ANCIDÉRITON ANTONIO DE CASTRO

TOXICITY OF INSECTICIDES TO LEPIDOPTERAN PESTS, SELECTIVITY TO PREDATORY STINKBUGS AND BEHAVIORAL ASPECTS OF THESE NATURAL ENEMIES

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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DEDICO

Ao meu pai José Mauro de Castro

À minha mãe Ivone Resende Coelho de Castro

Ao meu irmão Stefânio José de Castro

A todos os meus familiares e amigos.

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BIOGRAFIA

ANCIDÉRITON ANTONIO DE CASTRO, filho de José Mauro de Castro e Ivone Resende Coelho de Castro, nasceu em Cajuri, estado de Minas Gerais, Brasil, em 05 de setembro de 1985. Em março de 2004, ingressou no curso de graduação em Engenharia Agronômica, na Universidade Federal de Viçosa (UFV), graduando-se em janeiro de 2009. De agosto de 2005 à julho de 2008 foi bolsista de Iniciação Científica, na área de Controle Biológico de Insetos, Departamento de Biologia Animal, Universidade Federal de Viçosa, com bolsa do CNPq. Em março de 2009 ingressou no Programa de Pós-Graduação em Entomologia na Universidade Federal de Viçosa defendendo a dissertação em julho de 2010. Em agosto de 2010 ingressou no Programa de Doutorado em Entomologia pela mesma Universidade defendendo a tese em novembro de 2013. De novembro de 2012 à outubro de 2013 foi bolsista de doutorado sanduíche no United States Department of Agriculture – Agricultural Research Service, CMAVE, Florida A&M University, Center for Biological Control, Tallahassee, Flórida, Estados Unidos.

SUMMARY

ABSTRACT

CASTRO, Ancidériton Antonio de, D.Sc., Universidade Federal de Viçosa, December, 2013. **Toxicity of insecticides to lepidopteran pests, selectivity to predatory stinkbugs and behavioral aspects of these natural enemies.** Adviser: José Cola Zanuncio. Co-advisers: Jesusa Crisostomo Legaspi, José Eduardo Serrão, Germano Leão Demolin Leite and Teresinha Vinha Zanuncio.

The application of insecticides, often used erroneously and abusively, without considering the recommended limit, is the method of pest control used by most producers of soybean. Brazil is the world's largest consumer of pesticides and the use of these compounds has increased in other parts of the world in different cultures. The pest control in the soybean culture is based in conventional pesticides, including cyclodienes, organophosphates and pyrethroids. Biological control with parasitoids and predators and plant resistance to insects are important in integrated pest management (IPM) programs. Therefore, the use of insecticides should be compatible with the different control strategies to maintain the sustainability of agriculture. Predatory stinkbugs such as *Podisus maculiventris* (Say), *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) have potential for biological pest controls. The objective of this research was to assess the acute toxicity and behavioral sublethal response of the predators *P. nigrispinus* and *S. cincticeps* exposed to chlorantraniliprole, deltamethrin, methamidophos and spinosad; evaluate the survival, reproduction and life table parameters of *P. nigrispinus* fed on caterpillars of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae), a pest of soybean, exposed to some traditional insecticides (the pyrethroid deltamethrin and the organophosphate methamidophos) in addition to more recent compounds (the spinosyn spinosad and the diamide chlorantraniliprole); evaluate the toxicity of some botanical insecticides approved by the Organic Materials Review Institute (OMRI) and chlorantraniliprole against *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *P. maculiventris* under laboratory conditions for potential use in an integrated pest management. With the exception of deltamethrin for *S. cincticeps*, all insecticides showed higher acute toxicity to the prey than to these natural enemies providing effective control of *A. gemmatalis*. The recommended field concentration of deltamethrin, methamidophos and spinosad for controlling *A. gemmatalis* caused 100% mortality of *P. nigrispinus* and *S. cincticeps* nymphs. Chlorantraniliprole was the least toxic and the most selective insecticide to these predators resulting in mortalities lower than 10% when exposed to 10x the recommended field concentration for a period of 72 h. Behavioral pattern changes in predators were found for all insecticides, especially methamidophos and spinosad, which exhibited irritability (i.e., avoidance after contact) to both predator species. However, insecticide repellence (i.e., avoidance without contact) was not observed in any of the insects tested. The lethal and sublethal effects of pesticides on natural enemies is important for IPM. The pyrethroid and organophosphate insecticides should be substituted by chlorantraniliprole in IPM programs of *A. gemmatalis* in soybeans. Life table studies showed that spinosad and methamidophos are not compatible with *P. nigrispinus* in IPM programs in the soybean agro-ecosystem, whereas deltamethrin was slightly toxic and chlorantraniliprole the most promising due to lower toxicity to this predator. Entrust® and Coragen® showed higher toxicity to the pest when compared to the predator and PyGanic® and Azera® showed higher toxicity to the predator when compared to the pest using glass-vials bioassays. Coragen® also had the highest toxicity against *S. exigua* using diet incorporation bioassays, followed by Entrust®, PyGanic® and Azera®. The oral toxicity bioassays showed that Entrust® had the highest toxicity against *P. maculiventris* followed by PyGanic®, Azera® and Coragen®. The notion that natural compounds are safer than synthetic compounds to non-target species is refuted in the present study, which showed that the synthetic insecticide Coragen® was less toxic than the natural insecticides PyGanic®, Azera® and Entrust®. Therefore, certain bioinsecticides should not be exempted from risk assessment schemes, and non-target sub-lethal effects should not be neglected when considering potential insecticide use in integrated pest management programs.

RESUMO

CASTRO, Ancidériton Antonio de, D.Sc., Universidade Federal de Viçosa, dezembro de 2013. **Toxicidade de inseticidas para lepidópteros praga, seletividade a percevejos predadores e aspectos comportamentais desses inimigos naturais.** Orientador: José Cola Zanuncio. Coorientadores: Jesusa Crisostomo Legaspi, José Eduardo Serrão, Germano Leão Demolin Leite e Teresinha Vinha Zanuncio.

A aplicação de inseticidas, muitas vezes usado erroneamente e de forma abusiva, sem considerar o limite recomendado, é o método de controle de pragas utilizado pela maioria dos produtores de soja. O Brasil é o maior consumidor mundial de pesticidas e a utilização desses compostos aumentou em outras partes do mundo, em diferentes culturas. O controle de pragas em plantas de soja é baseada em pesticidas convencionais, incluindo ciclodienos, organofosfatos e piretróides. O controle biológico com parasitóides, predadores e resistência de plantas a insetos são importantes para programas de manejo integrado de pragas (MIP). Portanto, a utilização de inseticidas deverá ser compatível com as diferentes estratégias de controle para manter a viabilidade da agricultura. Percevejos predadores como *Podisus maculiventris* (Say), *Podisus nigrispinus* (Dallas) e *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) apresentam potencial para o controle biológico de pragas. Os objetivos dessa pesquisa foram avaliar a toxicidade e aspectos comportamentais dos predadores *P. nigrispinus* e *S. cincticeps* expostos aos clorantraniliprole, deltametrina, espinosade e metamidofós, inseticidas normalmente utilizados no controle da lagarta-da-soja. A sobrevivência, reprodução e os parâmetros de tabela de vida do predador *P. nigrispinus*, alimentado em lagartas de

Anticarsia gemmatalis (Hübner) (Lepidoptera: Erebidae) criadas em folhas da soja previamente expostas a quatro inseticidas utilizados nesta cultura (clorantraniliprole, deltametrina, espinosade e metamidofós), foram avaliados. A toxicidade de alguns inseticidas botânicos aprovados pelo Organic Materials Review Institute (OMRI) contra *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) para *P. maculiventris* em laboratório foi, também, avaliada. Todos os inseticidas, exceto a deltametrina para *S. cincticeps*, apresentaram maior toxicidade para a praga que a esses inimigos naturais, fornecendo controle efetivo de *A. gemmatalis*. As doses recomendadas de campo de deltametrina, metamidofós e espinosade, para o controle de *A. gemmatalis*, causaram 100% de mortalidade de ninfas de *P. nigrispinus* e *S. cincticeps*. Clorantraniliprole foi o menos tóxico e o inseticida mais seletivo para esses predadores, com mortalidades menores que 10% expostos a 10x a dose recomendada de campo por período de 72 h. Alterações do padrão de comportamento em predadores foram encontrados para todos os inseticidas, especialmente metamidofós e espinosade, os quais apresentaram irritabilidade (evitar após o contato) para ambas as espécies predadoras. No entanto, a repelência inseticida (evitar sem contato) não foi observada em nenhum dos insetos testados. Os efeitos letais e subletais de pesticidas sobre os inimigos naturais são de grande importância para o MIP, e nossos resultados indicam que a substituição de inseticidas piretróides e organofosforados em suas doses de campo por clorantraniliprole pode ser um fator chave para o sucesso de programas de MIP de *A. gemmatalis* em soja. Durante estudos de tabela de vida, espinosade e metamidofós não foram compatíveis com *P. nigrispinus* em programas de MIP em soja, enquanto deltametrina foi levemente tóxico e clorantraniliprole pode ser considerado o mais promissor devido à menor toxicidade para este predador. Entrust® e Coragen® apresentaram maiores toxicidade para a praga que o predador e PyGanic® e Azera® maiores toxicidade para o predador que a praga utilizando os bioensaios com frascos de vidro. Coragen® também demonstrou maior toxicidade contra *S. exigua* utilizando bioensaios de incorporação de dieta, seguido por Entrust®, PyGanic® e Azera®. Os bioensaios de toxicidade oral mostraram que Entrust® apresentou maior toxicidade contra *P. maculiventris* seguido por PyGanic®, Azera® e Coragen®. No presente estudo a noção de que os compostos naturais são mais seguros do que os compostos sintéticos para espécies não-alvo é refutada, o qual mostrou que o inseticida sintético Coragen® foi menos tóxico do que os inseticidas naturais PyGanic®, Azera® e Entrust®. Certos bioinseticidas não devem ser isentos de avaliações de risco e seus efeitos sub-letais não-alvo não devem ser negligenciados para a utilização de inseticidas em programas de manejo integrado de pragas.

INTRODUCTION

The soybean *Glycine max* (L.) Merril is one of the most important export crop in the world, especially to the United States (USDA, 2011) and Brazil (SMIL, 2000), with a world production of 264 million tons (USDA, 2012), and high economic and social value as a food source rich in protein of low cost (FORTUNATO et al., 2007) and vegetable oil for biofuel production (TEMUCIN, 2011). Soybean crops supply half of the global demand for vegetable oil and protein (OERKE & DEHNE, 2004). Brazil produced 69 million tons of soybean in 2009/2010, being the second largest producer after the United States with 91.4 million tons during the same period (USDA, 2012). Insect pests can reduce quality and yield of grains and seeds (OERKE, 2006; MACEDO et al., 2011). Soybean producers need to control phytophagous arthropods to reduce losses and to increase profits (ZALUCKI et al., 2009).

Insects can damage the soybean crop throughout its life cycle. *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Noctuidae) can attack soybean seedlings, *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae) and *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) and *Euschistus heros* (Fabricius), *Piezodorus guildinii* (Westwood) and *Nezara viridula* (L.) (Heteroptera: Pentatomidae) are major pests during the vegetative stage (EMBRAPA SOYBEAN, 2010).

The application of insecticides, often used erroneously and abusively, without considering the recommended limit, is the pest control method used by most producers of soybean (SONG & SWINTON, 2009). Brazil is the world's largest consumer of pesticides (CORRÊA-FERREIRA et al., 2010) and the use of these compounds has increased in other parts of the world in different cultures (SONG & SWINTON, 2009; MEISSLE et al., 2010). The pest control in the soybean culture is based in conventional pesticides, including cyclodienes, organophosphates and pyrethroids (BAUR et al., 2010). Biological control with parasitoids (AVANCI et al., 2005) and predators (BELORTE et al., 2004) and plant resistance to insects (MEISSLE et al., 2011) are important in integrated pest management (IPM) programs (MEDINA et al., 2005; BUENO et al., 2011a). Therefore, the insecticides used should be compatible with other control strategies to maintain the agriculture sustainability (ZALUCKI et al., 2009). In the early 1970s, before the implementation of IPM of soybean in Brazil, an average of six applications of broad-spectrum insecticides were used for the growing season. The implementation of IPM reduced pesticide use to two applications per season (BUENO et al., 2010). IPM and biological control are more sustainable, mainly by reducing dependence on expensive and harmful chemicals to the environment (KOGAN, 1998; BUENO et al., 2011a).

The introduction of the IPM with selective pesticides to protect natural enemies (FREWIN et al., 2012), allowed to consider the economic thresholds in crops for pest control (STERN et al., 1959; KOGAN et al., 1977). However, the use of this program in Brazil has declined in soybean (CORRÊA-FERREIRA et al., 2010) and insecticide applications reached four to six applications per crop cycle

(BUENO et al., 2010), with impact on the efficiency of biological control agents (CARMO et al., 2010). The overuse of insecticides, especially non-selective, reduces the efficiency of natural biological control, an essential component of IPM (CARMO et al., 2010). The preservation of natural enemies contributes to reducing insecticide use and environmental impact (BUENO et al., 2009).

Anticarsia gemmatalis is one of the major insect pests of soybean, occurring from Argentina to the United States (HOMRICH et al., 2008), including Florida (SOSA-GÓMEZ, 2004). The economic injury level for soybeans is different by regions of the world. In Brazil, control measures are initiated when observed 20 large larvae ($\geq$ 1.5 cm) per sample (one row of soybean 1 m) or 30% or 15% of defoliation at vegetative or reproductive stages, respectively. In the United States, soybean plants can withstand 35% defoliation until the flowering period. However, in this phase, defoliation greater than 20% decreases productivity (BUENO et al., 2011b). This insect causes high defoliation and can destroy the plant at high infestations because each caterpillar can consume up to 110 cm^2 of leaves (WALKER et al., 2000). Insecticide applications can cause resistance and pest control failures (AHMAD & ARIF, 2009; SILVA et al., 2011).

