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Chrysopid Predators and their Role in Biological Control

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ABSTRACT

The family Chrysopidae includes many species that could be considered important biological control agents. Chrysopid larvae and the adults of certain species are polyphagous predators and feed on several pests of economic importance. The use of chrysopids in biological control has been enabled by the development of efficient mass-rearing facilities for species bearing certain characteristics, such as *Chrysoperla* species. From the different approaches used in implementing biological control, augmentation release is the most commonly applied method in the case of chrysopids. To date, only some *Chrysoperla* sp. are commercially available for use in biological control. Food supplements, attractants and hibernation boxes are also available for enhancing conservation of chrysopids. Difficulties in the systematics of chrysopids have made the release of *Chrysoperla* sp. more problematic. The evaluation of the efficacy of chrysopid species that share common characteristics with *Chrysoperla* sp. as potential biological control agents, as well as further studying the performance of *Chrysoperla* species under field conditions could enhance their role in Integrated Pest management (IPM).

Key words: Chrysopidae, biological control, predators, augmentation, conservation

INTRODUCTION

Several chrysopid species are included among the most important aphidophagous predators. Their efficacy in biological control of aphids, as well as other arthropod pests has been well recognized for more than 250 years (Senior and McEwen, 2001). Due to the larval polyphagous feeding habits, chrysopids are considered to be important natural enemies of several pests besides aphids, such as whiteflies, thrips, lepidopteran pests and mites (Principi and Canard, 1984). In the context of biological control, most attempts have evaluated the efficacy of *Chrysoperla carnea sensu lato* in augmentation releases in the field or in the greenhouses (Ridgway and McMuphy, 1984; Hagen *et al*., 1999; Nordlund *et al*., 2001).

An important issue in the successfull use of chrysopids in biological control is the problematic systematics that still persists in the identification of *Chrysoperla* species (Tauber *et al*., 2000). Failures in chrysopid releases may, at least to some extent be explained by importations of wrongly identified species (Senior and McEwen, 2001).

In cases when the use of commercialy available chrysopids such as *Chrysoperla carnea* (Stephens) or *Chrysoperla rufilabris* (Burmeister) is problematic, other species could be exploited.

Species of the genera *Mallada* Navás, *Dichochrysa* Yang and *Ceraeochrysa* Adams might be proven efficient candidates for use in biological control (López - Arroyo *et al.*, 1999a, b; Tauber *et al.*, 2000; Daane, 2001). Data on the predation efficacy of other chrysopid species besides *Chrysoperla* are limited and mainly referred to laboratory conditions.

Research on chrysopid species occurs worldwide and comprises recently an important section in applied entomological research. The International Association for Neuropterology (IAN, www.neuroptera.com) consists a scientific forum that provides the opportunity for communication between researchers interested in the study of Neuropteroidea in general. Many of the IAN members are actively involved in the study of the biology and use of chrysopids in biological control programs. Their work has been well documented and summarized in the Proceedings of International Symposia organized by the Association. Ten Symposia have been held since 1982 and the Proceedings of most of them have been published or are currently in press (Gepp *et al.*, 1984, 1986; Mansell and Aspöck, 1990; Canard *et al.*, 1992, 1996; Panelius, 1998; Sziráki, 2002; Pantaleoni *et al.*, 2005 (2007).

Research on all aspects of the biology of chrysopids and their use in biological control has been comprehensively reviewed since 1984 in two books, the 'Biology of Chrysopidae' edited by Canard *et al.* (1984) and "Lacewings in the Crop Environment" by McEwen *et al.* (2001). In the present study, the integration of most important factors that could affect and shape the efficacy of chrysopid species in biological control, as well as considerations for further research have been reviewed.

FAMILY CHRYSOPIDAE: THE MOST IMPORTANT GENERA FOR USE IN BIOLOGICAL CONTROL

The family Chrysopidae contains about 1200 species that belong in 75 genera and 11 sub-genera. The larvae of all species, as well as the adults of certain species are predaceous and could be important biological control agents of several soft-bodied arthropods, such as aphids, coccids and mites. Due to their important role in biological control programs, the species of the family Chrysopidae are the most well studied among Neuroptera (Brooks and Barnard, 1990).

Chrysopid systematics have been problematic for a long time with a great number of species from different places of the world and different time periods to be wrongly referred to as species of the genus *Chrysopa* Leach (New, 2001). An important contribution to avert these conflicting and contradictory assumptions was the review of Brooks and Barnard (1990) that identified and presented 1200 species of the family with regard mainly to the morphology of male genitalia. These species have been categorized in three sub-families, namely Nothochrysinae, Apochrysinae and Chrysopinae based on adult morphology (Brooks and Barnard, 1990). The Chrysopinae includes almost 97% of well known chrysopid species and specifically all those species of great economic importance commonly attracted in houses by lights during night (Brooks and Barnard, 1990; De Freitas and Penny, 2001). The species that belong to the genera *Dichochrysa, Chrysopa, Ceraeochrysa* and *Chrysoperla* Steinmann are considered to be the most important and abundant biological control agents (Brooks and Barnard, 1990; Albuquerque *et al.*, 2001; Daane, 2001).

