The pollens consumed by common green lacewings *Chrysoperla* spp. (Neuroptera: Chrysopidae) in cabbage crop environment in western France

Johanna VILLENAVE[1], Dominique THIERRY[2], Abdullah Al MAMUN[3], Thierry LODÉ[4] and Elizabeth RAT-MORRIS[1]

[1] Institut National d'Horticulture, Unité de protection des plantes, 2 rue Le Nôtre, F-49045 Angers, France
[2] Université Catholique de l'Ouest, Département des sciences de la vie et de la terre, 44 rue Rabelais, F-49000 Angers, France
[3] Department of Zoology, University of Dhaka, Dhaka-1000, Bangladesh
[4]Laboratoire d'Ecologie Animale, U.F.R. Sciences, Université d'Angers, Campus de Belle-Beille, F-49045 Angers, France

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Abstract. The pollens consumed by common green lacewings *Chrysoperla* spp. in crop environment in western France, were analysed. The diverticulum contents including quantity of pollen of *Chrysoperla* adults were analysed to compare a feeding behaviour between the sexes. Females consume more pollen than males. The feeding behaviour of the two main species present was compared, yielding new insights into differences between species within the complex *Chrysoperla carnea* (Stephens).

INTRODUCTION

The need for food of high quality for the West European market has been significantly increasing so that Integrated Pest Management (IPM) and organic farming are now becoming a concern for agriculture. Crops must be harvested free of chemicals and their protection against phytophagous pests must be increasingly managed by alternative methods. In this way, naturally occurring generalist predators may play a key role in crop protection.

Barbosa (1998) emphasised the need to attract natural enemies to crop environments (Conservation Biological Control). Field crops are temporary habitats. Part of the year, they are inhospitable. The strategy of field-crop specialist insects can be migration within an heterogeneous habitat (Ronce and Kirkpatrick, 2001). A more heterogeneous vegetation bordering or surrounding cultivated areas supplies sites for oviposition and overwintering and a wider food choice for beneficial organisms, serving them as shelters (Thies and Tscharntke, 1999; Nicholls and Altieri, 2002) in part through diversification of plant species (Theunissen and Den Ouden, 1980; Baliddawa, 1985). Thus, Van Emden (1965) noted that Brassicacae bordering uncultivated fields were less damaged by *Brevicoryne brassicae* because syrphid predators were more abundant. The presence of weeds and wild plants in agroecosystems provides significantly more pollen and nectar as food for natural enemies in and around fields (Cowgill *et al.,* 1993; Colley and Luna, 2000).

Strategies to attract beneficial insects and enhance biological control of crop pests in adjacent fields (Kloen and Altieri, 1990; White *et al.,* 1995) have been studied through plant observation (Yeboah Gyan and Woodell, 1987; Kaitazov & Kharizanov, 1976) and food supply analyses (Bozsik, 1994). Most studies have been carried out on pollinators (Yeboah Gyan and Woodell, 1987; Aupinel *et al.,* 2001), pests (Lingren *et al.,* 1993), or entomophagous insects (Colley and Luna, 2000). Only a few such studies have been carried out on Neuroptera (Stelzl, 1991; Bozsik, 1992). The green and brown lacewings show many

favourable traits such as wide prey range and high voracity (Duelli, 2001). The green lacewings include *Chrysoperla* Steinmann species of the *carnea* Stephens group (Thierry *et al.,* 1992; Brooks, 1994; Henry *et al.,* 2001, 2002) and are widely used in IPM (New, 1975, 1999; Brooks, 1994). The most abundant of these species in western France (Thierry *et al.,* 2003) are *Ch. lucasina* (Lacroix) (Henry *et al.,* 1996) and *Ch. carnea* (Stephens) sensu Henry*, the so-called "common green lacewing" in cabbage crops (Villenave, unpubl. data). Their life history is complex: larvae are entomophagous while the adults are palynophagous and can live both in the arborescent and herbaceous strata. Adults do not show plant specificity (Monserrat & Marin, 1994; Gruppe & Schubert, 2001). Their migration is complex with three types of flight behaviour: migration flights to overwintering sites after diapause induction in late summer (Thierry *et al*., 1994), migration back to field crops in spring, and preoviposition migration flights to new habitats with aphid colonies (Duelli, 2001).

