KARINA SOLEDAD VILCA MALLQUI

COMPETIÇÃO E SUCESSO REPRODUTIVO DE CARUNCHOS DO FEIJÃO

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

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RESUMO

VILCA MALLQUI, Karina Soledad, D. Sc., Universidade Federal de Viçosa, agosto de 2013. C**ompetição e sucesso reprodutivo de carunchos do feijão.** Orientador: Raul Narciso Carvalho Guedes. Co-orientadores: Eugênio Eduardo de Oliveira, Nelsa Maria Pinho Guedes e Lessando Moreira Gontijo.

Os besouros da subfamília Bruchinae (Coleoptera: Chrysomelidae), conhecidos comumente como bruquínios, são insetos especialistas em alimentar-se da parte interna das sementes de leguminosas. Dentre estes *Acanthoscelides obtectus* (Say) e *Zabrotes subfasciatus* (Boheman) são considerados como principais pragas de feijão armazenado, ocorrendo em todas as regiões do planeta onde se armazena esta leguminosa e frequentemente co-ocorrem. No Brasil as perdas de grãos causadas por *Z. subfasciatus* variam de 7 a 40% , enquanto *A. obtectus* ocasiona perdas de 20 a 30% da produção total de feijão no país. Apesar de ambas as espécies serem pragas conhecidas e importantes, o impacto de sua co-ocorrência no desenvolvimento e colonização não foi pesquisado. Embora ambas as espécies apresentem variação semelhante de desenvolvimento, tamanho e razão sexual (1:1), as fêmeas de *A. obtectus* colocam seus ovos livremente e o primeiro instar larval penetra no grão de feijão, enquanto as fêmeas de *Z. subfasciatus* grudam seus ovos nos grãos e a larva penetra no feijão de dentro do ovo, sem exposição externa. Foram realizados experimentos de competição direita entre ambas as espécies usando uma série de aditiva, esperando co-existência sem limitação de alimentos, como ocorre geralmente em unidades de armazenamento de grãos. A não influência ou até efeito positivo de uma espécie sobre a outra era uma expectativa mais provável. A capacidade de competição de cada espécie na presença do seu competidor heterospecífico foi avaliada utilizando infecções mistas (adultos) em feijão. Os resultados indicam que as espécies co-occurrendo não apresentam interação negativa, e a presença de *A. obtectus* pode até favorecer *Z. subfasciatus*. O uso de inseticidas botânicos tem merecido destaque no controle de pragas e, entre eles, o nim, *Azadirachta indica*, tem sido testado em diferentes formulações incluindo extrato aquoso, óleo, pó de sementes e inseticidas comerciais baseados. Contudo são poucos os estudos realizados sobre os efeitos de inseticidas botânicos na reprodução de espécies pragas de grãos armazenados. Neste estudo, exploramos a hipótese de que a azadiractina, inseticida botânico obtido de extratos de nim, pode impactar negativamente a capacidade reprodutiva de *Z. subfasciatus*. Além disto investigamos se os adultos podem compensar efeitos subletais ajustando a alocação de seus esforços reprodutivos. Os resultados mostraram que as fêmeas de *Z. subfasciatus* aumentam sua fecundidade diária para compensar a diminuição da longevidade induzida pela azadiractina. Além disso, a matriz estruturada por fases revelou que as populações de *Z. subfasciatus* engendradas a partir de fêmeas expostas a azadiractina apresentaram uma maior taxa de crescimento populacional (r) e uma taxa líquida de reprodução superior (R_0) e finalmente, a análise da matriz de projeção mostrou densidades notavelmente mais elevados ao longo das gerações para populações de *Z. subfasciatus* expostas a azadiractina.

ABSTRACT

VILCA MALLQUI, Karina Soledad, D. Sc., Universidade Federal de Viçosa, August, 2013. **Competition and reproductive success in bean beetles.** Advisor: Raul Narciso Carvalho Guedes. Co-advisors: Eugênio Eduardo de Oliveira, Nelsa Maria Pinho Guedes and Lessando Moreira Gontijo.

Beeltes of the subfamily Bruchinae (Coleoptera: Chrysomelidae), also commonly known as bruchids, are insects specialized in feeding on the inner part of legume seeds. Among them, *Acanthoscelides obtectus* (Say) e *Zabrotes subfasciatus* (Boheman) are considered the main bests of stored common beans occurring in all regions were this legume is cultivated and they frequently co-occur. In Brazil, the losses caused by *Z. subfasciatus* range from 7 to 40%, while losses caused by *A. obtectus* range between 20 and 30% of the total common bean production in the country. Despite of well-known and of recognized importance of both bruchid species, the impact of their co-occurrence in their mutual development and colonization was not investigated. Although both species exhibit similar development, size and sex ratio (1:1), the females of *A. obtectus* lay their eggs loosely and the 1st instar larva burrows into the bean kernek, while females of *Z. subfasciatus* glue their eggs in the surface of the bean kernel and the larva burrows into the kernel from within the egg without external exposure. Direct competition experiments were carried out with both species using an additive series expecting a co-occurrence without food limitation as usually takes place in grain storage units. The lack of influence or even positive influence of one bruchid species over the other was the most likely expectation. The competition capacity of each species in the presence of its heterospecific competitor was assessed using (adult) mixed infestations in common bean seeds. The results indicate that the co-occurring species did not exhibit negative interaction and the presence of *A. obtectus* may even favor *Z. subfasciatus*. Botanical insecticides have been receiving attention for pest management and among them neem, *Azadirachta indica* has beem tested in different formulation including water extract, oil, seed powder and commercial insecticide formulations based on them. However, few studies are available exploring the effects of botanical insecticides in the reproduction of stored grain insect pests. Here we explored the hypothesis that azadirachtin, the active ingredient obtained from neem extracts, may negatively impact the reproductive capacity of *Z. subfasciatus*. Furthermore, we also investigated if the adults are able to compensate the sublethal effects of this botanical insecticide adjusting their reproductive efforts. The results obtained showed that females of *Z. subfasciatus* increase their daily fecundity to compensate a reduction in longevity caused by azadirachtin exposure. Furthermore, the structure matrix by developmental phases of the insect revealed that the populations of *Z. subfasciatus* engendered from females exposed to azadirachtin exhibited higher population growth rate (r) and net reproductive rate (R_o) and finally, an analysis of the projection matrix showed densities notably higher through subsequent generation for populations of *Z. subfasciatus* exposed to azadirachtin.

INTRODUÇÃO GERAL

Os besouros da subfamília Bruchinae (Coleoptera: Chrysomelidae), recentemente denominados de bruquíneos, são insetos especialistas em alimentar-se da parte interna das sementes de leguminosas. A subfamília tem aproximadamente 1.700 espécies (Johnson et al., 2004; Bouchard et al., 2011) e alguns dos besouros são pragas importantes em sementes de feijão armazenado (Moura, 1998).

Zabrotes subfasciatus (Boheman, 1833) e *Acanthoscelides obtectus* (Say, 1831), conhecidos como carunchos do feijão, são as principais pragas do feijão armazenado, causando grandes perdas qualitativas e quantitativas em grãos e sementes, especialmente nas regiões mais quentes do mundo. São consideradas espécies cosmopolitas, ocorrendo em todas as regiões do globo terrestre em que se faz o armazenamento de feijões, desde que fatores climáticos não limitem seu ciclo de vida, além de apresentar distribuição ampla nas regiões quentes e tropicais da América Latina. A espécie *A. obtectus* é a principal praga do grão de feijão armazenado nas regiões temperadas, com altitude em torno de 1500 m; já *Z. subfasciatus* normalmente predomina nas regiões tropicais em altitudes inferiores a 1500 m. *Acamthoscelides obtectus* e *Z. subfasciatus* atacam os cotilédones do feijão armazenado e, na fase larval, abrem galerias, podendo destruí-los completamente, ao que se soma a presença de ovos nos grãos, de orifícios de emergência dos adultos, de insetos mortos e de excrementos que afetam a qualidade do produto, e os grãos destinados à semeadura têm os embriões destruídos, ficando seriamente prejudicados. Somam-se ainda, os danos indiretos, que favorecerem a entrada de microrganismos e ácaros, e aquecimento dos grãos. No Brasil as perdas de grãos causados por *Z. subfasciatus* variam de 7 a 40%, nas diferentes regiões, enquanto *A. obtectus* ocasiona perdas de 20 a 30% da produção total de feijão no país (Celeste Filho & Almeida, 1980; Gallo et al, 2002; Rosolem & Marubayashi, 1994).

