LUIS OSWALDO VITERI JUMBO

BIORRATIONAL APPROACHES FOR CONTROLLING BEAN WEEVILS AND THE RED PALM MITE IN THE NEOTROPICAL REGION

Thesis presented to the Universidade Federal de Viçosa, as part of the requeriments of the Entomology Graduate Program, to obtain title of *Doctor Scientiae*.

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Eugênio Eduardo de Oliveira
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Dedication

I dedicate to my mother Francisca A. Jumbo, my father Angel O. Viteri, my sisters Bertha, Sonia, Jenny, Mireya and my brother Juan

Epígrafe

"The Education is the great engine of personal development. It is through education that the daughter of a peasant can become a doctor, the son of a miner can become the head of the mine, or the son of agricultural workers can become president of a great nation"//. *Nelson Mandela*.

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Abstract

VITERI JUMBO, Luis Oswaldo D.Sc., Universidade Federal de Viçosa, February, 2019. **Biorational approaches for controlling bean weevils and red palm mites in the Neotropical region**. Advisor: Eugênio Eduardo de Oliveira. Co-Advisors: Adenir Vieira Teodoro, Khalid Haddi and Lêda Rita D'Antonino Faroni.

The Neotropical region is characterized by having excellent climate conditions for producing a diversity of gramineous, legumes and fruits of economic importance. However, insect and mite pests have significantly constrained such agricultural production as these arthropods indirectly reduce yields or quality of the production. The control of such pests still is heavily dependent of synthetic compounds, which has threatened the environment and human health. Two relevant agricultural systems in the Neotropical region is the beans (e.g., *Phaseolus vulgaris* e *Vigna unguiculata*) and the coconut production, *Cocus nucifera* L., (and storage for the case of bean grains). Thus, in the present dissertation are described the investigations conducted aiming to provide novel biorational approaches for controlling insect pests that attack stored bean grains (i.e., the bruchid beetles *Acanthoscelides obtectus* (Say), *Zabrotes subfasciatus* (Boheman) and *Callosobruchus maculatus* (Fabricius)) as well as the red palm mite (i.e., *Raoiella indica* (Hirst)) that has caused severe losses in the coconut production in the Neotropical region. Firstly, the present dissertation dealt with the competitive abilities of bean weevils in different hosts. The next investigations were conducted aiming to evaluate the potential of essential oil extracted from clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomun zeylanicum*) for controlling these bean weevils (including potential side-effects associated with sublethal exposures), and were compared with a commercial product Deltamethrin (Decis-25 EC; 25 g a.i./L). Finally, experiments were conducted aiming to evaluate the potential of the lacewing *Ceraeochrysa caligata* (Banks) as biological agent for controlling the red palm mite *R. indica*. The results for the competition bioassays revealed that *C. maculatus* exhibits the best competitive abilities on cowpea beans, independently of its interspecific competitors (i.e., *A. obtectus* or *Z. subfasciatus*); although the weevil *Z. subfasciatus* also show hormetic responses in presence of moderate densities of the competitor *C. maculatus* on this host. On common bean grains the weevil *C. maculatus* can't produce offspring. The toxicological bioassays with essential oils revealed that *A. obtectus* exhibited equally susceptibility to clove (LD_{50}= 43.6 (38.9-49.3) μL of essential oil/kg bean) and cinnamon (LD_{50}= 46.8 (42.3-51.4) μL of essential oil/kg bean) essential oils. Furthermore, there was a trade-off between reproductive fitness and susceptibility to insecticides accordingly to the host where *A. obtectus* was being reared. Clove and cinnamon essential oils also exhibited insecticidal activities against *C. maculatus* similar to the synthetic insecticide Deltamethrin (Clove: LD_{50}= 78.2 (71.6-84.8)μL of essential oil/kg bean; cinnamon: LD_{50}= 131 (124.0-137.0)μL of essential oil/kg bean; deltamethrin: LD_{50}= 83.7 (80.6-86.6)μL of a.i/ kg^{-1} bean). Females of *C. maculatus* exposed to sublethal doses of clove (LD_{20}= 48.6μL^{-1}) and cinnamon (LD_{20}= 106.2μL^{-1}) significantly reduced oviposition. In the last part of this dissertation, the results revealed than the predatory behavior of *C. caligata* feeding upon *R. indica* is dependent of the larval development of the predator and prey size. It is clearly demonstrated that *C. caligata* (especially at the second-instar stage) has potential as a tool for the ecological management of the red palm mite. The results reveal that essential oils of clove and cinnamon, and the natural enemy *C. caligata* are alternatives for the integrated management of stored grain pests and the red palm mite respectively.

Resumo

VITERI JUMBO, Luis Oswaldo D.Sc., Universidade Federal de Viçosa, fevereiro de 2019. **Enfoques biorracionais para controlar gorgulhos do feijão e o ácaro vermelho das palmeiras na região neotropical**. Orientador: Eugênio Eduardo de Oliveira. Coorientadores: Adenir Vieira Teodoro, Khalid Haddi e Lêda Rita D'Antonino Faroni.

A região Neotropical é caracterizada por possuir excelentes condições climáticas para a produção de uma diversidade de gramíneas, leguminosas e frutas de importância econômica. No entanto, insetos e ácaros-praga tem restringido significativamente essa produção agrícola, ao passo que esses artrópodes indiretamente reduzem a produtividade ou a qualidade da produção. O controle de tais pragas ainda é fortemente dependente de compostos sintéticos, que ameaçaram o meio ambiente e a saúde humana. Dois sistemas agrícolas relevantes na região Neotropical são a produç ao de feijão (i.e., *Phaseolus vulgaris* e *Vigna unguiculata*) e coco, *Cocus nucifera* L. (e armazenamento para o caso de grãos de feijão). Assim, na presente dissertação são descritas investigações conduzidas com o objetivo de proporcionar novas abordagens bioracionais para controlar insetos-praga que atacam grãos armazenados feijão (i.e., o besouros bruquídeos *Acanthoscelides obtectus* (Say), *Zabrotes subfasciatus* (Boheman) e *Callosobruchus maculatus* (Fabricius)) assim como o ácaro vermelho da palmeira (*Raoiella indica* (Hirst)), que tem causado severas perdas na produção de coco na região Neotropical. Primeiramente, a presente dissertação trata das habilidades competitivas dos gorgulhos em diferentes hospedeiros. As seguintes investigações, foram realizados com o objetivo de avaliar o potencial do óleo essencial extraído de cravo da índia (*Syzygium aromaticum*) e canela (*Cinnamomun zeylanicum*) para controlar estes bruqídeos (incluindo potenciais efeitos colaterais associados com a exposição subletal), e foram comparados com um produto comercial deltametrina (Decis-25 DC; 25g a.i./L). Finalmente, foram conduzidos experimentos com o objetivo de avaliar o potencial do crisopideo *Ceraeochrysa caligata* (Banks) como agente biológico para o controle do ácaro vermelho das palmeiras, *R. indica*. Os resultados para os bioensaios de competição revelaram que *C. maculatus* apresenta as melhores habilidades competitivas em feijão-caupi, independentemente de seus competidores interespecíficos (ou seja, *A. obtectus* ou *Z. subfasciatus*); embora o caruncho *Z. subfasciatus* também mostre respostas horméticas na presene densidades moderadas do concorrente *C. maculatus* neste hospedeiro. Nos grãos de feijão comum, o gorgulho *C. maculatus* não teve descendentes. Os bioensaios toxicológicos com óleos essenciais revelaram que *A. obtectus* exibiu igualmente susceptibilidade a cravo (DL_{50}= 43,6 (38,9-49,3)μL de óleo essencial/kg de feijão) e canela (DL_{50}= 46,8 (42,3-51,4)μL de óleo essencial/kg feijão). Além disso, houve um trade-off entre a aptidão reprodutiva e a suscetibilidade aos inseticidas, de acordo com o hospedeiro onde ocorreu o desenvolvimento de *A. obtectus*. Óleos essenciais de cravo e canela também exibiram atividades inseticidas a *C. maculatus* semelhantes ao inseticida deltametrina (Decis-25 CE) (cravo: DL_{50}= 78,2 (71,6-84,8)μL de óleo essencial/Kg de feijão; canela: DL_{50}= 131 (124,0-137,0))μL de óleo essencial/kg de feijão; deltametrina: LD_{50}= 83,7 (80,6-86,6)μL i.a/kg^{-1} de feijão). Fêmeas de *C. maculatus* expostas a doses subletais de cravo (LD_{20}= 48,6μL^{-1}) e canela (LD_{20}= 106,2μL^{-1}) reduziram significativamente a oviposiç ao. Na última parte desta dissertação, os resultados revelaram que o comportamento predatório de *C. caligata* alimentando-se de *R. indica* são dependentes do desenvolvimento larval do predador e tamanho das presas. Está claramente demonstrado que *C. caligata* (especialmente no estágio de segundo instar) tem potencial como ferramenta para o manejo ecológico do ácaro vermelho da palmeira. Os resultados revelam que os óleos essenciais de cravo e canela, e o inimigo naturai *C. caligata* são alternativas para o manejo integrado de pragas de grãos armazenados e o ácaro vermelho da palmeira respectivamente.

Contents

General introduction

Legumes of the genus *Phaseolus* and *Vigna*a (Tomooka et al., 2014) possesses high agronomic and economic interest worldwide. Produced in over three-quarters of developing countries, the consumption of these legumes is surpassed only by the soy beans (*Glycine max* L.) and peanut (*Arachis hypogea* L.) (Gepts, 2001; González et al., 2006; Schmale et al., 2006). In 2014, the global estimated production of dry beans (*Phaseoulus vulgaris* L.) and cowpea beans (*Vigna unguiculata* L.) exceeded 24 and 5.5 million tonnes, respectively (Loke et al., 2016; Harouna et al., 2018). However, losses of beans caused by various stored products weevils under on-farm storage conditions could range from 70% up 100% in few months (Gołębiowski et al., 2008; Mbogo et al., 2009; Paul et al., 2009; Hall et al., 1997; Kang et al., 2013; de Melo et al., 2015). This situation forces farmer to sell his production as fast as it is harvested independently of price or to use protective insecticides against these weevils.

More than 1000 species of Bruchinae have the potential to cause damages to stored products, but only 30 species, are known to be adapted to attack and develop on human-domesticated and consumed legume seeds (Kunz et al., 2019; Pimentel, 2002). Although these species are spread throughout the world, each species has evolved different host preference and behaviors with different seed preferences for oviposition and , mating. For example, the weevils *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) prefer the common bean, while *Callosbruchus maculatus* is mainly found colonizing cowpea beans.

Under conditions of low or absence of the main hosts, these polyphagous species can compete with each other for colonization of less preferred resources (Sales et al., 2005; Savković et al., 2012; Souza et al., 2011) and such competition can affect the presence, distribution and abundance of these species.

In the search for hosts, factors such as variety/cultivar of beans, seed morphology, abundance of hosts jointly with the inter and intra-specific competition can affect positively or negatively the “fitness” of each species (Cope and Fox, 2003; Credland and Wright, 1989; Marteleto et al., 2009; Messina and Jones, 2009; rpd Szentesi, 2003; Teixeira et al., 2009; Wakil et al., 2013). Alterations in the fitness of insect pest populations are of great importance especially under confined conditions and where chemical applications are needed to control these pests like in the conditions prevailing in storage units. Indeed, the environmental stress imposed by xenobiotics applied to control such insect pest may be acting on the pre-existing genetic variability leading to a microevolutionary change favoring fitter genotypes in the new environment.

Although the three species of weevils (e.i., *C. maculatus*, *A. obtectus*, *Z. subfasciatus*) are common colonizers of beans, and could be targeted with essential oils; each specie has different strategies to ensure the survival of its offspring. For example, females of *C. maculatus* and *Z. subfasciatus* check the quality and quantity of food for the immature stage before laying the eggs firmly glued to the surface of the grains (Rees, 2007) while females of *A. obtectus* lay their eggs freely in the mass of the grains which allow the larvae of the first instar to look for and choose their hosts. In addition, other factors like beans abundance and morphology variety, competition, and environmentals stresses can push the females of these species to control the egg genesis in function of the action of one or more factors (Cope and Fox, 2003; do Valle Teixeira et al., 2016; Mallqui et al., 2013, 2014; Marteleto et al., 2009; Messina and Renwick, 1985).

The most common methods to control pest insects in stored products is the use of protective insecticides (pyrethroids) and fumigants (phosphine) (Sousa et al., 2008). However, the excessive use of these synthetic insecticides causes toxicological risks to human health (Lee et al., 2011) and environment environmental contamination (Moshi and Matoju, 2017; Wakil et al., 2013) and long-term use increases the risk of selection of resistant populations to these chemicals (Haddi et al., 2018; Pimentel et al., 2009; Song et al., 2011).

From this perspective, plant derived substances, particularly essential oils have drawn an increasing interest as powerful potential alternatives to chemical insecticides due to their safe and environment-friendly attributes (Laosinwattana et al., 2018). These compounds are involved in various ecological functions in plants, such as protection against herbivores and microbial diseases, attraction of pollinators and in allelopathy (Langenheim, 1994). As pesticides, essential oils have various modes of action. They can present neurotoxic activity (Regnault-Roger, 1997) act as growth regulators and molting inhibitors (Regnault-Roger et al., 2012; Tarelli et al., 2009) as well as oviposition inhibitors. They can have ovicidal, deterrents, attractants, or repellents effects (Park and Tak, 2016). They can act also as digestive enzymes blockers (Tarelli et al., 2009) and P450 cytochromes inhibitors (Regnault-Roger et al., 2012). Among essential oils that have been recently a subject of intensive investigations, the oils extracted from clove, *Syzygium aromaticum* (L.), and cinnamon, *Cinnamomum zeylanicum* (L.), plants have drawn particular interest because of their promising insecticidal activities against various pests of stored products (Correa et al., 2015; Haddi et al., 2015; Khalid Haddi, 2017; Silva et al., 2017; Jumbo et al., 2014).

Although, Leguminosae of grains are important source of protein in the food; fruits are mainly source of mineral and vitamins. One of the most important tropical crops are the banana (*Mussa* spp.) and coconut (*Cocos nucifera* L.) that provides direct food and vital revenue for millions of farmers across tropical and subtropical regions of the world. Coconut is a fruit from a perennial plant and is well-known for the diversity of its products, particularly within the realms of food, drink, structural material and energy supply (Nguyen et al., 2016). However, its plant is attack for various arthropods; standing out the red palm mite (*Raioella indica*) Hirst, (Acari: Tenuipalpidae) that found on the underside of the leaves of the host plant in very large numbers (20 – 300 individuals); this, because the reproduction is by parthenogenic arrhenotoky causing an significative increase on short time; resulting on severe damages that cause a 70% yield reduction (Kane, 2006; Jorge E. Peña, 2009). Although the chemical control has given efficient results in the control of this pest mite (Rodrigues and Peña, 2011); problems associated with the excessive use of these products are well known (Deknock et al., 2019; Suarez-Lopez et al., 2017). Therefore, is necessary to seek alternatives for its management.

Biological control is one of the most important components of integrate pest management with the potential to regulate populations of invasive species. Several predators arthropods including insects have been found associated with *R. indica* and has the potential to regulate populations of this species within environmental risk. Insects of genus *Ceraeochrysa* sp. (Neuroptera: Chrysopidae) also was reported that was more common and repeatedly found feeding upon *R. indica* and ovipositing on infested coconut leaves. Studies on the development and reproduction of these lacewing feeding on three phytophagous arthropods commonly found inhabiting coconuts in Florida [*R. indica*, *Nipaecoccus nipae* Maskell (Hemiptera: Pseudococcidae) and *Aonidiella orientalis* (Newstead) (Hemiptera: Diaspididae)] showed these lacewings active preyed upon *R. indica* during the first two instars and had similar developmental times compared to that observed with the two other diets (Carrillo et al., 2011).

Although botanical insecticides, particularly essential oils, have been extensively investigated and proposed as suitable alternatives for controlling insect pests, the vast majority of plant essential oil investigations have focused on their repellence and toxicity. However, the potential ecotoxicological risks or flaws of this pest control tools (including sublethal effects on the targeted pest generation and its subsequent progeny) have been frequently neglected. In fact, it could be hypothesised that a change in the host food source may impact the allocation of energy reserves and consequently the ability to mitigate the stress from compunds like insecticides and or essential oils used to control these pests; and should be considered when looking for control strategies in storage units.

Therefore, the objectives of the present work were: determine potential behavioral alterations of *C. maculatus*, *Z. subfasciatus* and *A. obtectus* on presence of heterospecific in two different hosts (*Phaseouls vulgaris* and *Vigna unguiculata*); evaluate the physiological cost and benefits in a population of *A. obtectus* reared in its preferred host (i.e., common bean) when exposed to stress from different classes of xenobiotics (pyrethroid deltamethrin and two essential oils); and finally explore the lethal and sublethal effects of clove and cinnamon oils as alternatives for the control of *C. maculatus* colonizing cowpea beans. Additionally also was evaluated the potential use of the lacewing *Ceraeochrysa caligata* (Neuroptera: Chrysopidae) as an organism to control the red palm mite, *Raoiella indica* (Acari: Tenuipalpidae).

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Chapter 1

Host-specific competitives abilities of Bruchids bean weevils (Coleoptera: Chrysomelidae: Bruchinae) on different hosts

Host-specific competitives abilities of Bruchids bean weevils (Coleoptera: Chrysomelidae: Bruchinae) on diferents hosts; Luis O. Viteri Jumbo, K. Haddi, Adenir Vieira Teodoro, Pedro Toledo, Sarah Miranda, E. E. Oliveira; **to be submited to Scientific Reports**.

Abstract

Bruchids beetles (Coleoptera: Chrysomelidae: Bruchinae) are pests of important legumes on underdeveloped countries. The *Callosobruchus maculatus* (Fabricius) uses seeds cowpea of genus *Vigna* as its main host, while, *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) prefer common beans of genus (*Phaseolus*). However, under conditions of limited resources and/or high densities, those species could compete to colonize alternatives hosts and thus could be positively or negatively affected. In this study, we evaluated the performance of those species in the main and alternative hosts on presence of various densities of heterospecifics competitors. Each species was fixed in an initial proportion 0.5 while increase proportions of the others was added: 0.00, 0.17, 0.24, 0.28, 0.38, 0.44, 0.50. After 45 days of competition (F_1) the number of adults emerged and the instantaneous rate of increase (r_i) were determined. The results show the competitive ability of *C. maculatus* on the main host and the susceptibility of *A. obtectus* when confronted with this specie independent of the host. Still *Z. subfasciatus* interferred in the performance of *C. maculatus*; and increase their offspring when competed with moderated densities on cowpea.

Key words bean weevil, cowpea, competition interspecific, Chrysomelidae

1.1 Introduction

Interspecific competition is an important ecological interaction than can affect the presence, distribution and abundance of species (Tilman, 1982; Schoener, 1983). When two or various species with common needs or habits compete with each other in a given environment, positive or negatives interactions may occur for each species. Potential effects based on reproductive inhibition between coexisting species is frequent. One species, could expel a second one from the shared space by inhibiting its reproductive potential in relation to their initial densities (Utida, 1953; Inbar et al., 1995; Gerla and Mooij, 2014). In these interactions the competitors can make use of physical, physiologicals or chemicals strategies.

The Bruchinae *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae: Bruchinae) are the main colonizing species of beans of the genus *Phaseolus*; and are responsible for the losses in stored beans in tropical and subtropical regions (Dobie et al., 1991; Hagstrum and Subramanyam, 2009; Viteri Jumbo et al., 2014; Mutungi et al., 2015; do Valle Teixeira et al., 2016). On the other hand, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Chrysomelidae: Bruchinae) is the main colonizer of beans belonging to genus *Vigna* which is cultivated in arid and semi-arid regions (Swella and MuShoBozy, 2009; Mbata and Payton, 2013; Musa et al., 2015; Massango et al., 2017). However, in conditions where scarcity or absence of the main hosts is verified, these species can inter-compete for the use and colonization of less preferred resources and hosts (Sales et al., 2005; Souza et al., 2011; Savković et al., 2012). Sometimes, at low densities, all individuals of a given species may aggregate in the preferred habitat, but when population density increases, some species could be forced to move to poorer habitats, where lowering their fitness.

These species of weevil exhibit various ovipositional behaviors to prevent mortality of their eggs (Panizzi, 2009). Mated females of *C. maculatus* and *Z. subfasciatus* deposit the individual fertilized eggs, sometimes several eggs per grain, firmly glued to the surface of the grains as femalies of these species to determine the most suitable food for the immature stage (Rees, 2007), while females of *A. obtectus* places the eggs freely in the mass of the grains and newly emerged larvae of the first instar are responsible to locate their hosts (Utida, 1967; Dendy and Credland, 1991; Credland, 1992; Schmale et al., 2002; Beck and Blumer, 2011). However, in their decision making process, these mated females respond not only the quality of the oviposition site, but also consider ecological factors such as the probability of intra or interspecific competition (Hindayana et al., 2001; Frechette et al., 2004). It has been shown, for example, that factors such beans variety, morphology, host abundance, intra or interspecific competition affect the fitness of *Z. subfasciatus* (Teixeira and Zucoloto, 2003; Marteleto et al., 2009; Teixeira et al., 2009; Oliveira et al., 2015), *A. obtectus* (Ramírez S. A., 2000; Szentesi, 2003; Mallqui et al., 2013) and *C. maculatus* (Messina and Renwick, 1985; Credland, 1987; Credland and Wright, 1989; Cope and Fox, 2003; Messina and Jones, 2009). Females of these species are able to control their ovogenesis in function of the presence or absence of one of the above mentionated factors.

Since these three weevil species may oc-occur in stored grain unites, their competitive capacity determine their survival, fitness and the colonization of mass grain. Therefore, this study was conducted with the aim of to determining the effect of binomial interspecific competition in the fitness of three weevil species (e.i., *Z. subfasciatus*, *A. obtectus* and *C. maculatus*) when competing at varying densities in two types of hosts (e.i., *Phaseolus vulgaris* and *Vigna* *unguiculate*) during one generation.

1.2 Material & Methods

1.2.1 Insects

Populations of *A. obtectus*, *Z. subfasciatus* were collected in the rural area of Viçosa (20°45′14"S, 42°52′55"W) Minas Gerais State, Brazil and population of *C. maculatus* was collected nearby the county of Fortaleza (03°43′02"S, 38°32′35"W) Ceara State, Brazil. Were maintained under laboratory conditions (27 ± 2°C, 75 ± 5% relative humidity, 12 h of scotophase). Each species was reared on the main host obtained from to local market: common beans (*P. vulgaris*) for *A. obtectus* and *Z. subfasciatus*, and cowpea beans (*V. unguiculata*) for *C. maculatus*. Grains of common bean and cowpea were placed at -10°C during ten days before offered to the insects on a 2 L glass jars (500g/container).

1.2.2 Experiments of competition

The bioassays of competition were carried out between the heteroespecific *A. obtectus* and *C. maculatus*; and *C. maculatus* versus *Z. subfasciatus* on two different hosts (e.i., *P. vulgaris* and *V. unguiculata*) using additives series as to suggested by (Snaydon, 1991). The initial number of insects of a specie was fixed on fifty individuals, and the other specie was increased progressively from 0 to 50; therefore, each specie with determinate number of insects competed in proportions of 0, 0.17, 0.29, 0.38, 0.44 and 0.50 against the other specie with a fixed total number of insects (Snaydon, 1991; Mallqui et al., 2013; Oliveira et al., 2007).

Each experimental unit consisted of a glass jar of 0.8 L with 100 g of common beans or cowpea beans; where non sexed adults insects (<24 h old) were added. Four replicates of each treatment (combination) were used; and the jars were maintained under controlled conditions (27 ± 2°C, 75 ± 5% relative humidity, 12 h of scotophase). After fifteen days both beans (each glass jars) was sieved to prevent infestation of fungus from dead insects; and after thirty days (45 day from beginning of the experiment) the number of emerged insects of each species was counted. The instantaneous rate of increase (r_i) was calculated for each species using the formula $r_i = [\mathrm{In}(N_f/N_i)]/\Delta T$, where N_f and N_i are the final and initial numbers of live (adults) insects, respectively, and ΔT is the duration of the experimental in days (Walthall and Stark, 1997).

1.2.3 Statistical analyses

The number of emerged adults insects and the instantaneous rate of increase of the three species in the two hosts, were subjected to analyses of covariance with the proportion of increase as the independent variable and the species with a proportional increase as a covariate (Proc GLM; SAS Institute, 2008). Complementary regression analyses were eventually carried out.

1.3 Results

1.3.1 Effects of competition on offspring production

The model of analysis of covariance for the total number of emerging insect for each species after 45 days of interspecific competition on cowpea and common bean showed significant effects of bean type, species with increasing proportion and interactions (Table 1.1).