The present study contributes to the clarification of chrysopid flight behaviour in the crop environment. Consideration of sources of pollen found in the lacewing diverticulum reveals amplitude and frequency of *Chrysoperla* migration in the crop environment in western France. The feeding behaviour is analysed, yielding new insights concerning the status and behaviour of species within the complex *Chrysoperla carnea*.

MATERIAL AND METHODS

Investigations were conducted in the Loire Valley in western France, in agricultural zones of vegetable crops (market gardening). The different sampling sites are managed with different cultural techniques: conventional, IPM, and strictly organic farming. All sites include cabbage crops, three sites had seed production and one site had vegetable production with organic farming. Lacewings were sampled regularly during the growing season, from March to August 2004. In spring, *Chrysoperla* spp. live and feed in the arborescent stratum. In summer, they stay in their resting places in the trees and fly to the low vegetation during the twilight for feeding, copulation and oviposition (Duelli, 1986). Therefore, every 15 days, from March to April, we collected lacewings during the day, and from June to August we collected during the day in the arborescent stratum, and during the twilight in the herbaceous stratum.

- Survey of Chrysopidae

For the herbaceous stratum, we used a mobile vacuum device for 10 minutes at each sampling occasion. In the canopy, we made two hundred hand net sweeps.

- Pollen analysis

Specimens collected were killed at –80°C and kept in a freezer. Thus, the pollen was not damaged. We extracted the oesophagal diverticulum of adults, which is the part of the gut where the pollen is stored and not yet digested (fig.1). The diverticulum was opened on a microscope slide. After lipid extraction with diethyl ether, the pollen grains from the diverticulum were placed on a slide with glycerin jelly (Kaiser fourn, Merck Eurolab) containing basic fuchsin as a stain.

* = *Chrysoperla affinis* (Stephens, 1836) sensu Thierry

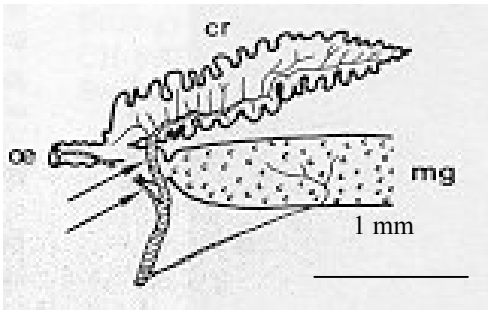

Fig. 1. Structures of gut of an adult chrysopid. Abbreviations : oe = oesophagus ; cr = crop (diverticulum) ; mg = midgut. (Canard *et al.,* 1990).

It is necessary to use only as much jelly as occupies exactly the space beneath the coverslip without undue pressure being applied (Hyde & Adams, 1958). Pollen grains were observed with an optic microscope x 400 (Olympus BH-2 with objective SPlan40, x40).
The pollen grains were determined to family or species level at x 400. To quantify the amount of pollen, the slides were observed at x 20 and the total number of pollen grains in the diverticulum was estimated, taking in account its size, which ranges from 2mm (empty crop) to 3mm (full crop).

- Statistical analysis

An analysis of variance was used to compare the means of two samples (Scherrer, 1984). The size of samples can be large or small. The logarithm of the quantity of pollen grains was analysed (to meet assumptions of normality in the analyses).

RESULTS AND DISCUSSION

Pollens in the food ingested

From March to August 2004, 157 *Chrysoperla* adults were collected, and therefore 157 slides were observed. The number of pollen grains varied from 0 to 12000 grains with a mean number of 432 (SD = 1647) per adult diverticulum. Pollen of about 40 plant species were observed (table 2), but the mean number of plant species observed was only 1.99 per adult (maximum six). The diverticula never contained a large variety of pollen.

Feeding during the flight periods and during the day

♦ From March to May

From March to May, *Chrysoperla* spp. adults feed in the arborescent and herbaceous strata. About 35 % of the specimens collected during this period had an empty diverticulum. The quantity of pollen grains found in diverticula adults collected from the two strata was not significantly different: 1715 (mean number) in the arborescent stratum and 575 in the herbaceous stratum (fig. 2) (after log transformation $Fc = 0.95 << F\alpha = 4.54$, $\alpha = 0.05$, df = 16). Among the lacewings with diverticula that contained pollen grains, we observed pollen of arbored plants in 89 % of the specimens collected in the trees, versus in only 37 % of those specimens collected in the herbaceous stratum. It seems difficult to conclude that the adults feed in the stratum they live in.
In the arborescent stratum, the main plant family represented by pollen grains in *Chrysoperla* diverticula was Rosaceae, and included such plants as *Prunus* sp., *Spiraea* sp., *Pyrus pyraster* and *Amelanchier* sp. (42 % of specimens with Rosaceous pollen contents). The other families represented were Caprifoliaceae (*Sambucus* sp), Betulaceae (*Corylus avellana*), Taxaceae and Pinaceae (*Pinus* sp.). In March-April, the flowering trees in the crop environment are mainly Rosaceae.
In the herbaceous stratum, the main plant families represented by the pollens found in *Chrysoperla* diverticula were firstly Brassicaceae (*Capsella* sp*., Brassica rapae* and *B. napus*