A infestação de *Z. subfasciatus* ocorre nos grãos armazenados onde os ovos são colocados aderidos aos grãos por um líquido pegajoso secretado pela fêmea no momento da oviposição. Após a emergência, as larvas penetram em um único grão, construindo galerias de um lado a outro e destruindo o cotilédone (Southgate 1979; Credland & Dendy 1992). Desta forma, as fêmeas se tornam responsáveis pela correta determinação da qualidade e quantidade de alimento para sua prole (Dendy & Credland, 1991), o que pode afetar o desempenho reprodutivo do adulto (e.g. Credland, Dick & Wright 1986; Grether, Hudon & Millie 1999; McGraw et al. 2002), uma vez que esta espécie não se alimenta na fase adulta (Abate & Ampofo, 1996; Teixeira & Zucoloto, 2003).

A infestação por *A. obtectus* representa um contraste, pois esta pode se dar no armazenamento ou campo, onde as fêmeas colocam os ovos nas vagens maduras e fendidas ou abrem uma pequena fenda ao longo da bainha da nervura central. No armazém os ovos são espalhados entre os grãos e em suas proximidades, podendo ser colocados isoladamente ou em grupos. As larvas que eclodem, deslocam-se à procura do hospedeiro e penetram nos grãos. Assim, as fêmeas determinam a área, mas não à semente particular, em que vai a desenvolver a sua descendência O desenvolvimento larval e a fase de pupa se dão no interior no grão, comportando-se de forma semelhante a *Z. subfasciatus* (Gallo et al., 2002; Carvalho & Rossetto, 1968; Wightman & Southgate, 1982).

A competição é uma interação biológica entre organismos ou espécies onde a capacidade ou habilidade é reduzida em consequência da presença do competidor, existindo uma limitação na quantidade de pelo menos um recurso necesaário por ambos organismos ou espécies; este recurso pode ser alimento, água, território e parceiros sexuais (Begon et al., 2006). A competição por recursos limitados tem sido amplamente reconhecida como um importante processo, responsável tanto pela seleção natural quanto pela regulação do tamanho populacional por sua influência sobre as taxas de natalidade, mortalidade ou movimentação (Nicholson, 1954; MacArthur & Wilson, 1967).

A competição pelos recursos locais pode conduzir à seleção da conduta adaptativa específica para superar as interferências prejudiciais entre os indivíduos (Sanz & Gurrea 2000; Alves-Costa & Knogge 2005), e isto pode ter consequências para a história de vida (Smith & Lessells 1985; Smith 1990, 1991). Uma das respostas comportamentais influenciadas pela competição é o comportamento reprodutivo. Muitas espécies desenvolveram a capacidade para avaliar fatores da adequação do hospedeiro, como o ovo ou a carga larval existente nele. Assim, fêmeas de *Callosobruchus maculatus* não depositam seus ovos ao acaso em grãos de caupi, mas comparam os grãos disponíveis quanto ao seu tamanho e carga de ovos/larvas que contém. O padrão não aleatório resultante da postura aumenta sobrevivência das larvas em 70% sobre o que ocorreria se os ovos fossem colocados aleatoriamente (Matthews & Matthews, 2010).

Devido à relação ecologia *versus* comportamento de competição, vários estudos utilizam resultados populacionais como: a relação entre número de indivíduos emergentes pelo número inicial de indivíduos, densidade inicial de ovos por grão, taxa de respiração e de alimentação, massa corporal e desempenho reprodutivo do adulto para determinar estratégias de competição. (Varley *et al.*, 1973; Bellows, 1982; Credland & Dick, 1987; Daniel & Smith, 1994; Guedes et al. 2003, 2007, 2010). Porém, a distinção entre processo de comportamento de competição e resultados populacionais da competição é de vital importância (Smith & Lessells, 1985; Smith, 1990; Guedes *et al.*, 2007), pois o comportamento de competição resulta de um processo intrínseco de seleção, enquanto que o seu resultado populacional também depende da quantidade de recurso exigido pelo organismo em relação a sua disponibilidade (Smith & Lessells, 1985). Resultados populacionais geralmente relacionados a um tipo de competição específico podem esconder a verdadeira estratégia de competição (Guedes *et al.*, 2007).

À primeira vista, a capacidade reprodutiva de uma espécie parece ser facilmente determinada. Na verdade, porém, registros de postura de ovos ou produção total de ovos nem sempre fornecem uma imagem verdadeira do potencial reprodutivo da espécie. Uma variedade de fatores, tanto internos como externos, influenciam a produção total de ovos. A nutrição é provavelmente o fator mais importante, pois, em pelo menos alguns casos, parece haver uma correlação entre a nutrição das larvas e a capacidade reprodutiva dos adultos. Deve-se considerar o esforço reprodutivo de um inseto, um termo que inclui não apenas o conteúdo calórico de óvulos e espermatozóides, mas toda a variedade de fenômenos envolvidos na produção de uma prole bem sucedida: a energia gasta em procura de companheiros, a procura de sítios apropriados de oviposição, cuidado dos ovos ou jovens, alimentação dos jovens, etc., e os riscos resultantes do desempenho destes comportamentos na reprodução dos insetos que tem uma relação direita com a população (Matthews & Matthews, 2010).

A competição por recursos limitados se torna particularmente importante em insetos granívoros devido ao fato destes, na fase imatura, passarem a vida dentro de um único grão. Este é um exemplo de um sistema fechado, no qual o indivíduo é incapaz de evitar a competição com os co-específicos ou os heteroespecíficos, já que várias larvas se desenvolvem dentro de um mesmo grão (Smith 1990, 1991; Colegrave 1994).

Nas ultimas décadas com o aumento dos problemas da resistência de insetos a inseticidas, a ressurgência e erupção de pragas e os problemas advindos do uso indiscriminado de inseticidas organo-sintéticos sobre inimigos naturais, o meio ambiente e o homem, os pesticidas botânicos têm sido apontados como uma alternativa atraente para o manejo de pragas na agricultura por serem considerados relativamente seguros ao meio-ambiente e pouco tóxicos a mamíferos (Isman 2006). Há uma extensa bibliografia científica mostrando os efeitos de compostos secundários extraídos de plantas contra várias espécies insetos e a árvore indiana de nim *Azadirachta indica* (A. Juss) (Meliacea) é uma das fontes mais bem estudadas desses compostos (Mordue et al. 2005, Morgan 2009).

O nim é quimicamente rico possuindo mais de 300 compostos secundários biologicamente ativos (Chawla et al. 1995, Koul 2004). A maioria desses compostos são terpenoides podendo ser encontrados em todas as partes da planta como frutos, sementes, ganhos, cascas, e raízes. O mais relevante, um triterpenoide denominado azadiractina, encontra-se predominantemente nas sementes, sendo utilizado como ingrediente de muitos produtos comerciais, inclusive inseticidas (Butterworth & Morgan 1968, Puri 1999). A azadiractina é um limonoide altamente oxidado com diversos grupos funcionais próximos um do outro (Blaney et al. 1994). A azadiractina é biologicamente interessante devido aos seus efeitos antialimentar, esterilizante e por agir como regulador de crescimento de uma variedade de espécies de insetos fitófagos (Isman et al. 1990). Esta substância, juntamente com uns poucos compostos estruturalmente relacionados, provê o mais poderoso pesticida natural descoberto desde a era dos pesticidas sintéticos. Seu espectro de atividade inclui também outros artrópodes e até mesmo anelídeos e nematódeos (Mordue & Blackwell 1993, Mordue et al. 2005).

