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HABITATS AND FOOD PREFERENCES CONCERNING SYRPHIDAE AND CHRYSOPIDAE AUXILIARIES.



HABITATS AND FOOD PREFERENCES CONCERNING SYRPHIDAE AND CHRYSOPIDAE AUXILIARIES.

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RESUME

La lutte biologique par conservation est un enjeu important ; cependant sa réussite nécessite la compréhension du mode de vie des insectes bénéfiques. Mais quel est l'aménagement du paysage, et quels sont les choix judicieux de végétation à mettre en place pour favoriser ces auxiliaires ? Les modèles choisis sont les Syrphidae (Diptera) et Chrysopidae (Neuroptera), qui sont palynophages au stade adulte. Afin de connaître la végétation la plus attractive de ces ennemis naturels, une étude du pollen consommé est effectuée. Des collectes ont été réalisées dans neuf sites de l'Ouest de la France entre 2004 et 2009, les auxiliaires sont ensuite identifiés et disséqués au laboratoire. Dans un souci de représentativité, cette analyse est ciblée sur les individus les plus communs que sont *Melanostoma spp.*, *Sphaerophoria spp.*, et *Chrysoperla spp.*. Ces insectes étant de bons indicateurs de la biodiversité, ils permettent de tester l'effet de différents milieux ou types de gestion sur l'attractivité des insectes. Grâce aux résultats de l'analyse du contenu du tube digestif, nous avons pu établir une liste des plantes les plus consommées par les syrphes et chrysopes. Cependant, si les Syrphidae sont sélectifs dans leur nourriture, les Chrysopidae sont plutôt opportunistes même si certaines plantes sont exclues de leur alimentation. Il apparaît également que les plantes les plus visitées ne sont pas obligatoirement les plus butinées ; par exemple *Lotus corniculatus* (Fabaceae) pour *Melanostoma spp.*.

ABSTRACT

Conservation biological control is an important issue; however its success necessitates the comprehension of beneficial insect's bioecology. But what kind of landscape, and which plants are favorable to attract those auxiliaries? The models chosen are Syrphidae (Diptera) and Chrysopidae (Neuroptera), pollen feeders while adults. So as to know the most attractive vegetation of those natural enemies, a pollen analysis is realized. Collects has been done in nine areas of Western France from 2004 to 2009, auxiliaries are then identified and dissected in laboratory. To be representative, this analysis is targeted on the most common individuals which are *Melanostoma spp.*, *Sphaerophoria spp.*, and *Chrysoperla spp.*. As those insects are good biodiversity indicators, they allow testing the impact of different environments or management way on the insect attractiveness. Thanks to gut content analysis results, we managed establishing a list of the most consumed plants by hoverflies and lacewings. However, if Syrphidae are selective in their nourishment, Chrysopidae are more opportunistic even if several plants are not foraged at all. It also appears that the more visited plants are not obviously the most gathered; for example *Lotus corniculatus* (Fabaceae) for *Melanostoma spp.*.

KEY WORD

Syrphidae, Chrysopidae, conservation biological control, food choice, land quality.

INTRODUCTION

The conservation biological control is a hopeful way for sustainable agriculture, obliged to find alternatives to pesticides. However the understanding of insect bio-ecology is indispensable to develop this method. Several studies have shown that the vegetation is a central point for the colonization by auxiliaries, which depends on the plant diversity and the landscape structure (Haslett, 2001). In France, 60% of the territory is agricultural land that is why the comprehension of the agriculture's impacts on the biodiversity is essential (Clergué *et al.*, 2004); in particular because the functioning of ecosystems is negatively influenced by a loss of biodiversity and can lead to a dysfunction of agronomical services as pollination and natural pest control (Albrecht *et al.*, 2007).

Currently, intensive farming seems contrary to a respectful activity which could improve biodiversity. In fact, agricultural intensification is characterized by pesticides use, little landscape heterogeneity and a deficit of habitat diversity is linked to species richness shortage (Hendrickx *et al.*, 2007). Regarding semi-natural areas, the ecosystems reality is totally different. The spontaneous ecological processes permit the installation of a balance between the livings. The varied plants present, the high vegetation and the complex landscapes characterizing those habitats appear to be one way to favor biodiversity, in particular auxiliaries' diversity (Sjödin *et al.*, 2008 ; Meyer *et al.*, 2009).

