Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae)

Author(s): Lessando M. Gontijo, Daiane Celestino, Obiratanea S. Queiroz, Raul Narciso C. Guedes and Marcelo C. Picanço

Source: Florida Entomologist, 98(1):59-64.

Published By: Florida Entomological Society

https://doi.org/10.1653/024.098.0111
URL: http://www.bioone.org/doi/full/10.1653/024.098.0111

Impacts of azadirachtin and chlorantraniliprole on the developmental stages of pirate bug predators (Hemiptera: Anthocoridae) of the tomato pinworm *Tuta absoluta* (Lepidoptera: Gelechiidae)

Lessando M. Gontijo[1,]*, *Daiane Celestino*[2], *Obiratanea S. Queiroz*[2],
Raul Narciso C. Guedes[2] *and Marcelo C. Picanço*[2]

Abstract

Conservation of natural enemies is an important approach for enhancing biological control. Selective insecticides have become important for managing arthropod pests, and the prospects for their use in combination with biological control agents are increasing. In addition, progress has been made in recent decades to develop reduced-risk insecticides that have novel modes of action and, therefore, likely to have a minimum non-target effect. In this study, we use a series of laboratory bioassays to investigate the impact of two reduced-risk insecticides, azadirachtin and chlorantraniliprole, on the egg, nymph and adult stages of two predatory pirate bugs, *Amphiareus constrictus* (Stal) and *Blaptostethus pallescens* Poppius (Hemiptera: Anthocoridae), important predators of the tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). All three stages were exposed to the label recommended field rate of these two insecticides, which is indicated for the control of *T. absoluta*. Neither azadirachtin nor chlorantraniliprole affected the mortality of adult predators or their egg hatchability, suggesting a safe acute toxicity for these stages. However, azadirachtin and chlorantraniliprole negatively decreased the capacity of predator nymphs to reach the adult stage. This decrease can directly affect the size of the predator population in the next generation, and may undermine the biological control of pests such as *T. absoluta*. In general, our results call for caution when using these reduced-risk insecticides in integrated programs of chemical and biological control of this pest.

Key Words: non-target effect, neem, chlorantraniliprole, predatory bug, tomato pinworm

Resumo

A conservação de inimigos naturais é uma estratégia importante para o controle biológico de insetos-praga. O uso de inseticidas seletivos também tem sido importante para o manejo de insetos-praga, e a prospectiva de ter seu uso combinado com agentes de controle biológico tem ganhado espaço ultimamente. Além disso, tem ocorrido um grande progresso nas ultimas décadas com relação ao desenvolvimento de inseticidas de risco reduzido que apresentam novos modos de ação, e possívelmente uma menor chance de causar efeitos não-alvo. No presente trabalho, nós conduzimos uma série de bioensaios em laboratório para investigar o impacto de dois inseticidas de risco reduzido, azadiractina e clorantraniliprole, sobre os estádios de ovo, ninfa e adulto de dois predadores hemípteras, *Amphiareus constrictus* (Stal) and *Blaptostethus pallescens* Poppius (Heteroptera: Anthocoridae), que são importantes inimigos naturais da traça do tomate *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Todos os três estádios foram expostos à dose de campo destes dois inseticidas, a qual é recomendada para o controle de *T. absoluta*. Tanto azadiractina como clorantraniliprole não afetaram a mortalidade dos predadores adultos ou a eclosão de seus ovos para qualquer espécie, sugerindo a ausência de uma toxicidade aguda para estes estádios de desenvolvimento. Porém, azadiractina e clorantraniliprole afetaram negativamente a capacidade de ninfas dos predadores alcançarem o estádio adulto. Essa redução na capacidade das ninfas alcançarem o estádio adulto pode afetar diretamente o tamanho da população de predadores na próxima geração, um impacto que poderia reduzir o controle biológico de pragas como *T. absoluta*. Em geral, nossos resultados sugerem a necessidade de cautela quando usar inseticidas de risco reduzido em programas que visam integrar o controle químico e biológico desta praga.

Palavras Chave: efeito não-alvo, azadiractina, clorantraniliprole, predadores, Tuta absoluta

Conservation of natural enemies is an important approach for enhancing the biological control of arthropod pests in many agroecosystems (Barbosa 1998; Landis et al. 2000). This conservation may be achieved, in part, through the use of selective insecticides that have minimum non-target effects on natural enemies. The concept of integrating chemical and biological control was first proposed by Stern et al. (1959), whose work and ideas helped to build a foundation for the construction of the integrated pest management (IPM) concept.