Os efeitos da azadiractina sobre a regulação do crescimento dos insetos já são bem documentados e conhecidos. Insetos tratados ou ingerindo alimento contaminado com azadiractina frequentemente apresentam mudas anormais, morte durante a ecdise, metamorfose incompleta resultando em intermediários larva-adulto, mudas atrasadas com prolongamento do desenvolvimento larval, pupas com apêndices da cabeça e tórax deformados, e adultos com reduzida longevidade, ausência de asas ou asas mutiladas (Schumutterer 1990, Mordue & Blackwell 1993, Mordue et al. 2005).

A base para o modo de ação da azadiractina é resultado da citotoxidade a diferentes tecidos e de sua interferência com os sistemas endócrino e neuroendócrino dos insetos (Sayah 2002). Reduções dos títulos de ecdisteróides, atrasos no aparecimento do pico de ecdisteróides, bloqueios da síntese do hormônio protoracicotrópico (PTTH), reduções da síntese hormônio juvenil (JH) e mudanças degenerativas estruturais nas células das glândulas endócrinas (glândula protorácica, corpus allatum, corpo cardiacum) frequentemente aparecem como principais eventos associados à influência da azadiractina (Schlüter et al. 1985, Garcia et al. 1990, Koul & Isman 1991, Banerjee & Rembold 1992, Meurant et al. 1994, Sayah 2002).

Os componentes do Azamax®, uma das formulações de nim disponíveis no mercado brasileiro, são responsáveis pela inibição da alimentação e a ecdise, redução do crescimento, reprodução, oviposição e longevidade dos insetos (Mordue & Blackwell, 1993, Schmutterer, 1990, Simmon Ascher, 1993), mais são, poucos os estudos realizados sobre os efeitos de inseticidas botânicos na reprodução de espécies pragas de grãos armazenados.

Diante desta situação e da importância dos carunchos do feijão no Brasil, o presente trabalho teve como objetivo estudar a habilidade competitiva de *A. obtectus* e *Z. subfasciatus* expostos à competição interespecífica em grãos de feijão vermelho (*Phaseolus vulgaris*) e avaliar o desempenho reprodutivo de *Z. subfaciatus* sob exposição de doses subletais da azadiractina.

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Artigo 1: *Journal of Stored Products Research (2013)* [In press]

Competition between the bean weevils *Acanthoscelides obtectus* and *Zabrotes subfasciatus* in common beans

Abstract

Bruchid pest species of common beans (*Phaseolus vulgaris* (L.)) in the Neotropics, the bean weevil *Acanthoscelides obtectus* and the Mexican bean weevil *Zabrotes subfasciatus*, exhibit niche and range overlaps, frequently co-occurring in bean storage facilities of south-central Brazil. Although both species are well-known and important pest species, the impact of their co-occurrence on their fitness has not been an area of interest. Here, we performed direct competition experiments between both of the species following an additive series. The competitive ability of each species in the presence of its heterospecific competitor was assessed using mixed (adult) insect infestations in common beans. The initial number of insects of one species was fixed at 50, whereas the other species had a number of insects ranging from 0 to 50. Therefore, each species with a variable number of insects started the competition at an initial proportion of 0, 0.17, 0.29, 0.38 and 0.50. The number of adults that emerged and the instantaneous rate of increase (r_i) for each species were determined after one generation under direct competition. Our results indicate that the competitive ability of *Z. subfasciatus* was favored by the presence of its heterospecific competitor (A. obtectus), while the opposite pattern did not occur. Thus, colonization by *A. obtectus* was not significantly compromised by *Z. subfasciatus*, while *Z. subfasciatus* seemed to actually benefit from the presence of A. obtectus under the conditions of our study. These results indicate that these co-occurring species do not exhibit negative interactions in storage facilities, and the presence of *A. obtectus* may even favor *Z. subfasciatus*.

Keywords: bruchids, *Phaseolus vulgaris*, population growth, interspecific competition.

Introduction

Severe competition in grain-feeding insects whose larvae are unable to leave the grain is a result of food limitation, and this competition is particularly strong among niche-overlapping species, which impacts their fitness and behavior (Smith and Lessells, 1985; Smith, 1990, 1991; Guedes et al., 2003, 2007; Guedes and Smith, 2008). However, a lack of resource limitations, which frequently occurs in product storage units, may allow the co-occurrence of potentially competing species. Although such a co-occurrence of different species in storage products may pose consequences for each species, the subject remains largely unexplored, with the exception of the cowpea beetles of the genus *Callosobruchus* Pic (Utida, 1953; Fujii, 1967; Bellows and Hassell, 1984; Lale and Vidal, 2001; Ishii and Shimada, 2008).

Bruchid pest species of common beans (*Phaseolus vulgaris* (L.)) in the Neotropics, the bean weevil *Acanthoscelides obtectus* (Say) and the Mexican bean weevil *Zabrotes subfasciatus* (Boheman), overlap in both niche and range, frequently co-occurring in bean stores of south-central Latin America, particularly in Brazil (Southgate, 1978; Alvarez et al., 2006; Paul et al., 2009; Teixeira and Zucoloto, 2012). Although both species are well-known and important pest species (Southgate, 1978; Baier and Webster, 1992; Abate and Ampofo, 1996), the impact of their co-occurrence on their fitness has not been researched. These bean weevil species are cosmopolitan insect pests that feed on wild and cultivated common beans (Abate and Ampofo, 1996; Alvarez et al., 2005, 2006; Paul et al., 2009; Thakur, 2012). These species are both of Neotropical origin with subsequent spread to Africa, Asia, and Europe (Southgate, 1978; Alvarez et al., 2005, 2006). Although both species exhibit similar range of development, size and sex ratio (1:1), females of *A. obtectus* lay their eggs loosely and the 1st intar larva burrows into the bean, while females of *Z. subfasciatus* attach their eggs to the bean and the larva penetrates the bean from inside the egg without external exposure (Howe and Currie, 1964; Southgate, 1978; Parsons and Crealand, 2003; Paul et al., 2009).

Here, we performed direct competition experiments between both of the species following an additive series. We were not expecting competitive exclusion but rather co-existence without food limitation, as is usually the case in product storage units, due to these species' common co-occurrence in south-central Latin America and their reported scramble larval competition within grain (Ohtsuka and Toquenaga, 2009;

Teixeira and Zucoloto, 2012). A neutral or even positive influence of one species on the other was a more likely expectation, which was assessed in the present study.

2. Material and methods

2.1. Insects

The original populations of *A. obtectus* and *Z. subfasciatus* were field-collected from small farm holders and established under laboratory conditions, starting with at least 500 individuals. The populations of both species were maintained for one year in the laboratory prior to the present experiments and were reared on whole common bean (*P. vulgaris*) grains (small red beans obtained from the local market) that were free of insecticides. They were maintained at controlled conditions reflecting the environmental conditions from their common area of co-occurrence (25 ± 2°C, 70 ± 5% relative humidity,14:10 L:D).

2.2. Competition experiments

Competition experiments were designed for the bean beetle *A. obtectus* and its heterospecific competitor *Z. subfasciatus* using an additive series, as suggested by Snaydon (1991). Mixed infestations were established on 100 g of common beans; the initial number of insects of one species was fixed at 50, whereas the other species had an increasing number of insects varying from 0 to 50. Therefore, each species with a variable number of insects started the competition at the initial proportion of 0, 0.17, 0.29, 0.38 and 0.50 against the second species, which exhibited a fixed total number of insects. Non-sexed adult insects (up to two days old) were placed in 0.25 L glass jars containing bean grains as experimental units. The sex ratios of both species is 1:1 and females exhibit multiple mating (Howe and Curie, 1964; Southgate, 1978; Parson and Credland, 2003; Teixeira and Zucoloto, 2012), therefore the non-sexing of the insects in our experiment does not represent a concern in this study. Three replicates (i.e., each corresponding to a jar with insects) were used for each treatment combination, and the jars were maintained under controlled conditions of temperature (25 ± 2°C), relative humidity (70 ± 5%) and photoperiod (14:10 L:D). The insects remained under competition for 42 days (one generation), after which the total number of (adult) insects of each species in each experimental unit was recorded. The instantaneous rate of population increase (r_i) for each species in each experimental unit was calculated using the formula $r_i = [\ln(N_f/N_i)]/\Delta T$, where N_f and N_i are the final and initial number of live (adult) insects, respectively, and ΔT is the duration of the experiment in days (Walthal and Stark, 1997).