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a major insect pest of vegetables and is widely distributed around the world (ZHENG et al., 2011; LAI et al., 2011). It damages many cultivated crops such as bean, corn, cotton, onion, peanut, potato, soybean, tomato and others. This insect is originally from Southeast Asia. It was first discovered in North America (Oregon) in 1876, and it was found in Florida in 1924 (CAPINERA, 2001).

The suborder Heteroptera presents predators in the families Pentatomidae, Reduviidae and Lygaeidae, with potential for pest suppression (COLL & HUGHES, 2008; CARSTENS et al., 2008; PEREIRA et al., 2009). The species of the subfamily Asopinae (Pentatomidae) are predators, and only about 10% of the 300 species are well studied (DE CLERCQ et al., 2002; GUEDES et al., 2009a; RIBEIRO et al., 2010; CASTRO et al., 2012), including *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) (CASTRO et al., 2012), *Podisus maculiventris* (Say) (SHAPIRO & LEGASPI, 2006; MONTEMAYOR & CAVE, 2011), *Podisus distinctus* (Stal) (GUEDES et al., 2009a), *Supputius cincticeps* (Stal) (ZANUNCIO et al., 2003), *Brontocoris tabidus* (Signoret) (ZANUNCIO et al., 2000; PIRES et al., 2011), *Alcaeorrhynchus grandis* (Dallas) (RIBEIRO et al., 2010) and *Tynacantha marginata* (Dallas) (MOREIRA et al., 1997).

The predator *P*. *nigrispinus* has a potential for IPM programs (ZANUNCIO et al., 1994; MATOS-NETO et al., 2002; LEMOS et al., 2005; ZANUNCIO et al., 2008; PIRES et al., 2011; CASTRO et al., 2012) and establishes, survives and reproduces in temporary agroecosystems (OLIVEIRA et al., 2002). They show rapid post-embryonic development (MEDEIROS et al., 2003 a,b), adaptation to different temperatures and prey (TORRES et al., 1998; LEMOS et al., 2003; VIVAN et al., 2003), generalist behavior (ZANUNCIO et al., 1994) and relative tolerance to insecticides (SMAGGHE & DEGHEELE, 1995; ZANUNCIO et al., 2003). *Podisus* *nigrispinus* was recorded in several countries of Central and South America as an important biological control agent on different crops (THOMAS, 1992; LEMOS et al., 2005; SILVA et al., 2009). However, fertility and fecundity of this predator may be affected by insecticides (TORRES et al., 2002; EVANGELISTA JÚNIOR et al., 2002; CASTRO et al., 2012).

Natural enemies are subject to contact with insecticides to control pests. Tarsal contact of predators with pesticide residues in plants is the main route of exposure of these natural enemies during foraging (MAHDIAN et al., 2007). However, predators can also be affected by direct contact of spray droplet, ingestion of insecticides or plant sap contaminated or by feeding on contaminated prey (MAHDIAN et al., 2007; CLOYD & BETHKE, 2011). To date, insecticide compatibilities have been demonstrated for methoxyfenozide, pyriproxyfen and spinosad with *Picromerus bidens* L. (Heteroptera: Pentatomidae) (MAHDIAN et al., 2007); deltamethrin and *Bacillus thuringiensis* with *P. maculiventris* (MOHAGHEGH et al., 2000); chlorantraniliprole and deltamethrin were slightly toxic to *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) (CAMPOS et al., 2011); and low permethrin doses were beneficial for *Podisus distinctus* (Stal) (Heteroptera: Pentatomidae) (ZANUNCIO et al., 2013). On the other hand, the pyrethroid gamma-cyhalothrin was toxic (PEREIRA et al., 2005) and the growth regulator diflubenzuron reduced *P. nigrispinus* fertility (CASTRO et al., 2012).

Life tables may be used to evaluate sublethal effects of pesticides on the demography of both target and non-target species (STARK & BANKS, 2003;

STARK et al., 2007). Sublethal effects on population dynamics go unnoticed because they can affect the fertility of individuals (PERVEEN, 2008) even with low mortality, as reported for *P. nigrispinus* with diflubenzuron (CASTRO et al., 2012).

Actions that influence the body's response to selective pressures of a particular insecticide determine behavioral mechanisms. These mechanisms increase the capacity of an insect population to escape the lethal effects of the insecticide and may be related to the learning ability of the insect (LOCKWOOD et al., 1984; FFRENCH-CONSTANT, 1994; LORINI & GALLEY, 1998; HOY et al., 1998). The population retains its intrinsic susceptibility to the insecticide, but change their behavior to avoid contact with the insecticide, which is an important behavioral tool in IPM (CAMPOS et al., 2011).

Behavioral mechanisms may be independent or stimulus-dependent (GEORGHIOU, 1972). Independent behavior stimulus-resistance includes behavioral pattern, which prevents exposure to a toxic substance, i.e. the individual does not require prior contact with insecticide (GEORGHIOU, 1972; LOCKWOOD et al., 1984). Stimulus-dependent behavioral resistance refers to the increase in the ability of the insect to detect toxic substances, irritant and repellent properties stimulates the escape response of the insect after detection of the substance (LOCKWOOD et al., 1984). Studies on the insecticides usually prioritize the effects of physiological and biochemical character, with little attention to behavioral responses of the body due to exposure to the insecticide (KONGMEE et al., 2004; GUEDES et al., 2009b).

Although pesticide use remains an important IPM tactic, efforts have been made in the search for compounds with reduced impact on natural enemies and other non-target arthropods. Studies have shown promising safety profiles of new compounds with low toxicity such as chlorantraniliprole to *D*. *luteipes* (CAMPOS et al., 2011) and *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammatidae) (PREETHA et al., 2009). However, more recent compounds such as chlorfenapyr were toxic against *D*. *luteipes* (CAMPOS et al., 2011). Futhermore, pyrethroids are generally toxic to natural enemies (PEREIRA et al., 2005; CORDEIRO et al., 2010; MACFADYEN & ZALUCKI, 2012), but deltamethrin showed low toxicity to *D*. *luteipes* (CAMPOS et al., 2011).

In this context, the objectives of this research were: 1- assess the acute toxicity and behavioral sublethal response of the predators *P*. *nigrispinus* and *S*. *cincticeps* exposed to chlorantraniliprole, deltamethrin, methamidophos and spinosad; 2- evaluate the survival, reproduction and life table parameters of *P*. *nigrispinus* fed on caterpillars of *A*. *gemmatalis*, a pest of soybean, exposed to some traditional insecticides (the pyrethroid deltamethrin and the organophosphate methamidophos) in addition to more recent compounds (the spinosyn spinosad and the diamide chlorantraniliprole); 3- evaluate the toxicity of some botanical insecticides approved by the Organic Materials Review Institute (OMRI) and chlorantraniliprole against *S*. *exigua* and *P*. *maculiventris* under laboratory conditions for potential use in an integrated pest management.

The introduction of this thesis is according to the ABNT. Chapters I and III follow the Chemosphere Journal instructions and chapter II is according to the Journal of Economic Entomology.

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Capítulo I

Survival and behavior of the insecticide-exposed predators *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae)

Survival and behavior of the insecticide-exposed predators *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae)

Abstract

Pentatomid stinkbugs are important predators of defoliating caterpillars in agricultural and forestry systems, and knowledge of the impact of insecticides on natural enemies is important information for integrated pest management (IPM) programs. Thus, we assessed the toxicity and behavioral sublethal response of the predators *Podisus nigrispinus* and *Supputius cincticeps* exposed to chlorantraniliprole, deltamethrin, methamidophos and spinosad, insecticides commonly used to control the velvetbean caterpillar (*Anticarsia gemmatalis*) in soybean crops. With the exception of deltamethrin for *S. cincticeps*, all insecticides showed higher acute toxicity to the prey than to these natural enemies providing effective control of *A. gemmatalis*. The recommended field concentration of deltamethrin, methamidophos and spinosad for controlling *A. gemmatalis* caused 100% mortality of *P. nigrispinus* and *S. cincticeps* nymphs. Chlorantraniliprole was the least toxic and the most selective insecticide to these predators resulting in mortalities lower than 10% when exposed to 10x the recommended field concentration for a period of 72 h. Behavioral pattern changes in predators were found for all insecticides, especially methamidophos and spinosad, which exhibited irritability (i.e., avoidance after contact) to both predator species. However, insecticide repellence (i.e., avoidance without contact) was not observed in any of the insects tested. The lethal and sublethal effects of pesticides on natural enemies is of great importance for IPM, and our results indicate that substitution of pyrethroid and organophosphate insecticides at their field rates by chlorantraniliprole may be a key factor for the success of IPM programs of *A. gemmatalis* in soybeans.

Keywords: *Anticarsia gemmatalis*, Asopinae, natural enemies, selectivity, toxicity.

Resumo

Percevejos predadores são importantes predadores de lagartas desfolhadoras em sistemas agrícolas e florestais, e o conhecimento do impacto de inseticidas sobre inimigos naturais é uma infomação importante para programas de manejo integrado de pragas (MIP). Dessa forma, a toxicidade e aspectos comportamentais dos predadores *Podisus nigrispinus* e *Supputius cincticeps* expostos aos clorantraniliprole, deltametrina, espinosade e metamidofós, inseticidas normalmente utilizados no controle da lagarta-da-soja (*Anticarsia gemmatalis*), foram avaliados. Todos os inseticidas, exceto a deltametrina para *S. cincticeps*, apresentaram maior toxicidade para a praga que para esses inimigos naturais, fornecendo controle efetivo de *A. gemmatalis*. As doses recomendadas de campo de deltametrina, metamidofós e espinosade para controle de *A. gemmatalis* causaram 100% de mortalidade de ninfas de *P. nigrispinus* e *S. cincticeps*. Clorantraniliprole foi o menos tóxico e o inseticida mais seletivo para esses predadores, resultando em mortalidades menores que 10% quando expostos a 10x a dose recomendada de campo por período de 72 h. Alterações do padrão de comportamento em predadores foram encontrados em todos os inseticidas, especialmente metamidofós e espinosade, os quais apresentaram irritabilidade (evitar após o contato) para ambas as espécies predadoras. No entanto, a repelência inseticida (evitar sem contato) não foi observada em nenhum dos insetos testados. Os efeitos letais e subletais de pesticidas sobre os inimigos naturais são de grande importância para o MIP, e nossos resultados indicam que a substituição de inseticidas piretróides e organofosforados em suas doses de campo por clorantraniliprole pode ser um fator chave para o sucesso de programas de MIP de *A. gemmatalis* em soja.

Palavras-chave: *Anticarsia gemmatalis*, Asopinae, inimigos naturais, seletividade, toxicidade.

1. Introduction

Insecticide selectivity and impact on natural enemies are key components of Integrated Pest Management (IPM) programs (Metcalf, 1980; Hardin et al., 1995; Desneux et al., 2007). Chemical control is the most common method used to control pests (Cooper and Dobson, 2007; Song and Swinton, 2009) and its use has increased in various cultures, notably in developing countries, despite of a few exceptions (e.g. China) due to increased use of transgenic crops (Song and Swinton, 2009; Meissle et al., 2010; Lu et al., 2012; Pedlowski et al., 2012). Simultaneously, changes in societal attitude has triggered the search for safer pesticides to humans and the environment, resulting in the development of compounds more specific to the target pest, i.e. for non-target organisms (Matsumura, 2004; Cordova et al., 2006; Nicholson, 2007). However, problems related to pollution by pesticides and overuse of these chemicals still remain.

Historically, crop protection has often resulted in the application of pesticides harmful to natural enemies (Wilson and Tisdell, 2001; Desneux et al., 2007). IPM aims to reduce the status of pests to tolerable levels with the use of effective, economically sustainable and ecologically sound management (Van Lenteren and Woets, 1988). Although pesticide use remains an important IPM tactic, efforts have been made in the search for compounds with reduced impact on natural enemies and other non-target arthropods. Thus, studies assessing lethal and sublethal effects of pesticides on these organisms are increasingly performed, though primarily at the population level (Stark and Banks, 2003; Desneux et al., 2007; Stark et al., 2007; Zanuncio et al., 2011; Biondi et al., 2012b; Castro et al., 2012; Seagraves and Lundgren, 2012). Exposure to a particular product may trigger adverse effects not necessarily resulting in the death of individuals (Desneux et al., 2007). These sublethal effects may comprise physiological parameters such as development, longevity and fecundity, as well as behaviors involved in mobility, foraging for hosts (or prey) and mates (Desneux et al., 2004 a,b; Kim et al., 2006; Harwood et al., 2007; Suma et al., 2009; Evans et al., 2010; Cabral et al., 2011; Caballero-López et al., 2012; Stara et al., 2011; He et al., 2012).

Arthropod predators are important in crops due to the ability to control phytophagous insects and mites (Symondson et al., 2002). Species of the subfamily Asopinae (Pentatomidae) are important predators of defoliating caterpillars (Zanuncio et al., 2003; Castro et al., 2012). These natural enemies can achieve significant populations feeding on other prey and plants before the arrival of pests (Zanuncio et al., 2004; Desneux and O'Neil, 2008; Holtz et al., 2011). They also display generalist behavior (Shapiro and Legaspi, 2006) with adaptation to different temperatures and prey (Vivan et al., 2003; Legaspi, 2004; Silva et al., 2012) and relative tolerance to insecticides (Smagghe and Degheele, 1995; Zanuncio et al., 2011; Castro et al., 2012), which emphasizes the importance of these for potential success of IPM programs (Zanuncio et al., 2008; Pires et al., 2011).

Anticarsia gemmatalis Hübner (Lepidoptera: Erebidae) is one of the major lepidopteran pests of soybeans occuring from Argentina to the United States, causing serious defoliation on plants during their vegetative and reproductive stages (Walker et al., 2000; Homrich et al., 2008). The use of insecticides is still one of the main methods for controlling this pest (Silva et al., 2011) and research is carried out to identify compounds with low toxicity to natural enemies in IPM programs of *A. gemmatalis*. We assessed the acute toxicity and behavioral sublethal response of the predators *Podisus nigrispinus* (Dallas) and *Supputius cincticeps* (Stal) (Heteroptera: Pentatomidae) exposed to deltamethrin, methamidophos, spinosad and chlorantraniliprole. These insecticides are used for *A. gemmatalis* control and this study may help optimizing combined use of pesticides and natural enemies for management of *A. gemmatalis,* while exhibiting low toxicity to natural enemies.