Beneficial insects as Chrysopidae (Neuroptera) and Syrphidae (Diptera) are known to be very useful because they can provide valuable ecosystem services; their larvae are notably control agents for a lot of crops because they can be zoophagous, mycophagous, saprophagous, and phytophagous (Sommagio, 1999 ; Meyer *et al.*, 2009). It is notably the case of genus as *Melanostoma*, *Sphaerophoria*, *Episyrphus* (Syrphidae) or *Chrysoperla* (Chrysopidae) which larvae eat aphids. The adults feed on pollen or nectar, in addition they can be considered as pollinators. As they have different environment requirements, they live in all kind of French ecosystems. Besides they are quite easily identifiable; these characteristics allow them to be good biodiversity indicators which means their richness reflects the habitat's quality (Sommagio, 1999 ; Clergué *et al.*, 2004 ; Thierry *et al.*, 2005). Flowers are indispensable to adults as they eat exclusively pollen or nectar and females are notably dependent of pollen for egg maturation (Wratten *et al.*, 2003).

The Syrphidae seems positively related to flower abundance and proximity (Kohler *et al.*, 2008) but are also sensible to landscape structure (Haslett, 2001). It appears that hoverflies exhibit floral constancy depending on the species, this means they choose their resource and use components disproportionately to their abundance in the habitat (Cowgill *et al.*, 1993 and Wratten *et al.*, 1995). The female adults need to balance their intake of both pollen and nectar, depending on their reproductive status (Sutherland *et al.*, 1999). About the *Chrysoperla* genus, their resource requirements are diverse as larvae are entomophagous while adults feed mainly on nectar, pollen grains, but also on insect honeydew (Villenave *et al.*, 2006). Those insects are appreciated in biological control because larvae present an impressive voracity. They seem to be opportunistic animals concerning pollen research; floral resources are essential for lacewing survival and can impact on their fecundity and oviposition date (Robinson, 2008).

In order to understand better which type of vegetation environment favor auxiliaries as Chrysopidae and Syrphidae, one way could be to compare biodiversity indices between

different landscapes. Here we will use Syrphidae and Chrysopidae defined as good biodiversity indicators, this will allow us testing the landscape and management impacts on auxiliaries. This first step will give results about the favorable habitat for conservation biological control's development. A broad work gathering 6 years of collect results which were done in nine different sites of France is here realized. This study is also important as it takes care of Syrphidae and Chrysopidae at the same time and will allow comparisons between those key auxiliaries. But the specificity of that investigation is that food choice is studied through an analysis of the pollen consumed by insects. This has not been very studied but in New Zeland concerning Syrphidae Wratten *et al.* (2003) and in France concerning Chrysopidae (Villenave *et al.*, 2005, 2006 ; Villenave, 2006). After the identification of favorable habitats, we will be able to discern some attractive plants which can be of interest in landscape management aiming for conservation biological control. In fact, investigating their foraging activity would make us capable to comprehend their floral needs and so the ecological processes crucial in the biodiversity field (Clergué *et al.*, 2004).

MATERIALS AND METHODS

Studied sites



This report is the culmination of analyses made in nine areas from 2004 to 2009. The studied sites were all located in western France, and can be classified in two different categories: farmlands and semi-natural landscapes (figure 1). Last ones can be defined as spontaneous meadows which were mown once a year. They were located near urban areas but were not exploited and had a free development. Each analysis has been carried out with at least three

Figure 1 : The nine areas studied in France from 2004 to 2009 with their GPS localisation: latitude/longitude.

- Red points are the farmlands: **Rennes** (35): 48,1/-1,6; **Tour-en-Sologne** (41): 47,5/1,5; **Saint Jean d'Angely** (17) : 45,9/-0,5; **Duhort-Bachen** (40) : 43,7/-0,3; **Angers** (49) : 47,4/-0,5 ; and **Lucs-sur-boulogne** (85) : 46,8/-1,5.
- Green ones are the semi-natural areas: **Beauvais** (60) : 49,4/2,0 ; **Saint Gilles Croix de vie** (85) : 46,7/-1,9; and **La Chapelle-sur-Erdre** (44) : 47,2/-1,5.

collect points, giving a more representative view of the whole site which size was comprised between 0,5 and 1ha. The different collect points were chosen relatively to their specific landscape, in order to represent each part of the place (for example, the same site could present one collect point in the hedge, one in the herbaceous stripe, one in the cultivated field). The collects were realized once a month in each site from April to October (favorable season to catch insects) during two or three consecutive years.