When *C. maculatus* competed in its main host (cowpea) against *Z. subfasciatus*; the total number of adults emerged of each specie was significantly affected by the increase of proportions (Table 1.1). The effect of initial proportion only affected *Z. subfasciatus*; and the interaction between increased and initial proportions was significant for *C. maculatus* (Table 1.1). As expected, a significant increase of adults emerged on each specie was observed in relation to proportions (Fig. 1.1A,B). Although *C. maculatus* was not influenced by the increase of proportions of its competitor *Z. subfasciatus*; the later showed hormetic response to the increase of proportions (at intermediated density) of the competitor *C. maculatus* (Fig. 1.1B).

Table 1.1: Analyses of covariance for the total number of live insects in cowpea and common beans after 45 days (one generation) of competition between *C. maculatus* and *A. obtectus* as well as *C. maculatus* and *Z. subfasciatus*

Sources of variation	Cowpea beans					Common beans		
		C. maculatus		*Z. subfasciatus*		*Z. subfasciatus*		
	df	*F*	*P*	*F*	*P*	*df*	*F*	*P*
Model	3	28.6	<0.0001*	15.4	<0.0001*	3	32.23	<0.0001*
Error	44	-	-	-	-	44	-	-
Specie w/ increased number of insects (S)	1	69.8	<0.0001*	33.9	<0.0001*	1	66.48	<0.0001*
Initial proportion (IP)	1	0.1	0.73	8.0	0.0070*	1	11.17	0.0017*
S x IP	1	14.6	0.0004*	3.7	0.006	1	17.66	0.0001*
Sources of variation		*C. maculatus*		*A. obtectus*		*A. obtectus*		
	df	*F*	*P*	*F*	*P*	*df*	*F*	*P*
Model	3	127.2	<0.0001*	14.7	<0.0001*	3	32.08	<0.0001*
Error	44	-	-	-	-	44	-	-
Specie w/ increased number of insects (S)	1	227.2	<0.0001*	21.1	<0.0001*	1	37.32	<0.0001*
Initial proportion (IP)	1	55.6	<0.0001*	2.0	0.17	1	0.11	0.74
S x IP	1	92.9	<0.0001*	20.3	<0.0001*	1	56.95	<0.0001*

**significant differences P<0.05*

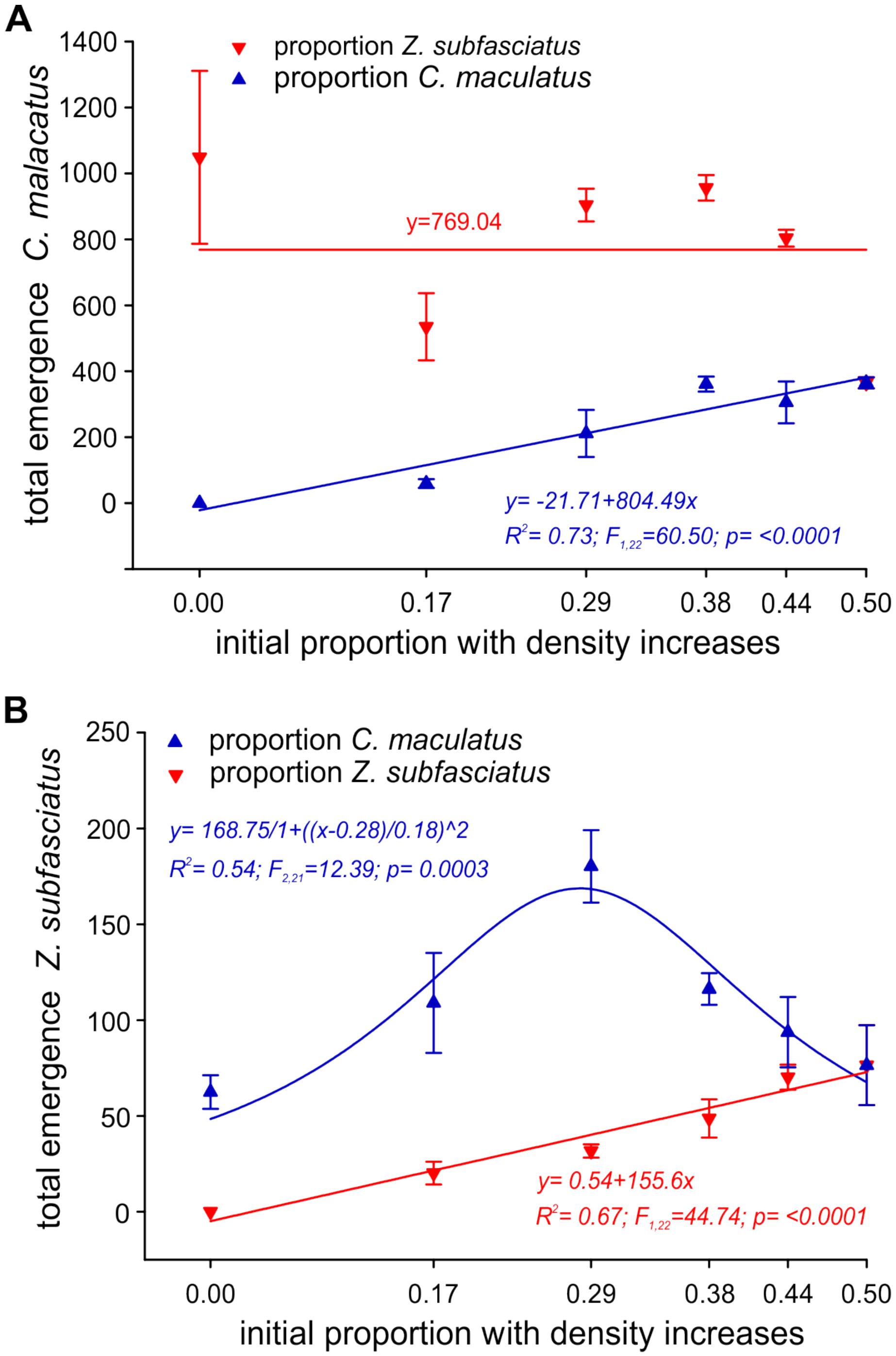

Figure 1.1: Total emergence of *C. maculatus* (A) and *Z. subfasciatus* (B) on interspecific competition after 45 days (one generation) on cowpea; the symbols represent the means of four treatments and the vertical bars represent the standard deviation.

Competition analysis between *C. maculatus* and *A. obtectus* in cowpea beans shows significative effects for the two species when the proportions are increased (Table 1.1). The effect of initial proportion was significant for *C. maculatus* and the interaction between increased initial proportions was significant for both species (Table 1.1). The proportions increase of *C. maculatus* resulted in a significant increase of the total number of emerged adults of this species, but is was reduced by the proportions increase of *A. obtectus* (Fig. 1.2A). Likewise, *A. obtectus* also was affected negatively with the increasing proportions of *C. maculatus* (Fig. 1.2B).

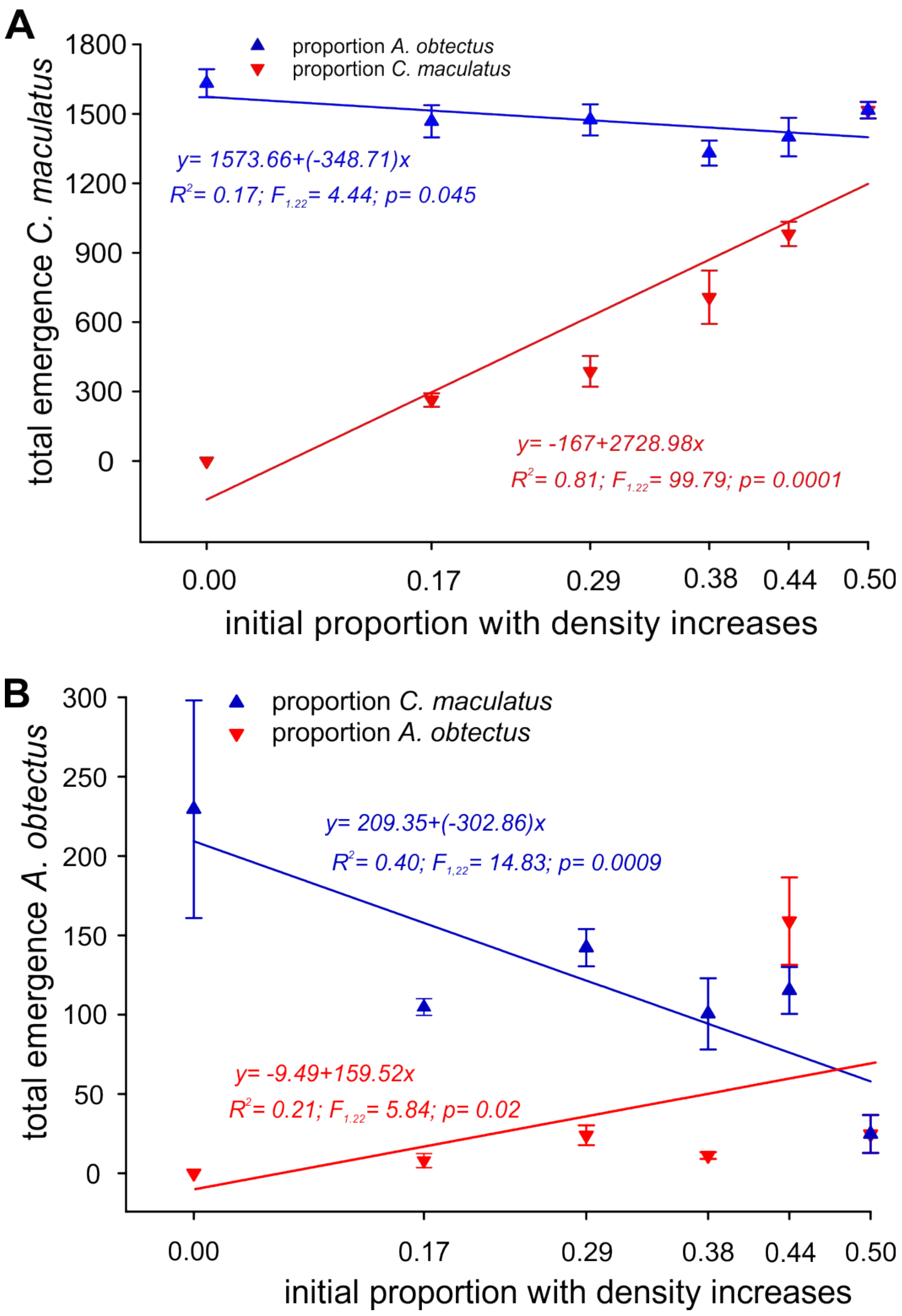

Figure 1.2: Total emergence of *C. maculatus* (A) and *A. obtectus* (B) on interspecific competition after 45 days (one generation) on cowpea; the symbols represent the means of four treatments and the vertical bars represent the standard deviation

Callosubruchus maculatus produced no offspring when it competed with *A.*

obtectus and *Z. subfasciatu*s in common beans which is the main host of the later species. The effect of the proportions increase was significant for the two species, and the effect of initial proportion was significant only for *Z. subfasciatus*; while interaction between these two factors affected both species (Table 1.1). The increase of proportions of *Z. subfasciatus* resulted in an increase of emerged adults of this species, and it was not affected by increasing of the competitor (Fig. 1.3A). Similarly, the number of emerged adults of *A. obtectus* was proportional to increased proportion but was negatively affected by the proportions increase of competitor (Fig. 1.3B).

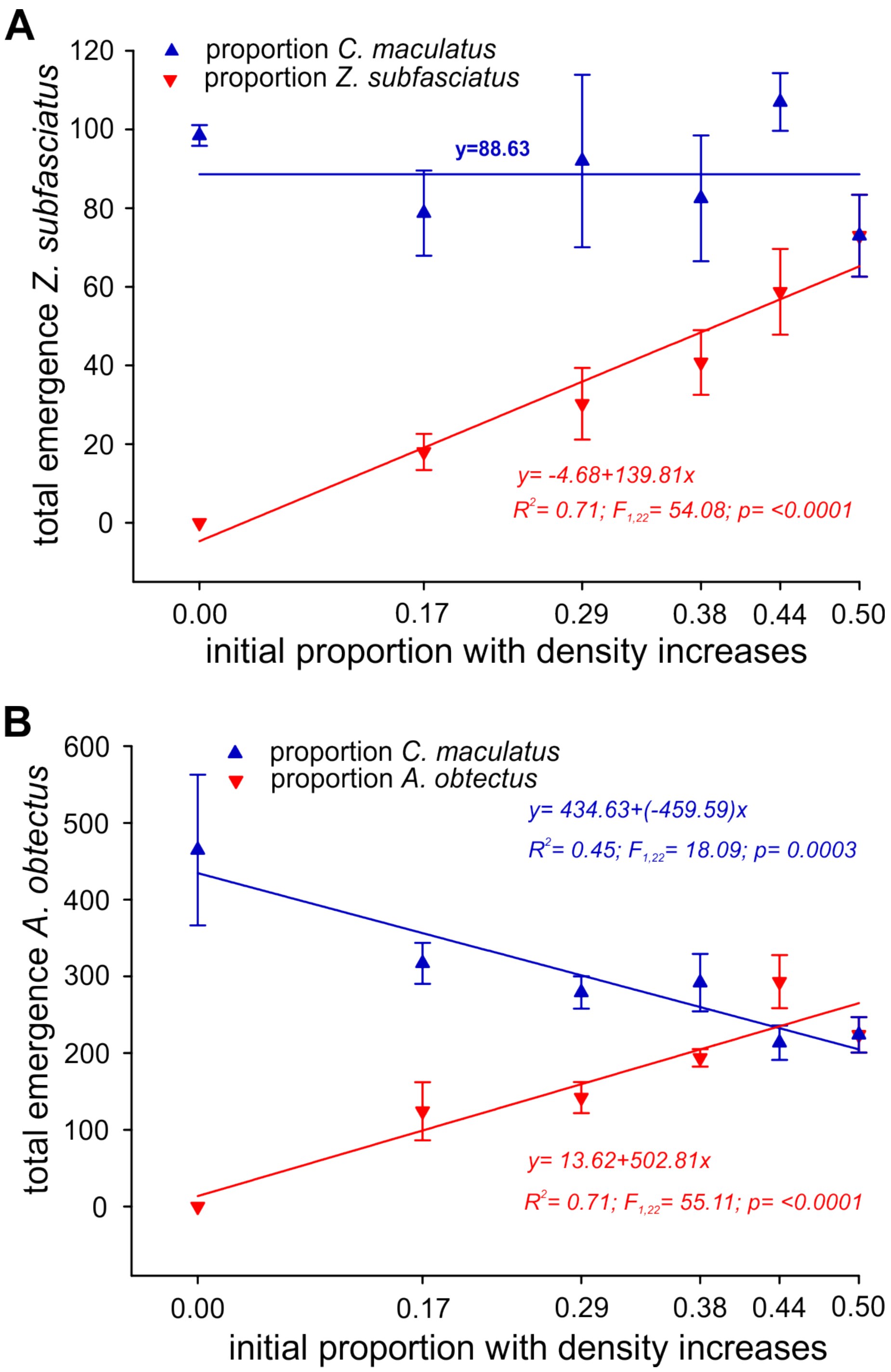

Figure 1.3: Total emergence of *Z. subfasciatus* (A) and *A. obtectus* (B) on interspecific competition with *C. maculatus* after 45 days (one generation) on common bean; the symbols represent the means of four treatments and the vertical bars represent the standard deviation.

1.3.2 Effects of competition on instantaneous rate of increase (r_i)

Covariance analyses performed for the instantaneous rate of increase (r_i) of each specie after 45 days of interspecific competition on common and cowpea beans showed significant effects of density and initial proportion as well as their interaction for *C. maculatus* and *A. obtectus* in both types of beans but not for *Z. subfasciatus* (Table 1.2). When *C. maculatus* competed with *Z. subfasciatus* in cowpea bean, there was significant effect of density (increasing proportions) for both species; while initial proportion and interaction of these two factors of variation affected the instantaneous rate of increase of *C. maculatus* (Table 1.2). The r_i of *C. maculatus* increased with increasing proportions but is was not affected by the increased proportions of the competitor (Fig. 1.4A); while the r_i of *Z. subfasciatus* was positively affected by increasing densities (Fig. 2.4B). When *C. maculatus* competed with *A. obtectus* in cowpea, significant effects of the increasing proportions, initial proportion and their interaction were found (Table 1.2).

Table 1.2: Analyses of covariance for the r_i of insects in cowpea and common beans beans after 45 days (one generation) of competition between *C. maculatus* and A. obtectus as well as *C. maculatus* and *Z. subfasciatus*

Sources of variation	Cowpea beans					Common beans		
		C. maculatus		*Z. subfasciatus*		*Z. subfasciatus*		
	df	*F*	*P*	*F*	*P*	*df*	*F*	*P*
Model	3	23.1	<0.0001*	1.82	0.1574	3	2.18	0.1043
Error	44	-	-	-	-	44	-	-
Specie w/ increased number of insects (S)	1	32.8	<0.0001*	3.84	0.05*	1	5.23	0.0270*
Initial proportion (IP)	1	11.4	0.0016*	1.49	0.23	1	0.07	0.7878
S x IP	1	24.1	<0.0001*	0.11	0.74	1	1.12	0.2950
Sources of variation		*C. maculatus*		*A. obtectus*		*A. obtectus*		
	df	*F*	*P*	*F*	*P*	*df*	*F*	*P*
Model	3	17.52	<0.0001*	6.49	<0.0010*	3	5.07	0.0042*
Error	44	-	-	-	-	44	-	-
Specie w/ increased number of insects (S)	1	9.4	0.0036*	9.23	0.0040*	1	0.33	0.57
Initial proportion (IP)	1	18.39	<0.0001*	5.15	0.03*	1	1.14	0.29
S x IP	1	24.11	<0.0001*	4.82	0.03*	1	13.67	0.0006*

**significant differences P<0.05*

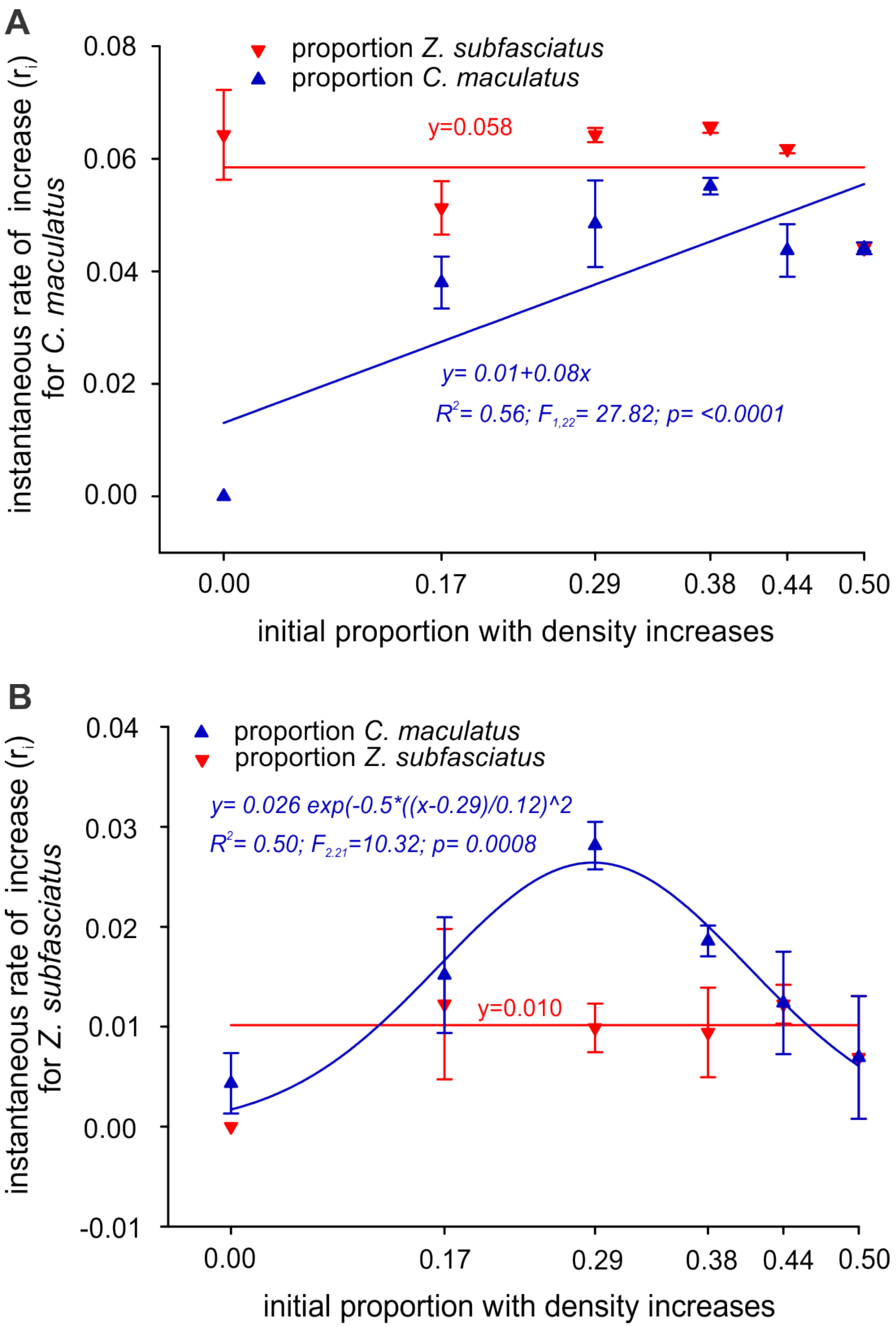

Figure 1.4: Instantaneous rate of increase (r_i) of *C. maculatus* (A) and *Z. subfasciatus* (B) on interspecific competition after 45 days (one generation) on cowpea; the symbols represent the means of four treatments and the vertical bars represent the standard deviation

Opposite to negative effect on the ri of *A. obtectus* with increase competitor proportions (Fig. 1.5A); *C. maculatus* not was affected by *A. obtectus* (Fig. 1.5B). Competition between *C. maculatus* and *Z. subfasciatus* and *A. obtectus* on common bean resulted in significant effects of the increase in proportions for *Z. subfasciatu*s, and significant effects of the interaction between density and inicial proportion for *A. obtectus* (Table 1.2).

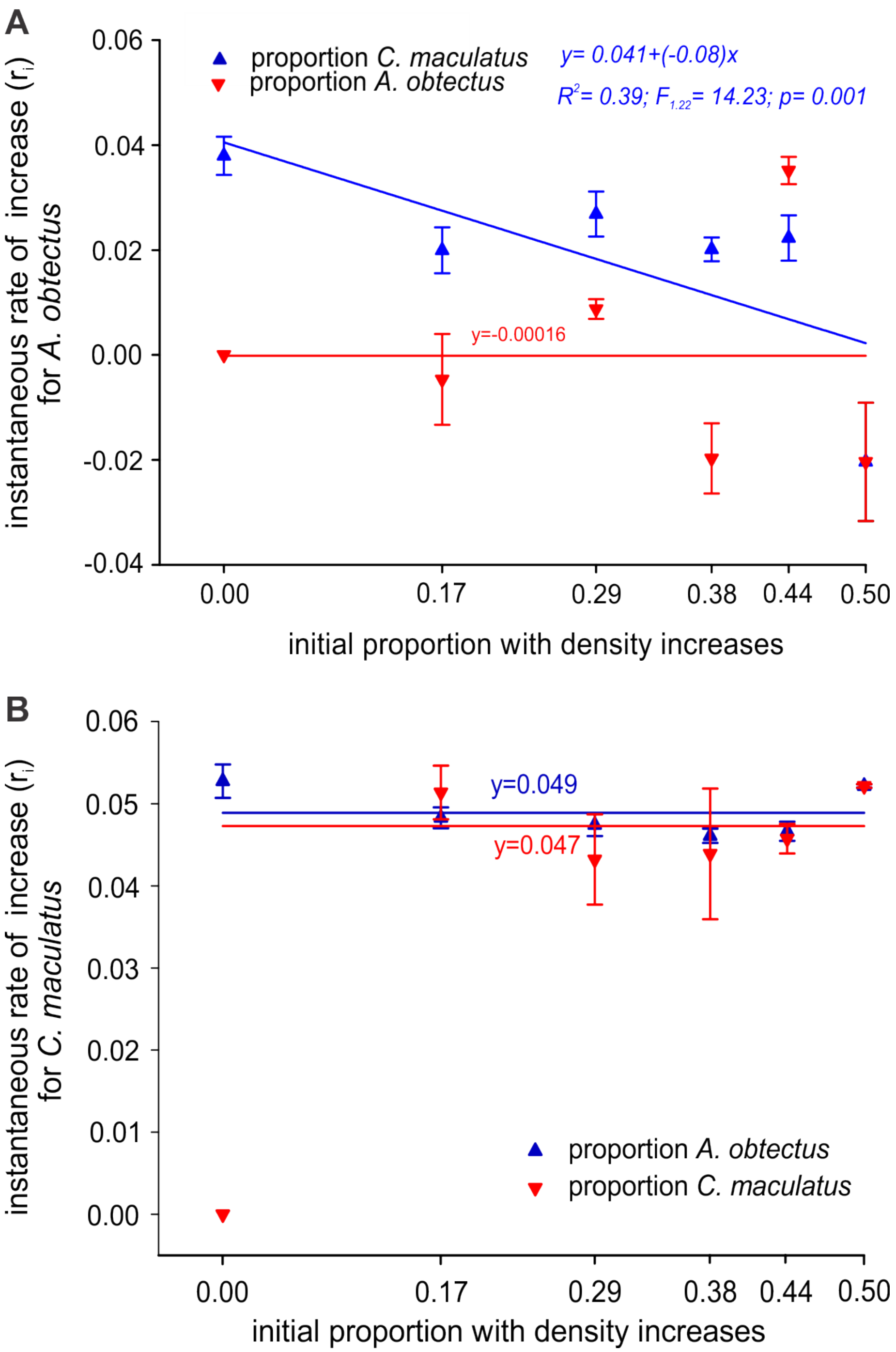

Figure 1.5: Instantaneous rate of increase (r_i) of *A. obtectus* (A) and *C. maculatus* (B) on interspecific competition after 45 days (one generation) on cowpea; the symbols represent the means of four treatments and the vertical bars represent the standard deviation

The r_i of *Z. subfasciatus* was not affected by competition of *C. maculatus* on common beans (Fig. 1.6A); while that r_i of *A. obtectus* was negatively affected by increased proportions of its competitor *C. maculatus* (Fig. 1.6B).

