

Identification of plant families associated with the predators *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) and *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) using pollen grain as a natural marker

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Abstract

The predators *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) and *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae), are frequently observed on vegetable crops, especially on tomato plants, as well as on flowers of several plants around crop fields. It is well known that when predators feed on pollen and nectar they can increase their longevity and reproductive capacity. The objective of this work was to identify plants that could be a pollen source for *H. convergens* and *C. externa* in order to develop strategies to attract and keep these predators in vegetable fields like the tomato crop. Adults of *C. externa* (53 individuals) and *H. convergens* (43 individuals) were collected in fields from 2004-2005 at Embrapa Hortaliças, Brasília, Federal District. The insects were processed by the acetolysis method and pollen from them was extracted and identified. A total of 11335 grains of pollen belonging to 21 families were extracted from *C. externa*. A total of 46 pollen grains belonging to ten families were extracted from *H. convergens*. The Poaceae family was the most abundant one for *C. externa* while Asteraceae was the commonest pollen for *H. convergens*. The importance of pollen from different plant species as a food resource for each predator species gives an indication of the importance of plant community structure inside and around crop fields for the establishment of these predator populations and to enhance conservation biological control.

Keywords: entomopalinology, nutritional ecology, natural biological control, predator.

Identificação de famílias de plantas associadas aos predadores *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) e *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) usando o pólen como marcador natural

Resumo

Os predadores *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) e *Hippodamia convergens* Guérin-Ménéville (Coleoptera: Coccinellidae) são frequentemente observados em cultivos de hortaliças, especialmente tomateiros, bem como sobre flores de diversas plantas próximas aos cultivos. Sabe-se que os predadores, quando se alimentam de pólen e néctar, aumentam a sua longevidade e a sua capacidade reprodutiva. O objetivo deste trabalho foi identificar as plantas que poderiam servir de fonte de pólen para as espécies selecionadas, com vistas a desenvolver estratégias para atrair e manter estas espécies em cultivos de hortaliças, especialmente de tomateiro. Adultos de *C. externa* (53 indivíduos) e *H. convergens* (43 indivíduos) foram coletados em 2005 no campo experimental da Embrapa Hortaliças, Brasília, Distrito Federal. Os insetos foram processados pelo método de acetólise e os grãos de pólen, extraídos e identificados. Um total de 11335 grãos de pólen, pertencentes a 21 famílias foi identificado para *C. externa*, enquanto que 46 grãos de pólen, pertencentes a dez famílias botânicas, foram identificados para *H. convergens*. O

pólen da família Poaceae foi o mais abundante para *C. externa*, e o pólen da família Asteraceae foi o mais comum para *H. convergens*. A importância relativa do pólen de diferentes espécies de plantas como recursos alimentares para cada espécie predadora dá indicações da importância da flora dentro e no entorno da cultura para o estabelecimento das populações desses predadores e incremento do controle biológico conservativo.

Palavras-chave: entomopalinologia, controle biológico natural, ecologia nutricional, predador.

1. Introduction

Entomopalynology is the study of how insects use pollen and how pollen grains adhere themselves to the body of the insects during their search for feeding resources. This study allows determining routes of migration, plants that are used for feeding, as well as plants which the insects pollinate (Jones et al., 1995). Pollen is very useful in those studies because they can be identified in family and genus; moreover, the identification of the pollen can supply much information on the geographic origin of the plant (Jones and Jones, 2001). However, the main limitation for this technique is precisely the lack of studies about identification and classification of pollen that are found in a given region of the country (Silberbauer et al., 2004). In the Distrito Federal, Brazil, this problem is not a constraint, since there is an important palynological study of the regional vegetation, the scrubland, including identification keys (Salgado-Laboriau, 1973), which makes studies involving palynological analysis possible.

Entomopalynology has been frequently used to study movements of bees and identification of alternative sources of feeding for pest insects. There are few studies on the floral resources exploited by predators considering the knowledge of the alimentary habits and migration movement of pest insects and natural enemies are important for the development of more effective methods to control crop pests (Jones and Jones, 2001, Silberbauer et al., 2004).