The climate of those sites was oceanic to semi-oceanic, characterized by cool and rainy summers, and cool summers (July temperature average between 18 to 22°C). The agriculture of the farmlands was more polyculture (gardening) except for Tour-en-Sologne and Saint Jean d'Angely situated in cereal regions.

Insects survey

To collect Chrysopidae and Syrphidae in the low layer, a hand net (pocket of 50 cm diameter and 80 cm depth) and a mobile individual vacuum were used. Each herbaceous sampling was done realizing one hundred hand net sweeps and five minutes of sucking up. Whereas in shrubby and arboreal strata samplings, two hundred hand net sweeps were carried out. The Syrphidae and Chrysopidae were then placed in 70° alcohol, followed by their laboratory

identification with a binocular glass. All the Chrysopidae were identified according to Aspöck *et al.* (1980)'s standards, also precised by Thierry *et al.* (1998) for the genus *Chrysoperla* Steinmann. Regarding the Syrphidae, their identification was realized using taxonomic keys (Perrier, 1983; van Veen, 2004).

Gut content analysis

The Chrysopidae esophageal diverticulum was extracted; this part of the gut served as a reserve where pollens and other contents were stored, but not yet digested. The diverticulum was opened on a slide in order to discharge the pollen. For Syrphidae, a simple stomach dissection on a slide permitted it. After lipid extraction with diethyl ether, the pollen was placed on a slide with glycerin jelly containing basic fuchsin as stain for pollen grains. Pollen grains were then observed with an optic microscope x 400 and determined at family or species level using in reference the INRA – Le Magneraud's pollen collection, protocol used by Villenave *et al.* (2005). The pollen grain quantity was estimated by observing at x 20 the total number of pollen grains in the diverticulum, the size of the latter was taken into account and ranged from 2mm (empty) to 3mm (full). Pollen identification was realized to the taxonomic specie level. Notice that the *Chrysoperla sp.* individuals were collected during the day, but they foraged during the night so they had digested for part the pollen grains in their stomach. This fact was not a problem to compare pollen quantity between the different Chrysopidae as they had forage about the same time, so the digestion was quite similar for each individual.

Flora survey

When the insects were gathered, the flora was listed in order to know which plants were blossomed at the moment. Each site was crossed and the plants presented were identified with a flora of the region, but no covering indication was realized. This permits easier pollen identification, and permits knowing if the pollens were susceptible to have been consumed on the premises or elsewhere.

The biodiversity indices

Three index of biodiversity were used during that survey to allow comparisons between different populations in diverse ecosystems: the richness Margalef's index (*M*), the standard Shannon-Weaver's diversity index (*H'*), and the Hurlbert's equitability index (*E*):

- *M* symbolized the species number represented. $MI = (F-1)/\text{Log}_2Q$ with F = Number of genus/species and Q = Total number of individuals sampled in the population.

- The *H'* index measured the specific diversity of the population; it also took in care the relative abundance of the species. $H' = -\sum p_i * \text{Log}_2(p_i)$ with p_i = relative abundance of specie_{*i*} in the sample and $p_i = q_i/Q$ with q_i = the number of individuals in the specie_{*i*}.

- The *E* index showed the equitability of the population as it used the maximal, the minimal, and the observed diversity. $E = (H' - H'_{\min}) / (H'_{\max} - H'_{\min})$ with $H'_{\max} = \text{Log}(F)$ and $H'_{\min} = \text{Log}Q - ((Q-F+1)\text{Log}(Q-F+1))/Q$.

Those indices were chosen as they were complementary and represented the biodiversity quality (Villenave J. *et al.*, 2006). Their calculation permitted making comparisons between different habitats (farmlands and semi-natural lands, or between various fallow land).

Analyses of Syrphidae and Chrysopidae food choice

Here, all the data collected in each site and at each date were used to give the most accurate view of the insects' foraging activity. However, only the more abundant species

were used in order to have representative data (more than 30 individuals). In fact, as the different species foraged variously, it was not possible to include all the insects dissected and to give a global conclusion. The Syrphidae chosen to realize the statistical analyses were *Melanostoma spp.* and *Sphaerophoria spp.* because they were the more common in the studied sites. As regards Chrysopidae, the *Chrysoperla* genus (including *Chrysoperla affinis*, *Chrysoperla lucasina* and *Chrysoperla carnea*) was used globally as the compartment and the food of the different species were quite similar (Villenave, 2006). Qualitative (pollen consumed or not, without quantity information) and quantitative analyses of the food choice were studied.

