MICHELA COSTA BATISTA

FEEDING ECOLOGY OF GREEN LACEWINGS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

VIÇOSA
MINAS GERAIS – BRASIL
2016

Ficha catalográfica preparada pela Biblioteca Central da Universidade Federal de Viçosa - Câmpus Viçosa

T
B333f
2016

Batista, Michela Costa, 1980-
Feeding ecology of green lacewings / Michela Costa Batista. – Viçosa, MG, 2016.
xi, 61f. : il. (algumas color.) ; 29 cm.

Orientador: Madelaine Venzon.
Tese (doutorado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Chrysopidae. 2. Inseto nocivo - Controle biológico. 3. Pragas agrícolas - Controle biológico. 4. Manjericão. I. Universidade Federal de Viçosa. Departamento de Entomologia. Programa de Pós-graduação em Entomologia. II. Título.

CDD 22. ed. 595.747

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APROVADA: 23 de março de 2016.

Maira Christina Marques Fonseca

Maria Augusta Lima Siqueira

Sebastião Martins Filho

Angelo Pallini Filho
(Coorientador)

Madelaine Venzon
(Orientadora)

"Compreendo mais do que nunca que os mínimos acontecimentos de nossa vida são dirigidos por Deus. É Ele que nos faz desejar e satisfaz nossos desejos."
(Sta. Teresinha do Menino Jesus)

"Uma nuvem não sabe porque se move em tal direção. Sente um impulso… é para este lugar que devo ir agora. Mas o céu sabe os motivos e desenhos por trás de todas as nuvens, e você também saberá, quando se erguer o suficiente para ver além dos horizontes."
(Richard Bach – Ilusões)

"Tudo é graça."

Ao I.C.M.
(*Totus tuus ego sum, et omnia mea tua sunt!*),
e a todos que me acompanharam nesta jornada
acadêmica, profissional, pessoal e espiritual, dedico
e ofereço.

AGRADECIMENTOS

A Deus, autor de todas as coisas... Aquele que primeiro me inspirou, que soprou no meu ouvido "não enterre seus talentos" e que plantou no meu coração o desejo de ir para "águas mais profundas".

À minha família que me apoiou nesta empreitada com encorajamento e amor. Que me ajudaram a suportar a distância, a saudade, as lágrimas e não me deixaram desistir (lembra mãe?). José Carlos, Irailde, Joelson, Ariadnny, Maria Alice e D. Raimundinha, MUITO OBRIGADA!

Às amigas que mesmo na distância estiveram presentes: para ouvir a mesma história, para chorar ou para rir comigo (bendito plano de 0,25 centavos a ligação). Gerbeli Salgado, Raquel Oliveira e Caroline Daher, vocês não têm noção do quanto a sua amizade tem sido importante, especialmente nos últimos quatro anos.

À Madelaine, por ter sido mais que uma orientadora. Obrigada pela amizade, pelo apoio, pela paciência... Há um propósito para cada pessoa que entra na nossa vida, e sei que ser sua orientada não foi um acaso. Obrigada por ter me aceitado como aluna... e como amiga.

Ao Angelo Pallini, pelo apoio e ajuda naqueles momentos mais críticos quando tudo parecia estar indo por água abaixo... obrigada por me mostrar que para tudo dá-se um jeito.

Ao Adenir V. Teodoro, por se dispor a continuar como meu coorientador durante o doutorado, e estar sempre pronto para corrigir dez vezes o mesmo trabalho. Obrigada por continuar me aturando.

À Universidade Federal de Viçosa (UFV) e ao Programa de Pós-Graduação em Entomologia pela oportunidade de realização do curso. Obrigada a todos os professores do programa que contribuíram com a minha formação e à Eliane de Castro Silva, pelo pronto auxílio nas questões burocráticas.

À Fapemig, à CAPES e ao CNPq, pela concessão das bolsas de doutorado e sanduíche, e recursos que financiaram a pesquisa.

À EPAMIG, pela oportunidade de desenvolver meus experimentos e pela a amizade dos funcionários e dos pesquisadores. Obrigada a todos!

Aos amigos de laboratório com quem troquei as mais variadas ideias, experiências e sentimentos. Como foram enriquecedoras nossas discussões que começavam com ciência e terminavam em reflexões filosóficas como àquela do espermatozoide vencedor, ou fazendo graça dos nomes estranhos de personagens históricos! André Lage, Elem Martins, Maíra Queiroz, Juliana Martinez, Erickson Canesche, obrigada por também se indignarem comigo, por me consolar e por me fazerem rir mesmo sem eu querer... pelo apoio técnico, intelectual e psicológico. Que essa amizade valiosa possa ser mantida depois que já formos todos "doutores".

Aos amigos que fiz em Viçosa e que tornaram essa jornada mais alegre. Em especial, meu obrigada à Lailla Gandra (Laillita), Marcus Vinicius (Marquito) e Fernanda Freitas (Fê).

À Juliana Maria de Oliveira. Ju, você foi um anjo que Deus providenciou para me proteger em Viçosa. Eu nunca vou poder agradecer o suficiente por tudo o que você fez por mim e jamais poderei ser uma amiga tão maravilhosa quanto você foi para mim.

À pesquisadora Maira C. M. Fonseca, pela orientação e suporte no início e ao longo de todo o trabalho com plantas aromáticas. Pela amizade e carinho e por sempre se mostrar disponível, tanto profissionalmente como pessoalmente.

Aos pesquisadores Débora Pires e David Andow pela valiosa contribuição ao artigo da qualificação (Capítulo I). Meu especial agradecimento ao David Andow também por ter intermediado meu contato com o professor George Heimpel (University of Minnesota) para o estágio sanduíche.

To George E. Heimpel, who opened the doors of his lab to me and provided me a unique experience of learning, working and friendship that was quite important for my professional and personal growth in the US. To all my labmates from Heimpel's lab and Andow's lab, and collegues from the Entomology Department (University of Minnesota) for all friendship and support. Special thanks to Hannah Grey and Elise Rosengren for bringing joy to our windowless office.

À minha "roommate" Renata Borba, a prova de que Deus providencia tudo com perfeição.

A todos os que não foram mencionados, mas que nesses quatro anos se fizeram presentes pessoalmente, ou via "Zap-zap" ou Facebook. Ainda que tenha sido uma única conversa, ela foi essencial naquele momento. Obrigada a todos pelas palavras de conforto, encorajamento, exortação ou por rezarem por mim... mesmo quando estive distante e/ou isolada jamais me faltou o carinho necessário para que eu seguisse em frente e vocês fizeram parte disso. Porque "a quem tem Deus nada falta".

BIOGRAFIA

MICHELA COSTA BATISTA, filha de José Carlos de Araújo Batista e Irailde Costa Batista, nasceu em Laranjeiras do Sul – PR, no dia 09 de julho de 1980. Em 2000, iniciou o curso de Ciências com Habilitação em Biologia na Universidade Estadual do Maranhão (MA), concluindo-o em 2005. Em setembro de 2010 concluiu o curso de Mestrado em Agroecologia na Universidade Estadual do Maranhão, sob orientação do professor Adenir Vieira Teodoro. Em 2012 iniciou o Doutorado em Entomologia na Universidade Federal de Viçosa, sob supervisão e orientação da pesquisadora Madelaine Venzon.

SUMÁRIO

RESUMO

BATISTA, Michela Costa, D.Sc. Universidade Federal de Viçosa, março de 2016. **Ecologia alimentar de crisopídeos.** Orientadora: Madelaine Venzon. Coorientadores: Adenir Vieira Teodoro e Angelo Pallini Filho.

O controle biológico é uma estratégia de manejo de pragas que se baseia na ação de inimigos naturais para controlar as populações de herbívoros, minimizando seus danos em cultivos agrícolas. Para que os inimigos naturais encontrem as plantas e se estabeleçam em uma área de cultivo para fornecer o serviço de controle biológico, eles precisam ser atraídos para o local, sobreviver, reproduzirem-se e serem capazes de predar as pragas presentes nesse cultivo. Geralmente, a atração de inimigos naturais está direta ou indiretamente ligada às necessidades alimentares. Uma vez atraídos para a área, as fontes de alimento no cultivo e arredores devem ser apropriadas para promover o crescimento e estabelecimento das populações desses inimigos naturais. Nosso objetivo foi entender aspectos chave da ecologia alimentar de crisopídeos, predadores generalistas encontrados naturalmente em agroecossistemas e comumente comercializados como agentes de controle biológico. No Capítulo I, avaliamos a atratividade de espécies de plantas aromáticas a *Ceraeochrysa cubana* Hagen, uma espécie de crisopídeo com ampla distribuição geográfica e que pode ser encontrada em diversos sistemas de cultivo. Adicionalmente, foram testados os efeitos dessas plantas aromáticas na sobrevivência e performance de larvas e adultos de *C. cubana*, a fim de elucidar a importância dessas plantas para o estabelecimento de populações de crisopídeos. Constatamos que plantas de *Ocimum basilicum* (manjericão) sem flores e não infestadas foram atrativas para *C. cubana*, e que as larvas podem sobreviver em *O. basilicum* por um período de tempo maior em comparação com as outras espécies de plantas aromáticas avaliadas. Adicionalmente, as flores de *O. basilicum* proporcionaram uma sobrevivência longa para larvas e adultos de *C. cubana*, em comparação com o controle negativo (água). Os resultados indicam que a utilização de *O. basilicum* como um componente de diversificação em áreas agrícolas pode ser benéfico para a atração e manutenção de populações de *C. cubana* para favorecer o controle biológico. No Capítulo II, foi estudada a amplitude de dieta de *Chrysoperla rufilabris* Burmeister, uma espécie de crisopídeo comumente usada e comercializada como agente de controle biológico, com 16 espécies de afídeos, avaliando-se a qualidade dessas espécies para a sobrevivência e o fitness desse predador generalista. Os resultados mostraram que *C.*

rufilabris se alimentou de todas as espécies de afídeo oferecidas. No entanto, esse crisopídeo se desenvolveu e produziu ovos apenas quando alimentado por sete das 16 espécies avaliadas, estando a maioria destas espécies agrupadas em um mesmo ramo filogenético. Também foi encontrado um forte sinal filogenético para a sobrevivência, consumo de afídeos e produção de ovos de *C. rufilabris*, indicando que a maioria das espécies apropriadas a *C. rufilabris* são filogeneticamente próximas, o que demonstra que essa espécie de crisopídeo é menos generalista do que se havia suposto. Assim, *C. rufilabris* pode não se beneficiar de uma grande amplitude de presas e isso deve ser levado em consideração no planejamento de estratégias de controle biológico que visem utilizar esse crisopídeo. Conclui-se que o conhecimento sobre a ecologia alimentar de agentes de controle biológico é essencial antes da escolha das espécies a serem liberadas ou atraídas e mantidas em uma área cultivada. Nesse sentido, *O. basilicum* é uma espécie de planta aromática promissora para atrair e manter populações de crisopídeos no campo. Além da atratividade, é importante considerar a filogenia das presas em estudos de amplitude de dieta de predadores generalistas a fim de se obter melhores resultados em programas de controle biológico.

ABSTRACT

BATISTA, Michela Costa, D.Sc. Universidade Federal de Viçosa, March, 2016. **Feeding ecology of green lacewings.** Adviser: Madelaine Venzon. Co-advisers: Adenir Vieira Teodoro and Angelo Pallini Filho.

Biological control is a pest management strategy that relies on the action of natural enemies to control the populations of herbivores, minimizing their damage on cultivated areas. For natural enemies to find and establish in a cropping area to provide biological control services they need to be attracted to the area, survive, reproduce, and be capable of preying on the pests present in the crop. Usually, natural enemy attraction is direct or indirectly linked with feeding needs. Once attracted to the area, the food resources in the crop and surroundings must be suitable to promote population growth and establishment of natural enemies populations. Therefore, the aim of this thesis was to understand key aspects of the feeding ecology of green lacewings, generalist predators naturally found in agroecosystems and commonly commercialized as biological control agents. On Chapter I, we assessed the attractiveness of aromatic plant species to *Ceraeochrysa cubana* Hagen, a lacewing species with a broad geographical range that can be found in several cropping systems. Additionally, we tested the effects of those aromatic plants on survival and performance of larvae and adults of *C. cubana*, in order to elucidate the importance of such plant species to the establishment of green lacewing populations. We found that non-flowering and uninfested *Ocimum basilicum* (basil) plants were attractive to *C. cubana*, and that larvae could survive for a longer period of time in *O. basilicum* leaves compared to the other aromatic plant species tested. Additionally, *O. basilicum* flowers promoted a long survival for larvae and adults of *C. cubana*, compared to the negative control (water). Results indicate that using *O. basilicum* as a diversification component in cultivated areas may be beneficial to attract and maintain *C. cubana* populations to support biological control. On chapter II, we studied the diet breadth of *Chrysoperla rufilabris* Burmeister, a green lacewing commonly used and commercialized as a biological control agent, over 16 aphid species, assessing the quality of those species on survival and fitness of this generalist predator. Results demonstrated that *C. rufilabris* preyed over all the aphid species, but could develop and produce eggs only in seven species, most of them from the same cluster in a phylogenetic tree. We also found a strong phylogenetic signal for survival, aphid consumption and egg load of *C. rufilabris*, indicating that most of the species more

suitable to *C. rufilabris* were closely related, which demonstrate that this green lacewing species is less generalist than it was supposed. Thus, *C. rufilabris* may not benefit from a broad prey range and that has to be taken into consideration when planning biological control strategies using this green lacewing species. In conclusion, knowledge on the feeding ecology of biological control agents is essential before choosing the species to be released or that to be attracted to and to maintain in the cropping system. In this sense, *O. basilicum* is a promising aromatic plant species to attract and maintain lacewing populations in the field. Additionally to attractiveness, it is important to consider prey phylogeny in the study of generalist predators diet breadth in order to have better results in biological control programs.

GENERAL INTRODUCTION

Biological control is a pest management strategy of using living organisms to reduce the population of prejudicial organisms, minimizing their damages and consequently economic losses for humans (Eilenberg *et al*. 2001, Hajek 2004, Delfosse 2005). Biological control brings no human health risk, it does not lead to the development of pest resistance, as it is common to chemical products, and usually has lower cost of implementation when compared to pesticides (Gurr *et al*. 2011). With the increasing need of a more sustainable agricultural production with less relying on pesticides, the need of improvement on biological control techniques and effective application demand deeper knowledge on the biology and ecology of biological control agents, the natural enemies of pests (Bale *et al*. 2008, Jonsson *et al*. 2008).