Takasu and Lewis (1995) showed that the female of parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), which are not fed, initially search and forage on plants and only after feeding themselves, they search for a host for parasitising it. This behaviour improves the retention and efficiency of search in the field, compared with the females that had been fed before being released, which shows the importance of food presence for parasitoids in the field. Wäckers (2004) in his study concluded that the mere presence of flowers in one agro ecosystem is not enough to guarantee nectar supplement for parasitoids. Besides attractiveness, it is also necessary that the resource is accessible. Therefore, these studies have shown that it is important to identify the plants used by the natural enemies for the development of handling strategies so that the natural enemies are kept within the fields.

Palynological analysis of insects can be applied to ecological study by the identification of the plant types used as food. From the agronomical point of view, the resultant information can be directly applied in the agro ecological delineation of cultivation, since the plant

species chosen to diversify the environment can be the predator insects preferred species.

The green lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) presents a wide geographical distribution and is native of the Neotropical Region (Albuquerque et al., 1994). The ladybeetle *Hippodamia convergens* Guérin-Ménéville, 1842 (Coleoptera: Coccinellidae) also presents a broad geographical distribution in North and South America. Both are predators frequently found in the tomato crop. Furthermore, *C. externa* was observed predating larvae of *T. absoluta* (França et al., 2000). Although it prefers to prey on aphids, it has a broad variety of prey in its diet. *Chrysoperla* spp. adults usually feed on pollen and nectar or plant secretions containing sugar (Freitas, 2002). At times of scarcity, it can feed on insect eggs, mites and small caterpillars (Hodek, 1973). Study in the laboratory has shown that *H. convergens* develops normally when it is fed on an egg diet of the *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Kato et al., 1999). As there is not much information on the complementary sources of feeding of these species, the objective of this work is to identify the families of plants that these predators use as a pollen source. For this, the pollen was used as a natural marker.

2. Material and Methods

2.1. Collection site

The study site was conducted at two different sites in different seasons: the rainy season (October to March) and the dry season (April to September). The Embrapa Hortaliças experimental field is located at Brasília, DF, Brazil. The vegetable crops have been under organic management since 2001. The organic area is situated at about 250 m from a riparian forest (Cerrado), and is intercropped with onion, carrot, broccolis, cucumber and garlic. These crops are rotated with corn and maize and sorghum are used as green fertilisers. It is surrounded by tracks of the Mexican sunflower *Tithonia diversifolia* (Hemsl.) (Asteraceae) and uncultivated areas are maintained with spontaneous vegetation, with Napier grass, *Pennisetum purpureum* Stapt. (Poaceae) and Brachiaria grass *Brachiaria decumbens* Schum (Poaceae) predominating.

Others collections were made at an organic farm located at PAADF, DF, Brazil. This farm is generally intercropped with more than twenty vegetable crops. These crops use maize and sorghum as green fertilizers and are bordered by the Mexican sunflower *Tithonia diversifolia* (Hemsl.) (Asteraceae). Uncultivated areas were main-

tained with spontaneous vegetation and were closer to the natural vegetation (Cerrado).

2.2. Populations

Adult *C. externa* and *H. convergens* were collected in organic vegetables crops at Programa de Assentamento Dirigido do Distrito Federal - PADF and at Embrapa Hortaliças Experimental Station. The PADF population was collected on 11/04/05 and Embrapa Hortaliças populations were collected from August to October 2005. Insects were collected from the plants using an entomological net or a vial of 75 × 25 mm. After field collection, the insects were spread out in a box containing ice, transported to Embrapa Hortaliças laboratory and stored in a freezer (−18 °C) until processing time.

The insects collected were taken to the laboratory where they were counted, placed in 5 mL glass vials and kept in a freezer. To identify the plant family, genus or species on which the insects were feeding when captured, the pollen grains contained in their intestines were examined using the Erdtman acetolysis process (Erdtman, 1960). The analyses were conducted in the Entomology Laboratory of Embrapa Vegetables, Brasília, Brazil.