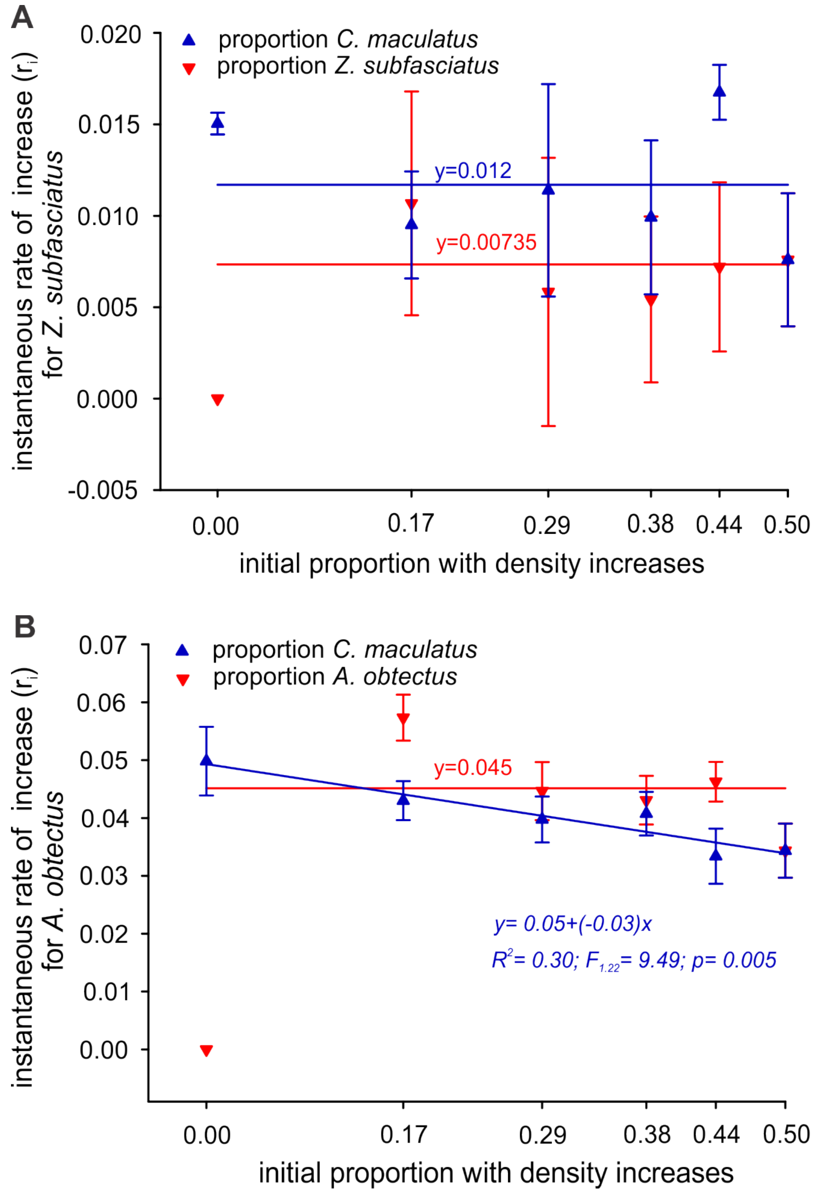

Figure 1.6: Instantaneous rate of increase (r_i) of *Z. subfasciatus* (A) and *A. obtectus* (B) on interspecific competition with *C. maculatus* after 45 days (one generation) on common bean; the symbols represent the means of four treatments and the vertical bars represent the standard deviation.

1.4 Discussion

Evidences suggest that when coexistence of different species occurs positive and negative interactions may take place and that their effects dependent on population density (Gerla and Mooij, 2014). Adults of *A. obtectus*, *Z. subfasciatus* and *C. maculatus* do not feed as adults on grain of bean, therefore, the interference of the quality of the bean is in different scales and effects of competition can affect the survival, growth and reproduction of these organisms. We showed that *A. obtectus* is affected negatively when exposed to different densities of its competitor *C. maculatus* independently of the host. Although *C. maculatus* obtain the best performance on cowpea, it was reduced by the presence of *Z. subfasciatus*, that also have biggest number of descendants on intermediates densities of this competitor.

The fitness of weevil *Z. subfasciatus* was not affected negatively by the presence of *C. maculatus* in common bean and cowpea and contrarily to what was expected, *Z. subfasciatus* doubled the offspring production in the presence of intermediates densities of the heteroespecific competitor in the cowpea beans.

Although this phenomenon was not expressed on common bean, we speculate that oviposition of *Z. subfasciatus* was stimulated in presence of moderate densities of potentials competitors in alternative hosts. In previous studies, *Z. subfasciatus* exhibited increasing matting in the presence of intermediate densities of conspecifics (do Valle Teixeira et al., 2016). Similarly, this species exhibited a better performance on the presence of moderate densities of *A. obtectus* (Mallqui et al., 2013). Competitive capacity of this specie could be due to the early mating that allows early oviposition and the possibility of choice of the better grain quality and hence a better use of resources by its larvae of this specie (Pajni and Jabbal, 1986).

Although increasing densities of adults causes a decrease in the females fecundity in bruchids (Yoshida, 1960; Giga, 1982); differences on the competitive capacity may be explained also by the oviposition habit of each species. Performance of *A. obtectus* was affected negatively on common bean and cowpea according to increasing densities of the heteroespecific *C. maculatus*. This specie also showed the competitive capacity to suppress *C. rhodesiensis* and *C. chinensis* when they coexist (Giga and Smith, 1985; Ishii and Shimada, 2008). Other factors that may affect the fitness of *A. obtectus* are linked to the fact that the females of this species avoid to oviposit in grains mass previously exposed to adults of conspecifics or that have evidence of infestation with larvae (Szentesi, 1981; Brzostek and Ignatowicz, 1990; Parsons and Credland, 2003; Nazzi et al., 2008). Such behavior may explain the negative effect of the *A. obtectus* in the presence of *C. maculatus* as the later have the capacity and habit of marking the grain with chemicals substances to avoid the oviposition of other bruchids (Messina et al., 1987).

Additionally, females of *A. obtectu*s have the habit to lay their eggs freely in the grain mass. The newly emerging first instar larvae may take few hours to days dispersing, selecting and perforating their host making them highly susceptible to mechanical damage (Simmonds et al., 1989; Thiéry et al., 1994).

Furthermore, reports indicate than females of *C. maculatus* mate after five minutes of emergence (Rup, 1986), and start laying eggs after one hour (Credland, 1987); with a maximum oviposition occurring between 24 and 48 hours from emergence (Dick and Credland, 1984; Credland and Wright, 1989; Fox, 1993). In *A. obtectus*, the maximum of oviposition is reached after 96 hours from emergence Larvae of *C. maculatus* respect to *A. obtectus* in the same host may increase by the strategy of early reproduction and obtain competitive advantages of these species (Bushnell and Boughton, 1940; Thanthianga and Mitchell, 1987).

The results of oviposition by *C. maculatus* change when looking at performance of this specie against the two competitors on cowpea. In this legume, the number of *C. maculatus* offspring was lower when competing with *Z. subfasciatus* compared to when *C. maculatus* competed with *A. obtectu*s. This competitive capacity of *C. maculatus* could be committed because females of *Z. subfasciatus* also delay the eggs added to grains , and have capacity of mate one hour after of emergence and begin to oviposit only after 2 hours with maxim delay after 48 hours (Pajni and Jabbal, 1986; do Valle Teixeira et al., 2016). In addition females of *C. maculatus* can discriminate grains infested with larvae through of sensorial receptors presents on the maxillary palps (Messina et al., 1987; Ignacimuthu et al., 2000).

Common beans, *P. vulgaris*, is the main host of *A. obtectus* and *Z. subfasciatus*, while *C. maculatus* prefers *Vigna* (Janković-Tomanić et al., 2015; do Valle Teixeira et al., 2016; Heidari et al., 2016). We show that the number of emerging adults of *A. obtectus* was higher on common beans compared with cowpea. Although protein contend (20-25%) is similar in these two bean species (Hamid et al., 2014; Jafari et al., 2016; do Evangelho et al., 2017); the diferences in grain size, diversity and concentration of other compounds can affect the quality of resources and specific nutrients required by larvae and thus influencing in the development of immature stages of each species (Benrey et al., 1998). Although, oviposition of *C. maculatus* was observed in *P. vulgaris*; no decendant emerged from this type of beans in concordance with previous reports (Karbache et al., 2011; Souza et al., 2011; Mainali et al., 2015). Failure in development of *C. maculatus* in *P. vulgaris* could be attributed to physical barriers (hardness of tegument) that difficult the entrance of neonate larvae during the perforation phase (Ernst, 1992). Furthermore, the presence of toxic proteins and others antinutritive compounds in the seeds of non hosts, may provoke repellence, deterrent; mortality and/or delay development of *C. maculatus* larvae (Seifelnasr, 1991; Machuka et al., 2000; Silva et al., 2004; Sadeghi et al., 2006; de Sá et al., 2014).

In conclusion, our study suggests that *C. maculatu*s is dominant on its main host; and could outcompete easily *A. obtectus*; while the *Z. subfasciatus* weevil could tolerate the competitive presence of *C. maculatus* or even have stimulatory advantages from its presence at moderates densities.

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Chapter 2

Changes in the insecticide susceptibility and physiological trade-offs associated with a host change in the bean weevil *Acanthoscelides obtectus*

Changes in the insecticide susceptibility and physiological trade-offs associated with a host change in the bean weevil *Acanthoscelides obtectus*; K. Haddi, L. O. Viteri Jumbo, M. S. Costa, M. F. Santos, L. R. A. Faroni, J. E. Serrão, E. E. Oliveira; **published in Journal Pest Science**.

Abstract

For most of the seed weevils (Coleoptera: Chrysomelidae: Bruchinae), the ability to survive environmental stresses like insecticide exposure and reproduce is variable and depends on the host. Here, we evaluated the physiological costs and benefits of a host shift from kidney beans of the landrace "Vermelho" to cranberry beans of the landrace "Manteigão" in the bean weevil *Acanthoscelides obtectus*. We assessed the susceptibility of *A. obtectus* to the commercial pyrethroid deltamethrin and clove and cinnamon essential oils as potential alternative insecticides and measured its reproductive performance (e.g., oviposition rate and the competitive abilities) on both hosts. Although the attraction of *A. obtectus* adult females to both hosts was similar, the females reared on kidney beans showed more sources of energy in the trophocytes and were less susceptible to both deltamethrin and clove essential oil . However, the females reared on cranberry beans had higher respiration and reproductive performances, indicating that more energy was expended on reproduction than on the mitigation of the insecticide actions. Thus, with a change in hosts, a trade-off between reproductive fitness and the susceptibility to insecticides was demonstrated for *A. obtectus*. These results should not only affect the management of *A. obtectus* in storage units but also on the understanding of these insects' host adaptiveness.

Key words: bruchids, host range, adaptation costs, trophocyte area, botanical insecticides, respiratory rates.

2.1 Introduction

Herbivorous insects generally specialize on one or a few closely related hosts, and for most of these insects, the use of a specific host and the detoxification of secondary metabolites may be negatively correlated with fitness in new environments (Gompert et al., 2015; J., 1990; Messina, 2004; Scheirs et al., 2005). The seed weevils in the coleopteran subfamily Bruchinae easily adjust to new nutritional or toxic environments (Sales et al., 2000), which is likely a primary reason for their adaptability to a wide range of hosts. However, the mechanisms for adaptation require alterations in physiological and behavioral processes that typically affect reproductive performance traits such as oviposition, emergence and population growth (Bifano et al., 2010; Milanović et al., 1991; Seslija and Tucic, 2003; Silva et al., 2001).

Is known that among the seed weevils, the bean weevil *Acanthoscelides obtectus* (Say) (Coleoptera: Chrysomelidae: Bruchinae) is a widespread pest that uses the seeds of the common bean, *Phaseolus vulgaris* (L.), as the primary host; however, these weevils reproduce in a wide range of ecological conditions, causing severe losses in several stored products (Alvarez et al., 2005; D., 2012; Mallqui et al., 2013). Females of *A. obtectus* scatter eggs among bean grains, but the host is selected by the early stage larvae and not the female adults (Parsons and Credland, 2003). The growth of larvae is restricted to the inside of mature seeds, which is typically the only source of nutrient reserves and resources used to reach maturity and to survive during the adult stage (Hamraoui A, 1995; Savković et al., 2012; Seslija and Tucic, 2003).

Adults of *A. obtectus* are facultatively aphagous and are capable of initiating their colonization in the fields or in storage facilities, where these insects cause major losses (Baier AH, 1992). Because the availability of a specific host is highly discontinuous and beans are treated with insecticides both in the field and in storage, the selection pressures on weevils are high, and the adaptations (Hamraoui A, 1995; Milanovic and Gliksman, 2004; Savković et al., 2012; Stojkovi et al., 2014) reflect the differential investment of energy between the contrasting processes of reproduction and defence against toxicants such as pesticides.

For instance, an adaptive allocation of energy between reproductive fitness and susceptibility to toxicants has been investigated for several insect species (Guedes et al., 2006; Huang et al., 2005; Messina and Fry, 2003; Oliveira et al., 2007; Santos et al., 2015; Seslija and Tucic, 2003), where it has often been described that lower susceptibility to insecticides may derive from higher energy investments favoring the detoxification of the toxic molecules at the expense of reproduction (Guedes et al., 2016). The respiration rate, an indicator of physiological stress, can be used to detect both reversible and irreversible toxicosis in insects (Karise et al., 2015; P., 1991; Mänd et al., 2005). Furthermore, the fat body in insects is composed mainly of cells called trophocytes which are characterized morphologically by the presence of lipid droplets, glycogen rosettes and protein components (de Oliveira PVT, 2003). As these trophocytes constantly exchange compounds with the hemolymph through absorption or exocytosis, the intensity of these exchanges can be morphologically estimated, which has allowed the use of the trophocytes morphology as an indicator of the availability and mobilization of energy reserves for organism maintenance, survival and reproduction during exposure to toxic compounds (Alves et al., 2010; Guedes et al., 2006; Nath, 2002).

Insect populations need to continuously adapt to alterations on their environments (Gbaye and Holloway, 2011; Gbaye et al., 2011). It is likely that the nature of available feeding substrates, through energy allocations, will play a crucial role on the reproduction capabilities especially in the presence of toxic compounds such as insecticides. Our hypothesis is that a change in the host food source will impact the energy reserves allocation and consequently the ability to mitigate the stress of xenobiotics like insecticides. Thus, this study was conducted aiming to evaluate the physiological costs and benefits of a host shift (i.e., from kidney beans of landrace "Vermelho" to cranberry beans of the landrace "Manteigão") in the bean weevil *A. obtectus*. We measured the susceptibility to insecticides (i.e., the pyrethroid deltamethrin and the essential oils of clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L.,) and the reproductive (e.g., oviposition, emergence rates and competitive ability) performance of the seed weevil *A. obtectus* on both bean types.

2.2 Material & Methods

2.2.1 Weevils and beans

The original population of *A. obtectus* was field-collected from beans-producing small farms in the Viçosa Region (Minas Gerais State, Brazil). These insects were collected in *P. vulgaris* beans of the landrace "Ouro Vermelho" (common name: "Feijão vermelho" and here referred to as "kidney beans"). Insect populations were started with a minimum of 500 individuals and developed and reared in laboratory conditions (27 ± 2°C, 75 ± 5% relative humidity, and 12 h of scotophase). Prior to the experiment beginning, we used the kidney beans obtained from a local market as substrates to rear the beetles for several generations. However, for the host-change experiments, we also used the *P. vulgaris* beans of the landrace "Manteigão" (common name: "Feijão manteigão" and here referred as "cranberry beans") obtained in the local market. The bean types were selected because of the size difference, with the kidney beans (25 ± 0.77 g/100 grains) being approximately half the size of the cranberry beans (40 ± 1.13 g/100 grains) (Oliveira et al., 2015). These beans also differ in terms of their nutritional contents. Accordingly to the beans provider (Cerealista Pereira, Cajuri, MG, Brazil), while in a portion of 60 g of beans, both bean types have similar amount of proteins (cranberry: 8.0 g. kidney: 11.0 g), the amount of lipids (cranberry: 0.8 g. kidney: 6.4 g) and carbohydrates (cranberry: 39.0g. kidney: 29g) are different. To avoid possible infestations from the field and reduce the potential effects of residual insecticides, the bean grains were stored at 10°C for 14 days before the addition of *A. obtectus*. The water content of the kidney and cranberry bean grains was 11.8%, which were offered ad libitum to the weevils.

2.2.2 Insecticide and plant essential oils

In order to assess the susceptibility to insecticides, we used synthetic pyrethroid deltamethrin and plant essential oils as insecticide probes. Deltamethrin is the most common compound used to control insect pests in fields and in storage units (Ceruti and Lazzari, 2005; Sehgal and Subramanyam, 2014). A commercial formulation of deltamethrin (Decis-25 CE; 25 g a.i./L, emulsifiable concentrate; Bayer CropScience, São Paulo, Brazil) was used. For the plant products, we used essential oils extracted from cinnamon bark and dried flower buds of clove, following a previously described procedure (Jumbo et al., 2014). Based on chromatographic analyses, eugenol (90%) and sesquiterpene $\beta-$caryophyllene (7%) are the primary constituents of clove and cinnamon essential oils, respectively. These two essential oils have been suggested as potential replacement of synthetic insecticides for controlling insects that infest stored beans (Jumbo et al., 2014).

2.2.3 Insecticide bioassays

To evaluate the effect of rearing host beans on the susceptibility of *A. obtectus* to insecticides, we assessed the mortality of newly emerged adults (<3 days old) when exposed to deltamethrin (5 μg of a.i./kg of beans; equivalent to the rate recommended for controlling weevil infestations in Brazilian storage units) and to the essential oils of clove (μL of essential oil/kg of beans: LD_{10}= 13.5; LD_{50}= 45.6; LD_{90}= 141.0) and cinnamon (μL of essential oil/kg of beans: LD_{10}= 17.9; LD_{50}= 46.8; LD_{90}= 122.4), based on their efficacies against *A. obtectus* (Jumbo et al., 2014). All the experiments were done under controlled conditions (27 ± 2°C, 75 ± 5% relative humidity).

For the bioassay with deltamethrin, the insecticide emulsion (0.5 mL, with distilled and deionized water as the solvent) was sprayed on 200 g of beans using an artist's air brush (Sagyma SW440A; Yamar Brasil, São Paulo, SP, Brazil) coupled to an air pump (Prismatec 131A Tipo 2VC; Itu, SP, Brazil). The insecticide was sprayed at a pressure of 0.7 kgf/cm^2. The grains remained in the container after spraying for 1 h to dry. Each replicate was 200 g of deltamethrin-treated bean grains (in 0.8 L jars) with 25 unsexed adult *A. obtectus*. In the bioassays with essential oil-treated beans, pure doses of each essential oil (i.e., without dilution in a solvent) were applied with a 25-μL Hamilton syringe (Hamilton, Reno, NV, USA) to 200 g of beans in 0.8-L glass jars. After the application, the jars were manually shaken for 60 s to ensure the complete distribution of the essential oil.

For all bioassays, 25 unsexed 1- to 3-day-old *A. obtectus* adults were placed in each jar. Four replicates were used for each dose, and the mortality was assessed after 24 h of exposure. The insects were recorded as dead when they did not respond to a touch with a fine probe. The control treatment consisted of untreated beans.

2.2.4 Cell morphometry of *A. obtectus* reared on both bean types

To evaluate the effects of bean type on the energy reserves of *A. obtectus*, the trophocyte size was measured in five males and five females recently emerged (less than 3 days old) from each bean type using a Periodic acid-Schiff (PAS) staining technique. This technique is commonly used to highlight structures with high percentage content of carbohydrates where the reaction of these sugars with periodic acids results in aldehydes. The aldehydes give a purple-magenta colour in the presence of Schiff reagent and the distinct staining densities demonstrate the tissue components to which the carbohydrates are attached (Guedes et al., 2006). Briefly, the insects were dissected in a saline solution (0.1

M NaCl, 20 mM KH2PO4, 20 mM Na2HP4), fixed in Zamboni's fixative solution (paraformaldehyde + picric acid), dehydrated in an ascending ethanol dilution series (70-95%) and embedded overnight in Leica historesin (Leica Biosystems, São Paulo, SP, Brazil). The samples were then embedded in historesin with hardener for microtomy. Sections of 4 μm were stained with hematoxylin and eosin (HE) and mounted in Eukitt mounting medium (Sigma-Aldrich Brasil, Sã Paulo, SP, Brazil). Using the Image Pro-Plus 4.5 software (Media Cybernetics, Rockville, MD, USA), the morphometrics of 10 fatty cells was measured for each individual, and the area of each cell was calculated (in μm^2).

2.2.5 Reproduction outputs and bean type preference assays

In order to investigate the effect of rearing host beans on the reproductive performances of *A. obtectus*, we measured the bean type preference, and the oviposition and emergence rates.

2.2.6 Bean type preference

In this experiment, the aim was to determine the bean type that was most attractive to *A. obtectus* (i.e., cranberry beans or kidney beans). Five circular, plastic containers (12 cm diameter and 8 cm height) were used as the apparatus in this experiment, with a central container (E) connected to four other containers (A, B, C and D) by plastic cylinders (12 cm long and 1 cm in diameter) (Mazzonettto F, 2003; Jumbo et al., 2014). The four side containers were filled alternatively with either bean type, and Five replicates of 50 newly emerged adults (< 12 h old) adult *A. obtectus* were released in the central container; the total number of insects per side container was recorded after 24 h. The attractiveness index was calculated using the following equation: AI= $(2 \times T)/(T + C) \times 100$, where AI= attractiveness index, C= number of insects in the containers filled with kidney beans, and T= number of insects in the containers filled with cranberry beans. The AI values indicate the level of attraction and range between 0 and 2: AI= 1 neutral activity; AI $>$ 1 repellence; and AI $<$ 1 attraction. As a margin of safety for this classification, the standard deviation (SD) of each treatment was added/subtracted from a value of 1 (indicative of neutrality). The kidney beans were used as the T factor in the equation because the insects used in these experiments all originated from kidney bean masses..

2.2.7 Oviposition and emergence rates assays

The oviposition rate of early-stage adult *A. obtectus* reared on each bean type was measured. Twenty newly emerged adults ($<$ 12 h old) were provided with 20 g of the bean type in which they were raised as immatures for oviposition (for 3 days). After this period, the identical groups of insects were transferred to another 20 g bean mass of the identical type for 3 more days. The eggs laid in the bean masses were counted for each 3 days period and for the total number of days (i.e., 6 days). Five repetitions were used for each type of bean (i.e., cranberry or kidney beans).

The bioassays for population density were conducted using groups of 50 recently emerged adults (less than 3 days old). These insects were allowed to colonize bean masses (100 g of beans) for 10 days. Thirty days after removal of the insects, the number of newly emerged adults was monitored at 3-days intervals for four weeks. Four repetitions were used for each type of bean mass.

2.2.8 Respirometry assay

In order to evaluate whether the host type would affect the respiratory responses of *A. obtectus*, we conducted respirometry bioassays using a CO_2 Analyzer TR2 (Sable Systems International, Las Vegas, NV, USA) following previously described methods (Guedes et al., 2006; Haddi et al., 2015a; Oliveira et al., 2005, 2007). The average rate of respiration (CO_2 production) was measured at 25°C(±2) for five replicates of 20 unsexed adults (less than 3 day old) that emerged from kidney bean or cranberry bean masses. The insects were placed in 25 mL chambers that were connected to a completely closed system. The chambers were connected to the system for 90 min before injecting CO_2-free air into the chambers for 2 min at a rate of 600 mL/min. The air current directed the CO_2 that was produced by insect respiration to an infrared reader that was connected to the system for the immediate quantification of the CO_2 produced. After determining the respiration rate, the insects were removed from the chambers and weighed on an analytical balance (BP 210 D; Sartorius, Göttingen, Germany). Respiration values were not normalized by body mass, in accordance with recommendations by (Packard and Boardman, 1999) and (JP, 2001).