---

[1] Instituto de Ciências Agrárias, Universidade Federal de Viçosa – Campus Florestal, Florestal, MG 35690-000, Brazil
[2] Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-000, Brazil
*Corresponding author; E-mail: lessandomg@ufv.br

Stern et al. (1959) noted several components that are essential for the integration of chemical and biological control. These components are as follows: 1) an understanding of the natural enemy and pest interactions at the ecosystem level; 2) sampling methods and prediction of pest occurrence; 3) enhancing the benefits of natural enemies through augmentation, importation and conservation; and 4) understanding the effects of pesticides on natural enemies and how to mitigate these effects via ecological (i.e., dose, timing and location of spray) and physiological (i.e., choice of toxicant) selectivity.

Progress has been made in recent decades in developing reduced-risk insecticides that act on specific biochemical sites present in certain insect pest groups. This effort has resulted in the discovery of important molecules that possess novel modes of action and therefore likely to have a minimum non-target effect as well as posing fewer threats to the environment or human health (Ishaaya et al. 2005). Two insecticides, azadirachtin and chlorantraniliprole, have been considered reduced-risk compounds (Isman 2006; Gentz et al. 2010). Both are recommended primarily for the control of lepidopteran pests. Azadirachtin is known to act as a growth regulator, feeding behavior and cellular growth inhibitor (Hanning et al. 2009; Isman 2006). In general, the efficacy of azadirachtin has been tested for various crop pests (Schmutterer 1985; Lynn et al. 2012; Pavela et al. 2013; Tomé et al. 2013), as well as for some natural enemies (Raguraman et al. 2004; Cordeiro et al. 2010; Celestino et al. 2014) showing to be relatively safer than most of the conventional synthetic insecticides. In contrast, chlorantraniliprole works as an activator of insect ryanodine receptors, causing rapid muscle dysfunction and paralysis (Cordova et al. 2006). Chlorantraniliprole has been tested primarily against lepidopteran pests (Haning et al. 2009; Wang et al. 2010) and also for some natural enemies (Dinter et al. 2008; et al. 2014), showing to be acutely safe for all the natural enemies tested. In addition, azadirachtin and chlorantraniliprole have been documented to have ovicidal effects on eggs of insect pests (Simmon Ascher 1993; Ioriatti et al. 2009).

The tomato pinworm *Tuta absoluta* (Meyrick) has been considered a major pest of tomato, initially restricted to Brazil and other South American countries (Barrientos et al. 1998, Estay & Bruna 2002; Guedes & Picanço 2012). The larvae of this pest attack leaves, stems and fruits of tomato plants, and the injuries can cause losses of as much as 80-100% (López 1991). In addition, feeding on the fruits by the pest renders the product unmarketable. This tomato pest has also become an exotic pest of international concern. Since this pest was documented in eastern Spain in 2006, it has invaded many other European countries and has spread throughout the Mediterranean basin (Desneux et al. 2010). In Brazil, the control of *T. absoluta* has been accomplished primarily by spraying insecticides, including many with a broad spectrum of activity (Bacci 2006; Guedes & Picanço 2012; Guedes & Siqueira 2012). This practice may threaten important natural enemies. A recent review has shown that some of the most abundant and promising predators to help managing *T. absoluta* are hemipterans, especially the Miridae and Anthocoridae families (Zappalá et al. 2013). In Brazil, two hemipteran predators, *Amphiareus constrictus* (Stal) and *Blaptostethus pallescens* Poppius (Heteroptera: Anthocoridae), have been observed relatively often feeding on *T. absoluta* in the field (Miranda et al. 1998; Bacci 2006). These predators have shown to be efficient in functional response studies at preying on *T. absoluta* eggs, and are promising biological control agents (Queiroz et al. *unpublished*). Thus, there is a need for the assessment of the selectivity of alleged reduced-risk insecticides, such as azadirachtin and chlorantraniliprole.

Selective insecticides have become the dominant approach for the management of insect pests, and the prospects for the use of these insecticides in combination with biological control agents are on the rise. When used in combination with effective natural enemies, these selective compounds may provide more comprehensive prophylactic and remedial treatments. Although many selectivity studies have been conducted, only a few of them have addressed the negative effects of insecticides on the early life stages (i.e., egg and or nymph/larva) of natural enemies. This lack of research is especially marked for natural enemies that have not yet been extensively studied, such as *A. constrictus* and *B. pallescens*, despite their perceived importance against the invasive tomato pinworm (Celestino et al. 2014; Pereira et al. 2014). Thus, our study investigates the impact of azadirachtin and chlorantraniliprole on the egg, nymph and adult stages of these important predators of *T. absoluta*. If conservation biological control measures are to be implemented, then prioritizing the insecticides that have the least non-target effect upon important natural enemies is an important step.