oleifera) (29 % of specimens with pollen contents), and secondarily Caryophyllaceae (*Cerastium* sp…) (24 %) and Asteraceae (*Senecio* sp…) (8 %) (tables 1 and 2).

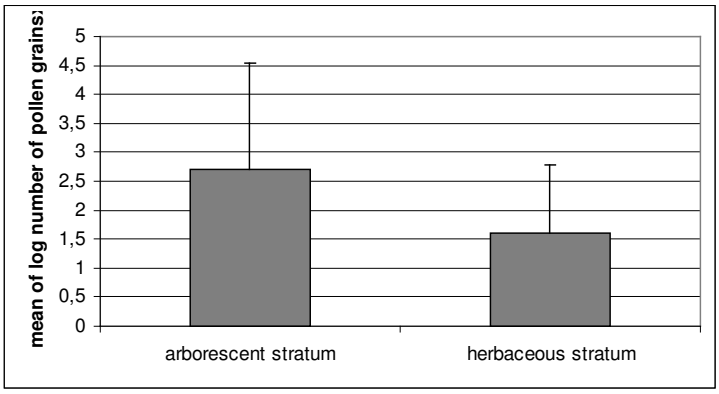

Fig. 2. Comparison of the mean of the log number of pollen grains observed in 32 *Chrysoperla* spp. adults diverticulum contents (50 % empty). Adults collected in the arborescent (mean of log number = 2.70, SD = 1.82) and herbaceous (mean of log number = 1.60, SD = 1.19) strata, from March to May 2004 in field environments in western France.

- From June to August

From June to August, the pollen analysis seems to confirm the observations of Principi & Canard (1984) and Duelli (1986): Chrysopidae are considered mainly nocturnal fliers in the herbaceous stratum whereas during the day the arborescent stratum is their resting place. The mean pollen quantity in specimens collected during the day is very low because the pollen have apparently been digested ($Fc = 5.26 >> F\alpha = 3.95$, $\alpha = 0.05$, $df = 85$) (fig.3).
About 24 % of lacewings collected by day in this period had empty diverticula. The major plant families found were: (i) for wild plants, Chenopodiaceae (54 % of *Chrysoperla* crops with pollen contents), Gramineae (33 %), Asteraceae (10 %), (ii) for field crops, Liliaceae (26 %: leek, *Allium porrum* present on the sites), Brassicaceae (19 %: *Brassica oleracea*) and Apiaceae (18 %: carrot, *Daucus carota* present on one site) (tables 1 and 2).

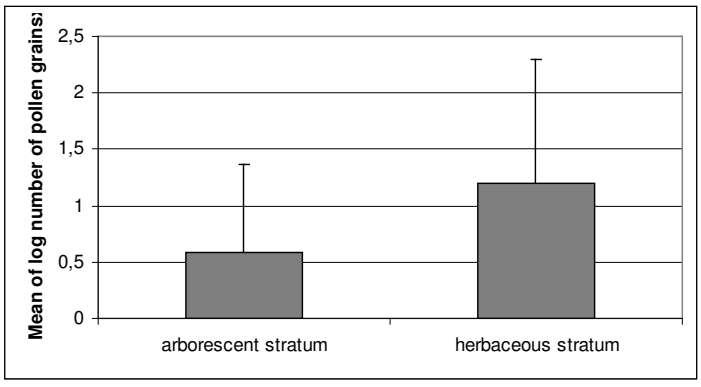

Fig. 3. Comparison between the diverticulum contents of 125 *Chrysoperla* adults (32 % empty) collected in the arborescent stratum (mean of log number = 1.20, SD =1.13) during the day and in the herbaceous stratum (mean log number = 0.59, SD = 0.60) during the twilight, from June to August 2004 in field environments in western France.

