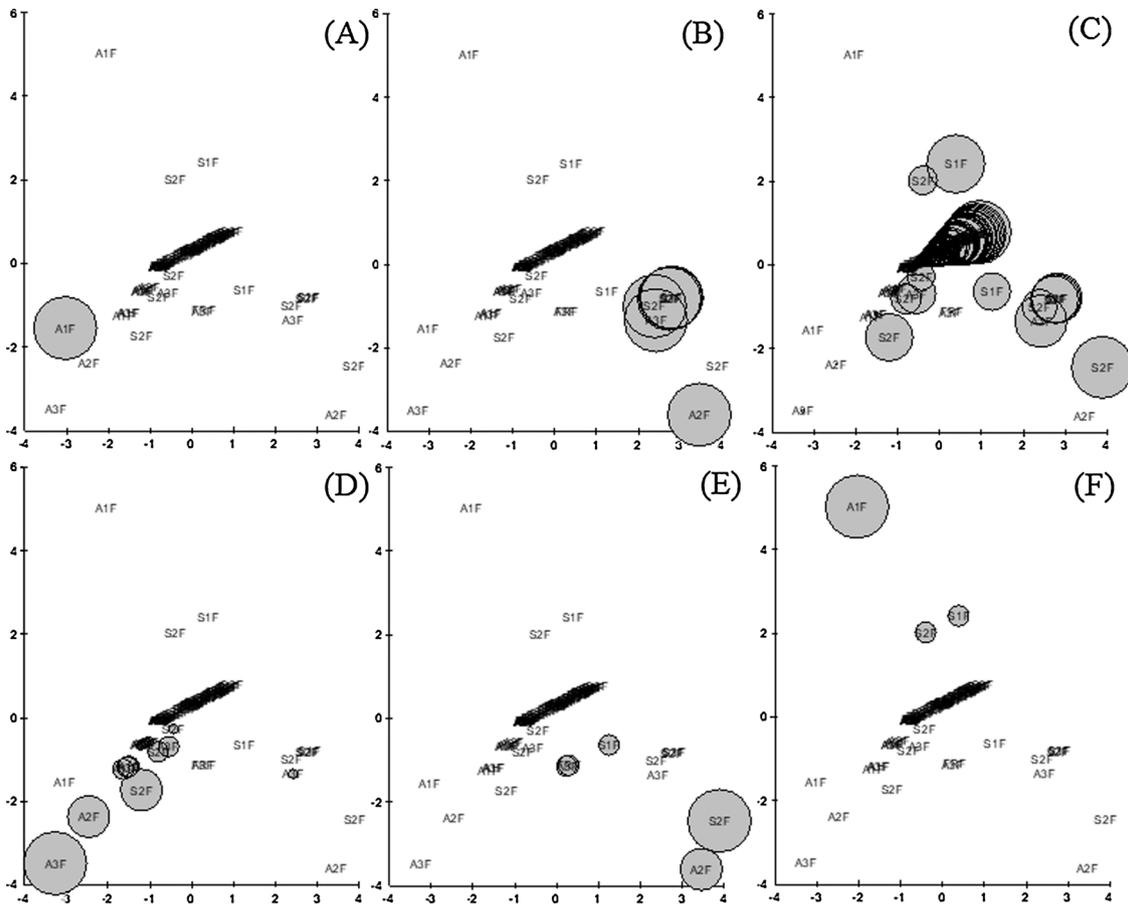
Fig. 7. PCA based on abundance of mites collected from feathers, (A) *Blattisocius dentriticus*, (B) *Blattisocius keegani*, (C) *Megninia ginglymura*, (D) *Tuccioglyphus setosus*, (E) *Cheyletus malaccensis*, (F) *Typhlodromus transvaalensis*. A_1, A_2, A_3 – automated systems; S_1, S_2 – semiautomated systems; FR– free range.
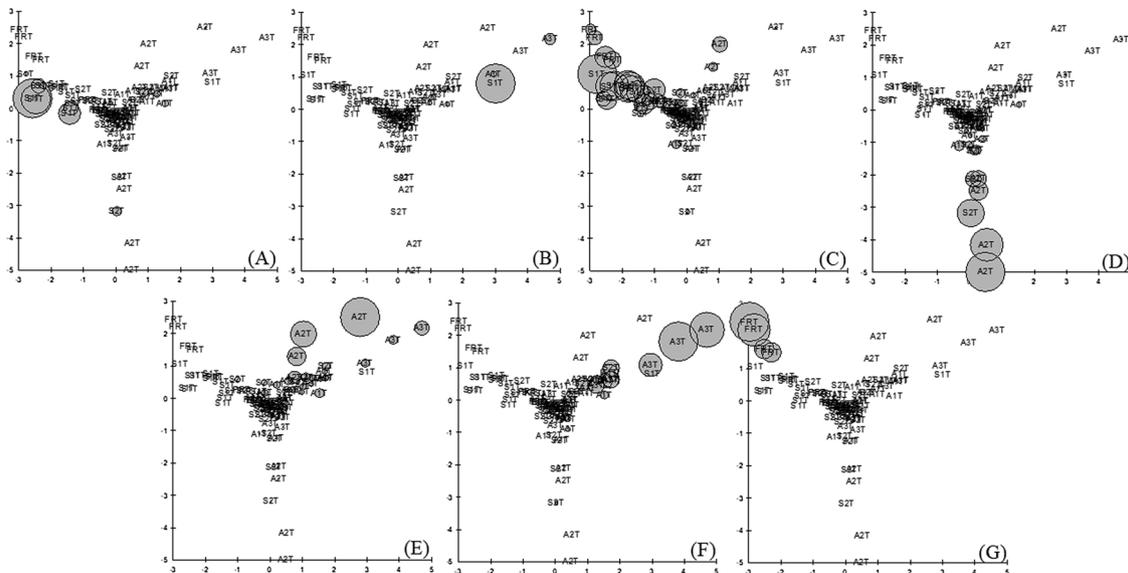