Materials and Methods

INSECTS AND INSECTICIDES

Colonies of *Amphiareus constrictus* (Stal) and *Blaptostethus pallescens* Poppius collected in open tomato fields in the counties of Coimbra and Viçosa (state of Minas Gerais, Brazil) in 2010, were established in the laboratory. Approximately 100 adults of *B. pallescens* maintained in glass jars (2.5 L) with the tops covered with organza fabric. Stems of cobbler's pegs (*Bidens pilosa* L.) immersed in water vials sealed with cotton were used as an egg-laying substrate. This substrate was replaced every other day, and the stems containing predator eggs were then transferred to clear plastic containers (250 ml), covered with organza to allow the nymphs to emerge. The emerged nymphs were maintained in these containers throughout their development and, the adults were transferred to larger glass containers (2.5 L) for mating and oviposition. Eggs of the Mediterranean flour moth *Anagastha kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Insecta Agentes Biologicos, Lavras, MG, Brazil) were provided every other day *ad libitum*, as a food source for the nymphs and adults. All the containers with either nymphs or adults were provided 2-3 small pieces of wet cotton, a water source. The same methodology described above was used for *A. constrictus*, except forusing shredded paper instead of the stems of cobbler`s pegs. Both species were maintained in a rearing room with environmental conditions of 27 ± 2°C, 70 ± 10% R.H. and a 12-h photoperiod.

The label recommended field rate for *T. absoluta* was used for both insecticides, which corresponded to 250 ml of AzaMax (azadirachtin) for 100 L of water, and to 1.5 ml of Premio (chlorantraniliprole) for 100 L of water (MAPA 2014). Thus, the terpenoid bioinsecticide azadirachtin was applied in the bioassay at a concentration of 0.03 mg a.i./mL (Azamax, emulsifiable concentrate, 12 g a.i./L, DVA Brasil, Campinas, SP, Brazil), and chlorantraniliprole was applied at 0.03 mg a.i./mL (Premio, suspension concentrate, 200 g i/L, DuPont do Brasil, Barueri, SP, Brazil) (MAPA 2014).

INSECTICIDE BIOASSAYS

In each treatment, between 27 and 31 adults (males and females) of *B. pallescens* or *A. constrictus* (< 3 days old) were individually exposed to the recommended field application rate of each insecticide as well as to a control treatment (distilled water). The experiment had a completely randomized design, with each insect representing a replicate. Prior to spraying, each insect was placed within a well (3.34 cm diameter) of a 6-well (flat bottom) acrylic cell culture plate (TTP Techno Plastic Products, Trasadingen, Switzerland). In addition, each cell had its bottom covered by filter paper and its wall treated with Teflon to prevent predator escape. Each individual predator received 200 µL of either insecticide or control solution applied with an artist's air brush (Sagyma SW440A, Yamar Brasil, São Paulo, SP, Brazil) attached to an air pump (Prismatec 131A Tipo 2 VC, Itu, São Paulo, SP, Brazil) at a pressure of 6.9×10^4 Pa. The sprayed insects were subsequently transferred individually to a glass test tube (9 x 1.5 cm; H x D) containing a small ball of humid cotton and eggs of the Mediterranean flour moth (provided *ad libitum*). The treated insects were maintained under the same controlled conditions used for their rearing, and mortality was assessed at 24 and 48 hours after the treatment was applied. The insects were considered dead if unable to move when prodded with a fine paintbrush.

The recently emerged nymphs (not older than 2 days) from untreated individuals of both species (26-31 nymphs/treatment) were subjected to the same treatments as described above. However, in addition to the direct spray exposure, the nymphs of both species were also exposed to insecticide via ingestion and residue contact. To do so, the first *A. kuehniella* eggs fed to the nymphs (1 g, first meal) and the interior walls and undersurface of the lids of clear plastic pots (100 ml) were sprayed once with the respective treatments, as described above, and allowed to dry under a fume hood. Thereafter, each nymph was maintained inside these plastic cups and provisioned *ad libitum* every other day (from the second meal on) with non-sprayed eggs of *A. kuehniella* and a small wet cotton ball. The mortality of the nymphs was evaluated daily. The nymphs were monitored until they died or reached the adult stage.

