







# FAUNISTIC ANALYSIS AND SEASONAL FLUCTUATION OF LADYBEETLES IN AN AGRO-ECOLOGICAL SYSTEM INSTALLED FOR ORGANIC VEGETABLE PRODUCTION

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## Abstract

Plans for an agro-ecological system for agricultural production must consider vegetal diversification in agricultural properties because, among other advantages, it can help the biological control of pests when it focuses on such an end. Predator ladybeetles (Coleoptera: Coccinellidae) can be found in different environments; they play an important role in biological control. The aims of the present study were to feature ladybeetle populations through faunistic analysis and determine their fluctuations in an agro-ecological system comprising seven sub-systems subjected to different cultivation systems in Seropédica County, RJ. The experiment was conducted from December/2018 to December/2019 at Módulo de Cultivo Orgânico Intensivo de Hortaliças (MCOIH), which is located at Sistema Integrado de Produção Agroecológica (SIPA). In total, 1,231 adult ladybeetles were captured, distributed into 13 species, 3 genera and 2 tribes of Coccinellidae, which resulted in  $S$  (taxon richness) = 19, Shannon-Wiener diversity index ( $H'$ ) = 0.65 (at 0 to 1 scale) and Margalef diversity index ( $\alpha$ ) = 2.53 (values lower than 2.0 represent low diversity sites). Equitability was low ( $E = 0.22$ ), since one of the ladybeetle species has prevailed: *Cycloneda sanguinea* (most frequent, dominating and constant) in MCOIH, as well as in each of the sub-systems. However, the simplest sub-systems installed for vegetable production (monoculture gardens) were not favorable for ladybeetle diversity, whereas sub-systems installed for polyculture of leafy vegetables recorded the greatest taxa diversity of ladybeetles, including species that predate in aphids that attack vegetables [*Coleomegilla maculata*, *Coleomegilla quadrifasciata*, *Cycloneda sanguinea*, *Eriopis connexa*, *Harmonia axyridis*, *Hippodamia convergens* and *Hyperaspis (Hyperaspis) festiva*]. The sub-system comprising gliricidia was used to produce fertilization biomass and favored the predominance of *C. sanguinea* in comparison to the other ladybeetle species in MCOIH. Spring was the season mostly favoring the occurrence of *C. sanguinea* and *H. convergens* adults; which were dominant species in ladybeetle assemblage in MCOIH; however, *C. sanguinea* was constant and *H. convergens* was accessory.

**Keywords:** Agroecological principles. Coccinellidae. Faunistic indexes. Population fluctuation. Vegetable diversity.

## 1. Introduction

Crop management forms in agro-ecosystems, i.e., the adoption of monocultures or polycultures, influence the abundance and richness of natural enemies to agricultural pests. Diversified agricultural systems tend to increase these ecological indexes, mainly when agricultural landscape diversity is taken into account (Root 1973; Vandermeer and Perfecto 1995; Altieri and Nicholls 2018). Such favorable environment is observed when there is greater availability of microhabitats used as refuge, reproduction and feeding sites by natural enemies. These favorable environments provide appropriate microclimate and alternative food sources for entomophagous insects (parasitoids and predator insects), some of which are natural enemies to agricultural pests (Andow 1991; Altieri et al. 2003; Aguiar-Menezes 2017; Altieri and Nicholls 2018).

Coccinellids (Coleoptera: Coccinellidae), commonly known as 'ladybeetles', are among entomophagous insects favored by diversified agro-ecosystems within, and around, agricultural properties (Resende et al. 2007, 2009; Lixa 2008, 2013; Snyder et al. 2009; Shanker et al. 2018). Ladybeetles are distributed in different terrestrial environments; moreover, they are important biological agents to control phytophagous mites (Arachnida: Acari), insects belonging to sub-order Sternorrhyncha (Insecta: Hemiptera) such as aphids, mealybugs and whiteflies. These insects can also feed on eggs and neonate Lepidoptera and Coleoptera larvae found in crops, but some ladybeetle species have mycophagous habit (Hagen 1962; Giorgi et al. 2009; Obrycki et al. 2009; Hodek and Evans 2012).

However, although communities of pest insects in agro-ecosystems can partially be balanced by increased populations of their natural enemies due to vegetal diversity, the effects shall not be generalized (Alhadidi et al. 2018; Altieri and Nicholls 2018). Ecological studies featuring populations of natural enemies in these systems can help better understanding such effects and, consequently, help planning management practices focused on vegetal diversity, whose premise follows agro-ecological principles, to control pests (Gliessman 2001; Altieri et al. 2003; Aguiar-Menezes 2017).

Insect populations can be featured through faunistic analysis by applying species richness, frequency, abundance, dominance and diversity indexes, as well as their variations between seasons. According to Rodrigues et al. (2008), understanding these faunistic indexes helps selecting more adapted and natural enemies capable of being used in biological control programs applied to the system; it also helps better understanding the local communities and their ecological structure. Nevertheless, such featuring is more often adopted as the basis for integrated pest management in agricultural and forest environments to control pest insects (Uramoto et al. 2005; Bernardi et al. 2011; Souza et al. 2020).

Based on the aforementioned, the following hypothesis was tested: an agro-ecological production system presenting diversified assembly of ladybeetle species shows population fluctuations that change between seasons due to changes in vegetation cover in space and time. Accordingly, the aims of the present study were to feature ladybeetle populations through faunistic analysis and to determine their fluctuations in an agro-ecological system comprising seven different sub-systems managed for intensive vegetable and/or biomass production for mulching and vegetal compound fermented for fertilization purposes in Seropédica County, RJ.