Fig. 8. PCA based on abundance of mites collected from traps, (A) *Blattisocius dentriticus*, (B) *Blattisocius keegani*, (C) *Megninia ginglymura*, (D) *Tuccioglyphus setosus*, (E) *Cheyletus malaccensis*, (F) *Typhlodromus transvaalensis*, (G) *Cheyletus eruditus*. A_1, A_2, A_3 – automated systems; S_1, S_2 – semiautomated systems; FR– free range.

when *Chortoglyphus arcuatus* Troupeau was present in large numbers, indicating a predator-prey association between them (Faleiro et al., 2015). In traps, the PCA analyses showed a relationship between this predator and *T. setosus* (Fig. 8). The abundances of these two mites were quite similar. This relationship needs to be elucidated, since little is known about *T. setosus*, as this species has been recently described

(Horn et al., 2017). *Cheyletus eruditus* was present only in *FR* in lower population. *Cheyletus eruditus* commonly occurs in stored foods, feeding on pest mites and reducing pest populations (Pulpan and Verner, 1965).

Typhlodromus transvaalensis was the second most abundant predator in the laying hen systems. Faleiro et al. (2015) found low populations of this species in laying hen farms associated to traps and nests of wild

birds. However, the real role of this predator in these environments is yet to be explained. Laboratory tests showed that it is capable of feeding on *M. ginglymura* (unpublished results - first author).

B. dentriticus and *B. keegani* were the predatory mites with the lowest abundance and populations during the sampling period. *Blattisocius keegani* is associated to beetles in stored products and shows potential for the control of the navel orangeworm *Amyelois transitella* Walker (Lepidoptera: Pyralidae) (Thomas et al., 2011) and *B. dentriticus* feeds on arthropod eggs and *T. putrescentiae* (Schrank) (Fenilli and Flechtmann, 1990). Three species of this genus were associated to laying hen farms: *B. dentriticus*, *B. keegani*, and *Blattisocius tarsalis* (Berlese); *B. dentriticus* was the most common in traps, coinciding with the populations of *D. gallinae* and *M. ginglymura* (Faleiro et al., 2015). *Blattisocius dentriticus* feeding on *M. ginglymura* had lower values than when the prey was *T. putrescentiae,* since the population of this predator increased about 7.53 times every 14.3 days (Silva et al., 2016). *Blattisocius dentriticus* had no relationship with *M. ginglymura* populations in the systems, only with populations of *T. setosus* in traps in S_1. *Blattisocius keegani* and *M. ginglymura* present in traps had a relationship in S_2 and this predator was not influenced by *T. setosus*.

The results obtained in the present study are corroborated by the data obtained by Faleiro et al. (2015), who found that *C. malaccensis* could be considered a natural enemy with potential for future biological control of ectoparasites associated with laying hens. In the laboratory, this predator proved to be effective in the biological control of *M. ginglymura*, resulting in a high fertility rate with more than 310 eggs/female (Granich et al., 2017). For a cleaner control without the use of synthetic chemical pesticides, inoculative releases would be a better choice to control poultry pest mites, involving the release of low numbers of this natural enemy several times prior to infestation periods (Faleiro et al., 2015). In addition, this management provides higher permanence, and the natural proliferation of predatory mites becomes a cleaner strategy, thus avoiding the use of synthetic chemical pesticides that are harmful to human and animal health, and considering that mites of sanitary importance have already shown resistance to these products.

5. Conclusions

Hypothesis 1 suggested that environmental conditions affect *M. ginglymura* and *T. setosus* populations. The multivariate analyses showed a direct relationship with temperature and *M. ginglymura*. *T. setosus* was related to relative air humidity.

Cheyletus malaccensis, *T. transvaalensis*, *B. keegani*, and *B. dentriticus* were the most common and abundant predatory mites, with population variation depending on the husbandry systems evaluated, and considered to have potential for the biological control of mites of sanitary importance in laying hen farms. On the other hand, even with low populations and having been found only in FR, *C. eruditus* was considered due to its recognized potential in biological control of *D. gallinae* in commercial laying hen farms (Lesna et al., 2009).

Hypothesis 2 that there is a relationship between M. ginglymura and the predators B. keegani and *T. transvaalensis*, was supported by the data shown in the multivariate analyses. In addition, *T. setosus* had relationship with the predator *C. malaccensis*.

Acknowledgements

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.06.002.

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