Because azadiractin and chlorantraniliprole have been documented to show some degree of ovicidal effect (Simmon Ascher 1993; Ioriatti C et al. 2009), we also exposed eggs of both predator species to the treatments described above. To do so, groups of predator eggs (25-35 eggs/treatment) were sprayed with their respective treatment using the equipment described above. However, prior to spraying, each group of eggs was covered in the well with a light organza fabric disc that allowed the eggs to come in contact with the treatment and prevented them from blowing away. After spraying, the eggs were transferred individually to small clear plastic cups (10 ml) with a filter paper disc on the bottom and a parafilm cover. The hatching of the predator eggs was evaluated daily.

STATISTICAL ANALYSES

Differences in adult mortality between treatments at 24 and 48 hours after exposure were tested with a non-parametric analysis (PROC GENMOD) in which mortality was represented as a binary response (dead or alive) because in this design the errors are binomially distributed. Differences in egg hatchability between treatments were tested with a Fisher's exact test (PROC FREQ). The effects of the treatments on nymph longevity were tested with a t-test (PROC TTEST). A survival analysis was conducted (PROC LIFETEST) to assess the effect of treatment on the proportion of nymphs that were able to reach the adult stage. In this survival analysis, the nymphs that either did not reach the adult stage or died before it were included as censored data. All analyses were performed in SAS (SAS Institute 2008).

Results

In general, neither azadirachtin nor chlorantraniliprole had a significant impact on the mortality of either adult predator species via direct exposure (Table 1), thereby suggesting a safe acute toxicity to adults. In addition, there was no significant increase in the mortality of adults from 24 to 48 hours for either treatment of either predator species (Table 1). There was no significant effect of azadirachtin on the egg hatchability of *A. constrictus* ($\chi^2 = 0.68$, $P = 0.43$) or *B. pallescens* ($\chi^2 = 2.23$, $P = 0.16$), when compared to their respective controls. Likewise, there was no effect of chlorantraniliprole on the egg hatchability of *A. constrictus* ($\chi^2 = 0.27$, $P = 0.66$) or *B. pallescens* ($\chi^2 = 0.47$, $P = 0.59$), when compared to controls. The egg hatchability for *A.constrictus* was 75% when treated with azadirachtin and 87.5% when treated with chlorantraniliprole; and for *B. pallescens* it was 60% when treated with azadirachtin and 92% when treated with chlorantraniliprole. Azadirachtin significantly reduced the longevity of nymphs of *A. constrictus* ($t = 2.53$, $P = 0.014$), unlike chlorantraniliprole, which had no significant effect ($t = 1.02$, $P = 0.32$) (Fig. 1). In contrast, the longevity of *B. palles-*

Table 1. Mortality (%) of adults of *Amphiareus constrictus* and *Blaptostethus pallescens* at 24 and 48 hours after insecticide exposure.

insecticide	**time after treatment (h)**	**control (%)**	**field rate (%)**	**χ^2**	**df**	**sig.**	***P***
Amphiareus constrictus							
Azadirachtin	24	7.14	10.00	0.15	1	n.s.	0.70
	48	14.28	23.33	0.78	1	n.s.	0.38
	num. predators	N = 28	N = 30				
Chlorantraniliprole	24	7.14	3.33	0.43	1	n.s.	0.51
	48	14.28	3.33	2.33	1	n.s.	0.13
	num. predators	N = 30	N = 30				
Blaptostethus pallescens							
Azadirachtin	24	3.70	0.00	1.37	1	n.s.	0.24
	48	3.70	0.00	1.29	1	n.s.	0.26
	num. predators	N = 27	N = 26				
Chlorantraniliprole	24	3.70	7.70	3.35	1	n.s.	0.53
	48	3.70	7.70	0.40	1	n.s.	0.53
	num. predators	N = 27	N = 28				

n.s. Mortality values (%) within dates are not significantly different at $P < 0.05$ (Pearson chi-square test).