## 2. Material and Methods

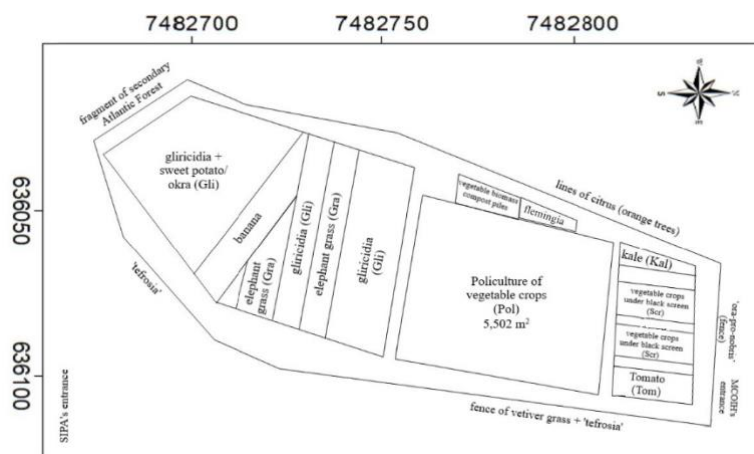
### Study site

The experiment was carried out from December 21st, 2018 to December 20th, 2019 at Módulo de Cultivo Orgânico Intensivo de Hortaliças (MCOIH) and covered the 2018/2019 crop season. MCOIH is located in Sistema Integrado de Produção Agroecológica (SIPA), also known as "Fazendinha Agroecológica Km 47", Seropédica County-RJ (22°45'S, 43°41'W; altitude, 33 meters above sea level). SIPA is a research unit on integrated vegetal and animal organic production presenting agro-ecological bases distributed within 70 ha. The intense and diversified cultivation of vegetables prevails in the unit, which is appropriate for vegetables, grass for green fertilization and soil coverage complexes. These cultures are applied through succession and/or simultaneous intercropping in different land plots that range from ½ to 1 ha. In total, 30 ha of preserved Atlantic Forest fragment was added to the site, as well as 14 ha of pasture (Neves et al. 2005). According to Köppen's classification, climate in the site is Aw, i.e., rainy season in summer and dry season in

winter. Annual rainfall reaches 1,213 mm, and the rainy season lasts from November to April; mean annual temperature is 24.5°C (Carvalho et al. 2006).

### MCOIH featuring

The module corresponds to 1-ha land plot installed at SIPA's entrance since 2010 (Figura 1); which is divided into seven sub-systems presenting different cultures managed to achieve intense vegetable and/or biomass production for mulching and fermented vegetal compounds for fertilization. It does not use animal-origin fertilization and was planned to assure labor and income for 4-people families (Silva et al. 2018).

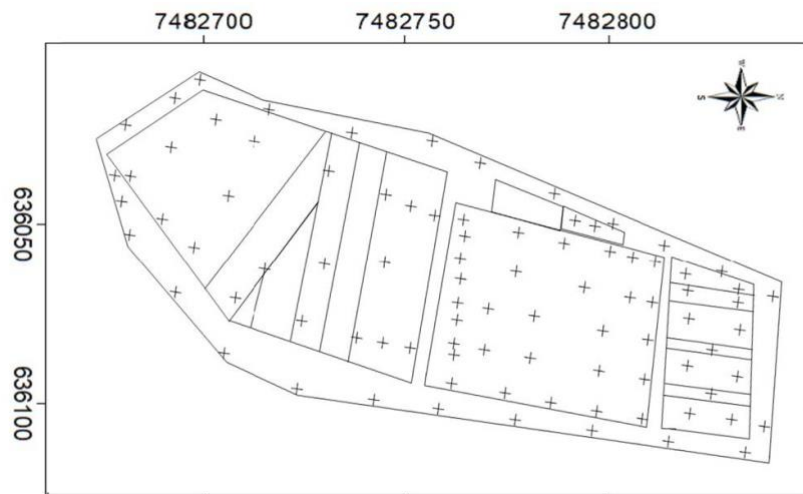


**Figure 1.** Plot of Módulo de Cultivo Orgânico Intensivo de Hortaliças (MCOIH) located in Sistema Integrado de Produção Agroecológica (SIPA), Seropédica County – RJ; and the borders of the different sub-systems.

Cultivation plan for the 2018/2019 cycle was divided into two cycles: spring/summer and fall/winter, which, in their turn, were divided into seven sub-systems. Sub-system 1 (Tom) was selected for tomato crops (*Solanum lycopersicum* L., cv. Perinha) grown from March to October at open field; it was followed by green manure production (gray mucuna - *Mucuna pruriens* L.) from November to March. Sub-system 2 (Scr) was selected for leafy vegetable crops under black screens with 50% shading from March to December; it was followed by *Crotalaria juncea* L. (crotalaria) intercropped with *Pennisetum americanum* (L.) K. Schum. (millet) - which would be used as green manure - from December to March. Sub-system 3 (Kal) was selected for kale (*Brassica oleracea* L. var. *acephala* DC.) crop grown from April to December at open field; it was followed by green manure production [jack bean, *Canavalia ensiformis* (L.) DC] from December to April. Sub-system 4 (Pol) was selected for the polyculture of leafy vegetables, tubers, cucurbits and beans from March to October at open field, which were subsequently replaced by maize crops (*Zea mays* L., cv. BRS Caatingueiro) intercropped with green manure (gray mucuna) from October to March. Sub-system 5 (Gli) was represented by gliricidia [*Gliricidia sepium* (Jacq.)] lines, which were subjected to three prunings (in April, July and December) and were intercropped with sweet potato (*Ipomoea batatas* L., local varieties), from March to September, and with okra (*Hibiscus esculentus* L.) from October to February. Sub-system 6 (Gra) was only represented by elephant grass (*Pennisetum purpureum* Schumach., cv. Cameroon) cultivated for six years (since model installation); it was subjected to three prunings a year (April, August and November). Sub-system 7 (Bor) was installed at the border of MCOIH - it referred to surrounding sites/fence; it was cultivated with some cropping lines with orange trees (*Citrus sinensis* L.), a fence of vetiver grass [*Chrysopogon zizanioides* (L.) Roberty] grown intercropped with 'tefrosia' (*Tefrosia cinapou* L.), and another fence of 'ora-pro-nobris' (*Pereskia aculeata* Mill.). This sub-system is adjacent to a fragment of secondary Atlantic Forest.

### Adult ladybeetle collection

MCOIH was georeferenced with the aid of GPS (Trimble® ProXT); in total, 88 georeferenced points were marked, which were distributed into sub-systems that represented the collection points of adult ladybeetles (Figure 2). Points have covered all the existing border lines and partitions. Point marking was random and aimed at distributing plants occurring in the site.



**Figure 2.** Distribution of georeferenced points (+) for ladybeetle collection in each sub-system in Módulo de Cultivo Orgânico Intensivo de Hortaliças (n = 88).

