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AGROECOSYSTEMS

Variations in community assemblages and trophic networks of aphids and parasitoids in protected crops

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Abstract. Greenhouse crops are thought to be simplified ecosystems because they often consist of monocultures that are relatively isolated from their environment. However, insect pests are still able to colonize these protected crops, which threaten their yields. Similarly, natural enemies of pests may be able to colonize greenhouses, providing a form of natural biological pest control. Protected strawberry crops are grown in several types of greenhouses that vary in their degree of openness. Crops often suffer from aphid outbreaks, which can be partly controlled by insect parasitoids immigrating from the surrounding environment. We investigated variations over space and time in both the aphid and parasitoid community diversity and species assemblages associated with protected strawberry crops. We sampled aphids and parasitoids in five regions of France in the spring and summer of two successive years. Despite the relative isolation of these protected crops, we identified a high aphid species richness in them, even at the greenhouse scale. Aphid community composition varied with spatial and temporal factors, but the species assemblages present were mostly determined by local factors. Parasitoid communities were mostly similar among the studied regions, but varied between seasons, with this temporal variation being related to changes in aphid species composition. The study of trophic interactions occurring between aphids and parasitoids allowed the most prevalent and efficient parasitoid species to be identified. The structures of food webs strongly varied in time and space, compromising any prediction of “natural” biological control. We also highlighted ecological factors that can disrupt aphid biological control, such as the occurrence of hyperparasitism or the possibility of apparent mutualism between aphid species. Finally, we showed that the degree of openness of greenhouses influenced both the aphid communities and the hyperparasitism rates in them. These results provide valuable information to improve aphid biological control in protected crops.

Key words: biological control; direct and indirect interactions; insect pests; natural enemies; spatiotemporal variation; strawberry crops.

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INTRODUCTION

In agriculture, crops can be cultivated in greenhouses to optimize growing conditions and

provide plant protection. Protected crops are often regarded as simplified ecosystems with very low biodiversity (Enkegaard and Brødsgaard 2006, Messelink et al. 2012). For instance,

greenhouse crops are often monocultures and held in closed systems, which prevents entry of crop pests from the local environment, but also restricts colonization by these pests' primary natural enemies (Perdikis et al. 2011). However, these systems are not completely pest-proof, as numerous harmful species still threaten greenhouse crops (Messelink et al. 2012). Phytophagous insect pests can colonize greenhouses directly, via openings in greenhouses, or indirectly, via contaminated plant material (Buitenhuis et al. 2016) or accidental transportation by workers between infested and uninfested crops. Once an insect pest colonizes a greenhouse, the crop system offers excellent conditions for its development, leading to rapid population growth (van Lenteren 2000). On the other hand, it has also been shown that, in some cases, natural enemies of pests can enter greenhouses, and may then control the crop pest populations (Castañé et al. 2004, Gabarra et al. 2004, Bosco et al. 2008). While it has been suggested that greenhouses are simplified ecosystems, they are still subject to unpredictable species colonization events that may generate complex ecological systems with high variation in species richness, community assemblages, and trophic network. Such ecological variations limit the ability of producers to predict both pest pressure on their crops and pest regulation by natural enemies.

Several studies have focused on the influence of environmental, ecological, and agricultural factors on both the diversity and relative abundances of pests and their natural enemies in greenhouses (Heinz 1998, Castañé et al. 2004, Gabarra et al. 2004, Bosco et al. 2008, Sanchez et al. 2011, Dong et al. 2019). However, few studies have considered the possibility of there being large-scale spatiotemporal variations in these systems' ecological metrics, such as species richness, community composition, and connectivity within their food webs. Investigating such variations would help to (1) determine whether greenhouse crops are simplified or complex and predictable or unpredictable systems; (2) understand the spatiotemporal dynamics of pests and their natural enemies in greenhouses; and (3) identify the most prevalent beneficial natural enemies of pests through food web analyses. Combining all of this information would be valuable for the development

and improvement of biological pest control programs.

Our study aimed to investigate the sources of variations in the diversity of both pest and beneficial species, the compositions of their respective communities, and the food webs involving these species in protected crops. For this purpose, we considered a large French network of greenhouses used for strawberry production. Cultivation of strawberries, *Fragaria × ananassa* Duchesne (Rosales: Rosaceae), in soilless substrate under protected structures is widespread globally (Rondon et al. 2005). In France, production is based on several coexisting geographic regions, which all include different climatic conditions and production systems. Strawberries are mostly grown under polytunnels or greenhouses, which vary in their degree of openness. Several arthropods can cause damage to strawberry crops (e.g., thrips, spider mites, *Drosophila suzukii*, and others), including aphids, which cause substantial losses to yield and quality by feeding on phloem sap, excreting honeydew, and transmitting plant pathogenic viruses (Dedryver et al. 2010). There is a particularly high diversity of aphids that are potential pest species of strawberry crops, which makes their control more complex (Cingolani and Greco 2018). To control aphid populations, strawberry producers have experimented with the limited use of insecticides and inundative releases of natural enemies of aphids for several years, but with insufficient results, especially concerning parasitoid releases (Turquet et al. 2019). Consequently, there is a need to study the ecology of species interacting in greenhouses before implementing biological control programs. By studying both aphid and parasitoid communities and food webs including these antagonists, our study's aim was to identify species of interest for the efficient biological control of aphids.

Herein, the relative frequencies of occurrence of aphid and parasitoid species in five French production regions during two successive years were measured in a large number of greenhouses. For each sampling year, the relative frequencies of different species in greenhouses were determined in both spring and summer. From the results of these insect collections, we analyzed the following patterns: (1) the diversity of aphid and parasitoid communities, which was

done to estimate whether and how greenhouse systems are simplified; (2) the degree of variability among and within years and regions in species richness, community assemblages, and food webs; (3) the beneficial species that could be candidates for use in aphid biological control programs; (4) the effect of the degree of openness of greenhouses on the studied ecological variables; and (5) the factors limiting the biological control of aphid pests. Given the nature of the crop systems investigated, we expected to find low diversity and simple insect communities at a local

scale, but with high interregional variability due to variation in production contexts, and also expected to find values of ecological variables indicating more complex systems (i.e., diversity and food webs) in more open greenhouses.

MATERIALS AND METHODS

Protected strawberry crops

For insect sampling, we considered five major regions of strawberry production in France, hereinafter denoted the West, Center, East, Southeast,

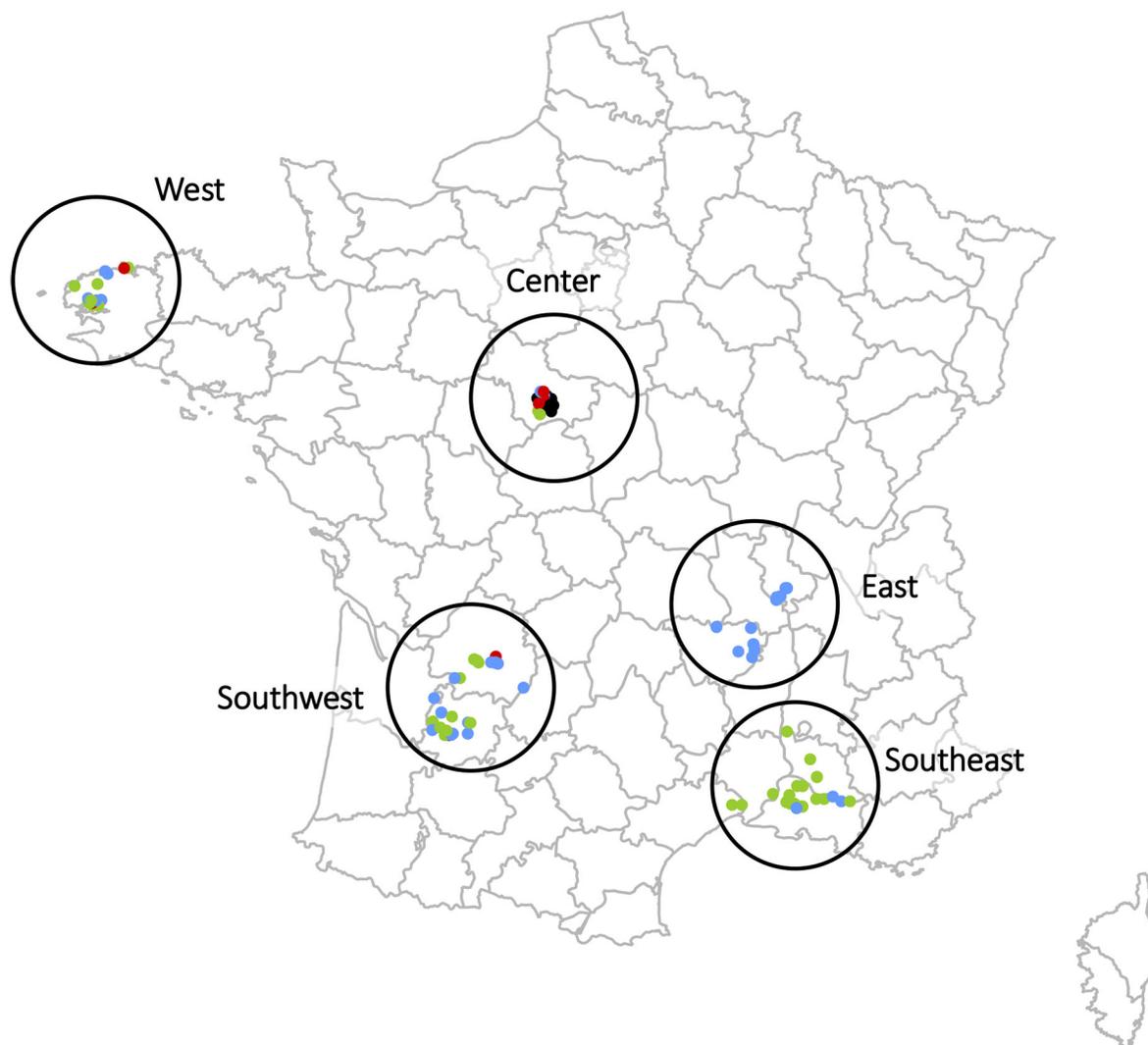


Fig. 1. Locations of sampling sites in France. The color of each dot represents the number of times the sampling site was sampled (from 1 to 4) as follows. Green, 1; blue, 2; red, 3; black, 4.