Dichochrysa is to date the largest in species number genus of the family Chrysopidae with at least 130 described species that can be found worldwidely but not at the Neotropics (Brooks and Barnard, 1990). According to morphological, biological and phylogenetic studies, many more *Dichochrysa* species will be discovered in the future in other places of the world (Aspöck *et al.*, 1980; Brooks and Barnard, 1990; Dong *et al.*, 2004). Many species formerly described in the genus *Mallada* by Brooks and Barnard (1990), are now considered to belong to *Dichochrysa* based on morphological features (Brooks, 1997), although differences in their biology remain to be defined (New, 2001). Furthermore, the genus *Anisochrysa* Nakahara is considered to be synonym to *Mallada* (Dong *et al.*, 2004). According to Daane (2001), species that belong to *Mallada* genus will prove to be efficient biological control agents in the future. *Dichochrysa* species have been recorded in several field crops such as fruit and nut orchards, vegetables, ornamentals, as well as in forests (Greve, 1984; New, 1984; Séméria, 1984; Zelený, 1984; Duelli, 2001; Szentkirályi, 2001a, b). Only the trash-carrier larvae are predaceous, whereas the adults feed mainly on pollen and honeydew (New, 2001).

Several species of the family Chrysopidae have been for a long time mistakenly considered to belong to the genus *Chrysopa*. According to Brooks and Barnard (1990), *Chrysopa sensu stricto* genus includes approximately 50 species and some sub - species. Both the adults and larvae are predaceous (New, 2001).

The genus *Ceraeochrysa* includes 46 species and is the dominant genus in the Neotropics (Brooks and Barnard, 1990; Tauber *et al.*, 2000).*Ceraeochrysa* species are recorded from Canada to Argentina and mainly in the tropics (De Freitas and Penny, 2001). They are commonly found in orchards and several agroecosystems where they are important biological control agents (Lopez-Arroyo *et al.*, 1999a, b; Tauber *et al.*, 2000; Albuquerque *et al.*, 2001).

The genus *Chrysoperla* includes approximately 36 species that can be found worldwidely (Brooks and Barnard, 1990). Their biology and ecology have been extensively studied and these are the species that are mainly used in biological control programs (Brooks, 1994; New, 2001). However, *Chrysoperla* systematics are currently problematic, especially for those species of the-*carnea* group (Brooks, 1994). The adults are not predaceous and feed on honeydew and pollen, whereas larvae are polyphagous predators (New, 2001).

GENERAL BIOLOGICAL ASPECTS OF CHRYSOPIDS

The biology of the most well studied chrysopid species has been reviewed by Canard and Principi (1984), Canard (2001) and Canard and Volkovich (2001).

Chrysopid eggs are oval, white, yellow or green, their size ranges from 0.7 to 2.3 mm and are usually laid on the edge of a silken stalk (with the exception of the species that belong to the genus *Anomalochrysa* McLachlan which lay unstalked eggs) (Gepp, 1984). The stalk is assumed to play an important defensive or nutritional role for the newly hatched larva and its length is a species specific characteristic that may vary from 2 to 26 mm and be influenced by the mother's body size, as well as environmental conditions (Tauber *et al.*, 2003). Recently, two silk genes expressed by *Mallada signata* (Schneider) females were identified and sequenced by Weisman *et al.* (2009).

Eggs are usually laid by the female singly or in clusters (Duelli, 1984; Gepp, 1984) on the underside or on the top of leaves or shoots where there is plenty of food facilitating its discovery by the young larvae (Duelli, 1984). Embryonic developmental time depends mainly on temperature. For example, *C. carnea* embryos develop in about 13 days at 15°C and 2.5 days at 35°C, whereas for *Chrysoperla externa* (Hagen) in 14 and 4 days, at 15.6 and 26.7°C, respectively (Canard and Principi, 1984; Albuquerque *et al.*, 1994).

Chrysopid larvae (three larval instars) can be divided in two groups in respect to their morphology and behavior (Gepp, 1984; Diaz-Aranda *et al.*, 2001; Tauber *et al.*, 2003). In the first group, the debris-carrying larvae or trash-carriers (e.g., larvae of the genera *Dichochrysa, Ceraeochrysa, Glenochrysa* Esben-Petersen, *Leucochrysa* McLachlan, *Italochrysa* Principi), collect with their mouthparts several pieces of plant material, exuviae or dead prey items and place them on their dorsa, thus forming a small 'packet' of 'trash' that is supposed to protect them from ants and other natural enemies (Eisner *et al*., 1978). In the second group, the larvae do not carry trash on their dorsa and are naked (e.g., *Chrysoperla* larvae).