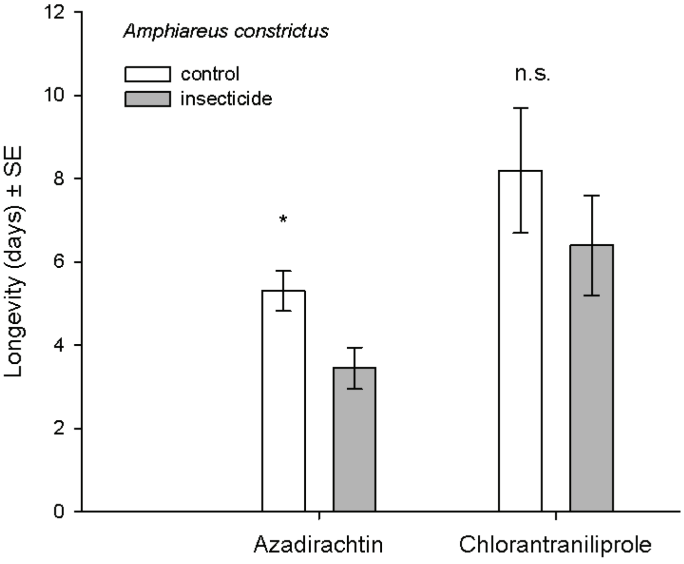

Fig. 1. Longevity (days ± SE) of nymphs of *Amphiareus constrictus* exposed to azadirachtin and chlorantraniliprole via three routes of exposure (ingestion, residue contact and direct spray). *Comparison between two bars is statistically significant (t-test, $P < 0.05$).

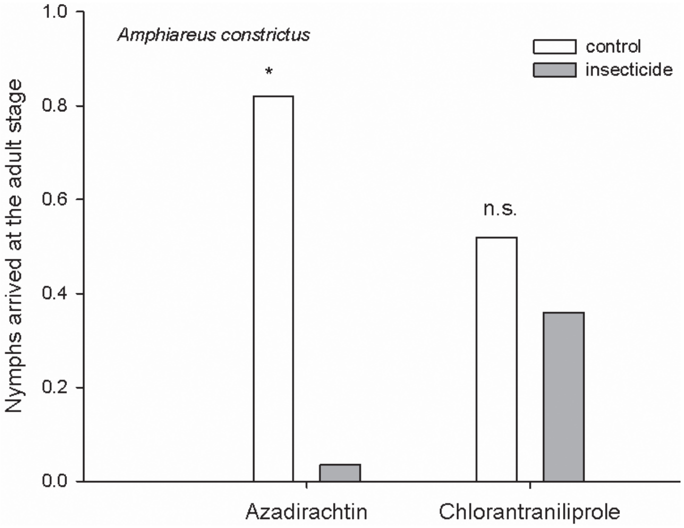

Fig. 3. Proportion of nymphs of *Amphiareus constrictus* that reached the adult stage after early exposure to azadirachtin and chlorantraniliprole via three routes of exposure (ingestion, residue contact and direct spray). *Comparison between two bars is statistically significant (survival analysis/log rank test, $P < 0.05$).

cens nymphs was not affected by either azadirachtin ($t = 0.75$, $P = 0.45$) or chlorantraniliprole ($t = 1.16$, $P = 0.25$) (Fig. 2).

Fewer nymphs of *A. constrictus* treated with azadirachtin were able to reach the adult stage (log-rank test, $\chi^2 = 14.75$, $P = 0.0001$), whereas no treatment difference was observed for those exposed to chlorantraniliprole (log-rank test, $\chi^2 = 0.10$, $P = 0.75$) (Fig. 3). The number of days taken by nymphs of *A. constrictus* to reach the adult stage when exposed to azadirachtin and to control ranged from 7 to 10 and 6 to 9, respectively, whereas the corresponding values for those exposed to chlorantraniliprole and to control ranged from 11 to 21 and 11 to 16, respectively. Both azadirachtin (log-rank test, $\chi^2 = 19.46$, $P < 0.0001$) and chlorantraniliprole (log-rank test, $\chi^2 = 7.26$, $P = 0.007$) negatively affected the capacity of the nymphs of *B. pallescens* to reach the adult stage (Fig. 4). The number of days taken by nymphs of *B. pallescens* to reach the adult stage when exposed to azadirachtin and to control ranged from 8 to 9 and 4 to 8, respectively, whereas the corresponding values for those exposed to chlorantraniliprole and to control ranged from 16 to 18 and 14 to 17, respectively. Because bioassays were conducted on different dates and had separate controls, these results suggest that the treatments appear to have affected the number of days required by either species to reach the adult stage. In addition, these results