For natural enemies to find and establish in a cropping area to provide biological control of pest populations some requisites are necessary. They need to be attracted to the habitat where pests are likely to colonize, to be able of surviving and reproducing in that area increasing their population, and be capable of preying on the pests present in the crop (Symondson *et al*. 2002, Tscharntke *et al*. 2007, Bianchi & Wäckers 2008). Usually, natural enemies attraction is direct or indirectly linked with feeding needs of the adults or of their offspring, thus these organisms may be attracted by plant cues that indicate herbivore attack or presence of floral resources, for instance, or other olfactory and visual attractants (Arimura *et al*. 2005, Maffei 2010, Hogg *et al*. 2011). But, once attracted to the area, food resources, such as pollen, nectar and prey, present in the crop and surroundings must be suitable to provide survival, development and reproduction of natural enemies and therefore promote their population growth and establishment (Hajek 2004, Driesche *et al*. 2008).

Regarding the attraction to natural enemies, recently, aromatic plant species have been calling attention for the results found in several field studies on the effects of these plants on arthropod structure when they are used in intercropped systems (Nagirnyaka & Krasavina 2005, Basedow *et al.* 2006, Song *et al.* 2011,Tang *et al*. 2013). Aromatic plants are species capable of producing essential oils that can be released naturally as volatile organic compounds (VOC's) which can repel or attract different arthropods (Bakkali *et al*. 2008, Barbosa *et al*. 2009, Mithöfer & Boland, 2012). Although the toxic and repellent effects of essential oils over herbivores as well as the benefits of intercropping with aromatic plants for the reduction of herbivore assemblage are well studied, it remains unclear how aromatic plants can attract and benefit the survival, development and reproduction of natural enemies (Bakkali *et al*. 2008; Zoubiri & Baaliouamer 2011, Song *et al*. 2011).

Diet requirements for natural enemies survival and performance (larval development and adult reproduction) varies according to the biology and life history of predators and parasitoids (Hajek 2004). Some species of natural enemies are very restrict in their diets, feeding on a narrow prey range, and thus are considered specialists, while generalist species have a broader diet breadth and thus they can supposedly take advantage of more prey resources available in the field. Despite being able of prey on several different species, in many cases, a generalist natural enemy has its diets restrict by physical, physiological or behavioral reasons. For generalist predator species, for instance, degrees of specialization on diet may apply and it is not an easy task to categorize them as a broad generalist due to the lack of information on diet breadth of most species (Futuyma & Moreno 1988, Symondson *et al*. 2002). Not all species eaten by a natural enemy may provide sufficient nutrients and energy to the growth of larvae and maintenance of adults, jeopardizing its performance. Therefore the quality of herbivore species present in the crop is essential to determine predation rate,

natural enemies' population growth and ultimately biological control and that may be assessed by evaluations of prey species on fitness parameters such as larval survival rates and developmental time, and adult fecundity (Bilde & Toft 2001, Driesche *et al*. 2008).

Consequently, before implementing biological control strategies on a cropping area, it is necessary to understand the feeding ecology of natural enemies present in the surroundings, or the ones intended to be released, to ensure an efficient control of herbivore populations. The growing interest in applying efficiently biological control strategies is increasing the necessity of more complete understanding on common natural enemies, such as generalist predators, easily found in the fields or which can be commercialized and used to suppress more than one pest population. In this sense, there is an increasing interest in lacewings (Tauber *et al*. 2000, McEwen *et al*. 2001, Pappas *et al*. 2011).

Lacewings (Neuroptera: Chrysopidae) are important biological control agents, with several predatory species at least in the larval stage (Canard 2001). These insects can be found naturally in different agroecosystems and are usually considered as highly polyphagous, which make them natural enemies of various common pest in crop systems (Albuquerque 2009, Pappas *et al*. 2011). Green lacewings are among the most used and commercialized biological control agents. Most common species commercialized in the United States and Europe are *Chrysoperla carnea* Stephens e *Chrysoperla rufilabris* Burmeister, while *C. externa* and more recently, *Ceraeochrysa cubana* Hagen, are the species used in Latin America (Pappas *et al.* 2011, Tauber *et al.* 2000). *Chrysoperla rufilabris* and *C. cubana* are predators in their larval stage and are known to feed primarily on aphids, while adults have a palyno-glycophagous feeding habit (Canard 2001). Although there are several studies on different aspects of the

biology and ecology of both species, information on their feeding ecology is still restricted.

Our aim was to understand key aspects of the feeding ecology of green lacewings used in support of biological control. On Chapter I, we assessed the attractiveness of aromatic plant species to *C. cubana* and their effects on survival and performance of larvae and adults, in order to elucidate the importance of such species to the establishment of green lacewing populations. Finally on chapter II, we studied the diet breadth of *C. rufilabris* over 16 aphid species, assessing the quality of those species on survival and fitness of this generalist predator.

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CHAPTER I

An aromatic plant species attracts and benefits survival of a generalist green lacewing predator

Abstract

Aromatic plants have been considered as suitable species for being used for diversification in cropping systems. They can affect arthropod community by reducing herbivore populations and attracting beneficial insects such as generalist predators. In order to select such plants for future use in horticulture fields, we assessed attractiveness of the aromatic plants *Ocimum basilicum* L. (basil), *Mentha piperita* L. (peppermint), *Melissa officinalis* L. (lemon balm) (Lamiaceae) and *Cordia verbenacea* DC ("erva-baleeira") (Boraginaceae) to adults of the green lacewing predator *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae). This predator is widely distributed over America and is commonly found in agroecosystems feeding on aphids, mites, small caterpillars, scales and insect eggs. We further tested the effect of these plant species on the survival, development and oviposition parameters of larvae and adults of *C. cubana*. Finally, considering the results achieved on attraction and survival on the previous experiments, we evaluated the survival of larvae and adults of *C. cubana* fed on flowers of basil. Females of *C. cubana* were attracted to *O. basilicum* but not to remaining aromatic plants. Larval survival was higher when individuals had access only to *O. basilicum* leaves than when they had access to leaves of *M. piperita*, *M. officinalis*, *C. verbenacea* or water. Adult survival on leaf treatments and on water was no longer than three days. Flowers of *O. basilicum* enhanced *C. cubana* larvae survival, yet they did not reach adulthood. Adults fed on *O. basilicum* flowers lived longer compared with water, but they did not reproduce. *Ocimum basilicum* is a promising aromatic plant species to be considered for conservation biological control programs. Besides being

attractive to adults of the generalist predator, it benefits larvae and adults by providing nutritional resources when prey or other resources are absent.

Key words – Basil. Chrysopidae. Conservation biological control. Companion plants.

Introduction

Conservation biological control (CBC) is a pest management practice that relies on maintaining and increasing the populations of herbivore natural enemies (NEs), which are already present in a given area by modifying the habitat and adopting practices that benefit their establishment and performance as biological control agents (Landis *et al.* 2000, Tscharntke *et al.* 2007). Implementing diversification of vegetation in agricultural areas and surroundings is one of the strategies used in CBC, which can be achieved by managing non-crop plants or intercropping with beneficial plant species (Amaral *et al.* 2013, Wyckhuys *et al.* 2013).

Although plant diversity in cultivated areas may benefit NEs, this strategy not always result in an improvement in biological control (Andow 1991, Cullen *et al.* 2008). Some factors, such as provision of inadequate resources for the development of these organisms, can lead to failure of plant diversification in increasing biological control and thus it is important to verify the attractiveness and suitability of resources before choosing the plants that will compose the agricultural landscape or intercropping (Landis *et al.* 2000, Bianchi *et al.* 2006, Fiedler *et al.*, 2008). In this sense, aromatic plant species have been considered suitable for diversification as intercropping with these plants generally reduces pest populations (Nagirnyaka & Krasavina 2005, Basedow *et al.* 2006, Song *et al.* 2011, Tang *et al.* 2013).

Aromatic plants are capable of producing essential oils that are released spontaneously as volatile organic compounds (VOC's) and which are characterized by a strong noticeable odor (Bakkali *et al.* 2008). The properties of aromatic plant essential oils as insect repellents, deterrents and insecticides are well studied (Zoubiri & Baaliouamer 2014, Bakkali *et al.* 2008) as well as the benefits of intercropping with these plants for

regulation of arthropod community structure (Basedow *et al.* 2006, Kianmatee & Ranamukhaarachchi 2007, Song *et al.* 2010). However, few studies focused on the potential of aromatic plants species in attracting herbivore predators (Schader *et al.* 2005, Song *et al.* 2010, 2011, Tang *et al.* 2013, Wan *et al.* 2015), and even fewer addressed directly to this matter (Togni *et al.* 2016).

It is unclear how aromatic plant species can attract herbivore predators. NEs may be attracted to essential oil VOC's release (Resende 2012, Togni *et al.* 2016) or to the provision of food resources such as alternative prey, pollen and nectar provided by the flowers (Song *et al.* 2010; Tang *et al.* 2013). Several NEs take advantage of pollen and nectar as food in their mature stage or even as an alternative food source in their immature stages (Ambrosino *et al.* 2006, Fiedler *et al.*, 2008, Kopta *et al.* 2012). Additionally, these plant species may provide shelter and oviposition sites (Togni *et al.* 2016). Nevertheless, not all flowering species produce suitable resources for the best performance of NEs (van Rijn & Wackers 2016), thus its yet not clear whether aromatic plant flowers can benefit NEs fitness and thus be attractive for these arthropods.

Therefore, our aim was to investigate the possible mechanisms underlying field responses regarding the attraction of aromatic plant species to NEs (Schader *et al.* 2005, Basedow *et al.* 2006, Song *et al.* 2010, 2013, Wan *et al.* 2015). In this sense, we first explored the attraction of non-flowered aromatic plants to females of a generalist predator commonly found in cultivated crops, the green lacewing *Ceraeochrysa cubana* Hagen (Neuroptera: Chrysopidae) (Freitas & Penny 2001, Albuquerque *et al.* 2001). This lacewing has been studied for biological control purposes, it has a wide geographic range along the American Continent, and its trash-carrying larvae prey on several herbivore species (Venzon & Carvalho 1993; Lópes-Arroyo *et al.*, 1999, Albuquerque *et al.* 2001, Freitas *et*

al. 2009, Sosa & Freitas 2010). As one of our hypothesis for attraction relied on essential oils released as VOC's, we select aromatic plant species based on the production of essential oils and on research assessing essential oils against herbivore species. Following those criteria, we chose *Ocimum basilicum* L. (basil), *Melissa officinalis* L. (lemon balm) and *Mentha piperita* L. (peppermint) from the family Lamiaceae and *Cordia verbenacea* DC ("erva-baleeira"), a Brazilian species of aromatic plant, from the family Boraginaceae. All these plant species have high concentration of essential oils (Bakkali *et al.* 2008, Zoubiri & Baaliouamer 2014, López *et al.* 2008) and these oils have been tested against insect pests (e.g. Oshaghi *et al.* 2003, Yi *et al.* 2007, Popovic *et al.* 2013, Silva *et al.* 2014).

After testing the aromatic plants for attraction to *C. cubana*, we investigated the possible benefits those plants could provide to the predator. Firstly, we tested the oviposition preference of *C. cubana* to the aromatic plants in order to evaluate oviposition as a possible mechanism of attraction. Afterwards, we performed experiments to explore whether *C. cubana* larvae and adult would be able to survive in the presence of sole leaves of the aromatic plants tested, as well as to assess whether the presence of leaves could reduce the developmental time of larvae and/or stimulate adult fecundity and fertility. Díaz (2014) tested the survival of *C. cubana* larvae in flowers and leaves of non-crop plant. The author found that larvae were able to develop until the adult stage in some treatments with only leaves, suggesting that larvae from this species could have fed on leaf material, regardless of its carnivorous feeding habit. Regarding adults, the presence of certain plants, even in their vegetative stage, may benefit some aspects of predator biology such as ovipositon (Landis *et al.* 2000). Finally, we selected the aromatic plant with the most promising results to test the effect of its flowers on the survival of larvae and adults of the predator to examine the possible benefits of flowers on predator survival and development.

That may give us elements to evaluate the importance of the aromatic plant species for maintenance of predator population in crops. We hypothesized that: 1) non-flowering aromatic plants may attract the generalist lacewing predator *C. cubana* possibly due to essential oils released as VOC's; 2) aromatic plants that are attractive to *C. cubana* will be preferred for oviposition; 3) leaves of aromatic plant species may benefit *C. cubana* larvae by increasing their survival or shortening developmental time; and 4) flowers of aromatic plants that are attractive to *C. cubana* will benefit larvae and adult fitness.

Material and Methods

Experiments were carried out in a greenhouse and in the laboratory, in Viçosa (20º 45' 14" S, 42º 52' 55" W, 648 m a.s.l.), State of Minas Gerais, Brazil.

Predator rearing

Ceraeochrysa cubana was obtained from the stock colony kept in the laboratory (25 ± 2ºC, 70 ± 10% RH and 14 hours of photophase) of the Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG), EPAMIG Sudeste, in Viçosa, State of Minas Gerais, Brazil. Rearing followed the methodology of Venzon et al. (2006). Adults were kept in PVC tubular cages (15 x 15 cm) wrapped in white paper towels. Cages were supported in plastic trays lined with white paper towel and closed on their superior end with PVC film. Individuals were fed with diet made of brewer's yeast and honey (1:1) placed on a piece of plastic paraffin (Parafilm^{TM}) and hung on the superior part of the cage. Water was supplied in a glass vial (15 mL) filled with a wet cotton pad. Paper towel, diet and water vials were replaced once a week. The surface where the eggs were laid, i.e. paper towel and PVC film, were kept in plastic containers (14 cm diameter, 10 cm height) after

being removed from the cages, and checked every two days until egg hatching. Afterwards, larvae were individualized in plastic vials (2.5 cm diameter, 7 cm height) closed with PVC film and fed with *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs every three days until pupation. *Anagasta* eggs are considered a nutritious food for green lacewing larvae and they are commonly used for their mass-rearing (Lopez-Arroyo *et al.* 1999, Tauber *et al.* 2000).