2.5. Statistical analysis

The number of live insects and the instantaneous rate of increase of each species 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 performed when necessary (PROC REG; SAS Institute, 2008). The assumptions of normality and homogeneity of variance were checked, and no data transformation was necessary (UNIVARIATE procedure, SAS Institute, 2008).

3. Results

3.1. Competition and the total number of insects

The models of the analyses of covariance for the total number of live insects of each species after 42 days of competition were significant (Table 1). The total number of adults of *Z. subfasciatus* was significantly affected by the increasing proportion and the presence of the other species but not by the interaction between these two sources of variation (Table 1). The increase in the initial proportion of *Z. subfasciatus* led to a significant increase in the number of *Z. subfasciatus,* as expected (Fig. 1A). Intriguingly, the number of *Z. subfasciatus* also increased with an increased proportion of its heterospecific competitor *A. obtectus* (Fig. 1A).

The analysis of covariance for the total number of adults of *A. obtectus* indicates significant effects of one species exhibiting an increased proportion and the presence of that species on the other co-occurring species (Table 1). The effect of the initial proportion of insects was not significant for the number of *A. obtectus* that emerged (Table 1). Curiously, neither a greater initial proportion of *A. obtectus* nor a greater proportion of *Z. subfasciatus* significantly increased the number of *A. obtectus* that emerged (Fig. 1B).

3.2. Competition and the instantaneous rate of population increase (r_i*)*

Only the model of analysis of covariance for the population growth rate of *Z. subfasciatus* was significant, while no significant difference was observed for *A. obtectus* (Table 2). Although the interaction between the presence of the species that exhibited an increased proportion and the effect of the increasing proportion was marginally significant, only the proportion of *A. obtectus* was actually significant when the growth rate of *Z. subfasciatus* was assessed (Table 2; Fig. 2).

4. Discussion

Direct competition between the bean weevil (*A. obtectus*) and the Mexican bean weevil (*Z. subfsciatus*) occurs within their range in the Neotropics, where they coexist. Such coexistence and the apparent scramble larval competition of both of these species (Ohtsuka and Toquenaga, 2009; Teixeira and Zucolo, 2012) led us to suggest that their competitive exclusion was unlikely, particularly in bean storage units. Indeed, the direct-competition experiments carried out with adults from both species, following an additive series, confirmed this expectation. The bean weevil *A. obtectus* was not apparently affected by its heterospecific, while the Mexican bean weevil *Z. subfasciatus* seems actually favored by the presence of *A. obtectus*.

The mechanisms determining the competitive outcome among bruchid species are still unresolved. Larval stages of these insect species spend their lives within a single kernel and cannot leave until they reach adulthood. The impacts of early-life foraging decisions are especially significant for organisms that do not feed as adults, which is the case for *A. obtectus* and *Z. subfasciatus* (Alvarez et al., 2005, 2006). Within this context, one would expect that a species that shows better performance during larval stages would present a better outcome under competition. However, this pattern is not always the case. Competition studies with the bruchids *C. chinensis* and *C. maculatus* confirmed that the latter is a superior larval competitor than the former (Bellows and Hassell, 1984; Fujii, 1967; Ishii and Shimada, 2008), although larval competition does not always reflect on the final competition outcome. *Callosobruchus chinensis* usually competitively excludes *C. maculatus* (Bellows and Hassell, 1984; Fujii, 1967), but the exclusion of *C. chinensis* by *C. maculatus* has also been documented (Ishii and Shimada, 2008; Utida, 1953).

Egg-laying behavior and larval behavior are likely important in determining the outcome of competition between *A. obtectus* and *Z. subfasciatus*, allowing their co-existence in bean stores. These species show strikingly different egg-laying behavior, which under mixed infestation conditions might determine, for example, differential egg mortality, thereby favoring one of the species over the other. Eggs are laid on the bean surface by *Z. subfasciatus* and scattered on or among beans by *A. obtectus* (Howe and Currie, 1964; Parsons and Credland, 2003). Higher mortality therefore seems more likely in the early larval development of *A. obtectus* than that of *Z. subfasciatus* because the first instar larvae of *A. obtectus* must move among the beans and choose a host themselves before boring into one (Howe and Currie, 1964; Parsons and Credland, 2003). Moreover, *A. obtectus* eggs might suffer mechanical injuries, resulting in high egg mortality, as reported for *C. chinensis* for which adult density-dependent trampling is the most plausible explanation of egg mortality (Fujii, 2009).

Host selection performed by early larvae and not female adults is a key behavioral difference between *A. obtectus* and *Z. subfasciatus*. While the loose egg-laying behavior of *A. obtectus* females is likely to lead to higher early larval mortality due to the larva's need to find a suitable host and burrow into the kernel (Parsons and Credland, 2003), such larvae may take advantage of previously bored channels. Larvae of *A. obtectus* may act as "pioneers," directly burrowing into the grain as larvae of *Z. subfasciatus* do, or act as "followers," taking advantage of entrance holes already created by either a pioneer larva of the same species or of a different species (e.g., *Z. subfasciatus*) (Ohtsuka and Toquenaga, 2009). The number of pioneer larvae tends to decrease with decreasing larval density, demonstrating that being a pioneer incurs high costs (Ohtsuka and Toquenaga, 2009). Therefore, larvae of *A. obtectus* may adjust their behavior to the follower mode, minimizing their early cost of establishment under competition with *Z. subfasciatus,* which is unable to make such an adjustment.

The positive interference of *A. obtectus* on *Z. subfasciatus* when under competition is more likely a consequence of their behavioral strategies of larval competition. Multiple adults usually emerge from a single bean kernel in both of the species, suggesting that their larvae exhibit a scramble type of competition, which seems to prevail among bruchids, with a few exceptions (Smith and Lessells, 1985; Smith, 1990, 1991; Guedes et al., 2003, 2007). For instance, a larva of *Z. subfasciatus* may take advantage of the tunnels created by heterospecific larvae to position itself in suitable grain parts. However, little is known about the behavior during larval competition of *A. obtectus* and *Z. subfasciatus*, which is usually inferred based on the outcome of larval competition and is subject to potentially mistaken interpretation, as detected in the maize weevil *Sitophilus zeamais* (Guedes et al., 2010). X-ray studies of the larval behavior within the grain are likely to shed led light on this issue and are currently underway with *A. obtectus* and *Z. subfasciatus.*

Although this investigation did not attempt to elucidate the mechanisms determining the competition between *A. obtectus* and *Z. subfasciatus*, it did present information about their competitiveness and co-existence. In summary, our results indicate a lack of negative interaction between *A. obtectus* and *Z. subfasciatus.* Furthermore, the competitive ability of *Z. subfasciatus* was favored by the presence of its heterospecific competitor (*A. obtectus*), while the opposite pattern did not occur. These results indicate that these co-occurring species do not exhibit negative interactions in storage facilities, and the presence of *A. obtectus* may even favor *Z. subfasciatus*.

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Table 1. Analyses of covariance for the total number of live insects after 42 days (one generation) of competition between *A. obtectus* and *Z. subfasciatus*. The asterisk indicates significant difference at $P \leq 0.05$.

SOURCES OF VARIATION	**df**	***Z. subfasciatus***		***A. obtectus***	
		F	***P***	**F**	***P***
Model	3	4.23	0.01*	6.04	0.002*
Error	32	-	-	-	-
Species with increasing density	1	6.67	0.01*	12.74	0.001*
Initial proportion	1	5.71	0.02*	0.24	0.96
Interaction	1	1.00	0.33	8.10	0.008*

Table 2. Analyses of covariance for the instantaneous rate of population growth (ri) after 42 days (one generation) of competition between *A. obtectus* and *Z. subfasciatus*. The asterisk indicates significant difference at P ≤ 0.05.