RESULTS

The biodiversity indices results:

Collects of 357 individus of Syrphidae and Chrysopidae, from 33 different species permitted biodiversity comparisons between the two landscapes (semi-natural and farmlands). All the biodiversity indices of semi-natural lands were more important than those of farmlands, which means semi-natural lands offered a better biodiversity of Syrphidae and Chrysopidae (table 1). To go further in this biodiversity study, it was possible to consider all the semi-natural and farmland sites, then to select those of which were fallow lands and to sort them by management manner (table 2). Notice that all the fallow lands of the semi-natural areas offered spontaneous meadows.

Table 1: biodiversity indices results for the three semi-natural lands and the three farmlands studied in western France from 2004 to 2009. In bold are the highest indices, and in italic the lowest.

Indices	Semi-natural lands	Farmlands	All
MI (Margalef Index)	2,34	<i>1,13</i>	1,76
H' (Shannon-Weiner Index)	3,20	<i>2,19</i>	2,61
E (Hurlbert Index)	0,67	<i>0,63</i>	0,62

Table 2: biodiversity indices results depending on the kind of fallow land met in the nine western French areas studied from 2004 to 2009. In bold are the highest indices, and in italic the lowest.

Indices	Spontaneous	Sowed (Fabaceae)	Sowed (Poaceae)	Sowed (various plants)	All
MI	2,01	1,39	<i>0,61</i>	1,41	2,10
H'	2,63	2,45	<i>1,15</i>	2,50	2,74
E	<i>0,57</i>	0,69	<i>0,38</i>	0,72	0,60

Results of the analyses concerning Syrphidae and Chrysopidae food choice:

The results presented here were carried out on a total of 357 insects collected, 201 of which being Chrysopidae and 156 Syrphidae. *Melanostoma spp.* individuals had eaten pollen of 18 different plant families (fig. 2), and *Sphaerophoria spp.* of 15 plant families (fig. 3). *Chrysoperla spp.* had eaten pollen from 30 plant families (fig. 5), and notice that *Chrysoperla affinis* which presented the most numerous individus of the *Chrysoperla spp.* had consumed almost all plant families presented. Only Araliaceae, Ericaceae and Tiliaceae families were not consumed by that specie, but by others *Chrysoperla spp.*

Figure 2 : Qualitative analysis of the *Melanostoma spp.* consumption according to plant families.

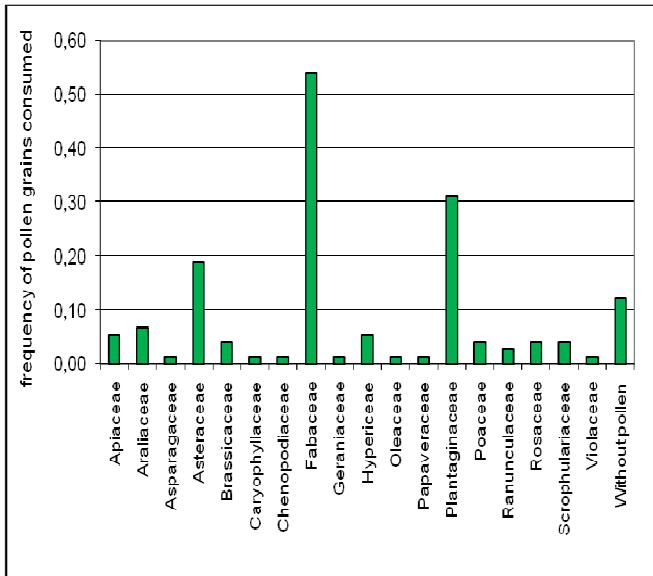


Figure 3 : Quantitative analysis of the *Melanostoma spp.* consumption according to plant families, and standard deviation.