and Southwest regions (Fig. 1). All five regions present contrasting production conditions in terms of climate, landscape context, and greenhouse types. Two types of greenhouses are used by growers to produce strawberries: closed greenhouses, which consist of plastic greenhouses, glass greenhouses, or high tunnels with insect-proof nets, and open greenhouses, which are high tunnels without insect-proof devices. Strawberries are grown in rows, mainly in soil-less substrate, but also in soil on plastic-covered hills (87% and 13% of crops, respectively, in our study). Plants are produced in nurseries and planted every year after a fallow period of several weeks in greenhouses. Early season cultivars are planted in late autumn to early winter and produce fruit during the spring, while everbearing cultivars are planted in late winter and produce fruit from early summer to early autumn. Pollination relies mainly on bumblebee hives in closed greenhouses and can be enhanced by other pollinators in open greenhouses. Management of aphids sometimes relies on inundative biological control using predators and parasitoids, and chemical insecticides are usually also used at the beginning of the growing season and when aphid population densities become high.

In each sampled greenhouse, we recorded the aphid management practices used, and we noted cases in which parasitoid releases for aphid biological control occurred.

Insect sampling

Aphids colonizing protected strawberry crops and their associated parasitoids were sampled in greenhouses in two consecutive years (2017 and 2018) in the five different regions described above. For these two consecutive years, data were collected during the spring (from April to May) and the summer (from August to September). Given the differential phenology of different strawberry crops, season and region were not fully crossed factors herein. In the Southeast region, sampling occurred only in spring, while we sampled insects in the East region during summer only. To account for intraregional variations in response variables, 6–13 sites were sampled within each region and in each season of a given year (see Table 1 for the sampling effort within regions and Fig. 1 for the geographical positions of sampling sites). Herein, each sampling site represented a unique greenhouse in which we collected insects. As far as was possible, we sampled the same greenhouses across

Table 1. Percentage of sites (greenhouses) in each spatiotemporal context (region, year, and season) in which different aphids and parasitoids were detected, and the total numbers of occurrences of aphid and parasitoid species used in subsequent analyses.

Year and season	Region	No. sites	Sites with detected aphids (%)	Sites with detected parasitoids (%)	No. aphid occurrences	No. parasitoid occurrences
2017						
Spring	West	11	100	45	197	28
Spring	Center	11	100	64	211	42
Spring	Southwest	11	100	82	178	80
Spring	Southeast	13	100	31	295	27
Summer	West	6	100	83	134	27
Summer	Center	12	100	33	155	8
Summer	Southwest	11	100	82	193	26
Summer	East	11	100	45	162	25
2018						
Spring	West	12	100	42	226	14
Spring	Center	13	100	77	257	56
Spring	Southwest	12	100	100	240	104
Spring	Southeast	11	100	27	220	17
Summer	West	6	100	67	78	9
Summer	Center	12	92	33	101	4
Summer	Southwest	12	92	42	150	8
Summer	East	11	91	18	68	7
Total		175	98	53	2865	482

seasons and years. To test the effect of the degree of openness of greenhouses on the values of ecological variables measured in them, we collected insects in both closed and open greenhouses.

The objective of insect sampling was to identify the diversity of aphid species colonizing strawberry greenhouses, the parasitoid species that used these pests as hosts, and the compositions of their respective communities. We selected between 25 and 30 sampling locations distributed throughout each of the monitored greenhouses. A sampling location consisted of a portion of a crop row about 2 m long (about ten strawberry plants) that was examined for the presence of both aphids and mummies (i.e., dead aphids containing a primary or secondary immature parasitoid). The aphid sampling design comprised collecting all of the aphid species present at each sampling location. Each time a different aphid species was found in a sampling location, this was recorded as an additional aphid occurrence there. We collected aphid individuals in 1.5-mL plastic tubes filled with 96% ethanol for further verification of their species identity. If possible, we preferentially sampled adult individuals to allow for the more accurate identification of species, since aphid identification is easiest for adults (Blackman et al. 2006). The parasitoid sampling design comprised collecting the mummies found at each sampling location. The collected mummies were placed individually into 1.5-mL plastic tubes sealed with cotton wool until parasitoid emergence. After emergence, the parasitoid species and the aphid species they parasitized were identified.

From these observations, we obtained data on the diversity of aphid species in a given greenhouse, the parasitoid species that used these aphid species as hosts, and their respective relative frequencies of occurrence (i.e., number of occurrences of a species divided by the total number of occurrences). Insect abundances (i.e., the actual numbers of aphids and parasitoids in a greenhouse) were not estimated in this sampling design, so parasitism rates could not be calculated.

Insect identification

To avoid species misidentification in the greenhouse, aphid and parasitoid species identities were subsequently verified after sampling under

a binocular microscope using identification keys (aphids, Remaudière and Seco Fernandez 1990, Blackman 2010; parasitoids, Tremblay and Penacchio 1985, Tobias and Jakimavicius 1986, Mescheloff and Rosen 1990, Kavallieratos et al. 2003, Starý 2006). The aphid species used by parasitoids as hosts (i.e., aphid mummies) were also identified based on their morphological traits. For mummies of species of the aphid genus *Aphis*, identification to species level was not possible due to high species similarity, so these data were analyzed at the level of the *Aphis* genus. As morphological species identification of parasitoids belonging to the genus *Lysiphlebus* was uncertain, especially for the *L. fabarum* species complex (Tomanović et al. 2018), specimens of this genus were analyzed at the level of the *Lysiphlebus* genus. Hyperparasitoids (i.e., secondary parasitoids) emerging from aphid mummies were identified to the genus level using identification keys (Powell 1982).

Data analyses

Aphid and parasitoid community analyses.—For each combination of region, year, and season, the occurrence and co-occurrence of different species of aphids or parasitoids at the individual level were visualized using the R package Mondrian (Siberchicot et al. 2016). We further studied the variations in community assemblages across regions, years, and seasons by assessing the relative frequencies of these insect species within the sampling sites (i.e., greenhouses). The dissimilarity between these insect communities (β -diversity) was quantified by calculating the Bray–Curtis dissimilarity index values between all pairwise combinations of greenhouses. The resultant Bray–Curtis dissimilarity matrix was then ordinated using non-metric multidimensional scaling (nMDS), which projected the multidimensional data of the dissimilarity matrix onto a minimal-dimensional space (Paliy and Shankar 2016). A two-dimensional ordination was used, and the position of each greenhouse on this scatter graph depended on its dissimilarity from all other greenhouses. The effects of the region, year, and season sampled on insect community dissimilarity were tested by performing a permutational multivariate analysis of variance (PERMANOVA) on the Bray–Curtis dissimilarity

matrix. Pairwise comparisons between levels of factors were performed using pairwise Adonis tests with Bonferroni corrections (Martinez Arbizu 2017). Both nMDS and PERMANOVA were implemented using the R package *vegan* (Oksanen et al. 2019), and the graphical representation of the nMDS was generated using the R package *ggplot2* (Wickham 2016).

Aphid–parasitoid food web analyses.—Quantitative graphical representations of food webs (Memmott et al. 1994) were generated based on the identified aphid mummies and the respective parasitoids emerging from them for each combination of region, year, and season to illustrate the extent of the links between aphids and parasitoids. These quantitative food webs were drawn using the R package *bipartite* (Dormann et al. 2008). Considering only food webs containing at least two parasitoids species, we then calculated six different metrics associated with the structure of the food web in a given region, season, and year. These metrics were (1) connectance, which describes the overall complexity of a food web; (2) H2, which describes the level of specialization within a food web (from 0—no specialization—to 1—perfect specialization); (3) nestedness, which describes the extent to which specialist species interact with subsets of the species with which generalist species interact (from 0—high nestedness—to 100—chaos); (4) aphid species richness; (5) parasitoid species richness; and (6) values of the Pianka index, which is an index of trophic overlap (from 0—completely different patterns of aphids exploited by parasitoid species—to 1—an identical pattern of aphid exploitation by parasitoid species). To study the variation in food web structure among regions, years, and seasons, we performed a principal components analysis (PCA) on the values of the six food web metrics described above. Based on the result of the PCA, a hierarchical clustering analysis using the Ward method was performed to classify the food webs. These analyses were performed, and their associated graphs were generated using the R packages *FactoMineR* (Lê et al. 2008) and *factoextra* (Kassambara and Mundt 2017), respectively.