One trap supported by a rebar, tight with wire, was installed 10 cm from culture canopy in each georeferenced point; every time the culture reached height taller than 1.0m in the sub-system, the trap was installed 1.0m from soil surface. Traps consisted of yellow sticky cards (COLORTRAP®, Isca Tecnologias Ltda, Ijuí, RJ) – cut in size 10 x 13 cm – based on the methodological efficiency shown by Stephens & Losey (2004). They were replaced every 14 days; insects belonging to family Coccinellidae (captured from the cards every two days) were collected to avoid their escape from the traps. The collected adults were stored in 2.0-ml centrifuge microtube (in separate) and taken to the Integrated Pest Management Center of Federal Rural University of Rio de Janeiro (CIMP/UFRRJ) for screening, counting and identification of ladybeetles at tribe, genus and species level – whenever possible –, based on the collection of CIMP reference ladybeetles captured at SIPA and on support from online resource sources (Bugguide 2020).

### Faunistic analysis of ladybeetle assemblage

Adult ladybeetle populations in MCOIH were featured by calculating frequency, dominance and constancy faunistic indexes recorded for the identified taxa. The frequency of each ladybeetle taxon was expressed in percentage; it was calculated based on the number of individuals in each taxon, which was divided by the total number of collected adults – it corresponded to all collected ladybeetle taxa – and multiplied by 100. Taxon was featured as dominant when they presented frequency higher than 1/S; if the opposite happened, the taxon was not dominant. The value recorded for S referred to taxa richness (identified tribe, species and genus), i.e., the number of captured taxa throughout the whole study period (Southwood 1995; Uramoto et al. 2005). Three classes were taken into account for constancy index: constant (found in more than 50% of the collection process), accessory (found in 25% to 50% of the collection process) or accidental (found in less than 25% of the collection process) (Uramoto et al. 2005). Species presenting the largest number of captured adults in comparison to the total number were referred to as predominant species, which have also recorded the highest frequencies; therefore, these species were classified as dominant and constant.

Diversity of ladybeetle taxa in MCOIH and in its sub-systems was quantified through Shannon-Wiener ( $H'$ ) and Margalef ( $\alpha$ ) diversity indexes. Species diversity in a system can be quantified through several ways; the simplest ones ignore species uniformity, such as the  $\alpha$  index, whereas the most useful ones are those that take into consideration species uniformity, such as the  $H'$  index (Gliessman 2001).

The  $H'$  index was calculated through equation  $H' = -\sum p_i \times \log p_i$ ; wherein,  $p_i$  is the ratio between the number of adults in the taxon  $i$  ( $n_i$ ) in comparison to the total number of adults in it – which corresponds to all ladybeetle taxa collected in the system –; logarithm at basis-10 was used. This index measures the degree of uncertainty by randomly predicting the species a given collected individual will belong to, based on a random sample comprising a population of  $S$  species and  $N$  individuals (Margurran 1988; Gliessman 2001).

The  $\alpha$  index was calculated according to equation  $\alpha = S - 1 / \ln N$ ; wherein  $S$  is taxa richness in the sampled site and  $N$  is the number of total adults, by taking into account all taxa of ladybeetles collected in

the site (Margalef 1972; Southwood 1995). This index represents species niche-use pattern, whose high values (higher than 5.0) represent high biological richness, whereas values lower than 2.0 correspond to low diversity sites (overall, as results of anthropogenic effects) (Margalef 1972).

Equitability (E) of ladybeetle assemblage in MCOIH and in its sub-systems was determined by using the ratio between H' index and maximum index (Hmax), which results from the assumption that all species present the same abundance. In this case,  $H_{max} = \ln S$ ; therefore, equitability was calculated through equation  $E = H' / \ln S$ ; wherein, S is taxa richness of collected ladybeetles. This index represents the uniformity in the number of individuals between species based on values ranging from 0 to 1 – the highest value is recorded when all species present the same relative frequency (Pinto-Coelho 2000).

### Analysis of ladybeetle seasonal fluctuation

Fluctuations in adult populations were determined for the taxa of the most abundant constant and accessory class (higher Fr); they were represented in graphs plotted in Excel® (Microsoft Office 356 Home) for each sub-system. The number of adults captured in the traps at each collection day (n = 26) was based on each season of the year (summer, fall, winter and spring).

## 3. Results and Discussion

### Faunistic analysis

In total, 1,231 adult ladybeetles were captured in MCOIH, 1,064 adults belonged to 13 species, 141 of them were distributed into three genera and 44 adults were divided into two tribes (Table 1). Most species were aphidophagous, but the two tribes encompassed species that feed on mealybugs (coccidophagous); *P. confluens* was the only mycophagous species (Resende et al. 2006; Lixa 2008; Giorgi et al. 2009; Hodek and Evans 2012; Escalona et al. 2017).

*Cycloneda sanguinea* was the most frequent ladybeetle species; it was followed by *H. festiva* and together they represented 63.52% of the total number of collected adults. Both species were considered dominant and constant. The opposite was observed in experiment conducted in other SIPA land plots subjected to the same methodology (yellow sticky cards) (Resende et al. 2007, 2009; Lixa 2008). In monoculture of kale and in the intercropping of kale with green manures [*Crotalaria spectabilis* L. and *Mucuna deeringiana* (Bort) Merr.], Resende et al. (2007) showed that *Hyperaspis (H.) festiva* was more frequent (higher than 70% in monoculture, and 80% in the intercropping, in comparison to all collected ladybeetle species); and *C. sanguinea* was the second most frequent species. The experiment base on kale intercropped with coriander (*Coriandrum sativum* L.) showed that *H. festiva* represented more than 50% of the total number of collected adult ladybeetles in kale monoculture; however, it was the second most frequent species (31.6%) in the intercropping system - it was preceded by *Scymnus (Pullus) sp.3* (52.5%) (Resende et al. 2009). *Cycloneda sanguinea* was the sixth species in this experiment, in descending order of frequency. However, Lixa (2008) observed that, overall, *C. sanguinea* was the most frequent species (42.2% of collected species); it was followed by *H. convergens* (28.9%) in the experiment with aromatic plants conducted in 2007 in the assessed site, which nowadays corresponds to MCOIH.