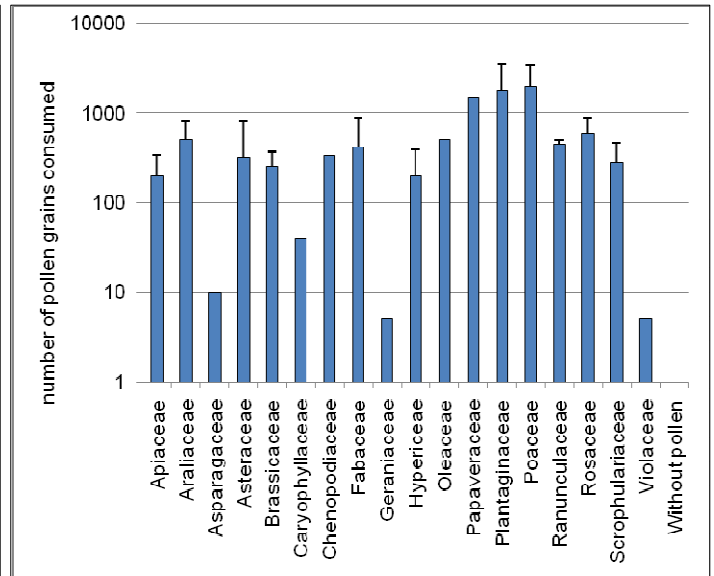


Figure 4 : Qualitative analysis of the *Sphaerophoria spp.* consumption according to plant families.

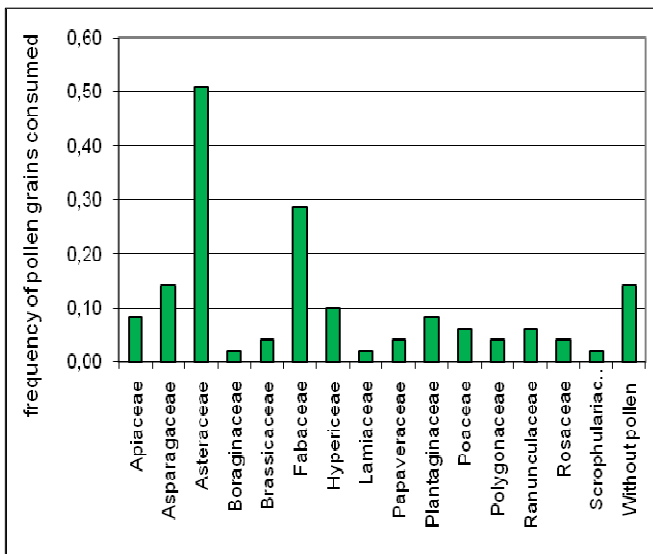
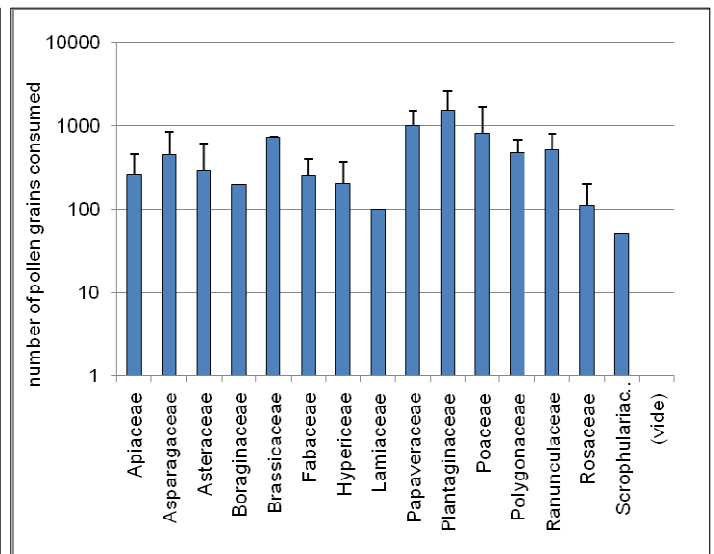


Figure 5 : Quantitative analysis of the *Sphaerophoria spp.* consumption according to plant families, and standard deviation.



Concerning Syrphidae; *Melanostoma spp.* visited more often the plants from Fabaceae and Plantaginaceae families but quantitative differences were not observed, only Asparagaceae, Geraniaceae and Violaceae families were significantly less consumed in quantity (fig. 2 and 3). And more *Sphaerophoria* specimens were visiting Asteraceae and Fabaceae plants but any quantitative differences were neither detected (fig. 4 and 5). Concerning *Chrysoperla spp.*, the trends were dissimilar. Even if Caryophyllaceae and Poaceae plants were more consumed qualitatively (figure 6), the average of individuals number visiting those plants did not overtake 0,20. But the quantitative analysis of figure 7 showed quantitative differences in the number of pollen grains consumed. Ericaceae, Polygonaceae and Solanaceae appeared here quite eaten. More generally, the Syrphidae studied did not eat all the plants presented on the site, but *Chrysoperla spp.* consumed a broader range of flowers. Hoverflies were particularly attracted by *Leucanthemum vulgare*, *Lotus corniculatus* and *Plantago sp.*

(appendix 1) as more than 5% of all the individuals had eaten those plants. However, lacewings which ate many flowers also showed some preferences for *Daucus carota*, *Brassica sp.*, *Chenopodium sp.* and Poaceae (appendix 2).