Larval development is mainly influenced by temperature (Canard and Principi, 1984). In most cases, developmental time is shorter in *Chrysoperla* sp. in relation to *Dichochrysa* sp. or *Nothochrysa* McLachlan sp. For example, the larval developmental time for *C. externa* ranged from 46.5 days at 15.6°C to 12.2 days at 26.7°C (Albuquerque *et al*., 1994), whereas for *Dichochrysa prasina* Burmeister ranged from 85 to 19.7 days at 15 and 30°C, respectively (Pappas *et al*., 2008a). All chrysopid larvae are polyphagous predators with a broad range of prey, such as aphids, coccids, cicadellids, whiteflies, thrips, psyllids, eggs and larvae of Lepidoptera, Coleoptera, Diptera or Neuroptera and eriophyid or tetranychid mites (Canard and Principi, 1984; Canard, 2001). Prey location is usually random but there are also cases when it has been mediated to a certain extent by the honeydew produced by the prey (Canard, 2001). There are also chrysopid species that are highly specific to a certain prey species, such as *Chrysopa slossonae* Banks larvae which are only associated with the wooly alder aphid, *Priciphilus tesselatus* (Fitsch) (Tauber and Tauber, 1987; Milbrath *et al*., 1993). Larval prey quantity and quality may have a great influence on preimaginal development, as well as on the reproductive potential of adults (Principi and Canard, 1984; Canard, 2001).

Upon the completion of larval development, the third instar fully grown larva spins a cocoon and remains inside it until adult emergence (Canard and Principi, 1984). Cocoons are usually placed on the plant, inside curled leaves, on the leaves or in the soil (Canard and Volkovich, 2001). The development of the cocoon usually lasts for one or two weeks and depends on several factors, mainly temperature and sex. For example, cocoon development in *C. externa* was completed in 7.1 days at 24°C, whereas for *Chrysopa pallens* (Rambur) in 12.7 days at 20°C (Grimal and Canard, 1990; Carvalho *et al*., 1998).

Most of chrysopid adults are not predaceous and feed on plant derived food, such as nectar and pollen, as well as with insect honeydew (e.g., aphid or coccid honeydew). Pollen grains have been found in relatively high quantities in the digestive trunk of *C. carnea* and *D. prasina* adults (Bozsik, 1992, 2000; Villenave *et al*., 2005). The adults of species of the genera *Anomalochrysa*, *Atlantochrysa* Hölzel and *Chrysopa* are considered to be predaceous (Brooks and Barnard, 1990). Besides several insects, such as aphids, coccids and mites, other food type like pollen grains, fungi spores and yeast have also been identified in the midgut of predaceous chrysopid adults (Principi and Canard, 1984; Bozsik, 1992; Canard, 2001).

To date, published research articles have mainly focused on the effects of several factors on certain biological aspects of chrysopids relative to their use in biological control. A great number of researchers have studied the effects of several abiotic factors, such as temperature (Honěk and Kocourek, 1988; Tauber *et al*., 1987, 2006; Albuquerque *et al*., 1994; Volkovich and Arapov, 1996; López-Arroyo *et al*., 1999a; Nakahira *et al*., 2005; Mantoanelli *et al*., 2006; Silva *et al*., 2007; Pappas *et al*., 2008a, c), photoperiod (Tauber and Tauber, 1972; Hodek and Honěk, 1976; Nechols *et al*., 1987; Canard, 1988, 1990, 1997, 2005; Grimal, 1988; Canard and Grimal, 1988; Chang *et al*., 1995; Volkovich and Arapov, 1996; Volkovich and Blumental, 1997; Fujiwara and Nomura, 1999; Macedo *et al*., 2003; Nakahira and Arakawa, 2005), relative humidity (Tauber and Tauber, 1983; Pappas *et al*., 2008b) or CO_2 (Gao *et al*., 2010) on certain life-history traits and predation capacity of chrysopids.

In Integrated Pest Management (IPM) programs, biological pest control with chrysopids could be efficiently combined with the use of selective pesticides. Vogt *et al.* (2001) have reviewed the effects of different management practices, such as the use of pesticides and transgenic *Bacillus thuringiensis* (*Bt*) plants on *C. carnea sensu lato*. There are many studies focusing on the effects of pesticides commonly used in agriculture on certain life-history traits of chrysopid species (Liu and Chen, 2000; Qi *et al.*, 2001; Carvalho *et al.*, 2002, 2003; Chen and Liu, 2002; Medina *et al.*, 2003; Bueno and Freitas, 2004; Godoy *et al.*, 2004; Ferreira *et al.*, 2006; Silva *et al.*, 2005, 2006; Nadel *et al.*, 2007; Rezaei *et al.*, 2007; Giolo *et al.*, 2009; Mandour, 2009; Schneider *et al.*, 2009) and on pesticide resistance development (Pathan *et al.*, 2008, 2010; Venkatesan *et al.*, 2009), mostly suggesting the relatively broad tolerance of chrysopids to many pesticides. Furthermore, there are several research papers reporting the effects of *Bt* on the performance of chrysopid species (Hilbeck *et al.*, 1998a, b, 1999; Meier and Hilbeck, 2001; Dutton *et al.*, 2002; Romeis *et al.*, 2004; Pilcher *et al.*, 2005; Rodrigo-Simón *et al.*, 2006; Mellet and Schoeman, 2007; Sharma *et al.*, 2007; Sisterson *et al.*, 2007; Guo *et al.*, 2008; Lawo and Romeis, 2008, Li *et al.*, 2008, 2010; Mason *et al.*, 2008).