Of the 19 collected ladybeetle taxa, 73.68% of the collected species in MCOIH were dominant and 26.32% were non-dominant (Table 1). According to Odum (1983), the pattern of few dominant species recording the largest number of individuals is characteristic of the structure of communities in the tropics, which have defined seasons.

Different from results in the present study, Lixa (2008) observed that *C. maculata* and a non-identified species of Chilocorini were non-dominant species in the three assessed aromatic plants (coriander, dill and fennel). The other ladybeetle species in this class were *E. connexa* (in the two first aromatic species), *C. quadrifasciata* (in coriander) and *H. axyridis* and *Olla v-nigrum* (Mulsant, 1866) (Coleoptera: Coccinellinae) (in fennel). *Olla v-nigrum* was not collected in the current study. Resende et al. (2009) observed that *Brachiacantha* sp. and *Z. bimaculosus* had different behaviors depending on the culture management type; they were non-dominant in the kale monoculture and dominant in the kale/coriander intercropping, whereas both species were non-dominant in the present study.

**Table 1.** List of ladybeetle taxa (Coleoptera: Coccinellidae) distributed in Módulo de Cultivo Orgânico Intensivo de Hortaliças; number of captured adults (N); their relative frequency (Fr), dominance class (D=dominant, N=non-dominant) and constancy. Seropédica County, RJ, from December/2018 to December/2019.

Taxa	N	Fr (%) <sup>a</sup>	Dominance <sup>a</sup>	Constancy
<i>Cycloneda sanguinea</i> (Linnaeus, 1763)	520	42.24	D	constant
<i>Hyperaspis</i> ( <i>Hyperaspis</i> ) <i>festiva</i> (Mulsant, 1850)	262	21.28	D	constant
<i>Scymnus</i> ( <i>Pullus</i> ) sp.1	98	7.96	D	constant
<i>Psyllobora confluens</i> (Fabricius, 1801)	79	6.42	D	constant
<i>Hippodamia convergens</i> Guérin-Meneville, 1842	60	4.87	D	accessory
<i>Coleomegilla maculata</i> DeGeer, 1775	42	3.41	D	constant
tribo Chilocorini	40	3.25	D	constant
<i>Eriopis connexa</i> Germar, 1824	24	1.95	D	accessory
<i>Scymnus</i> ( <i>Pullus</i> ) sp.2	22	1.79	D	accessory
<i>Hyperaspidium</i> sp.	16	1.31	D	accessory
<i>Coleomegilla quadrifasciata</i> Schönherr, 1808	15	1.22	D	accessory
<i>Exoplectra miniata</i> (Germar, 1824)	15	1.22	D	accessory
<i>Harmonia axyridis</i> (Pallas, 1773)	11	0.89	D	accessory
<i>Hyperaspis silvani</i> Chrotch, 1874	7	0.57	D	accidental
<i>Azya luteipes</i> Mulsant, 1850	5	0.41	N	accidental
<i>Brachiacantha</i> sp.	5	0.41	N	accidental
<i>Zagreus bimaculosus</i> Mulsant, 1850	4	0.32	N	accidental
tribo Hyperaspini	4	0.32	N	accidental
<i>Hyperaspis quadrina</i> Mulsant	2	0.16	N	accidental
Total	1231	100	-	-
S	19			
H'	0.64			
$\alpha$	2.53			
E	0.22			

With respect to constancy, 36.84% of collected taxa belonged to the accessory class; the other ones were classified as constant or accidental: 31.58% of each class (Table 1). According to the experiment conducted by Lixa (2008) in SIPA, *C. sanguinea* and *H. convergens* were the most frequent in coriander and fennel; both species were considered constant. However, according to the present study, *H. convergens* is the most frequent species among the accessory ones.

The highest faunistic frequency, constancy and dominance indexes recorded for *C. sanguinea* and *H. festiva* have pointed out that they are well-succeeded species in the community of coccinellids distributed in SIPA. The larvae of these ladybeetles were observed preying aphids (*Lipaphis pseudobrassicae* Davis, 1914) infesting leaves of kale in SIPA (Resende et al 2006). Therefore, these species must contribute to the natural biological control of aphid populations in this agro-ecological system.

As for the present study, taxa richness (S) was lower than the number observed by Resende et al. (2009), who captured 24 ladybeetle taxa in yellow sticky cards, within a three-month period-of-time (from August to October/2006) (16 collections, twice a week) in SIPA. Nevertheless, Resende et al. (2007) collected 13 ladybeetle species by using the herein adopted methodology within a four-month period-of-time (from July to October/2003) (three collections per week) in the kale/green manure intercropping and kale monoculture experiments in another SIPA land plot. Lixa (2008) recorded taxon richness similar to seven throughout six-month collection through adult removal, from September/2007 to January/2008 (seven years before MCOIH installation). Six of these species and one unidentified species belonged to tribe Chilocorini were captured during the present study, except for *O. v-nigrum*. A study with tangerines cv. Poncã conducted in SIPA, from August 2003 to January 2005, by Rodrigues et al. (2008), collected individuals belonging to six Coccinellidae species (by removal): one phytophagous species [*Mada* (= *Ladoria*) *desarmata* (Mulsant, 1850)] and five predating species, of which *Tenuisvalvae* (= *Hyperaspis*) *notata* (Mulsant, 1850), *Pentilia egena*

Mulsant, 1850 (coccidophagous species) and *Stethorus* sp. (acaridophagous species) were not collected during the present study.

Thus, based on previous studies and on the herein recorded results, it is possible observing the presence of a complex of Coccinellidae species in SIPA, which totaled 29 taxa. As for the current research, the number of taxa found in SIPA corresponded to 65.5% of the total after 1-year survey. On the other hand, Resende et al. (2009) have shown greater taxa richness ( $S=21$ ) after three months of kale/coriander intercropping, mainly when part of the planted coriander was allowed to bloom. Results in the current study suggest that the vegetal diversity observed in MCOIH did not favor diversity increase in this group of predators. According to Southwood and Way (1970), 'functional' diversity is the most important one, rather than diversity *per se*, and it evidences the need of systematic research on the effects of "quality" of plant diversification on pests and natural enemies. Thus, it is worth highlighting that coriander is much more attractive to ladybeetles, mainly at blooming - when flower resources such as pollen and nectar are complementary food in their diet (Patt et al. 1997; Medeiros 2007; Lixa et al. 2010; Resende et al. 2010; Togni et al. 2010).