Aromatic plants

Four aromatic plant species were tested: *O. basilicum* (basil), *M. officinalis* (lemon balm), *M. piperita* (peppermint), and *C. verbenacea* ("erva-baleeira"). *Ocimum basilicum* is an aromatic annual herb from family Lamiaceae, native to Asia and Africa. This plant grows about 60 cm height and it is cultivated worldwide with medicinal e culinary purposes. It has a long flowering period that last for three to four months and its flowers are considered a good source of nectar with high yield of sugar. Basil produces a high content of essential oil, ranging from 0.2% to 5.2%, and its predominant constituents are estragole and linalool the, substances with repellent and insecticide properties (López *et al.* 2008, Kwee & Niemeyer 2011). *Melissa officinalis* is an aromatic perennial bushy herb from family Lamiaceae, originated in the Mediterranean region but, as *O. basilicum*, it can be cultivated in many regions of the world. This plant grows from 70 to 150 cm height and it is mainly used for medicinal and spice purposes. Flowers of *M. officinalis* are attractive to honey bees as source of nectar and they bloom for 45-50 days. Essential oil produced by *M. officinalis* ranges from 0.02% to 0.3% and it is mostly constituted by citral and citronellal, monoterpenes with repellant properties (Mimica-Dukic *et al.* 2004, Moradkhani *et al.* 2010). *Mentha piperita* is an aromatic herbaceous perennial plant from family Lamiaceae, native to Europe and can be grown in several places over the world. This plant grows from

30-90 cm height and it can be used for medicinal and culinary purposes. Peppermint essential oil content ranges from 0.7% to 3.0%, and it is composed mainly by the monoterpenes menthol and menthone, both with pesticide properties (Shah & D'Mello 2004, McKey & Blumberg 2006). *Cordia verbenacea* is a bushy perennial aromatic plant species from family Boraginacea, native from the Brazilian Atlantic Rainforest. It can grow from 80 to 200 cm and it is mainly used for medicinal purposes. Essential oil contents range from 0.2% to 1.5% and the major components are α-pinene, which is repellant to insects, and β-felandrene (Fernandes *et al.* 2007, Rodrigues *et al.* 2012).

Plant seedlings

Stem cuttings of each of the plant species were collected from an organic cultivation in the Fazenda Experimental Vale do Piranga, from EPAMIG, located in the municipality of Oratórios (20º 25' 50" S e 42º 48' 20" W, 492m als), Minas Gerais, Brazil, and taken to the greenhouse. Cuttings were placed in 1 L pots filled with soil substrate to root. Seedlings were watered daily and N-fertilized every 15 days. Pruning was conducted whenever necessary to keep plants with similar sizes and/or number of leaves. After a pruning, at least 15 days passed before using plants in the attractiveness experiments.

Release-recapture experiment

In order to assess attractiveness of the aromatic plants to *C. cubana* we performed a series of release-recapture experiments (Pallini *et al.* 1997, Venzon *et al.* 2001) using seedlings of the same age, without flowers (to avoid effects of attraction due to floral resources) or signs of herbivore infestation (to avoid effects of attraction due to herbivore-induced volatiles). Each experiment tested one of the following plant combination: (I) *O. basilicum vs. C. verbenaceae*; (II) *M. piperita vs. M. officinalis*; (III) *O. basilicum vs. M.*

piperita; and (IV) *M. officinalis vs. C. verbenacea*. Each plant species was considered as a treatment and each experiment was replicated four times. Combination I and II were chosen randomly. Combinations III and IV were taken from the plant species that attracted more (III) and less (IV) lacewings in the first two replicates of I and II.

As not all plant species had similar architecture or leaf size, care was taken to use plants with similar sizes and/or number of leaves/leaf surface in each trial, in order to minimize such effects on insect's choice. Plants sizes ranged from 20 to 40 cm, and all plants used had a minimum of 30 leaves. Potted plants were allocated equidistantly in a hexagon (60 cm diameter) inside a PVC framed cage covered with fine mesh (100 cm x 100 cm x 80 cm). Each hexagon consisted of six potted plants, three pots per plant species, displayed interspersed. Each plant position was occupied by one treatment in half of the replicates, and the other treatment in the other half in order to control for any unforeseen directionality in the predators choice (Janssen 1999).

About 100 mated females of *C. cubana*, aging 10-15 days, were individualized, without food for 18 hours, in plastic vials (2.5 cm diameter, 7 cm height) with a wet cotton pad inside. We assumed that mated and starved females were more prone to find suitable patches for reproduction and feeding (Desouhant *et al.* 2005). Vials were closed with PVC film. Females were released in the center of the plant hexagon by unplugging the vials. Plants were checked for the presence of *C. cubana* one hour after release and the ones found on the plants were counted and removed from the cage (recapture) using an aspirator. This process was repeated every hour during 7h (Venzon *et al.* 2001). Afterwards, counting and recaptures were carried out after 24, 48 and 72h after releasing. These times for evaluation were set after prior tests to observe the general behavior of females, after releases. Mean temperature and relative humidity inside the cage were registered using a thermohigrometer (Incoterm, São Paulo, Brazil).

Oviposition preference experiment

Ceraeochrysa cubana females were tested regarding their oviposition preference towards aromatic plant species, following the same species combinations and plant conditions from the previous experiment. Two potted plants, one of each species, were placed inside a wooden framed cage (70 cm x 67 cm 67 cm) covered with fine mesh. Ten mated females, aging about 10 days, were individualized as described previously and starved for 18h before release. Previous observations revealed that *C. cubana* females are able to lay eggs during 48h after a period of 18h of starvation. Thus, 48h hours after releasing, we recaptured the females and counted the eggs on the plants and on the cage internal surface. Each plant species combination was replicated six times, alternating plant position in each replication. Mean temperature and relative humidity inside de cages were also registered.

***Ceraeochrysa cubana* survival and performance on leaves of aromatic plant species**

We evaluated whether larvae and adults of *C. cubana* could survive, develop and reproduce in the presence of leaves of the four aromatic plant species in order to assess any benefits that could be provided by non-flowered and non-infested plants to this predator, once the attraction experiment was conducted with plants without any food for either adults or its larvae.

Larvae survival

Newly molted second instar larvae of *C. cubana*, fed previously only on *A. kuehniella* eggs, were individualized in transparent plastic containers (20 x 10 cm) and covered with a fine mesh. Second instar larvae were used, because in the first instar, larvae

are more prone to die due to instar-related causes than diet (Díaz 2014). Each larva was subjected to the following food sources (treatments): (a) *O. basilicum* leaf plus water; (b) *M. piperita* leaf plus water; (c) *M. officinalis* leaf plus water; (d) *C. verbenacea* leaf plus water; (e) *A. kuehniella* eggs plus water (positive control); (f) water (negative control); (g) *O. basilicum* leaf plus *A. kuehniella* eggs plus water. This last treatment was set to assess whether the presence of *O. basilicum* leaf could boost the development of larvae, by shortening the developmental time for instance, when compared to the sole presence of suitable food (i.e., *A. kuehniella* eggs). *Ocimun basilicum* was chose based on the results of the release-recapture experiments. All leaves used were taken from plants at same age. Leaves were gentle washed in tap water, dried and observed under a stereoscopic microscope to check for eggs or tiny arthropods that could be eaten by larvae. The leaf petioles of the aromatic plants were placed in a Petri dish (2.5 cm diameter) filled with water and covered with Parafilm^{TM} in order to keep the leaves turgid. Water was offered to the larvae in a wet piece of cotton, laid in a Petri dish (2.5 cm diameter). Leaves and *A. kuehniella* eggs were replaced every three days and water was supplied daily. Each treatment was replicated 20 times. Developmental time and survival of larvae were evaluated until death or pupation. After 13 days, all pupae that still did not reach adulthood were observed daily under a stereoscopic microscope and the dead ones were discarded.

Adult survival and oviposition

We evaluated adult survival, fertility and fecundity in the same treatments described above, except for treatments "e" and "g", for which, instead of *A. kuehniella* eggs, we used a diet of yeast and honey (1:1). For each treatment, a newly emerged *C. cubana* couple was placed in a PVC cage (10 cm diameter x 10 cm height) supported by a Petri dish (14 cm diameter), both lined with towel paper. A small branch with three to four

leaves of one of the aromatic plant species was placed in a small glass vial (10 mL) filled with wet cotton, from where the adults could also assess water. Procedures regarding leaves were the same described in larval survival experiment. Cages were covered with PVC plastic film and each treatment was replicated 12 times. Adult survival, female pre-oviposition, oviposition period and effective days of oviposition (i.e. number of ovipositing days during the oviposition period) were recorded and oviposition rate was evaluated daily. To assess the viability of eggs, we took a sample of 20 eggs from each replicate every three days until eight samples were reached, totaling 160 eggs. Eggs were individualized in microcentrifuge tubes (1.5 mL) with a small piece of wet cotton in the bottom to avoid desiccation and observed for hatching for a period of 15 days.

Ceraeochrysa cubana survival on basil flowers

Since pollen and flower nectar can benefit both adult and larval survival of *C. cubana* (Díaz 2014), we also tested predator survival in the presence of flowers of the most attractive plant from the release-recapture experiment.

For larval survival, the following treatments were used: (a) *O. basilicum* inflorescence plus water; (b) *O. basilicum* leaf plus water; (c) *A. kuehniella* eggs plus water (positive control); (d) water (negative control). Experiment settings regarding larvae age and containers followed the same procedures described for the experiment for larval survival in leaves. Eggs and small arthropods present in inflorescences were removed before the experiment under stereoscopic microscope. Each basil inflorescence had six flowers and they were replaced every two days. Basil leaves and *A. kuehniella* eggs were replaced every three days and water was supplied daily. Each treatment was replicated 20 times. Survival of larvae was evaluated until death or pupation. After 13 days, all pupae

that still did not reach adulthood were observed daily under a stereoscopic microscope and the dead ones were discarded.

Adult survival was evaluated in the same treatments described above, except for treatments "c" which, instead of *A. kuehniella* eggs, we used a diet of yeast and honey (1:1). Experiment setting regarding age of couples and cages followed the same procedures described for the experiment for adult survival on leaves. Each basil inflorescence had 15 flowers (7.5 flowers per individual in the cage) and was replaced every two days. Diet and basil leaves were replaced every three days and water was supplied daily. Each treatment was replicated 12 times. As there was no oviposition in the flower treatment, we did not perform further analysis, besides the survival.

Statistical analysis

Data from the release-recapture experiments from each plant species combination were considered altogether, i.e., the counts for each evaluation were pooled and time was not considered in the analysis. Thus, data was fitted using generalized linear models (GLM's) functions with quasi-Poisson error distribution (Crawley 2007) and submitted to ANOVAs. To assess differences among positions, we performed *a posteriori* contrasts (Crawley 2007).

To test the effect of each treatment on larval and adult survival, data were analyzed using Cox proportional hazard model (Cox PHM) and statistical significances were assessed by Likelihood ratio test (Cox 1972, Crawley 2007). Cox PHM are semi-parametrical models used to access the effects of covariates on survival without discriminating the form of the baseline hazard and calculate the relative likelihood of death among subjects, at a certain time, based on their covariates values (Fox 2001, Crawley 2007, Cook 2009). Differences among treatments were assessed *a posteriori* contrasts of

the means (Crawley 2007). Kaplan-Meier survivorship function was used to illustrate the effect of treatment on survival (Crawley 2007).

Duration of larvae instars was also analyzed by ANOVAs using GLM function with quasi-Poisson error distribution and *a posteriori* contrasts of means to assess differences among treatments. ANOVAs were also applied to oviposition data, using GLM's function with quasi-Poisson (counting data) or quasi-Binomial (proportion data) error distribution.

We performed residue analysis to assess the appropriateness of all models and error distributions used (Crawley 2007). All statistical analyses were carried out in the software R (R Development Core Team 2015).

Results

Release-recapture experiment

In the first and second series of trials, females of *C. cubana* were significantly more attracted to *O. basilicum* in comparison to *C. verbenaceae* ($F_{1,22} = 6.073$; $P = 0.022$; Fig 1a) and *M. piperita* ($F_{1,22} = 29.895$; $P < 0.001$, Fig 1b). In the third and fourth series of trials, *C. cubana* did not prefer *M. officinalis* over *M. piperita* ($F_{1,22} = 0.402$; $P = 0.532$; Fig 1c) neither *M. officinalis* over *C. verbenaceae* ($F_{1,22} = 0.390$; $P = 0.538$; Fig 1d).

The positions of plants in the cage had a significant influence on females choice in the following combinations of aromatic plants: *O. basilicum vs. M. piperita* ($F_{5,17} = 3.307$; $P = 0.029$); *M. officinalis vs. M. piperita* ($F_{5,17} = 4.892$; $P = 0.006$); and *M. officinalis vs. C. verbenaceae* ($F_{5,17} = 5.140$; $P = 0.005$). In the experiments where *O. basilicum* were tested against *C. verbenaceae*, position of plants had no significant influence on females choice ($F_{5,17} = 2.045$; $P = 0.123$). As a general pattern, Northeast and North positions were more

attractive to females of *C. cubana* compared to East/Southeast, South, Southwest and West/Northwest ($F_{5,90} = 5.374$; $P < 0.001$).

