

BIOLOGICAL CONTROL

Suitability of Leguminous Cover Crop Pollens as Food Source for the Green Lacewing *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae)

MADELAINE VENZON¹, MARIA C. ROSADO¹, DENISE E. EUZÉBIO¹, BRÍGIDA SOUZA² AND JOSÉ H. SCHOEREDER³

¹Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Centro Tecnológico da Zona da Mata (CTZM)
Vila Gianetti 46, Viçosa, MG; venzon@epamig.ufv.br

²Depto. Entomologia, Univ. Federal de Lavras, Lavras, MG

³Depto. Biologia Geral, Univ. Federal de Viçosa, Viçosa, MG

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Adequação do Pólen de Leguminosas Utilizadas em Adubação Verde como Fonte de Alimento para *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae)

RESUMO - A diversificação das culturas com plantas que forneçam pólen nutricionalmente adequado para predadores pode reduzir a população de pragas pelo aumento da efetividade dos predadores. Neste trabalho, avaliou-se a viabilidade nutricional de polens de leguminosas utilizadas como adubação verde, para o crisopídeo *Chrysoperla externa* (Hagen), comumente encontrado em agroecossistemas cafeeiros. Os adubos verdes testados foram guandu e crotalária, leguminosas utilizadas em sistemas orgânicos de café. Adultos de *C. externa* recém-emergidos foram criados em dietas contendo pólen de guandu, de crotalária e de mamona (controle). O sucesso reprodutivo de *C. externa* foi avaliado nas dietas de pólen e quando mel foi adicionado ao pólen, para se verificar a necessidade do predador por fonte extra de carboidratos. A taxa de crescimento populacional do predador foi semelhante nas dietas de pólen de guandu e de crotalária. No entanto, a taxa aumentou significativamente quando mel foi adicionado às dietas. Fêmeas alimentadas com pólen de guandu e mel e com pólen de crotalária e mel tiveram crescimento superior ao das alimentadas com pólen de mamona e mel. Fêmeas alimentadas somente com pólen de mamona ou somente com mel não ovipositaram. Os polens de guandu e de crotalária foram igualmente adequados para *C. externa*, especialmente quando foram complementados com mel. Os resultados sugerem que para aumentar a efetividade dos predadores, os sistemas orgânicos de café deveriam ser diversificados com plantas que forneçam pólen em combinação com plantas que forneçam néctar.

PALAVRAS-CHAVE: Controle biológico conservativo, predador, café, *Cajanus cajan*, *Crotalaria juncea*, crisopídeo

ABSTRACT - Diversification of crops with species that provide suitable pollen for predators may reduce pest population on crops by enhancing predator effectiveness. In this paper we evaluated the suitability of leguminous cover crop pollens to the predatory green lacewing *Chrysoperla externa* (Hagen). The predator is commonly found in coffee agroecosystems and the plant species tested were pigeon pea and sunn hemp, which are used in organic coffee systems. Newly emerged females and males of *C. externa* were reared on diets containing pollen of pigeon pea, sunn hemp, or castor bean, used as a control. The reproductive success of *C. externa* was evaluated when females fed the pollen species and when honey was added to the diets, to verify the predator need for an extra carbohydrate source. Similar intrinsic growth rates were found for females fed on pigeon pea pollen and on sunn hemp pollen but these rates increased significantly when honey was added to the diets. Females fed with pigeon pea pollen plus honey and with sunn hemp pollen plus honey had higher intrinsic growth rates than those fed with castor bean pollen plus honey. Females fed on castor bean pollen only or on honey only, did not oviposit. Leguminous pollen species were equally suitable for *C. externa* especially when they were complemented with honey. The results suggest that to successfully enhance predator effectiveness, organic coffee plantation should be diversified with plant providing pollen in combination with plant providing nectar.

KEY WORDS: Conservation biological control, predator, coffee, *Cajanus cajan*, *Crotalaria juncea*

Conservation biological control involves the manipulation of the environment of natural enemies for enhancing their survival and performance, thereby resulting in increased effectiveness (Barbosa 1998, Gurr & Wratten 1999). The diversification of plants within the crop area may favour natural enemies by providing protection from environmental factors and alternative food sources such as nectar, pollen and honeydew (Landis *et al.* 2000, Gurr *et al.* 2003).