With regard to taxa diversity, the ladybeetle community presented several relatively high Shannon-Wiener ( $H'$ ) index if one takes into consideration that this index is calculated based on a basis-10 logarithm that ranges from 0 to 1 (Rodrigues et al. 2008). However, the recorded  $H'$  was almost twice lower than the highest value recorded by Resende et al. (2009) in the kale/coriander intercropping system ( $H' = 1.31$ , based on the Neperian logarithm). This index is a combination of species richness index to equitability; the lowest values correspond to lower diversity and it means lower uncertainty of random collections of individuals belonging to a certain species (Margurran 1988; Gliessman 2001); this outcome, in its turn, highlights greater chances of a certain species to prevail in the system.

The predominance of species reduces equitability, since species diversity is associated with the combination of richness (number of species) and uniformity (distribution of the number of individuals between species) (Walker 1989; Gliessman 2001). Therefore, it is likely that the highest proportion of *C. sanguinea* and *H. festiva* individuals must have influenced the observed  $H'$  value.

The recorded Margalef index can be considered median, because, according to Margalef (1972), the index often ranges from 1.5 to 3.5, and rarely exceeds 4.5 – low values (lower than 2.0) result from the predominance of some species to the detriment of the majority; values higher than 5.0 highlight great biological richness (Begon et al. 1996). The list of recorded taxa (Table 1) shows the predominance of *C. sanguinea*, which represented more than 40% of the total number of collected adult ladybeetles, and of *H. festiva*, which corresponded to approximately 20% of it. This outcome points out that there are few ladybeetle species presenting populations encompassing several individuals.

Because this index represents the niche-use pattern adopted by different species (Margalef 1972), the predominance of these two ladybeetle species may have resulted from improvements in their ability to explore niches available in SIPA. Lixa (2008) has observed that the lowest Margalef index was recorded for assemblage of ladybeetles collected on coriander ( $\alpha = 1.13$ ), dill ( $\alpha = 0.73$ ) and fennel ( $\alpha = 1.29$ ) – together, *C. sanguinea* and *H. convergens* corresponded to 71.1%, 83.5% and 78.5% of the total number of adult individuals collected in these plants, respectively, so they were considered dominant. The predominance of species in classical experiments conducted in SIPA was also observed by Resende et al. (2007), who also recorded intermediate values for the kale/coriander intercropping ( $\alpha = 2.85$ ) and for the kale-single crop ( $\alpha = 2.67$ ). These authors observed that two ladybeetle species [*Symnus (Pullus) sp.3* and *H. festiva*] accounted for more than 80% of the captured adults, all together.

According to Odum (1975), the mean maximum uniformity under high diversity conditions reached 80%. Thus, the recorded equitability values could be considered low and pointed towards the fact that a ladybeetle species will always present itself as dominant in the system.

By assessing the MCOIH system (Table 2), it was possible seeing that the composition of species in ladybeetle assemblage changed among sub-systems, likely due to differences in vegetal-cover composition and to management intensity in each one. However, all species, genera and tribes of ladybeetles collected in the MCOIH were already reported to occur at the SIPA (Resende et al. 2006; Rodrigues et al. 2008; Lixa et al. 2010; Resende et al. 2010).

Only three of the ladybeetle species collected in MCOIH occurred in a single sub-system: *A. luteipes* in subsystem 7 (on the border of MCOIH), and *H. quadrina* and *H. silvani* in sub-system 5 (gliricidia + sweet potato and okra). According to Ricklefs (2003), none of the organisms can compose all habitat types and consume all preys available; however, biodiverse environments require individuals to make choices that allow the optimized use of the habitat.

**Table 2.** Occurrence of adult individuals belonging to the ladybeetle species (Coleoptera: Coccinellidae) captured in each sub-system in Módulo de Cultivo Orgânico Intensivo de Hortaliças (MCOIH). Seropédica County, RJ. From December/2018 to December/2019.

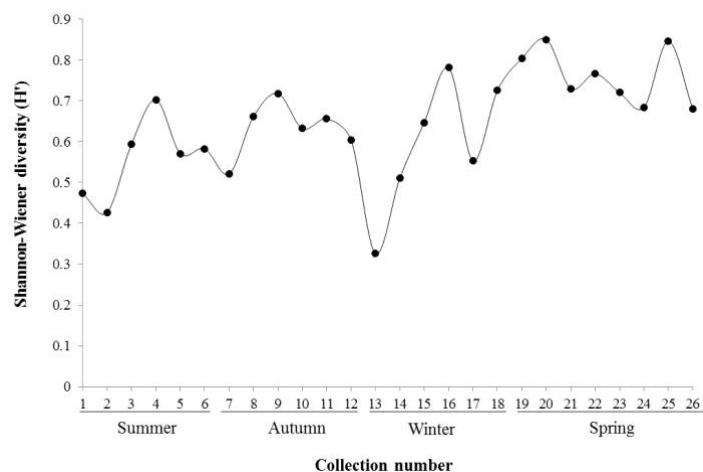
Species	Number of individuals per sub-system (s) <sup>a</sup>						
	s1 (Tom)	s2 (Scr)	s3 (Kal)	s4 (Pol)	s5 (Gli)	s6 (Gra)	s7 (Bor)
<i>Azya luteipes</i>	0	0	0	0	0	0	5
<i>Brachiacantha</i> sp.	0	0	0	0	0	3	2
<i>Coleomegilla maculata</i>	0	12	6	22	1	1	0
<i>Coleomegilla quadrifasciata</i>	0	9	1	5	0	0	0
<i>Cycloneda sanguinea</i>	6	74	21	133	159	34	76
<i>Eriopis connexa</i>	0	5	1	13	0	1	3
<i>Exoplectra miniata</i>	0	1	0	1	1	0	12
<i>Harmonia axyridis</i>	1	3	1	4	0	1	1
<i>Hippodamia convergens</i>	0	15	12	26	3	4	4
<i>Hyperaspis (Hyperaspis) festiva</i>	7	63	18	75	50	0	53
<i>Hyperaspis quadrina</i>	0	0	0	0	2	0	0
<i>Hyperaspis silvani</i>	0	0	0	0	6	0	0
<i>Psyllobora confluens</i>	8	7	6	3	24	4	37
<i>Zagreus bimaculosus</i>	0	0	0	3	1	0	0
<i>Hyperaspidium</i> sp.	0	0	0	4	6	6	0
<i>Scymnus (Pullus) sp1</i>	0	0	0	6	25	5	63
<i>Scymnus (Pullus) sp2</i>	0	0	0	2	3	0	17
tribo Chilocorini	0	1	0	0	0	1	30
tribo Hyperaspini	1	0	0	0	0	2	9
Total	23	190	66	297	281	62	312
S	5	10	8	13	12	11	13
H'	0.59	0.69	0.72	0.72	0.61	0.63	0.89
$\alpha$	1.28	1.72	1.67	2.11	1.95	2.42	2.09
E	0.37	0.30	0.35	0.28	0.25	0.25	0.35