Aphid and parasitoid species co-occurrence.—To analyze patterns of intraguild (i.e., aphid–aphid and parasitoid–parasitoid) and interguild (i.e., aphid–parasitoid) species co-occurrences, we

conducted a co-occurrence analysis with the R package *cooccur* (Griffith et al. 2016). We used the probabilistic model of Veech (2013) to determine whether pairs of insect species were more or less frequently encountered in the same greenhouses than would be expected by chance.

Aphid species exploitation by parasitoids.—To test whether aphid species were exploited at similar rates by parasitoids, the relative frequency of live aphids of each species and the relative frequency of parasitized aphids of each species were compared using chi-square tests for each combination of region, year, and season. With these tests, we assessed whether the exploitation rate of each aphid species was higher or lower than would be expected under the hypothesis of random exploitation of aphid species by parasitoids. If an aphid species was overexploited in proportion to its frequency of occurrence in the aphid community, this was a preferred target of parasitoids. If a species was proportionally underexploited, it was partially rejected/ignored by parasitoids. If no deviation from the null hypothesis was found, the proportional exploitation of this species was based solely on its proportional occurrence in the aphid community.

Hyperparasitoid pressure.—The effects of region, season, and year on hyperparasitism rate (i.e., the number of secondary parasitoids found divided by the total number of secondary and primary parasitoids emerging from aphid mummies) were analyzed using a generalized linear mixed model (GLMM), assuming a binomial error and using a logit-link function. As some mummies were collected in the same greenhouses, the sampling site was included in the model as a random factor to account for data non-independence. Generalized linear mixed model analysis was conducted using the R package *lme4* (Bates et al. 2015).

Greenhouse openness and diversity, assemblages, and hyperparasitism.—The effect of greenhouse openness on the species diversity (i.e., species richness and Shannon diversity index values), community assemblages (i.e., the relative frequencies of occurrence of the dominant species in the aphid and parasitoid communities), and hyperparasitism rate in a given greenhouse was analyzed using linear mixed-effect models with region, year, and season included as random effects. For species richness data, we used

GLMM analyses with a Poisson error distribution and a log-link function, and checked for overdispersion. For both the relative frequencies of dominant species and hyperparasitism data, we performed GLMM analyses with a binomial error distribution and a logit-link function. In these binomial models, since the presence/absence of a given species or the hyperparasitism statuses of individuals were noted on individuals originating from the same greenhouse, there was spatial dependency in the dataset. Hence, the sampling site (i.e., greenhouse) number was included as a random factor in these models to account for the substructuring of the data. For Shannon diversity index data, general linear mixed models (LMM) were used. In all models, the significance of the greenhouse openness factor was determined using a likelihood ratio test, and, if significant, the associated parameter estimate was analyzed to interpret the fitted models. LMM and GLMM analyses were conducted using the *lme4* package in R (Bates et al. 2015).

All analyses were conducted using R software (R Core Team 2017, version 3.4.2).

RESULTS

Species richness and insect occurrence frequencies

Table 1 lists the numbers of occurrences of both the aphid and parasitoid species collected in the 175 greenhouses sampled during two consecutive years in the five regions studied. Overall, 2865 occurrences of aphids were observed, and aphids were detected in almost all (98%) of the greenhouses monitored. The number of aphid occurrences ranged from 0 to 30 per greenhouse and from 68 to 257 per region/year/season (Table 1). Thirteen aphid species were identified, and the majority of aphids belonged to five species: *Acyrtosiphon malvae* (Mosley; 22.7%), *Rhodobium porosum* (Sanderson; 16.7%), *Macrosiphum euphorbiae* (Thomas; 22.8%), *Chaetosiphon fragaefolii* (Cockerell; 17%), and *Aphis gossypii* Glover (11.2%). The other minor aphid species found were *Aulacorthum solani* (Kaltenbach; 3.3%), *Aphis forbesi* Weed (3%), *Aphis nasturtii* Kaltenbach (1.5%), *Myzus persicae* (Sulzer; 1%), *Macrosiphum rosae* (Linnaeus; 0.5%), *Amphorophora rubi* (Kaltenbach; 0.2%), *Aphis fabae* Scopoli (0.1%), and *Myzus ornatus* Laing (0.1%). Overall,

parasitoids emerged from 61% of the collected mummies, and 482 parasitoid occurrences were recorded in the 175 monitored greenhouses. Among these greenhouses, aphid mummies were present in 93 (53%) of them, and only eight were subjected to a parasitoid release for aphid regulation (of these, at least one of the released species was found in only four greenhouses). The number of parasitoid occurrences ranged from 0 to 15 per greenhouse and from 4 to 104 per region/year/season (Table 1). All parasitoid individuals emerging from mummies were identified, among which 380 were primary parasitoids belonging to eight species or genera, specifically: seven species/genera belonging to the subfamily Aphidiinae (Braconidae) and one belonging to the family Aphelinidae. Most of the parasitoids belonged to three species: *Aphidius ervi* (Haliday) (37.4% of the primary parasitoids found), *Praon volucre* (Haliday) (33.7%), and *Aphidius eglanteriae* Haliday (16.6%). Other parasitoid individuals belonged to the taxa *Lysiphlebus* Foerster (5.5%), *Aphidius colemani* Viereck (3.2%), *Aphelinus abdominalis* Dalman (2.9%), *Aphidius matricariae* Haliday (0.3%), and *Toxares* Haliday (0.3%).

Aphid and parasitoid communities

Fig. 2 presents the frequencies of occurrence and co-occurrence of aphid species in the sampled greenhouses in different regions, years, and seasons. Several aphid species were often observed per greenhouse (from 0 to 7 species per greenhouse). Aphid community assemblages varied greatly among greenhouses, and with both time and space, as follows: the results of the PERMANOVA based on the Bray–Curtis dissimilarity matrix showed that there were significant variations in aphid community composition among sampling regions (Fig. 3a), seasons (Fig. 3b), and years (Fig. 3c). In addition, the interactions between these three factors were also significant. Overall, these factors accounted for a small part of the variance in the data ($R^2 = 0.27$; Table 2a). Pairwise comparisons between regions found few significant differences (Appendix S1: Table S1): Differences occurred mainly between the East region and the four others. In this particular region, very few aphids were observed, and communities were largely dominated by *M. euphorbiae*. The significant interaction between region and season resulted from there