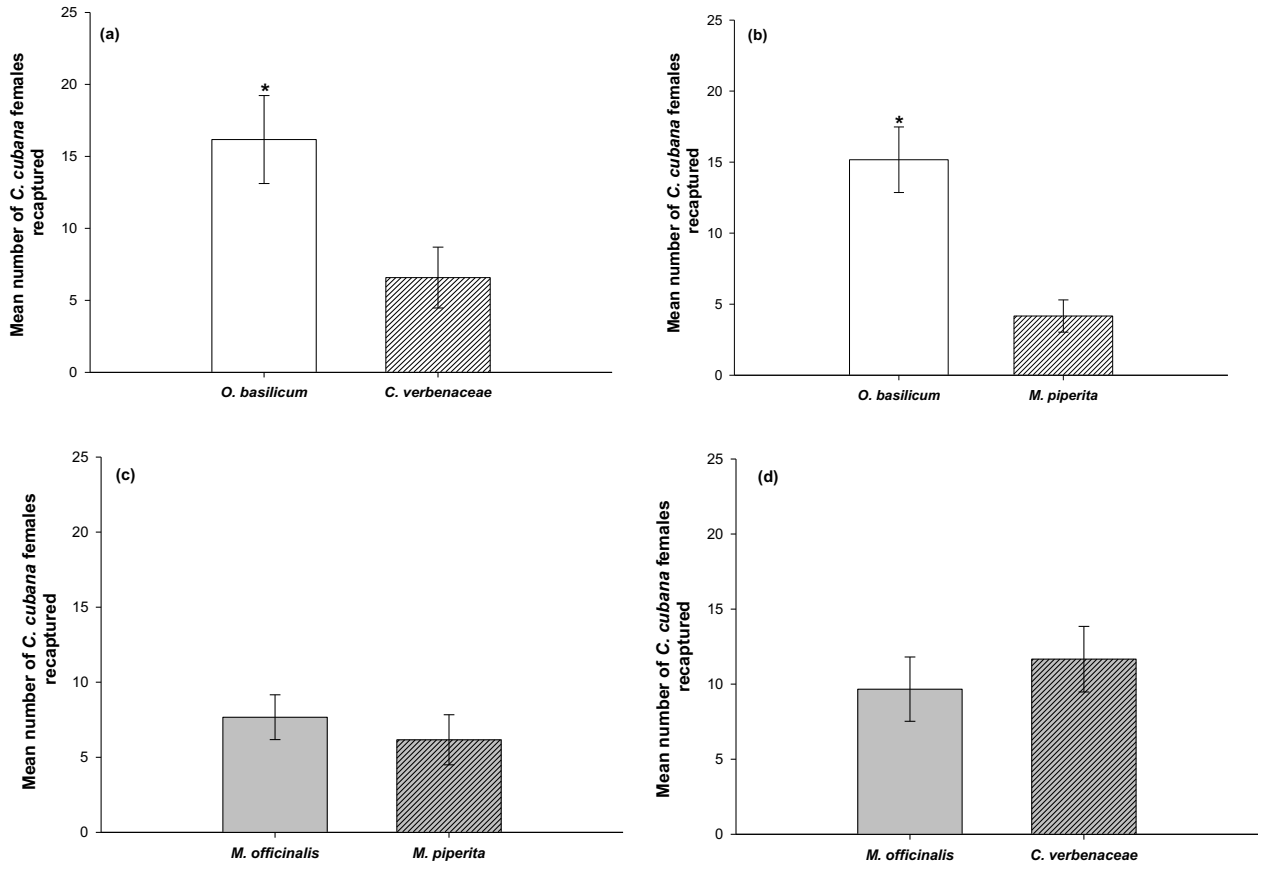


Figure 1 – Mean number of females of the green lacewing *Ceraeochrysa cubana* recaptured per plant after 72 hours, in each series of aromatic plant species. Mean ± standard errors are presented. *Indicates that a treatment is significantly different from the other (*a posteriori* contrasts, $P < 0.05$).

In the experiments where *O. basilicum* were tested against *C. verbenaceae*, position of plants had no significant influence on females choice ($F_{5,17} = 2.045$; $P = 0.123$). As a general pattern, Northeast and North positions were more attractive to females of *C. cubana* compared to East/Southeast, South, Southwest and West/Northwest ($F_{5,90} = 5.374$; $P < 0.001$).

Oviposition preference experiment

In all series of oviposition experiments, females of *C. cubana* laid the majority of eggs on the cage surface (> 90%). Females laid only 1% and 4.8% of the eggs on *O. basilicum* and *C. verbenaceae* leaves, respectively. Also, only 0.8% and 1.2% of the eggs were laid on the leaves of *O. basilicum* and *M. piperita*, respectively. Females laid just 1.1% and 1.6% of the eggs on the leaves of *M. officinalis* and *M. piperita*, respectively. Finally, only 2.5% and 2.6% of the eggs were laid on the leaves of *M. officinalis* and *C. verbenaceae,* respectively. As there was an apparent difference in the oviposition in the combination of *O. basilicum* and *C. verbenacea*, we performed a statistical analysis only for the data of this trial, and found that, even considering only the eggs laid on the plants, there was no significant difference between treatments ($F_{1,10} = 2.437$; P = 0.149). Due to the very low number of eggs laid on the leaves of the remaining aromatic plants, statistical analyses could not be performed.

Larval survival on leaves of aromatic plant species

Larval survival of *C. cubana* differed among treatments (Likelihood ratio test = 155.4, df = 6, P < 0.001). As expected, larvae lived longer in treatments where *A. kuehniella* eggs were offered compared to remaining diets (Fig 2); most larvae in these treatments pupated between days 7 to 11 however, one larvae died, before pupating, in day 12 causing the abrupt decline observed in the *A. kuehniella* survival curve. There were no significant differences in the survival of larvae regarding the presence or absence of a leaf of *O. basilicum* plus *A. kuehniella* eggs ($X^2 = 0.0613$, P = 0.804; Fig 2). Although larvae lived shorter when only a leaf was offered, compared to those with *A. kuehniella* eggs, we found that larvae lived longer in *O. basilicum* leaves when compared to *M. piperita*, *M. officinalis*, *C. verbenaceae* leaves and the water control ($X^2 = 6.834$; P < 0.01; Fig 2).

Mentha piperita, *M. officinalis*, *C. verbenaceae* and water did not differ from each other regarding larval survival (X^2 = 1.922; P = 0.166; Fig 2).

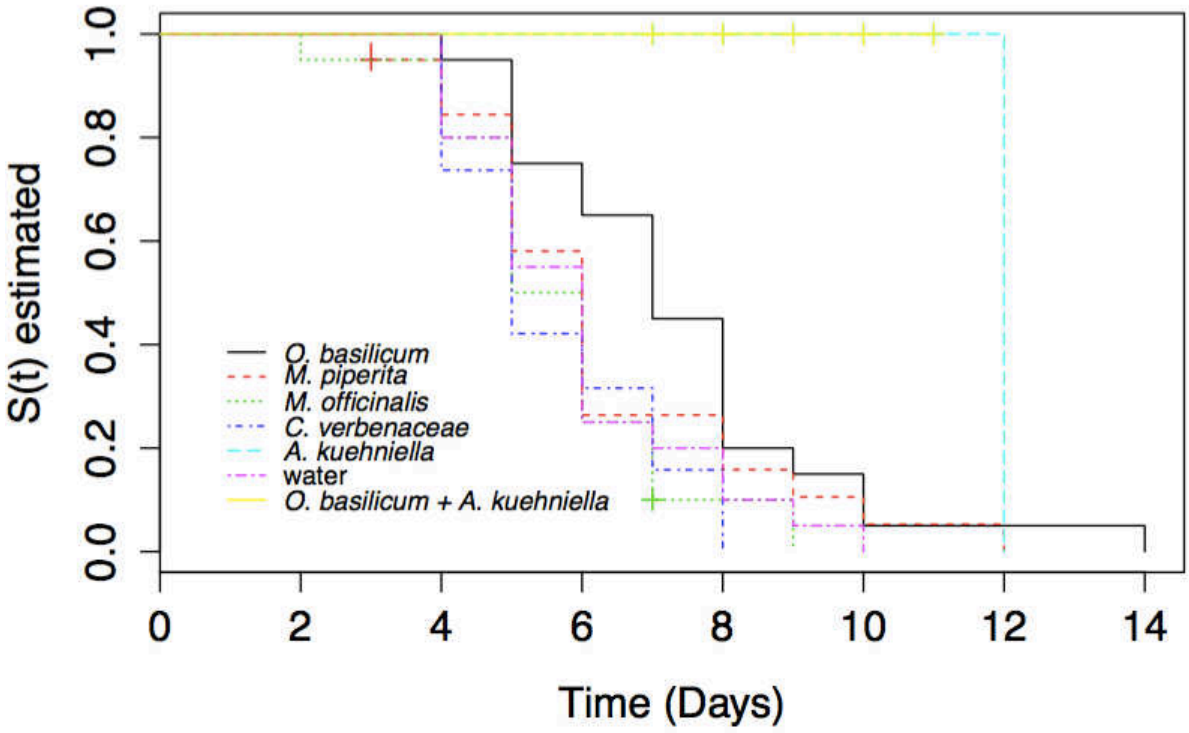


Figure 2 – Larval survival of green lacewing *Ceraeochrysa cubana* (S(t)) on *Ocimum basilicum*; *Mentha piperita*; *Melissa officinalis*; *Cordia verbenacea*; *Anagasta kuehniella* eggs; water; *O. basilicum* plus *A. kuehniella* eggs. Kaplan-Meier survival plot. Statistical significance was determined by Likelihood ratio test with a Cox proportional hazard model.

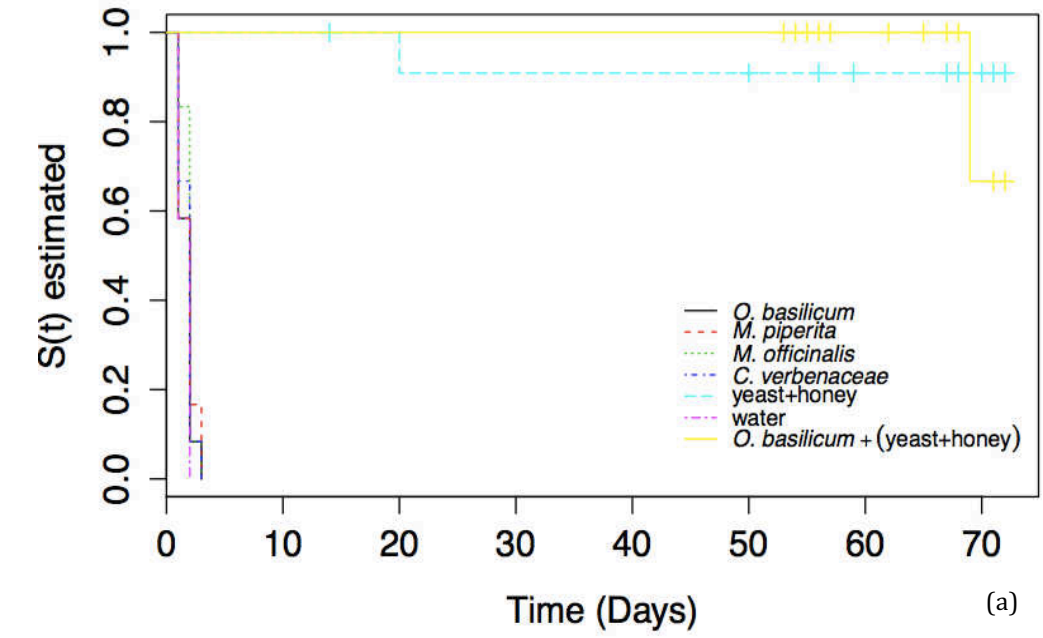
All larvae in the negative control (water) died before reaching the third instar and pupae stage was reached only when larvae fed *A. kuehniella* eggs. Thus, to evaluate the effects of treatments on the developmental time of *C. cubana* larvae, we excluded from statistical analysis all replicates where the larvae died before molting to the third instar, and evaluate pupae stage only for the treatments with *A. kuehniella* eggs. Duration of second instar was significantly influenced by diets ($F_{5,54}$ = 2.486; P < 0.05). Second instar lasted shorter in the treatments with *A. kuehniella* eggs both with (n = 20) and without *O. basilicum* leaves (n = 19) offered along with eggs, *O. basilicum* leaf (n = 9), *M. officinalis* (n = 3) and *C. verbenaceae* (n = 2), compared to *M. piperita* (n = 7), which had the longest

second instar duration. Third instar duration was not affected by the diets offered ($F_{5,54}$ = 0.844; P > 0.5). Only larvae from the treatments with *A. kuehniella* eggs pupated, and sole the ones that emerged as adults were included in the analysis for pupae duration. There was no significant differences on pupae duration between treatments where eggs were offered, with or without a *O. basilicum* leaf (n = 15 and n = 11, respectively; $F_{1,24}$ = 2.837; P = 0.105).

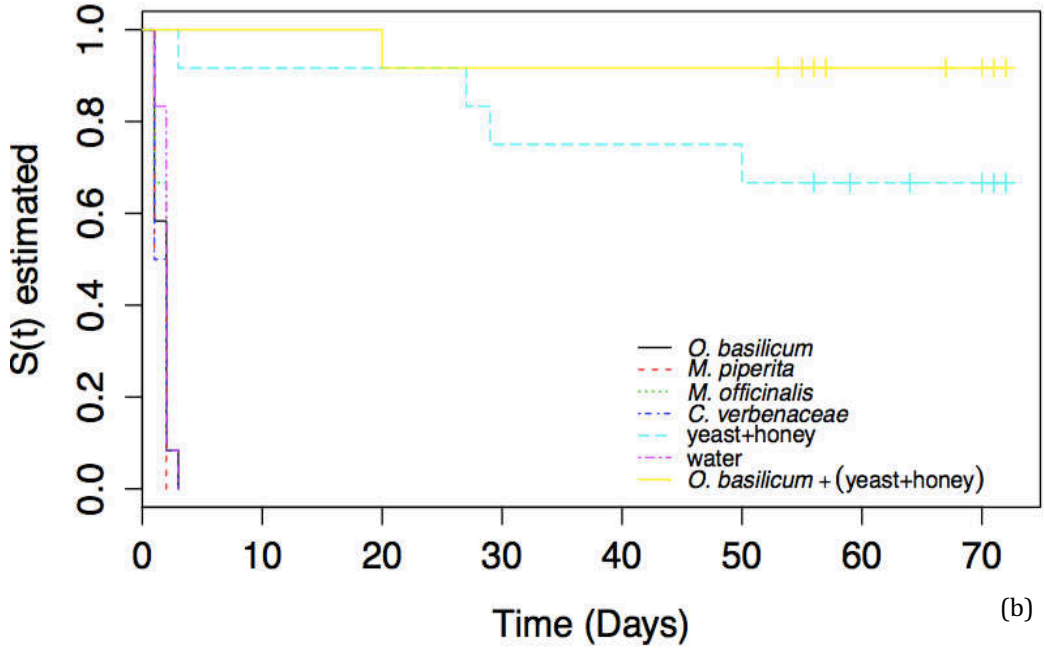
Adult survival and fecundity in the presence of leaves of aromatic plant species

Diets influenced life span of adults of *C. cubana* (Females: Likelihood ratio test = 97.31, df = 6, P < 0.001; Males: Likelihood ratio test = 93.74, df = 6, p < 0.001; Fig 3a,b). Both males and females lived longer when yeast plus honey were offered compared to the remaining treatments (Females: X^2 = 95.83, P < 0.001; Males: X^2 = 91.73, P <0,001), and the addition of *O. basilicum* leaves to this diet did not influence the survival of adults (Females: X^2 = 0.005, P = 0.944; Males: X^2 = 2.181, P = 0.14). *Ocimum basilicum, M. piperita, M. officinalis* and *C. verbenaceae* did not differ among themselves and did not differ from the treatment with only water regarding male and female survival (Females: X^2 = 0.403 P = 0.525; Males: X^2 = 0.868, P = 0.351).