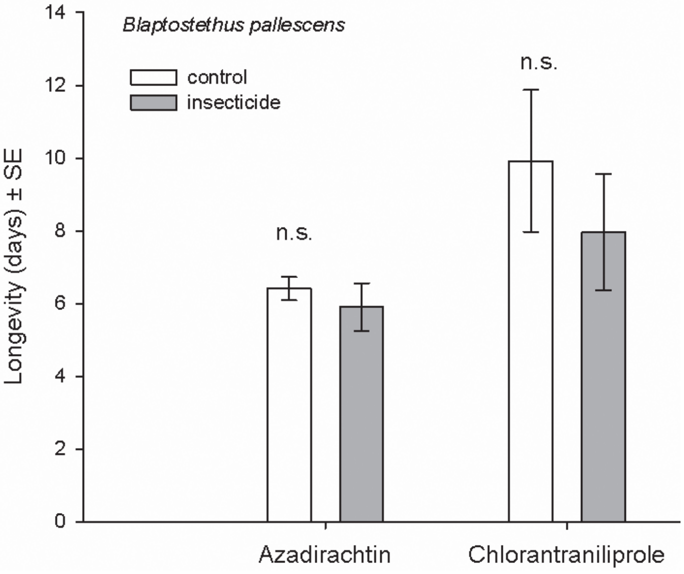

Fig. 2. Longevity (days ± SE) of nymphs of *Blaptostethus pallescens* exposed to azadirachtin and chlorantraniliprole via three routes of exposure (ingestion, residue contact and direct spray). *Comparison between two bars is statistically significant (t-test, $P < 0.05$).

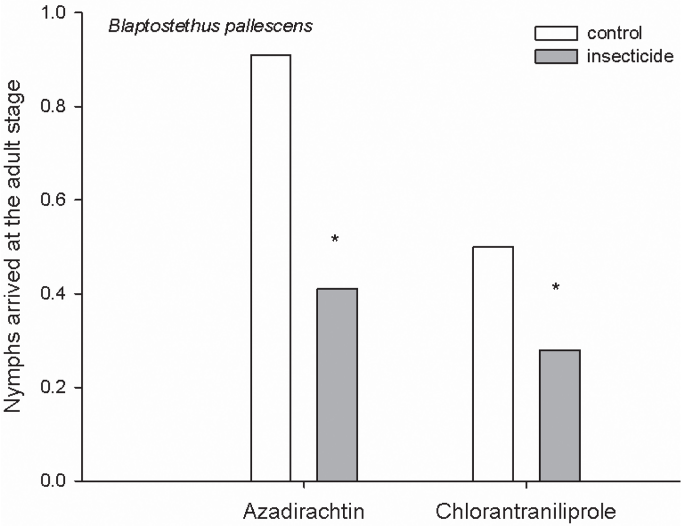

Fig. 4. Proportion of nymphs of *Blaptostethus pallescens* that reached the adult stage after early exposure to azadirachtin and chlorantraniliprole via three routes of exposure (ingestion, residue contact and direct spray). *Comparison between two bars is statistically significant (survival analysis/log rank test, $P < 0.05$).

indicate that the pesticides do not affect the time to reach the adult stage for those that survive the nymphal stage.

Discussion

The general aim of our study was to examine the toxicity of reduced-risk insecticides toward important field predators of *Tuta absoluta*. The United States Environmental Protection Agency defines reduced-risk pesticides as those exhibiting low toxicity to mammals and non-target organisms, low potential for underground water contamination, low potential to select for pest resistance, or compatibility with IPM tactics (EPA 2014). Azadirachtin and chlorantraniliprole insecticides might be considered reduced-risk pesticides because of their low toxicity to mammals and non-target organisms (Isman 2006; Sattelle et al. 2010).

Our results show that both of azadirachtin and chlorantraniliprole are acutely safe for both predators, adults of *A. constrictus* and *B. pallescens*. Azadirachtin is known to be harmless to other predators at the adult stage regardless of the means of exposure (spray contact, residue or ingestion) (Medina et al. 2004). Likewise, in other selectivity studies chlorantraniliprole did not affect the mortality of adult natural enemies after contact or ingestion exposure (Brugger et al. 2010). Nevertheless, further studies are needed to investigate possible sublethal effects on adult reproduction because both azadirachtin and chlorantraniliprole have been documented to affect adult reproductive characteristics under sublethal doses (Singh 2003; Smagghe et al. 2013; Celestino et al. 2014).

Although several other studies have observed an ovicidal effect of azadirachtin and clorantraniliprole (Simmon Ascher 1993; Ioriatti et al. 2009), our study did not find any significant impact of these insecticides on the hatchability of predator eggs based on the recommended field rate. However, we did not follow up on any sublethal effects that these insecticides might show on newly emerged nymphs from treated eggs.