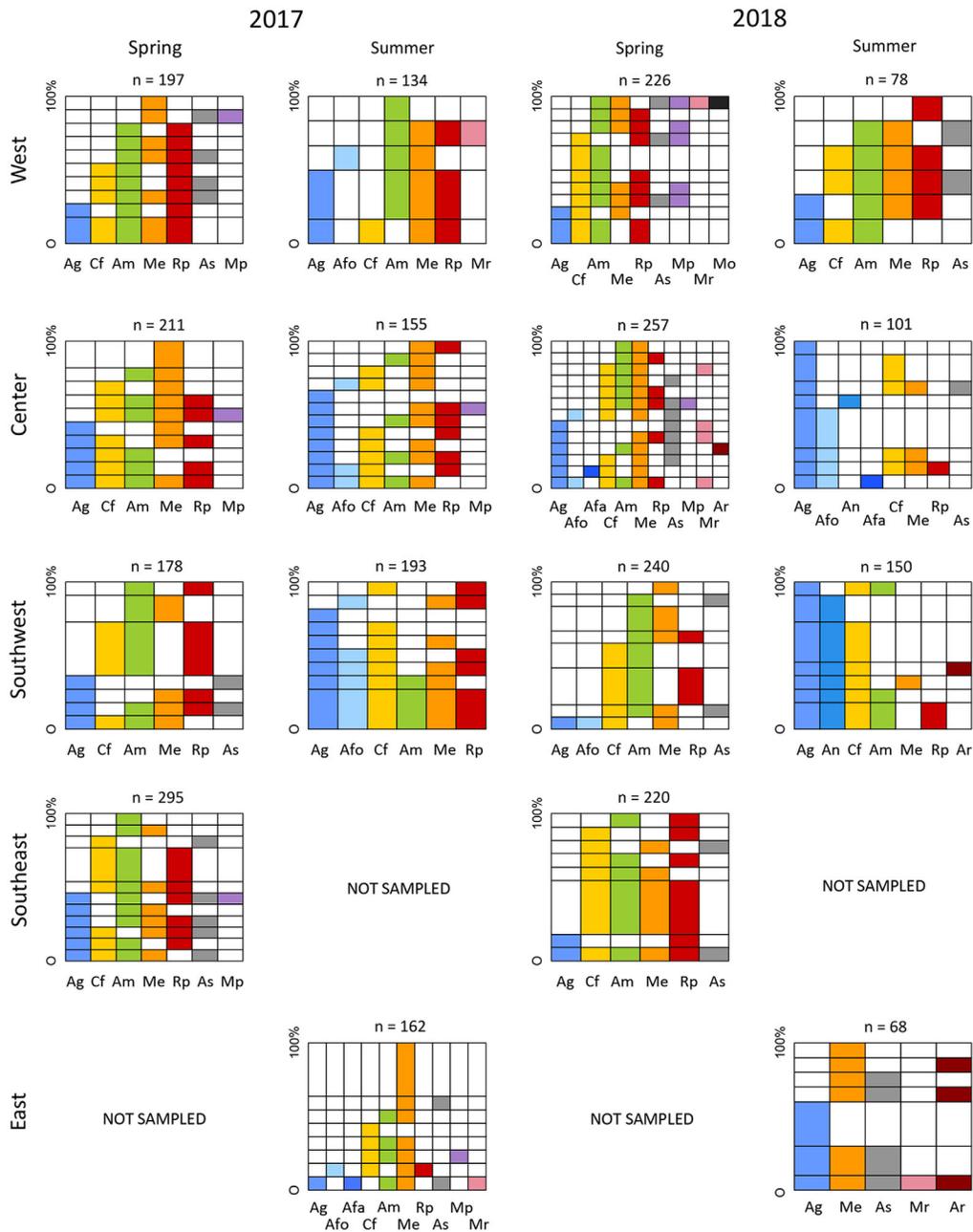


Fig. 2. Variations in aphid community assemblages among sampling regions, years, and seasons. In each graph, one column represents one aphid species, and different sampling sites are represented by different horizontal lines. For each column, the colored area corresponds to the total percentage of sites presenting the corresponding aphid species. When colored areas are present in different columns, this indicates aphid species co-occurrence. If two sampling sites presented the same community assemblage, these are represented by identical horizontal lines. Sampling sites without aphids are not represented here. Abbreviations are Ag, *Aphis gossypii*; Afo, *Aphis forbesi*; An, *Aphis nasturtii*; Afa, *Aphis fabae*; Cf, *Chaetosiphon fragaefolii*; Am, *Acyrtosiphon malvae*; Me, *Macrosiphum euphorbiae*; Rp, *Rhodobium porosum*; As, *Aulacorthum solani*; Mp, *Myzus persicae*; Mr, *Macrosiphum rosae*; Ar, *Amphorophora rubi*; Mo, *Myzus ornatus*.

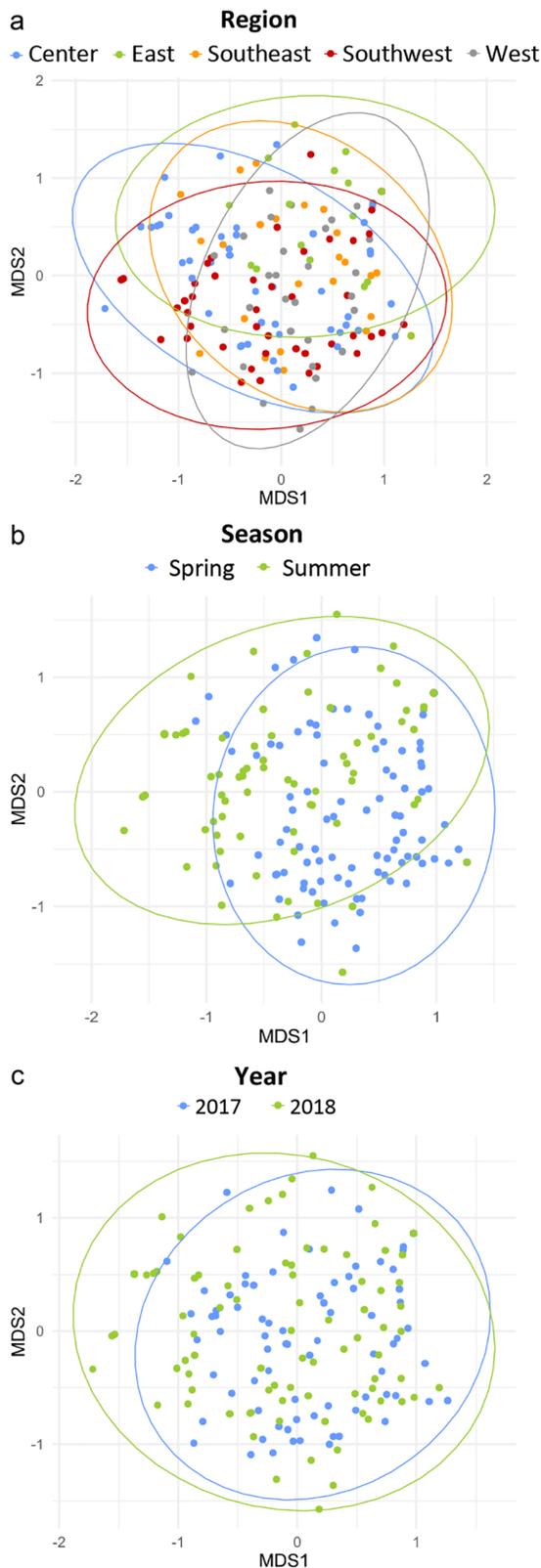


Fig. 3. Non-metric multidimensional scaling (nMDS) plot comparing aphid communities from different regions, years, and seasons. Each dot represents the aphid community identified from a single sampling site. The analysis was performed using Bray–Curtis dissimilarity index values. The stress of the two-dimensional nMDS representation was 0.19. Plots differentiate among spatiotemporal contexts as follows: (a) sampling region; (b) sampling season; and (c) sampling year. Ellipses represent the 95% confidence interval around the centroid.

being both interregional differences in aphid community assemblages during the summer only and strong seasonal variations within each region (Appendix S1: Table S1). During the spring, *A. malvae*, *M. euphorbiae*, *R. porosum*, and *C. fragaefolii* dominated the communities in four regions, while *A. gossypii* and other *Aphis* species (*A. forbesi* and *A. nasturtii*) dominated aphid communities during the summer (Fig. 2). The interaction between season and year was significant, aphid community assemblages were significantly different between the two summers, but not between the two springs. Finally, the

Table 2. Results of permutational multivariate analysis of variance based on the Bray–Curtis dissimilarity distance matrix produced between all pairwise combinations of sampling sites for (a) aphid and (b) parasitoid community assemblages.

Covariates	df	F	R ²	P
(a) Aphid				
Region	4	3.74	0.07	0.001
Season	1	15.41	0.07	0.001
Year	1	2.14	0.01	0.063
Region:Season	2	4.35	0.04	0.001
Region:Year	4	3.32	0.06	0.001
Season:Year	1	2.87	0.01	0.017
Residuals	158		0.73	
Total	171		1	
(b) Parasitoid				
Region	4	3.1	0.1	0.001
Season	1	14.2	0.12	0.001
Year	1	3.5	0.03	0.006
Region:Season	2	3.01	0.05	0.002
Region:Year	4	0.93	0.03	0.52
Season:Year	1	4.32	0.04	0.001
Residuals	78		0.64	
Total	91		1	

significant interaction between region and year resulted from the difference in aphid community assemblages between the East region in 2018 and all other combinations of regions and years. This corresponded to the modality wherein the frequencies of aphids found were particularly low (Appendix S1: Table S1).

Fig. 4 presents the frequencies of occurrence and co-occurrence of the parasitoid species emerging from the aphid mummies collected in the sampled greenhouses across different seasons, years, and regions. Several parasitoid species were often observed per greenhouse (from 0 to 3 species per greenhouse). Parasitoid communities varied significantly in their composition among sampling regions (Fig. 5a), seasons (Fig. 5b), and years (Fig. 5c). PERMANOVA results showed there were significant effects of region, season, and year, as well as their interactions, on the structures of parasitoid communities, and these factors accounted for 36% of the variance in the data (Table 2b). Pairwise comparisons between regions showed that there were no significant differences, except between the West region and the four others (Appendix S1: Table S2). The significant interaction between region and season suggested there were no intraseasonal differences between regions, but that significant differences between regions occurred between the spring and summer seasons. For the Center and the Southwest regions, parasitoid community assemblages varied between spring and summer similarly (Appendix S1: Table S2): During the spring, these communities were dominated by *A. ervi*, *P. volucre*, and *A. eglanteriae*, while during summer, the genus *Lysiphlebus* was prevalent (related to there being a concurrently higher occurrence frequency of the genus *Aphis*, its aphid host). The significant interaction between year and season showed that, in both years, parasitoid communities presented different assemblages from spring to summer: Compared to spring, summer had a very low occurrence frequency of parasitized aphids, which was particularly marked in 2018 and resulted in a very low diversity of parasitoid species being found. Also, the composition of the parasitoid community did not vary between the two springs and between the two summers (Appendix S1: Table S2). Overall, the numbers of parasitoid

individuals found were low, so these results need to be interpreted with caution.

Aphid–parasitoid food webs

The global (i.e., for all samples) aphid–parasitoid food web generated in this study is shown in Fig. 6. This web indicates that *A. ervi* and *P. volucre* were the most frequent and most generalist species observed in the sampled protected strawberry crops. They both parasitized *A. malvae*, *M. euphorbiae*, and *R. porosum*. At the regional scale, these two dominant species did not always use these three aphid species as hosts, even if they were present in greenhouses. For example, the parasitoid *A. ervi* was never found parasitizing *M. euphorbiae* during the spring of 2017 (Appendix S1: Fig. S1a). *A. ervi* was the most widespread parasitoid species, except in three regions in the summer of 2018, when parasitoid abundances in general were extremely low. *P. volucre* was observed in fewer cases, as it was absent in the West region and scarce during summer. Interestingly, the aphid *C. fragaefolii* was exclusively parasitized by *A. eglanteriae*, and aphids belonging to the genus *Aphis* were mainly parasitized by parasitoids of the genus *Lysiphlebus*, but also by *A. matricariae* and *A. colemani*. Rare aphid species were seldom parasitized, except *A. solani*, which was parasitized by *A. ervi* in one site and by *P. volucre* in one case.