Although carnivorous, many predators feed on plants that provide food. The nutritional value of such food varies with predator species and circumstances in which they find themselves. For some predaceous species non-prey items are essential during a non carnivorous life stage (Principi & Carnard 1984, Hickman & Wratten 1997), and for others plant-food may act as a supplement until nutritional optimal prey is located or as a complement of a suboptimal prey (Eubanks & Denno 1999, Evans *et al.* 1999, Beckman & Hurd 2003, Patt *et al.* 2003). Green lacewings are polyphagous predators whose larvae are carnivorous and adults have varied feeding habits with species being either carnivorous or glyciophagous and polliniphagous (Principi & Carnard 1984). *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) is a widespread species native from the Neotropical Region (Albuquerque *et al.* 1994). Adults feed on plant-provided food and on honeydew and their larvae prey on a variety of soft-body arthropods (Carvalho & Souza 2000). In Brazil, *C. externa* was reported to occur in several agroecosystems (Souza & Carvalho 2002, Figueira *et al.* 2003, Santos *et al.* 2003), including coffee crops where its larvae are found to preying on mites, young caterpillars, and on pupae of *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae), the main coffee pest (Ecole *et al.* 2002).

One possibility to enhance the abundance and to increase the effectiveness of *C. externa* for controlling coffee pests would be the provision of extra food for adult predators within the coffee plantation via intercropping (Barbosa 1998, Landis *et al.* 2000). In orchards, the increase of plant diversification provided by cover crop species has been shown to improve natural enemy effectiveness (Bugg & Waddington 1994, Grafton-Cardwell *et al.* 1999). However, the value of a plant as a food source for predators may depend on plant species. For instance, pollen nutrient content varies with plant species (Todd & Bretherick 1942, Roulston *et al.* 2000). Thus, not all cover crop producing pollen may be suitable for conservation biological control purposes. In this paper, we tested the suitability of two leguminous cover crop species as pollen source to *C. externa*. The plant species tested are pigeon pea (*Cajanus cajan* (L.) and sunn hemp (*Crotalaria juncea* L.) which are used in organic coffee systems for improving soil properties and reducing weed competition (Guimarães *et al.* 2002, Santos *et al.* 2002). Since the content of carbohydrate in the diet may influence chrysopid fecundity (Sheldon & MacLeod 1971) and pollen carbohydrate content varies with plant species (Todd & Bretherick 1942, Roulston *et al.* 2000), we also tested whether the addition of an extra carbohydrate source to the leguminous cover crop pollen diet would increase the reproductive success of *C. externa*.

Material and Methods

Predators. Adults of *C. externa* used in the experiment originated from a rearing kept at the Laboratório de Entomologia of the Universidade Federal de Lavras, Lavras, MG, Brazil. Individuals were kept in cages consisted of a PVC tube (8 x 11 cm) covered with nylon gauze and placed on a petri dish (15 cm of diameter) (Carvalho & Souza 2000). They were fed with a diet that consisted of yeast and honey (1:1) offered on a parafilm stripe hanged inside the cage. Water was provided on a piece of cotton soaked and placed inside a 10 ml vial. Food and water were replaced twice a week. Eggs of *C. externa* were collected from the cages by cutting their pedicel and transferring them to glass tubes (2.5 x 8.5 cm). Newly emerged larvae were fed with an ample supply of eggs of the flour moth *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) until pupation. The rearing unit was kept at 25 ± 2°C, 70 ± 10% RH and 14h photophase.

Pollen. Pigeon pea (*C. cajan*) and sunn hemp (*C. juncea*) were grown from seeds in pots in a greenhouse at the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), Viçosa, MG, Brazil. At the time of flowering, flowers were collected daily and taken to the laboratory for pollen collection. Pollen was collected with the aid of a brush. Pollen of castor bean (*Ricinus communis*) was used in the experiment for comparison with leguminous pollen as it has been shown to be nutritionally suitable for other Chrysopidae species (Krishnamoorthy 1984, Gautam & Paul 1988). It was obtained by collecting flowers from plants at the campus of the Universidade Federal de Viçosa (UFV). In the laboratory, flowers were placed on a tray covered with filter paper and after three days the detached pollen was collected. All three pollen species were stored for a short period under dry conditions in a refrigerator at 4°C (van Rijn & Tanigoshi 1999). The pollen protein content of leguminous species and of castor bean were analysed following the official methods of AOAC International (Cunniff 1998).