2.2.9 Statistical analyses

All statistical analyses were conducted using either the SAS (Institute, 2008) or the Sigma Plot (SPSS, Chicago, IL, USA) software. The data of mortality bioassays of essential oils were analyzed with three-way analysis of variance (ANOVA), followed by pairwise multiple comparisons using the Holm–Sidak method. The bean types (2), oil types (2) and doses (3) were the factors in the analyses. The data obtained in the bioassays for the deltamethrin susceptibility, and oviposition rates were analyzed with one-way ANOVA, followed by pairwise comparisons (based on Duncan's method) to distinguish the treatments. The bean type preference was analyzed using Likelihood Chi Square test. Using the curve fitting procedure of the Sigma Plot 8.0 software, we conducted regression analyses to determine the emergence rate. The data of the respiration rate assay (μL of CO_2/h/insect) were analyzed with ANOVA on Ranks, followed by pairwise comparisons using Dunn's method. The data for cell morphometry were submitted to ANOVA, and the averages were tested with t-tests at 0.05 probability. The assumptions of normality and homogeneity of variance were tested, and no data transformations were required (UNIVARIATE procedure).

2.3 Results

2.3.1 Insecticide bioassays

The insects that were reared on cranberry bean masses were more susceptible ($F_{1,7}$= 32.4; P= 0.001) to deltamethrin than were their co-specifics that were reared on kidney bean masses (Fig. 2.1A) In the bioassays with essential oils, the mortality increased with each increase in dose for both essential oils. However, although the bean type did not affect the mortality caused by cinnamon essential oil at any dose (Fig. 2.1B), the application of clove LD_{90} caused higher mortality ($F_{1,63}$= 9.0; P= 0.024) in the insects reared on cranberry bean masses than in those from kidney bean masses (Fig. 2.1C).

2.3.2 Cell morphometry of *A. obtectus* reared on two bean varieties

Morphologically, the fat body of males and females of *A. obtectus* reared on both beans types (i.e., cranberry beans or kidney beans) was uniformly opaque white. The cytoplasm had heavy vacuolization, and the nucleus was variable in size and location (Fig. 2.2) Although the bean type did not affect the trophocyte area of males ($F_{1,19}$= 1.3; P= 0.27), the trophocyte size in females reared on kidney beans was significantly larger ($F_{1,19}$= 8.4; P= 0.005) than that in those reared on cranberry beans (Fig. 2.2). Furthermore, no significant differences were found in the size of the trophocytes between males and females reared on cranberry beans ($F_{1,19}$= 3.5; P= 0.063) or on kidney beans ($F_{1,19}$= 0.1; P= 0.71) (Fig. 2.2). However, in the histochemical tests with cells of the fat body, the intensity of the reaction to carbohydrates was the highest in females reared on kidney beans, followed by that of females reared on cranberry beans. The intensity of the reaction to

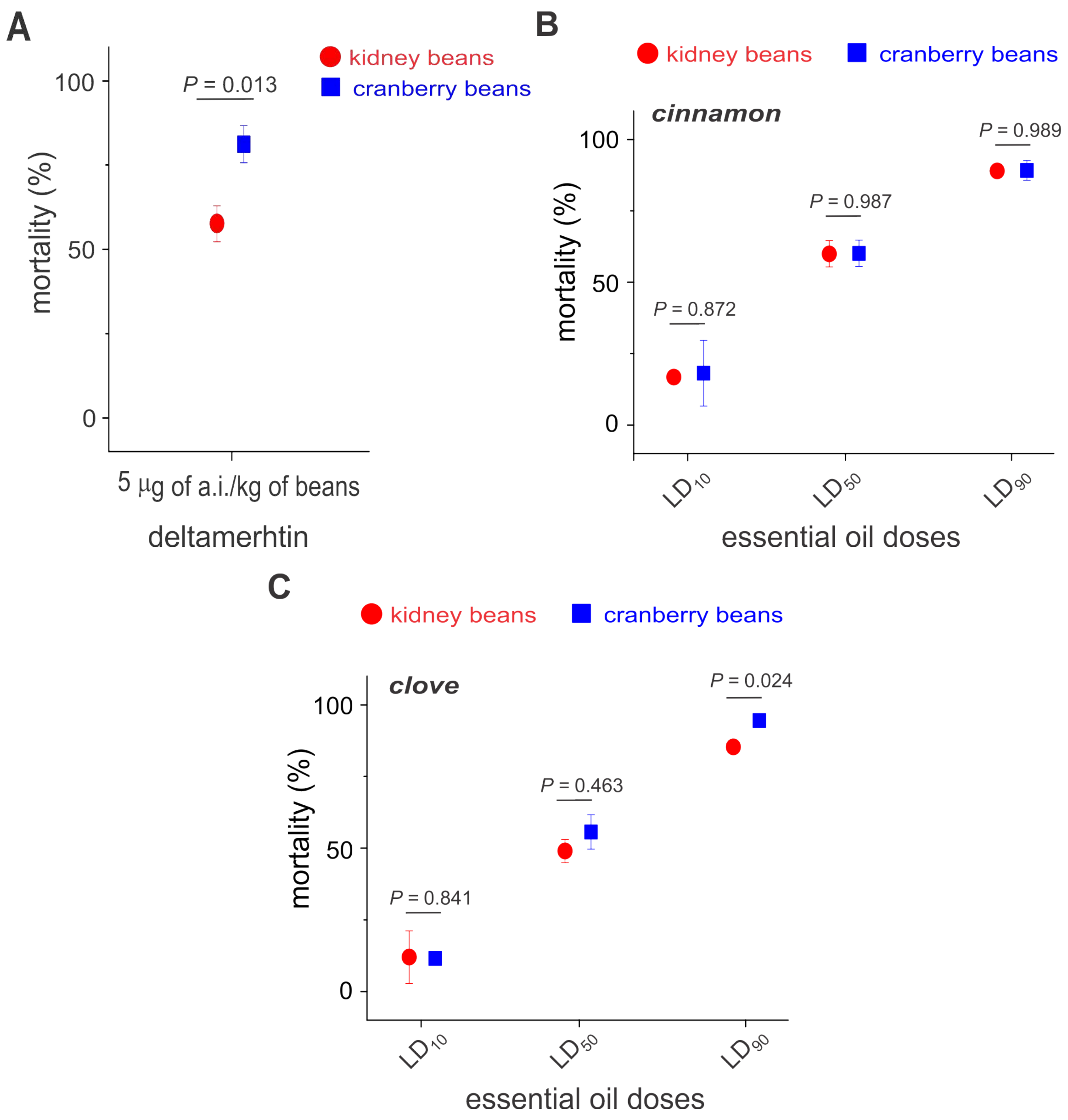

Figure 2.1: Susceptibility (mean ± SD; n= 5) of *Acanthoscelides obtectus* tus reared on kidney beans or on cranberry beans to the pyrethroid insecticide deltamethrin (A) and to essential oils of cinnamon (B) and clove (C). For deltamethrin, we used the recommended field rate (i.e., 5μg of a.i./kg of beans). For the essential oils, we used the LD$_{10}$, LD$_{50}$ and LD$_{90}$ values [cinnamon (inμL of essential oil/kg of beans): LD$_{10}$= 17.9; LD$_{50}$= 46.8; LD$_{90}$= 122.4; clove (in μL of essential oil/kg of beans): LD_{10}= 13.5; LD$_{50}$= 45.6; LD$_{90}$= 141.0

Table 2.1: Intensity of PAS reaction conducted on the histological sections of the trophocytes of *Acanthoscelides obtectus* reared on kidney beans or cranberry beans

Gender	Bean type	
	Cranberry	Kidney
Male	+ -	+ -
Female	+	+ +

+ -, weak reaction intensity; +, intermediate reaction intensity;
+ +, strong reaction intensity.

carbohydrates in male fat body cells was low for both bean types (Table 2.1).

2.3.3 Reproduction outputs and bean preference assays

2.3.4 Bean type preference assay

In the preference assays, 46.6% (± 2.84) of *A. obtectu*s adults chose the containers filed with cranberry beans, while 53.1% (± 2.62) of the insects preferred containers filled with kidney beans, demonstrating that the attraction of the two bean types to *A. obtectus* adults was not significantly different (df= 4; X^2= 18.6; P= 0.17). Furthermore, the average values for the kidney bean attractiveness (estimated by the AI) over the attractiveness of cranberry beans was of 0.91 (± 0.17), which was not statistically different from 1.

Oviposition and emergence rates assays

In the oviposition assays, the number of eggs laid was significantly different on each bean type after the first 3 days ($F_{1,9}$= 15.8; P= 0.004), for the second 3-day period ($F_{1,9}$= 11.5; P= 0.01) and for the total period of colonization ($F_{1,9}$= 15.2; P= 0.004; (Fig. 2.3). The number of the eggs laid on cranberry beans was always higher than the number of eggs laid on the kidney beans.

The total number of *A. obtectus* that emerged after one generation (42 days)

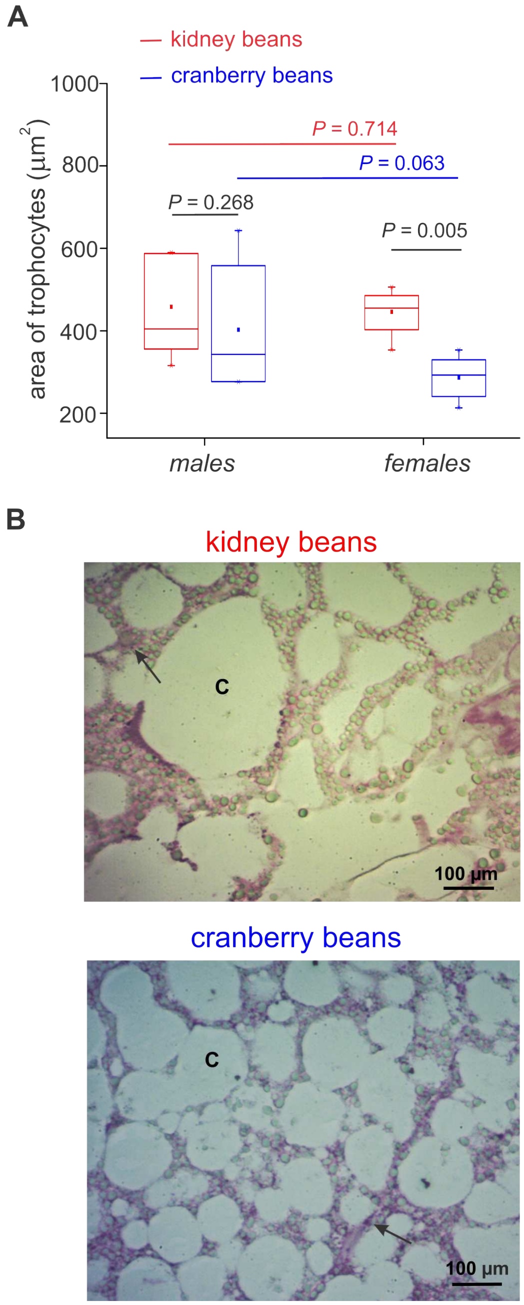

Figure 2.2: Trophocyte (fat body) area of adult males and females of *A. obtectus* reared on kidney beans (in red) or from cranberry beans (in blue). a The box plots indicate the median and the dispersion (lower and upper quartiles) of the mean trophocyte area. The filled rectangles inside the box plots indicate the mean values. The horizontal lines indicate the plots that were compared with t tests at 0.05 probability. b Fat body of adult females of *A. obtectus* reared on kidney beans (upper panel) or from cranberry beans (bottom panel); the cytoplasm (c) and nucleus arrows are indicated.

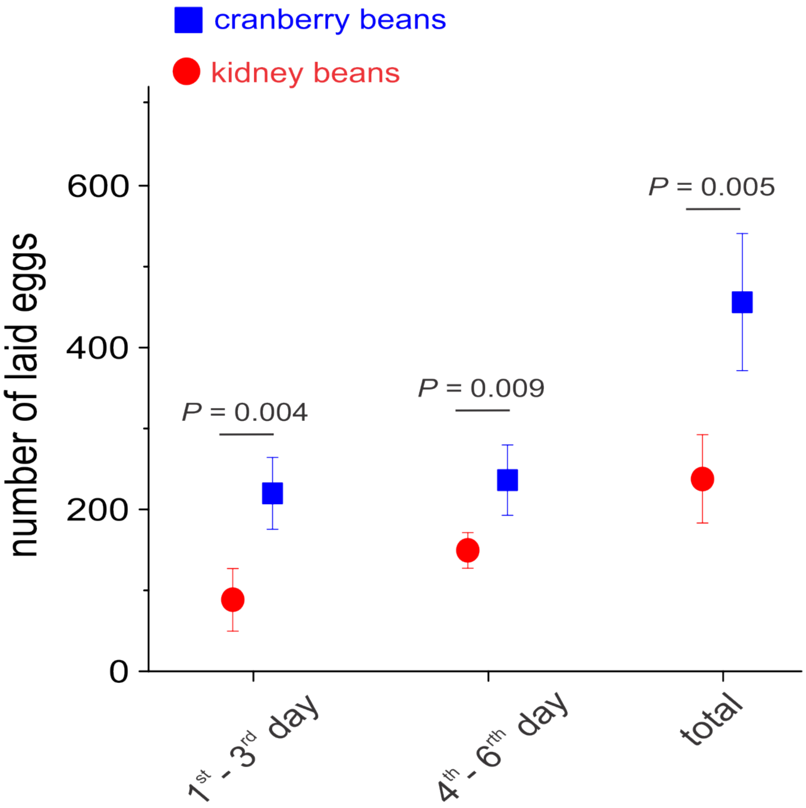

Figure 2.3: Number of eggs (mean ± SD; n = 5) laid by females of *A. obtectus* reared on kidney bean or from cranberry bean masses during the first 6 days of adulthood

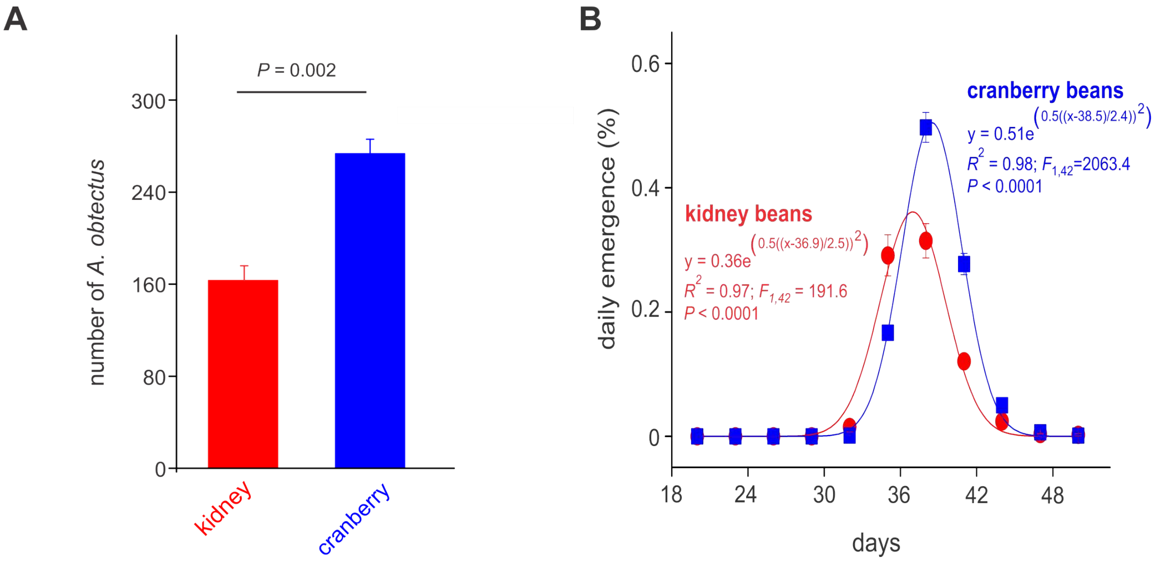

Figure 2.4: Effect of bean type on the emergence of *A. obtectus*. a Total number (mean ± SD; n= 4) of adults emerged after one generation (42 days). b Daily emergence of A. obtectus reared on kidney bean or from cranberry bean masses

from cranberry beans was significantly higher ($F_{1,9}$= 50.9; P= 0.002) than that from kidney beans (Fig. 2.4A). The rate of emergence (i.e., the daily emergence) confirmed this finding, and a significantly higher peak of emergence from the cranberry beans than that from kidney beans was observed on approximately day 40 (Fig. 32.4B).

2.3.5 Respirometry bioassays

The body mass of adults that emerged from each bean type was not significantly different ($P < 0.05$). However, the respiration rates were significantly higher (H= 7.67; df= 1; P= 0.01) for the insects that were reared on cranberry beans (0.022 ± 0.002μL CO_2/h/insect) than for those reared on kidney beans (0.016 ± 0.001 μL CO_2/h/insect).

2.4 Discussion

Here, we evaluated whether the investment of energy between the contrasting processes of reproduction and protection against insecticides on *A. obtectus* would be affected after a host change (i.e., from kidney beans to cranberry beans). Compared with those reared on kidney beans, the insects reared on cranberry beans had higher susceptibility to insecticides (i.e., deltamethrin and clove essential oil at LD_{90}), a smaller amount of energy in the fat body cells, higher oviposition and emergence rates, and higher respiratory activity.

As observed for many other herbivorous insects, the interactions between bruchids and their seed hosts are complex and are shaped by a co-evolutionary arms race between insect and host (Awmack and Leather, 2002; Gbaye et al., 2012; Huang et al., 2005; Lewis et al., 2012; Oyeniyi et al., 2015; Sales et al., 2000). For *A. obtectus*, the development of larvae within different types of legume seed affects adult performance by altering various physiological, morphological, behavioral and life-history traits (Hamraoui A, 1995; Janković-Tomanić et al., 2015; Khelfane-Goucem et al., 2014; Stojković B, 2012; Stojkovi et al., 2014). The physical and primarily chemical characteristics of each bean type are also likely important because legume grains have a diverse array of toxins that can influence insect development (Gbaye and Holloway, 2011; Lattanzio et al., 2005; Madodé et al., 2012; Vamosi, 2005). For example, the arcelins proteins that are differentially found in beans and have insecticidal properties toward bruchid pests as well as inhibitory effects on larval development (Osborni et al., 1988; Cardona C, 1990). However, arcelins appear to have no effect on the bean bruchid, *A. obtectus*, (Cardona C, 1990; Paes et al., 2000).

Although grain size did not affect the preference of *A. obtectus* females, the large size of cranberry beans as well as their smaller amount of carbohydrates likely favored larval performance, as indicated by the higher rate of emergence from cranberry beans than from kidney beans. The development of *A. obtectus* is strongly affected by the carbohydrate fraction (especially the arabinose and fucose contents), while the protein fraction has little or no effect (Gatehouse AMR, 1987).

To survive the challenges imposed by a change in host or environmental stressors (e.g., insecticides), insects must adapt their arsenal of behavioral, metabolic and physiological defenses (Correa et al., 2015; Guedes et al., 2006; Haddi et al., 2015b; Liu et al., 2013; Oliveira et al., 2015; Park et al., 2013). However, because energy is a limited resource, the energy allocated to conflicting processes (e.g., reproductive fitness or protection against toxicants) is an essential factor in determining the adaptation and survival of a species. In this study, we observed a host type-mediated trade-off in the relationship between reproductive fitness and susceptibility to insecticides for *A. obtectus*, which might be an indication of the effect of the host on the adaptive allocation of energy in this weevil, as reported for several other insect species (Guedes et al., 2006; Messina and Fry, 2003; Messina et al., 2009; Oliveira et al., 2005; Santos et al., 2015; Seslija and Tucic, 2003).

A. obtectus females reared on cranberry beans apparently mobilized more of their energy reserves for reproduction than for mitigating the negative effects of insecticides; these females had a less intense reaction to energy molecules, smaller trophocytes and higher rates of oviposition but were more susceptible to insecticides (i.e., deltamethrin and clove essential oil) than were those reared on kidney beans. The respiratory activity was higher for *A. obtectus* females reared on cranberry beans than for those reared on kidney beans, indicating higher metabolism, which might reflect an increase in cost for reproduction or for adaptation to a different environment (Correa et al., 2015; Guedes et al., 2006;

Haddi et al., 2015a; Unkiewicz-Winiarczyk and Gromysz-Kałkowska, 2012).

Although not addressed in detail in this study, the high susceptibility of *A. obtectus* reared on cranberry beans to deltamethrin and clove essential oil might also be the result of an impaired detoxification system. Physiological adaptations to a different host can trigger modifications in detoxification processes (e.g., phosphatase-dependent processes) (Basiouny A, 2010; Yan et al., 2011) and may reduce protection against xenobiotic compounds. For example, Janković-Tomanić et al. (2015) reported a decline in phosphatase enzymatic activity following the long-term adaptation of *A. obtectus* to a secondary host, which might partially explain the high susceptibility to insecticides observed in this study. Although the relationships between phosphatase activity and energy allocation trade-offs in *A. obtectus* have not been investigated, the close relationship between phosphatase and energy demands and between these enzymes and the development of the embryonic stages of insects has been established (Hashem et al., 2014; Kaur et al., 2012; Oliveira et al., 2013; Zahia K, 2009).

In the present investigation, morpho-physiological techniques and toxicological and reproductive bioassays were combined to provide valuable information not only for the management of *A. obtectus* in storage units but also to increase the understanding of the adaptability of these insects to their hosts. Understanding the dynamics of pest invasion and establishment in a particular bean type in relation to that bean type's nutritional, physical and chemical contents may help in decision-making for a better insecticide selection and application both in field and in storage units. It may also help with the establishment of a pest monitoring program for the most susceptible bean types. Future experiments that clarify the mechanisms that drive the evolution of such adaptations (e.g., mutation accumulations or antagonistic pleiotropy) or that examine the effect of different hosts on other immune functions (e.g., defense against pathogens, parasitoids or predators) remain essential.

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Chapter 3

Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils

Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils. Luis Oswaldo Viteri Jumbo, Khalid Haddi, Lêda Rita D. Faroni, Fernanda F. Heleno, Frederico G. Pinto, Eugênio E. Oliveira; **published in PLOS-ONE**.

Abstract

The use of plant essential oils has been shown to efficiently control insect pests of stored beans, significantly reducing the threats associated with synthetic insecticides. Here, we evaluated the potential of applications of essential oils of clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L., to control *Callosobruchus maculatus*, considered as one of the most cosmopolitan pests of stored beans. Using four combinations of couples (i.e., unexposed couples, exposed females, exposed males, and exposed couples), we also evaluated how sublethal exposure to these essential oils impacted *C. maculatus* oviposition. Bioassays results revealed that both essential oils exhibited insecticidal activities similar to the synthetic pyrethroid insecticide deltamethrin. Furthermore, oil dosage increments proportionately decreased the growth rate and reduced the losses in bean weight caused by cowpea weevils, and offspring emergence was almost abolished when parents were exposed to the LD_{20} of each essential oil. Finally, significant oviposition impairments were perceived only in couples where females were exposed (i.e., females exposed and exposed couples) to the LD_{20} of cinnamon and clove essential oils. Thus, by exhibiting similar insecticidal activities as synthetic insecticides and by significantly affecting the oviposition of sublethally exposed *C. maculatus* females, the cinnamon and clove essential oils represent valuable tools with potential of integration into the management of *C. maculatus* infestations.

Key words: Botanical insecticides; *Syzygium aromaticum*; *Cinnamomum zeylanicum*; cowpea weevil; sublethal effects.

3.1 Introduction

Plant essential oils have gained a reputation as being potentially bioactive compounds against many insect species, which has portrayed them as safer tools in terms of the environment and human health (Isman and Grieneisen, 2014; Regnault-Roger et al., 2012; Stevenson et al., 2017; Isman, 2016; Nerio et al., 2010; Pavela, 2015) . Despite the potential of essential oils to control pests of stored products, few studies have addressed the physiological and biological responses of stored product pests when the exposure occurred at sublethal levels.

It has been well recognized that stored product pests when sublethally exposed to synthetic insecticides can exhibit not only detrimental (what is somehow expected), but under certain circumstances also positive responses on their physiology and behavior (Desneux et al., 2007; Haddi et al., 2016; Correa et al., 2015; Guedes et al., 2016; Cutler and Guedes, 2017). Although the mechanisms explaining such sublethal responses (i.e., positive or detrimental) are still not well understood, it has been described that individuals sublethally exposed to synthetic insecticides show alterations in relevant life traits (e.g., the development time, longevity, fertility, fecundity, immune capacities, locomotion, navigation, sexual communication, oviposition and feeding) (Desneux et al., 2007). It is worthy to note, however, that similar life trait alterations seem to be elicited by sublethal exposure to botanical insecticides, which in their turn can trigger insect responses that either increase (Teodoro et al., 2017; Freitas et al., 2016; Haddi K, 2017; Oliveira et al., 2017; de Araújo et al., 2016) or compromise the efficacy of these alternative control tools (Correa et al., 2015; Haddi et al., 2015; Silva et al., 2017).