2. Material and methods

2.1. Insects

The predators *P. nigrispinus* and *S. cincticeps* and the prey *A. gemmatalis* were obtained from mass-reared cultures from the Laboratory of Biological Control of Insects (LCBI) of the Institute of Biotechnology applied to Agriculture (BIOAGRO), at the Federal University of Viçosa (UFV), Viçosa, Minas Gerais State, Brazil. These natural enemies are reared with pupae of the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) under controlled environmental conditions (25 ± 2 °C, 70 ± 5% relative humidity, and 12:12 light:dark photoperiod) (Molina-Rugama et al., 1997; Zanuncio et al., 2000). Yellow mealworm adults and larvae are reared on a plastic tray containing wheat flour mixed with yeast (≈5%) and vegetables such as carrot, sweetpotato, and cassava, as food and moisture supplied once a week. More details on producing yellow mealworms can be obtained in Zamperline et al. (1992). Caterpillars of *A. gemmatalis* are reared on artificial diet (Greene et al., 1976) and their adults in wooden cages (30 x 30 x 30 cm) with screened sides, glass covers and fed cotton soaked in nutrient solution at the bottom of the cages. Nymphs of *P. nigrispinus* and *S. cincticeps* and larvae of *A. gemmatalis* larvae were observed daily to obtain third-instar insects for use in the bioassays.

2.2. Insecticides

All of the insecticides used are registered for controlling *A. gemmatalis* in Brazilian soybean fields (Agrofit, 2012). The insecticides used and their respective commercial formulations were: the pyrethroid deltamethrin (Decis® 25 EC; 25 g a.i./L; Bayer CropScience Ltd.; São Paulo-SP), the organophosphate methamidophos (Tamaron® BR SC; 600 g a.i./L; Bayer CropScience Ltd.; Belford Roxo-RJ), the diamide chlorantraniliprole (Premio® CS; 200 g a.i./L; DuPont Brasil S.A.; Barra Mansa-RJ) and the spinosyn spinosad (Tracer® 480 CS; 480 g a.i./L; Dow AgroSciences Industrial Ltd.; São Paulo-SP).

2.3. Concentration-mortality bioassays

The concentration-mortality bioassays were carried out using Petri dishes (9.0 cm diameter x 2.0 cm high) with the bottom completely covered with soybean leaves of the cultivar "BRSMT pintado" treated with insecticide solutions. For each treatment, the soybean leaves were immersed for five seconds at different concentrations of each insecticide solution (diluted in water) and the leaves were let to dry in shade for an hour before placement in the Petri dishes (Castro et al., 2012). Each Petri dish received ten third-instar larvae of *A. gemmatalis* or ten third-instar nymphs of *P. nigrispinus* or *S. cincticeps*. Bioassays were established following a completely randomized design with five to eight concentrations and six replicates. The concentrations used were established through preliminary bioassays with a 10-fold range of dilutions for each insecticide and species to allow recognition of the concentration range leading to mortality variation between 0% and 100%. Mortality was assessed after 72 h of exposure and the insects were considered dead if they did not move when prodded with a fine hair brush. Predators were not fed during the exposure to the insecticide in this bioassay since they can survive to over 14 d without prey as a food source (Lemos et al., 2001).

2.4. Time-mortality bioassays under insecticide field rates

The acute (lethal) toxicity towards predatory stinkbugs of the maximum recommended insecticide concentrations for the control of *A. gemmatalis* (chlorantraniliprole-13.3 μg a.i./mL, deltamethrin- 50 μg a.i./mL, spinosad- 240 μg a.i./mL and methamidophos- 1500 μg a.i./mL) was estimated using third-instar nymphs of *P. nigrispinus* and *S. cincticeps*. Ten nymphs of each species were placed over the insecticide-impregnated filter paper glued (with synthetic white water-based glue resin) to the bottom of a Petri dish (9 cm diameter x 2 cm high), whose inner walls were covered with Teflon® PTFE (DuPont, Wilmington, DE, USA) to prevent insect escape. The filter paper disc was considered treated when soaked for 5 s with 1 mL of solution corresponding to each recommended field concentration of insecticide. Five replicates were used for each combination of insecticide and predator species, in addition to a control treatment were only water (distilled and deionized) was applied to the filter papers. Insect mortality was observed every 30 min during the initial 24 h exposure and at 5 h intervals afterwards until the death of all insects or until they reached the adult stage. *Tenebrio molitor* pupae were provided *ad libitum* to the predatory stinkbug nymphs throughout the bioassays. The nymphs were recorded as dead if they were unable to move when dorsally prodded with a fine brush. All bioassays were carried out simultaneously under the same conditions of the insect rearing following a completely randomized design.

2.5. Behavioral bioassays

Two behavioral locomotory bioassays were carried out with third-instar *P. nigrispinus* and *S. cincticeps* nymphs – one using arenas fully-treated with insecticide and the other using half-treated arenas (Guedes et al., 2009; Corrêa et al., 2011). Filter papers (Whatman No. 1; 9 cm diameter) were treated with insecticide (or water) as previously described (Section 2.4). The insecticide concentrations used were the same field rates used for the time-mortality bioassays since no mortality was observed during the exposure time (10 min) in any treatment including the control. The inner walls of each Petri dish were coated with Teflon® PTFE to prevent insect escape. Arenas with individual (third-instar nymphs) *P. nigrispinus* or *S. cincticeps* were used for each insecticidal treatment in each behavioral bioassay (fully- and half-treated arenas). Twenty insects (i.e. replicates) were used for each combination of insecticide treatment and predator species (including the control) in the bioassays with fully- and half-treated arenas. In each trial, the filter paper was replaced, and the side on which the insect was released in the arena was randomly established in each trial.

The insect movement within each arena was recorded for 10 min and digitally transferred to a computer using an automated video tracking system equipped with a CCD camera (ViewPoint Life Sciences Inc., Montreal, Canada). The arena images were either undivided (for the bioassays on insecticide fully-treated arenas) or divided into two symmetrical zones (one treated and the other untreated, for the bioassays on half-treated arenas). The parameters recorded were: distance walked (cm), walking velocity (cm/s), resting time (s) and the number of stops in the arena, and proportion of time spent in each half of the arena (for the half-treated arenas). The insects spending less than 1 s on the insecticide-treated half of the arena were considered repelled, while the ones remaining less than 50% of the time on such treated half were considered irritated (Cordeiro et al., 2010).

2.6. Statistical analyses

The results of the time-mortality bioassays were subjected to Probit analysis using PROC PROBIT (SAS Institute, 2008), generating concentration-mortality curves and the selectivity and toxicity rates were calculated. To measure the selectivity of insecticides on predator species, we calculated the differential selectivity with 95% confidence intervals based on the values of LC_{50} of insecticides for pest (*A. gemmatalis*) and for predators (*P. nigrispinus* and *S. cincticeps*) (Robertson and Preisler, 1992). The time-mortality data were subjected to survival analysis using the non-parametric procedure LIFETEST (SAS Institute, 2008). This procedure allows the estimate of survival curves obtained through Kaplan-Meier estimators generated from the proportion of third-instar nymphs surviving from the beginning to the end of the experiment. The overall results for locomotory bioassays were subjected to multivariate analysis of variance (PROC GLM using the MANOVA statement; SAS Institute, 2008). Each parameter was subsequently subjected to univariate analysis of variance, and Tukey's HSD test ($p< 0.05$), when appropriate (PROC UNIVARIATE, SAS Institute, 2008). Pairwise differences in the time spent in each half of half-treated arenas (i.e., insecticide avoidance) were tested using paired Student's *t* test ($p< 0.05$) for each insecticide and species. Homogeneity of variance and normality of errors were checked and data were transformed when necessary (PROC UNIVARIATE; GPLOT PROC, SAS Institute, 2008).

3. Results

3.1. Concentration-mortality bioassays

Concentration-mortality curves for the pest *A. gemmatalis* and the predators *P. nigrispinus* and *S. cincticeps* showed low χ^2 values (<11.00) and high *p*-values (>0.09), indicating the data adequacy to the PROBIT model used to estimate the mortality curves. This allowed the estimation of the LC_{50}'s (Table 1).

Spinosad had the highest toxicity to *A. gemmatalis* followed by chlorantraniliprole, methamidophos and deltamethrin, with relative toxicity of 32.20, 739.43 and 1074.07, respectively (Table 1). The insecticides spinosad (LC_{90} = 0.16 (0.09-0.35)), chlorantraniliprole (LC_{90} = 8.90 (4.40-27.51)), deltamethrin (LC_{90} = 44.40 (33.41-66.64)) and methamidophos (LC_{90} = 50.86 (28.90-158.95)) are probably effective in controlling *A. gemmatalis* because the LC_{90} of these insecticides in our experimental conditions were lower than the field label rate. Chlorantraniliprole was safe to *P. nigrispinus* and *S. cincticeps*, making it impossible to estimate the LC_{50} for this insecticide because predators showed no mortality greater than 10% at concentrations 10 times higher than the field label rate (i.e., 133.4 µg a.i./mL). Methamidophos and deltamethrin had the highest toxicity, respectively, in relation to spinosad for *P. nigrispinus* nymphs (Table 1). Against *S. cincticeps*, deltamethrin was the most toxic insecticide followed by methamidophos and spinosad (Table 1). Spinosad and particularly chlorantraniliprole showed higher toxicity to the pest than to the predators, unlike deltamethrin and methamidophos whose toxicity to the pest species was similar to those of both predators (Table 1).

3.2. Time-mortality bioassays

The survival analysis of predatory stinkbugs exposed to dried insecticide residues indicated significant differences among treatments for both species, *P. nigrispinus* (Log-rank test, $\chi^2 = 259.91$, d.f. = 4, $p < 0.001$) and *S. cincticeps* (Log-rank test, $\chi^2 = 297.48$, d.f. = 4, $p < 0.001$). The survival of *P. nigrispinus* and *S. cincticeps* nymphs was 100% in the control (without insecticide exposure) after 500 h of exposure, while the insecticides methamidophos, spinosad and deltamethrin led to 100% mortality of *P. nigrispinus* after 55, 60 and 150 h, respectively, and *S. cincticeps* after 60, 100 and 280 h, respectively (Fig. 1). Chlorantraniliprole led to 25% mortality of *P. nigrispinus* and 30% for *S. cincticeps* after 500 h exposure (Fig. 1). Such differences were reflected in the median survival time (LT_{50}) observed for each insecticide, with chlorantraniliprole leading to higher LT_{50}'s. The LT_{50}'s to *P. nigrispinus* were 13.52, 14.60, 24.61 and 442.61 h for the insecticides methamidophos, spinosad, deltamethrin and chlorantraniliprole, respectively, and LT_{50}'s to *S. cincticeps* 17.12, 17.98, 19.30 and 366.77 h for methamidophos, deltamethrin, spinosad. The median survival time was not estimated for insects without insecticide exposure because of the 0% mortality observed.

3.3. Behavioral bioassays

3.3.1 Behavioral bioassays in fully-treated arenas

The mobility parameters of *P. nigrispinus* and *S. cincticeps* in arenas fully-treated with insecticides showed significant differences among insecticides

($df_{num/den}$ = 16/620.81; Wilks' lambda = 0.8491; $F = 2.13$; $p = 0.0061$), predators ($df_{num/den}$ = 4/203; Wilks' lambda = 0.8928; $F = 6.09$; $p < 0.0001$) and the interaction of predators x insecticides ($df_{num/den}$ = 16/620.81; Wilks' lambda = 0.8097; $F = 2.78$; $p = 0.0002$). Univariate analyses of variance for mobility parameters varied for walked distance ($F_{(9;206)} = 1.93$; $p = 0.04$), walking velocity ($F_{(9;206)} = 2.70$; $p = 0.005$), resting time ($F_{(9;206)} = 3.71$; $p = 0.0002$) and number of stops ($F_{(9;206)} = 3.39$; $p = 0.0007$). The locomotor activity of *P. nigrispinus* when exposed to surfaces treated with spinosad was significantly lower compared to deltamethrin (Fig. 2). As for *S. cincticeps*, the results were distinct from *P. nigrispinus* and all insecticides caused decreased locomotor activity compared to the control treatment (with water) (Fig. 2).

3.3.2 Behavioral bioassays in half-treated arenas

The time spent in each half of the arena half-treated with insecticides showed significant differences for *P. nigrispinus* with the insecticides methamidophos ($T_{(14)} = 2.42$; $p = 0.03$) and spinosad ($T_{(14)} = 2.26$; $p = 0.04$) and, for *S. cincticeps*, with methamidophos ($T_{(17)} = 2.52$; $p = 0.02$), spinosad ($T_{(17)} = 2.13$; $p = 0.04$) and deltamethrin ($T_{(23)} = 3.00$; $p < 0.01$). The proportion of time in each half of the arena did not differ between the treated and untreated half of the arena for *P. nigrispinus* with deltamethrin and chlorantraniliprole and for *S. cincticeps* with chlorantraniliprole ($p > 0.05$) (Fig. 3).

Tracks representative of the typical walking behavior of third instar from both predatory stinkbugs species on arenas partially impregnated with dried insecticide residues are shown in Fig. 4. Behavioral avoidance to insecticide-treated surfaces was recognized through its two components – insecticide repellence (i.e., avoidance without contact) and insecticide irritability (i.e., avoidance after contact). Insecticide repellence was not observed in any of the insects used in this bioassay. However, insecticide irritability occurred in both predator species to the insecticides methamidophos and spinosad. In addition, *S. cincticeps* also showed irritability to deltamethrin.