		March		April		May		June		July		August	
Family	Species \ week rank	12	14	16	18	20	22	24	26	28	30	32	34
Apiaceae	*Daucus carota*								■	■			
	Apium, Aethusa												■
Amaranthaceae	*Amaranthus* sp								■	■	■	■	■
Aristolochiaceae	*Aristolochia clematitis*			■									
Asteraceae	*Cirsium arvense*									■			
	Senecio sp			■							■		
	Helianthus annuus										■		
Betulaceae	*Corylus avellana*			■									
	others												
Brassicaceae	*Capsella bursa-pastoris*		■	■									
	Brassica oleracea										■	■	■
	Brassica rapae				■								
Caprifoliaceae	*Sambucus* sp			■									
Caryophyllaceae	*Cerastium* sp		■	■									
	Stellaria sp			■									
Chenopodiaceae	*Chenopodium* sp								■	■	■	■	■
Pinaceae	*Pinus* sp				■				■			■	■
Ericaceae	*Vaccinium* sp											■	
Fabaceae	*Coronilla* sp												■
	Medicago sativa												■
	others										■		■
Gramineae									■	■	■	■	
Lamiaceae	*Lamium purpureum*			■									
Liliaceae	*Allium porrum*										■	■	
Ranunculaceae	*Ranunculus bulbosus*		■										
	others											■	
Rosaceae	*Amelanchier* sp			■									
	Malus domestica			■									
	Prunus avium		■	■	■								
	Pyrus pyraster			■									
	Spiraea sp			■									
	others	■	■	■									
Solanaceae	*Lycopersicum esculentum*											■	
Taxaceae	*Taxus baccata*			■									
Tiliaceae	*Tilia* sp											■	
Urticaceae	*Urtica dioica*										■		

Table 1. Plant species consumed by *Chrysoperla* spp. from March to August 2004 in western France: black squares indicate the periods in which pollen grains of a particular plant species were found in the diverticula of adult lacewings.

Food according to sex status

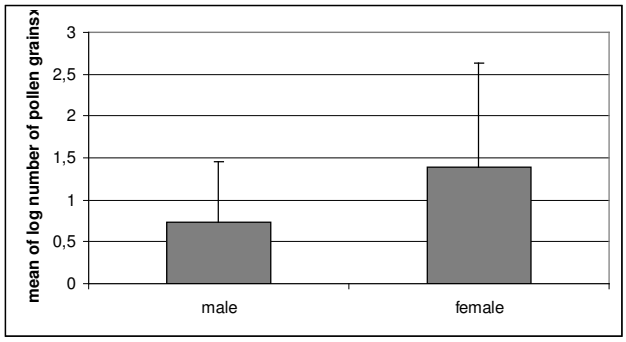

Fig. 4. Comparison between the mean number of pollen grains present in the diverticula of male (SD = 0.72) and female (SD = 1.23) of 157 *Chrysoperla* (77 males, 80 females) collected from March to August 2004 in field environments in western France.

Diverticula of *Chrysoperla* females contained significantly more pollen grains (mean number = 1137) than did those of males (mean number = 44) (after log transformation Fc = 8.87 >> Fα = 3.96, α = 0.05, df = 84, significant difference) (fig. 4). Furthermore, 59 % of empty diverticula belong to males. The results of pollen analyses suggest that male energetic needs are limited to precopulatory searching.