With the attempt to evaluate the quality of certain prey species, as well as the predation efficacy of chrysopids, there are a lot of published papers mostly focusing on the performance of larvae and less on adults (Zheng *et al.*, 1993a, b; Legaspi *et al.*, 1994; Giles *et al.*, 2000; Chen and Liu, 2001; Limburg and Rosenheim, 2001; Patt *et al.*, 2003; Miller *et al.*, 2004; Pappas *et al.*, 2007, 2008c, 2009; Cheng *et al.*, 2009, 2010; Huang and Enkegaard, 2010). The efficiency of chrysopids has also been also evaluated in the context of IPM revealing the biotic potential of this group of aphidophagous predators in agricultural practice (Reddy, 2001; Corrales and Campos, 2004; Furlong *et al.* 2004; Santos *et al.*, 2007; Mirmoayedi and Maniee, 2008, 2009; Turquet *et al.*, 2009).

MASS-REARING CHRYSOPIDS FOR USE IN BIOLOGICAL CONTROL

To date, the mass-rearing of commercially available chrysopids is mainly based on eggs of lepidopteran species of the genera *Sitotroga*, *Ephestia* (*Anagasta*) and *Corcyra* that have been proved as nutritionally superior and of low cost food to produce, in comparison to other artificial diets tested (Tauber *et al.*, 2000; Riddick, 2009). Until now, any attempts to develop a sufficient and low cost artificial diet that could replace the use of prey species eggs were unsuccessful (Ridgway *et al.*, 1970; Cohen and Smith, 1998).

Although most research projects have been focused on the development of the best food for rearing larvae, mass-rearing of adults has not received much attention (Nordlund *et al.*, 2001). Non predaceous chrysopid adults (e.g., *Chrysoperla* adults) are usually reared on a proteinaceous liquid diet consisting of a mixture of protein hydrolysate, sucrose and water (Hagen and Tassan, 1970). However, when considering predaceous adults (e.g., *Chrysopa* adults), their mass-rearing is difficult and very costly (Tauber *et al.*, 2000).

The evaluation of artificial and factitious foods on the performance of several chrysopid species has been the subject of many published research articles. From the mass-rearing of chrysopids perspective, several methods for the production of larvae by offering an artificial diet (Cohen and Smith, 1998; Vasquez *et al.*, 1998; Zaki and Gesraha, 2001; Sattar *et al.*, 2007; Sattar and Abro, 2009) or a factitious food (Singh and Varma, 1989; Pappas *et al.*, 2007, 2008a; Matos and Obrycki, 2006; Sattar and Abro, 2009) have been tested for different chrysopid species. Mass-rearing techniques have been reviewed in extent by Tulisalo (1984), Tauber *et al.* (2000) and Nordlund *et al.* (2001).

The automation of mass-rearing systems of *Chrysoperla* species is still in progress with the aim to save production costs and space (McEwen *et al*., 1999). Such systems include suitable techniques for maintaining chrysopid adults, automatically feeding larvae and collecting eggs for mass- rearing a large number of larvae (Tauber *et al*., 2000). The automation of one of more steps in the mass-rearing of *Chrysoperla* species could result in the reduction of production costs and the enhancement of their commercial availability (Norlund and Correa, 1995; Nordlund *et al*., 2001).

The short term storage of eggs and diapausing adults of certain *Chrysoperla* species could be achieved at low temperatures (8 to 13°C) without reduction of their quality (Tauber *et al*., 1993, 1997; López-Arroyo *et al*., 2000). Afterwards, depending on market demands and appropriate adjustment in the mass - rearing system, the immediate, predictable and synchronous completion of post - diapause development and commencement of egg laying by females could be feasible.

Another important aspect in the commercialization of chrysopid species and other natural enemies is the quality control of the individuals to be released. A first important step in the mass - rearing is the correct identification of the species to be reared, as well as the verification of it at various time periods after setting up the rearing. *Chrysoperla* sp. colonies are known to deteriorate after rearing for a long period of time under controlled laboratory conditions (Jones *et al*., 1978; Chang *et al*., 1996). Regular quality control inspections of the stock colony in terms of survival and reproduction performance combined with proper handling of chrysopids life - cycle (i.e., maintenance in diapause or at low temperatures) seems to be crucial for the successful marketing of chrysopids. A few studies have noted the need for setting up certain standards in the commercialization of chrysopids (Gardner and Giles, 1996; Daane and Yokota, 1997; O'Neil *et al*., 1998; Silvers *et al*., 2002).

CHRYSOPID SPECIES THAT HAVE BEEN USED IN BIOLOGICAL CONTROL

The efficiency of chrysopids as biological control agents against aphids was studied in 1742 for the first time (Senior and McEwen, 2001). Since then, the vast majority of published research articles concerning the evaluation of certain chrysopid species for use in biological control programs have been mainly focused on *Chrysoperla* species. These species are considered to be important biological control agents in certain agroecosystems worldwidely and they are the most commonly released commercially available chrysopids (Tauber *et al*., 2000). Besides *Chrysoperla* sp., there is also some scattered information concerning other important chrysopid species tested for use in biological control belonging to other genera, as well, such as *Chrysopa*, *Mallada* and *Ceraeochrysa* (Table 1).