SOURCES OF VARIATION	**df**	***Z. subfasciatus***		***A. obtectus***	
		F	***P***	**F**	***P***
Model	3	6.98	0.002*	1.24	0.32
Error	32	-	-	-	-
Species with increasing density	1	2.91	0.10	2.08	0.16
Initial proportion	1	14.32	0.001*	1.64	0.21
Interaction	1	4.50	0.05*	0.13	0.72

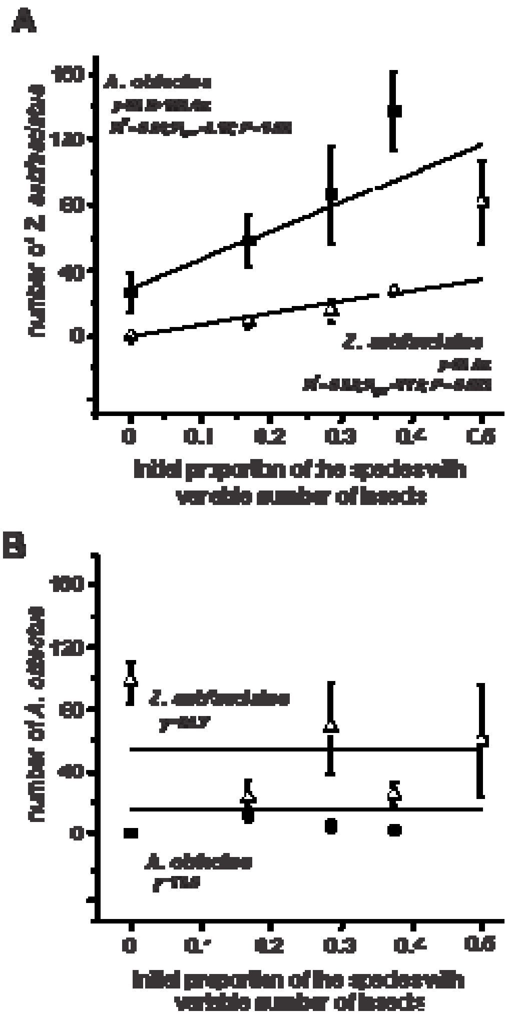

Fig. 1. Total number of live insects (A*: Z. subfasciatus*; B: *A. obtectus*) after three experiments, each lasting 42 days (one generation), involving competition between A. obtectus and Z. subfasciatus. The curves refer to the species with a fixed number of insects and the other species with a variable number of insects, which is indicated on each curve. The symbols represent the mean of three replicates and the vertical bars represent the standard deviation.

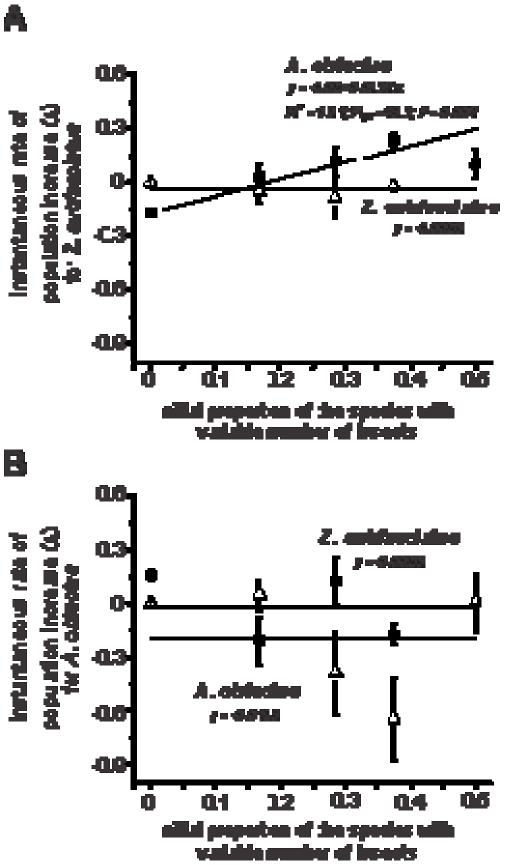

Fig. 2. Instantaneous rate of population increase (ri) of *Z. subfasciatus* (A) and *A. obtectus* (B) after three experiments, each lasting 42 days (one generation), involving competition between *A. obtectus* and *Z. subfasciatus*. The curves refer to the species with a fixed number of insects and the species with a variable number of insects, which is indicated on each curve. The symbols represent the mean of three replicates and the vertical bars represent the standard deviation.

Artigo 2: *Journal of Pest Science (2013)* [Submitted]

Azadirachtin-mediated shift in fecundity-longevity trade-off in the Mexican bean beetle *Zabrotes subfasciatus*

Abstract

Insecticides can have lethal or sublethal effects upon targeted pest species, and sublethal effects may even favor pest outbreaks if insecticide-induced hormesis occurs. Hormesis is a biphasic dose-response of a given chemical compound that is stimulatory at low doses and toxic (or inhibitory) at high doses. The former response may result from the disruption of animal homeostasis leading to trade-off shifts between basic ecophysiological processes (e.g., between maintenance and reproduction). A growing interest in the use of biorational (botanical) insecticides such as azadirachtin to control stored-product pests raises concerns about potential sublethal effects (i.e., insecticide-induced population growth), especially on pests that have been targeted by a small number of insecticides. In this study, we explored the hypothesis that azadirachtin can negatively impact the reproductive capacity of the Mexican bean beetle *Zabrotes subfasciatus* (Boheman) (Chrysomelidae: Bruchinae), a key stored-bean pest of worldwide distribution. Additionally, we investigated whether adults of this species could compensate for any sublethal effect that might have affected any of their reproductive parameters by adjusting the allocation of its reproductive efforts. The results showed that females of *Z. subfasciatus* increased daily fecundity to compensate for azadirachtin-induced decreased longevity. In addition, a stage-structured matrix study revealed that populations of *Z. subfasciatus* engendered from females exposed to azadirachtin exhibited a higher rate of population increase (*r*) and a higher net reproductive rate (R_O). Finally, a projection matrix analysis showed notably higher densities along the generations for azadirachtin-exposed *Z. subfasciatus* populations. Thus, our study provides empirical evidence for the capacity of insect pests to adapt to sublethal effects caused by biorational insecticides; consequently, this study highlights the importance of understanding this phenomenon when devising pest management strategies.

Keywords: Neem, hormesis, hormoligosis, *Phaseolus vulgaris*, biorational insecticides.

Introduction

Insecticides are widely used to control arthropod pests in many agroecosystems (Cowan and Gunby 1996). The benefits of using insecticides include the rapid control response and easy application, and most insecticides are readily available to growers. Nonetheless, the use of insecticides may cause many problems if used improperly. In general, the overuse of insecticides can contaminate the environment, select for resistant pests, and even promote arthropod pest outbreaks (Chelliah and Heinrichs 1980; Roush 1987; Pimentel et al. 1993; Hardin et al. 1995). The latter phenomenon may be caused by either the deleterious effects of insecticide upon the pest's natural enemies (Godfray and Chan 1990) or by hormesis in the pest species (Guedes et al. 2010; Yu et al. 2010; Cordeiro et al. 2013).

Hormesis is an adaptive response characterized by a biphasic dose-response that promotes a stimulatory response at low doses and a toxic (or inhibitory) response at high doses. The stimulatory response may result from the disruption of the animal homeostasis leading to trade-off shifts between basic ecophysiological processes (e.g., between maintenance and reproduction) (Calabrese 2002; Kendig et al. 2010; Jager et al. 2013). Pesticide-induced hormesis has often been documented among arthropod pest species and their natural enemies (Gerson and Cohen 1989 Guedes et al. 2009, 2010; Cutler 2013). For instance, Yu et al. (2010) observed direct stimulation of the fertility of the aphid *Myzus persicae* when exposed to sublethal doses of imidacloprid. Nonetheless, the role played by trade-off shifts induced by insecticides in increasing population growth has not been extensively investigated, and such knowledge should be obtained to devise appropriate pest management tactics for use with chemical control.