Reproductive success. The experiment was carried out at the laboratory of EPAMIG, Viçosa, MG. A newly emerged female and a male of *C. externa* were isolated in a cage consisted of a PVC tube (10 cm of diameter and 10 cm high) covered with plastic film and placed on a petri dish (10 cm of diameter). They were fed with one of the following diets: a) pigeon pea pollen; b) pigeon pea pollen plus honey; c) sunn hemp pollen; d) sunn hemp pollen plus honey; e) castor bean pollen; f) castor bean pollen plus honey; and g) honey. Honey was added to pollen diets to test the need for carbohydrate other than provided by pollen. Pollen and honey were offered separately inside rubber lids (0.5 cm of diameter) and replaced every two days. Water was provided in pieces of cotton soaked and placed inside a 10 ml vial. Cages were kept inside climate boxes at 25 ± 1°C, 70 ± 10% RH and 14h photophase. Every day cages were checked for eggs and for predator survival.

Pre-oviposition and oviposition period, oviposition rate and longevity were determined. Viability and incubation

period of predator eggs were measured by collecting five eggs from each replicate every five days. Eggs were isolated in plastic tubes (2 cm of diameter and 3.5 cm of high) covered with plastic film, and monitored daily. Each replicated consisted of a PVC cage with one female and one male of *C. externa* and six replicates of each treatment were conducted. Data on egg viability were square root transformed before statistical analysis.

Data were analysed using analysis of variance (ANOVA) followed by a priori defined contrasts (Crawley 2002). Because *C. externa* females did not oviposit when fed only on castor bean pollen and only on honey, these treatments were excluded from the analysis. Thus, we had five levels of the treatment and we carried out three planned contrasts, based on three hypotheses: (1) pigeon pea pollen changes life history parameters in relation to sunn hemp pollen (level *a* is different from level *c*); (2) the addition of honey to leguminous pollen changes life history parameters in relation to only leguminous pollen (levels *b* and *d* are different from *a* and *c*) (3) leguminous pollen plus honey changes life history parameters in relation to castor bean pollen plus honey (level *b* and *d* are different from level *f*). Analyses were carried out using the software R (Ihaka & Gentleman 1996), and followed by residual analyses.

Data on reproduction, longevity, and juvenile development and survival of *C. externa* were used to estimate the intrinsic growth rate (r_m) of the predator on various diets. As we did not measure the survivorship, we used data from Aun (1986) for *C. externa* larvae fed on *A. kuehniella* eggs under similar experimental conditions (larvae development took 9.6 days with 62.6% of juvenile survival and pupae stage took 10.5 days with viability of 79.5%). The sex ratio was assumed 0.47 (Ribeiro *et al.* 1997). For each diet, the r_m of *C. externa* was calculated and expressed as the number of females per day using the Lotka equation (Carey 1993):

$$\sum_{x=0}^T l_x m_x e^{-r_m(x+1)}$$

where T is the oldest age class, l_x is the proportion of surviving females from birth to age *x* and m_x is the number of female progeny produced per female at the midpoint of the interval *x* to *x*+1.

Jackknife estimates of intrinsic growth rates and their variance were calculated as described by Maia *et al.* (2000). Differences of intrinsic growth rates among treatments were compared by analysis of variance, followed by the same planned contrasts carried out in the above analysis.

Results

The analysis of protein content in pollen from different plant species revealed that the total percentage of protein was 24.9% for pigeon pea, 25.6% for sunn hemp and 37.5% for castor bean.

Females of *C. externa* that fed on pollen of castor bean and honey diet failed to oviposit. The longevity of females and males fed with these diets was, respectively, 6.5 ± 3.3 and 10.0 ± 6.4 days, for castor bean pollen and 64.8 ± 11.7 and 39.5 ± 21.6 days for honey.

Pre-oviposition period of *C. externa* did not differ significantly among diets ($F = 1.68$; $df = 4, 25$; $P = 0.186$) (Table 1). The oviposition rate of *C. externa* also did not differ among diets ($F = 1.81$; $df = 4, 25$; $P = 0.158$), however, the total number of eggs laid by *C. externa* was influenced by the diet treatments ($F = 3.75$; $df = 4, 25$; $P = 0.016$). When honey was added to the pollen of leguminous species, females laid significantly more eggs than when pollen was offered alone, independently of plant species ($t = 3.153$; $P = 0.004$). However, no difference was found in the total number of eggs laid by females fed with the three pollen species offered with honey ($t = 0.50$; $P = 0.62$) and among females fed with pigeon pea pollen and with sunn hemp pollen ($t = 0.08$; $P = 0.94$) (Table 1).

The egg hatching period ($F = 2.06$; $df = 4, 25$; $P = 0.12$) and egg viability ($F = 1.56$; $df = 4, 25$; $P = 0.215$) of *C. externa* fed with various pollen diets did not differ

Table 1. Life history parameters of adults *C. externa* subjected to different diet treatments.