Table 1: Some chrysopid species which have been studied for use in biological control under field or laboratory conditions*

Chrysopa sensu stricto	***Chrysoperla* sp.**
Chrysopa formosa Brauer	*Chrysoperla carnea* (Stephens)
Chrysopa kulingensis Navas	*Chrysoperla rufilabris* (Burmeister)
Chrysopa nigricornis Burmeister	*Chrysoperla externa* (Hagen)
Chrysopa oculata Say	*Chrysoperla sinica* (Tjeder)
Chrysopa pallens (Rambur)	*Chrysoperla comanche* Banks
Chrysopa perla (L.)	*Chrysoperla lucasina* (Lacroix)
Chrysopa septempunctata Wesmael	*Mallada* sp.
***Ceraeochrysa* sp.**	***Mallada signata* (Schneider)**
Ceraeochrysa cubana (Hagen)	*Mallada basalis* (Walker)

*Reported by Ridgway and McMuphy (1984), Hagen *et al*. (1999), Albuquerque *et al*. (2001), Daane and Hagen (2001), Maisonneuve (2001), Senior and McEwen (2001)

Table 2: Pest species which have been successfully controlled by *Chrysoperla* species

Pest	References
Aphis gossypii Glover	Zaki *et al*. (1999)
Aphis pomi (DeGeer)	Hagley (1989)
Aulacorthum solani (Kaltenbach)	Ter-Simonjan *et al*. (1982)
Bemisia argentifolii Bellows and Perring	Legaspi *et al*. (1996)
Bemisia tabaci Gennadius	Breene *et al*. (1992)
Chaetosiphon fragaefolii (Cockerell)	Easterbrook *et al*. (2006)
Erythroneura elegantula Osborne	Daane and Yokota (1997)
Erythroneura variabilis Beamer	Daane *et al*. (1996), Daane and Yokota (1997)
Heliothis sp.	Ridgway and Jones (1968, 1969), Van den Bosch *et al*. (1969), López *et al*. (1976), Stark and Whitford (1987)
Leptinotarsa decemlineata (Say)	Nordlund *et al*. (1991)
Macrosiphum euphorbiae (Thomas)	Shands *et al*. (1972)
Myzus persicae (Sulzer)	Scopes (1969), Shands *et al*. (1972)
Pectinophora gossypiella (Saunders)	Irwin *et al*. (1974)
Pseudococcus longispinus (Targioni Tozzetti)	Goolsby *et al*. (2000)
Pseudococcus maritimus (Ehrhorn)	Doutt and Hagen (1949, 1950)
Pseudococcus obscures Essig	Doutt and Hagen (1949, 1950)
Rhopalosiphum padi L.	Rautapaa (1977)
Tetranuchus ludeni Zacher	Reddy (2001)
Tetranychus urticae Koch	Hagley and Miles (1987), Gomaa and Eid (2008)
Toxoptera citricida (Kirkaldy)	Michaud (2001)

Chrysoperla species have been released to control aphids in pepper, eggplant pea, potato and cotton fields. They have also been used to control *Leptinotarsa decemlineata* (Say) in eggplants, *Panonychus ulmi* (Koch) in apple orchards and *Heliothis virescens* (Fabricius) in cotton (Nordlund *et al*., 2001). In the greenhouse, *C. carnea*, *Chrysopa septempunctata* Wesmael, *Chrysopa formosa* Brauer and *C. perla* have been successfully used for aphid control in several crops, such as pepper, cucumber, eggplant, lettuce (Tulisalo, 1984). Chrysopids released in North America for biological control include mainly *C. carnea* to control several pests, such as mealybugs, aphids cicadellids and aphids, *C. rufilabris* to control chrysomelids, whiteflies, aphids and cicadellids, *Chrysoperla externa* (Hagen) against noctuids (Daane and Hagen, 2001) and *Chrysoperla plorabunda* (Fitsch) to control aphids (Michaud, 2001 and references therein). Some examples of the earliest till the latest attempts to use *Chrysoperla* species in biological control both in field crops as well as in greenhouses are shown in Table 2.

In the Neotropics, according to Albuquerque *et al*. (2001), *C. externa* and *Ceraeochrysa* species (e.g., *Ceraeochrysa cubana* (Hagen)) are considered to be of primary importance for the implementation of biological control programs.

In the Australian region among the very few indigenous lacewing species, *M. signata* is the most promising candidate for use in biological control (New, 2002), whereas in China *Chrysoperla sinica* (Tjeder) has already been used in augmentative release biological control programs with success (Senior and McEwen, 2001).

CHRYSOPERLA CARNEA SIBLING SPECIES PROBLEM: IMPLICATIONS FOR BIOLOGICAL CONTROL

Many species of the genus *Chrysoperla*, such as *C. carnea*, *C. rufilabris* and *C. externa* are important biological control agents (Albuquerque *et al*., 1994; Legaspi *et al*., 1994). Among the above mentioned species, *C. carnea* is the most studied species that has also been extensively used in releases in the context of biological control. According to recent studies, *C. carnea* comprises a complex of species that is generally referred to as *Chrysoperla carnea* Stephens *sensu lato* (Thierry *et al*., 1992; Henry *et al*., 2001). *C. carnea* species is considered to be a mega - species referred to as - *carnea* - group, which is one of the four species groups of the genus *Chrysoperla*. Although there are morphological differences between the species of the other three groups (*comans* -, *nyerima* - and *pudica* - group), the species belonging to the *carnea* - group are morphologically uniform (Brooks, 1994).