<sup>a</sup>s1 (Tom) = tomato/mucuna, s2 (Scr) = leafy vegetables/green manures under black screens, s3 (Kal) = kale/green manures, s4 (Pol) = polyculture of leafy vegetables, tubers, cucurbits and beans/corn + mucuna, s5 (Gli) = gliricidias + sweet potato/okra; s6 (Gra) = elephant grass; s7 (Bor) = MCOIH borders, i.e., surrounding sites / fence (citrus / vetiver + 'tefrosia' / 'ora-pro-nobis').

The occurrence of *A. luteipes* on the borders of MCOIH, mainly in sites close to the orange trees, was expected to happen, since this is a predator species of mealybug that infests citrus, as observed by Rodrigues et al. (2008). Besides *A. luteipes* in orange orchard (var. Valência), Silva et al. (2005) collected *C. sanguinea* and *H. festiva*, which were also captured in sub-system 7 – both studies collected a relatively larger number of *C. sanguinea* than of the other two species. Spolidoro et al. (2004) assessed the diversity of predators in tangerine cv. Poncã orchards, in SIPA, and found lower diversity of natural enemies, among them coccinellids such as *A. luteipes* and *C. sanguinea*, in August ( $H' = 0.01$ ), as well as greater diversity of them in September ( $H' = 0.84$ ). These data partially corroborate results recorded for MCOIH, because the lowest diversity of species was observed in July/13<sup>th</sup> collection ( $H' = 0.33$ ) and the greatest diversity was recorded in September/20<sup>th</sup> collection ( $H' = 0.85$ ) (Figure 3).



Experiments conducted by Rodrigues et al. (2008) in SIPA, based on the organic cultivation of tangerine (*Citrus reticulata* Blanco) interspersed with papaya (*Carica papaya* L.) and gliricidia, showed richness ( $S = 6$ ) lower than that found in the current study, when it was compared to sub-system 7. However, species such as *A. luteipes*, *C. sanguinea* and *Scymnus* sp. were observed in both studies. High diversity means balance; however, when there is lower diversity, it is possible noticing prevalence of the system by few species.



**Figure 3.** Diversity index (Shannon-Wiener) recorded for ladybeetle taxa (Coleoptera: Coccinellidae) captured in Módulo de Cultivo Orgânico Intensivo de Hortaliças in each collection. Seropédica County, RJ, from December/2018 to December/2019.

Sub-system 1 (Tom = tomato/mucuna) presented the lowest taxa diversity among the other sub-systems, and it may have resulted from the fact that only one vegetal species has composed the site under culture rotation system. Togni et al. (2010) observed that monoculture of tomato (variety Pollyana) presented lower abundance and richness of natural enemy species (among them *H. convergens* and *E. connexa*) than the intercropping of tomato with coriander (variety Verdão) in an experiment conducted in an organic production site in Brasília, DF. Harterreiten-Souza et al. (2012) observed greater richness of ladybeetle species ( $S = 9$ ) in tomato crops than that observed in the current research; however, they collected specimens in tomato intercropped with coriander and other cultures. These authors also observed that ladybeetle species prevailed in this system (*H. convergens*). Subsystem 1 (Tom) was the only one where no *H. convergens* representatives were captured, as well as *E. connexa*, that was also not captured in sub-system 5 (Gli).

Seven aphidophagous species (*C. maculata*, *C. quadrfasciata*, *C. sanguinea*, *E. connexa*, *H. axyridis*, *H. convergens* and *H. festiva*) were common to sub-systems 2 (Scr = vegetables/green manure under black screens), 3 (Kal = kale/green manure at open field) and 4 (Pol = polyculture of vegetables/maize+mucuna at open field). These species are predators of aphids attacking vegetable crops (Hoffmann and Fordsham 1993; Resende et al. 2006). The occurrence of three to four species of predator ladybeetles is common in at least one habitat, they feed on the same prey species. Nevertheless, two ladybeetle species belonging to the same genus can feed on different prey species, even when they are in the same habitat (Hagen 1962; Hodek 1973).

Furthermore, *C. sanguinea* was found at relatively larger numbers than other species collected in these three sub-systems. This ladybeetle, along with *P. confluens*, were the only species found in all sub-systems in MCOIH (Table 2) – both species were considered dominant and constant (Table 1). *P. confluens* is a mycophagous species (Giorgi et al. 2009) likely feeding on phytopathogenic fungi that caused powdery in okra trees in MCOIH. Santos-Cividanes and Cividanes (2009) were the first ones to record the occurrence of *P. confluens* in okra infested by powdery mildew (caused by *Erysiphe cichoracearum* De Candolle) in Western São Paulo State.

However, among all sub-systems, *C. sanguinea* was mostly collected in sub-system 5 (Gli), which was followed by sub-system 4 (Pol) – one of its border is adjacent to sub-system 5 – (Figure 1). The greatest relative abundance of *C. sanguinea* in sub-system 5 possibly resulted from the presence of prey (aphids) in

gliricidias, whose green sprouts are often infected by *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae), as observed by Resende et al. (2006).