2.3. The acetolysis process

Acetolysis is a chemical process that dissolves the insect's body but not the pollen grains. The predators were taken from the freezer one day before being subjected to acetolysis, cleansed with 99.3% ethylic alcohol and then placed on absorbent paper to dry. Each insect was then put in a 1.5 mL Eppendorf tube and crushed with a toothpick. The following steps were conducted in an exhaustion hood: for each set of 48 tubes, a solution of acetic anhydride and sulfuric acids in the proportion of 9:1 (27:3 mL), respectively, were prepared. About 0.5 mL of this solution was poured in each Eppendorf tube and brought to boil at 120 °C for seven minutes in Termolyne®. Glacial acetic acid was added to each Eppendorf tube after boiling to stop the acetolysis reaction. Each group of 16 tubes was then centrifuged for 3 minutes in a horizontal centrifuge at the maximum speed, 5,000 rpm. After discharge of the supernatant, the pollen grains deposited in the bottom of the tubes were washed with distilled water three times and centrifuged again after each washing. Before each centrifugation the solution was stirred with a toothpick. After the last wash, the excess in each tube was discharged and two drops of the colorant Saffrin solution + ethylic alcohol was added. Following that, the solution was centrifuged once more for three minutes, the supernatant was discharged and eight drops of glycerin were added. After this process, each set of 16 tubes was heated for 16 hours at 32 °C for the alcohol to evaporate (Jones and Coppedge, 1996).

2.4. Pollen identification

Each tube of the resulting solution from the above treatment contained the pollen grains ingested by one predator. Two drops of this solution were placed on a microscope slide, covered with a coverslip and fixed at

the sides with nail polish. The slides were photographed using an Axophot optic microscope equipped with camera. The identification of the pollen grains was made using identification keys for pollen types of the Cerrado flora and other pertinent publications (Heusser, 1971; Salgado-Labouriau, 1973, 1982, 1984; Franz et al., 1986; Faegri and Iversen, 1989; Salgado-Labouriau and Rinaldi, 1990). The taxonomic classification of the pollen types to the lowest taxonomic ranking possible was made by MAM and MLSL.

2.5. Statistical analyses

Data from each species and between them were statistically compared by applying the Percentage of Similarity and Simpson Diversity Index tests. The programme used was Ecological Methodology (Krebs, 2002).

3. Results

A total of 53 individuals of *C. externa* were processed by the acetolysis method and in 45 insects (85%) a total of 11,353 grains of pollen of 21 botanical families were found (Table 1 and 3). The number of grains found per individuals varied from one to 4,469. Poaceae pollen was present in 38 individuals (86% of the processed total), in an average of 214 grains of pollen per insect, 97% of the grains were of this family. A total of 43 individuals of *H. convergens* was processed and only 22 (51%) presented pollen (Table 2 and 3). Forty-six pollen grains belonging to the nine botanical families were found. The predator presented an average of 1.0 grain of pollen per individual and the number of grains ranged from 1 to 10.

For *C. externa* a high similarity between the samples was observed (Index of Renkonen = 98.1). On the other hand, for *H. convergens* differences between the samplings were observed (Index of Renkonen varied from 23.3 to 45.0). The most similar samplings were the ones from September and October (Index of Renkonen = 45.0) and the most divergent ones were the ones from April and October (Index of Renkonen = 23.3). Although *C. externa* and *H. convergens* presented common pollen from eight botanical families, the similarity compared between the species was low (Index of Renkonen = 14).

The diversity index for *C. externa* was very low (Index of Simpson = 0.070 with a trend of 0.001) and for *H. convergens* was higher (Index of Simpson = 0.810).

4. Discussion

For *C. externa*, pollen of plants from the Poaceae family dominated (probably *P. purpureum* and *B. decumbens*, present at the collection site) not only in frequency of occurrence but also in total number of grains (Table 1). Some families such as Moraceae, Smilacaceae and Myrtaceae presented more than one type of pollen, showing variability which is presented within the fami-

Table 1. Pollen grains and botanical family, genus and species found in the digestive tract of *C. externa*. DF, 2005-2006.