According to several studies, *C. carnea* consists of many reproductively isolated species that have no morphological differences and produce courtship songs of low frequency by vibrating their abdomen on the substrate when they are ready to mate. The songs are produced both by females and males, they are a prerequisite for the mating and according to some researchers they are species - specific and could be considered as a reliable index of the species identity (Henry *et al*., 2001; Noh and Henry, 2010).

To date, the songs of four cryptic species recorded in North America (*C. plorabunda*, *Chrysoperla adamsi* Henry, Wells and Pupedis, *Chrysoperla johnsoni* Henry, Wells and Pupedis and *Chrysoperla downesi* (Banks) *sensu stricto*) (Henry, 1979b, 1993; Henry *et al*., 1993), six in Europe and Western Asia (*Chrysoperla mediterranea* Hölzel, *Chrysoperla lucasina* (Lacroix), *C. carnea* (Stephens), *Chrysoperla pallida* Henry, Brooks, Duelli and Johnson, *Chrysoperla agilis* Henry, Brooks, Duelli and Johnson, *Chrysoperla zastrowi arabica*) (Henry *et al*., 1996, 1999, 2002, 2003, 2006), one in Africa (*Chrysoperla zastrowi zastrowi* (Esben - Petersen)) (Henry *et al*., 2006) and two in Eastern Asia (*Chrysoperla nipponensis* (Okamoto) types A and B) (Henry *et al*., 2009) have been described in detail. The species *C. plorabunda* was confirmed to be a distinct species from *C. carnea* although it was considered to be its synonym for a long time (Henry, 1979a, 1983, 1985a, b; Henry and Wells, 1990; Henry *et al*., 1993). There is a small variation between these songs even for populations originating from regions thousands of kimometers away (Henry and Wells, 1990). However, Tauber *et al*. (2000) argue that the description of new species based only on their songs is not reliable, since there are not enough data concerning songs variation in different seasons and geographical areas or connecting distinct songs with different biological traits.

In any case, when considering the use of *Chrysoperla* species in biological control there is an urgent need for a correct identification of the species to be released. There is a speculation that since *C. carnea* has been considered as a single species for a long period of time, sibling species may have been introduced as biological control agents in areas where another species of the - *carnea* group predominated (Senior and McEwen, 2001). Due to the well documented variation between the chrysopid species in certain life trait characteristics such as habitat and prey preference, seasonal life cycles and tolerance to several abiotic environmental factors (e.g., temperature or relative humidity) (Tauber *et al*., 2000), such releases of the inappropriate sibling species could be considered at least to some extent, as the causal factor of the recorded failures in the attempt to use chrysopids in biological control through augmentation releases.

THE USE OF CHRYSOPIDS IN BIOLOGICAL CONTROL

Field application: When considering the different biological control tactics used to control pests, four different methods could be used when releasing chrysopid species:

- Classical biological control, by importation of a new natural enemy
- Augmentation by means of inoculative releases
- Augmentation by means of inundative releases
- Conservation

In general, chrysopids have not been widely used in classical biological control and most work has been focused on their augmentation and conservation in the agroecosystems (Hagen *et al.*, 1999). The large numbers of different chrysopid species occurring worldwidely have rendered the importation of an exotic chrysopid species to another country, useless.

Augmentation of natural enemies by means of inoculative releases aims to the provisional pest control for a long period of time through the reproduction and establishment of the natural enemy in the crop. Due to the great ability of chrysopid adults to disperse and the occasional need of immediate response in pest control, augmentation of chrysopids by means of inundative releases (mainly based on the ability of the individuals released to suppress pest populations) has been most commonly used in cases where release cost is not restrictive. A few researchers have also documented the efficacy of chrysopids in augmentation biological control (Ridgway and Jones, 1969; Daane *et al.*, 1996; Daane and Yokota, 1997; Ehler *et al.*, 1997; Knutson and Tedders, 2002).

Conservation techniques in biological control aim to the establishment of increased numbers of a chrysopid species within the field through the enhancement and manipulation of their habitat (e.g., crop fields). Research related with chrysopids has been mainly focused on the evaluation of certain chemicals or blends that could be sprayed on plants and act as attractants for the chrysopids and on the use of food supplements, such as artificial honeydews or pollen (Tauber *et al.*, 2000; Nentwig *et al.*, 2002; James, 2003, 2006; Tóth *et al.*, 2006, 2009; Venzon *et al.*, 2006; Zhang *et al.*, 2006; Yu *et al.*, 2008). Other conservation methods that have application in the manipulation of chrysopids are the use of hibernation shelters for the protection of overwintering adults (McEwen and Sengonca, 2001; Wennemann, 2003; Weihrauch, 2008), cultural methods, such as intercropping (Senior and McEwen, 2001) or the use of flowering plants (Shrewsbury *et al.*, 2004).

Release methods: Chrysopids are most commonly released as eggs or larvae, but there are also cases that adults have been used in biological control. Eggs have been tested most extensively in relation to their application in the field (Tauber *et al.*, 2000). The release of adults has been considered to be problematic, due to the fact that they usually disperse and leave the target field before ovipositing (Duelli, 1984). This problem might be confronted by releasing adults previously fed in the insectary during preoviposition period, so as to be ready to oviposit after release in the field (Nordlund *et al.*, 2001).