4. Discussion

In this study we assessed the efficacy of residues of four insecticides to control the velvetbean caterpillar (*A. gemmatalis*), and subsequently evaluated the toxicity of these compounds to the predatory stinkbugs *P. nigrispinus* and *S. cincticeps* constantly reported in crops such as soybean and eucalyptus in Brazil (Matos-Neto et al., 2002; Zanuncio et al., 2004; Silva et al., 2009; Pires et al., 2011). The insecticides methamidophos (organophosphate) and deltamethrin (pyrethroids) were less toxic to *A. gemmatalis* and more toxic to predators; more recent compounds such as the bioinsecticide spinosad and, mainly chlorantraniliprole that showed the highest toxicity to this pest and lower toxicity to predators. Higher toxicity of the insecticides methamidophos and deltamethrin is mainly due to the wide action spectrum of these insecticides that, in general, have lower selectivity in favor of non-target species (Desneux et al., 2007; Cordeiro et al., 2010; Biondi et al., 2012a).

Spinosad showed better safety profile than deltamethrin and methamidophos, but its selectivity to non-target arthropods is disputable. Biondi et al. (2012b) reported that 71% of the reviewed studies indicated significant lethal effect of spinosad on predators (under laboratory conditions). In addition, the mortality of *P. maculiventris* adults increased from 20% in 24 h to 84% in 48 h and 100% in 72 h when exposed to residues of spinosad on glass surfaces (Viñuela et al., 2001), which also confirm results that pesticides are more toxic on inert materials than vegetable substrates (plant) (Desneux et al., 2005; Dagli and Bahsi, 2009). Plant enzymes may reduce the toxicity of the insecticide (Schuler, 1996), which can be absorbed by the waxy cuticle layer of leaves making them less available for natural enemies (Desneux et al., 2005).

The diamide chlorantraniliprole showed low toxicity to *P. nigrispinus* and *S. cincticeps* nymphs after 500 h exposure to dried residues of this insecticide and showed no mortality greater than 10% using 10x the recommended label rate after 72 h exposure. This lower toxicity for these predators was expected for chlorantraniliprole because of its high affinity towards Lepidoptera ryanodine receptors due to the conformation and structure of the insecticide molecule (Nauen, 2006; Lahm et al., 2009). Chlorantraniliprole was also reported showing great selectivity to parasitoids, predators and mites (Dinter et al., 2008; Preetha et al., 2010; Campos et al., 2011; Biondi et al., 2012a).

Effects on behavior arising from neurotoxic compounds are not surprising and should be considered, since nerve interactions can be affected by sublethal amounts of insecticides and trigger distinct behavioral responses in comparison to individuals not exposed to insecticides (Haynes, 1988; Desneux et al., 2007; Braga et al., 2011). The insecticides used reduced the locomotor activity of *S. cincticeps* nymphs which may be an adaptive behavior that allows a lower direct exposure of predators to toxic residue (Campos et al., 2011), which did not occur in *P. nigrispinus* nymphs. Pesticides causing behavioral locomotory changes have been described in other species and can result in significant reduction in capture efficiency of the pest and its mating in areas sprayed with pesticides (Cordeiro et al., 2010; Evans et al., 2010; Griesinger et al., 2011; Biondi et al., 2012a,b; He et al., 2012).

Behavioral avoidance to insecticides is desirable in natural enemies because it reduces the exposure and increases survival in field conditions (Haynes, 1988; Desneux et al., 2007; Cordeiro et al., 2010; Campos et al., 2011). Insecticide repellence was not observed. However, predators showed significant insecticide irritability to the insecticides methamidophos, spinosad and, in the case of *S. cincticeps*, also to deltamethrin, which under field conditions can increase the survival of these predators to these insecticides because they are extremely toxic in the tested conditions in the laboratory (Cordeiro et al., 2010). However, despite of the arthropod predators avoiding insecticide contact, changes in locomotory behavior can affect the population dynamics, foraging and reproductive success of those individuals (Evans et al., 2010; Griesinger et al., 2011; He et al., 2012). Pesticides can affect the chemical communication between arthropods and reduce the ability of predators to locate their partners for mating (Griesinger et al., 2011) and consumption of pests (He et al., 2012).

In summary, we assessed the lethal and sublethal (mobility) effects of four insecticides used to control *A. gemmatalis* towards two pentatomid predators, *P. nigrispinus* and *S. cincticeps*. The compounds of the new generation of insecticides, especially the chlorantraniliprole, were more toxic to *A. gemmatalis* and less toxic to predators than those traditional insecticides such as organophosphates and pyrethroids. This pattern, though less obvious, was also found in behavioral walking bioassays where predators had more abrupt behavioral changes when exposed to residues of methamidophos and deltamethrin. The same pattern may also take place with other behavioral traits relevant for predator population growth and biological control (e.g., mating behavior, prey foraging etc), which deserves more attention. Thus, our results reinforce the need for replacement of the insecticides methamidophos and deltamethrin by more selective compounds such as chlorantraniliprole, which have lower toxicity to non-target organisms and hence allowing more sustainable IPM programs.

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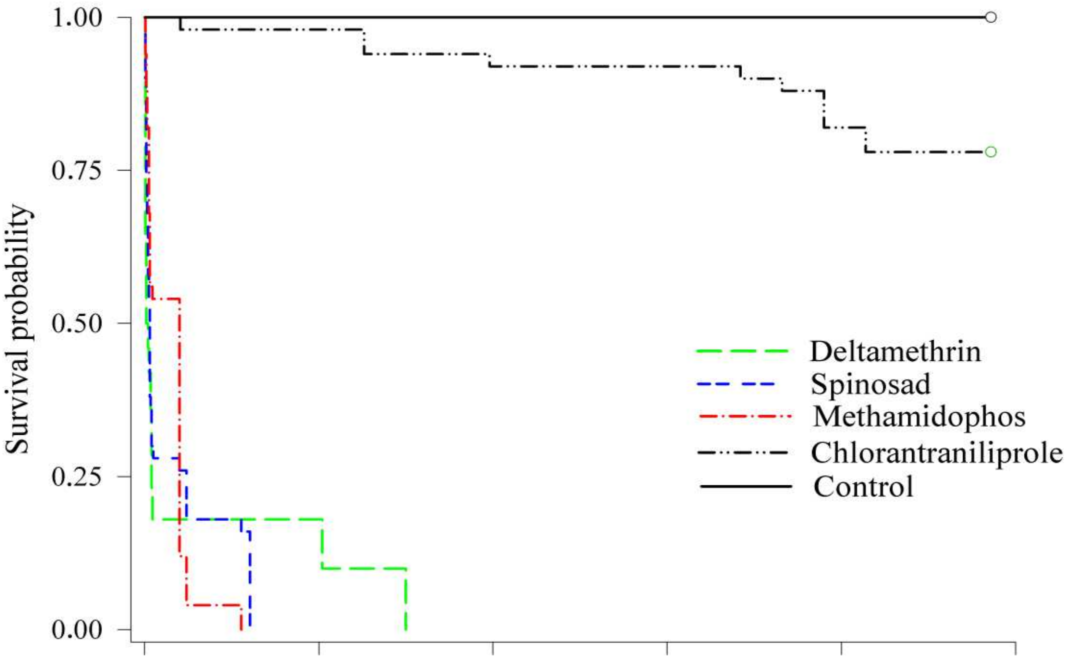
Table 1. Relative toxicity of four insecticides to third-instar velvetbean *Anticarsia gemmatalis* (Lepidoptera: Erebidae) and relative toxicity and selectivity (related to the velvetbean toxicity data) of four insecticides to third-instar *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae)

Insect	Insecticides	No. insects	Slope (SE)	LC_{50} (95% FL) µg a.i./mL	Relative toxicity (95% CI)	Differential selectivity (95% CI)	χ^2	*P*
Anticarsia gemmatalis	Spinosad	224	1.23 (0.16)	0.01 (0.01-0.02)	1.00 (0.56-1.79)	-	5.63	0.34
	Chlorantraniliprole	256	0.99 (0.13)	0.46 (0.30-0.69)	32.20 (18.24-56.84)	-	2.25	0.90
	Methamidophos	256	1.87 (0.30)	10.50 (6.68-16.32)	739.43 (436.76-1251.83)	-	10.87	0.09
	Deltamethrin	256	2.76 (0.29)	15.25 (12.72-18.73)	1074.07 (683.17-1688.62)	-	5.69	0.46
Podisus nigrispinus	Chlorantraniliprole	256	-	-	-	-	-	-
	Methamidophos	288	2.14 (0.23)	18.45 (15.04-22.80)	1.00 (0.75-1.33)	1.76 (1.19-2.59)	5.14	0.64
	Deltamethrin	160	1.83 (0.33)	36.04 (25.77-61.47)	1.95 (1.25-3.05)	2.36 (1.52-3.66)	2.82	0.42
	Spinosad	224	2.19 (0.26)	49.86 (39.29-62.28)	2.70 (2.00-3.66)	3512.54 (2199.33-5609.84)	4.73	0.45
Supputius cincticeps	Chlorantraniliprole	256	-	-	-	-	-	-
	Deltamethrin	192	1.83 (0.24)	8.36 (6.18-10.96)	1.00 (0.68-1.48)	0.55 (0.39-0.77)	3.14	0.53
	Methamidophos	256	1.74 (0.22)	19.80 (15.34-25.44)	2.37 (1.64-3.43)	1.89 (1.25-2.84)	1.47	0.96
	Spinosad	256	2.01 (0.21)	47.98 (38.18-60.29)	5.74 (4.02-8.19)	3379.88 (2116.67-5396.98)	6.09	0.41

(A) *Podisus nigrispinus*



(B) *Supputius cincticeps*

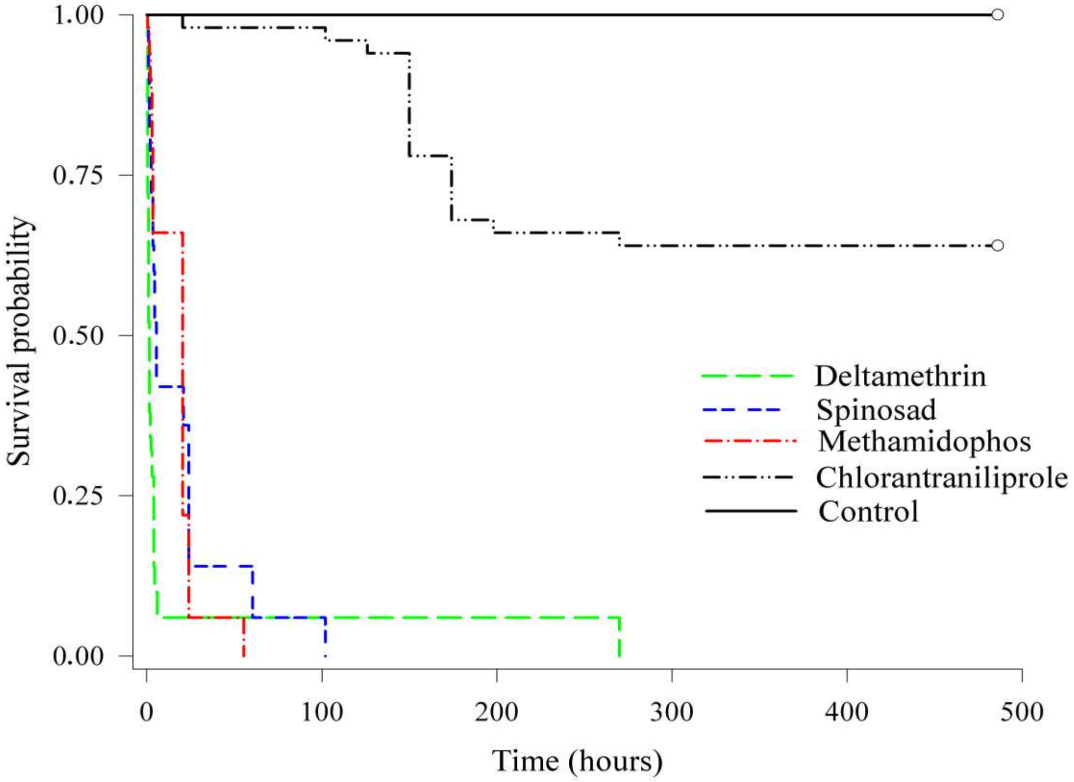


Figure 1. Survival curves of two predatory stinkbug species, *Podisus nigrispinus* (A) and *Supputius cincticeps* (B) (Heteroptera: Pentatomidae), exposed to chlorantraniliprole, deltamethrin, methamidophos, spinosad, and water (control).