Based on the PCA of the six metrics associated with the structure of the food web in a given region, year, and season (Fig. 7a), the hierarchical clustering results separated the food webs formed between aphids and primary parasitoids into three distinct groups (Fig. 7b). One cluster included food webs with high connectance and high Pianka index values. The other two groups included food webs with high values of H2, but distinct parasitoid richness. These three clusters were not related to any patterns in regional, seasonal, and annual differences.

Aphid and parasitoid species co-occurrence

Among the 210 potential insect species pair combinations, 98 pairs were removed from the co-occurrence analysis because they were expected to have less than one co-occurrence. Among the 112 pairs of species analyzed, 15% were positively associated, 12% were negatively associated, and 72% were randomly associated

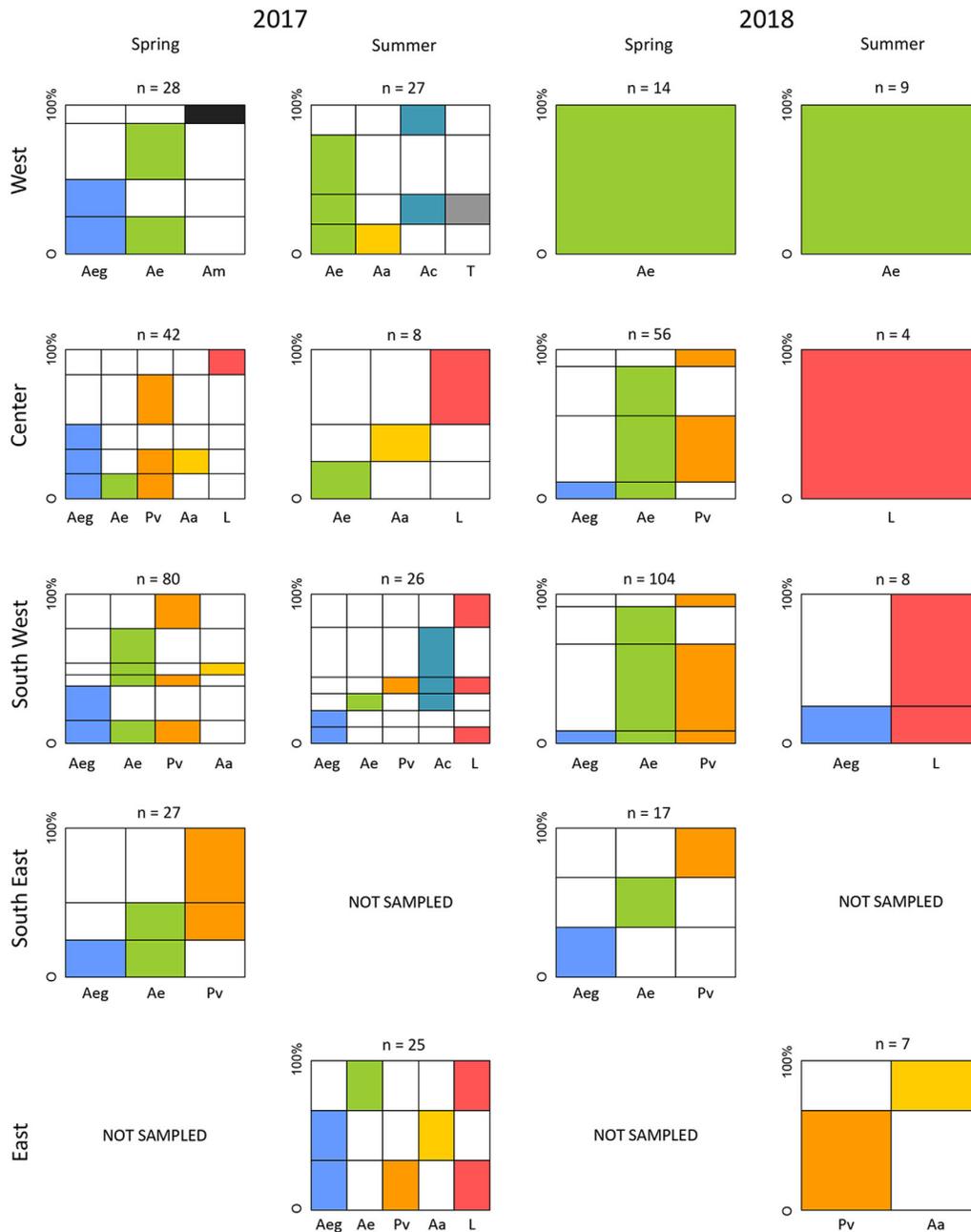


Fig. 4. Variations in parasitoid community assemblages among sampling regions, years, and seasons. In each graph, one column represents one parasitoid species, and different sampling sites are represented by different horizontal lines. For each column, the colored area corresponds to the total percentage of sites presenting the corresponding parasitoid species. When colored areas are present in different columns, this indicates parasitoid species co-occurrence. Sampling sites without parasitoids are not represented here. If two sampling sites presented the same community assemblage, these are represented by identical horizontal lines. Abbreviations are Aeg, *Aphidius eglanteriae*; Ae, *Aphidius ervi*; Pv, *Praon volucre*; Aa, *Aphelinus abdominalis*; L, *Lysiphlebus* spp.; Ac, *Aphidius colemani*; Am, *Aphidius matricariae*; T, *Toxares* spp.

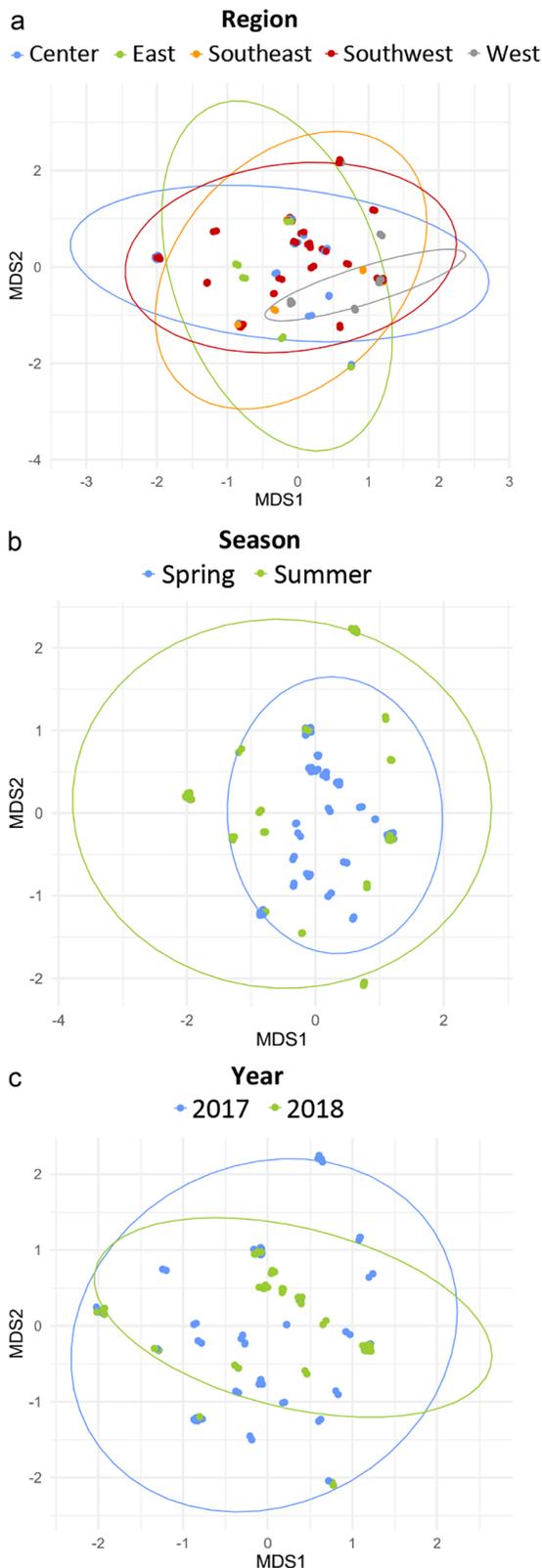


Fig. 5. Non-metric multidimensional scaling (nMDS) plot comparing parasitoid communities from different regions, years, and seasons. Each dot represents the parasitoid community identified from a single sampling site (when present). The analysis was performed using Bray–Curtis dissimilarity index values. The stress of the two-dimensional nMDS representation was 0.07. Plots differentiate among spatiotemporal contexts as follows (a) sampling region; (b) sampling season; and (c) sampling year. Ellipses represent the 95% confidence interval around the centroid.

(Fig. 8). Among aphid species, *A. malvae*, *R. porosum*, and *C. fragaefolii* were all positively associated, while *A. gossypii* was positively associated with *A. forbesi* and *A. nasturtii*. The three aforementioned *Aphis* species were either randomly or negatively associated with the other aphid species. Among parasitoid species, *A. ervi* was positively associated with *P. volucre*, while it was negatively associated with *Lysiphlebus* spp. Finally, among aphid–parasitoid co-occurrences, *A. eglanteriae* was positively associated with *C. fragaefolii*; *A. ervi* was positively associated with *M. rosae*, *R. porosum*, and *A. malvae*; and *P. volucre* was positively associated with *A. malvae*. The genus *Lysiphlebus* was positively associated with *A. gossypii*, *A. forbesi*, and *A. nasturtii*, and negatively associated with *A. malvae*.