Both females and males could not survive longer than three days in the treatments without yeast plus honey diet and therefore it was not possible to perform further analysis. Treatments with yeast plus honey with or without *O. basilicum* leaves did not differ in pre-oviposition ($F_{1,22}$ = 0.164; P = 0.69), oviposition ($F_{1,22}$ = 1.248; P = 0.276) periods or the number of days of effective oviposition ($F_{1,22}$ = 0.649; P = 0.429). The total number of eggs laid was not affected by the presence of a leaf of *O. basilicum* along with the diet offered ($F_{1,22}$ = 1.006; P = 0.327) as well as daily ovipositon ($F_{1,22}$ = 0.03, P = 0.863) and the proportion of viable eggs ($F_{1,22}$ = 3.034, P = 0.095).



(a)



(b)

Figure 3 – Female (a) and male (b) survival of green lacewing *Ceraeochrysa cubana* (S(t)) on *Ocimum basilicum*; *Mentha piperita*; *Melissa officinalis*; *Cordia verbenacea*; yeast plus honey; water; *O. basilicum* plus yest + honey. Kaplan-Meier survival plot. Statistical significance was determined by Likelihood ratio test with a Cox proportional hazard model.

***Ceraeochrysa cubana* survival on basil flowers**

Survival of larvae of *C. cubana* in basil flowers differed among treatments (Likelihood ratio test= 127.5, df = 3, P < 0.001, Fig 4). Larvae lived longer in treatments where flowers were offered compared to remaining diets (Fig 4), although only three larvae pupated: two did not reach adulthood, and the third one produced a deformed adult which died after emerging. Larvae fed on flowers could survive for 71 days, in average, and four individuals survived for more than 100 days without pupating. The treatment where a sole *O. basilicum* leaf was offered as food source differed from the negative control (X^2 = 10.132, P < 0.01, Fig 4). Larvae had completed all the larval stages and turned into viable adults only when fed *A. kuehniella* eggs. Most larvae on *A. kuehniella* treatment pupated between days 9 to 11, however two larvae which survived more than 11 days without pupating caused the abrupt decline in the survival curve of this treatment.

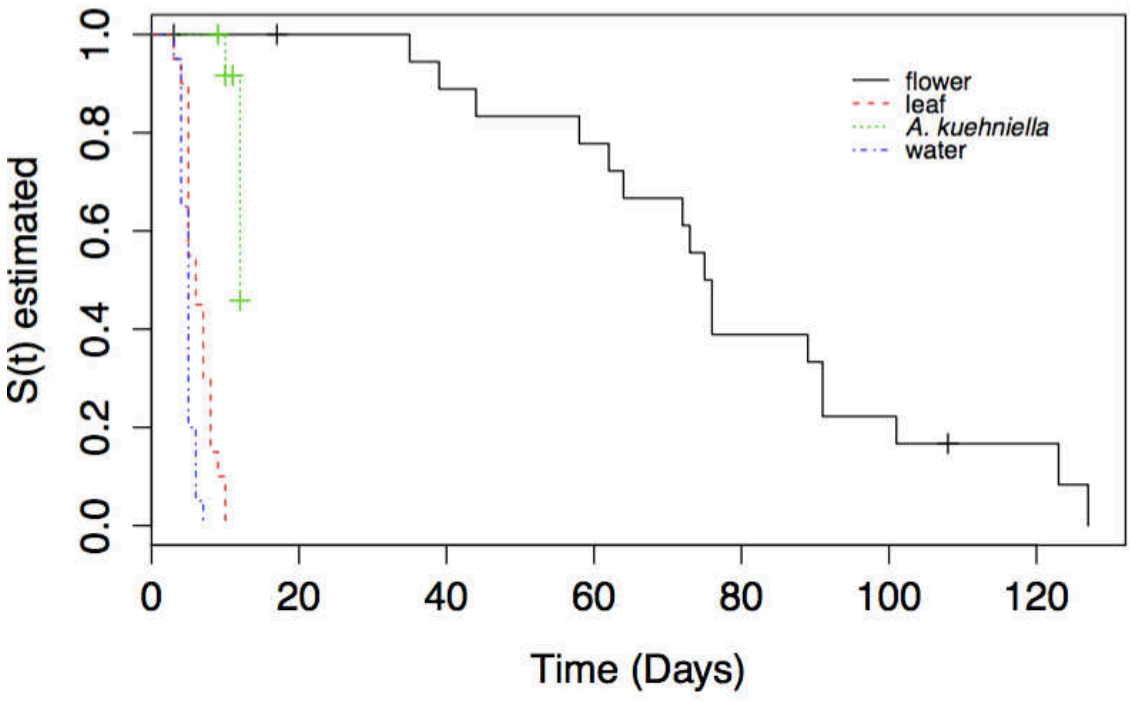
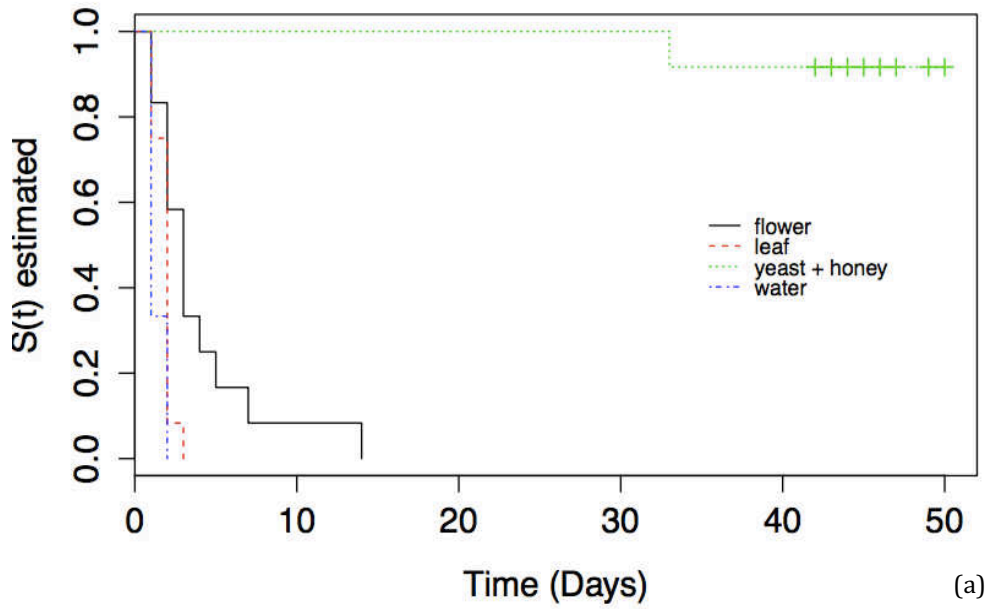
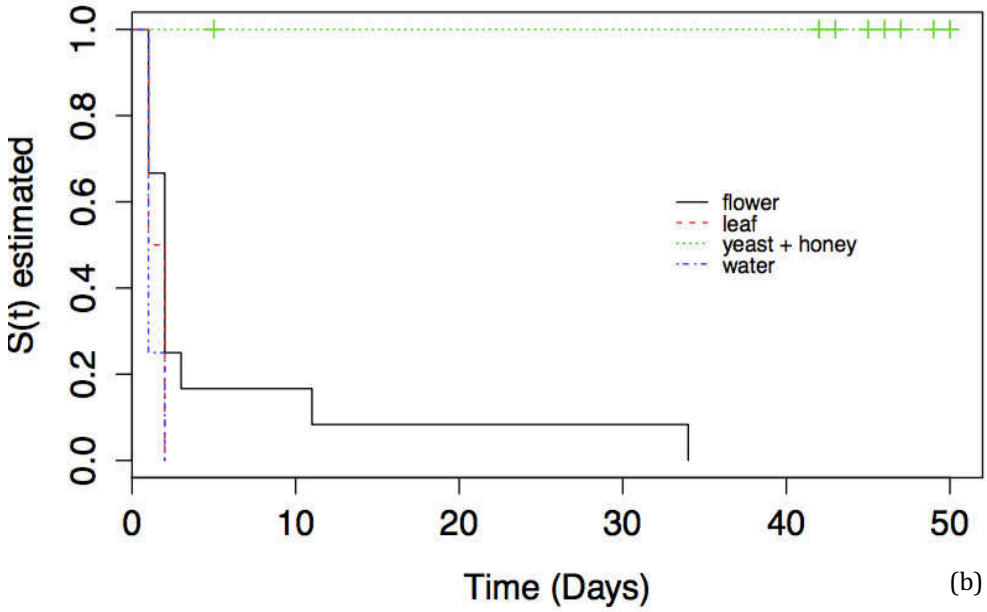


Figure 4 – Larval survival of green lacewing *Ceraeochrysa cubana* (S(t)) on flower of *Ocimum basilicum*; leaf of *O. basilicum*; eggs of *Anagasta kuehniella*, and water. Kaplan-Meier survival plot. Statistical significance was determined by Likelihood ratio test with a Cox proportional hazard model.

Survival of females (Likelihood ratio test= 65.25, df = 3, $P < 0.001$; Fig 5a) and males (Likelihood ratio test = 55.98, df = 3, $P < 0.001$; Fig 5b) of *C. cubana* in basil flowers also differed among treatments. Survival of adults in the diet of yeast plus honey was longer for both sexes compared to the remaining treatments (Figs 5a,b). Life span of adults feeding on flowers was longer than the adults exposed to leaves and the ones in the negative control (Females: $X^2 = 12.467$; $P < 0.001$; Males: $X^2 = 5.484$, $P < 0.05$). However, females fed with either these three diets did not oviposit. Adult survival in the treatment with basil leaves was not significantly different from the negative control (Females: $X^2 = 2.851$, $P = 0.091$; Males: $X^2 = 0.87$, $P = 0.351$).



(a)



(b)

Figure 5 – Female (a) and male (b) survival of green lacewing *Ceraeochrysa cubana* (S(t)) on flower of *Ocimum basilicum*; leaf of *O. basilicum*, yeast plus honey, and water. Kaplan-Meier survival plot. Statistical significance was determined by Likelihood ratio test with a Cox proportional hazard model.

Discussion

Adult females of the lacewing *C. cubana* were attracted to and its larvae benefited from longer survival on basil plants in the absence of suitable food (prey, pollen, nectar),

opening an interesting path in the use of aromatic plant species in programs of biological control. Although no aromatic plant tested had a significant effect on the survival and oviposition parameters of adults, and *O. basilicum* presence as a vegetal material along with eggs or diet did not improve larvae development or adult oviposition parameters, the increase of larval survival in the presence of leaves of *O. basilicum* seems remarkable as there is no record of *C. cubana* larvae feeding on leaf material. Additionally, survival of both larvae and adults of *C. cubana* in *O. basilicum* flowers reinforces the benefits that this plant species can offer to a generalist predator.

In our study, *C. cubana* females preferred *O. basilicum* plants over *C. verbenaceae* or *M. piperita*, and showed no preference for *M. piperita, M. officinalis* or *C. verbenaceae* when they were tested against each other. Field studies have shown the potential of *O. basilicum* plants in reducing pest incidence in commercial crops in intercropping systems (Schader *et al.* 2005, Basedow *et al.* 2006, Kianmatee & Ranamukhaarachchi 2007; Tang *et al.* 2013) and laboratory trials had demonstrated the negative effects of its essential oil on pest physiology and survival (Aslan *et al.* 2004, Kostic *et al.* 2008, Kiradoo & Srivastava 2010). Although several studies referred to a possible *O. basilicum* attractiveness to generalist natural enemies (Schader *et al.* 2005, Basedow *et al.* 2006, Kianmatee & Ranamukhaarachchi 2007; Song *et al.* 2010, 2011, 2013, Tang *et al.* 2013, Wan *et al.* 2015) there was no study that addressed directly *O. basilicum* attractiveness to predators, an important component to avoid pest outbreaks in sustainable agriculture systems (Symondson *et al.* 2002, Whyckhuys *et al.* 2013), which make our study a novelty.

Our study clearly demonstrated *C. cubana* female preference for non-flowering, *O. basilicum* plants, which flowers could serve as food for adults, larvae or pests. Therefore we hypothesized that this attractiveness may be due to VOC's naturally released by basil. Lacewings in general are more active by dawn and at night when luminosity is low. So,

they rely more on olfactory cues than on visual stimuli to find suitable patches to forage, shelter and oviposit (New 2001, Resende 2012). On the other hand, *O. basilicum* produces a relatively high content of essential oil, ranging from 0.23% to 5.22% (López *et al.* 2008), which is stored on leaf surface in foliar trichomes and released as odor.

The main compounds of *O. basilicum* essential oil are the monoterpenes linalool and estragol (Popovic *et al.* 2013), which may be toxic, repellent and deterrent to herbivores (e.g. Pascual-Villalobos *et al.* 2004, El-Seedi *et al.* 2012). These monoterpenes could also have an important role in predator attraction, which remains to be investigated. Tabata *et al.* (2011) comparing volatiles released by squash plants infected and non-infected by powdery mildew (*Podosphaera* sp.) and their effects on behavioral response of the mycophagous ladybug *Psyllobora vigintimaculata* Say (Coleoptera: Coccinellidae) found that infected plants had an elevated emission of various compounds that were also present in the volatiles of uninfected plants among them, linalool. These authors hypothesize that the increase in such compounds could reflect the induction of plant defense against the fungus and thus they could be an important part in the volatile blend that attracts the fungi-feeding ladybug. Therefore, it seems likely that plants with high concentrations of linalool, or other monoterpenes like estragole, in an easily volatile essential oil form could attract foraging predators, which associate these substances with the possible presence of their prey. None of the other aromatic plants tested have linalool in their composition (Scavroni *et al.* 2005, Rodrigues *et al.* 2012, Jalal *et al.* 2015).