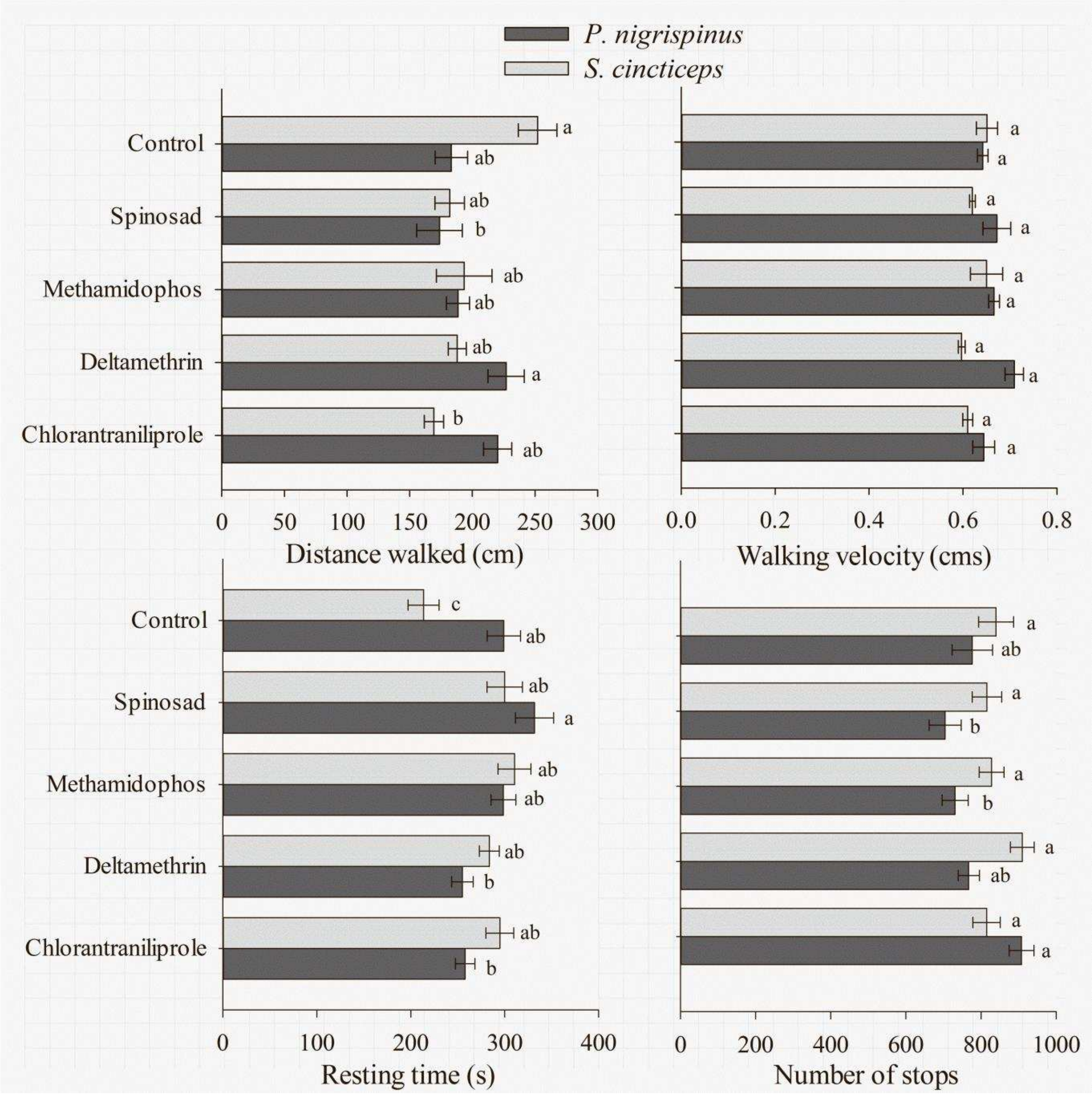


Figure 2. Distance walked (±SEM), walking velocity (±SEM), resting time (±SEM) and number of stops (±SEM) during 10 min exposure of third-instar *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae) on filter paper arenas (9 cm diameter) fully-treated with dried insecticide residues. Bars with the same letter do not differ significantly (Tukey's HSD test at $p < 0.05$).

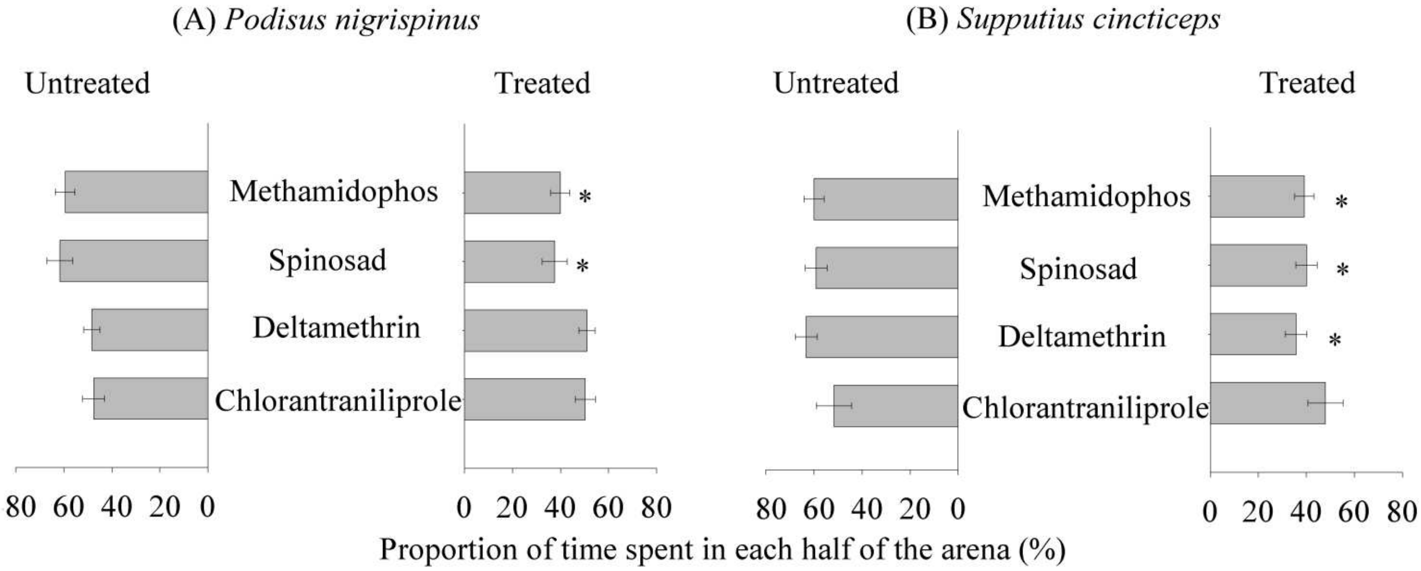


Figure 3. Proportion of time spent by third-instar *Podisus nigrispinus* (A) and *Supputius cincticeps* (B) during 10 min exposure in each half of filter paper arenas (9 cm diameter) half-treated with dried insecticide residues. An asterisk in the bar indicates significant difference between the insecticide-treated and untreated halves of the arena (paired Student's *t* test at $p < 0.05$).

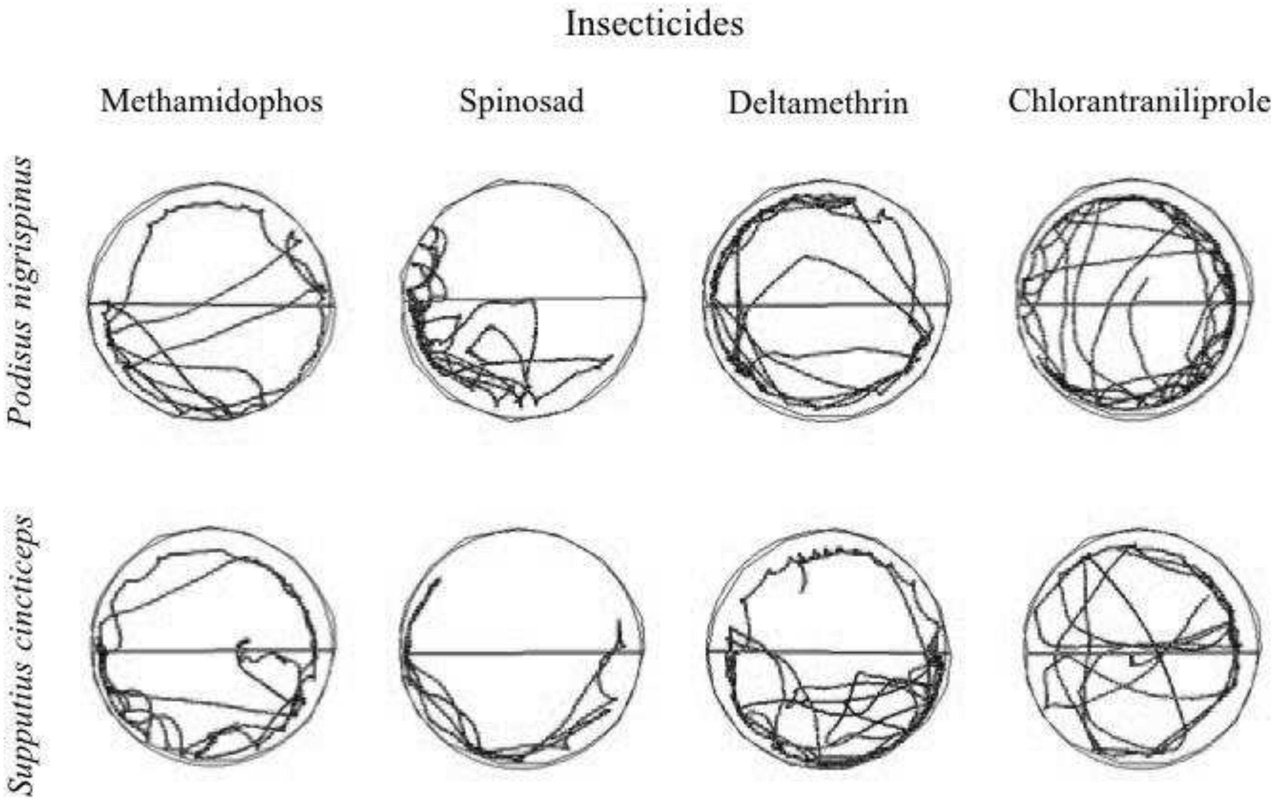


Figure 4. Representative tracks showing the movement of individual predatory stinkbug third-instar *Podisus nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae), over a 10 min period on paper-filter arenas (9 cm diameter) half-impregnated with dried insecticide residues (upper half of each arena).

Capítulo II

Life table of the insecticide-exposed predator *Podisus nigrispinus* (Heteroptera: Pentatomidae): Implications for IPM

Life table of the insecticide-exposed predator *Podisus nigrispinus* (Heteroptera: Pentatomidae): Implications for IPM

Abstract

The predator *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) shows potential for Integrated Pest Management programs of defoliating caterpillars in agricultural and forestry systems. Insecticides can indirectly affect caterpillar predators through consumption of treated prey. The survival, reproduction and life table parameters of *P. nigrispinus* fed on caterpillars of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae) reared on soybean leaves previously exposed to four insecticides widely used in this crop (chlorantraniliprole, deltamethrin, methamidophos and spinosad) were evaluated. Caterpillars of *A. gemmatalis* were fed for 12 h with treated soybean leaves and offered to *P. nigrispinus* adults over five consecutive days. Spinosad and methamidophos were not compatible with *P. nigrispinus* in IPM programs in the soybean agro-ecosystem. Deltamethrin was slightly toxic and chlorantraniliprole can be considered the most promising insecticide due to lower toxicity to this predator.

Keywords: *Anticarsia gemmatalis*, Asopinae, IPM, Predatory stinkbugs, Risk assessment

Resumo

O predador *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) demostra potencial para programas de manejo integrado de pragas de lagartas desfolhadoras em sistemas agrícolas e florestais. Inseticidas podem afetar indiretamente os predadores através do consumo de presas tratadas. A sobrevivência, reprodução e os parâmetros de tabela de vida do predador *P. nigrispinus* alimentado em lagartas de *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Erebidae) criadas em folhas da soja previamente expostas a quatro inseticidas utilizados nesta cultura, como clorantraniliprole, deltametrina, espinosade e metamidofós, foram avaliados. Lagartas de *A. gemmatalis* foram alimentadas por 12 h com folhas de soja tratadas e oferecidas a adultos de *P. nigrispinus* durante cinco dias consecutivos. Espinosade e metamidofós não são compatíveis com *P. nigrispinus* em programas de MIP em soja, enquanto deltametrina foi levemente tóxico e clorantraniliprole o mais promissor devido à menor toxicidade para este predador.

Palavras-chave: *Anticarsia gemmatalis*, Asopinae, avaliação de risco, MIP, percevejos predadores.

1. Introduction

Generalist predators are known worldwide for their ability to control insect pests in cultivated crops (Symondson et al. 2002). For example, most Asopinae (Heteroptera: Pentatomidae) are predatory stinkbugs with key role in management of pests such as lepidopteran larvae (Ribeiro et al. 2010, Zanuncio et al. 2008) in greenhouses and field, even against herbivorous Pentatomid species (De Clercq et al. 2002). These predators can build up their populations before pests arrive using host plants (Coll and Guershon 2002) and alternative prey as food sources (Zanuncio et al. 2005). *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), a generalist predator native to Central and South America (Thomas 1992, Silva et al. 2009), has potential for the Integrated Pest Management (IPM) programs (Matos-Neto et al. 2002, Zanuncio et al. 2008).

In the soybean agro-ecosystem, and despite the potential effectiveness of biological control, many producers commonly use pesticides noxious to beneficial arthropods (Desneux et al. 2007) as the main pest control method (Song and Swinton 2009). These insecticides include cyclodienes, organophosphates and pyrethroids (Baur et al. 2010). New compounds developed (Nauen and Bretschneider 2002) with biopesticides and biorational pesticides are receiving attention (Rosell et al. 2008, Chandler et al. 2011).

An alternative to conventional pest control is IPM, which aims to reduce the pest population to tolerable levels with different methods (Van Lenteren and Woets 1988). Biological control with parasitoids, social wasps (Prezoto et al. 2006) and predators (Silva et al. 2009), plant resistance (Meissle et al. 2011) and pesticides when required are combined in IPM. The insecticides should be selectively with control strategies to maintain agriculture sustainability (Zalucki et al. 2009). IPM and biological control can enhance sustainability by reducing dependence on chemicals (Kogan 1998, Bueno et al. 2011).

The compatibility of pesticides with natural enemies is important in IPM programs (Arnó and Gabarra 2011). Insecticide compatibilities have been demonstrated for methoxyfenozide, pyriproxyfen and spinosad with *Picromerus bidens* L. (Heteroptera: Pentatomidae) (Mahdian et al. 2007); deltamethrin and *Bacillus thuringiensis* with *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) (Mohaghegh et al. 2000); chlorantraniliprole and deltamethrin were slightly toxic to *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) (Campos et al. 2011); and low permethrin doses were beneficial for *Podisus distinctus* (Stal) (Heteroptera: Pentatomidae) (Zanuncio et al. 2013). On the other hand, the pyrethroid gamma-cyhalothrin was toxic (Pereira et al. 2005) and the growth regulator diflubenzuron reduced *P. nigrispinus* fertility (Castro et al. 2012).