MCOIH, even each of its assessed sub-systems, are biodiverse agro-ecosystems that enable the diversity of ladybeetles that explore different habitat types. Accordingly, it is important considering that spontaneous plants are also part of agro-ecological systems for organic production, where plants are managed based on the completion limits of cultivated plants (Pereira et al. 2008). Oliveira and Rando (2017) assessed natural enemies in host plants at times close to maize cultivation and observed greater *Cenchrus echinatus* L. diversity ( $\alpha = 1.86$ ), which was mainly represented by ladybeetles.

Median values recorded for different sub-systems in MCOIH corroborated the results found by Oliveira and Rando (2017), since only sub-system 1 (Tom) presented low value ( $\alpha = 1.28$ ) (Table 2). *Cenchrus echinatus* was one of the spontaneous plants found in this sub-system by Silva (2018), who quantified 35 spontaneous species belonging to 17 botanical families in the seed bank of the MCOIH soil.

Aguiar-Menezes (2004) highlight the importance of selective weeding to conserve ladybeetles in SIPA, they show the visitation of adult *C. sanguinea* to plants of *Sonchus oleraceus* L., where these specimens find alternative prey. According to Blaauw and Isaacs (2015), spontaneous plants in sites adjacent to *Vaccinium myrtillus* L. (blueberry) production had positive effect on the density and diversity of ladybeetles. Some spontaneous-vegetation plant species have been used to maintain native coccinellids in agricultural cultivation sites (Amaral et al. 2013; Shanker et al. 2018).

### Seasonal population fluctuation

The population of adult *C. sanguinea* (constant species) individuals has fluctuated throughout the whole experiment (total of 26 collections) in all MCOIH – it was not possible capturing individuals during collections 7 and 8 (Figures 3A and B). However, *H. convergens* (accessory species) was only captured from the 17<sup>th</sup> collection on, and this period corresponds to late winter and early spring (Figure 3D); but, when each sub-system was assessed in separate (Figures 3A to E), it was not possible capturing representatives of both species in certain times of the year. With respect to the pattern of the *C. sanguinea* population, it was possible observing that it was captured at collection 3, only in sub-system 5 (Gli = gliricidia + sweet potato/okra), in sub-system 7 (borders of MCOIH) and at collection 5 in sub-system 5 (Gli) – both in summer.

The fluctuation of *C. sanguinea* population showed that sub-system 1 was the one presenting the lowest population fluctuation. Species occurrence only started at the end of the tomato cycle and at the beginning of mucuna planting in the site (early spring) (Figure 3A). As for sub-system 6 (Gra = elephant grass pasture), population peak of *C. sanguinea* was observed in the collection prior to springtime (Figure 3F).

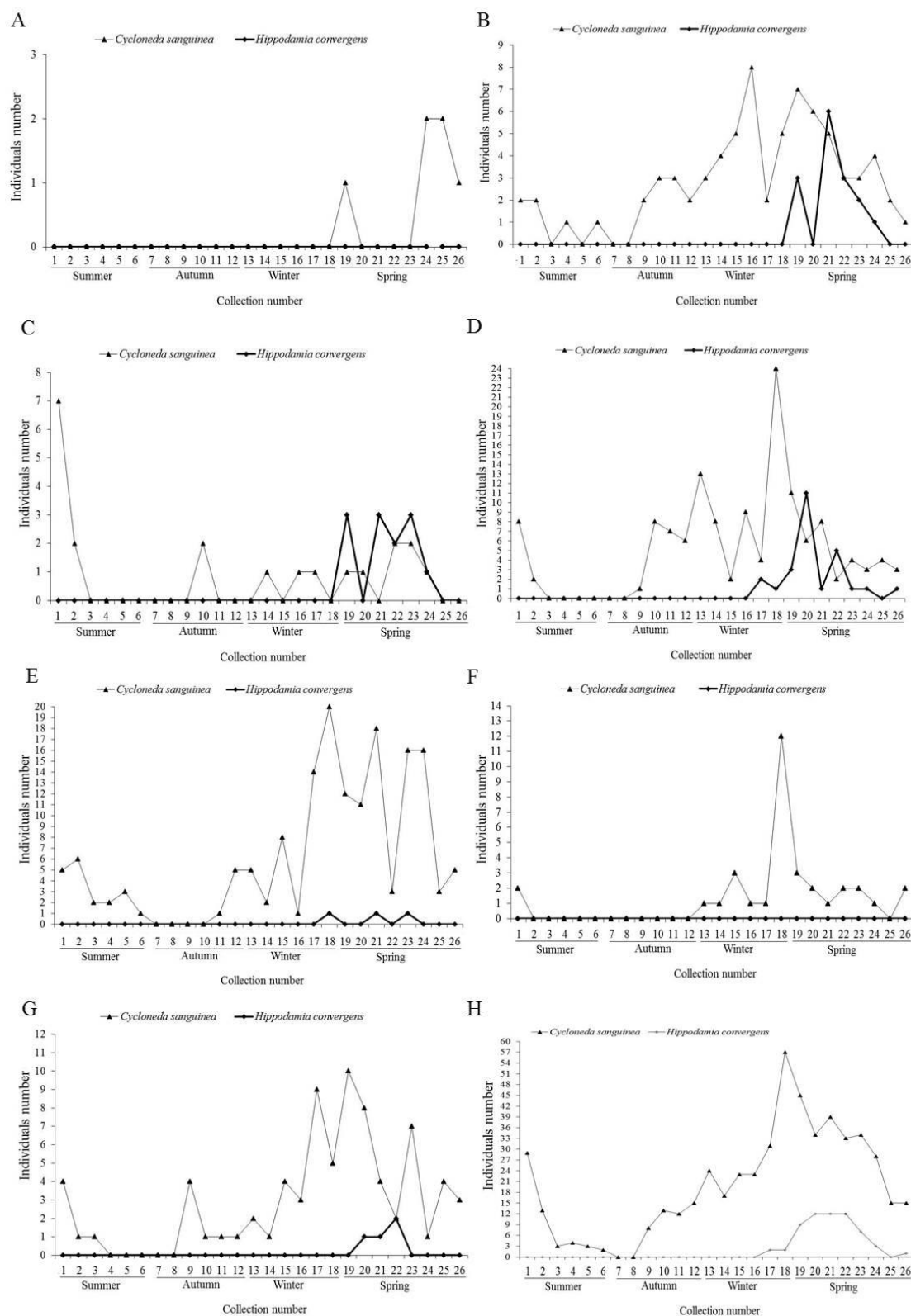
*Hippodamia convergens* presented population peak in two collections after the population peak of *C. sanguinea* in sub-systems 2 (Scr = leafy vegetables/green manure under black screens) and 4 (Pol = polyculture of leafy vegetables/maize+mucuna, at open field). The number of collected individuals was higher than that of species recording the highest frequency in MCOIH (Figures 3B and D, respectively). Although Gott et al. (2010) observed that *C. juncea* is the host of coccinellids, among them *C. sanguinea*, and that this culture favors the production of these predators, sub-system 2 (Scr) recorded the smallest number of individuals collected in the presence of crotalaria (Figure 3B).