	August	September	Total
Amaranthaceae			
<i>Alternanthera</i>	1	-	1
Apiaceae	3	-	3
Apocynaceae	1		
<i>Laseguea acutifolia</i>		1	2
Aristolochiaceae			
<i>Aristolochia</i>	-	1	1
Asteraceae			
Tipo <i>Aspilia</i>	10	1	11
Bromeliaceae	1	-	1
Caryophyllaceae	-	10	10
Chenopodiaceae	2	-	2
Cucurbitaceae			
<i>Cerathosanthus</i>	-	1	1
Euphorbiaceae		1	
<i>Tragia lagoensis</i>	1		2
Fabaceae			
<i>Zornia</i>	6	-	6
Smilacaceae	5	1	
Tipo <i>Smilax</i>		1	7
Melastomataceae-Combretaceae	1	10	11
Myrtaceae	-	52	52
Moraceae	3	6	
Tipo <i>Brosimum</i>	1		10
Opiliaceae			
<i>Agonandra brasiliensis</i>	2	-	2
Pinaceae	-	1	1
Poaceae	7088	4071	11159
Pteridophyta*	-	1	1
Styracaceae			
Tipo <i>Styrax</i>	2	-	2
Trigoniaceae	48	-	48
unidentified	4	2	6
Total	7178	4165	11336

*spore

lies. The majority of the individuals showed only one type of pollen grain. However, there was an individual that presented nine types of pollen. This shows that the insect seeks the pollen by visiting a variety of plants.

The most common pollen grain found in *H. convergens* belonged to the Asteraceae family, which was present in all samples, followed by Apiaceae, Leguminosae and Poaceae. Some families such as Asteraceae and Leguminosae presented more than one type of pollen, which indicated that the insects had fed on different plants. The families Arecaceae, Caryophyllaceae and Moraceae were found exclusively in individuals collected in the PADF. The majority of the *H. convergens*

individuals presented only one type of pollen, but there was an individual that presented five types of pollen (Table 2). A more equitable pollen distribution was observed for the four most common types of pollen collected in *H. convergens*.

By comparing the pollen types set used by *C. externa* in the months of August and September, a high similarity between the samples was observed. This shows that the standard use of the pollen in August and September was similar, or else, it presented great abundance of grass and numerous rare species. This result can present the influence of space and time proximity between the samples, but it is also possible that it is common for the species

Table 2. Pollen grains and botanical family, genus, species found in the digestive tract of *H. convergens*. DF, 2005-2006.

	April	August	September	October	Total
Amaranthaceae					
<i>Alternanthera</i>	-	-	1	-	1
Apiaceae	-	3	-	3	6
Arecaceae	3	-	-	-	3
Asteraceae					
<i>Aspilota</i> Type 1	1	1	2	8	
<i>Aspilota</i> Type 2				4	16
Caryophyllaceae	2	-	-	-	2
Chenopodiaceae	2	-	-	2	4
Leguminosae	-	1	3	1	6
<i>Mimosa</i>				1	
Moraceae	1	-	-	-	1
Pinaceae	-	1	-	-	1
Poaceae	3	2	-	1	6
Pollen grains (Total)	12	8	6	20	46

Table 3. Total number of adult predators analysed, percentage of pollen grains found in the digestive tract of the insects in each experimental area and number of plant families identified.

	<i>C. externa</i>	<i>H. convergens</i>
Number predator analysed	53	43
Number predator with pollen	45	21
% pollen	84.9	51
Number pollen grains	11336	46
Number pollen grains / Number predator with pollen	252	2
Botanical Family	21	9

that present great association with grass (Albuquerque et al., 1994). For *H. convergens*, the differences between the samplings were observed. The most similar samplings were the ones from September and October and the most divergent ones were from April and October, which represented different regions and periods of flowering (Almeida et al., 1998).