Another possibility is *C. cubana* being attracted to *O. basilicum* due to its own foraging needs. Some volatiles are present in both flowers and leaves and non-predacious adults of lacewings may recognize plants where they may find suitable pollen and nectar for feeding even in plant vegetative stage (Zhu *et al.* 2005, Resende 2012). Our results on survival of adults on *O. basilicum* flowers demonstrated that these flowers could provide

food for adults keeping them alive for a period of time when there is no other food source available. It is possible that, in this case, females could be attracted to the plant for some volatile shared by leaves and flowers and prefer *O. basilicum* plants for food for themselves (Zhu *et al.* 2005).

Finally, during our experiment, we observed that females landed on *O. basilicum* leaves were more unnoticeable than the ones landed in the other plant species and we speculate that probably basil is a better shelter to avoid intraguild predation compared to remaining tested species. *Ocimum basilicum* leaves are light green, almost the same color as *C. cubana*, while the other plant species have dark green leaves. Nevertheless, based on our results on *C. cubana* larvae survival, we suppose that *O. basilicum* choice by female is linked with larvae benefit.

In fact, larvae of *C. cubana* which was offered only *O. basilicum* leaves had a longer life span in comparison to water control and to larvae fed on other aromatic plant leaves, although they lived shorter when compared to larvae fed on *A. kuehniella* eggs. It is well known that lacewing larvae are mainly predacious, feeding on different prey species (Albuquerque 2009, Papas *et al.* 2011), but occasionally these insects may consume plant-based food such as pollen and floral or extrafloral nectar to supplement their diet (Limburg & Rosenheim 2001, Albuquerque 2009). However, there are no records of lacewing larvae feeding on leaf tissues as observed for some omnivorous generalist predators (Coll & Guershon 2002, Ghabeish *et al.* 2010). Limburg and Rosenheim (2001) studying extrafloral nectar consumption by *Chrysoperla plorabunda* Fitch (Neuroptera: Chrysopidae) in cotton leaves and its effects on larvae survival, observed a small proportion of larvae in treatments with leaf only (with no access to extrafloral nectaries or water supply) piercing leaf veins with its mandibles. Despite this behavior, authors could find no evidence of larvae feeding on leaves once in this treatment average survival was 1-2 days and leaf only treatment did

not differ from treatments with no leaf at all or leaf only plus water supply. On the other hand, Díaz (2014) testing the effect of different non-crop plant species on survival and development of *C. cubana* larvae observed that 50% of larvae reared only in the presence of leaves of *Ageratum conyzoides* L. (Asteraceae) plus water survived for 51 days, and 13% from those pupate and achieved adulthood, suggesting that this species could be feeding on leaf tissues. We could not find any other visible indication to support feeding on leaf tissues hypothesis in addition to larvae survival. One possibility could be the presence of small eggs of mites, fungi, or honeydew from which larvae could have fed on but all leaves used in the trials were washed with water and carefully observed under a stereoscopic microscope before been offered to larvae. As information on *Ceraeochrysa* spp. is generally scanty (Albuquerque *et al.* 2001, Sosa & Freitas 2010) and most work on larvae addressed only prey food (Santa-Cecília *et al.* 1997, Lopez-Arroyo *et al.* 1999, Auad *et al.* 2001, Alcântara *et al.* 2008), it is conceivable that leaf tissue feeding for certain plant species had passed unnoticed. If *C. cubana* larvae are capable of somehow feeding on *O. basilicum* leaves, it leads us for the question: why can they not feed on the other leaves? One possibility lies in the leaf surface: *O. basilicum* leaf surface is "softer" with few trichomes in comparison to the other plant leaves and maybe this facilitated leaf piercing by larvae mandibles. In any case, further studies are necessary to elucidate whether larvae feed on *O. basilicum* leaves and which features in the leaf surface or composition allow *C. cubana* larvae survival.

Although larvae survived longer on *O. basilicum* leaves compared to water, the same was not observed for adults, which could not survive more than three days when fed only leaves of aromatic plant species and no yeast plus honey diet. Adults of *C. cubana* are glyco-pollenophagous, meaning that their main source of food is nectar both floral and extrafloral, pollen from flowering species and honeydew produced by aphids (Venzon &

Carvalho 1992, Albuquerque *et al.* 2001, Canard 2001, Papas *et al.* 2011). Their mouthparts are adapted to fluid foods and pollens as they do not present any incisor part in their mandibles and their lacinia is shaped as a spoon, proper to collect food that do not need to be cut (Canard 2001). Thus, it is not surprisingly that adults could not take any nutrients from leaves, as it seems larvae did. However, adults could benefit from blooming plants, feeding on pollen and nectar, as shown by the experiment on survival on *O. basilicum* flowers.

Ocimum basilicum flowers can be an alternative food source for *C. cubana* adults and larvae. In the absence of prey, larvae of *C. cubana* can survive on sole flowers of *O. basilicum* for a long period of time. Larvae could not complete their development feeding on flowers of *O. basilicum*, as they can do in pollen from other flowering plant species (e.g. Oliveira *et al.* 2010), which implies that sole *O. basilicum* flowers do not provide a suitable source of nutrients for the full development of *C. cubana*. Nevertheless, larva can live for 71 days on average in the absence of other food source than flowers. Adults also can benefit from basil flower resources, although for a shorter period of time when compared to larvae. These results indicate biological control could be enhanced when *O. basilicum* is present as a companion plant, as this plant may allow the establishment of predator population before pest outbreak by supporting larva and adults even when food is scarce or absent (Bianchi *et al.* 2006, Amaral *et al.* 2013).

Even choosing *O. basilicum* to land and stay in, females did not demonstrated preference for laying eggs on leaves of any of the aromatic plants tested in the oviposition preference trials inside cages. One explanation is that the number of plants inside the cage was insufficient. To test this possibility, we set a pilot trial with one replica of each plant combination, using two plants of each species, instead of one, to increase plant surface inside the cage. Even so, females laid much more eggs on the cage surface than on leaf

plants. In addition to a possible ineffective experiment set-up, we have to consider the possibility that *C. cubana* do not present a plant preference to oviposit. Lacewing oviposition preferences varies according to the species, and not always seems to have a clear pattern towards larval food needs (Medeiros *et al.* 2010), sometimes reflecting more the adult feeding needs specially for non-predatory adults (Duelli 1987). Moreover, none of these two criteria were present in our tests once oviposition preference was tested using uninfested non-flowering plants.

We did not find any effect from the addition of *O. basilicum* leaves along with eggs/yeast plus honey diet on larvae survival and development and on survival and oviposition parameters of adults. The presence of some plant species may improve egg laying by predators (Ballal & Singh 1999, Landis *et al.* 2000) due to complementary resources supplied by the plant or as a strategy to avoid larvae predation (Evans 2003). Nevertheless, *O. basilicum* did not provide any improvement on development/oviposition parameters for both larvae and adults. Contrasting with larval survival on leaf treatments, it seems that *O. basilicum* leaf benefits larval survival providing nutrients, this "food" has no synergetic effect along with prey and it is only used in absence or scarcity of other food sources.

The aromatic plant species *O. basilicum* is promising to be used in conservation biological control programs, not only for its well-known negative effects on pests, but also for its attractiveness to generalist predatory insects such as *C. cubana*. Besides attracting adults, basil benefits larvae survival even in its vegetative stage, which is a desirable aspect to an effective biological control. To control pest populations in commercial crops, predators have to be near the crop before pest outbreaks which only can be achieved by attracting these organisms earlier and giving predators conditions to establishment (Bianchi *et al.* 2006, Tscharntke *et al.* 2007, Amaral *et al.* 2013). Additionally to VOC's release and

shelter to adults, *O. basilicum* also offers flower resources, which benefit larvae and adult survival.

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CHAPTER II

Diet breadth of the aphid predator *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae)

Abstract

Generalist predators feed on more than one species, but the extent to which diet impacts their performance (development and reproduction) influences their ability to establish in a crop area and to suppress pest populations. The aim of this study was to evaluate the diet breadth of *Chrysoperla rufilabris* Burmeister over 16 species of aphids and to assess the effects of each aphid species on the survival, larval development time, prey consumption, pupae weight and egg load of adult females and to determine whether these traits are more similar for more closely related aphid species. Aphid species affected significantly all predator traits evaluated, except pupae weight. Significant phylogenetic signals were found for survival rates, aphid consumption and egg load, but not for developmental time or pupae weight. From the 16 aphid species tested, *C. rufilabris* preyed on all of them, but complete development, with adult emergence and egg load production was achieved only in seven species. As a general pattern, the best performances were achieved for the aphid species clustered with *Aphis glycines*, and for *Myzocallis asclepiadis*, an aphid species more distantly related to *A. glycines*. Nevertheless, the phylogenetic relation found for most of the aphid species suitable for *C. rufilabris* indicates a level of specialization in the diet breadth of this green lacewing. Therefore, host phylogeny is an important aspect to be considered when planning to use *C. rufilabris* in biological control programs, especially in those based on natural enemy releases.

Key words: Host range. Phylogeny. Biological control agent. Generalist predator. Specialization.

Introduction

Arthropod generalist predators are important biological control agents that can benefit commercial crops by controlling various pest populations preventing pest outbreaks (Symondson *et al.* 2002; Tscharntke *et al.*, 2007). Although generalist predators may feed on more than one species, the extent to which diet impacts their performance (development and reproduction) can influence their ability to establish in an area and to suppress pest populations (Bilde & Toft 2001, Driesche *et al.* 2008). Prey species that are more closely related to one another may have more similar suitability traits for a given predator species than prey species that are more distantly related, as was observed for herbivores and parasitoids (Jaenike 1990, Agrawal & Kotanen 2003, Desneux *et al.* 2012). Thus, the phylogeny of prey species may provide information on various aspects of predator-prey relationships.

The green lacewing *Chrysoperla rufilabris* Burmeister (1839) is a generalist predator that can be naturally found in various commercial crops. Its larvae are back-naked, i.e. it does not carry debris, and may prey on several soft-bodied pest species, but it is known to feed primarily on aphids. This green lacewing species is well distributed over North America and it is one of the most common commercialized biological control agent in North America and Europe (Tauber *et al.* 2000, Chen & Liu 2001, Pappas *et al.* 2011).

Although *C. rufilabris* is often described as a generalist aphid feeder, not much is known about the phylogenetic relations among species in its diet breadth. Usually, the diet breadth of generalist species comprises distantly related prey/host species, while specialists tend to feed on closely related ones, once close related species are presumed to share characters that can make them suitable for a given consumer (Futuyama & Moreno 1988, Jaenike 1990, Desneux *et al.* 2012). Therefore, studies on phylogenetic relations in the diet breadth of *C. rufilabris* help to improve the knowledge about its associations with species

attacked and thus, help to increase the effectiveness of biological control strategies applied to the field. Nevertheless, most studies on *C. rufilabris* diet is restricted to a few aphid species with no focus on phylogeny (Legaspi *et al.* 1994, Giles *et al.* 2000, Chen & Liu 2001).

Therefore, our objective was to evaluate the diet breadth of *C. rufilabris* over 16 species of aphids and to assess the effects of each aphid species on the development time of larvae and egg load of adult females, in order to determine whether a broad prey range can be beneficial to the establishment and effectiveness of this green lacewing species as a generalist biological control agent. In addition to testing for significant differences among aphid species in various suitability and performance traits we determined whether these traits are more similar for more closely related aphid species, and thus verify in which extend *C. rufilabris* is a generalist predator.

Material and Methods

Experiments were carried at the University of Minnesota, Saint Paul, Minnesota, United States (44° 94'42" N, 93° 09' 36" W, 214m asl)

Chrysopid

Chrysoperla rufilabris eggs were acquired from Beneficial Insectary Inc. (Redding, California, USA) every 15 days. They were transferred to plastic containers (5 cm height, 11 cm diameter) among layers of paper towel and observed daily until hatch. Newly emerged larvae were kept individually in plastic vials (2.5 cm diameter, 7 cm height), provided with *Ephestia kuehniella* Zeller (Lepidoptera: Pyrallidae) eggs *ad libidum* and kept in a growth chamber at 25ºC, 60% RH and 16:8-h light:dark (L:D) until reaching the second instar. Eggs of *E. kuehniella* are known to be an optimal food source for

Chrysoperla spp. larvae (Tauber *et al.* 2000, Papas *et al.* 2007). Second-instar larvae were used for the experiments because first-instar larvae are more prone to die due to instar-related causes than diet (Díaz 2014).

Aphids

Sixteen aphid species (Table 1) were tested as prey for *C. rufilabris* larvae. All aphid species were reared in plant growth chambers on their respective host plants at 25ºC, 65% relative humidity (RH) and 16:8-h light:dark (L:D). These aphid species were chosen to encompass a wide breadth of aphid taxonomy and contained species in two subfamilies – Aphidinae and Calaphidinae, with members of two tribes within the Aphidinae (Aphidini and Macrosiphini). To test the impact of host plant on prey suitability, we compared the survival and performance of *C. rufilabris* fed on the cotton aphid, *Aphis gossypii,* reared in its common host plant *Gossypium hirsutum* (cotton) and on milkweed (*Asclepias syriaca*), which produces toxic cardenolides (Martel and Malcom 2004).

Experimental set-up

A Petri dish (9 cm diam) was lined with filter paper and a young leaf of the host plant was placed inside the dish with a wet cotton pad around its petiole to keep it turgid. A leaf of the same host infested with *ad libidum* individuals of a single aphid species was placed over the experimental leaf and a newly molted second instar larvae of *C. rufilabris*, fed previously only on *E. kuehniella* eggs, was placed onto the leaf. The Petri dish was then sealed with Parafilm™ around the lid to maintain humidity and to avoid lacewing larvae or aphids from escaping. Lacewing larvae were checked daily for survival and aphids were replenished every other day, by adding a new infested leaf. Excess of humidity inside the Petri dish was dried if necessary during daily evaluations. Every three days, the Petri dish

was cleaned and a new filter paper, clean host leaf and infested host leaf were provided. Daily observations were made until *C. rufilabris* pupation.

Table 1. Aphid species and host plant on which they were cultured, and number of replicates. Control replicates included.