Azadirachtin significantly reduced the longevity as well as the number of nymphs of *A. constrictus* that reached the adult stage. In fact, azadirachtin has been documented to reduce the longevity of certain insect species in the orders Diptera e Hemiptera (Simmon Ascher 1993). In contrast, neither azadirachtin nor chlorantraniliprole affected the longevity of *B. pallescens*, but both significantly reduced the number of nymphs of this species. A deleterious effect of azadirachtin on the immature forms of predators may be expected because this insecticide acts as a growth regulator, suppressing the peaks of ecdysteroids, hormone that regulates the molt and thus the development of immature insects (Pilar Marco et al. 1990). In contrast, only a few cases of the growth inhibitory effects of chlorantraniliprole are known (Barrania 2013). To our knowledge, the current study is the first to report on the growth inhibition effect of chlorantraniliprole on natural enemies. Nevertheless, the results of this study do not allow us to determine specifically whether those nymphs that died before reaching the adult stage did so because of molt disruption or due to other direct physiological impairment caused by the insecticide. In any case, an observation that may help explain the greater effect of these two insecticides on the nymphal stage than on the adult stage is that our experiment with nymphs exposed them concomitantly to three routes of exposure (ingestion, residue contact and direct spray), which may potentiate the deleterious effects of those insecticides. For example, Banken & Stark (1998) observed that the cumulative effects of three routes of exposure greatly increased the toxicity of insecticides to the predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae).

Our results are somewhat discouraging regarding several of the negative effects that these insecticides may have upon these predators, especially nymphs. This reduction in the number of nymphs reaching the adult stage can directly affect the size of the predator population in the next generation, and such effects may ultimately and negatively impact the biological control of pests such as the tomato pinworm *T. absoluta*. Although our study shows compelling evidence for certain deleterious effects caused by azadirachtin and chlorantraniliprole, further studies are needed to investigate, in addition, the sublethal effects of these insecticides on the reproductive and behavioral characteristics of these predators (i.e., fecundity, foraging behavior). Because our experiments were conducted only in the laboratory, it is also necessary to conduct field studies because the outdoor conditions may affect the level of exposure of the predators to insecticides. In any case, the results of our study call for caution when spraying not only the broad spectrum insecticides but also the so called reduced-risk insecticides.

Acknowledgments

We thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Programa Ciências sem Fronteiras/Atração de Jovens Talentos) for funding and support. We extend special thanks to Guilherme Braoios for helping with some of the data collection.

References Cited

Bacci L. 2006. Fatores determinantes do ataque de *Tuta absoluta* ao tomateiro. D.S. Dissertation in Horticulture, Universidade Federal de Viçosa.

Barbosa P. Ed. 1998. Conservation biological control. San Diego, CA. Academic. 396 pp.

Barrania AA. 2013. Antifeedant, growth inhibitory and toxicity effects of chlorantraniliprole, thiamethoxam and novaluron against the cotton leaf worm, *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae) in cotton fields. The Egyptian Journal of Agricultural Research 91(3): 903-911.

Banken JAO, Stark JD. 1998. Multiple routes of pesticide exposure and the risk of pesticides to biological controls: A study of neem and the sevenspotted lady beetle (Coleoptera: Coccinellidae). Journal of Economic Entomology 91(1): 1-6.

Barrientos ZR, Apablaza HZ, Norero SA, Estay PP. 1998. Temperatura base y constante térmica de desarrollo de la polilla del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). Ciencia Investigación Agraria 25: 133-137.

Brugger KE, Cole PG, Newman IC, Parker N, Scholz B, Suvagia P, Walker G, Hammond TG. 2010. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Management Science 66: 1075-1081.

Celestino D, Braoios GI, Ramos RS, Gontijo LM, Guedes RNC. 2014. Azadirachtin-mediated reproductive response of the predatory pirate bug *Blaptostethus pallescens*. Biocontrol DOI 10.1007/s10526-014-9601-z

Cordeiro EMG, Corrêa AS, Venzon M., Guedes RNC. 2010. Insecticide survival and behavioral avoidance in the laccewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81:1352-1357.

Cordova D, Benner EA, Sacher MD, Rauh JJ, Sopa JS, Lahm GP. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pesticide Biochemistry and Physiology 84:196-214.

Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narvéz-Vasquez CA, Gonzáles-Cabrera J, Ruescas DC, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T, Urbaneja A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, geographic expansion and prospects for biological control. Journal of Pest Science 83: 197-215.