Among the essential oils that have been shown to adequately control insect pests, the oils extracted from clove, *Syzygium aromaticum* (L.), and cinnamon, *Cinnamomum zeylanicum* (L.), plants have drawn particular interest because of their promising insecticidal activities against various pests of stored products such as the maize weevil *Sitophilus zeamais* and the red flour beetle *Tribolium castaneum* (Regnault-Roger et al., 2012; Teodoro et al., 2017; Freitas et al., 2016; Haddi K, 2017; Oliveira et al., 2017; de Araújo et al., 2016). In insects, these essential oils have neurotoxic action both as fumigants and or contact insecticides and there metabolites can act upon variety of molecular targets including inhibition of acetylcholinesterase or disturbing the functions of GABAergic and aminergic systems (Jankowska et al., 2017).

The cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae), is a cosmopolitan pest of legume seeds and is among the most serious pests of stored products in tropical countries (Kang et al., 2013; Ebiamadon et al., 2011; Massango et al., 2016). The insect larvae represent the most destructive stage, as adult cowpea bruchid do not feed (Ileke KD, 2013; Lima et al., 2001). However, as the availability of a specific host is highly discontinuous and because these adult insects have to live in hosts that are normally treated with insecticides (Gbaye et al., 2011a; Haddi et al., 2017; Gbaye et al., 2011b), these insects might have to face insecticidal sublethal exposures prior to deciding where they are going to lay eggs.

Most of the cowpea, *Vigna unguiculata* (L.) Walp, production occurs in tropical countries with high contribution of small farmers. In this context, the control of *C. maculatus*, when accomplished, is mainly achieved by the application of a small group of synthetic molecules (e.g., phosphine and pyrethroid insecticides, such as deltamethrin). Dependence on a small group of synthetic molecules raises the risk of selecting resistant populations as well as increases the hazard risks to human health and to the environment (Regnault-Roger et al., 2012; Isman, 2006).

Thus, it is urgently needed to develop alternatives to the chemical control of *C. maculatus* that not only can reduce the concerns outlined above but also can be prone of embracing the actual trend in developing new botanical-derived insecticides based on the inclusion of the active ingredient (i.e. EOs) in stable delivery systems (nanoparticles, nanoemulsion, etc) (Kah et al., 2018; Campolo et al., 2017). Therefore, we investigated the chemical composition of clove and cinnamon essential oils and evaluated whether these oils would adequately control *C. maculatus*. We also characterized the biological responses (e.g., oviposition, offspring emergence and population growth) of *C. maculatus* exposed to sublethal amounts of each type of essential oil.

3.2 Material & Methods

3.2.1 Insect rearing

The original population of *C. maculatus* was field-collected from small farms in the Viçosa region (Minas Gerais State, Brazil) during the year 2015, and the population was maintained on pest- and insecticide-free cowpea beans under laboratory conditions ($27 \pm 2°C, 75 \pm 5\%$ RH, 12 h scotophase). The bean grains had a water content of 12% and were offered ad libitum. The farm-owners gave permission to collect samples of *C. maculatus* from their fields and since *C. maculatus* is not an endangered or protected species in Brazil, no specific permissions were required for such collection.

3.2.2 Extraction and chemical characterization of essential oils

Locally purchased cinnamon bark and dried flower buds of clove were used for essential oil extraction, as described by (Jham GN, 2005). Briefly, the primary material was ground and sieved to obtain a fine powder (less than 1 mm) that was extracted at room temperature by constant percolation with hexane, followed by hydrodistillation for 6 h. Then, the distillate was extracted twice with dichloromethane and dried over anhydrous sodium sulfate. The distilled oils were stored in airtight screw-capped vials at -10°C until use.

The components of the cinnamon and clove essential oils were determined by gas chromatography-mass spectrometry (GCMS-QP2010, Shimadzu). The separation was done on a capillary column of fused silica (30 m × 0.22 mm) with stationary phase RTX5 (0.25-m-thick film). The initial column temperature was 60°C for 2 min, followed by increase of 3°C min^{-1} up to 240°C, and this temperature was maintained for 15 min. The temperatures of the injector and detector were maintained at 220°C and 240°C, respectively.

The carrier gas was helium with a flow of 1.8 mL min^{-1}. Samples were diluted in dichloromethane and injected in a 1.0μL split ratio of 1:20. Data acquisition was made in full-scan mode, with a scanning range between 29 and 400 m/z. Experimental mass spectra were compared with known mass spectra (The National Institute of Standards and Technology (NIST 14) Mass Spectra Library, 2017). The arithmetic index (AI) was calculated according to R.P. (1997), using the retention times of the essential oil compounds and a homologous series of C_8-C_{26} n-alkane standards following the formula: AI (x) = 100 $P_z + 100[(t\ (x) - t\ (P_z))/t\ (P_z{+}1) - t\ (P_z))]$; where x: compound at time t; P_z: alkane before x; and P_z+1: alkane after x. The relative percentage of each compound was calculated by the integral ratio of its respective peak area and the total area of all the compounds of the sample. The calculated AI for each compound was compared with values reported in the literature (R.P., 1997).

3.2.3 Insecticidal activity

We conducted dose-mortality bioassays to determine the lethal doses of the cinnamon and clove essential oils to adult *C. maculatus*. These bioassays followed procedures previously described elsewhere (Jumbo et al., 2014). Briefly, each essential oil was pure (i.e., without diluents) and was applied using a 25-μL microsyringe (Hamilton, Reno, NV, USA) to 50 g of beans that were placed in 0.8-L glass jars. After the application, the jars were manually shaken for 60 s to ensure a complete distribution of the essential oils. Twenty unsexed 1-2-day-old *C. maculatus* adults were placed in each jar, and the jars were sealed with a fine porous cloth to allow ventilation; jars were kept under controlled conditions (27±°C, 75 $\pm$ 5% relative humidity, 12 h scotophase). The insect mortality was recorded after a 24-h exposure period. Insects were considered dead if they did not respond to fine paintbrush stimuli (i.e, two subsequent touches in 2 min intervals). Five doses of each essential oil were tested in the bioassays (e.g., Cinnamon: 20, 60, 120, 160 and 200 μl kg^{-1}. Clove: 20, 40, 80, 120, 160 μl kg^{-1}). Five replications were used per dose, and the control treatment did not receive any oil application. As a positive control, we used the pyrethroid insecticide deltamethrin (25 g L^{-1}; EC; Bayer Crop Science, SP, Brazil) diluted in distilled water to obtain the desired doses (e.g., 64, 72, 80, 88, 96, 104 μl a.i kg^{-1}). The application and the conditions of bioassays were similar to those described for the essential oils.

3.2.4 Effects of essential oils on the biological development and bean-mass losses

Effects on instantaneous rate of population growth (r_i) and bean-mass losses. The instantaneous rate of increase (r_i) test was carried out in 0.8-L glass jars, where 20 unsexed 1-2-day-old adults of *C. maculatus* were allowed to colonize 50 g of beans treated with an essential oil based on the concentration-mortality results previously obtained (see Results section). We measured the instantaneous rate of increase (r_i) of groups of *C. maculatus* that were subjected to different sublethal exposures to cinnamon (LD$_{20}$= 106.2, LD$_{40}$= 123.0, LD$_{60}$= 139.4, μL kg^{-1} of bean) and clove (LD$_{20}$= 48.6, LD$_{40}$= 67.6, LD$_{60}$= 90.2 μL kg^{-1} of bean) essential oils. Five replicates were used for each combination of concentration and essential oil. All the glass jars were maintained at 27 ± 2°C, 75 ± 5 % relative humidity and 12 h scotophase. The control treatment did not receive any essential oil application. The number of F_1 insects was counted after 45 days, and the instantaneous rate of increase for each population was calculated as follows: $r_i = \ln{(N_f/N_i)}/\Delta t$, where N_f is the final number of observed adults, N_i is the initial number of *C. maculatus*, and Δt is the duration of the experiment (Walthall and Stark, 1997).

The grain masses provided for insect colonization were weighed at the start (day 0) and at the end (day 45) of the bioassays to calculate the percentage of grain loss.

3.2.5 Effects on average and cumulative emergence

The bioassays for the average emergence were conducted using the same experimental procedures described for the instantaneous rate of increase (r_i). The progeny formed by the adult *C. maculatus* emerging from the beans were assessed in two days intervals starting from the 20^{th} day after treatments began until no adult emergence was observed (i.e., 20 days after emergence of the 1^{st} adults). After each assessment, the emerged adults were removed.

3.2.6 Effects of sublethal exposure to essential oils on the *C. maculatus* oviposition

Newly emerged (< 48 h old) groups of *C. maculatus* adult males and females were exposed separately to clove and cinnamon-essential-oil-treated beans at the LD_{20} values for clove (i.e., 48.6 μL kg^{-1} of beans) and for cinnamon (i.e., 106.2 μL kg^{-1} of beans) essential oils. After a 24-h exposure period, we paired *C. maculatus* couples in four combinations (i.e., unexposed couple, exposed female, exposed male, and exposed couple) and allowed each couple to oviposit in 20 grain of untreated beans. At 3-day intervals, the couples were moved to new 20 grain bean masses, and this process was repeated for a total period of 9 days. The number of oviposited eggs was assessed under microscope after 3, 6 and 9 days. Twenty couples were used for each treatment combination.

3.2.7 Statistical analyses

Dose-mortality data were subjected to probit analysis (Institute, 2008), and 95% confidence intervals for toxicity ratios were estimated following Robertson and Preisler (Robertson and Preisler, 1991); the values were considered significant if the range did not include the value 1. Regression analyses were performed to detect trends in cumulative and average emergence that resulted in each treatment over time. Regression analysis was performed using the curve-fitting procedure of Sigma Plot 12.0. The regression model was chosen based on parsimony, lower standard errors, and steep increases in R^2 with increases in model complexity. The regression models for each treatment were considered different from each other if the confidence limits of their parameters did not overlap. We also conducted linear regression to assess the effects of increasing lethal exposure to essential oils on the r_i and grain-mass losses of *C. maculatus*. The data on the number of eggs used in each treatment combination were submitted to repeated measures ANOVA. The assumptions of normality and homogeneity of variance were tested for all parameters, and no data transformations were necessary (PROC UNIVARIATE, SAS Institute Inc., Cary, NC, USA).

3.3 Results

3.3.1 Chemical composition of the essential oils

The chemical analyses showed that the two main components of cinnamon and clove essential oils were eugenol and β-caryophylene (Table 3.1). However, the cinnamon essential oil additionally contained a wide range of other compounds in smaller amounts, including acetyleugenol, benzyl benzoate, linalool, cinnamyl acetate and cinnamaldehyde.

Table 3.1: Chemical composition of clove and cinnamon essentials oils.

Compound	**Arithmetic index**				**Concentration**	
	S. aromaticum		***C. zeylanicum***		***S. aromaticum***	***C. zeylanicum***
	a	b	a	b	(%)	
eugenol	1363	1356	1364	1356	87.4	73.1
$\beta-$caryophylene	1415	1417	1414	1417	11.5	7.7
$\alpha-$humulene	1447	1452	1447	1452	1.1	0.4
$\alpha-$pinene	-	-	931	932	-	0.7
$\alpha-$phellandrene	-	-	1004	1002	-	0.3
$p-$cymene	-	-	1022	1020	-	1.0
limonene	-	-	1026	1024	-	0.5
eucalyptol	-	-	1028	1026	-	0.7
linalool	-	-	1100	1095	-	2.6
(*E*)-cinnamaldehyde	-	-	1268	1267	-	2.3
methyleugenol	-	-	1405	1403	-	0.6
cinnamyl acetate	-	-	1444	1443	-	2.5
acetileugenol	-	-	1528	1521	-	3.6
caryophyllene oxide	-	-	1576	1582	-	0.5
benzil benzoate	-	-	1760	1759	-	3.4

^{a} calculated, ^{b} tabulated

3.3.2 Insecticidal activity

The mortality levels obtained in the dose-mortality bioassays were satisfactorily described by the probit model [goodness-of-fit tests exhibiting low x^2-values (<10) and high P-values (> 0.05)]. The toxicity ratios (TR) were estimated relative to the LD_{50} for deltamethrin. The toxicities of the clove and cinnamon essential oils were similar to the pyrethroid-based insecticide deltamethrin (Table 3.2).

Table 3.2: Toxicity of clove and cinnamon essential oils and deltamethrin on adults of *Callosobruchus maculatus*

Insecticide	**Slope$\pm$SD**	**LD$_{20}$ (95% FL)**	**LD$_{40}$(95%FL)**	**LD$_{50}$(95% FL)**	**LD$_{60}$(95% FL)**	X^2	***P***	**TR$_{50}$(95% CL)**
Clove	4.00.32	48.6 (42.0–54.0)	68.0 (62.0–74.0)	78.2 (71.6–84.8)	90.0 (84.0–98.0)	5.25	0.15	0.94 (0.9–1.0)
Cinnamon	9.31.02	106.4 (96.0–114.0)	124.0 (116.0–130.0)	131.0 (124.0–137.0)	138.0 (132.0–146.0)	3.90	0.14	1.56 (1.5–1.6)
Deltamethrin	13.91.25	72.8 (68.8–76.0)	80.0 (76.8–83.2)	83.7 (80.6–86.6)	87.2 (84.0–90.3)	9.91	0.08	1.00 (0.9–1.0)

SD standard deviation; LD: Lethal dose (μL kg^{-1}); FL= Fiducial limits; x^2= Chi-square for lack-of-fit to the probit model, and P= Probability associated with the chi-square statistic; TR$_{50}$= Toxicity ratio determined by LD$_{50}$ of each the essential oil /LD$_{50}$ of deltamethrin; CL= Confidence limits of TR$_{50}$.

3.3.3 Effects of essential oils on the biological development and bean-mass losses

Effect on instantaneous rate of population increase (r_i) **and bean weight loss.** The instantaneous rate of population increase (r_i) decreased in a dose-dependent manner for the two essential oils used (Fig. 3.1). The extinction stage (negative r_i) was reached when the *C. maculatus* insects were in contact with concentrations equal to or higher than LD_{60} for clove (i.e., 67.6 μL kg^{-1} of bean) and cinnamon (i.e., 139.4 μL kg^{-1} of bean) essential oils.

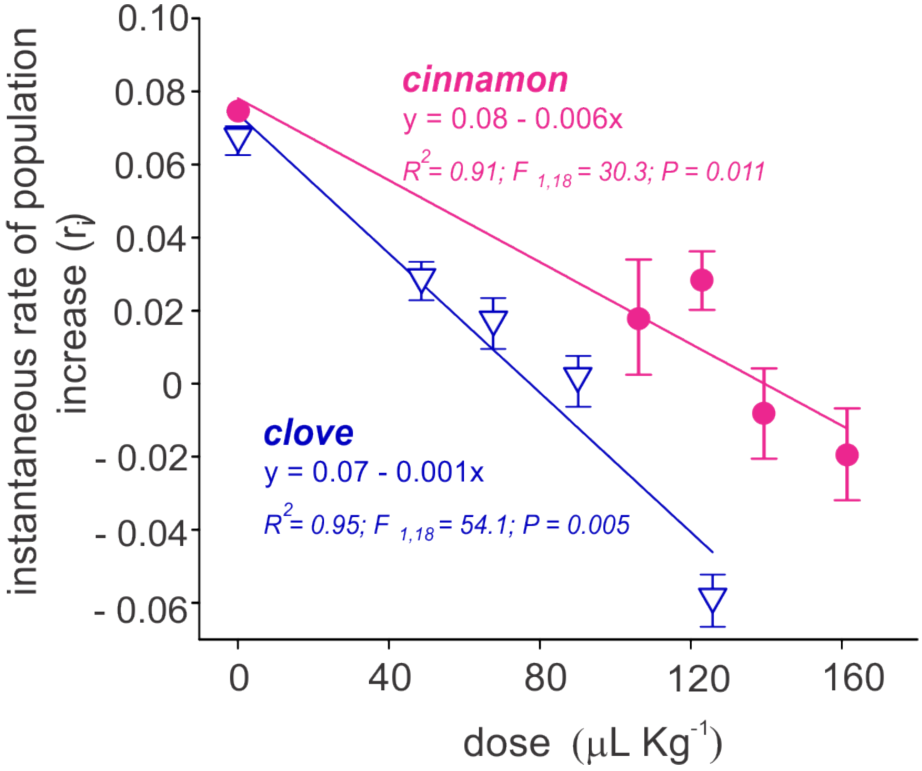

Figure 3.1: Instantaneous rate of population increase of *C. maculatus* exposed to clove and cinnamon essential oils. The symbols represent the means of five replicates of the LD_0 (control), LD_{20}, LD_{40}, LD_{60} and LD_{80} for each oil. The doses are expressed in μL of essential oil/kg beans. The vertical bars represent the SD.

Similar trends were observed for the bean mass losses, where the LD_{20} concentrations of clove (F= 21.3; $P < 0.001$) and cinnamon (F= 69.8; $P < 0.001$)

essential oils significantly reduced grain loss when applied as a contact treatment (Fig. 3.2). These losses were reduced from approximately 15%, when the beans were incubated with *C. maculatus* insects in the absence of essential oils, to less than 6%, when the bean was treated with clove and cinnamon essential oils.

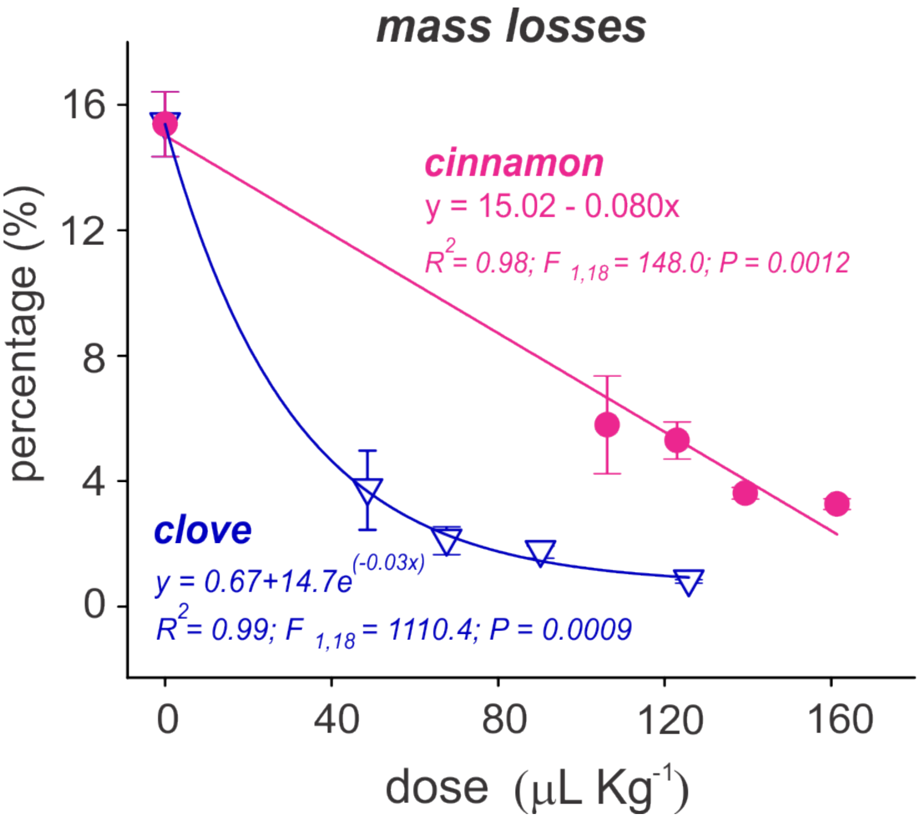

Figure 3.2: . Bean weight losses caused by *C. maculatus* exposed to clove and cinnamon essential oils. The symbols represent the means of five replicates of the LD_0 (control), LD_{20}, LD_{40}, LD_{60} and LD_{80} for each oil. The doses are expressed in μL of essential oil/kg beans. The vertical bars represent the SD.

Positive correlations were observed between the instantaneous rate of increase of *C. maculatus* and the mass losses in grain masses treated with clove ($R^2 = 0.72; P < 0.001$) and cinnamon (R^2= 0.83; $P < 0.001$) essential oils, between the instantaneous rate of increase and the total number of emerged adult of *C. maculatus* for clove (R^2= 0.75; $P < 0.001$) and cinnamon (R^2= 0.85; $P < 0.001$) essential oils, and between the total number of emerged adult of *C. maculatus* and the grain mass losses for clove ($R^2 = 0.98$; $P = 0.03$) and cinnamon ($R^2 = 0.99$; $P < 0.001$) essential oils.

3.3.4 Effects on average and cumulative emergence

The average emergence of new *C. maculatus* insects was negatively and severely impacted after treatment by either clove or cinnamon essential oil, as observed in the differences between the emergence curves (Fig. 3.3).

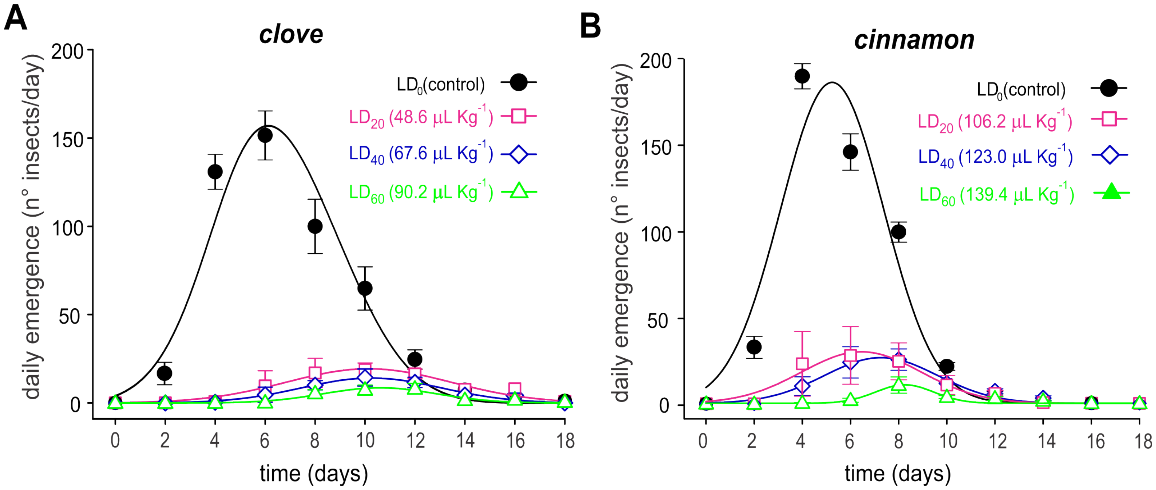

Figure 3.3: Average emergence of *C. maculatus* exposed to clove (A) and cinnamon (B) essential oils. The symbols represent the means of four replicates of the LD_0 (control), LD_{20}, LD_{40}, and LD_{60} for each oil. The doses are expressed in μL of essential oil/kg beans. The vertical bars represent the SD.

Treatments with concentrations starting at the LD_{20} for both essential oils resulted in a near abolition of emergence. Moreover, the total emergence of *C. maculatus* was significantly delayed by treatments of almost all concentrations of the two essential oils when compared to the control, as shown by the lack of overlap between the cumulative emergence curves (Fig. 3.4).

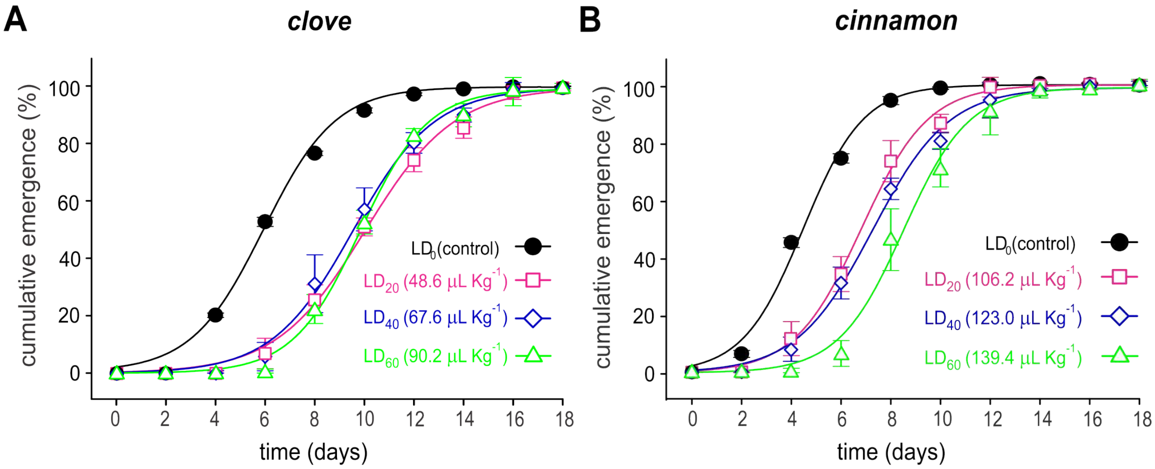

Figure 3.4: Normalized cumulative emergence of *C. maculatus* exposed to clove (A) and cinnamon (B) essential oils. The symbols represent the means of four replicates of the LD_0 (control), LD_{20}, LD_{40} and LD_{60} for each oil. The doses are expressed in μL of essential oil/kg beans. The vertical bars represent the SD.