Tarsal contact of predators with pesticide residues on plants is the main exposure route of these natural enemies during foraging (Mahdian et al. 2007). However, direct contact of spray droplet, ingestion of insecticides or plant sap contaminated or by feeding on contaminated prey (Mahdian et al. 2007, Cloyd and Bethke 2011) can also affect natural enemies. Life tables may be used to evaluate sublethal effects of pesticides on the demography of the target and non-target species (Stark and Banks 2003, Stark et al. 2007). Sublethal effects on population dynamics may be unnoticed because they can affect the fertility of individuals (Perveen 2008) even with low mortality, as reported for *P. nigrispinus* with diflubenzuron (Castro et al. 2012). The aim of the present work was to evaluate the survival, reproduction and life table parameters of *P. nigrispinus* fed on caterpillars of *A. gemmatalis*, a pest of soybean, exposed to some traditional insecticides (the pyrethroid deltamethrin and the organophosphate methamidophos) in addition to more recent compounds (the spinosyn spinosad and the diamide chlorantraniliprole).

2. Materials and Methods

2.1. Insects

The predator *P. nigrispinus* and the prey *A. gemmatalis* were obtained from mass-reared cultures from the Laboratory of Biological Control of Insects (LCBI) of the Institute of Applied Biotechnology in Agriculture (BIOAGRO) at the Federal University of Viçosa (UFV) in Viçosa, Minas Gerais State, Brazil. This predator is reared with the yellow mealworm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) pupae under controlled environmental conditions (25 ± 2 °C, 70 ± 5% relative humidity, and 12:12 light: dark photoperiod) (Zanuncio et al. 2005). Caterpillars of *A. gemmatalis* are reared on artificial diet (Greene et al. 1976) and their adults in wooden cages (30 x 30 x 30 cm) with screened sides, glass covers and fed cotton soaked in nutrient solution at the bottom of the cages.

2.2. Insecticides

All of the insecticides used are registered to control *A. gemmatalis* in Brazilian soybean fields (Agrofit 2012). The insecticides used and their respective commercial formulations were: the pyrethroid deltamethrin (Decis® 25 EC; 25 g a.i./L; Bayer CropScience Ltd.; São Paulo-SP), the organophosphate methamidophos (Tamaron® BR SC; 600 g a.i./L; Bayer CropScience Ltd.;

Belford Roxo-RJ), the diamide chlorantraniliprole (Premio® CS; 200 g a.i./L; DuPont Brasil S.A.; Barra Mansa-RJ) and the spinosyn spinosad (Tracer® 480 CS; 480 g a.i./L; Dow AgroSciences Industrial Ltd.; São Paulo-SP).

2.3 Reproduction and life table bioassays

Males and females *P. nigrispinus* were individualized for three days after their emergence until sexually maturation (Castro et al. 2012). Afterwards, fifteen pairs of *P. nigrispinus* were placed individually per treatment in plastic pots (500 mL) with water provided through 2.5 mL tubes. Males of the same treatment and conditions substituted those that died before their respective females.

Soybean leaves of the cultivar "BRSMT pintado" were immersed for five seconds in a solution with one of the insecticides: chlorantraniliprole (13.3 µg a.i./mL), deltamethrin (50 µg a.i./mL), spinosad (240 µg a.i./mL) and methamidophos (1500 µg a.i./mL) and then the leaves were let to dry in shade for an hour. Third-instar *A. gemmatalis* caterpillars were fed on the treated soybean leaves for 12 h and presented to each *P. nigrispinus* couple for five days following the mating period (one caterpillar per day) (Castro et al. 2012). The control had third-instar *A. gemmatalis* caterpillars fed on soybean leaves dipped in water. Following the five day trial, each *P. nigrispinus* couple was fed two *T. molitor* pupae every other day until their natural death.

The egg masses of *P. nigrispinus* were removed from the plastic pots and nymph hatch observed, daily. The preoviposition, oviposition and post-oviposition periods; the number of eggs and nymphs per egg mass; the total number of eggs, nymphs and egg mass per female; egg viability; incubation period and, longevity of *P. nigrispinus* female were grouped into three days age classes and used to construct a life table for this predator. Data of the reproductive parameters were subjected to the analysis of variance (ANOVA) and the means compared using the Tukey's test ($P < 0.05$).

The life table parameters were calculated with Krebs formulas (1994): (1) the net reproductive rate (R_0) (number of females produced per female during its life), $R_0 = \sum_{x=0}^{y} l_x m_x$; where l_x is the probability of survival from birth to age x per day per age class during immature and adult stages, and m_x is the number of females produced per female of age x and the following older class y; (2) generation duration (D) (time between the birth of the parents to that of their progeny), $D = \ln(R_0) / r_m$; (3) intrinsic rate of population increase (r_m) (population rate of increase per unit of time), $r_m = \ln(R_0) / D$; and (4) the time necessary for the *P. nigrispinus* population to double in size (T), $T = \ln(2) / r_m$. These parameters were analyzed ($P < 0.05$) using the SAS statistical program (SAS Institute 2000) and the Jackknife procedure to compare the parameters with a t-test (Maia et al. 2000).

3. Results

Reproduction and life table parameters of *P. nigrispinus* were not obtained for spinosad and methamidophos due to high mortality of females of this predator: 90% and 95% after three and four days of feeding on caterpillars treated with these insecticides, respectively.

3.1 Effects on reproduction

The pre, post and oviposition periods, incubation period, longevity, egg viability, numbers of eggs and nymphs per egg mass and of egg masses were similar with chlorantraniliprole, deltamethrin and the control (Table 1).

The numbers of eggs (F= 5.308; df= 2,30; P= 0.0106) and nymphs (F= 5.35; df= 2,30; P= 0.010) per *P. nigrispinus* female were higher with chlorantraniliprole and the control than with the deltamethrin (Table 1). The number of eggs and nymphs per female per day of *P. nigrispinus* showed a peak at the beginning of their reproductive cycle of females for chlorantraniliprole, deltamethrin and control (Figs. 1 a, b). Low peaks of egg production at the end of the female reproductive life cycle were also observed (Figs. 1 a, b).

The survival curves of *P. nigrispinus* were similar in the control and with the chlorantraniliprole (Fig. 1c), indicating that this insecticide does not increase or decrease longevity of this predator. Futhermore, the chlorantraniliprole did not reduce reproductive parameters of this predator (Table 1). The survival curve with deltamethrin was also similar to the control, but this insecticide reduced the eggs and nymphs production of this predator (Table 1, Fig. 1).

3.2 Life table parameters

The generation duration (*D*) and the time necessary for the *P. nigrispinus* population to double in size (*T*) were similar with chlorantraniliprole, deltamethrin and the control (Table 2). However, the net reproductive rate (R_0) and intrinsic rate of population increase (r_m) were lower with deltamethrin (62.5 and 0.12 respectively) than with chlorantraniliprole (95.9 and 0.13 respectively) and the control (115.2 and 0.14 respectively) (Table 2).

4. Discussion

The survival, reproduction and life table parameters of the predatory stinkbug *P. nigrispinus* are important to determine the safety of insecticides registered for controlling *A. gemmatalis*. Older insecticides like the organophosphate methamidophos and the newer compound, the spinosyn spinosad were harmful to the predator *P. nigrispinus*. In contrast, the pyrethroid deltamethrin was slightly harmful. However, a promising safety profile was observed for chlorantraniliprole, a novel compound available on the market.

The greatest number of eggs and nymphs per *P. nigrispinus* female with chlorantraniliprole and the control than with deltamethrin are in agreement with studies reporting that the offspring of *Orius laevigatus* (Fieber) (Hemiptera: Anthocoridae) with chlorantraniliprole did not differed from the control (Biondi et al. 2012a) and, this insecticide has shown selectivity to natural enemies (Campos et al. 2011, Preetha et al. 2009, De Castro et al. 2013). This low toxicity was expected for chlorantraniliprole, because of its high affinity for ryanodine receptors due to the structure and conformation of the insecticide molecule (Nauen 2006, Lahm et al. 2009). In contrast, pyrethroids are usually very toxic to beneficial arthropods (Croft 1990, Cordeiro et al. 2010). Indeed, the deltamethrin disrupted the ability of *Anagrus nilaparvatae* (Pang et Wang) (Hymenoptera: Mymaridae) to perceive host-plant odor cues (Liu et al. 2012). The broad-spectrum neurotoxic insecticides deltamethrin could affect the reproduction of *P. nigrispinus* but not the novel insecticides chlorantraniliprole.

The similar peaks pattern for the number of eggs and nymphs per surviving female per day for chlorantraniliprole, deltamethrin and the control has been previously observed in *P. nigrispinus* fed on caterpillars of *A. gemmatalis* reared on soybean leaves exposed to diflubenzuron (Castro et al. 2012) and fed on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) (Vivan et al. 2002). Non-social insects show during the adult stage a preoviposition period, followed by reproductive stage maximum and a decline with insect age. In addition, ovary activation in predatory stinkbugs occurs after mating (7-d old females) with a reproductive peak in 21-d old females (Lemos et al. 2009). Sousa-Souto et al. (2006) reported that multiple matings are important for the reproductive success of *P. nigrispinus* females and the constant availability of males enables females to increase their fertility by up to 50%. Thus, numbers of egg and nymph peaks at the end of the reproductive stage of *P. nigrispinus* could be related to the replacement of males that died before their female mates.

The survival curves of *P. nigrispinus* showed that spinosad and methamidophos caused elevated mortality of *P. nigrispinus* females, 90% and 95% after three and four days of feeding on caterpillars treated with these insecticides, respectively. Spinosad has caused controversy in relation to its toxicity to natural enemies. The U.S. Environmental Protection Agency (EPA) classifies spinosad as a low risk toxicological and environmental insecticide (EPA 1997). A total of 71% and 34% of the studies indicated lethal effect of spinosad on predators under laboratory and, field and semi-field conditions, respectively (Biondi et al. 2012b). Spinosad caused 10% mortality of *Geocoris punctipes* (Say) (Heteroptera: Pentatomidae) after 72 h treatment of feeding with *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) caterpillars fed for six hours on treated soybean leaves (Boyd and Boethel 1998) and low mortality of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on prey treated with this insecticide (Galvan et al. 2006). However, selectivity of spinosad on predators is under discussion because earwigs *Doru taeniatum* (Dohrn) (Dermaptera: Forficulidae) suffered 86% mortality/intoxication 72 h after feeding on spinosad-treated *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae) larvae (Cisneros et al. 2002). Futhermore, 72 h after treatment, spinosad at the maximum concentration recommended (800 mg a.i. litre^{-1}) reduced the number of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) adults by 39.8% and 87.2% in topical and ingestion treatments (Medina et al. 2003). Mortality of *P. maculiventris* nymphs was found when treated via ingestion and topical treatments of spinosad from 15 and 50 mg a.i. litre^{-1} onwards, respectively (Viñuela et al. 1998). The safety profile of spinosad is unclear, although some differences might be explained because results in the laboratory can be different from those obtained in the field (Biondi et al. 2012b). Organophosphates are toxic to insects because of their ability to inactivate acetylcholinesterase (Fukuto 1990). The high mortality of *P. nigrispinus* with methamidophos is mainly due to the broad action spectrum of this insecticide rendering it as not compatible with natural enemies (Bacci et al. 2007, Preetha et al. 2009, Wang et al. 2012). Therefore, organophosphates should be replaced with relatively safe plant-protection products in IPM programs.

The survival and fertility rates of *P. nigrispinus* showed no impact of chlorantraniliprole, but the life table parameters showed reduction in the reproductive capacity of *P. nigrispinus* with deltamethrin. The sublethal effects of the insecticides chlorantraniliprole and the deltamethrin on *P. nigrispinus* can be explained by using life table parameters that show how its population dynamics may be affected (Castro et al. 2012). The reduced fertility shown by *P.*

nigrispinus exposed to deltamethrin resulted from a reduction in the number of eggs and nymphs per female and, other life table parameters such as R_0 and r_m. The positive values of R_0 (>1.0) and r_m with chlorantraniliprole and deltamethrin indicate a potential for population increase of this predator with these insecticides (Medeiros et al. 2000, 2003; Castro et al. 2012). However, the lower net reproductive rate (R_0) and intrinsic rate of population increase (r_m) of *P. nigrispinus* fed on caterpillars exposed to deltamethrin demonstrate a serious effect of this insecticide on the capacity for population increase of this natural enemy, similar to that found for *P. nigrispinus* fed on caterpillars exposed to diflubenzuron (Castro et al. 2012). Thus, deltamethrin adversely affects the reproduction of this predator and its use in IPM programs should be studied further.

The lethal and sublethal effects of traditional pesticides and newer compounds on the generalist predator *P. nigrispinus* via treated prey varied widely. *Podisus nigrispinus* was susceptible to spinosad and methamidophos, notably because of high mortality observed in adults. Spinosad and methamidophos were incompatible with this predator for IPM. Deltamethrin was less toxic but still reduced offspring. Finally, chlorantraniliprole was harmless with mortality and reproductive capacity levels similar to that of the untreated control group. Furthermore, chlorantraniliprole can be the most promising insecticide for IPM programs because of its lower toxicity to this predator. Consequently, specific risk assessment and field studies to assessment of the safety of these compounds to predatory stinkbugs should be undergone before implementing any IPM programs.

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Table 1. Reproductive parameters (Mean ± SEM) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) females fed on *Anticarsia gemmatalis* (Lepidoptera: Erebidae) caterpillars reared on soybean leaves treated with chlorantraniliprole (13.3 ppm), deltamethrin (50 ppm) and untreated leaves (Control)

Reproductive parameters	Chlorantraniliprole	Deltamethrin	Control
Number of eggs/female	300.18 ± 34.99 a	177.55 ± 30.61 b	318.18 ± 33.89 a
Number of nymphs/female	274.64 ± 34.82 a	159.64 ± 27.73 b	290.27 ± 29.53 a
Preoviposition period (days)^{ns}	9.00 ± 0.74	10.73 ± 0.52	8.45 ± 0.87
Oviposition period (days)^{ns}	24.73 ± 3.34	15.64 ± 3.45	24.00 ± 3.04
Post-oviposition period (days)^{ns}	3.09 ± 0.73	2.09 ± 0.37	1.55 ± 0.28
Longevity (days)^{ns}	36.82 ± 3.60	28.45 ± 3.80	34.00 ± 2.96
Egg viability (%)^{ns}	90.41 ± 1.61	88.79 ± 2.24	94.45 ± 1.65
Incubation period (days)^{ns}	5.01 ± 0.02	5.01 ± 0.01	5.00 ± 0.01
Number of eggs/egg mass^{ns}	19.83 ± 1.76	17.37 ± 1.22	19.92 ± 1.86
Number of nymphs/egg mass^{ns}	17.91 ± 1.58	15.70 ± 1.17	18.26 ± 1.74
Number of egg masses^{ns}	15.91 ± 1.83	10.36 ± 1.85	17.55 ± 2.62

^{ns} Not significant. Means followed by the same letter within rows, do not differ by Tukey's test at 5%.