Botanical insecticides have long been portrayed as a plausible alternative to synthetic insecticides because they supposedly pose little threat to the environment or to human health. Among botanical insecticides, neem oil has received substantial attention and has been the target of various studies (Rosell et al. 2008). The main active component of neem oil is azadirachtin, which is known to act as a growth regulator, antifeedant and cellular growth inhibitor (Isman 2006). Although less frequently recognized, azadirachtin has also been documented to reduce the longevity of adult insects (Simmon Ascher 1993). In general, the efficacy of azadirachtin against various crop pests has been tested (Schmutterer 1985; Lynn et al. 2012; Pavela et al. 2013;

Tomé et al. 2013), although similar studies regarding stored products are scarce. Nevertheless, azadirachtin has shown to be detrimental to the growth and development of the stored-cowpea pest species *Callosobruchus maculatus* (Coleoptera:Bruchidae) (Makanjuola 1989).

Another stored-product pest is the Mexican bean weevil *Zabrotes subfasciatus* (Boheman, 1833) (Coleoptera: Chrysomelidae); this pest feeds on stored grains and is of significant economic and social importance because it attacks the bean *Phaseolus vulgaris*, a major staple food in Latin America and Africa (Minney et al. 1990). Females of *Zabrotes subfasciatus* oviposit on the external surface of beans; after hatching, the young larvae burrow into the grain and molt four times before pupating (Howe and Currie 1964). Young adults emerge out of the grain by biting and pushing out a 'window' in the seed coat with their mandibles. The adults are more exposed to chemical treatments than the larvae, and thus their reproductive behavior should influence their fitness. In this study, we explored the hypothesis that the botanical insecticide azadirachtin can negatively impact the reproductive capacity of *Z. subfasciatus*, which could have broad implications for its population dynamics. In addition, a potential interaction between azadirachtin and *Z. subfasciatus* gender is examined. Concomitantly, we also investigate whether the adults can respond to sublethal exposures that may have affected reproductive parameters by adjusting the allocation of their reproductive efforts.

Material and methods

Insects

The insects used in the experiments were obtained from laboratory colonies maintained in a growth room at 26 ± 1 °C, a relative humidity of 60 ± 5% and under fluorescent lights set at a photoperiod of 14:10 L:D. The colonies were maintained inside 2-L glass jars 3/4 filled with pesticide-free seeds of the common bean *P. vulgaris* ('vermelho' cultivar).

Azadirachtin exposure and insect reproduction

All of the experiments described here were conducted between October 2012 and May 2013. The experiment used to evaluate the impact of azadirachtin on the reproductive capacity of males and females of *Z. subfasciatus* was conducted twice at different dates. This experiment used four experimental treatments. Each treatment examined one *Z. subfasciatus* pair, in which azadirachtin was applied to both, one or neither of the genders. In the first experiment, each treatment was replicated 9-10 times whereas in the second experiment, each treatment was replicated 20-24 times. The treatments were conducted according to a completely randomized design. Each replicate consisted of a Petri dish (90 x 12 mm, d:h) containing 20 grams of untreated *P. vulgaris* seeds ('vermelho' cultivar) and enclosing a pair of *Z. subfasciatus*. At the beginning of each experiment, 48-72 -h old females and/or males of *Z. subfasciatus* were exposed for 24 h to the residue from a one-hour treatment of *P. vulgaris* seeds with control/water or azadirachtin".; the insects were then transferred to the Petri dish described above (in replicate) and maintained in a growth room under the same environmental conditions as those described above. To apply the treatments, 500 g of *P. vulgaris* seeds were sprayed with 1 ml of either water (control) or azadirachtin insecticidal solution (Azamax®, DVA Brazil, Campinas, São Paulo, Brazil) using an aerograph sprayer attached to a vacuum pump (Prismatec, Itu, SP, Brazil) at a pressure of 10 psi; thereafter, the seeds were allowed to dry for one hour. Then, males and females of *Z. subfasciatus* were individually enclosed in a glass vial (22 x 60 mm, d:h) that was enclosed by organza fabric on the top and filled with *P. vulgaris* seeds; the insects were then exposed for 24 h to their respective treatments. Because Azamax® is not yet approved for use against *Z. subfasciatus* or other stored-product beetles, we tested the highest field rate registered (this rate is recommended for another pest, the darkwinged fungus gnat *Bradysia impatiens* (Johannsen) (Diptera: Sciaridae) (Ministério da Agricultura, Pecuária e Abastecimento 2013)). This recommended field rate is equivalent to a concentration of 0.06 ml of active ingredient per liter of solution. Daily, and for each experiment, we evaluated the male and female survivorship/longevity after treatment. In addition, female fecundity, egg fertility and F_1 sex ratio were also evaluated. The fertility was calculated as the percentage of eggs that hatched successfully into larvae.

Stage-structured matrix model for Zabrotes subfasciatus

To better evaluate the significance of the small differences observed in the reduced longevity and increased fecundity on *Z. subfasciatus* population growth, a stage-structured matrix model was developed for azadirachtin-treated and -untreated female beetles. The life cycle stages included in the models were egg, larva, pupa and adult. The resultant projection matrices consisted of a set of upper diagonal elements

(P_i) representing the probability of an individual surviving and remaining in that life stage and a set of lower diagonal elements (G_i) representing the probability of an individual surviving and transitioning to the subsequent life stage (Caswell 2001). These probabilities are dependent upon the survival (σ_i) and development (γ_i) rates of each life stage (*i*) in relation to the time step of the projection (1 day), such that $P_i = \sigma_i (1 - \gamma_i)$ and $G_i = \sigma_i \gamma_i$.

For the stage-structured matrix investigation, the longevity and survivorship of eggs, larvae and pupae were evaluated in a test that was independent from the reproduction experiment. For this test, 48- to72-hour-old mated females of *Z. subfasciatus* were exposed individually to the treatments described above (15 females per treatment) and thereafter allowed to oviposit on clean *P. vulgaris* seeds. The survivorship and longevity of the eggs, larvae and pupae were assessed by examining them every other day inside the seed (one individual per seed) using digital X-ray equipment (Faxitron Corporation, Tucson, AZ).

Data analyses

The assumption of normality for the longevity, fecundity and fertility data was assessed by evaluating the variance equality (plotting residuals versus estimates), conducting a Levene's test and plotting studentized residuals against normal quantiles to look for outliers.

The effect of treatment on longevity, fecundity (total and daily) and fertility was assessed using an analysis of variance (PROC GLM). In addition, specific contrasts were used to compare treatments where only one gender was treated with treatments where both genders and neither gender were treated, and to compare the latter two groups. Treatments were combined for treated and untreated females once males were found not to exhibit any effect, and thereafter, the data were analyzed using a t-test (PROC TTEST). Differences in sex ratio were analyzed within each treatment using a chi-square test (PROC FREQ). All analyses were conducted in SAS (SAS 2008).

The data regarding daily fecundity, F_1 sex ratio, survivorship and the duration of immature stages were used to parameterize a stage-structured matrix model using the software PopTools (PopTools version 3.2.3, Hood 2010). This analysis allows an estimate of the rate of increase (*r*), net reproductive rate (R_0) and a projection of the recoveries of treated and control populations.

Results

All longevity, fecundity and fertility data were found to satisfy normality. A significant effect of insecticide was observed on the longevity of the beetles (df = 3, *F* = 20.11, *P* = <0.001). Azadirachtin-treated female and male beetles tended to live shorter lives than untreated beetles (Figure 1). No treatment effect was observed on total fecundity (df = 3, *F* = 0.75, *P* = 0.52), even when the data for treated and untreated females were combined (df = 110, *t* = 0.06, *P* = 0.95) (Table 1). In contrast, a marginal treatment effect on daily fecundity was observed (df = 3, *F* = 2.45, *P* = 0.06). In the contrasts in the pairwise analyses, significant differences were only seen when comparing the treatment of 'untreated females coupled with untreated males' with the treatment of 'treated females coupled with untreated males' (df = 1, *F* = 5.38, *P* = 0.02) and when comparing the former with the treatment of 'treated females coupled with treated males' (df = 1, *F* = 5.00, *P* = 0.03); these findings suggest that the insecticide affected only females. In fact, treatments with azadirachtin-treated females showed higher daily fecundity than untreated females, regardless of whether the male had been treated. Likewise, combined data also showed that treated females had higher daily fecundity than untreated females (df = 101, *t* = 2.33, *P* = 0.02) (Figure 2). No effect of treatment was seen on egg fertility (df = 3, *F* = 0.99, *P* = 0.40). Likewise, no significant difference was observed for the F_1 sex ratio within treatments ('untreated females coupled with untreated males', df = 1, χ^2 = 1.48, *P* = 0.22; 'treated females coupled with treated males', df = 1, χ^2 = 0.11, *P* = 0.74; 'untreated females coupled with treated males', df = 1, χ^2 = 0.20, *P* = 0.65; and 'treated females coupled with untreated males', df = 1, χ^2 = 0.97, *P* = 0.32).