The low similarity found between *C. externa* and *H. convergens* is due to the differences in the relative abundance of each botanical family, especially for the great abundance of Poaceae in relation to *C. externa*. The diversity of plants used by the *C. externa* was very low, which showed sample homogeneity. Despite the great number of pollen grains found, the grasses were the dominant ones. For *H. convergens*, the diversity was higher resulting from the higher equitability in the abundance of pollen grains between species, although it presented about half of the taxa richness observed for *C. externa*. These differences have direct implications in the way that *H. convergens* use the resource as will be argued later on.

The frequency of use and the relative importance of pollen as an alimentary resource was different for

C. externa and *H. convergens*. The most likely explanation for this fact is that either *H. convergens* only occasionally exploits pollen as a food source or maybe it is more exploited by the females. Wratten et al. (1995) observed a greater proportion of females of the *Melangyna novaezelandiae* predator (Macquart) (Diptera: Syrphidae) containing pollen. Irvin et al. (1999) have shown that there are some differences in pollen consumption by the syrphid flies according to the season of the year and sex; therefore, the phenology of the syrphid flies is directly associated with the seasonal distribution of pollen. As the pollen grains are important for the sexual maturation of the syrphid eggs of the studied species, the presence of floral resources can increase the population of these species in agroecosystems.

Grasses are an important source of resource for *C. externa*, yet a great diversity of plants can also be used by them. Our studies indicated that pollen ingestion must be of greater importance for *C. externa*, to obtain energy not only to activate reproduction as occurs in the majority of generalist predators as, for example, *H. convergens*. In general, the adults of predators have the same feed-

ing habit of immature stages, as in the coccinellid case. However, neuroptera and many Diptera change their alimentary habits when adults and present similar nutritional requirements to herbivores (Garcia, 1991). It is important to clarify that little is known about the preferential food of each species (Freitas, 2002). However, in accordance with Principi and Canard (1984), the *Chrysoperla* genus, when adult, only eats pollen and nectar; the data found for *C. externa* are compatible, showing herbivorous alimentary habits. Comments on the behaviour of foraging of larvae of Neuroptera have shown that floral resources are used in the larval diet of *Chrysoperla plorabunda* (Fitch.) (Neuroptera: Chrysopidae) in cotton; and that the consumption increases when the local availability of prey diminishes. The authors concluded that the larva of *C. plorabunda* is omnivorous, instead of being carnivorous as had been affirmed in the literature (Limburg and Rosenheim, 2001). Yee (1998 apud Silberbauer et al., 2004) analysed the grains of pollen ingested by neuropteran *Mallada signatus* (Schneider) of the Chrysopidae family and also found great amounts of pollen in the gut, especially of *Eucalyptus* spp. (Myrtaceae). The work of Patt et al. (2003) have shown that the inclusion of pollen and sucrose in low quality diets for larvae of *Chrysoperla carnea* Stephens diminished the developmental time and generated bigger adults. Therefore, the evidence that the ingestion of supplementary alimentary items improve the survival, growth and the fecundity of omnivores, especially under conditions of scarcity of prey, increases. Anyway, the fact that *C. externa* and *H. convergens* feed themselves on pollen, to a greater or lesser amount, is advantageous, mainly at times of food scarcity. Eubanks and Denno (1999) verified that the predator chinch-bug *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae) feeds itself on more than a trophic level, which allows its survival when the resources are of low quality (green-flies) or unavailable. Moreover, the population dynamic of omnivorous insects is intimately associated with variations in its host plants and not only with the density of its prey. On the other hand, the study of Cottrell and Yeargan (1998) with the ladybug *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) showed that the abundance of pollen increases the larval population, yet it did not increase predation. This suggests that abundance of pollen diverts *C. maculata* from predatory acts.

Another important aspect for the ecology of *Chrysoperla* is that since the adult feeds on pollen and nectar, the place chosen by the females for oviposition often does not follow an orientation of feeding adjusted for the future larvae. Therefore, eggs can be laid in places where there is no prey for the larvae (Duelli, 1987). In corn, the presence of a great amount of chrysopid eggs is common (more than five eggs/plant on average); however few larvae are seen. The factors that influence larval survival are not very well known (Andow, 1996). However, analyses of pollen over a long period will be able to disclose much about the nutritional needs of the chrysopids.