Exploitation rates of aphid species by parasitoids

The aphid mummies found were identified to belong to the most frequent aphid species in the sampled greenhouses. However, the rate of exploitation of a given aphid species by parasitoids was not related to its relative frequency of occurrence ($P < 0.001$). Considering all of the data, *Aphis* spp. and *A. malvae* were underexploited by parasitoids, while *M. euphorbiae* and *R. porosum* were overexploited. The under- or overexploitation of these aphid species by parasitoids was also observed at the local scale (i.e., per each region–year–season combination; Appendix S1: Fig. S2).

Hyperparasitoid pressure

Overall, the hyperparasitism rate was about 22%, meaning that around 1/5 of the parasitoids

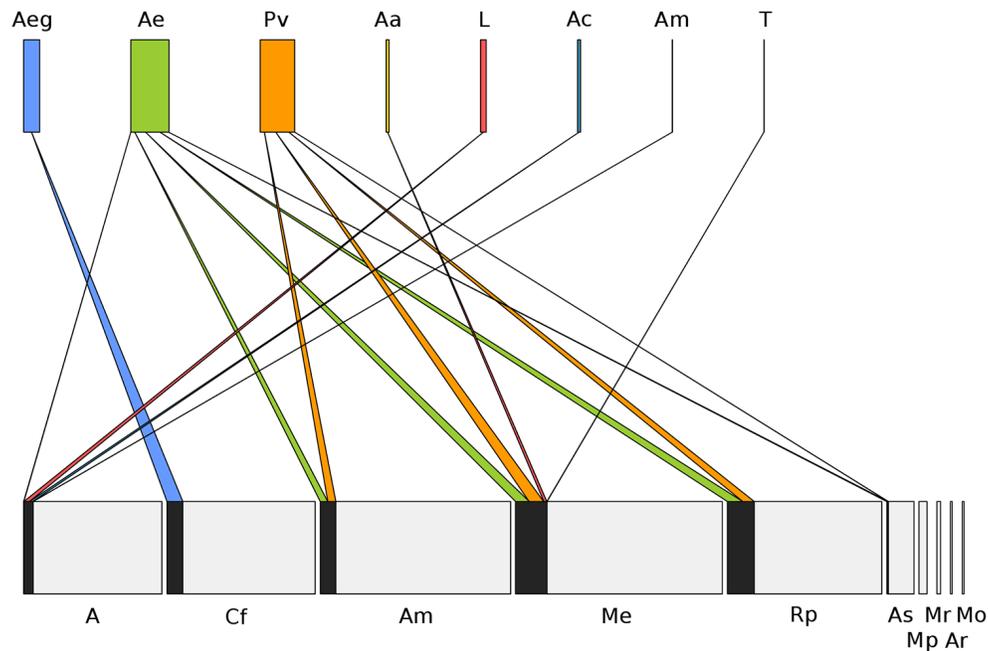


Fig. 6. Global aphid–parasitoid quantitative food web built with the overall field data. Upper bars represent the relative frequencies of each parasitoid species (hyperparasitoids are not represented). Lower bars represent the relative frequencies of each aphid species. Black, parasitized aphids; gray, living aphids. The thickness of the arrows between the bars is proportional to the interaction strength between each pair of aphid/parasitoid species. Aphid abbreviations are A, *Aphis* spp; Cf: *Chaetosiphon fragaefolii*; Am, *Acyrtosiphon malvae*; Me, *Macrosiphum euphorbiae*; Rp, *Rhodobium porosum*; As, *Aulacorthum solani*; Mp, *Myzus persicae*; Mr, *Macrosiphum rosae*; Ar, *Amphorophora rubi*; Mo, *Myzus ornatus*. Parasitoids abbreviations are Aeg, *Aphidius eglanteriae*; Ae, *Aphidius ervi*; Pv, *Praon volucre*; Aa, *Aphelinus abdominalis*; L, *Lysiphlebus* spp.; Ac, *Aphidius colemani*; Am, *Aphidius matricariae*; T, *Toxares* spp.

that emerged from aphid mummies were secondary parasitoids. The hyperparasitoids collected belonged to the genera *Asaphes*, *Coruna*, *Dendrocerus*, *Pachyneuron*, and *Syrphophagus*. Hyperparasitism rates did not vary significantly among regions ($\chi^2 = 2.92$, $df = 4$, $P = 0.57$), seasons ($\chi^2 = 0.08$, $df = 1$, $P = 0.77$), or years ($\chi^2 = 0.0043$, $df = 1$, $P = 0.94$).

Greenhouse openness and insect diversity, insect assemblages, and hyperparasitism

The degree of openness of greenhouses influenced some of the measured ecological variables in these systems (Table 3). Aphid species diversity (i.e., species richness and Shannon diversity index values) did not vary with the degree of openness of greenhouses. However, the relative occurrence frequencies of some dominant aphid species were significantly affected by greenhouse

openness: The occurrence frequency of *A. gossypii* in the aphid community was higher in open greenhouses (open 15.4%; closed 6.8%), while both *A. malvae* and *R. porosum* had higher relative occurrence frequencies in closed greenhouses (*A. malvae*, open 16.4%, closed 29.2%; *R. porosum*, open 7.7%; closed 26.1%). Primary parasitoid richness and community assemblages were not affected by greenhouse openness (Table 3). Secondary hyperparasitoid activity in greenhouses depended on the degree of openness, in that the hyperparasitism rate was about three times higher in open greenhouses than in closed ones (open 32.3%, closed 10.6%).

DISCUSSION

Herein, we studied aphid and parasitoid community composition and the trophic interactions

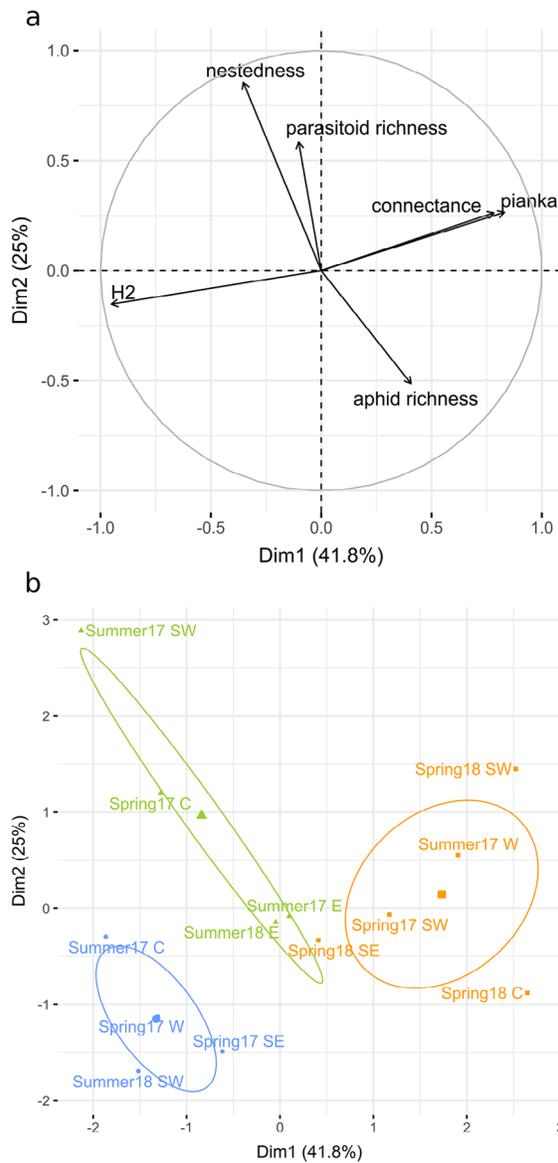


Fig. 7. Principal components analysis (PCA) based on six metrics summarizing the structures of aphid-parasitoid food webs (see *Materials and methods* for details). Each point is a food web at a given combination of region, year, and season. Plots show (a) correlations of food web metrics used in PCA partitioning; and (b) results of hierarchical clustering performed on PCA partitioning results using Ward's criterion. A *k*-means consolidation was performed on the clustering results. Ellipses represent the 95% confidence interval around the centroid. Regions are represented as follows: W, West; SW, Southwest; C, Center; E, East; SE, Southeast.

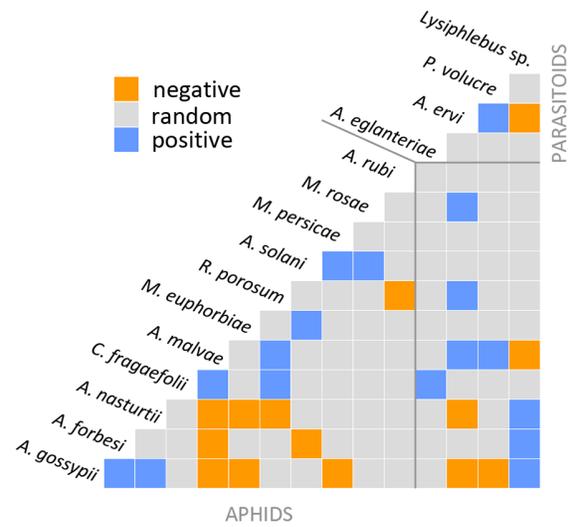


Fig. 8. Patterns of pairwise co-occurrence between aphid and parasitoid species. Different colors indicate species that were negatively, randomly, and positively associated with each other, respectively. Species that did not have any positive or negative associations with any other species are not shown.

between these antagonistic species in protected strawberry crops at different time (intra- vs. interannual) and spatial (intra- vs. interregional) scales. Our results showed that there is a high species richness of these insects in strawberry crops and much variability in insect communities and food webs among greenhouses. Only a small part of this variability could be explained by broadscale factors (i.e., regional, annual, and seasonal factors). Thus, if a seasonal shift in insect communities was observed, both species assemblages and food webs would be more likely to have been determined by local environmental filters. This study provides essential information to help improve biological control strategies used against aphids in greenhouses.