Aphid species	Subfamily/Tribe	Host plant	No. of replicates
Schizaphis graminum Rondani	Aphidinae/ Aphidini	*Hordeum vulgare*	20
Rhopalosiphum padi L.		*Hordeum vulgare*	20
Rhopalosiphum maidis Fitch		*Hordeum vulgare*	25
Aphis monardae Oestlund		*Monarda fistulosa*	32
Aphis oestlundi Gillette		*Oenothera biennis*	35
Aphis gossypii Glover		*Gossypium hirsutum*	39
		Asclepias syriaca	30
Aphis glycines Matsumara		*Glycine max*	27
Aphis asclepiadis F.		*Asclepias syriaca*	45
Aphis nerii Boyer de Fonscolombe		*Asclepias incarnata*	20
Aphis craccivora Kock		*Vicia fabae*	25
Aphis fabae Scopoli		*Rumex altissimus*	17
Uroleucon obscuricaudatus Olive	Aphidinae/ Macrosiphini	*Heliopsis helianthoides*	12
Uroleucon sonchii L.		*Sonchus* sp.	40
Sitobion avenae F.		*Hordeum vulgare*	15
Myzus persicae Sulzer		*Rumex altissimus*	17
Myzocallis asclepiadis Monell	Calaphidinae/ Panaphidini	*Asclepias syriaca*	38
Ephestia kuehniella eggs (control)	-	-	71

As it was not feasible to test all the aphid species at the same time, experiments were carried out by group of species, testing six or 12 aphid species (treatments) in each

group, with two replicates per species. Every ten days (the average time until pupation) a new group with six or 12 treatments was set up, again with 2 replicates per group. After 12 replicates, all treatments in which more than 50% of larvae died before adulthood were not used for further replicates. More replicates of the other more suitable treatments were obtained until the emergence of ten females in order to obtain information on egg load (see below).

For each block of six or 12 treatments, two control replicates were established using frozen *E. kuehniella* eggs as food source for the lacewings. These controls were established in a Petri dish (9 cm diameter) lined with filter paper and provided with a wet piece of cotton. Approximately 50 mg of *E. kuehniella* eggs was offered to the larvae every other day along with water. Eggs were acquired from Beneficial Insectary Inc.

Evaluations

Larval survival and development

Chrysoperla rufilabris larvae were checked daily for survival and the number of days of each stage (second instar, third instar and pupae) was recorded. Additionally, pupae were weighed using an analytical balance (precision 0.1 mg). Only individuals that reached adulthood were considered for analysis of development (see below).

Aphid consumption

For a subsample of ten replicates, we counted the number of aphids killed per larva. To recognize an aphid as having been consumed by *C. rufilabris,* we observed the larva killing aphids in a preliminary trial to see how each aphid species looked after being consumed by *C. rufilabris* larvae. Lacewing larvae feed by piercing the prey with both mandibles, injecting salivary secretions into the prey to liquefy the internal tissues and then

extracting the resulting fluid (Carnard, 2001). This feeding process makes the attacked prey appear dry and shriveled. Thus, dried and shriveled aphids similar to the ones observed in the preliminary trial were considered attacked by *C. rufilabris*. Counting was carried out under a dissecting microscope every three days until *C. rufilabris* pupation.

Egg load

The egg load of ten *C. rufilabris* females per each aphid species from which more than 50% of individuals survived until adulthood was counted. After emergence, adults were sexed and females were kept inside plastic vials (2.5 cm diameter, 7 cm height). Honey was provided by brushing it onto the walls of the vial and water was provided by a piece of wet cotton placed in the bottom of the vial. Honey and water were replenished every other day. After ten days, females were frozen and dissected to count the egg load. Mature eggs (fully yoked and chorionated oocytes) were green and of approximately the same size as deposited eggs, while immature eggs with yolk (yolked oocyte) were also green, but smaller than the mature ones. The sum of mature and immature eggs was used for statistical analyses.

Statistical analyses

The experiment took 225 days to accomplish. Therefore, prior to statistical analysis, the data were separated in three blocks comprising 75 days each.

Data on *C. rufilabris* survival were analyzed using the Cox proportional hazard model (Cox PHM) and statistical significances were assessed by Likelihood ratio tests (Cox 1972, Crawley 2007). Cox PHM are semi-parametric models used to assess the effects of variables on survival without specifying the form of the baseline hazard. The model calculates the relative likelihood of death among subjects at a certain time as affected by

the variables (Cox 1972, Fox 2001, Crawley 2007, Cook 2009). Differences among treatments were assessed by contrast analysis.

Data on the time for larval development, aphid consumption and egg load were fitted using general linear models (GLMs) functions with Quasi-Poisson error distribution while pupal weight was fitted using linear models and Gaussian error distribution. We performed residue analyses to assess the appropriateness of models and error distribution (Crawley 2007). Differences among treatments were assessed using Analysis of Variance (ANOVA) and means were compared by *a posteriori* contrasts (Crawley 2007).

All statistical analyses were carried out in the software R (R Development Core Team 2016).

Phylogenetic analyses

A phylogeny of the 16 aphid species assessed in the study was used to determine whether some of the experimental outcomes showed phylogenetic signal. The phylogeny used for these analyses was pruned from one presented by Desneux et al. (2012) although branch lengths are not included in the analyses reported here. Experimental outcomes analyzed for aphid phylogenetic signal were: (i) survivorship of *C. rufilabris* from the 2nd instar to the adult stage, (ii) number of aphids consumed by *C. rufilabris* larvae, (iii) the summed development time of *C. rufilabris* larvae and pupae, and (iv) the egg load of *C. rufilabris* females. Since all aphid species did not survive to contribute to all data sets, the sample size (and therefore the phylogeny) was not the same for all of these analyses. Thus, the test on survivorship and aphid consumption included all 16 aphid species, but the test on development time included 11 species and the test on egg load included only eight. Tests of phylogenetic signal were conducted using the 'Analysis of Traits' module in the software package Phylocom (Webb *et al.* 2007), which randomizes trait values across the

tips of the phylogeny to detect significant clustering across the phylogeny, which is considered phylogenetic signal. Runs of 10,000 randomizations were run in analysis of traits for each analysis.

Results

Larval survival and development

Larval survival of *C. rufilabris* was significantly affected by the prey aphid species (Likelihood ratio test = 306.5, df = 19, $P < 0.001$), with the highest survival achieved on *A. oestlundi*, *A. monardae*, the soybean aphid, *A. glycines* and *M. asclepiadis*. Of these four species, the first three are closely related but *M. asclepiadis* is distantly related from the others and is part of a different subfamily. Larval mean age (until death or adulthood) is presented in Figure 1. Larval-adult survival showed significant phylogenetic clustering (Phylocom Analysis of traits; $P < 0.001$) (Fig. 1; note that *E. kuhniella* was not included in the phylogenetic analysis but is included in the figure to facilitate comparison).

From the 16 aphid species tested, only 11 species could provide for the complete development of *C. rufilabris* (from 2nd instar until adult emergence). For those 11 species, the time to molt to each larval stage was also influenced by the aphid species (Figure 2; from 2nd to 3rd instar – $F_{12, 232} = 2.677$, $P < 0.01$; from 3rd to pupae – $F_{12, 232} = 8.552$, $P < 0.0001$; from pupae to adult – $F_{12, 232} = 3.678$; $P < 0.0001$; total developmental time - $F_{12, 232} = 5.914$; $P < 0.0001$) and by block (from 2nd to 3rd instar – $F_{2, 230} = 3.016$, $P < 0.05$; from 3rd to pupae – $F_{2, 230} = 4.871$, $P < 0.01$; from pupae to adult – $F_{2, 230} = 45.455$; $P < 0.0001$; total developmental time - $F_{2, 230} = 17.289$; $P < 0.0001$). The interaction between aphid and block was statistically significant for the 2nd to 3rd instar ($F_{20, 210} = 1.762$; $P < 0.05$), for the 3rd instar to pupae ($F_{20, 210} = 2.169$; $P < 0.01$) and for total developmental time ($F_{20, 210} = 2.541$; $P < 0.01$), while there was no significant interaction for pupae to adult ($F_{20, 210} =$

1.442; P > 0.1). No significant phylogenetic signal was found for the time taken to reach the third instar or the pupal stages (P > 0.2 for both responses).

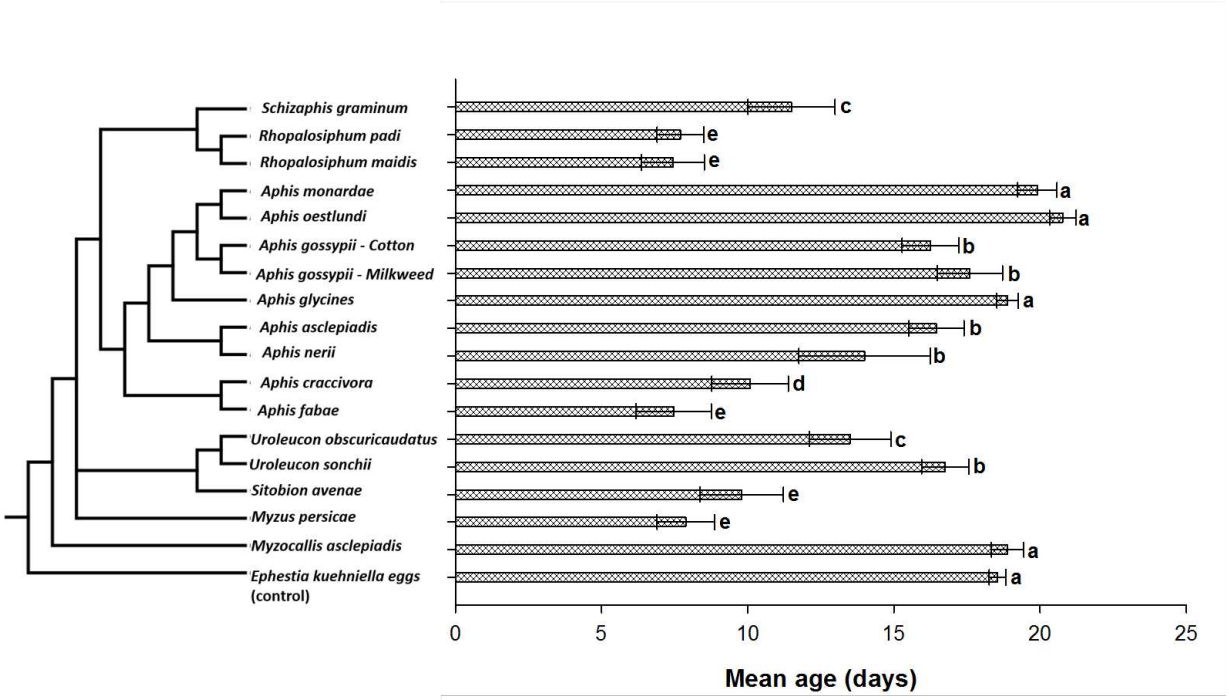

Figure 1 – Mean age (from second instar until death or adulthood) of *Chrysoperla rufilabris* larvae provided different aphid species. Mean ± standard errors are presented. Different letters indicate statistical differences among treatments (*a posteriori* contrasts P < 0.05). Phylogeny pruned from one presented by Desneux et al. (2012). *Ephestia kuhniella* was not included in the phylogenetic analysis but it was included in the statistics and is presented in the figure to facilitate comparison.

The highest total developmental time was observed for larvae provided with *A. oestlundi*. Intermediate developmental times were registered when larvae were fed on *A. gossypii* (both on cotton and milkweed), *A. monardae* and *U. sonchii*. Larval developmental time was lower for *S. graminum*, *R. maidis*, *A. glycines*, *A. asclepiadis*, *A. craccivora*, *S.* avenae and *M. asclepiadis*, compared to the other species (Fig 2). No

significant differences among aphid species consumed were found for *C. rufilabris* pupae weight (Mean = 10.48 ± 0.09, $F_{12, 232} = 1.426$, $P > 0.1$), and no phylogenetic clustering was found for pupal weight either (Phylocom Analysis of Traits $P > 0.05$).

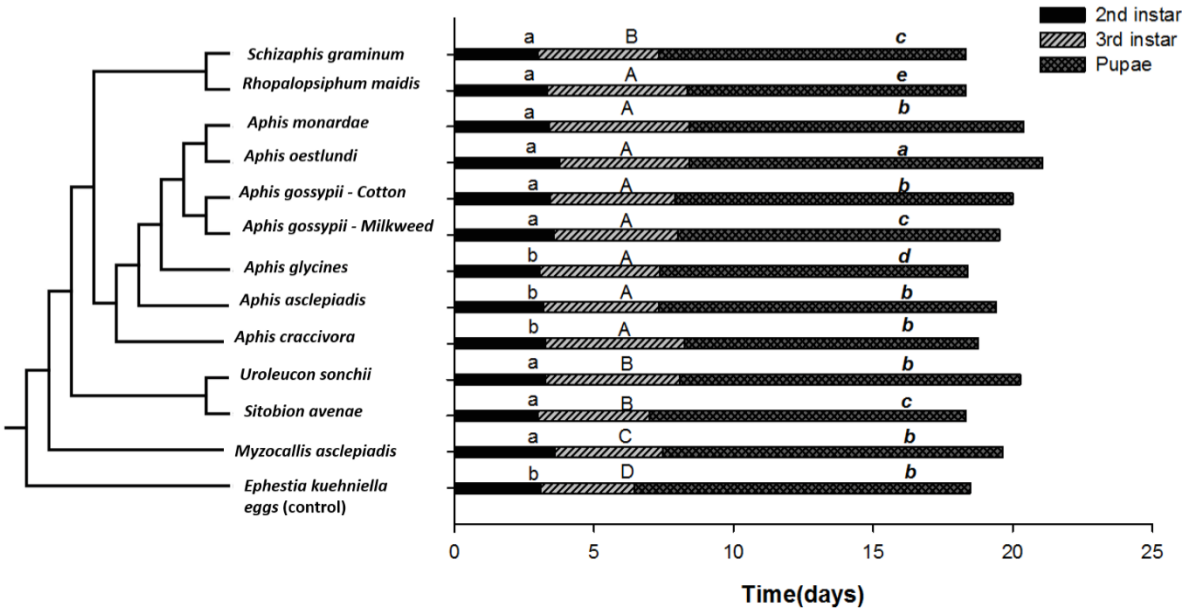

Figure 2 – Mean time (days) for each *C. rufilabris* development stage duration. Different regular lower case letters in the same vertical direction indicate statistical differences among treatments for the second instar duration; different upper case letters in the same vertical direction indicate statistical differences for the third instar duration; different bold italic lower case letters in the same vertical direction indicate statistical differences for the pupae stage duration (*a posteriori* contrasts $P < 0.05$). Phylogeny pruned from one presented by Desneux et al. (2012). *Ephestia kuhniella* was not included in the phylogenetic analysis but it was included in the statistics and is presented in the figure to facilitate comparison.