3.3.5 Effects of sublethal concentrations of essential oils on *C. maculatus* oviposition

The results of repeated measures ANOVA showed that there was a significant interaction (Wilks' lambda= 11.77; *df*= 6; $P < 0.001$) between oil types, couple combinations and evaluation times (Fig. 3.5). The sublethal exposure to clove and cinnamon essential oils mediated the effects on *C. maculatus* oviposition, resulting in significant (F= 25.1; *df*= 3; $P < 0.001$) differences in the total number of eggs among the four combinations of treatments. When only females were exposed (i.e., exposed females or exposed couples) or when both females and males were exposed (i.e., exposed couples), the total number of laid eggs decreased dramatically in comparison with both the untreated couples and the couples where only males were treated. The two essential oils showed significantly (F= 4.19; *df*= 1; $P = 0.04$) different inhibiting effects on oviposition, and this difference was more evident in the oviposition of couples where only females were treated, as the decrease was more important for cinnamon compared to clove and untreated couples. Moreover, the differences between the effects of sublethal exposure to both essential oils were significantly (F= 136.2; df= 2; $P < 0.001$) higher during the first 3 days of the oviposition period.

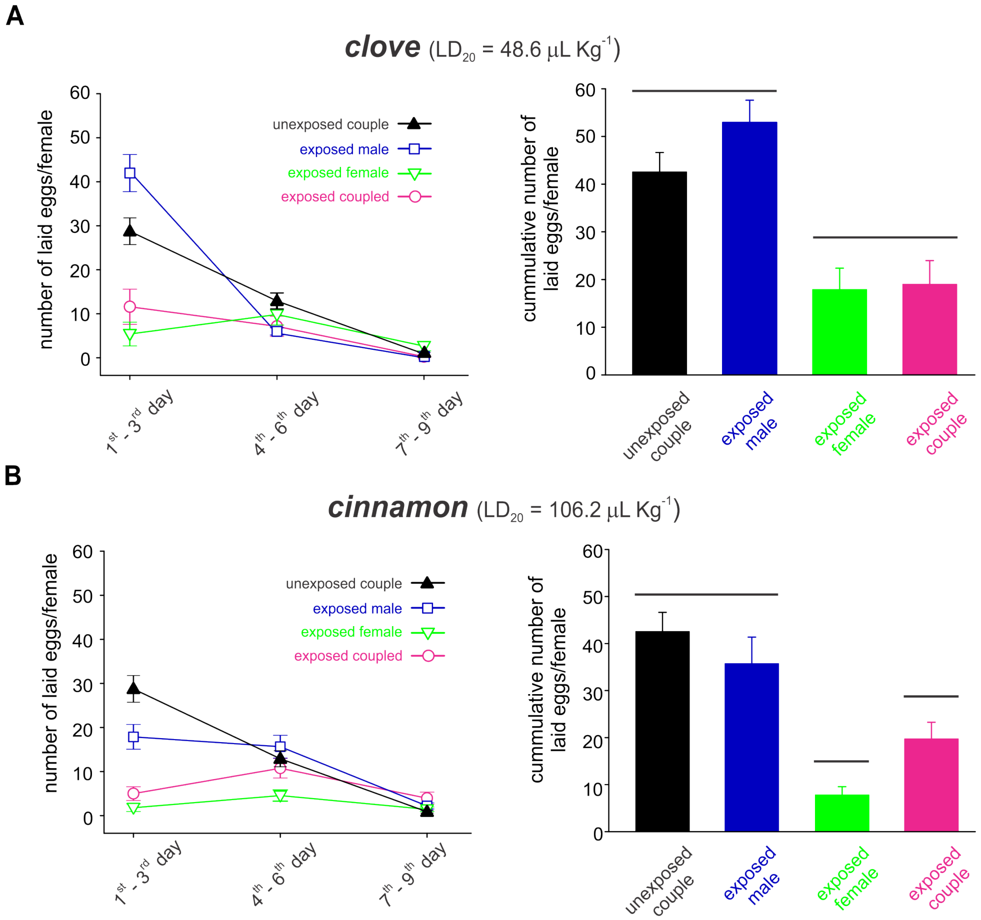

Figure 3.5: Effects of sublethal exposure to essential oils on *C. maculatus* oviposition. Number of eggs of *C. maculatus* females that were sublethally exposed to clove (A) or cinnamon (B) essential oils and coupled with essential oil-treated or essential oil-untreated partners. The symbols represent the means of 20 replicates (±SD) for the number of eggs laid by females of *C. maculatus* at three-day intervals (left panels) and the cumulative number of eggs (right panels) laid on cowpea bean masses during the first 9 days of adulthood. On the right panels, the treatments grouped by the same horizontal line did not differ according to a Tukey HSD test ($P < 0.05$).

3.4 Discussion

Plant essential oils are among the most interesting options for cheaper, safer and eco-friendly replacements (or to be used as adjuvants) for synthetic insecticides (Regnault-Roger et al., 2012; Stevenson et al., 2017; Isman, 2016; Pavela, 2015). Here, we demonstrated that applications of clove and cinnamon essential oils not only adequately controlled C. *maculatus* on stored cowpea beans but also were capable of reducing the oviposition and population growth of C. *maculatus* even at sublethal dosages.

Essential oils, such as clove and cinnamon oils, are very complex natural mixtures and can contain various compounds at different concentrations with two or three major components that will determine the biological properties of the essential oil (Bakkali et al., 2008). However, synergistic effects between the components of essential oils have been frequently reported in previous studies (Fornari et al., 2012; Joffe et al., 2011; Koul et al., 2013; Miresmailli et al., 2006; Omolo et al., 2005; Kanda et al., 2016). Our chemical analyses of cinnamon and clove essential oils revealed that their primarily components were eugenol ($>$ 70.0%), followed by the sesquiterpene $\beta-$caryophyllene (between 7.0 and 12%). These results are in concordance with previous studies that reported similar compositions (Jumbo et al., 2014; Bakkali et al., 2008; Chaieb et al., 2007; Dayan et al., 2009; Fichi et al., 2007a,b; Park et al., 2000). It is worth noting that cinnamon essential oil, despite its major components, also contained a range of other compounds, including acetyleugenol, benzyl benzoate, linalool, cinnamyl acetate and cinnamaldehyde (between 2 and 4%), lending more evidence to the hypothesis that essential oil biological activities may be shaped by the potential synergistic and antagonistic interactions among all these molecules and not only by major essential oil compounds (Bakkali et al., 2008; Koul O, 2008).

Several studies have reported the insecticidal toxicity of clove and cinnamon essential oils and their primary compounds that successfully control stored product pests (Correa et al., 2015; Silva et al., 2017; Haddi et al., 2017; Jumbo et al., 2014; Brari J, 2015; Athanassiou CG, 2014; Pérez S, 2010) and other insects (Park et al., 2000; Chang and Cheng, 2002; Cheng et al., 2009; Khater et al., 2009; Wang et al., 2011). The vast majority of these investigations have attributed these essential oil insecticidal activities to their major constituents (i.e., eugenol and β-caryophyllene), as these compounds are known to act on the insects' nervous system by disturbing the functions of GABAergic (Tong and Coats, 2012; Bloomquist et al., 2008) and aminergic (Enan, 2005a,b; Kostyukovsky et al., 2002) systems and by inhibiting the actions of acetylcholinesterase (Keane S, 1999; Lopes et al., 2010; Abdelgaleil et al., 2015).

Negative effects on developmental traits, such as rates of growth and progeny emergence of bruchid insects such as *C. maculatus*, have been reported for various essential oils and their components (Massango et al., 2016; Ileke KD, 2013; Haddi et al., 2017; Pérez S, 2010; Paranagama and Gunasekera, 2011; Jumbo et al., 2014; Elhag, 2000; R., 2011; Ekeh FN, 2013). The present investigation demonstrated that treating cowpea bean masses with sublethal dosages (i.e., as lower as their LD_{20}) of these essential oils leds to significant decreases in the *C. maculatus* instantaneous rates of population growth and the bean mass losses, and the application of the essential oils almost abolished *C. maculatus* offspring emergence. Such biological impairments caused by clove and cinnamon essential oils on bruchids can be the result of the direct mortality of adults, repellency, oviposition deterrence or progeny and growth inhibition (Athanassiou CG, 2014). However, our oviposition results (i.e., females sublethally exposed to these essential oils decreased their ability to lay eggs even when they were offered to mate with untreated sexual partners in untreated bean masses) revealed an even more complex scenario and potential effects on sexual fitness (e.g., locomotory activities, mating behavior) or on the physiological basis of oviposition (e.g., respiratory activities, oogenesis or hormonal disruption) may also contribute to the reduced performance of essential oil sublethally exposed insects. For instance, similar physiological impairments (e.g., repellence, emergence inhibition, altered respiratory activities and transgenerational behavior changes) have been reported in stored product pests (e.g., *S. zeamais*) exposed to essential oils of cinnamon and clove (Correa et al., 2015; Freitas et al., 2016; Haddi et al., 2015; Silva et al., 2017).

Furthermore, as biosynthesis and release of mating signals as well as the production of eggs may be influenced by atmospheric volatiles and gases (Hilker and Meiners, 2011; Stadler E, 2003). plant extracts compounds, such as terpenes, can influence the site-choice of egg-laying female insects (Hilker and Meiners, 2011). Although future experiments are required to isolate the effects of the exposure to essential oils on the physiological basis of oviposition and on the mating behavior, a potential energy trade-off between the detoxification process and oogenesis might be an explanation for the inhibited oviposition observed here (Hashem et al., 2014; Kaur et al., 2012; Oliveira et al., 2013).

In our study we have used drops of pure essential oils on bean masses and although such technique showed good biological activities on *C. maculatus* under laboratory conditions and may have potential applications at small farmer's level, this delivery system may suffer from draw backs inherent to the volatile nature of essential oils in larger storage facilities. In fact, rapid biodegradation of these compounds due to their poor physicochemical stability, high volatility, and thermal decomposition will require some controlled-release system such as nanotechnological formulations to optimize the action of their active ingredients (Pavela and Benelli, 2016; de Oliveira et al., 2014).

Thus, our findings revealed adequate insecticidal activities of clove and cinnamon essential oils against *C. maculatus* and demonstrated that, even at sublethal doses, these botanical compounds impaired the ability of *C. maculatus* to damage cowpea bean masses, which make them suitable tools that can be integrated into management programs of *C. maculatus*, especially for storage facilities. Further work is also needed to test the applicability and efficacy of nanofomulations of these essential oils under broader stored products conditions.

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Chapter 4

The lacewing *Ceraeochrysa caligata* as a potential biological agent for controlling the red palm mite, *Raoiella indica*

The lacewing *Ceraeochrysa caligata* as a potential biological agent for controlling the red palm mite, *Raoiella indica*; Luis O. Viteri J., Adenir V. Teodoro, Adriano S. Rêgo, Khalid Haddi, Andréia S. Galvão, E. E. Oliveira; **submited to PeerJ**.

Abstract

The use of naturally occurring biological agents to control invasive pests seems to pose lower threats to the environment and human health than do chemical control practices. Here, we assessed the capacities of the lacewing *Ceraeochrysa caligata* (Neuroptera: Chrysopidae) to prey upon different developmental stages of the red palm mite, *Raoiella indica* (Acari: Tenuipalpidae), which is an invasive and one of the most destructive pests of palm trees worldwide. Our results revealed that the capacity of *C. caligata* to feed upon *R. indica* increased with the larval development of the predator. Higher feeding levels and shorter handling times were recorded for the first and second instars of *C. caligata* when preying upon the eggs and immature-stage individuals of *R. indica*. Furthermore, individuals of different stages of the predator exhibited differential functional responses according to prey type (i.e., eggs, immatures or adults of *R. indica*). Second-instar *C. caligata* individuals exhibited a sigmoid increase in consumption rate with increasing prey availability (i.e., a type III functional response) when preying upon immature stages of *R. indica*. However, when preying upon *R. indica* adult females, second-instar *C. caligata* individuals exhibited a type II functional response (i.e., an increase in consumption rate as prey availability increased before reaching a plateau). Individuals of the predator's first and third larval instars exhibited a type II functional response for all prey types. Collectively, our findings demonstrate that *C. caligata* (especially at the second-instar stage) has potential as a tool for the ecological management of the *R. indica*.

Key words: invasive pests, functional response, insect predators

4.1 Introduction

The red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), is an invasive pest. Although the red palm mite has a myriad of hosts (Carrillo et al., 2012a), it prefers palm trees (de Assis et al., 2013). Native to the Old World, the mite has invaded the New World and is currently distributed in North America (Roda et al., 2012) , the Caribbean (Kane et al., 2012) and South America (Kane et al., 2012; Navia et al., 2011). The mite is a multivoltine and gregarious species whose attack symptoms include yellowing of the lower leaves, which can lead to reduced yields of important agricultural crops such as coconut, *Cocos nucifera* (L.), and banana, *Musa* spp. (Peña et al., 2012).

Despite the need for frequent application and the risks associated with synthetic acaricides (e.g., threats to human health, environmental contamination, and selection of resistant individuals), their application continues to be the most common strategy used to control *R. indica* (Jayaraj et al., 1991; Rodrigues, 2012). However, this control strategy does not show high efficacy against *R. indica* as the mites live and feed under the abaxial leaf surfaces of their hosts, which typically limits their exposure to applied acaricides. Furthermore, coconut palm trees may reach over 10 m high, which makes the control of *R. indica* on these hosts through pesticide sprays particularly challenging (Domingos et al., 2013).

The use of naturally occurring biological control agents represents a plausible alternative strategy for controlling *R. indica*. Some investigations have demonstrated the potential of several mites and insects capable of preying on *R. indica* (Carrillo et al., 2012b; Hoy, 2012; Peña et al., 2009). Regarding predatory mites, species of the genus *Amblyseius* (Acari: Phytoseiidae) have drawn attention due to their abundance throughout the year and their feeding upon all developmental stages of the red palm mite (Carrillo et al., 2012b; Vásquez and de Moraes, 2012). For predator insects, twelve species from five families have been reported in association with *R. indica* (Carrillo et al., 2012b), including *Oligota* sp. (Coleoptera: Staphylinidae) (Somchoudhury, 1987), *Aleurodothrips fasciapennis* (Thysanoptera: Phlaeothripidae) and *Ceraeochrysa* sp. (Neuroptera: Chrysopidae) (Peña et al., 2009). Lacewings (Neuroptera: Chrysopidae) of the genus *Ceraeochrysa* have particular potential as biological control agents of *R. indica* as these predators have high reproductive capacity (Carvalho and Souza, 2000), high ecological plasticity (Khuhro et al., 2014), relative tolerance to pesticides (Carvalho et al., 2011; Ono et al., 2017; Rugno et al., 2015) and can be readily mass-reared under controlled conditions (López-Arroyo et al., 1999).

The predatory capacity of biological control agents can be influenced by several factors, including environmental conditions (e.g., temperature, humidity, and light) and the diverse bioecological interactions (e.g., sizes, behaviors and densities) among predators and prey (Aljetlawi et al., 2004; Laws, 2017; Luff, 1983; Solomon, 1949). The present investigation aimed to investigate the capacity of the lacewing *Ceraeochrysa caligata* (Banks, 1946) to prey upon *R. indica*. We evaluated the potential of *C. caligata* as a biological control agent of *R. indica* by conducting functional response bioassays [i.e., evaluating changes in predator feeding behavior in response to changes in prey density (Solomon, 1949)] with individuals of all three predator larval instars presented with mites at the immature and adult stages.

4.2 Material & Methods

4.2.1 Rearing of predators and collection of red palm mites

Eggs of the lacewing *C. caligata* were collected in pesticide-free coconut plantations at experimental fields of the “Embrapa Tabuleiros Costeiros” in Aracaju (10°56′46″S, 37°03′12ȷ*W*), Sergipe State, Brazil. The eggs were maintained in Petri dishes, and the emerged larvae were individualized and fed *ad libitum* with eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) during development. The larval feeding occurred three generations before the bioassays. Adults were maintained in plastic containers (20 cm diameter, 30 cm height) with open tops covered by fine tissue (i.e., organza) for ventilation. Adults were fed *ad libitum* with an artificial diet consisting of a mixture of bee honey and brewer’s yeast (1:1) and provided cotton wool soaked with distilled water, which were replaced every two days (de Freitas, 2001). The rearing units were maintained under controlled conditions of temperature (27 ± 2°C), relative humidity ($65 \pm 5\%$) and photoperiod (12:12 L:D).

We collected eggs, immature-stage individuals (larvae, protonymphs, and deutonymphs) and adult females of *R. indica* from pesticide-free coconut plantations at experimental fields of the “Embrapa Tabuleiros Costeiros.”

4.2.2 Functional response bioassays

The functional responses of individuals of the three larval instars of *C. caligata* to eggs, immatures and females of *R. indica* were assessed under laboratory conditions using experimental procedures described elsewhere (Hassanpour et al., 2011, 2009). Briefly, the assay arena consisted of Petri dishes (5 cm diameter) containing a cleaned section (15 cm^2) of coconut leaflet placed upsidedown on a layer of solidified agar (7.0 cm^2) of free area. Adult females or immatures of *R. indica* were gently transferred to the section of coconut leaflet with a fine brush, while pieces of leaflets containing eggs were cut and placed into the arena. Surplus eggs were removed to adjust the density of the prey. The prey densities were 20, 30, 40, 70, 100 and 150 individuals for the first instar (L1) of *C. caligata* and 100, 150, 250, 350, 450 and 600 for the second (L2) and third (L3) instars. The maximum and minimum prey densities for each *C. caligata* instar were determined in preliminary tests. Larvae of *C. caligata* ($<$ 12 h old) were starved for 12 hours before the bioassays. Then, using a brush, *C. caligata* larvae were transferred individually to the experimental arenas of various prey densities. The Petri dishes were covered with perforated Parafilm to prevent the predators from escaping. Predator larvae that did not feed in the first five minutes were excluded from the experiment. The number of prey consumed was recorded after six hours, and prey were not replaced. Each prey density was replicated 10 times for each *C. caligata* larval instar. The Petri dishes with prey and predators were maintained at 27 ± 2°C, and 65 ± 5% relative humidity and 12 h scotophase.

4.2.3 Statistical analyses

The functional responses were estimated by determining the general shape of each functional response curve based on logistic regression of number of prey consumed as a function of mite stage and density using the CADMOD procedure of SAS statistical software (Institute et al., 2008). The cubic model was initially tested due to its capacity to detect the most possible functional response graph variations (Juliano, 2001), and a polynomial function was fitted.

$$\frac{N_e}{N_o} = \frac{exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{1 + exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)} \tag{4.1}$$

Where (N_e) is the number of attacked mites; (N_0) is the offered density; and P_0, P_1, P_2 and P_3 are the intercept, linear, quadratic and cubic coefficients, respectively, related to the slope of the curve. The signs of P_1 and P_2 are used to determine the type of functional response. When the linear coefficient is significantly negative $(P_1 < 0)$, the predator displays a type II functional response which indicates the proportion of prey consumed declines monotonically with the initial prey density. When the linear coefficient is significantly positive $(P_1 > 0)$, the predator presents a type III functional response (Juliano, 2001).

As our experiments were conducted with prey depletion, we used the random predator equation (Juliano, 2001; Rogers, 1972) to describe the functional responses of type II and III.

$$N_e = N_0\{1 - exp[\alpha(T_h N_e - T)]\} \tag{4.2}$$

$$N_e = N_0\{1 - exp[(d + bN_0)(T_h N_e - T)/(1 + cN_0)]\} \tag{4.3}$$

where N_e= number of prey attacked; T= exposure time (6 h); N_0= initial prey density; = attack rate, a constant rate of successful search; and T_h= handling time. The coefficients of , b, c and d are constants related to the attack rate. Subsequently, T_h (handling time) and (attack rate) parameters of the functional response were estimated with nonlinear least square regression using the PROC NLIN procedure of SAS (Institute et al., 2008) as described elsewhere

(Juliano, 2001). Differences in consumption among predator larval stages were determined by nonparametric Kruskal-Wallis test ($P < 0.05$).

4.3 Results

The functional response curves for individuals of the three *C. caligata* larval instars revealed that the number of eggs, immatures or adult females of *R. indica* consumed by *C. caligata* rapidly increased with increasing prey density (Fig. 4.1).

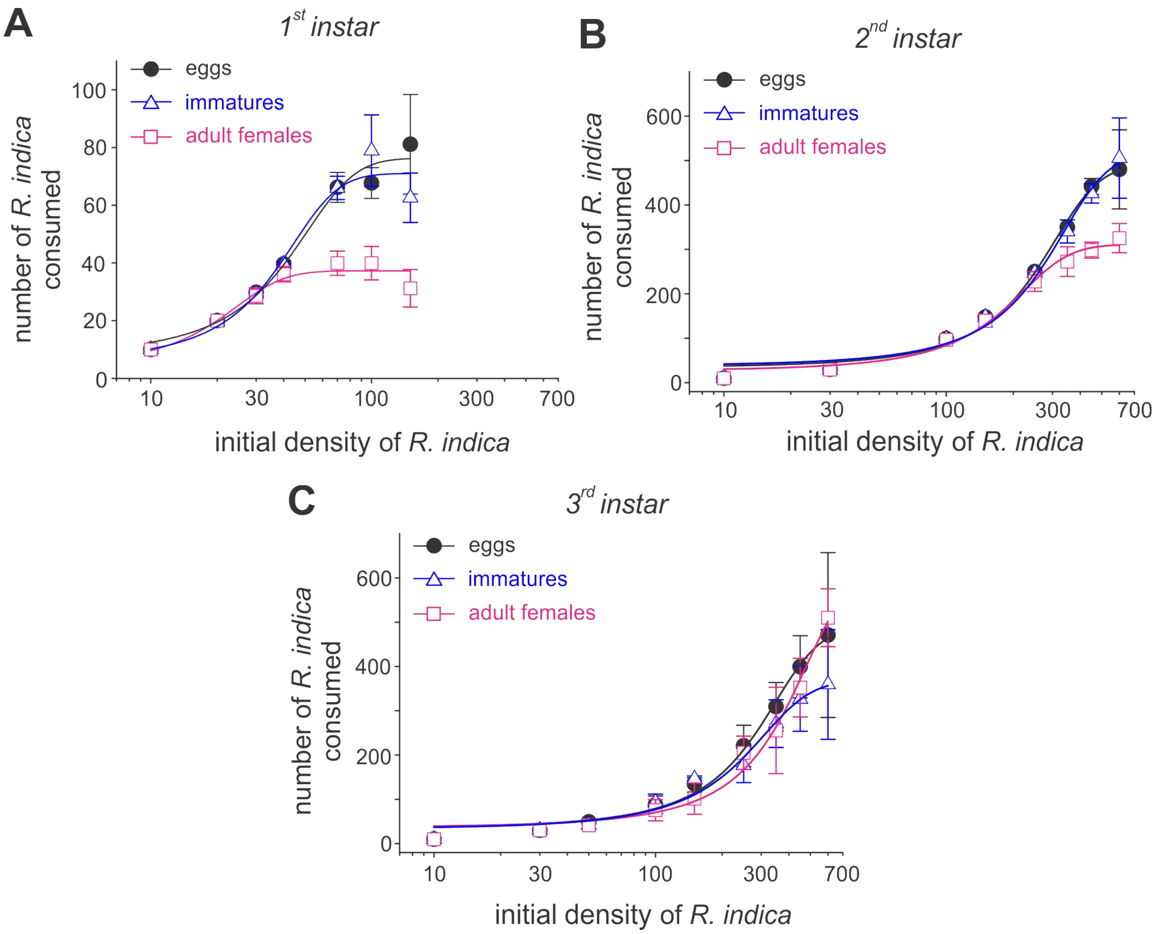

Figure 4.1: Mean numbers $(\pm SD)$ of eggs, immatures and females of *R. indica* consumed by first (A), second (B) and third (C) larval instars of the lacewing *Ceraeochrysa caligata* in relation to prey density over a period of 24 h.

Furthermore, whereas logistic regression yielded a significant $(P < 0.001)$ negative linear parameter (i.e., type II functional response) for individuals of the first and third instars of *C. caligata* feeding upon all prey stages (Table 4.1), second-instar individuals of *C. caligata* exhibited a type III functional response (i.e., a positive linear coefficient) when feeding upon eggs and immatures of *R. indica* but a type II functional response when preying upon adult females of *R. indica* (Table 4.1). A type II functional response was also evident in the proportion of prey consumed (N_e/N_o) by first-, second- and third-instar individuals of the predator (Fig. 4.2).