In relation to the type of plant used, *C. externa* preference for plants of the Poaceae family, which constitute one of the largest and most widely distributed families of vascular plants, was evident; plus, they are dominant in many environments, as well as in some physiognomic types of scrubland. Grass blossoms occur during the whole year, yet there is a peak of pollen production from April to June. In accordance with Salgado-Laboriau and Rinaldi (1990), the grain size of pollen varies from 17.5 up to 60.6 μm maximum diameter. A small difference in ornamentation together with grain size allows the distinction of groups or species. In this work, the measure of the grains of grass was not carried out. However, due to the great difference in size which was found, it is very likely that at least two types of grass have been explored: native species, which present smaller grains of pollen and represented 90% of the pollen of Poaceae and the cultivated species of grass, for example, corn, which presents bigger grains of pollen and constituted 10% of the Poaceae pollen. Another interesting aspect is the diversity of the plants explored by *C. externa* which ingested Pteridofita spores (trilete - spores) and other angiosperms (among others). The work of Venzon et al. (2006) has shown that the female of *C. externa*, when fed with pollen of forage plants likes Pigeon pea (*Cajanus cajan*) or Sunn hemp (*Crotalaria juncea*), displayed better development when complemented with honey. This suggests that the organic systems of coffee could increase the effectiveness of this predator if it is diversified with plants that supply pollen and nectar. For comparative effect, it would be interesting that similar work included grasses as a source of resources.

The diversity of pollen found in the digestive system of *H. convergens* suggests that it forages actively in different plants, with fast capacity of movement on the vegetation. A palynological analysis over a long period for both species would be important to determine the use of floral resources throughout the year. Haslett (1989) made an analysis of pollen for six species of syrphid flies, concluding that some of the studied species are highly selective in their diet of pollen, while others are generalists.

Pollen analysis is important for the identification of plants that are frequently used by predators, so that these plants are included in agroecosystems as a way to keep the predators in the environment. Prasifka et al. (1999) have shown that some predators, as for example, *H. convergens*, moves within parcels next to cotton and sorghum grain. This indicates that sorghum grain can be used as predator maintaining the source in the cotton field. An advisable practice would be to form around the cultivated area, cultivation protection barriers with the species of the more abundant families, that is, grass for *C. externa* and *H. convergens* which could be Asteraceae combined with Apiaceae or Leguminosae or Poaceae. If it is possible, it can be synchronised with the added species period of budding with the most critical period of the culture, that is, when the damage caused by the insect pest may result in bigger losses of productivity. The

planning of environmental diversification is important so that there is always pollen availability. In this way, several strategies are possible to be implemented in a way to guarantee an ecological handling of the environment, as for example, polycultivation (so that one of the plants does not necessarily need to be aimed at production); moreover, the establishment of bands of cultivation to attract beneficial insects inside or around the cultivation area; furthermore, surrounding vegetation that includes the botanical families that are present in the samples in the cultivation adjacencies.

This work has shown that although pollen grains are very important for predators, different species will present different necessities. For a better understanding of the role of the plants as the predator alimentary resource, it is important to determine the habitat use and local movements by studying the ingested pollen by generalist predators, throughout the seasons of the year, inside and outside the cultivated areas, as well as in its surrounding vegetation. The types of pollen which are found in the insects depend on the period of the year, where they are collected, as well as of the presence and the variety of the budding, which is seasonal (Silberbauer et al., 2004). The understanding of the space and seasonal movement of herbivores is useful for the handling of insect-pests (Gregg et al., 2001). In the case of predators, they can be manipulated by adequate foraging supply when necessary (Silberbauer et al., 2004). In accordance to Ambrosino et al. (2006), the use of plants in agro ecosystems as source of resources for natural enemies is a practice which has increased, but the attractiveness of the candidate plants has not been tested for the main arthropods. To know the form by which the plant species are used by predators could be useful for the adequate handling of the insect community, attracting the beneficial insects for the cultivation places by means of inclusion of its favourite plants next to or within the cultivation area.

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