Aphid consumption

All aphid species offered to *C. rufilabris* were accepted as prey and the total

number of aphids consumed differed significantly among species (Figure 3; $F_{16, 155}$ = 164.058, P < 0.001) and blocks ($F_{2, 153}$ = 3.629, P < 0.05); and there was no interaction between aphid species and block ($F_{16, 137}$ = 1.633, P > 0.05). There was significant phylogenetic clustering for aphid consumption (Phylocom Analysis of Traits P < 0.001).

The aphid species *A. monardae* and *A. oestlundi* were consumed at the highest rate, followed by *A. gossypii* (both in cotton and in milkweed), *A. glycines*, *A. nerii*, *A. craccivora* and *A. fabae*. Consumption of *S. graminum*, *R. padi, A. asclepiadis, S. avenae*, *M. persicae* and *M. asclepiadis* were intermediate while the lowest consumption was observed for *R. maidis*, *U. obscuricaudatus* and *U. sonchii.*

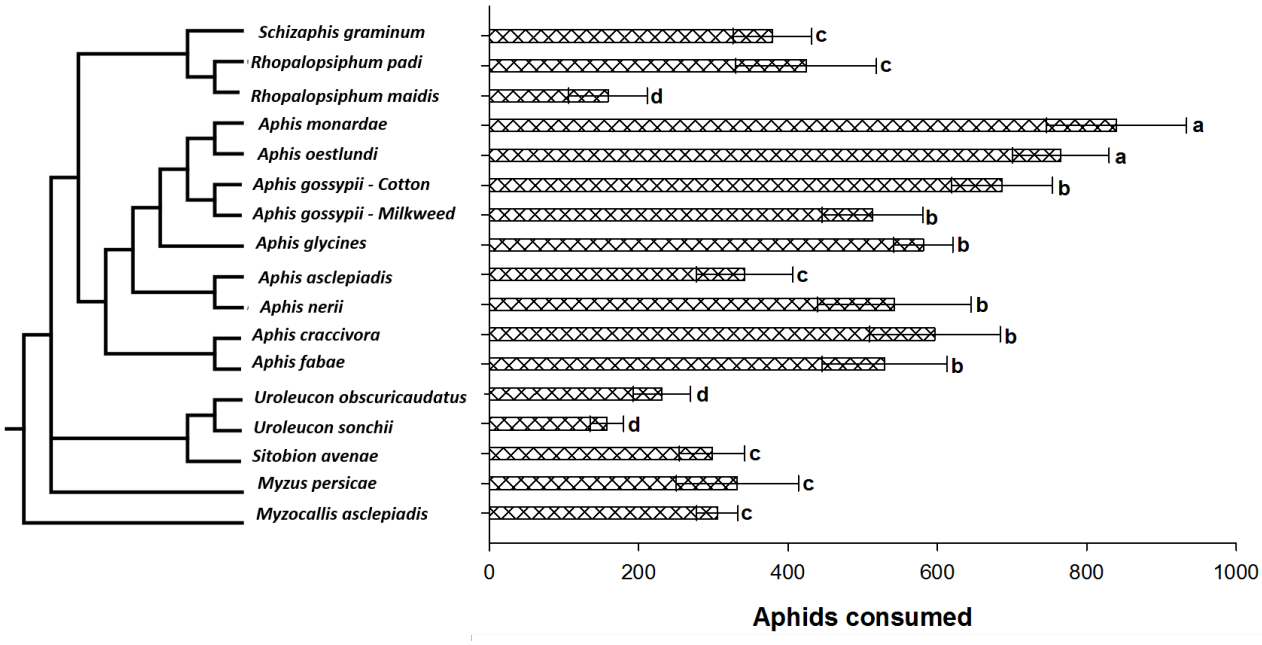

Figure 3 – Mean number of total aphids consumed by *C. rufilabris* larvae during the second and third instar. Mean ± standard error are presented. Different letters indicate statistical differences among treatments (*a posteriori* contrasts P < 0.05). Phylogeny pruned from one presented by Desneux et al. (2012).

Egg load

Although 11 aphid species could provide for the complete development of *C. rufilabris*, only seven species had more than 50% of replicates surviving until the adult stage and therefore only these species were tested for egg load.

Total egg load (i.e. the sum of immature eggs with yolk and mature eggs) for emerged females was significantly affected by the prey offered to the larvae (Figure 4; $F_{8, 95} = 8.556$, $P < 0.001$). Neither block ($F_{2, 93} = 1.585$, $P > 0.1$) nor the interaction between aphid species and block ($F_{16, 77} = 1.377$, $P > 0.1$) affected the egg load of *C. rufilabris*. Significant phylogenetic clustering was found for *C. rufilabris* egg load as well (Phylocom Analysis of Traits; $P < 0.001$).

From the aphid species tested, the highest egg load was found in the soybean aphid, *A. glycines*, while the lowest one was found in *A. monardae* and *A. oestlundi*. *Aphis gossypii* (both in cotton and in milkweed), *A. asclepiadis*, *U. sonchii* and *Myzocallis asclepiadis* had intermediate egg loads, compared to the other aphid species.

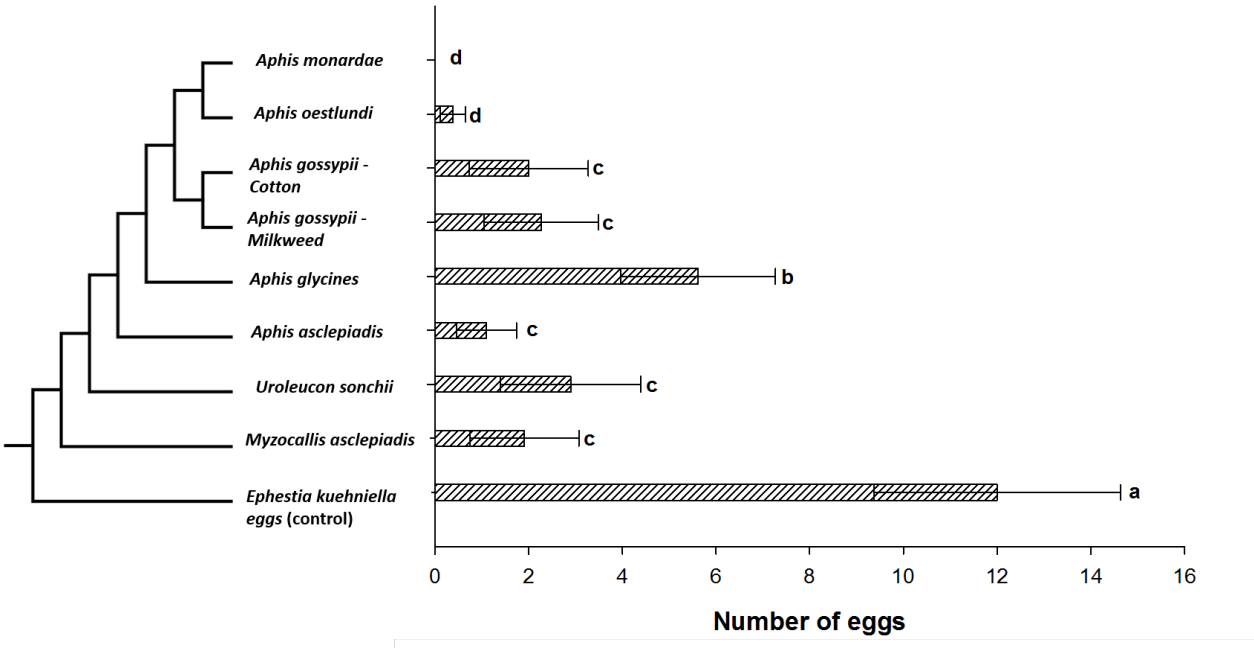

Figure 4 – Mean number of eggs (the sum of immature eggs with yolk and mature eggs) counted in the ovaries of dissected females of *C. rufilabris*. Mean ± standard error are presented. Different letters indicate statistical differences among treatments (*a posteriori* contrasts $P < 0.05$). Phylogeny pruned from one presented by Desneux et al. (2012). *Ephestia kuhniella* was not included in the phylogenetic analysis but it was included in the statistics and is presented in the figure to facilitate comparison.

Discussion

Although *C. rufilabris* is considered a generalist aphid predator and indeed it can prey on a broad range of aphid species under laboratory conditions, our results demonstrate that the survival and performance of this green lacewing species is limited by a narrower aphid prey range. As a general pattern, the best survival rates and performance results were observed in aphids with closer related phylogeny, mostly in the aphids from the genus *Aphis*, meaning *C. rufilabris* may be less generalist than it has been reported (Legaspi *et al.* 1994, Cohen & Smith 1998, Tauber *et al.* 2000).

The highest survival rates of *C. rufilabris* were achieved when larvae were fed on a clade that includes the soybean aphid *A. glycines* and the cotton aphid *A. gossypii*, and on *M. asclepiadis*, which demonstrated the suitability of these prey species for this green lacewing. The highest rates of aphid consumption were also found in the soybean aphid clade. Chen and Liu (2001) tested the relative consumption of *A. gossypii, M. persicae* and *Lypaphis erysimi* (Kalt.) (Hemiptera: Aphididae) on the survival and development of *C. rufilabris* larvae, and they found higher survival and consumption rates for *A. gossypii* and *M. persicae*. In our study, except for *U. sonchii*, species that experienced lower consumption rates were also the ones associated with lower larval survival of *C. rufilabris*, such as *R. maidis* and *R. padi*. Surprisingly, some species considered as suitable food for *C. rufilabris* and other green lacewing species, such as *M. persicae* had a negative effect on *C. rufilabris* survival in our study (Chen & Liu 2001, Pappas *et al.* 2007, Khuhro *et al.* 2012).

Some aphid species can sequester secondary metabolites from their host plants and thus affect the survival and development of their natural enemies (Helms *et al.* 2004, Desneux *et al.* 2009). To test this possibility, we evaluated the performance of *C. rufilabris* larvae on *A. gossypii* reared in cotton and in milkweed, and there were no significant differences between the two host plants for larval survival, aphid consumption, developmental time or egg load. In fact, *C. rufilabris* had a good performance on the three aphid species reared in milkweed, despite the toxins from this plant species that could be sequestered by those aphids (Helms *et al.* 2004, Mooney *et al.* 2008).

Developmental time of *C. rufilabris* larvae was also affected by the aphid species. Some of the shortest total developmental times were observed in three species of *Aphis*, including the soybean aphid, and in *M. asclepiadis*. A suitable prey species may increase the survival rates of the predator and promote a shorter preimaginal development (Canard & Volkovich 2001, Pappas *et al.* 2007). Although *S. avenae*, *S. graminum*, *R. maidis* also

promoted a shorter preimaginal development, they had a low survival rate, as well as *A. craccivora*. For those species less than 20% of replicates survived until adult emergence, therefore they were not suitable for *C. rufilabris* larvae. The highest mean developmental times were observed in larvae fed on *A. monardae, A. oestlundi* and *A. gossypii*, all four species with high survival rates. Even not shortening the preimaginal development of *C. rufilabris*, those aphid species can be considered beneficial to this green lacewing considering the survival promoted by them. In the field, a longer developmental time combined with a high survival rate, may be useful once it can represent a longer period of predation and thus of biological control (Legaspi *et al.* 1994). *Chrysoperla rufilabris* could not complete its development in five aphid species: *A. fabae*, *A. nerii*, *M. persicae*, *R. padi* and *U. obscuricaudatus*. Both *A. fabae* and *A. nerii* are seem to be inadequate for lacewing development, so failure in completion of larval development is not unusual (Daane 2001, Pappas *et al.* 2007). That does not seem to be the case for *R. padi* and *M. persicae*, species that were reported as suitable for green lacewing species (Daane 2001, Chen & Liu 2001, Khuhro *et al.* 2012, Pappas *et al.* 2007).

Finally, of all 16 aphid species tested, only seven could be assessed for egg load, based on the survival rates. Five of those species were in the genus *Aphis* and closely related to *A. glycines*, the species that provided for the highest egg load in *C. rufilabris*. Desneux *et al.* (2009), in a series of experiments on host specificity of the aphid parasitoid *Binodoxys communis* (Hymenoptera: Braconidae) found a similar result, with many aspects of host use by *B. communis* being related to phylogenetic proximity of aphid species to *A. glycines*.

Our significant results of phylogenetic signal for survival, aphid consumption and egg load with the clustering on the *Aphis* genus indicates that *C. rufilabris* may have a more restricted diet than it was supposed until now (Cohen & Smith 1998, Legaspi *et al.* 1994,

Tauber *et al.* 2000). Close related species are presumed to share characters that can make them suitable for a given consumer, being an indication of certain level of specialization. In fact, it is expected that specialists feed on closely related species, while generalists feed on more distantly related ones (Futuyma & Moreno 1988, Jaenike 1990).

Although *C. rufilabris* fed on all aphid species tested, development could not be completed in all those species, which limits the extent to which a broad prey range can benefit this green lacewing species. Such finding is important for planning the use of this green lacewing species in biological control programs, especially those based on natural enemy releases. Additionally, the narrower prey range of *C. rufilabris* and the phylogenetic relation found for the most suitable aphid species open a new perspective on the study of generalist predators found naturally in the field, which can improve the knowledge and consequently the efficacy of some strategies adopted in conservation biological control.

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GENERAL CONCLUSIONS

Our results support concluding that:

i. *Ocimum basilicum* is a promising plant species to be used in programs of conservation biological control due to its attractiveness to the predatory lacewing *C. cubana*. After attracting *C. cubana*, *O. basilicum* can provide resources for the larval and adult survival which may facilitate the establishment of *C. cubana* populations in cropping systems before pest outbreaks;

ii. Some generalist species may be limited in their diet breadth and thus do not benefit from a broad host range. Knowledge on the diet breadth associated with the phylogeny of prey range may be an important factor on planning for biological control strategies, especially those based on natural enemy releases;

iii. *Chrysoperla rufilabris* demonstrated a level of specialization on *Aphis* spp. species with high survival and consumption rates, while it performed poorly when fed aphids such as *S. graminum* and *S. avenae*. This has to be taken into consideration when choosing *C. rufilabris* as a biological control agent to be released in a crop field.