Comparison between the sibling species

The ecological demands and biological traits are similar for the two species of lacewings present in crops of western France. *Chrysoperla lucasina* is a holomediterranean species (Henry *et al.,* 1996; Thierry *et al.,* 1996; Aspöck *et al.,* 2001) and is more adapted to high temperatures, while the other species is a North European species (Thierry *et al.,* 1996). Both species feed in low vegetation (field and herbaceous stratum) beginning in later spring. Our results show a difference in food consumed between these green lacewings (table 2). The number of different plant species consumed is very low compared to the total possible range. According to Monserrat and Marìn (1994), *Chrysoperla carnea s. lat.* has no preference for plant family or species because it has been found in all types of vegetation. However, separating sibling species of the *carnea*-complex reveals differences in feeding habits and behaviour. The high ecological versatility of these lacewings facilitates colonisation of various habitats (arborescent and/or herbaceous strata). However, we can see a difference in the range of plant species chosen by the two species, with the common green lacewing feeding on more species of plants than does *Ch. lucasina.* We found a total of 22 plant families and about 40 different species for the common green lacewing, versus 11 plant families and about ten species for *Ch. lucasina* (table 2). So C*h. carnea* sensu Henry is a more polyphagous species than is *Ch. lucasina*.
Both species consume the pollen of flowering plants such as leek (*A. porrum*), carrot (*D. carota*), cabbage or rape (*Brassica* sp) (table 2). However, *Ch. lucasina* seems to prefer field crops and other herbaceous plants while *Ch. affinis* seems to consume the pollens of trees and herbaceous plants. Furthermore, 88 % of *Ch. lucasina* were collected in June when the low vegetation is flowering. *Ch. lucasina* is then specialised in field crops. In previous studies, e.g. Monserrat & Marìn (1994), it was not possible to separate the species among *carnea* group, and that may explain the differences in our results versus those of these previous studies.
Our results for feeding behaviour corroborate the studies on the *carnea* complex : life history and biology (Tauber & Tauber, 1973; Duelli *et al.,* 1997; Henry *et al.,* 1999; Ventura *et al.,* 2000), ecophysiology (Thierry *et al.,* 1994) and ecology (Henry *et al.,* 1996, 2001; Paulian, 2001), characterised, for example, by the way of overwintering (Thierry *et al.,* 1998 ; 2002).
According to Paulian (2001), *Ch. lucasina* is more frequent and abundant in field crops and on herbaceous plants, where it is the first species to appear in spring, while the common green lacewing is rather a more frequent tree canopy inhabitant.
Because much variability occurs among individuals in all analyses, our conclusions must confirmed by further studies. In addition, the presence of pollen in diverticula can also be misleading because lacewings adults feed frequently on honeydew in which pollen grains have become stuck and are therefore also consumed (Duelli, 1999).

Alternative methods to attract beneficial organisms for conservation biological control may consist in growing plants that are preferred as pollen sources for green lacewings in the field neighbourhood. These might include Rosaceae, Caprifoliaceae, Betulaceae and Pinaceae as trees and Caryophyllaceae, Asteraceae, Gramineae and Chenopodiaceae as herbaceous plants

for *Ch. carnea* sensu Henry; and mainly herbaceous plants as Brassicaceae, Gramineae, Apiaceae and Asteraceae for *Ch. lucasina*.

We assume that the adult chrysopids feed on a narrow range of pollen species, that occur mainly in the habitats in which they spend most time. Chrysopidae seem to feed near their resting-place with the bordering crop-vegetation being reservoir habitats for *Chrysoperla* spp. To test this hypothesis further, relations between plants occurring in the agroecosystems and the pollens consumed by *Chrysoperla*, and the possible choice or selection of food by these lacewing species will be investigated. In addition, pollen analysis can be used to study the *Chrysoperla* migration flight from March to October.

	Chrysoperla carnea		*Chrysoperla lucasina*	
	Plant family observed in diverticulum (%)	% of consuming adults	Plant family observed in diverticulum (%)	% of consuming adults
Apiaceae	9.65	4.17	4.65	2.03
Aquifoliaceae	0.88	0.004	---	---
Aristolochiaceae	1.75	0.05	---	---
Asteraceae	5.26	3.15	**18.60**	0.27
Betulaceae	1.75	0.03	---	---
Brassicaceae	**18.42**	16.49	9.30	33.67
Caprifoliaceae	0.88	0.01	---	---
Caryophyllaceae	7.89	4.28	---	---
Chenopodiaceae Amaranthaceae	**20.18**	5.12	**39.53**	1.43
Ericaceae	---	---	2.33	0.02
Euphorbiaceae	2.63	0.03	---	---
Fabaceae	6.14	0.58	11.63	0.26
Gramineae	**16.67**	0.75	**23.26**	0.09
Lamiaceae	0.88	0.20	---	---
Liliaceae	5.26	0.05	**27.91**	**62.10**
Pinaceae	7.02	0.06	---	---
Plantaginaceae	0.88	0.02	---	---
Polygonaceae	0.88	0.24	---	---
Ranunculaceae	1.75	0.49	---	---
Rosaceae	12.28	**62.40**	4.65	0.08
Solanaceae	2.63	1.83	---	---
Taxaceae	0.88	0.004	---	---
Tiliaceae	---	---	2.33	0.002
Urticaceae	4.39	0.03	6.98	0.05

Table 2. Comparison of diverticulum contents (pollen) of 114 adults of *Chrysoperla carnea* sensu Henry and 43 adults of *Ch. lucasina*: plant families and species observed; proportion (%) of adults consuming the family plant. Plant families ranked by alphabetic order.

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