A greenhouse: a simplified agroecosystem?

Greenhouse crops are often considered simple ecosystems because a unique plant species is cultivated in a system isolated from the environment (van Lenteren 2000, Enkegaard and Brødsgaard 2006). While we expected to find a simple ecological network in these systems, we actually found a large diversity of insects colonizing strawberry crops in the sampled

Table 3. Estimated parameters, *z*-values, and *P*-values associated with the effect of the greenhouse openness factor on aphid species diversity, relative occurrence frequencies of the dominant aphid species, primary parasitoid species diversity, relative occurrence frequencies of the dominant primary parasitoid species, and the rates of hyperparasitism in each greenhouse.

Species and response	Parameter	Estimate	<i>z</i> Value	<i>P</i>	Openness effect
Aphids					
Species richness	Intercept	1.141	11.905	<0.001	
	Open	0.001	0.008	0.993	No effect
Shannon index	Intercept	0.807	12.861	<0.001	
	Open	0.026	0.362	0.717	No effect
% <i>Aphis gossypii</i>	Intercept	-3.914	-4.393	<0.001	
	Open	1.019	2.088	<0.05	Open > Closed
% <i>Acyrtosiphon malvae</i>	Intercept	-2.110	-2.782	<0.01	
	Open	-1.065	-2.041	<0.05	Open < Closed
% <i>Chaetosiphon fragaefolii</i>	Intercept	-3.094	-7.188	<0.001	
	Open	0.7753	1.695	0.09	No effect
% <i>Macrosiphum euphorbiae</i>	Intercept	-2.319	-2.617	<0.01	
	Open	0.3289	0.607	0.543	No effect
% <i>Rhodobium porosum</i>	Intercept	-2.0099	-6.404	<0.001	
	Open	-2.4369	-5.428	<0.001	Open < Closed
Primary parasitoids					
Species richness	Intercept	-0.446	-1.441	0.156	
	Open	-0.194	-0.877	0.383	No effect
Shannon index	Intercept	0.234	3.596	<0.001	
	Open	-0.008	-0.102	0.9185	No effect
% <i>Aphidius eglanteriae</i>	Intercept	-8.492	-5.047	<0.001	
	Open	1.061	0.692	0.489	No effect
% <i>Aphidius ervi</i>	Intercept	-1.031	-0.811	0.417	
	Open	-0.231	-0.304	0.761	No effect
% <i>Praon volucre</i>	Intercept	-2.725	-1.166	0.244	
	Open	-0.661	-0.844	0.398	No effect
Secondary parasitoids					
Rate of hyperparasitism	Intercept	-2.803	-6.381	<0.001	
	Open	1.5475	3.279	<0.01	Open > Closed

greenhouses, specifically comprising thirteen aphid species, eight parasitoid species/genera, and five hyperparasitoid genera. In comparison with other open-field and greenhouse crops, protected strawberry crops were more diverse in terms of the aphid species they harbored (Table 4), even at the greenhouse scale (i.e., up to seven aphid species were found in a single protected crop). This aphid species richness could be partly explained by the planting strategies of growers (Buitenhuis et al. 2017), as plant materials with several different geographical origins usually coexist in a single greenhouse, diversifying the possibilities of aphid introduction to them. Contrary to other agroecosystems, parasitoid species richness was found herein to be lower than aphid diversity (Table 4). Usually,

natural enemy richness is positively correlated with their abundance (Letourneau et al. 2009), and the occurrence frequency of parasitized aphids was very low in many greenhouses sampled in this study, which suggested low parasitoid abundances (Table 1). This relationship between diversity and abundance in parasitoids would explain the fact that the highest diversity was observed in the Southwest region, the production area where the largest number of aphid mummies was found herein.

A greenhouse: a predictable agroecosystem?

In addition to insect species richness, we examined variations in aphid and parasitoid community assemblages and how these antagonistic species interacted within greenhouses across

Table 4. Number of species of aphids, primary parasitoids, and secondary parasitoids observed in several agroecosystems.

Crop	Type of crop	No. aphid species	No. primary parasitoid species	No. secondary parasitoid species	Reference
Wheat	Open field	3	6 genera	5 genera	Gagic et al. (2011)
Wheat, barley, triticale	Open field	3	6	3 genera	Tougeron et al. (2018)
Melon	Greenhouse	1	8	5	Dong et al. (2019)
Pepper	Greenhouse	5	7	1	Sanchez et al. (2011)
Wheat	Open field	2	3	Not measured	Alhmedi et al. (2011)
Pea	Open field	2	4	Not measured	Alhmedi et al. (2011)
Wheat	Open field	3	6	Not measured	Derocles et al. (2014)
Rapeseed	Open field	2	1	Not measured	Derocles et al. (2014)
Pea	Open field	1	6	Not measured	Derocles et al. (2014)
Triticale	Open field	1	6	Not measured	Derocles et al. (2014)

regions, years, and seasons. For both aphids and parasitoids, community assemblages differed among the sampled regions. For aphids, we recorded low interregional variation in communities in spring. During this season, we often found *M. euphorbiae*, *A. malvae*, *R. porosum*, and *C. fragaefolii* at high frequencies, with the three latter species co-occurring positively in the strawberry greenhouses. This insect community assemblage homogeneity could be the result of climate conditions, which are more convergent across regions in spring than in summer (CLIMATIK platform, <https://www6.paca.inrae.fr/agroclim/Les-outils>). In addition to this, the sources of aphid infestations could also be similar among regions because of plant material exchanges. While plant material exchanges favor diversity at the greenhouse scale, they may homogenize communities among regions since plant material with a single origin can be used in various regions. In summer, the system is less predictable, but there is clearly a shift in aphid community composition, with increasing frequencies of occurrence of species of the genus *Aphis* (i.e., *A. gossypii*, *A. forbesi*, and *A. nasturtii*), and with *A. gossypii* co-occurring positively with other *Aphis* species. It has been shown that *A. gossypii* is more resistant to high temperatures (Gao et al. 2016) than other species (Barlow 1962,

Beetge and Krüger 2019). This might also be the case for *A. forbesi* and *A. nasturtii*. For parasitoids, their communities were similar among regions, except for the West region, where both diversity and occurrence frequencies of parasitoids were very low (i.e., only one parasitoid species was found in 2018). Both *A. ervi* and *P. volucre* predominated in the parasitoid communities in spring, and co-occurred in greenhouses positively. The seasonal shift in parasitoid communities was mostly driven by both the collapse of parasitoid populations in summer and the higher frequency of occurrence of members of the genus *Lysiphlebus* in these communities. Most species of this parasitoid genus are associated with aphids of the genus *Aphis*, which is the most frequently occurring aphid genus in the summer (Stary 2006).

Our results suggested the existence of some insect assemblage similarities among the five French regions sampled, but the impact of interregional differences on insect communities accounted for a low percentage of assemblage variation (i.e., about 20%; see Table 2). In addition, the analysis of food webs containing aphids and parasitoids showed no clear evidence of either spatial or temporal patterns: If different types of trophic networks were found, they did not cluster in accordance with any consistent

region, sampling year, or season. All of these findings suggest that the differentiation of communities and trophic networks is driven by local environmental factors, making these ecological variables (i.e., diversity, assemblage structure, and food webs) difficult to predict. Immigration and local population extinctions, among other factors, can be sources of uncertainty for the prediction of pest pressure in greenhouses. In open fields, one major factor driving aphid population dynamics is immigration by winged individuals (Fievet et al. 2007). In greenhouses, aphid population development can be initiated via the introduction of infected plant materials, but winged aphid inputs from local environments would also lead to some colonization events. Compared to open fields, aphid immigration would be limited in greenhouses, but further studies are needed to quantify the inputs of winged immigrants to such protected crops. In open fields, aphid populations can also experience high extinction rates, or failures in population establishment due to pressure from both natural enemies and weather conditions (Fievet et al. 2007). In greenhouses, pressure from natural enemies would be reduced compared to that in open fields, and climatic conditions are more stable, making extinction events less likely. Predicting insect diversity at the greenhouse scale would require analyzing all factors shaping communities, including the management practices used (e.g., pesticide treatment programs, chemical insecticide use), the surrounding landscape (e.g., possible sources of insect food provisioning, presence of alternate hosts for parasitoids), and the presence of other natural enemies (e.g., predators or fungal pathogens).

Community composition and food web structure at a given local scale can also be seen as the results of environmental filters selecting for species capable of survival and persistence at this location (Kraft et al. 2015). Classically, environmental filtering has been viewed as a set of sequential mechanisms that filter a large pool of potential species to produce the subset that actually occurs within a community (Weiher and Keddy 1995). Herein, we considered two filters that may shape community composition and trophic networks in greenhouses: insect ecological specialization and the openness of greenhouses.