All predators in the spinosad and methamidophos treatments died before oviposition.

Table 2. Life table parameters (Mean ± SEM) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) females fed on *Anticarsia gemmatalis* (Lepidoptera: Erebidae) caterpillars reared on soybean leaves treated with chlorantraniliprole (13.3 ppm), deltamethrin (50 ppm) or untreated leaves (control)

Treatments	R_0	D	T	r_m
Chlorantraniliprole	95.94 ± 11.18 a	35.59 ± 1.68 a	5.40 ± 0.18 a	0.13 ± 0.004 a
Deltamethrin	62.50 ± 10.77 b	33.55 ± 1.25 a	5.60 ± 0.15 a	0.12 ± 0.003 b
Control	115.21 ± 12.27 a	34.19 ± 1.05 a	4.99 ± 0.10 a	0.14 ± 0.003 a

Means per column followed by the same letter do not differ (test at 5% probability). R_0- number of females produced per female during its life; D- generation duration; T- time necessary for the *P. nigrispinus* population to double in size; r_m- population rate of increase per unit of time.

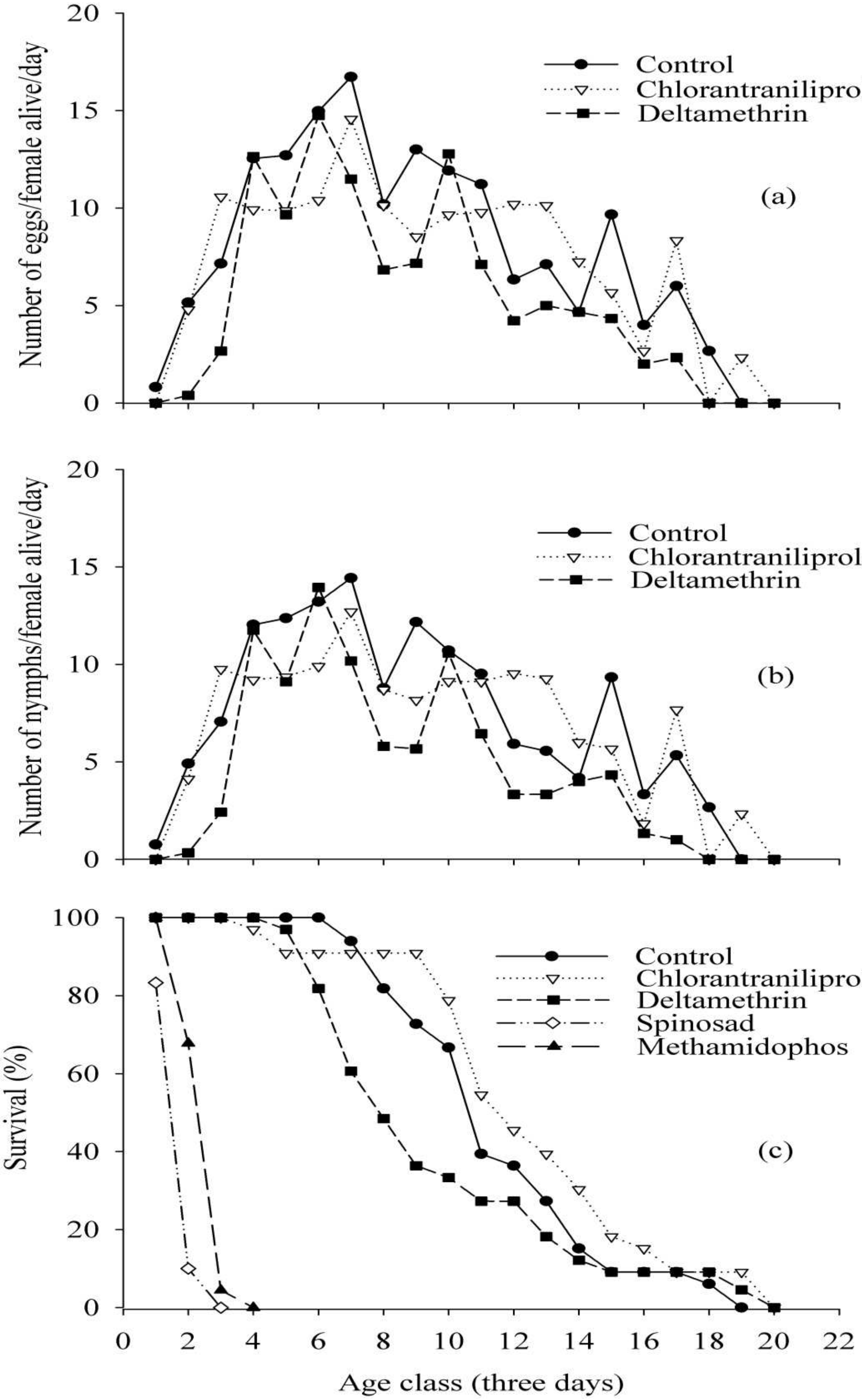

Figure 1. Number of eggs (a), nymphs (b) and survival (c) of *Podisus nigrispinus* (Heteroptera: Pentatomidae) fed on *Anticarsia gemmatalis* (Lepidoptera: Erebidae) caterpillars reared on soybean leaves exposed to insecticides and untreated control.

Capítulo III

Evaluation of organically acceptable insecticides and chlorantraniliprole for the *Spodoptera exigua* (Lepidoptera: Noctuidae), and its predator, *Podisus maculiventris* (Heteroptera: Pentatomidae)

Evaluation of organically acceptable insecticides and chlorantraniliprole for the *Spodoptera exigua* (Lepidoptera: Noctuidae), and its predator, *Podisus maculiventris* (Heteroptera: Pentatomidae)

Abstract

The beet armyworm, *Spodoptera exigua* (Hübner) is one of the major insect pests of vegetables around the world, and resistant to various classes of chemical insecticide. Selective insecticides are required to control *S. exigua* in integrated pest management (IPM) programs. In addition, biological control of this pest using predatory stinkbugs has shown promise as a control tactic. The toxicity of botanical insecticides approved by the Organic Materials Review Institute (OMRI) against *S. exigua* and *P. maculiventris* was evaluated under laboratory conditions. Insecticides evaluated were Azera® (pyrethrin and azadirachtin), PyGanic® (pyrethrin), Entrust® (spinosad) and one non-OMRI-listed formulation, chlorantraniliprole Coragen® (diamine). Entrust® and Coragen® showed higher toxicity to the pest compared to the predator and PyGanic® and Azera® showed higher toxicity to the predator compared to the pest using glass-vials bioassays. Coragen® also had the highest toxicity against *S. exigua* using diet incorporation bioassays, followed by Entrust®, PyGanic® and Azera®. The oral toxicity bioassays showed that Entrust® had the highest toxicity against *P. maculiventris* followed by PyGanic®, Azera® and Coragen®. The notion that natural compounds are safer than synthetic compounds to non-target species is refuted, which showed that the synthetic insecticide Coragen® was less toxic than the natural insecticides PyGanic®, Azera® and Entrust®. Therefore, certain bioinsecticides should not be exempted from risk assessment schemes, and non-target sub-lethal effects should not be neglected when considering potential insecticide use in integrated pest management programs.

Keywords: Beet armyworm, biological control, botanicals, integrated pest management, natural enemies, OMRI

Resumo

Spodoptera exigua (Hübner) é uma das maiores pragas de vegetais em todo o mundo, e tem sido documentada resistente a várias classes de inseticidas. Inseticidas seletivos são necessários para programas de manejo integrado de pragas (MIP) para controlar *S. exigua*. Além disso, o controle biológico dessa praga utilizando percevejos predadores tem se mostrado promissor como uma tática de controle. A toxicidade de inseticidas botânicos aprovados pelo Organic Materials Review Institute (OMRI) contra *S. exigua* e *P. maculiventris* em condições de laboratório foi avaliada. Inseticidas avaliados foram Azera® (piretrina e azadiractina), PyGanic® (piretrina), Entrust® (espinosade) e uma formulação não listada no OMRI, Coragen® (diamina). Entrust® e Coragen® apresentaram maiores toxicidade para a praga que para o predador e PyGanic® e Azera® maiores toxicidade para o predador que para a praga utilizando os bioensaios com frascos de vidro. A toxicidade do Coragen® foi, também, maior contra *S. exigua* utilizando bioensaios de incorporação de dieta, seguido por Entrust®, PyGanic® e Azera®. Os bioensaios de toxicidade oral mostraram maior toxicidade do Entrust®contra *P. maculiventris* seguido por PyGanic®, Azera® e

Coragen®. A noção de que os compostos naturais sejam mais seguros que os compostos sintéticos para espécies não-alvo é refutada, sendo o inseticida sintético Coragen® menos tóxico que os naturais PyGanic®, Azera® e Entrust®. Portanto, certos bioinseticidas não devem ser isentos de avaliações de risco, e os efeitos sub-letais não-alvo não devem ser negligenciados quando se considera a utilização de inseticidas potenciais em programas de manejo integrado de pragas.

Palavras-chave: controle biológico, inimigos naturais, inseticidas botânicos, manejo integrado de pragas, OMRI

1. Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is a major insect pest of vegetables and widely distributed around the world (Zheng et al., 2011; Lai et al., 2011). It damages many cultivated crops such as bean, corn, cotton, onion, peanut, potato, soybean, tomato and others. This insect is originally from Southeast Asia. It was first discovered in North America (Oregon) in 1876, and it was found in Florida in 1924 (Capinera, 2001). Insecticide application is the most common method to control this pest species, however, the control achieved using chemicals is not completely successful due to resistance to various classes of chemical insecticide (Brewer and Trumble, 1989; Moulton et al., 2000; Osorio et al., 2008; Ahmad and Arif, 2010; Lai and Su, 2011). Therefore, alternative tools for *S. exigua* control are required for use with integrated pest management (IPM) programs. Promising control tactics against *S. exigua* larvae under field and greenhouse conditions are the use of natural enemies and alternative chemicals that are effective against this pest, safe to humans and wild life, environmentally friendly and compatible with biocontrol agents.

Natural enemies have adapted to attack *S. exigua*, including parasitoids and predators. The most common predators are the minute pirate bugs, *Orius* spp. (Hemiptera: Anthocoridae); big-eyed bugs, *Geocoris* spp. (Hemiptera: Lygaeidae); damsel bugs, *Nabis* spp. (Hemiptera: Nabidae); and a predatory spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) (Capinera, 2001). *Podisus maculiventris* is a generalist predator used in augmentative releases to control pests in agricultural and forest ecosystems (Biever and Chauvin, 1992; Tipping et al., 1999). This natural enemy prey on eggs and larvae of over 100 Coleoptera and Lepidoptera species (McPherson, 1980). This predator is an important biological agent because of its high reproductive capacity, voracious feeding habits (Hough-Goldstein, 1988; Hough-Goldstein and McPherson, 1996), and selectivity to insecticides (Smagghe and Degheele, 1995; Mohaghegh et al., 2000). *Podisus maculiventris* has demonstrated potential against important pests, including the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Biever and Chauvin, 1992); the tomato looper, *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae) (De Clercq et al., 1998); the viburnum leaf beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae) (Desurmont and Weston, 2008); and the yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Coleoptera: Chrysomelidae) (Montemayor and Cave, 2012).

The organic acceptable insecticides against *S. exigua* are poorly studied. Tactics of pest management and formulations approved by the Organic Materials Review Institute (OMRI) could potentially be used to control *S. exigua*. PyGanic® Crop Protection EC 5.0_{II} is an OMRI-listed formulation of pyrethrin with efficacy against *M. ochroloma* and *L. decemlineata* (Barcic et al., 2006; Balusu and Fadamiro, 2012). Entrust® SC is a natural insect control product with spinosad as its active ingredient (Dow Chemical Company, 2001) with efficacy against chrysomelid and lepidopteran pests (Balusu and Fadamiro, 2012; De Castro et al., 2013). Azera® is an OMRI-listed formulation of pyrethrin and azadirachtin with a quick knockdown and acting in the sodium channel inhibitor, disrupting insects nervous system and, as insect growth regulator (MGK Company, 2012). Coragen® (chlorantraniliprole) is an insecticide of the anthranilic diamide class with broader insecticidal activity, against Lepidoptera, Coleoptera, Diptera, Isoptera and Hemiptera pests (Sattelle et al., 2008; Lahm et al., 2009; De Castro et al., 2013).

Efforts are employed to search compounds with low toxicity to biocontrol agents to devise IPM of *S. exigua*. Therefore, it is critical to establish the susceptibility levels of natural enemies and pest populations at the outset even before the widespread use of insecticides. The current study evaluated the toxicity of botanical insecticides approved by the Organic Materials Review Institute (OMRI) and chlorantraniliprole against *S. exigua* and *P. maculiventris* under laboratory conditions for potential use in an integrated pest management.

2. Materials and Methods

2.1. Insects

The predator *P. maculiventris* was obtained from the United States Department of Agriculture, Agricultural Research Service, CMAVE (Center for Medical Agriculture and Veterinary Entomology), Tallahassee, Florida, USA. This natural enemy was fed on the yellow mealworm larvae, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) in a laboratory at 25 ± 2°C, 70 ± 5% relative humidity and a 12:12 light: dark photoperiod. A colony of *S. exigua* was obtained from the CMAVE, USDA-ARS, Gainesville, Florida, USA. *S. exigua* larvae were reared on artificial diet (Guy et al., 1985).