Figure 6 : Qualitative analysis of the *Chrysoperla spp.* consumption according to plant families.

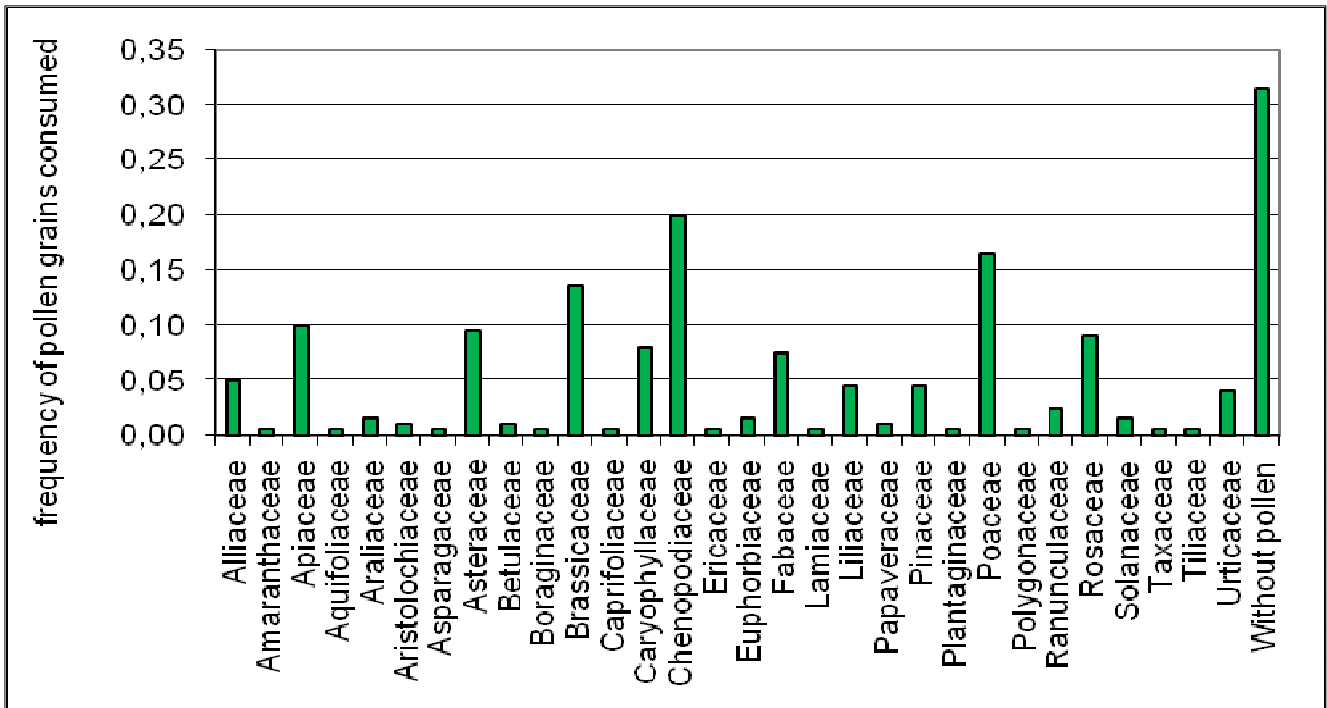
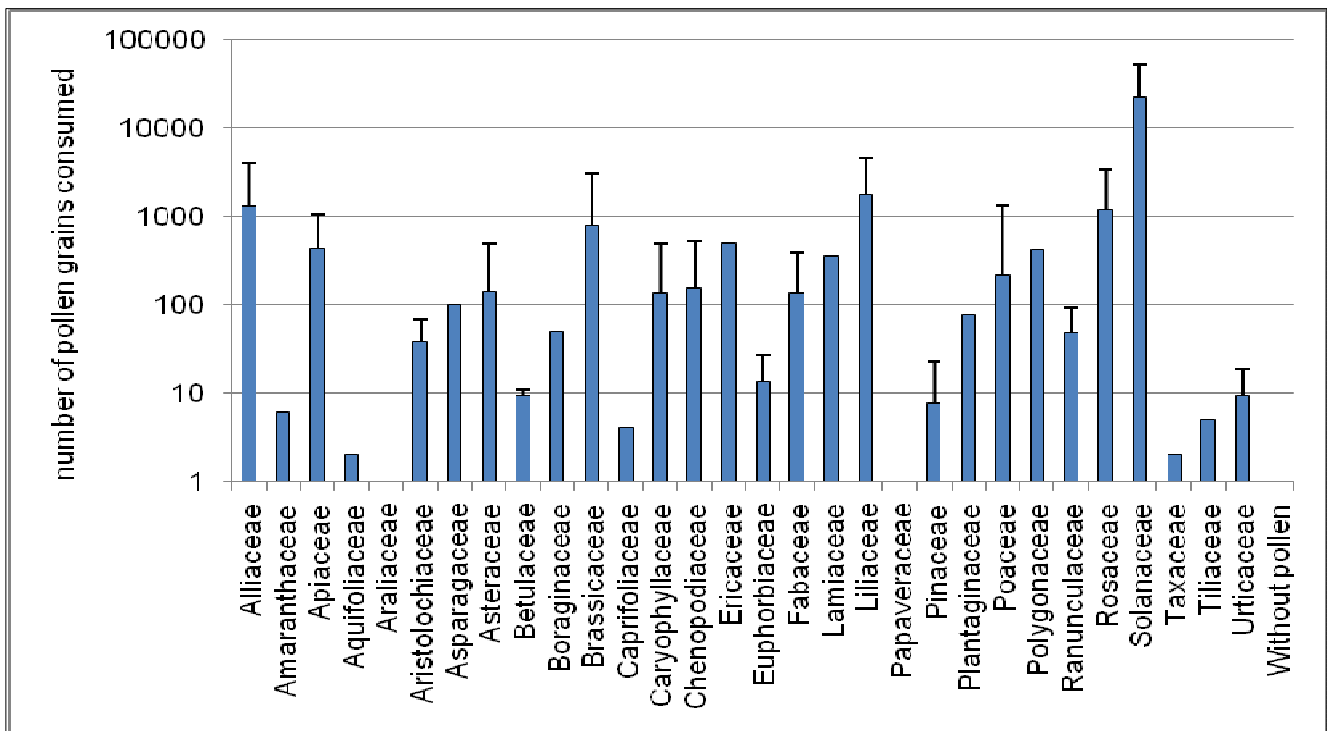