Diets	Pre-oviposition (days) ^{n.s.}	Oviposition rate (eggs/day) ^{n.s.}	Total number of eggs per female	Egg stage (days) ^{n.s.}	Egg viability (%) ^{n.s.}	Female longevity	Male longevity
Pigeon pea pollen	6.5 ± 1.87	7.2 ± 2.27	161.5 ± 90.10 a	4.8 ± 0.12	70.4 ± 21.91	33.0 ± 10.08 a	32.4 ± 8.56 a
Pigeon pea pollen and honey	4.7 ± 0.82	11.9 ± 4.52	667.5 ± 411.65 b	4.8 ± 0.18	81.8 ± 8.97	84.0 ± 14.59 b	69.5 ± 36.00 b
Sunn hemp pollen	7.7 ± 1.75	6.3 ± 2.20	174.2 ± 57.16 a	5.0 ± 0.04	38.2 ± 14.61	39.0 ± 5.76 a	41.0 ± 8.00 a
Sunn hemp pollen and honey	5.3 ± 1.21	11.0 ± 7.93	444.3 ± 303.47 b	4.9 ± 0.16	57.4 ± 24.28	59.2 ± 28.94 b	96.2 ± 4.02 b
Castor bean pollen and honey	9.4 ± 3.78	6.4 ± 5.07	301.0 ± 288.1 a	4.9 ± 0.08	52.5 ± 35.63	65.3 ± 28.90 a	42.2 ± 23.07 a

Means (\pm SD) within a column followed by the same letter do not differ significantly (ANOVA, $P < 0.05$); ^{n.s.} non significant.

significantly. On the other hand, significant differences were found for the longevity of females ($F = 6.308$; $df = 4, 25$; $P = 0.001$) and males ($F = 10530$; $df = 4, 25$; $P < 0.001$) among the various diets. Adults that fed on leguminous pollen supplemented with honey lived longer than those fed on only pollen (females: $t = 2.725$; $P = 0.011$; males: $t = 5.623$; $P < 0.001$) (Table 1). However, longevity did not differ when adults fed with the three pollen species offered with honey (females: $t = 1.26$; $P = 0.22$; males: $t = 1.71$; $P = 0.10$) and when they fed with pigeon pea pollen and with sunn hemp (females: $t = 0.52$; $P = 0.61$; males: $t = 1.16$; $P = 0.26$) (Table 1).

The intrinsic growth rate (r_m) of *C. externa* differed when females were fed with the various pollen diets ($F = 112.96$; $df = 4, 25$; $P < 0.001$). When honey was added to the diet, the intrinsic growth rate of females increased significantly ($t = 9.07$; $P < 0.001$) (Fig. 1). Females fed with leguminous pollen species plus honey showed a higher intrinsic growth rates than those fed with castor bean pollen plus honey ($t = 18.47$; $P < 0.001$). The intrinsic growth rate of *C. externa* did not differ significantly when females fed on pollen of pigeon pea and sunn hemp ($t = 2.034$; $P = 0.053$) (Fig. 1). Despite of the lack of significance, small differences in this rate may represent an increase of the population after some generations.

Discussion

Pollen of the leguminous cover crops pigeon pea (*C. cajan*) and sunn hemp (*C. juncea*) are suitable for the reproductive success of the green lacewing *C. externa*. The results showed that the intrinsic growth rate of *C. externa* increased 1.5 and 1.2 fold, respectively, when honey was added to the pollen. The higher number of eggs produced by females fed on the diets supplemented with honey accounted for the increase of the growth rate.

The low carbohydrate content on the pollen of leguminous species might explain why the addition of honey increased *C. externa* reproductive success (Todd & Bretherick 1942). Possibly, *C. externa* requires a higher concentration of carbohydrates than present on these pollens to express its full reproductive potential. The analysis of protein content revealed that protein concentration in pollen of pigeon pea and sunn hemp pollens was lower than those of castor bean. Likely, there is a negative correlation between protein and carbohydrate content for the pollen of some plant species (Todd & Bretherick 1942). Low carbohydrate concentration could explain why females of *C. externa* failed to oviposit when fed on pollen higher protein content. Indeed, the other studies showed that castor bean supplemented with honey increased the fecundity of another chrysopid species, *Chrysopa scelestes* Banks (Krishnamoorthy 1984, Gautam & Paul 1988).