2.2. Insecticides

The insecticides tested included commercial formulations of diamide chlorantraniliprole (Coragen® SC; 18.4% a.i.; DuPont™; Wilmington, DE, USA), the spinosyn spinosad (Entrust® SC; 22.5% a.i.; Dow AgroSciences, Indianapolis, IN, USA), PyGanic® Crop Protection EC 5.0_{II} (5.0% pyrethrins; McLaughlin Gormley King Company®, Minneapolis, MN, USA) and Azera® (1.20% azadirachtin and 1.40% pyrethrins; McLaughlin Gormley King Company®, Minneapolis, MN, USA).

2.3. Glass-vial bioassays

The procedure in this bioassay was that of Kanga et al. (1995). In this procedure, 20-mL glass scintillation vials were treated with a 0.5 mL solution of each of the test insecticides in water. The vials were rolled until the water evaporated and the insecticides coated on the inner surfaces. Vials treated with water were used as the control. A dilution ratio of insecticides to water from 1:1 to $1{:}10^5$ were tested. All insecticides were diluted in distilled water to get the desired concentrations. Three third-instar *P. maculiventris* nymphs or third-instar *S. exigua* larvae were treated at each dose of the insecticides at room temperature (25 ± 2°C and 70 ± 5% RH), and mortality was determined after 24 h exposure. About 210 *P. maculiventris* nymphs and 210 third-instar *S. exigua* larvae were tested per insecticide. *Podisus maculiventris* nymphs or *S. exigua* larvae unable to walk a short distance (up to 10 mm) when released were considered dead.

2.4. Diet incorporation bioassays

The insecticide susceptibility to the beet armyworm was assayed with third-instar *S. exigua* larvae using a diet incorporation method. Seven concentrations per insecticide using serial dilutions were prepared with distilled water. After preparing the diet, 0.5 mL of each diluted insecticide was mixed thoroughly with 1.5 g of artificial diet in a plastic cup (1 oz.). Five third-instar larvae were placed in each cup, and 10 cups prepared per concentration. 350 larvae were used for each insecticide. The cups were covered with paper lids and kept in a room (25 ± 2°C temperature, 70 ± 5% relative humidity and a 12:12 light: dark photoperiod). Larval mortality was evaluated after 24h. Larvae were recorded as dead if they did not respond with head movements or peristaltic contractions when touched with a camel hair brush.

2.5. Oral toxicity bioassays

A dilution ratio of insecticides from 1:1 to $1:10^5$ were diluted in distilled water to get the desired concentrations for the bioassays. Third-instar *P. maculiventris* nymphs were individually (one third-instar nymph per cup) exposed to the concentrations of each insecticide for ingestion through treated drinking water. The insecticide solution was offered to the predators through 0.5 mL cylindrical tubes, inserted in the cover of each plastic cup (1 oz.) at room temperature (25 ± 2°C and 70 ± 5% RH), and mortality was determined after 24 h exposure. About 210 *P. maculiventris* nymphs were tested per insecticide. Control groups received only water. No food was provided prior to the beginning (24 hours) of this experiment and during the test to stimulate the drinking behavior of *P. maculiventris*. Mortality individuals were those without movements.

2.6. Statistical Analyses

The concentration–mortality (glass-vial), diet incorporation and oral toxicity mortality data were subjected to Probit analysis (Russell et al., 1977). Percentage mortality was also adjusted for control mortality (Abbott, 1925). Differences among insecticides were considered significant if the 95% confidence level of the LC_{50} does not overlap (Robertson and Preisler, 1992).

3. Results

Concentration-mortality curves for the pest *S. exigua* and the predator *P. maculiventris* showed low χ^2 values (< 19.00) and high *p*-values (> 0.97), indicating the data adequacy to the PROBIT model used to estimate the mortality curves. This allowed the estimation of the LC_{50}'s (Table 1, 2 and 3).

3.1. Glass-vial bioassays

Coragen® had the highest toxicity to *S. exigua* followed by Entrust®, PyGanic® and Azera®, with relative toxicity of 10.99, 16.75 and 28.19, respectively (Table 1).

Coragen® was safe to *P. maculiventris* what made impossible to estimate its LC_{50} in this route of exposure because no mortality was observed at concentrations up to 500 µg a.i./vial. PyGanic® and Azera® had the highest toxicity, respectively, than Entrust® for *P. maculiventris* with treated glass-vials (Table 1). Entrust® and particularly Coragen® showed higher toxicity to the pest than to the predator and PyGanic® and Azera® showed higher toxicity to the predator than to the pest (Table 1).

3.2. Diet incorporation bioassays

Coragen® also had the highest toxicity to *S. exigua* with diet incorporation bioassays, followed by Entrust®, PyGanic® and Azera®, with relative toxicity of 5.24, 11.98 and 15.13, respectively (Table 2).

3.3. Oral toxicity bioassays

The oral toxicity bioassays showed the highest toxicity of Entrust® to *P. maculiventris* followed by PyGanic®, Azera® and Coragen® with relative toxicity of 2.63, 2.87 and 10.89, respectively (Table 3).

4. Discussion

In this study we assessed the efficacy of residues of some OMRI-approved insecticides and one non-OMRI-listed formulation against the beet armyworm *S. exigua* and its predator, the spined soldier bug. The insecticides Entrust® and Coragen® showed the highest toxicity to the pest and lower toxicity to the predator, which is generally needed for IPM programs. Chlorantraniliprole showed low toxicity to the predators *P. nigrispinus* and *Supputius cincticeps* (Heteroptera: Pentatomidae) after exposure to dried residues of this insecticide and mortality lower than 10% using 10x the recommend label rate after 72h exposure (De Castro et al., 2013). This insecticide was also harmless to the bumble bees *Bombus impatiens* (Cresson) (Hymenoptera: Apidae) (Gradish et al., 2009) and great selectivity to parasitoids and mites (Dinter et al., 2008). Low toxicity of chlorantraniliprole is mainly due to the conformation and structure of the insecticide molecule that has high affinity towards Lepidoptera ryanodine receptors (Nauen, 2006; Lahn et al., 2009).

The efficacy of Entrust® to control *S. exigua* by contact (glass-vial) or ingestion (diet incorporation) agrees with the fact that it reduced infestations of lepidopteran pests of cole crops *Plutella xylostella* (L.), *Pieris rapae* (L.), and *Trichoplusia ni* (Hübner) in Alabama (Maxwell and Fadamiro, 2006). The Entrust® has broad-spectrum activity, multiple modes of entry and residual effect and its active ingredient, spinosad, is a contact and stomach poison (Liu et al., 1999; Balusu and Fadamiro, 2012). However, the selectivity of Entrust® to non-target species is arguable because it showed higher toxicity to *P. maculiventris* by ingestion in contaminated water than by contact to its residues on the glass-vial. The effect of spinosad on predators has been reported and 71% of the studies reviewed indicated lethal effect under laboratory conditions (Biondi et al., 2012). In addition, predatory stinkbugs can present insecticide irritability (i.e., avoidance after contact) to spinosad, what can increase its survival (De Castro et al., 2013).

The highest toxicity of the OMRI-approved insecticides PyGanic® and Azera® to *P. maculiventris* and lower values to *S. exigua* agrees with its rapid knockdown which contributed to its efficacy (Balusu and Fadamiro, 2012). Futhermore, azadirachtin, the other active ingredient of Azera®, is the main insecticidal component from neem plant with broad use against insect-pests (Mordue (Luntz) et al., 2005). The selectivity of azadirachtin to the predators is controversial and its safety to biocontrol agents has been questioned (Viñuela et al., 2000; Qi et al., 2001; Medina et al., 2004). The mortality of lacewings by azadirachtin was high (100%) (Cordeiro et al., 2010) and it caused malformations in the predator *P. maculiventris* (Viñuela et al., 2000).

In summary, the toxicity of three botanical insecticides approved by the Organic Materials Review Institute (OMRI) and one non-OMRI-listed formulation against *S. exigua* and *P. maculiventris* was assessed. The insecticide Entrust® and, especially Coragen® were more toxic to *S. exigua* and less toxic to this predator. However, Entrust® via drinking water was highly toxic to *P. maculiventris*. PyGanic® and Azera® were more toxic to this predator and less toxic to the pest. Thus, the notion that natural compounds are safer than synthetic compounds to non-target species is refuted in the present study. Bioinsecticides should not be exempted from risk assessment, and non-target sub-lethal effects not to be neglected when considering its use in integrated pest management.

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Table 1. Relative toxicity of different insecticide formulations to third-instar beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) and relative toxicity and selectivity (related to the beet armyworm toxicity data) of different insecticide formulations to third-instar *Podisus maculiventris* (Heteroptera: Pentatomidae) in glass-vials bioassays

Insect	Insecticides	No. insects	Slope (SE)	LC_{50} (95% FL) µg a.i./vial	Relative toxicity (95% CI)	Differential selectivity (95% CI)	χ^2	*P*
Spodoptera exigua	Coragen	288	0.97 (0.10)	0.35 (0.24-0.54)	1.00 (0.57-1.76)	-	10.00	0.99
	Entrust	252	1.63 (0.17)	3.93 (2.98-5.13)	10.99 (6.81-17.73)	-	7.95	0.99
	PyGanic	252	1.45 (0.16)	5.99 (4.48-8.08)	16.75 (10.25-27.39)	-	8.04	0.99
	Azera	252	1.66 (0.17)	10.07 (7.71-13.12)	28.19 (17.52-45.35)	-	8.56	0.99
Podisus maculiventris	Coragen	300	-	-	-	-	-	-
	PyGanic	234	1.47 (0.19)	8.51 (6.09-11.34)	1.00 (0.65-1.53)	1.42 (0.94-2.16)	4.63	0.99
	Azera	288	1.26 (0.14)	40.85 (29.73-58.61)	4.80 (3.07-7.51)	4.06 (2.66-6.18)	9.16	1.00
	Entrust	180	2.22 (0.29)	66.62 (52.92-85.35)	7.83 (5.36-11.44)	16.96 (11.93-24.12)	6.72	0.99

Table 2. Relative toxicity of different insecticide formulations to third-instar beet armyworm *Spodoptera exigua* (Lepidoptera: Noctuidae) in diet incorporation bioassays

Insecticides	No. insects	Slope (SE)	LC_{50} (95% FL) µg a.i./cup	Relative toxicity (95% CI)	χ^2	*P*
Coragen	300	1.21 (0.13)	0.86 (0.61-1.17)	1.00 (0.64-1.57)	12.42	0.99
Entrust	350	1.63 (0.15)	4.48 (3.56-5.62)	5.24 (3.55-7.74)	9.34	1.00
PyGanic	450	0.88 (0.08)	10.24 (7.26-14.91)	11.98 (7.44-19.29)	18.40	0.99
Azera	400	1.63 (0.14)	12.94 (10.37-16.28)	15.13 (10.25-22.34)	18.29	0.99

Table 3 Relative toxicity of different insecticide formulations to third-instar *Podisus maculiventris* (Heteroptera: Pentatomidae) in oral toxicity bioassays

Insecticides	No. insects	Slope (SE)	LC_{50} (95% FL) μg a.i./mL	Relative toxicity (95% CI)	χ^2	*P*
Entrust	210	1.39 (0.17)	17.91 (12.91-25.80)	1.00 (0.62-1.61)	9.40	0.97
PyGanic	210	1.33 (0.17)	47.07 (33.27-66.37)	2.63 (1.64-4.22)	3.17	1.00
Azera	210	0.95 (0.15)	51.42 (32.47-81.98)	2.87 (1.65-4.99)	5.03	0.99
Coragen	150	2.10 (0.31)	195.00 (149.49-266.83)	10.89 (7.05-16.82)	3.64	0.99

General conclusions

The lethal and sublethal (mobility) effects of chlorantraniliprole, deltamethrin, methamidophos and spinosad used to control *Anticarsia gemmatalis* on the pentatomid predators, *Podisus nigrispinus* and *Supputius cincticeps* were assessed. The compounds of the new generation of insecticides, especially the chlorantraniliprole, were more toxic to *A. gemmatalis* and less toxic to predators than traditional organophosphates and pyrethroids insecticides. This pattern, though less obvious, was also found in behavioral walking bioassays when the predators had more abrupt behavioral changes when exposed to methamidophos and deltamethrin. Thus, our results reinforce the need for replacing the insecticides methamidophos and deltamethrin by more selective compounds such as chlorantraniliprole, which had lower toxicity to the predators tested and hence allowing more sustainable IPM programs.

The lethal and sublethal effects of chlorantraniliprole, deltamethrin, methamidophos and spinosad on the generalist predator *P. nigrispinus* via treated prey varied widely. *Podisus nigrispinus* was susceptible to spinosad and methamidophos, notably with high mortality observed in adults. Spinosad and methamidophos were incompatible with this predator for IPM. Deltamethrin was less toxic but still reduced offspring of *P. nigrispinus*. The chlorantraniliprole was harmless with mortality and reproductive capacity levels similar to that of the untreated control group. Furthermore, chlorantraniliprole can be the most promising insecticide for IPM programs because of its lower toxicity to this predator. Specific risk assessment and field studies to assess the safety of these compounds to predatory stinkbugs should be undergone before implementing any IPM programs.

The toxicity of three botanical insecticides (Azera®, Entrust® and PyGanic®) approved by the Organic Materials Review Institute (OMRI) and one non-OMRI-listed formulation (Coragen®) against *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) was assessed. The insecticide Entrust® and, especially Coragen® were more toxic to *S. exigua* and less toxic to this predator via glass-vials. However, Entrust® via drinking water was highly toxic to *P. maculiventris*. PyGanic® and Azera® were more toxic to this predator and less toxic to the pest via glass-vials. Thus, the notion that natural compounds are safer than synthetic compounds to non-target species is refuted in the present study. Bioinsecticides should not be exempted from risk assessment, and non-target sub-lethal effects not to be neglected when considering its use in integrated pest management.