Chrysopid eggs mixed with rice hulls or vermiculite, attached on oviposition substrates or destalked are usually dispersed manually to ensure their uniform distribution in the field. Solid mediums (e.g., rice hulls) as carrying agents of the eggs have the disadvantage of not offering a good retention on the plant in comparison with liquid mediums (e.g., agar solutions) that help eggs to be easily attached on the leaves (Tauber *et al.*, 2000). Chrysopid larvae can be released manually by placing rearing units on the plant or with the help of a paintbrush. They are also formulated mixed with rice hulls inside bottles and applied on the plants (Nordlund *et al.*, 2001).

Advances in release methods of chrysopid eggs include the development of mechanical devices by commercial companies that use low pressure air and a liquid medium for the distribution and efficient adherence of the eggs on the plants. Furthermore, aerial application by means of model airplanes or helicopters, have been tested for use in augmentative biological control with chrysopids. The mechanical application of chrysopid eggs and larvae has been the subject of several studies mainly in terms of the effects of automation on their viability (Nordlund *et al.*, 2001 and references therein).

Commercially available chrysopid species: To date, the most important and commonly used commercially available chrysopids are *Chrysoperla* species. The species *C. carnea*, *Chrysoperla comanche* Banks and *C. rufilabris* are being mass - reared and commercially sold by many companies in North America since years. Furthermore, *C. carnea*, *C. externa* and *C. sinica* are commercially available in Europe, Latin America and Asia, respectively (Tauber *et al.*, 2000; Nordlund *et al.*, 2001).

Concerning the different stages that could be purchased by a grower, chrysopid eggs, larvae, cocoons and adults are all commercially available by several companies and in different formulations. Eggs and larvae, usually sold in thousands, are cheaper than cocoons and adults. The top seller of all available species is *C. carnea* although there is a lot of concern whether this is the true *C. carnea* or not. According to Tauber *et al.* (2000), the identification of the species of the *Chrysoperla* stock should be the first crucial step at the onset of the culture to be mass - reared.

Besides different chrysopid formulations, food supplements, attractants as well as hibernation boxes are also available commercially to be used in conservation biological control. Food supplements for adults include bottles or bags containing yeast or pollen and nectar substitutes that could be mixed with water and applied as a paste or sprayed on the plants. By enhancing the availability of food for chrysopid adults, the companies that provide these supplements claim the increase in oviposition of the adults, their maintenance in the target field and subsequent successful pest control. Eggs of *Ephestia kuehniella* Zeller, is a factitious food commonly used for mass - rearing chrysopids, as well as other predatory insects. It is commercially available as a supplementary food for predatory insects within the context of biological control. In the case of chrysopids, it could be used as supplementary larval diet. However, to our knowledge there are no studies related to the benefits of using *E. kuehniella* eggs on the population of chrysopids under field conditions. Hibernation boxes also known as 'lacewing chambers' and several attractants are currently commercially available.

CHARACTERISTICS OF THE EFFICIENT BIOLOGICAL CONTROL AGENTS OF THE FAMILY CHRYSOPIDAE

The most efficient biological control agents of the genus *Chrysoperla* species share some common characteristics (presented below) that enhance their role in biological control, in relation to other chrysopid species.

Larval characteristics:

- **Broad prey range:** *Chrysoperla* sp. larvae are considered to be polyphagous and especially the larvae of commercially available species are recommended for use against several crop pests (Canard and Principi, 1984)
- **Easy mass - rearing:** *Chrysoperla* larvae are easily reared on several factitious foods, such as *E. kuehniella* eggs and their mass - rearing has been automated by companies (Tauber *et al.*, 2000)

- **Resistance to insecticides:** Chrysopid larvae have been found to be highly resistant to the effects of pesticides in relation to other predatory insects (Nordlund *et al.*, 2001). This advantage could enhance their use in IPM programs
- **Short developmental times:** The relative rapid development of *Chrysoperla* larvae (Principi and Canard, 1984) is a desirable trait for their use in biological control, as it ensures the establishment of the released insects in the target crops
- **Predation efficiency:** Chrysopid larvae consume a large amount of food so as to complete their development (Canard, 2001). For example, each *C. carnea* larva consumed on average 140 second instar nymphs of *Myzys persicae* Sulzer or 946 *E. kuehniella* eggs to complete its development (El-Arnaouty *et al.*, 1996). Among larvae of chrysopid species, those of the third instar are the most voracious (Principi and Canard, 1984)
- **Low dispersal ability:** Both chrysopid eggs and larvae are easily released in the field and the settlement of the population could be achieved due to low dispersal ability of the larvae. Furthermore, it is easier to achieve the desired number of released individuals when chrysopids are applied as larvae that are not suffering from predation by other predators as much as the eggs (Nordlund *et al.*, 2001)