The stage-structured matrix model showed that insecticide-treated females exhibited a higher net reproductive rate value (R_o = 2.32) than untreated females (R_o = 1.92). Likewise, a higher rate of population increase was observed for populations of insecticide-treated females (*r* = 0.071) than for the control population (*r* = 0.055). In addition, the projection matrix showed that populations of *Z. subfasciatus* engendered by insecticide-treated females tended to be larger in subsequent generations than those engendered by untreated females (Figure 3).

Discussion

Adult females and males of *Z. subfasciatus* exposed to azadirachtin residue on bean seeds had shorter longevity. The effect of azadirachtin on the longevity of other insect species has been investigated, and the results vary. For example, Okumu et al. (2007) observed a shorter longevity for adults of *Anopheles gambiae* that were fed as larvae on a diet treated with azadirachtin. In contrast, Kraiss and Cullen (2008) observed an increase in larval period for azadirachtin-treated *Harmonia axyridis*. Nonetheless, to our knowledge, our work is the first to report the finding that azadirachtin residue induces shorter adult insect longevity. This sublethal effect may reduce the pest population in subsequent generations, especially by decreasing the number of eggs laid by females due to a decrease in reproductive time.

Although azadirachtin decreased longevity, total fecundity was not affected. In contrast, daily fecundity was significantly increased for azadirachtin-treated females. This finding suggests that treated females may shift their trade-off balance by increasing their daily fecundity when longevity is reduced. Few studies have investigated such trade-off responses in arthropods exposed to insecticides (Calabrese 2005; Guedes et al. 2009; Cutler 2013; Jager et al. 2013). In addition, this phenomenon is difficult to distinguish from other, confounding effects. Such trade-off responses may favor one basic physiological process, such as reproduction, while compromising another, such as maintenance (or longevity, as here). This shift in resource allocation (energy) can thus lead to hormetic responses (Calabrese 2002, 2005; Jager et al. 2013) such as the increased fecundity seen here. In a separate study, a higher dose of azadirachtin (100 times greater than the dose tested here) was highly detrimental to the survival of *Z. subfasciatus* (*unpublished data*), thereby characterizing a biphasic response of *Z. subfasciatus* and indicating an azadirachtin-induced hormesis.

The higher daily fecundity shown by the azadirachtin-treated females did not appear to be influenced by the presence of males, treated or untreated. This result suggests that azadirachtin can affect male longevity without compromising male reproductive capacity. In addition, the shorter male longevity and its potential effect in reducing egg fertilization might also have been mitigated by males copulating more frequently at the early adult stage (at the beginning of the experiments). However, more studies are necessary to confirm this hypothesis.

The stage-structured matrix study revealed that populations of *Z. subfasciatus* engendered by females exposed to azadirachtin residue have higher rates of increase (*r*) as well as higher net reproductive rates (R_o). This finding suggests that the growth of treated populations of *Z. subfasciatus* is more rapid. Furthermore, the projection matrix estimates also showed notable quantitative differences between azadirachtin-exposed and control populations. Thus, populations of *Z. subfasciatus* exposed to bean seeds that were coated with azadirachtin are more likely to experience outbreak events. Therefore, these results illustrate that biorational insecticides such as azadirachtin, which are increasingly being adopted in agricultural landscapes, may also contribute and/or facilitate the development of arthropod pests outbreaks; hence, this insecticide should be used with caution.

In summary, the results show an adaptive reproductive strategy adopted by females of *Z. subfasciatus* that appears to increase their daily fecundity to compensate for azadirachtin-decreased longevity. These findings are of particular importance for those seeking to use biorational insecticides such as azadirachtin to control stored-product pests. It is also worth noting that the use of higher doses could subsequently render grain residues with different ages (and thus residue levels); these residues could in turn induce the pest phenomenon observed here. Furthermore, this study provides empirical evidence for those interested in understanding insecticide-induced trade-off shifts in biological processes that induce hormetic responses in arthropods.

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Table 1. Total fecundity (± SE) and egg fertility (± SE) of *Zabrotes subfasciatus* females. Means followed by the same letters within columns do not differ statistically at $P < 0.005$ (LSMEANS, PROC GLM). Lowercase letters indicate pooled data.

Treatments	**Total fecundity**	**Fertility**
Untreated females and males	25.18 ± 1.81 A	84.88 ± 2.33 A
Untreated females and Azadirachtin-treated males	23.89 ± 2.21 A	82.57 ± 3.79 A
Azadirachtin-treated females and males	26.96 ± 2.44 A	75.21 ± 6.22 A
Azadirachtin-treated females and untreated males	22.27 ± 1.82 A	82.88 ± 4.15 A
Pooled data by untreated females	24.44 ± 1.59 a	83.80 ± 2.15 a
Pooled data by azadirachtin-treated females	24.57 ± 1.43 a	79.26 ± 3.67 a

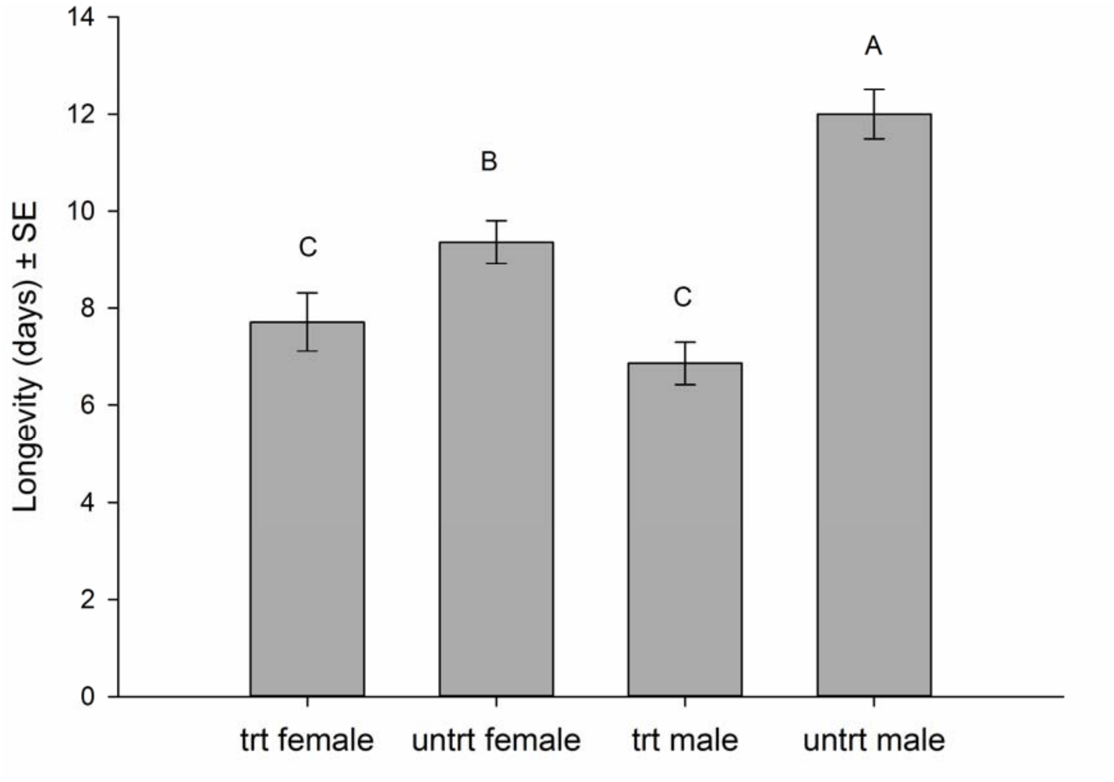

Figure 1. Mean longevity (days ± SE) of azadirachtin-treated and -untreated females or males of *Zabrotes subfasciatus*. Bars followed by different letters differ significantly at $P < 0.005$ (LSMEANS, PROC GLM). (trt = azadirachtin-treated *Z. subfasciatus*, and untrt = untreated *Z. subfasciatus*).