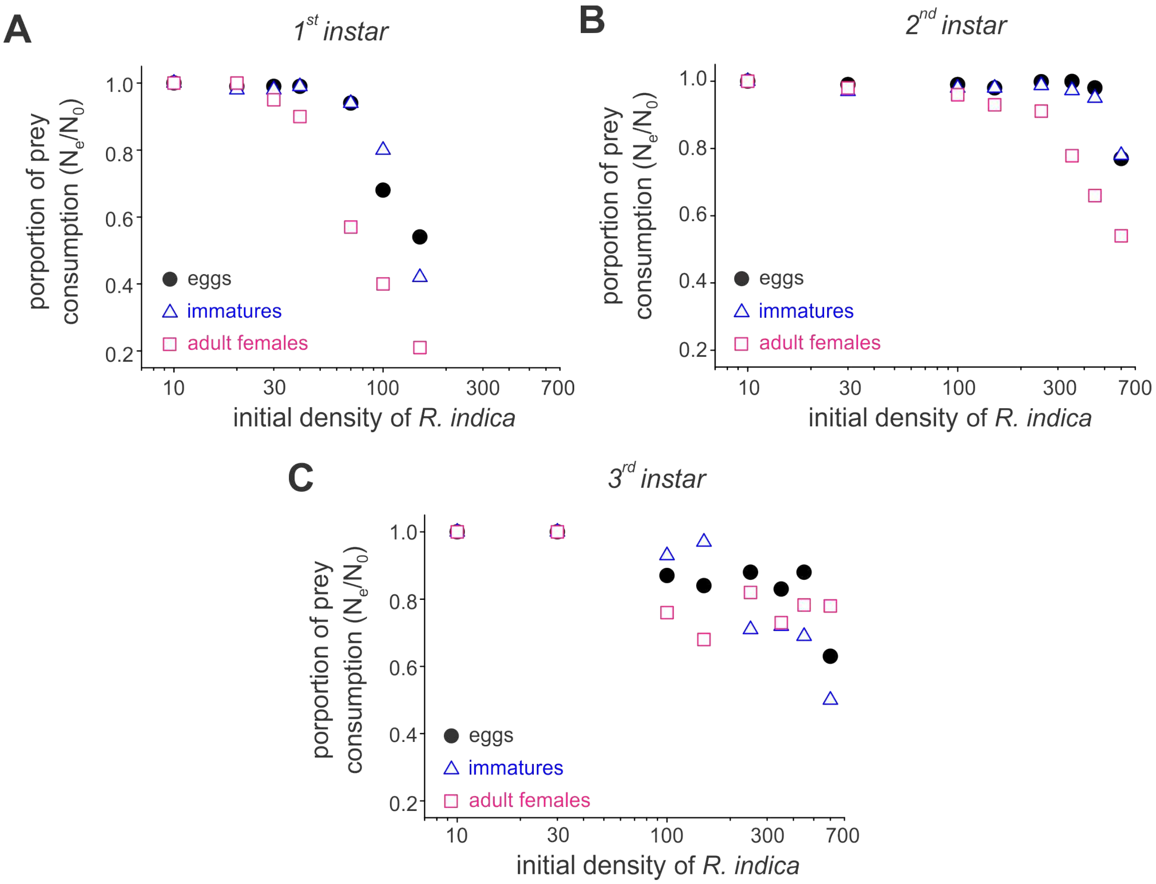

Figure 4.2: Proportions (N_e/N_o) of eggs, immatures and females of *Raoiella indica* consumed by first (A), second (B) and third (C) larval instars of *Ceraeochrysa caligata* according to prey density.

Table 4.1: Estimated parameters of the logistic regression of the proportion of eggs, immatures and females of Raoiella indica consumed by the first, second and third larval instars of *Ceraeochrysa caligata*

		Larval instar of *Ceraeochrysa caligata*											
		1^{st}				**2^{nd}**				**3^{rd}**			
prey stage	parameter	Estimate	SD	X^2	p	Estimate	SD	x^2	p	Estimate	SD	X^2	p
eggs	P_0	10.883	0.857	161.5	<0.0001	2.328	0.366	40.50	<0.0001	3.351	0.230	211.34	<0.0001
	P_1	-0.160	0.015	109.3	<0.0001	0.023	0.002	120.4	<0.0001	-0.015	0.002	34.33	<0.0001
	P_2	$6x10^{-4}$	$7x10^{-5}$	82.90	<0.0001	$-4.0x10^{-6}$	$2.0x10^{-7}$	260.4	<0.0001	$4.8x10^{-5}$	$7.7x10^{-6}$	38.99	<0.0001
	P_3	$6.8x10^{-6}$	$2.3x10^{-9}$	8.70	0.0033	$6.0x10^{-8}$	$2.9x10^{-8}$	4.10	0.0418	$-4.9x10^{-8}$	$7.1x10^{-9}$	48.30	<0.0001
immatures	P_0	5.729	0.505	128.7	<0.0001	-3.545	0.859	17.05	<0.0001	4.057	0.213	364.01	<0.0001
	P_1	-0.049	0.009	26.11	<0.0001	0.099	0.011	89.46	<0.0001	-0.024	0.002	123.88	<0.0001
	P_2	$6x10^{-5}$	$4.3x10^{-5}$	1.97	0.1605	$-3x10^{-4}$	$3.2x10^{-5}$	90.05	<0.0001	$6.3x10^{-5}$	$6.5x10^{-6}$	94.23	<0.0001
	P_3	$4.7x10^{-6}$	1.8x10-6	6.60	0.0102	$2.3x10^{-7}$	$2.8x10^{-8}$	81.69	<0.0001	$-5.6x10^{-8}$	$5.8x10^{-9}$	88.84	<0.0001
adult female	P_0	8.387	0.774	117.42	<0.0001	4.509	0.167	726.84	<0.0001	1.796	0.148	148.30	<0.0001
	P_1	-0.222	0.030	56.78	<0.0001	-0.012	8.1x10-4	216.74	<0.0001	-0.009	0.002	29.85	<0.0001
	P_2	0.002	0.001	31.87	<0.0001	$7x10^{-6}$	$9x10^{-8}$	70.60	<0.0001	2.7x10-5	$5.3x10^{-6}$	25.84	<0.0001
	P_3	$-5.9 X 10^{-6}$	$1.2x10^{-6}$	23.84	<0.0001	$2.9x10^{-9}$	$7.9x10^{-9}$	14.43	0.0001	$-2.2x10^{-8}$	$4.9x10^{-9}$	19.86	<0.0001

Negative and positive linear terms (P_1) denote type II and III functional responses, respectively.

The attack rate (α) of each *C. caligata* larval instar did not vary by prey type (Table 4.2). However, significant differences in the time spent by the predator handling the prey were observed among prey types (Table 4.2). Individuals of *C. caligata* first and second larval instars exhibited longer handling times (First instar: T_h= 0.146 ± 0.0050. Second instar: T_h= 0.018 ± 0.0004$)$ with adult females than with other types of *R. indica*. In contrast, *C. caligata* individuals of the third larval instar exhibited the shortest handling time with adult females $(T_h$= 0.010 ± 0.0021$)$ (Table 4.2). As shown in (Fig. 4.3) the average number of prey consumed independent of prey type generally increased with the developmental stage of the predator. Furthermore, predator individuals of the first (Fig. 4.3A, H= 22.2, df= 2, $P<$ 0.001) and second (Fig. 4.3B, H= 9.9, df= 2, P= 0.007) instars consumed significantly fewer adult females than eggs or immatures of *R. indica*. However, no significant differences among prey types were observed in the average number of prey consumed by *C. caligata* individuals of the third larval instar (Fig. 4.3C, H= 3.4, df= 2, P_4= 0.188).

Table 4.2: Attack rate (α) and handling time (T_h) of the first, second and third larval instars of *Ceraeochrysa caligata* feeding upon eggs, immatures and adult females of *Raoiella indica*.

Predator stage	**Prey stage**	**Parameter**						
		Attack rate	**Asymptotic 95% CI**		**Handling time**	**Asymptotic 95% CI**		R^2
		α	**Lower**	**Upper**	t_h	**Lower**	**Upper**	
1^{st}	Egg	0.032±0.0108 a	0.0107	0.0539	0.076±0.0025 b	0.071	0.0810	0.98
	Immature	0.050±0.0283 a	-0.0067	0.1067	0.082±0.0032 b	0.075	0.0879	0.94
	Adult Female	0.021±0.0023 a	0.0158	0.0252	0.146±0.0050 a	0.136	0.1561	0.82
2^{nd}	Egg	0.023±0.0366 a	-0.0499	0.0965	0.013±0.0007 a	0.011	0.0141	0.99
	Immature	0.006±0.0018 a	0.0020	0.0090	0.010±0.0005 a	0.009	0.0120	0.99
	Adult Female	0.005±0.0007 a	0.0034	0.0063	0.018±0.0004 a	0.017	0.0189	0.99
3^{rd}	Egg	0.003±0.0009 a	0.0011	0.0047	0.011±0.0011 a	0.009	0.0163	0.99
	Immature	0.003±0.0007 a	0.0013	0.0041	0.016±0.0010 a	0.013	0.0176	0.97
	Adult Female	0.001±0.0003 a	0.0006	0.0017	0.008±0.0016 a	0.005	0.0120	0.98

Values within columns followed by the same letter are not significantly different as determined by confidence interval $(CI \pm 95)$

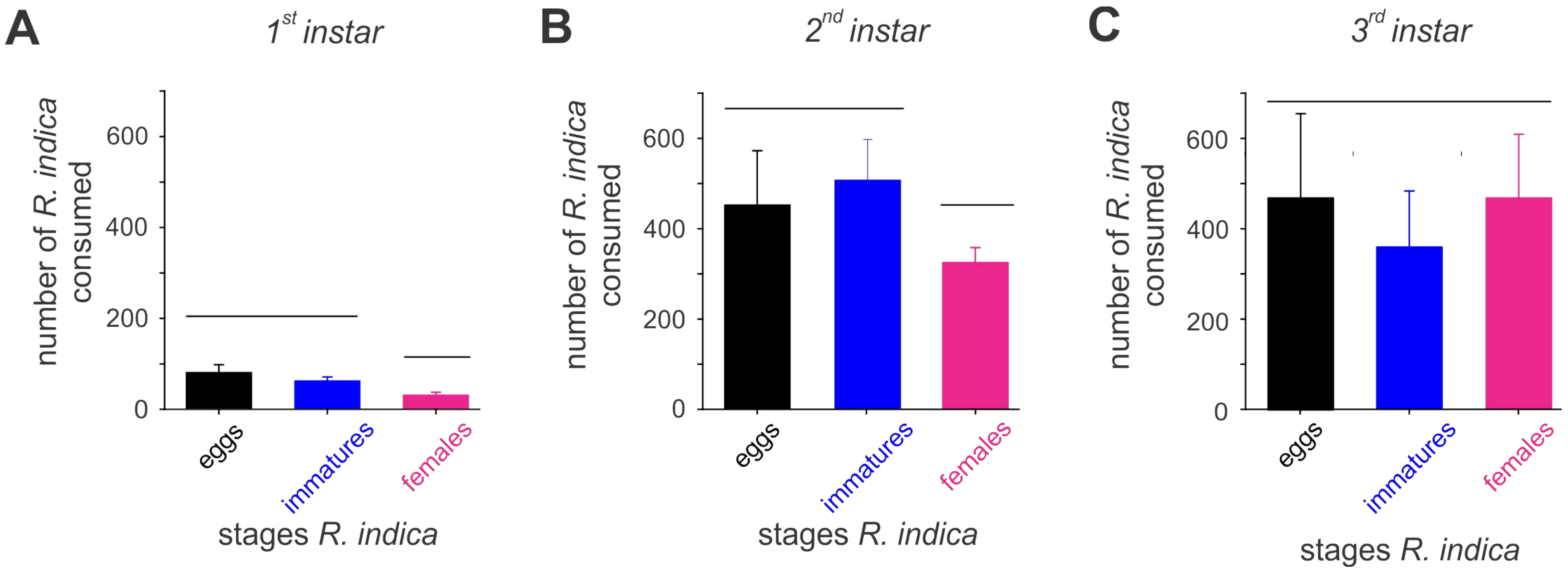

Figure 4.3: Average consumption (±*SD*) of eggs, immatures and females of *R. indica* by first (A), second (B) and third larval instars (C) of *Ceraeochrysa caligata*. Bars grouped under the same horizontal line do not differ according to a Kruskal-Wallis test $(P < 0.05)$.

4.4 Discussion

Functional responses are well-established parameters used to estimate the potential use of natural enemies as biological agents for controlling arthropod pests (Cuthbert et al., 2018; Ebrahimifar et al., 2017; Milonas et al., 2011; Solomon, 1949). Here, we demonstrated that all larval instars of the lacewing *C. caligata* are capable of preying upon all developmental stages (i.e., eggs, immatures and adults) of the red palm mite *R. indica*. Our findings revealed that although immature individuals of *C. caligata* exhibited mostly type II functional responses (which are highly relevant when prey densities are low), second-instar individuals of the predator might be more capable of suppressing *R. indica* populations, as they exhibited a type III functional response when preying upon eggs and immature individuals of *R. indica*.

Although three types of functional responses may be exhibited by predators (Holling, 1959); type II is the most common in insects (Begon et al., 1999), including chrysopids (Montoya-Alvarez et al., 2010; Parajulee et al., 2006; Stewart et al., 2002). The type II functional response is characterized by an increase in consumption rate with increasing prey availability until reaching a plateau, at which consumption rate stabilizes (Holling, 1959), as well as negative values estimated for the linear parameters (Juliano, 2001). This kind of functional response is generally limited only by handling time (i.e., the time required to subdue, consume, and digest the prey), which allows the predator to effectively control the prey population when prey density is low (Munyaneza and Obrycki, 1997; Santos, 1975). However, some mites and insect predators exhibit type III functional responses; in theory, such predators are more efficient than are those with type II responses in suppressing prey populations in biological control programs (Holling, 1966; Huffaker et al., 1971) as they exhibit positive density-dependent behaviors (Fernández-arhex and Corley, 2003; Pervez, 2005).

In the present investigation, as previously demonstrated for other lacewing species (Hassanpour et al., 2011, 2009; Sultan and Khan, 2014), immature-stage individuals of the lacewing *C. caligata* showed mostly type II functional responses. Second-instar *C. caligata* individuals preying upon eggs and immatures of *R. indica* showed type III responses.) Variation in functional response type can be partially explained by variation in the sizes and densities of both prey and predator (González-Suárez et al., 2011; Hassanpour et al., 2015, 2011; Hassell et al., 1977; Kabissa et al., 1996; Milonas et al., 2011; Sultan and Khan, 2014). Therefore, it is reasonable to argue that second-instar individuals of *C. caligata* would be more capable of suppressing *R. indica* populations than would *C. caligata* at other stages due to their greater control of this mite at the egg and immature stages. However, the capacity of *C. caligata* at other larval instars, for which type II functional responses were observed, should not be ignored, as first- and second-instar *C. caligata* can efficiently control red palm mite populations when these invasive pests are at low densities.

A valuable indicator of consumption rate and predator efficacy is handling time, which is defined as the cumulative time invested in capturing, killing and digesting prey (Veeravel and Baskaran, 1997). Attack rate is another relevant parameter when considering potential biological agents and indicates the predator's capture success, which is influenced by processes such as searching, detecting and encountering prey as well as by prey size (Aljetlawi et al., 2004; Ball et al., 2015; Holling, 1959). In this study, prey type did not affect the attack rates of individuals of each instar of *C. caligata*. However, the attack rate was higher for first-instar *C. caligata* individuals than for individuals of the other instars, which suggests that if *C. caligata* initially prefers to feed upon smaller sizes of *R. indica* (i.e., eggs and immature-stage individuals), this feeding preference is reversed as the predator developmentally advances. Prey size has been reported to influence the feeding preferences of other lacewings (Aqueel et al., 2014; Nordlund and Morrison, 1990). For instance, Nordlund and Morrison (1990) reported that individuals of the last larval instar of *Chrysoperla rufilabris* preferentially fed on larger prey when they were offered the choice between the caterpillar *Heliothis virescens* and the aphid *Aphis gossypii*.

Individuals of the second larval instar of *C. caligata* exhibited higher consumption of *R. indica* eggs and immatures than did those of the first larval instar, but prey consumption did not differ between the predator's second and third larval instars. Although some investigations reported no increase in prey consumption rate as the predator developed (Atlıhan et al., 2004; Chen and Liu, 2001; Fonseca et al., 2000; Huang and Enkegaard, 2010), our findings are in accordance with some previous results (Jose-Pablo et al., 2017; Sultan and Khan, 2014). The buccal apparatus of *C. caligata* is equipped with a sclerotized, elongate, acutely pointed and serrated jaw (McEwen et al., 2007) that can easily penetrate all developmental stages of *R. indica*. However, eggs and immatures of *R. indica* may have been easily preyed upon as such individuals are immobile (eggs) or exhibit low mobility relative to adult *R. indica* females.

Alternatively, the higher consumption of *R. indica* eggs and immatures than of adult *R. indica* by *C. caligata* may be related to differences in biomass and nutrient contents among the developmental stages of *R. indica*. As the eggs and immature-stage individuals of *R. indica* are of smaller biomass than are adults and may have different nutrient constitutions, *C. caligata* individuals of the second larval instar may had to increase their consumption of eggs and immatures to overcome these difficulties. Although not addressed here, the absence of differences in the feeding capacities of *C. caligata* between the second and third larval instars may have resulted from an absence of significant differences between these stages in predator voracity, the need for energy storage, or the ability to locomote or handle prey (Atlıhan et al., 2004; Bressendorff and Toft, 2011; Hassanpour et al., 2011; McEwen et al., 2007; Schmidt et al., 2012).

From a pest management perspective, the findings described in the present investigation indicate that *C. caligata* has potential as a biological agent to control all stages of the red palm mite, *R. indic*a: Individuals of the last larval instar of *C. caligata* can regulate low-density populations of red palm mites, and the high rates of consumption of *R. indica* eggs and immatures especially by this predator at the two first larval instars can prevent the emergence and reproduction of new *R. indica* individuals.

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Final considerations

Here was evaluated the performance of three-weevil species (i.e. *C. maculatus*, *A. obtectus* and *Z. subfasciatus*) in conditions of low, moderate and high densities of heterospecific competitors on two host (common bean and cowpea). Moreover, the potential use of essential oils of clove *S. aromaticum* L., and cinnamon, *C. zeylanicum* L. as an alternative to pyrethroid deltamethrin to control *A. obtectus* and *C. maculatus* also was evaluated. In both cases the costs and benefits of reproductive performance (oviposition, increase rate instantaneous) when exposed to sublethal doses also were measured.

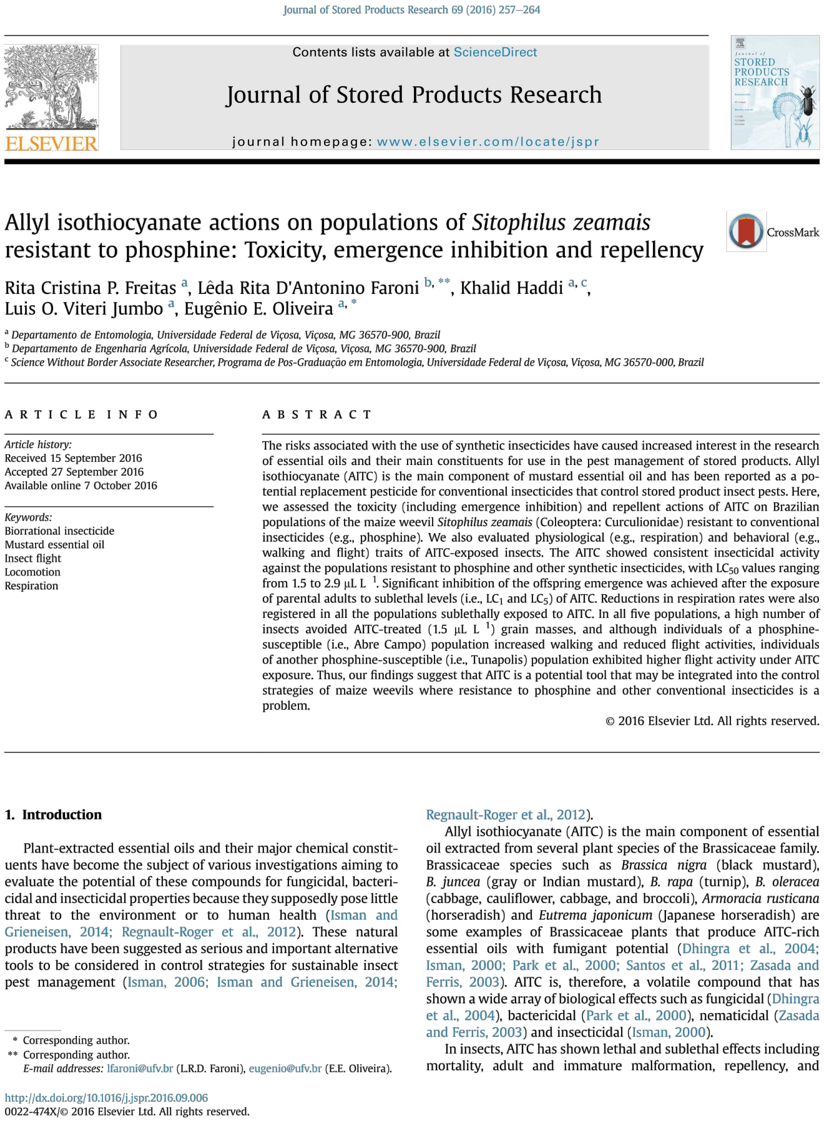
The weevil *C. maculatus* is dominant on its main and alternative host when compited whit *A. obtectus*; and could displace easy to its specie. While weevil *Z. subfasciatu*s could tolerate or have advantages competitives when the competitor *C. maculatus* is present in moderated densities.

Was also observed that susceptibility to insecticides and fitness of *A. obtectus* is dependent on the host; showing these weevil an effect trade-off on survival and reproduction rate. This is an important factor than must be considered when establish control strategies for this weevil.

Botanical compounds as essential oils of clove, *S. aromaticum* L., and cinnamon, *C. zeylanicum* L., exhibited insecticidal action to*C. maculatus*; reducing the descendents number when the seeds be treated and consequently the loss of mass of the grains. Further work is also needed to test the applicability and efficacy of nanofomulations of these essential oils under broader stored products conditions.

Lacewing *C. caligata* is a potential regulator od *R. indica*; the functional response as well as the capacity predatory of these predator varies with the prey and predator stages. Collectively, our findings demonstrate that all isntar of *C. caligata* has potential as a tool for the ecological management of the red palm mite.

Appendix



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Allyl isothiocyanate actions on populations of *Sitophilus zeamais* resistant to phosphine: Toxicity, emergence inhibition and repellency

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ABSTRACT

The risks associated with the use of synthetic insecticides have caused increased interest in the research of essential oils and their main constituents for use in the pest management of stored products. Allyl isothiocyanate (AITC) is the main component of mustard essential oil and has been reported as a potential replacement pesticide for conventional insecticides that control stored product insect pests. Here, we assessed the toxicity (including emergence inhibition) and repellent actions of AITC on Brazilian populations of the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) resistant to conventional insecticides (e.g., phosphine). We also evaluated physiological (e.g., respiration) and behavioral (e.g., walking and flight) traits of AITC-exposed insects. The AITC showed consistent insecticidal activity against the populations resistant to phosphine and other synthetic insecticides, with LC_{50} values ranging from 1.5 to 2.9 $\mu L\ L^{-1}$. Significant inhibition of the offspring emergence was achieved after the exposure of parental adults to sublethal levels (i.e., LC_1 and LC_5) of AITC. Reductions in respiration rates were also registered in all the populations sublethally exposed to AITC. In all five populations, a high number of insects avoided AITC-treated (1.5 $\mu L\ L^{-1}$) grain masses, and although individuals of a phosphine-susceptible (i.e., Abre Campo) population increased walking and reduced flight activities, individuals of another phosphine-susceptible (i.e., Tunapolis) population exhibited higher flight activity under AITC exposure. Thus, our findings suggest that AITC is a potential tool that may be integrated into the control strategies of maize weevils where resistance to phosphine and other conventional insecticides is a problem.

1. Introduction

Plant-extracted essential oils and their major chemical constituents have become the subject of various investigations aiming to evaluate the potential of these compounds for fungicidal, bactericidal and insecticidal properties because they supposedly pose little threat to the environment or to human health (Isman and Grieneisen, 2014; Regnault-Roger et al., 2012). These natural products have been suggested as serious and important alternative tools to be considered in control strategies for sustainable insect pest management (Isman, 2006; Isman and Grieneisen, 2014; Regnault-Roger et al., 2012).