Pollen starch content also influenced chrysopid fecundity (Sheldon & MacLeod 1971, Venzon & Carvalho 1992). For instance, the high percentage of carbohydrate in corn pollen is mainly due to its high starch content (Todd & Bretherick 1942). Sheldon & MacLeod (1971) reported that *Chrysoperla carnea* Stephens showed a lower level of oviposition compared when honey was supplemented to the diet. Moreover, the authors found a large proportion of unaltered starch in *C. carnea* feces. Alternatively, as pointed out by Patt et al. (2003), apart from the nutritional value of macro-nutrients found in pollen, vitamins, mineral nutrients and sterols are important for digestion and other metabolic processes (Stanley & Linskens 1974, Waldbauer & Friedman 1991). Thus, differences in the digestibility and assimilation of nutrients from different pollen species are expected to have influence on predator fecundity.

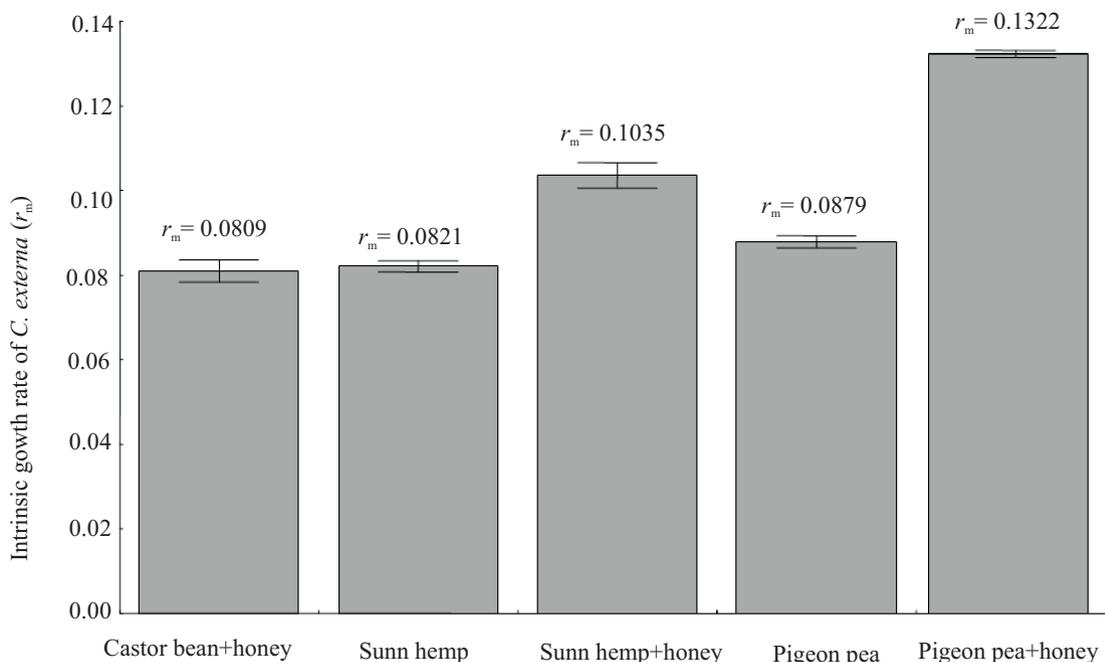


Fig. 1. The intrinsic growth rate (r_m) of *C. externa* subjected to different diet treatments.

The fact that reproductive success of *C. externa* was higher when pigeon pea and sunn hemp pollen were supplemented with honey has practical implications for the success of conservation biological control in coffee. In order to enhance the population of *C. externa* in coffee crops, cover crop plants might be introduced to increase food resources for this insect. The use of intercrop plant techniques in coffee crops increased the leaf miner predation by predatory wasps (Amaral *et al.*, unpublished data). Furthermore, the provision of extra food sources such as nectar may also benefit parasitoids (Wackers 2004), which are important natural enemies of coffee pests in Brazil (Reis Jr. *et al.* 2000, Reis *et al.* 2002).

Finally, care should be taken when selecting plant providing food for conservation biological control because herbivores may also feed on pollen (van Rijn *et al.* 2002) and nectar (Baggen *et al.* 1999). For instance, the coffee leaf miner, *L. coffeella*, has higher fecundity when fed with sucrose solution (Nantes & Parra 1985) and it may benefit from plant providing sugar. Although this possibility exists, Baggen *et al.* (1999) pointed out that factors such as floral architecture and color and balance of sugar may impede lepidopterans access and utilization of nectar. Thus, increasing plant diversity in organic coffee system may reduce pest population through enhanced natural enemy effectiveness provided that food-plants do not benefit herbivores.

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