Figure 7 : Quantitative analysis of the *Chrysoperla spp.* consumption according to plant families, and standard deviation.



DISCUSSION

Firstly, the use of Syrphidae and Chrysopidae as biodiversity indicators shows that semi-natural areas present a better biodiversity than farmlands. Those figures consolidate previous studies which advise farmers to tolerate linked fallow lands nearby their crops to enhance the auxiliaries' biodiversity (Kohler *et al.*, 2007). Notice that the good indices obtained for the spontaneous fallow land underlines one advantage of the semi-natural areas, which presents this type of vegetation. Remark that the E index alone does not always show exactly the same results as the two others; this is due to the fact that the equitability of a settlement can be good even if the diversity is not important. This index cannot explain alone the habitat quality.

However, when the indices were calculated for each site separately, the conclusions were less visible between the two kinds of landscape and between the different vegetations. In fact, the indices could vary a lot depending on the site's environment (hedges or not, meadows, crops or urban structure) and its management (choice of the vegetation, mow frequency, pesticide use). That is why the choice of a global view was done here, pooling the results of all the semi-natural sites, or farmlands. To underline better the impact of the site environment (or of the management), sites should be chosen because they only differ for one aspect. Otherwise it is not possible to know exactly which element is related to the insect attractiveness.

Furthermore, interactions between the landscape's (farmland, semi natural areas) and the flora's impacts should be studied. But it is difficult to give conclusions here, as the vegetation could be quite similar in the two different landscapes. In fact if the vegetation is spontaneous in a farmland, it does not necessary vary a lot from spontaneous vegetation in semi-natural areas. But the environment around the site can still have an impact on the insect biodiversity.

In conclusion, even if the semi-natural lands seem favorable to insect biodiversity; the flora composition could also have an impact on it. In fact, the use of flowering vegetation has been widely advocated as a strategy for providing parasitoids and predators with nectar and pollen (Winkler *et al.*, 2009). This is relevant as the insects often forage around their collection point, especially lacewings which do not fly very well. This seems true in the pollen results because the plants observed on the sites were very often those found in the stomach. A radioactive tag of the pollen could be a good way to control that hypothesis.