Diversity in a greenhouse: specialist or generalist species?

The aphid communities associated with protected strawberry crops were composed of species with various degrees of plant specialization. Three species (*C. fragaefolii*, *A. forbesi*, and *R. porosum*) are oligophagous or specialists feeding on the genus *Fragaria*. *Acyrtosiphon malvae* is a polyphagous species, but some subspecies are associated with particular host plants, such as the subspecies *A. malvae* ssp. *rogersii* (Theobald), which is associated with plants of the genus *Fragaria* (Blackman et al. 2006). Other species, like *A. gossypii*, *A. nasturtii*, *M. euphorbiae*, and *A. solani*, are highly polyphagous (Blackman et al. 2006). Plant specialization in aphids can influence the number of parasitoid species occurring in the crop and the breadth of their aphid host ranges. Indeed, polyphagous aphid species tend to harbor more parasitoid species compared with mono- and oligophagous species (Stadler 2002), and these parasitoids tend to be more generalist (Gagic et al. 2016). This is the case for *M. euphorbiae*, as this most generalist aphid species found herein was attacked by the largest number of parasitoids. Conversely, *C. fragaefolii*, one of the most specialized aphids found, was parasitized by only a single parasitoid species, *A. eglanteriae*. This latter oligophagous species has mainly been described feeding on aphids of *Chaetosiphon* spp. living on plants of *Fragaria* spp. and *Rosa* spp. (Kavallieratos et al. 2004, Barjadze et al. 2010). The small size of *C. fragaefolii* compared with other aphid species and the presence of capitate hairs on its body that act as physical defenses may be factors that have led to the specialization of *A. eglanteriae* (Stireman and Singer 2003). Metrics of aphid–parasitoid food web structure suggested that there was high specialization in these trophic networks. This high specialization may be explained by the presence of various scarce parasitoid species (i.e., *Lysiphlebus* spp., *A. abdominalis*, *A. colemani*, *A. matricariae*, and *Toxares* spp.) that mainly used aphid hosts in the genus *Aphis*. Overall, the food webs found included two common parasitoid species, *A. ervi* and *P. volucre*, with overlapping host ranges (Appendix S1: Table S3). Specifically, both of these parasitoids used the three aphid species most frequently found in the spring (*A. malvae*, *R. porosum*, and *M. euphorbiae*) as hosts, and co-occurred in the same greenhouses (Fig. 8).

Diversity in a greenhouse: a question of openness?

The degree of greenhouse openness can filter species by their ability to enter the greenhouse or to become established in certain conditions. We expected to find higher aphid diversity in open greenhouses, but the degree of openness of the greenhouses sampled did not have a significant effect on their aphid species richness. Therefore, even in closed greenhouses, a high number of aphid species was able to colonize strawberry crops. On the other hand, the relative occurrence frequencies of particular aphid species were related to greenhouse openness. Indeed, *A. malvae* and *R. porosum* were found with higher relative frequency in closed greenhouses. A possible explanation for this is that these aphid species can be introduced into greenhouses via contaminated plant material, and are then particularly favored by the more stable temperature conditions of closed greenhouses in the spring. To the contrary, *A. gossypii* was favored in open greenhouses. This species, which was particularly abundant in the summer, may enter greenhouses from the surrounding landscape.

Primary parasitoid richness and community assemblages were not affected by greenhouse openness, suggesting that all parasitoid species were equally able to locate aphids in more or less closed greenhouses. Even though we identified eight parasitoid species/genera, all the sampled communities were dominated by *A. ervi* and *P. volucre*. The aphids we found in strawberry crops, especially *M. euphorbiae* and *A. malvae*, can be parasitized by a large range of parasitoid species (Starý 1966, Starý et al. 1971, Michelen Saval and Moscardo 1987, Kavallieratos et al. 2004, Starý 2006). Thus, it may be hypothesized that both *A. ervi* and *P. volucre* would likely be more able to overcome greenhouse barriers compared with other species.

Food webs in greenhouses: valuable information for biological control?

For biological control purposes, it is essential to know the natural enemies that can be used to achieve an adequate level of pest control and identify factors disrupting control by them. In this study, we identified three groups of parasitoids based on both their host ranges and the

phylogeny of their hosts (Desneux et al. 2012) as follows: (1) a group composed of two generalist parasitoids, *A. ervi* and *P. volucre*, associated with aphids in the tribe Macrosiphini; (2) a group of minor parasitoid species mainly associated with aphids in the tribe Aphidini (*Aphis* spp.); and (3) a parasitoid species, *A. eglanteriae*, strongly specialized on using *C. fragaefolii*. To improve the biological control of aphid populations, the presence of these species in greenhouse crops could be favored directly, by inundative biological control, or indirectly, by conservation biological control. Inundative biological control is already used against aphids in strawberry crops and relies on the use of mixes of several parasitoid species (de Menten 2011). Since using parasitoid mixes allows aphid control without species identification, this approach does not target the specific aphid species infesting particular crops. While some species applied in parasitoid mixes were never found in our samples (e.g., *Ephedrus cerasicola*), other species collected (*A. eglanteriae* and *Lysiphlebus* spp.) were absent from the mixes used in control programs (i.e., these species are not commercially produced). Also, in our survey, when parasitoids had been released in greenhouses by producers, the species released were rarely found in our samples, suggesting that collapses of parasitoid populations had occurred. These results underline the relative contribution of spontaneous indigenous colonization compared with inundative releases of parasitoids to biological control. Perez-Alvarez et al. (2019) demonstrated the important role of interactions between released biological control agents and the indigenous natural enemies of pests, with the effectiveness of inundative biological control being modulated by the complexity of the surrounding landscape. Conservation biological control would thus be a way to enhance the natural regulation of aphids in greenhouses. A way to promote colonization by key indigenous parasitoid species would be to provide them with habitat and food inside or outside of greenhouses (Cotes et al. 2018, Rodríguez et al. 2018). Indeed, impacts of landscape complexity on the natural enemies of crop pests have been widely studied (Chaplin-Kramer et al. 2011), especially for parasitoid communities (Krueger 2003, Thies et al. 2003, Rand et al. 2012). However, few studies have been done on the role of

landscape composition in conservation biological control in greenhouses (but see Dong et al. 2018).

As several aphid species with shared parasitoids coexisted in strawberry greenhouses, apparent competition and apparent mutualism could occur between these pests (Messelink et al. 2012). Apparent competition between aphid species would be beneficial for producers, as the presence of one aphid species could help to control the population of another species. On the other hand, apparent mutualism would be detrimental for biological control, as the presence of one pest could decrease the efficiency of a natural enemy to control another pest (Messelink et al. 2008), for example, if one pest species is preferred by a natural enemy over another. Saeed et al. (2018) showed that the presence of two less preferred aphid species decreased the frequency of parasitism of *A. ervi* on the grain aphid, *Sitobion avenae*. In our study, the three dominant aphid species, *A. malvae*, *M. euphorbiae*, and *R. porosum*, shared the two dominant generalist parasitoids. Even if *A. malvae* co-occurred with *A. ervi* and *P. volucre*, this species was proportionally less often parasitized than the two other species, suggesting it was less suitable for use by parasitoids. It would then benefit from apparent mutualism and could become a dominant species in greenhouses. Such processes may alter biological control efficiency, and their incidence thus has to be evaluated.

Finally, natural enemies of aphids can also be consumed by other predators or parasitoids, which might be detrimental to biological control efforts. This type of consumption is well-known for parasitoids, in the form of so-called hyperparasitism. Greenhouse crops may be particularly more favorable for some hyperparasitoid species compared with outdoor crops due to the higher temperatures therein (Prado et al. 2015). In our study, even though the average hyperparasitism rate in greenhouses was quite low (about 22%), at the local scale the pressure exerted by hyperparasitoids on primary parasitoids was sometimes high (up to 100%). Interestingly, our results showed that there were higher hyperparasitism rates in open greenhouses than in closed ones, indicating that biological control with parasitoids is more likely to be disrupted in open greenhouses by hyperparasitoids coming into the crop from the surrounding environment. This raises

the issue of the importance of the surrounding landscape to biological control, as highlighted by Dong et al. (2018). Because hyperparasitism may lead to the local collapse of primary parasitoid populations and increases in pest populations (Sullivan and Völkl 1999), one should consider these organisms when planning or applying any biological control program with parasitoids.

CONCLUSION

Understanding how local and large-scale factors affect communities and food webs is a key challenge in both biodiversity management and biological pest control programs. Our study provided insights into the diversity of the aphid pest and parasitoid species colonizing protected crops and sources of variation in their communities and trophic interactions. This information will be useful for the management of aphid pest species. Despite there being a high variability in species composition among greenhouses, the same species dominated the communities in all the sampled regions, especially in the spring, when aphids are a serious issue for crop producers. Thus, it could be possible to identify similar strategies for biological control at the national scale. These strategies may include the production of local strains of the key parasitoid species attacking the main pests of crops, possibly associated with the use of predators as well. It will also be important to consider the management of the surroundings of greenhouses to preserve the populations of released and indigenous natural enemies of pests in them.

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supervision of this study. All authors contributed critically to the drafts and gave final approval for publication. The authors declare no conflict of interest.

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