There was *C. sanguinea* (in summer) in presence of jack beans at the beginning of the blooming stage in sub-system 3 (Kal = kale/green manure at open field). Nevertheless, there was *C. sanguinea* from kale crop introduction on, as well as *H. convergens* population fluctuation higher than, or equal to, that recorded for *C. sanguinea* – individuals were not captured in the 20<sup>th</sup> collection (Figure 3C). Kale plants presented caterpillar eggs and *L. pseudobrassicae* aphids from the 10<sup>th</sup> collection on, and it may have contributed to the presence of ladybeetles.

Sub-system 5 (Gli), where gliricidias were inserted, was subjected to pruning at collections 7, 15 and 24; therefore, it was possible observing lower fluctuation of individuals belonging to *C. sanguinea* in subsequent collections (Collections 16 and 25). With respect to *H. convergens*, only few individuals were captured in scattered collections.

Silva et al. (2005) assessed seasonality of coccinellids species found in citrus orchards in Montenegro County – RS, at different times of the year and found that *C. sanguinea* was the most frequent species in the site; it occurred in all seasons of the year. Results recorded for sub-system 7 (Bor) partially corroborated

results recorded by Silva et al. (2005), according to whom, the presence of *C. sanguinea* in citrus orchards was not observed in the last two summer collections and in the first fall collection (Figure 3G). Overall, *C. sanguinea* and *H. convergens* presented the largest number of individuals collected in spring (Figure 3H); the population of *H. convergens* presented the smallest number of collected individuals.



**Figure 4.** Population fluctuation of adult *Cycloneda sanguinea* and *Hippodamia convergens* individuals in Módulo de Cultivo Orgânico Intensivo de Hortaliças. Seropédica County, RJ, from December/ 2018 to December/2019. A) Sub-system 1 (Tom): tomato/mucuna; B) Sub-system 2 (Scr): leafy vegetables/green manure under black screens; C) Sub-system 3 (Kal): leaf cabbage/green manure; D) Sub-system 4 (Pol): polyculture of leafy vegetables and beans/maize+mucuna; E) Sub-system 5 (Gli): gliricidias + sweet

potato/okra; F) Sub-system 6 (Gra): elephant grass pasture; G) Sub-system 7 (Bor): surrounding sites/fence (citrus/vetiver + 'tefrosia'/'ora-pro-nobis'); H) General: total in MCOIH.

The infestation of aphid *A. craccivora* in sub-system 5 was observed in sprouts of gliricidia (*G. sepium*) and it was associated with ladybeetles, mainly with *C. sanguinea* (Figure 5). Bennett (1985) and Stary and Cermeli (1989) reported association between *A. craccivora* and gliricidia in Trinidad and in Venezuela, respectively. *Aphis craccivora* is considered one of the occasional gliricidia pests under semiarid condition in Northeastern Brazil, which can damage its sprouts (Drumond and Carvalho Filho 2005). Resende et al. (2006) observed adult *Diomus* sp. and *Scymnus (Pullus)* sp. (Coleoptera: Coccinellidae) feeding on *A. craccivora* infesting the green sprouts of gliricidia cultivated in other land plots in SIPA.



**Figure 5.** Adults of *Cycloneda sanguinea* in association with *Aphis craccivora* in the sprouts of *Gliricidia sepium* in Módulo de Cultivo Orgânico Intensivo de Hortaliças. Seropédica County, RJ. (Source: Elen de Lima Aguiar-Menezes).

Therefore, it was observed that sub-system 5 (Gli) played an essential role in MCOIH, because it hosted prey for ladybeetles and favored the conservation of these natural enemies in the production system. Sub-system 7 shall also be taken into account, because it can work as refuge, and entrance and exit point, for adult ladybeetles in the site, although this role needs further investigation. Experiments conducted by Lixa (2013) about the recovery of pollen ingested by ladybeetles captured in MCOIH showed that these individuals fed on the pollen of *Averrhoa carambola* L. (start fruit) located approximately 300 meters from the site - this distance is within the dispersion limit set for adult coccinellids (Hodek 1967; Evans 2003). It is important highlighting that sub-system 7 is the one located closer to the secondary forest site by MCOIH.

Strategies based on the conservation of coccinellids must be developed in the MCOIH according to conservative biological control requirements of integrated pest management. It must be done in order to keep the population density of these insects in this agro-ecological system, so that they can fulfil their ecological function in the production area.

#### 4. Conclusions

One complex of ladybeetles (13 species, 3 genera and 2 tribes of Coccinellidae) is found in Módulo de Cultivo Orgânico Intensivo de Hortaliças (MCOIH), but cultivation plan for the 2018/2019 cycle favors greater individual proportion of *C. sanguinea* and *H. festiva*, which are dominant and constant species.

Areas covered with monoculture of vegetable crops at open field in MCOIH (tomato or kale crops, both followed by green manures) do not favor ladybeetle diversity; actually, the polycultures of vegetable crops (under black screens or at open field) lead to the opposite result and host the same ladybeetle species that fed on aphid species attacking vegetables (*C. maculata*, *C. quadrifasciata*, *C. sanguinea*, *E. connexa*, *H. axyridis*, *H. convergens* and *H. festiva*).

The cultivation of gliricidia, mainly when there are infestation of aphid *A. craccivora*, favors *C. sanguinea* prevalence in comparison to other ladybeetle species in MCOIH.

Spring favors the greater occurrence of *C. sanguinea* and *H. convergens* adults in MCOIH, and the population peak of *C. sanguinea* occurs at late winter.

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