Contrary to Chrysopidae, Syrphidae species were quite more selective. The interest of studying the pollen preferences seems accurate to improve conservation biological control since it has been shown that the nutritional state of predators in adjacent crops can be enhanced by providing specifically chosen flowers in field margins (Lee *et al.*, 2006; Olson & Wäckers, 2007 in Winkler *et al.*, 2009). Previous investigations have given a list of attractive plants for hoverflies; it is the case of *Taraxacum sp.* (Wratten *et al.*, 1995). Also *Cirsium sp.*, *Matricaria perforata*, *Silene alba*, *Leontodon autumnalis*, the *Sonchus* genus and *Aethusa cynapium* are consumed selectively according to Cowgill *et al.* (1993). Those plants can be added to the list given here (appendices), as different studies can give various conclusions depending on the plants present during the feeding of natural enemies (Colley *et al.*, 2000). That is one reason why some plants appear less consumed. It is notably the case in this study for Asparagaceae which are not quantitatively eaten because this family is only present in one of the nine areas studied. But for other families, as the Geraniaceae and Violaceae which are commonly present everywhere, their little consumption is not due to their scarcity

on the site studied. It is even more the case for families consumed by *Chrysoperla spp.* in the same sites but which are not eaten at all by Syrphidae (as Alliaceae and Apiaceae). Several leads for that selectivity have been proposed like (i) the nectar presence, (ii) the pollen availability, (iii) the morphological adaptations or the flower colors (Gilbert, 1983 ; Haslett, 1989). What can be notice is that the pollen consumed by more than 5% of all the hoverflies individuals selected was originating from yellow flowers (appendix 1). In fact, they have strong preferences for yellow flowers, with many anthers but the flower size is not very important (Goulson *et al.*, 1998; Golding *et al.*, 1999; Hichman *et al.*, 2001). Wavelengths could have been studied here to really know if the flowers where in the range of preferred colors. What is also essential is that the qualitative plant choice is not obviously correlated to the pollen grains quantity consumed. Even if Syrphidae choose plants selectively, the more visited are not always the more eaten. Two hypotheses can be given; (i) natural enemies are attracted by those plants but do not like them (taste or nutritive quality); (ii) the pollen is nourishing so they do not need a lot of pollen grains (Jacobs, 2004).

Concerning *Chrysoperla sp.*, lacewings appear to be opportunistic as they visited 30 plant families which is important in comparison to Syrphidae, this is also noted by Villenave J. (2005). However, some plants are never foraged as Hypericaceae, Oleaceae and Scrophulariaceae but consumed by Syrphidae in the same sites. Several hypotheses can be put forward: (j) they are repulsive, (jj) they are too rare on the studied sites, (jjj) they are not available due to plant phenology (van Impe *et al.*, 2002 in Villenave J.*et al.*, 2006) because their flowers close at twilight during the activity period of *Chrysoperla* adults.

Some pollen collected on the insects' stomach can be surprising, such as Pinaceae or Poaceae pollen. It is then difficult to know if those plants are really foraged or if it is a contamination. Indeed, it could be possible that insects collected Pinaceae pollen on another plant family while gathering its pollen. The small quantities of grains found could favor this contamination hypothesis.

To conclude, this report which tried to find good landscapes and suitable management way to enhance the conservation biological control give some important results. Semi-natural landscapes seem efficient to increase the insect biodiversity, and the implantation of attractive and nourishing plants (as those listed) can be a good method to attract and conserve those predators.

What should be done to complete that research could be to use the covering percentage of each plant in order to make calculations indicating the selectivity or opportunistic foraging activity of insects (relation between the percentage of presence of each plant, and its consumption frequency by insects). However, the pollen productivity of flowers differs among species (from 0,005 mg to 55 mg per flower), so the abundance of flowers differs from the pollen availability (Cowgill *et al.*, 1993). In addition, quantitative comparisons between Chrysopidae and Syrphidae consumption are difficult here as natural enemies are collected in daytime; but lacewings eat during the night and present digested pollen contrary to hoverflies which forage during the day. This fact is revealed by the important error margins result from high differences in the number of pollen grains found in the diverticulum of different *Chrysoperla sp.*. In fact, depending on the digestion stage of the Chrysopidae, the pollen can be totally digested (more than 30% of the *Chrysoperla sp.* dissected do not present pollen as everything has already been digested) or not.

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