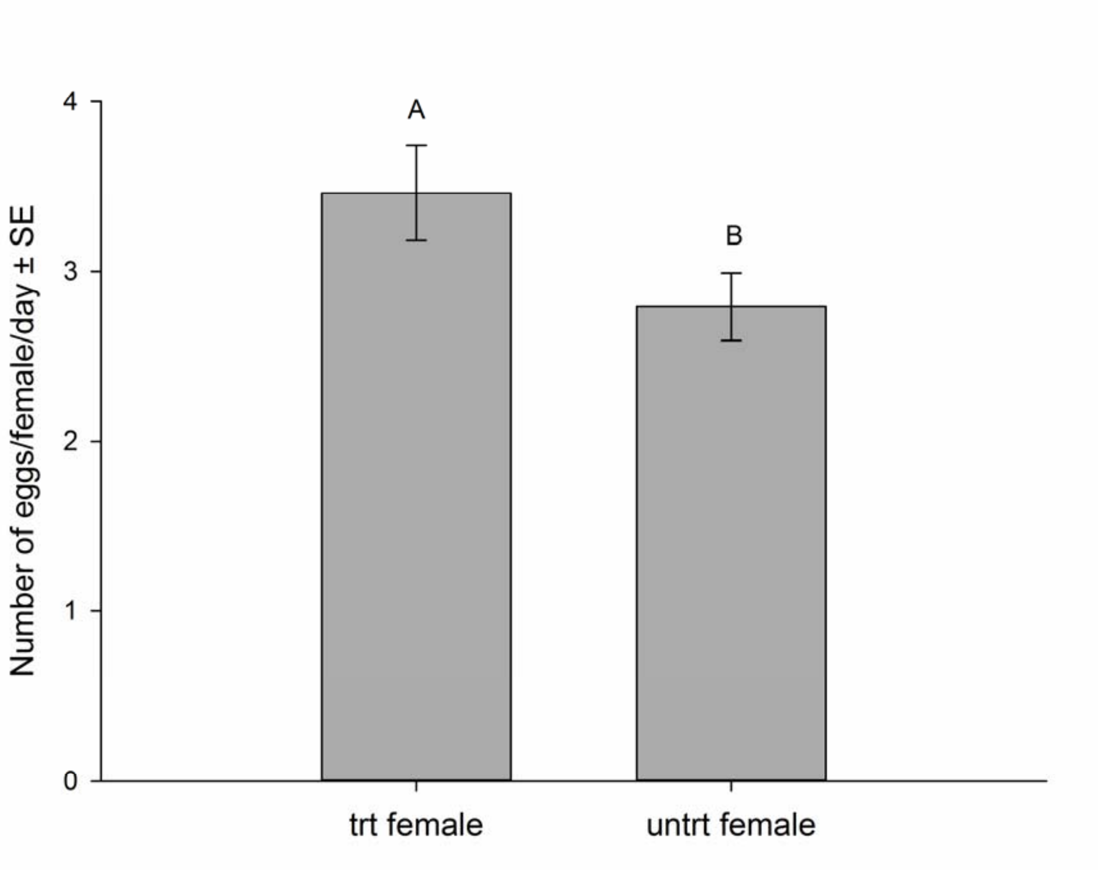

Figure 2. Daily fecundity (mean number of eggs/female/day ± SE) of azadirachtin-treated and -untreated females of *Zabrotes subfasciatus*. Bars followed by different letters differ significantly at $P < 0.05$ (PROC TTEST). (trt = azadirachtin-treated *Z. subfasciatus*, and untrt = untreated *Z. subfasciatus*).

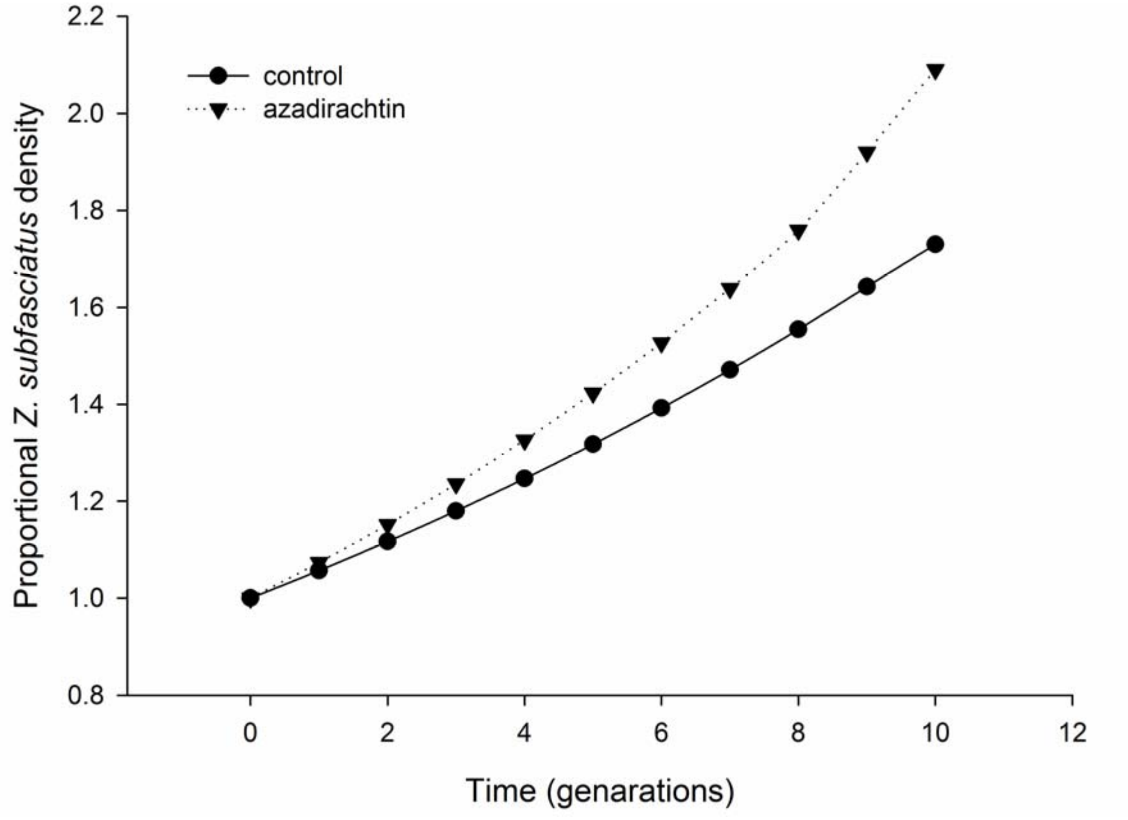

Figure 3. Population growth projections for azadirachtin-treated and -untreated females of *Zabrotes subfasciatus* based on a stage-structured matrix model. Population density includes all stages of *Z. subfasciatus* (egg, larva, pupa and adult).

CONCLUSÕES GERAIS

As observações realizadas e os resultados populacionais indicam que e *A. obtectus* e *Z. subfasciatus* coexistem em condições de armazém, exibindo interações positivas como consequência de sua estratégia de competição larval e que a habilidade competitiva *de Z. subfasciatus* é favorecida pela presença de *A. obtectus*.

Os dados obtidos neste trabalho demostram que existe uma estratégia reprodutiva adaptativa em fêmeas de *Z. subfasciatus* que parecem incrementar sua fecundidade diária para compensar a menor longevidade induzida pelos resíduos subletais de azadiractina a que foram expostos.