Adult characteristics

- **Easy mass-rearing:** *Chrysoperla* sp. adults are not predaceous and can be easily mass - reared on a liquid diet that consists mainly of honey or pollen. This trait facilitates their rearing since there is no need for culturing an additional prey species and/or plants for the adults
- **High reproductive potential:** The number of eggs laid by chrysopid females is affected by the quality and quantity of the larval and adult food, as well as by the environmental conditions (e.g. temperature, photoperiod, relative humidity) (Principi and Canard, 1984). In general, *Chrysoperla* females have a high reproductive potential. For example, the females of *C. mediterranea* laid approximately 2160 eggs during their lifetime at 20°C and of *C. externa* 2304 eggs at 25°C (Lee and Shih, 1982; Carvalho *et al.*, 1996)
- **Attraction to protein hydrolysates:** Mixing protein hydrolysates (mainly of yeast) with honey or sugar has been used as artificial honeydew of high quality (Hagen, 1950; Tauber *et al.*, 2000). Furthermore, chrysopid adults are attracted to protein hydrolysates (Canard, 2001) and this behavior has been used in conservation biological control

Besides *Chrysoperla* species, all of the above characteristics have been recorded in species of other genera, such as *Ceraeochrysa* (López-Arroyo *et al.*, 1999a, b; Albuquerque *et al.*, 2001), *Mallada* (Daane, 2001; New, 2002) and *Dichochrysa* (Principi, 1956; Pappas *et al.*, 2007, 2008a, c, 2009). Furthermore, the larvae of the above mentioned genera could be protected by ants and other natural enemies by carrying 'trash' on their dorsa (Principi, 1956; Eisner *et al.*, 1978). Due to the common desirable characteristics of *Dichochrysa*, *Mallada* and *Ceraeochrysa* with *Chrysoperla* species and their abundance is several crops, they could be assumed to also play an important role in biological control (López-Arroyo *et al.*, 1999a, b; Tauber *et al.*, 2000; Daane, 2001).

FUTURE ASPECTS AND CONSIDERATIONS IN THE USE OF CHRYSOPIDS IN BIOLOGICAL CONTROL

Within *C. carnea* complex many species are included that are not sufficiently studied in terms of their performance in the field as biological control agents. Given the commercial success of

C. carnea, there is an urgent need other species such as *C. agilis*, C. *lucasina*, C. *mediterranea* or *C. pallida* in Europe to be included in screening studies for efficient natural enemies. Furthermore, the biology of species of the *carnea* compex is poorly studied, although there is scattered data to suggest their considerable variation in their performance in different habitats and biotic or abiotic factors (Tauber *et al.*, 2000).

In order to enhance the role of Chrysopidae in biological control, the importance of other species than *Chrysoperla* should be evaluated. Among chrysopid species, *Chrysoperla* sp. are almost the only ones which are well studied and commercially available for use in biological control. By contrast, there are limited data on the biology of species belonging to the genera *Dichochrysa*, *Mallada* and *Ceraeochrysa* which could be also used commercially for release in biological control programs. For example, from the 130 known *Dichochrysa* species, only the biology of *D. prasina*, has been extensively studied under laboratory conditions (Pappas *et al.*, 2007, 2008a, b, c, 2009). It has been found that *D. prasina* has those desirable traits that could support its amenability for mass - rearing and future commercialization (Pappas *et al.*, 2007, 2008a, b, c, 2009). This is also the case for *Ceraeochrysa* and *Mallada* species. *Ceraeochrysa cubana* is considered a promising biological control agent in the Neotropics (López-Arroyo *et al.*, 1999a, b; Albuquerque *et al.*, 2001). *Mallada signata* is the only chrysopid species that has gained attention as an important indigenous predator in Australia (Horne *et al.*, 2001), while the role of other chrysopids has been neglected (New, 2002).

Feeding habits of chrysopid species intended to be used in biological control should also be studied. They are all considered to be polyphagous predators of several soft - bodied arthropods. However, prey quality seems to be crucial for the successful completion of their immature development and subsequent adult performance (Principi and Canard, 1984). Therefore, prey preference or prey specificity of candidate chrysopid species should be further studied in detail so as they could be exploited for the control of the most suitable pests.

Intraguild Predation (IP) could severely affect the success of chrysopid releases in the field. A limited number of studies have focused on IP of *C. carnea*, *C. rufilabris* or *C. prolabunda* (Cisneros and Rosenhein, 1997; Lucas *et al.*, 1997, 1998; Phoofolo and Obrycki, 1998; Dinter, 1998, 2002; White and Eigenbrode, 2000; Michaud and Grant, 2003; Mochizuki *et al.*, 2006; Jazzar *et al.*, 2008). The expansion of such studies to other combinations of chrysopid predators and prey species, as well as to field conditions could cast light on the optimization of chrysopid releases for biological control.

When considering the commercialization of chrysopid species, methods and rates of release should be evaluated both under laboratory and field conditions. There is limited data on the economics of chrysopid use in biological control in relation to release costs and subsequent pest populations suppress (Tauber *et al.*, 2000). In a few studies (Gardner and Giles, 1996; Legaspi *et al.*, 1996; Daane and Yokota, 1997), several factors, such as intraguild predation, release methods and rates that could influence the outcome of field releases have been identified. Such factors should be considered in future studies on the evaluation of the efficacy of chrysopids in the field. Studies on the improvement of conservation methods could also enhance the efficacy of naturally occurring and released chrysopids in biological control.

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