Allyl isothiocyanate (AITC) is the main component of essential oil extracted from several plant species of the Brassicaceae family. Brassicaceae species such as *Brassica nigra* (black mustard), *B. juncea* (gray or Indian mustard), *B. rapa* (turnip), *B. oleracea* (cabbage, cauliflower, cabbage, and broccoli), *Armoracia rusticana* (horseradish) and *Eutrema japonicum* (Japanese horseradish) are some examples of Brassicaceae plants that produce AITC-rich essential oils with fumigant potential (Dhingra et al., 2004; Isman, 2000; Park et al., 2000; Santos et al., 2011; Zasada and Ferris, 2003). AITC is, therefore, a volatile compound that has shown a wide array of biological effects such as fungicidal (Dhingra et al., 2004), bactericidal (Park et al., 2000), nematicidal (Zasada and Ferris, 2003) and insecticidal (Isman, 2000).

In insects, AITC has shown lethal and sublethal effects including mortality, adult and immature malformation, repellency, and

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ORIGINAL PAPER

Toxicity and metabolic mechanisms underlying the insecticidal activity of parsley essential oil on bean weevil, *Callosobruchus maculatus*

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Abstract Control of the bean weevil, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), in stored cowpea beans, *Vigna unguiculata* (L) Walp., when accomplished, is mainly achieved by applications of phosphine. However, the long-term use of this insect control practice has contributed to the selection of resistant populations, enhancing the need to develop alternative control tools. Due to their diverse biologically active compounds, the essential oils of aromatic plants such as parsley, *Petroselinum sativum* L., have been suggested as suitable alternatives for controlling insect pests. Here, we evaluated the toxicity (including the metabolic mechanisms underlying the insecticidal activity) of parsley essential oil against *C. maculatus*. Parsley essential oil controlled *C. maculatus* in a concentration-dependent manner (concentration increases resulted in lower weight losses and better germination levels of beans) with a fumigant toxicity (LC_{50}: 489.5 μL L^{-1} air) significantly lower than that observed for phosphine (LC_{50}: 35.7 μL L^{-1} air). However, when applied at their LC_{10} values (*Parsley essential oil*: 399.3 μL L^{-1} air. *Phosphine*: 18.6 μL L^{-1} air), the parsley essential oil caused significantly higher reductions in the emergence of *C. maculatus* compared with phosphine. Additionally, application of the synergists piperonyl butoxide (PBO), triphenyl phosphate (TPP) and diethyl maleate (DEM) demonstrated that glutathione S-transferases enhanced the parsley essential oil toxicity, while cytochrome P450-dependent monooxygenases formed part of the resistance mechanisms used by *C. maculatus* to mitigate the toxicity of the essential oil. Our findings suggest that parsley essential oil may be integrated into the control of *C. maculatus* only when synergized with PBO.

Keywords Botanical insecticides · *Petroselinum sativum* · Reproduction responses · Piperonyl butoxide · Triphenyl phosphate · Diethyl maleate

Key message

- Parsley essential oil controlled *C. maculatus* in a concentration-dependent manner.
- Despite its lower fumigant toxicity (LC_{50}: 489.5 μL L^{-1} - air) comparatively to phosphine (LC_{50}: 35.7 μL L^{-1} air), the parsley essential oil caused significantly higher reductions in the emergence of *C. maculatus* compared with phosphine.
- The enzymes glutathione S-transferases enhanced the essential oil toxicity.
- The enzymes cytochrome P450-dependent monooxygenases formed part of the resistance mechanisms used by *C. maculatus* to mitigate the essential oil actions.

Introduction

The bean weevil *Callosobruchus maculatus* (F.) is a cosmopolitan field-to-store pest that is capable of severely damaging the seeds of cowpea beans, *Vigna unguiculata* Walp.,

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Entomologia Experimentalis et Applicata

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Progeny of the maize weevil, *Sitophilus zeamais*, is affected by parental exposure to clove and cinnamon essential oils

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Key words: transgenerational effects, botanical insecticides, insecticide-induced hormesis, *Syzygium aromaticum*, *Cinnamomum verum*, sublethal effects, Coleoptera, Curculionidae, Lauraceae, Myrtaceae

Abstract

The interest in and utilization of botanical insecticides, particularly essential oils, has become increasingly relevant to the control of insect pests. However, the potential ecotoxicological risks or flaws (including sublethal effects on the targeted pest generation and its subsequent progeny) of this pest control tool have been neglected frequently. Here, we evaluated the effects of sublethal exposure to clove, *Syzygium aromaticum* (L.) Merrill & Perry (Myrtaceae), and cinnamon, *Cinnamomum verum* J. Presl (Lauraceae), essential oils on adult (F0) maize weevils, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), and the physiology (e.g., body mass, respirometry, and grain consumption) and population dynamics (e.g., daily emergence and sex ratio) of their progeny. Longevities of the parents were negatively affected by the essential oils in a concentration-dependent manner. Parental sublethal exposure to clove oil (0.17 µl cm^{2}) accelerated offspring emergence but delayed the emergence of females compared to males. Parents that were sublethally exposed to clove (0.17 µl cm^{2}) or cinnamon (0.35 µl cm^{2}) essential oils produced heavier offspring. Parental sublethal exposure to cinnamon essential oil accelerated offspring emergence (at 0.70 µl cm^{2}), delayed female emergence (at 0.17 µl cm^{2}), and enhanced grain consumption (at 0.35 and 0.70 µl cm^{2}) of the progeny. Thus, our findings indicate that sublethal exposure to clove and cinnamon essential oils is capable of promoting transgenerational effects in *S. zeamais* that can negatively impact the control efficacy of such products.

Introduction

Pesticides of any nature (i.e., synthetic or natural products) are potential sources of environmental stress that can not only influence the target organisms but also the performance of their progeny. In addition to genetically based changes, parental exposure to pesticides may alter progeny performance through transgenerational, non-genetic effects (Bonduriansky & Day, 2008; Gressel, 2011; Manikkam et al., 2012; Collotta et al., 2013; Cutler, 2013). Such transgenerational effects mediated by sublethal exposure to synthetic insecticides have been reported in many arthropods (Cutler et al., 2005; Zalizniak & Nugegoda, 2006; Vogt et al., 2007; Yin et al., 2009; Suhett et al., 2011; Campos et al., 2012; Liang et al., 2012; Ayyanath et al., 2013; Guo et al., 2013). The consequences of such transgenerational effects may lead to failures in the management of key agricultural pests or to the resurgence/outbreaks of secondary pests (Desneux et al., 2007; He et al., 2013; Guedes & Cutler, 2014; Qu et al., 2015; Santos et al., 2016a,b).

Although botanical insecticides, particularly essential oils, have been extensively investigated and proposed as a suitable alternative for controlling insect pests (Isman, 2006; Bakkali et al., 2008; Regnault-Roger et al., 2012; Isman & Grieneisen, 2014), the vast majority of plant essential oil investigations have focused on their repellence

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ORIGINAL PAPER

Changes in the insecticide susceptibility and physiological trade-offs associated with a host change in the bean weevil *Acanthoscelides obtectus*

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Abstract For most of the seed weevils (Coleoptera: Chrysomelidae: Bruchinae), the ability to survive environmental stresses like insecticide exposure and reproduction is variable and depends on the host. Here, we evaluated the physiological costs and benefits of a host shift from kidney beans of the landrace "Vermelho" to cranberry beans of the landrace "Manteigão" in the bean weevil *Acanthoscelides obtectus*. We assessed the susceptibility of *A. obtectus* to the commercial pyrethroid deltamethrin and clove and cinnamon essential oils as potential alternative insecticides and measured its reproductive performance (e.g., oviposition and emergence rates) on both hosts. The females of *A. obtectus* reared on kidney beans were less susceptible to both deltamethrin and clove essential oil and showed more sources of energy in the trophocytes although the attraction of adult females to both hosts was similar. However, the females reared on cranberry beans had higher reproductive performances and respiration rates, indicating that more energy was expended on reproduction than on the mitigation of the insecticide actions. Thus, with a change in hosts, a trade-off between reproductive fitness and the susceptibility to insecticides was demonstrated for *A. obtectus*. These results should not only affect the management of *A. obtectus* in storage units but also the understanding of these insects' host adaptativeness.

Keywords Bruchids · Host range · Adaptation costs · Trophocyte area · Botanical insecticides · Respiratory rates

Key messages

- *Acanthoscelides obtectus* reared on cranberry beans exhibited higher susceptibility to synthetic and botanical insecticides and smaller trophocytes areas.
- Host change seems to affect the energy investment between the contrasting processes of reproduction and protection against insecticides in *A. obtectus.*
- Host type affected the oviposition and emergence rates as well as the respiratory activity of the bean weevil *Acanthoscelides obtectus.*

Introduction

Herbivorous insects generally specialize on one or a few closely related hosts, and, for most of these insects, the use of a specific host and the detoxification of secondary metabolites may be negatively correlated with fitness in new environments (Gompert et al. 2015; Jaenike 1990; Messina 2004; Scheirs et al. 2005). The seed weevils in the coleopteran subfamily Bruchinae easily adjust to new nutritional or toxic environments (Sales et al. 2000), which

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General Entomology/Entomologia Geral

Dinâmica populacional e parasitismo natural de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) em pomares de citros em Sergipe

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Resumo. O psilídeo, *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Liviidae), por ser vetor da bactéria causadora do Hunglongbing (HLB), tornou-se uma praga-chave dos citros no Brasil. Além dos citros, a planta ornamental conhecida como murta-de-cheiro, *Murraya paniculata* (L.) Jack, também é hospedeira do psilídeo. O presente trabalho teve como objetivo estudar a dinâmica populacional do psilídeo em pomares de citros e o seu parasitismo em citros e murta-de-cheiro no estado de Sergipe, o quinto maior produtor de citros do Brasil. As avaliações foram realizadas quinzenalmente durante onze meses em sete pomares de laranjeira Pera *Citrus sinensis* (L.) Osbeck localizados em dois municípios (Boquim, e Umbaúba). As populações de ovos, ninfas e adultos de *D. citri* foram comparadas entre todos os pomares e todas as fases de vida foram relacionadas com os fatores abióticos, temperatura, umidade relativa e precipitação de cada localidade. O psilídeo *D. citri* foi classificado como espécie acessória (pouco abundante) nos pomares de Sergipe, apresentando maior densidade populacional nos meses de novembro, dezembro e março. A precipitação foi o único fator abiótico que contribuiu para o aumento populacional de adultos do psilídeo. Altas taxas de parasitismo (55 %) de ninfas do psilídeo pelo parasitoide exótico *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) foram detectadas em plantas de murta-de-cheiro. Com base nos resultados, caso a bactéria seja detectada em Sergipe, para o manejo do vetor, amostragens de menor intervalo deverão ser realizadas nos meses da primavera e verão. Adicionalmente, o parasitoide *T. radiata* poderia ser liberado inundativamente em programas de manejo integrado do vetor.

Palavras-chave: Ecologia; Fatores abióticos; Monitoramento populacional; Psilídeo-dos-citros; *Tamarixia radiata*.

Population dynamics and natural parasitism of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) on citrus orchards in Sergipe state

Abstract. The Asian citrus *psyllid, Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Liviidae), which is the vector of the Hunglongbing (HLB) bacterium, has become a key citrus pest in Brazil. In addition to citrus, the ornamental plant known as Orange Jasmine, *Murraya paniculata* (L.) Jack, also hosts the psyllid. The present work aimed at studying the population dynamics of the psyllid in citrus orchards and its parasitism in citrus and *M. paniculata* in the state of Sergipe, the fifth largest citrus-producing state in Brazil. The evaluations were performed fortnightly for eleven months in seven Pera *Citrus sinensis* (L.) Osbeck orange orchards located in two municipalities of Sergipe state (Boquim, and Umbauba). The populations of eggs, nymphs and adults of *D. citri* were compared among all orchards and all developmental stages were related to the abiotic factors temperature, relative humidity and precipitation, in each locality. The psyllid was classified as an accessory species (not very abundant) in the orchards of Sergipe, showing a higher population density in November, December and March. Precipitation was the only abiotic factor that contributed to the population increase of adults of the psyllid. High rates of parasitism (55%) of psyllid nymphs by the exotic parasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) were detected in *M. paniculata* plants. Based on the results, if the HLB bacterium is detected in Sergipe, shorter samplings should be performed in the spring and summer months aiming at vector management. In addition, *T. radiata* could be released inundatively in integrated vector management programs.

Keywords: Abiotic factors; Asian citrus psyllid; Ecology; Population monitoring; *Tamarixia radiata*.

O psilídeo-dos-citros, *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Liviidae), foi registrado no Brasil em 1942 (C L 1942) e até 2004 era considerado uma praga secundária, causando danos diretos somente em alta densidade populacional (G LL *et al.* 2002). Após o registro do Huanglongbing (HLB) em 2004, o psilídeo tornou-se a principal praga dos citros em São Paulo, por ser vetor da bactéria causadora dessa doença, que se encontra disseminada nas regiões produtoras desse estado (B 2006; B L *et al.* 2010).

O HLB, conhecido anteriormente por *greening*, é a mais importante e destrutiva doença da citricultura mundial e é causado por uma bactéria que habita o floema da planta hospedeira (B 2006; G L G C & B

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Diversity and convergence of mechanisms involved in pyrethroid resistance in the stored grain weevils, *Sitophilus* spp.

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Target-site mutations and changes in insect metabolism or behavior are common mechanisms in insecticide-resistant insects. The co-occurrence of such mechanisms in a pest strain is a prominent threat to their management, particularly when alternative compounds are scarce. Pyrethroid resistance among stored grain weevils (i.e., *Sitophilus* spp.) is an example of a long-standing concern, for which reports of resistance generally focus on a single mechanism in a single species. Here, we investigated pyrethroid resistance in maize and rice weevils (i.e., *Sitophilus zeamais* and *S. oryzae*), exploring potential knockdown resistance (*kdr*) mutations in their sodium channels (primary site for pyrethroid actions) and potential changes in their detoxification and walking processes. Resistance in pyrethroid-resistant rice weevils was associated with the combination of a *kdr* mutation (L1014F) and increases in walking and detoxification activities, while another *kdr* mutation (T929I) combined with increases in walking activity were the primary pyrethroid resistance mechanisms in maize weevils. Our results suggest that the selection of pyrethroid-resistant individuals in these weevil species may result from multiple and differential mechanisms because the L1014F mutation was only detected in Latin American rice weevils (e.g., Brazil, Argentina and Uruguay), not in Australian and Turkish rice weevils or Brazilian maize weevils.

The overuse of dichlorodiphenyltrichloroethane (i.e., DDT) up to the 1980's and more recently of other synthetic insecticides (e.g., pyrethroids) for controlling stored product insect pests has contributed to the selection of insecticide-resistant strains, leading to severe economic losses in storage facilities worldwide. Regarding the pyrethroid insecticides, the resistance management is complicated because resistance occurs in a variety of forms, including reduced insecticide penetration, metabolic resistance (through detoxification enzymes), behavioral resistance and target-site alterations[2] . Although the pyrethroid insecticides exert their toxicity primarily by disrupting the function of the voltage-gated sodium channels in excitable cells[3–8], these compounds also have secondary action targets (e.g., ionic imbalance and osmoregulatory dysfunction) that contribute to their activity[91] .

Multiple and distinct pyrethroid resistance mechanisms have been investigated in toxicological studies with focus on the contribution of the major mechanism, which includes target-site mutations (known as knockdown (*kdr*) resistance) and/or metabolic-based resistance[12 17]. The co-occurrence of distinct and multiple pyrethroid resistance mechanisms threatens resistance management strategies, with the threat particularly acute when alternative compounds are scarce, as is the case with stored grain weevils. Thus, it is essential to evaluate the potential of other classes of insecticides such as neonicotinoids, oxidiazines and spinosyns to control resistance populations of stored grain weevils.

Most of the losses in stored grains are caused by insect pests among which the grain weevils of the genus *Sitophilus* (e.g., the maize weevil *Sitophilus zeamais* Motsch. and the rice weevil *Sitophilus oryzae* L.) are

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PLOS | ONE

RESEARCH ARTICLE

Toxicity to, oviposition and population growth impairments of *Callosobruchus maculatus* exposed to clove and cinnamon essential oils

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Abstract

The use of plant essential oils has been shown to efficiently control insect pests of stored beans, significantly reducing the threats associated with synthetic insecticides. Here, we evaluated the potential of applications of essential oils of clove, *Syzygium aromaticum* L., and cinnamon, *Cinnamomum zeylanicum* L., to control *Callosobruchus maculatus*, considered as one of the most cosmopolitan pests of stored beans. Using four combinations of couples (i.e., unexposed couples, exposed females, exposed males, and exposed couples), we also evaluated how sublethal exposure to these essential oils impacted *C. maculatus* oviposition. Bioassays results revealed that both essential oils exhibited insecticidal activities similar to the synthetic pyrethroid insecticide deltamethrin. Furthermore, oil dosage increments proportionately decreased the growth rate and reduced the losses in bean weight caused by cowpea weevils, and offspring emergence was almost abolished when parents were exposed to the LD_{20} of each essential oil. Finally, significant oviposition impairments were perceived only in couples where females were exposed (i.e., females exposed and exposed couples) to the LD_{20} of cinnamon and clove essential oils. Thus, by exhibiting similar insecticidal activities as synthetic insecticides and by significantly affecting the oviposition of sublethally exposed *C. maculatus* females, the cinnamon and clove essential oils represent valuable tools with potential of integration into the management of *C. maculatus* infestations.

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Introduction

Plant essential oils have gained a reputation as being potentially bioactive compounds against many insect species, which has portrayed them as safer tools in terms of the environment and human health [16]. Despite the potential of essential oils to control pests of stored products, few studies have addressed the physiological and biological responses of stored product pests when the exposure occurred at sublethal levels.

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Artículo Original

Bioactividad de aceites vegetales a *Orthezia praelonga* (Hemiptera: Sternorrhyncha: Orthezidae) y selectividad a su predador *Ceraeochrysa caligata* (Neuroptera: Chrysopidae)

Bioactivity of vegetable oils to *Orthezia praelonga* (Hemiptera: Sternorrhyncha: Orthezidae) and selectivity to its predator *Ceraeochrysa caligata* (Neuroptera: Chrysopidae)

http://opn.to/a/07XD3

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RESUMEN: Los aceites vegetales abundan en plantas oleaginosas y pueden ser una alternativa para el control de plagas en sistemas de manejo integrado. Los ácidos grasos presentes en estos aceites muestran bioactividad a plagas y pueden ser una alternativa al uso de insecticidas recomendados para plantaciones de cítricos. Aunque estos aceites son de origen natural, el impacto en especies benéficas debe ser extensamente evaluado. Este estudio fue conducido a fin de evaluar la toxicidad y la repelencia de aceites crudos de algodón, dendé, soja desgomado y coco a una plaga clave de cítricos, *Orthezia praelonga* (Hemiptera: Sternorrhyncha: Ortheziidae), y su selectividad al predador *Ceraeochrysa caligata* (Neuroptera: Chrysopidae). Se realizaron bioensayos de concentración-mortalidad en condiciones de laboratorio y se estimaron concentraciones letales de los aceites sobre hembras adultas de *O. praelonga*. Adicionalmente, se evaluó el efecto repelente de cada aceite a la CL_{50} y CL_{80} después de 1, 24 y 48 h. Los cuatro aceites vegetales presentaron toxicidad a *O. praelonga*: algodón CL_{50}=1,92 µl/ml, dendé CL_{50}=2,54 µl/ml, soja CL_{50}=3,18 µl/ml y coco CL_{50}=5,02 µl/ml; su selectividad a *C. caligata* fue verificada (CLs_{50} >80 % de vivos). Se demostró el efecto repelente del aceite de algodón (CL_{50}=1,92 µl/ml; CL_{80}=5,99 µl/ml) y dendé (CL_{80}=10,22 µl/ml) con una hora de exposición. Contrariamente, el aceite de soja desgomado (CL_{80}=12,50 µl/ml) repelió a *O. praelonga* con 24 y 48 horas después de haber sido expuesta. Los resultados de toxicidad, repelencia y selectividad muestran al aceite vegetal de algodón, dendé y soja desgomado como los más adecuados para el control de *O. praleonga*.

Palabras clave: ácidos grasos, *Ceraeochrysa caligata*, cochinilla, toxicidad, repelencia.

ABSTRACT: Vegetable oils are abundant in oleaginous plants and they can be an alternative for the pest control in integrated management systems. Fatty acids present in these oils are bioactive to pests and they can be an alternative to the use of insecticides suggested to crops of citrus. Although these oils are of natural origin, the impact on beneficial species must be widely evaluated. Here, we evaluated the toxicity and repellency of crude oils of cotton, palm, soybean and coconut to a key citrus pest, *Orthezia praelonga* (Hemiptera: Sternorrhyncha: Ortheziidae), and its selectivity to *Ceraeochrysa caligata* (Neuroptera: Chrysopidade). Bioassays of concentration-mortality were performed under laboratory conditions and lethal concentrations of oils were estimated on *O. praelonga* adult females were estimated. Additionally, the repellent effect of each oil to the LC_{50} and LC_{80} was evaluated after 1.24, and 48 h. The four vegetable oils showed toxicity to *O. praelonga*: cotton LC_{50} = 1.92 µl/ml, palm oil LC_{50} = 2.54 µl/ml; soybean LC_{50} = 3.18 µl/ml and coconut LC_{50} = 5.02 µl/ml; and its selectivity to *C. caligata* was verified (CLs_{50} >80 % alive). Repellent effects of cotton oil (LC_{50} = 1.92 µl/ml, LC_{80} = 5.99 µl/ml), and palm oil (LC_{80} = 10.22 µl/ml) were demonstrated for one hour of exposure. In contrast, the soybean oil (LC_{80} = 12.50 µl/ml) repelled *O. praelonga* after 24 and 48 h of exposure. Results of toxicity, repellence and selectivity show the cotton, palm, and soybean oils as the most suitable for the control of *O. praleonga*.

Key words: coccid, fatty acids, toxicity, repellence, *Ceraeochrysa caligata*.

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Break Throught Plus	121,00	1	121,00
Editoracão artigo	821,00	1	821,00
material repele insetos	19,40	1	19,40
Material didáctico	564,70	-	564,65
Material didactico	332,00	-	332,00
Software figuras CoreDraw	600,00	1	600,00
Feijão vermelho (12 unidades)	4,60	12	55,08
Cola silicone (2)	12,50	8	100,00
Feijão cowpea (18 unidades)	2,00	25	49,75
Total			**2662,88**
Ano 2			
Balança digital	104,00	1	104,00
Material didactico	495,00	-	495,00
Contador estatistico analógico	49,90	-	49,90
Congresso cientifico CBE (2016)	1064,00	1	1064,00
Notebook pessoal	1700,00	-	1700,00
Total			**3412,9**
Ano 3			
Material didactico	82,00	-	82,00
Material didactico	92,00	-	92,00
Ovos de Anagasta	304,30	-	304,30
Ovos de Anagasta	304,30	-	304,30
Ovos de Anagasta	220,00	-	220,00
Ovos de Anagasta	356,00	-	356,00
Ovos de Anagasta	315,00	-	315,00
Estagio EMBRAPA (passages: BH-SE-BH)	1100,00	-	1100,00
Total			**2773,6**

Continua..................

Ano 4			
Rubro	**Valor**	**Quantidade**	**(R$)**
Placas petri	37,52	-	37,52
Editoracão de artigo Scientific Reports	908,78	-	908,78
Publicação de artigo Scientific Reports	7190,00		7190,00
Publicação de artigo plos One	6125,00	-	6125,00
Sementes cevada (pulgões)	55,00	-	55,00
Sementes cevada (pulgõoes)	88,90	-	88,90
Material didactico	28,00	-	28,00
Material didactico	175,00	-	175,00
Material didactico	65,00	-	65,00
Micropipetas	3203,97	-	3203,97
Material didactico	870,00	-	870,00
Ovos de Anagasta	540,00	-	540,00
Sementes de cevada	88,90	-	88,90
Agitador magnetico	25,00	-	25,00
Agitador magnetico	50,00	-	50,00
Editoração artigo Biological control/PeerJ	887,25	-	887,25
Sementes cevada (pulgões)	27,00	-	27,00
Material miniestufa criação	41,80	-	41,80
Material miniestufa criação	66,00	-	66,00
Placas petri (10 paquetes)	59,50	-	59,50
Placas petri (10 paquetes)	59,50	-	59,50
Plastico micro-estufa	150,00	-	150,00
Congress Scientific CBE (2018)	1356,00	-	1356,00
Ovos de Anagasta	210,00	-	210,00
Material cubre estufa criação	97,00	-	97,00
Publicação de artigo PeerJ	5625,00	-	5625,00
Valor título Doutor entomologia	95,00		95,00
Impressão teses	200,00		200,00
Total			**28325,12**
Outros	**1000,00**		**1000,00**
Ano 1 + Ano 2 + Ano 3 + Ano 4			**38174,50**