Dinter A, Brugger K, Bassi A, Frost NM, Woodward MD. 2008. Chlorantraniliprole (DPX-2Y45, DuPont™ Rynaxypyr®, Coragen® and Altacor® insecticide) – a novel anthranilic diamide insecticide – demonstrating low toxicity and low risk for beneficial insects and predatory mites. IOBC/WPRS Bulletin 35.

Environmental Protection Agency [EPA], USA. 2014. *Pesticides: Regulating Pesticides*. Washington, DC, US: US EPA. [cited 19 July 2014]. Available from: http://www.epa.gov/opprd001/workplan/reducedrisk.html.

Estay P, Bruna A. 2002. Insectos y a´caros asociados al tomate en Chile, pp. 9-22 *In* Estay P, Bruna A [eds.] Insectos, ácaros y enfermedades asociados al tomate en Chile. Centro regional de Investigación INIA La Platina, Santiago, Chile.

Gentz, MC, Murdoch G, King GF. 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biological Control 52: 208-215.

Guedes RNC, Picanço MC. 2012. The tomato borer *Tuta absoluta* in South America: pest status, management and insecticide resistance. Bulletin OEPP/EPPO Bulletin 42: 211-216.

Guedes RNC, Siqueira HAA. 2012. The tomato borer *Tuta absoluta*: insecticide resistance and control failure. CAB Reviews 7, no. 056. Doi: 10.1079/PAVSNNR20127055

Hanning GT, Ziegler M, Marçon PG. 2009. Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. Pest Management Science 65: 969-974.

Ioriatti C, Anfora G, Angeli G, Mazzoni V, Trona F. 2009. Effects of chlorantraniliprole on eggs and larvae of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae). Pest Management Science 65: 717-722.

Ishaaya I, Kontsedalov A, Horowitz R. 2005. Biorational insecticides: Mechanism and cross-resistance. Archives of Insect Biochemistry 58: 192-199.

Isman M. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology 51: 45-66.

Landis DA, Wratten SD, Gurr GM. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45: 175-201.

López E. 1991. Polilla del tomate: Problema crítico para la rentabilidad del cultivo de verano. Empresa y Avance Agrícola 1:6-7.

Lynn OM, Kim JE, Lee KY. 2012. Effects of azadirachtin on the development and gene expression of fifth instar larvae of Indianmeal moth, *Plodia interpunctella*. Journal of Asia-Pacific Entomology 15: 101-105.

Medina P, Budia D, Del Estal P, Vinuela E. 2004. Influence of azadirachtin, a botanical insecticide, on *Chrysoperla carnea* (Stephens) reproduction: toxicity and ultrastructural approach. Journal of Economic Entomology 97 (1): 43-50.

[MAPA] Ministério da Agricultura, Pecuária e Abastecimento. 2014. Agrofit. Coordenação Geral de Agrotóxicos e Afins/DFIA/DAS. (http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons).

Miranda MMM., Picanço MC, Zanuncio JC,Guedes RNC. 1998. Ecological life table of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Biocontrol Science and Technology 8: 597-606.

Pavela R., Zabka M, Kalinkin V, Kotenev E, Gerus A, Shchenikova A, Chermenskaya T. 2013. Systemic applications of azadirachtin in the control of *Corythucha ciliata* (Say, 1832) (Hemiptera, Tingidae), a pest of *Platanus* sp. Plant Protection Science 49: 27-33.

Pereira RR, Picanço MC, Santana Jr. PA, Moreira SS, Guedes RNC, Corrêa AS. 2014. Insecticide toxicity and walking response of three pirate bug predators of the tomato leaf miner *Tuta absoluta*. Agriculture and Forest Entomology 16: 293-301.

Pilar Marco M, Pascual N, Belles X, Camps F, Messeguer A. 1990. Ecdysteroid depletion by azadirachtin in *Tenebrio molitor* pupae. Pesticide Biochemistry and Physiology 38: 60-65.

Raguraman S, Ganapathy N, Venkatesan T. 2004. Neem versus entomopathogens and natural enemies of crop pests: The potential impact and strategies, pp. 125-182 *In* Koul O, Wahab S [eds.], Neem: Today and in the New Millennium. Kluwer Academic Publishers, Netherlands.

Sattelle D, Cordova D, Cheek T. 2008. Insect ryanodine receptors: molecular targets for novel pest control chemicals. Invertebrate Neuroscience 8: 107-119.

Schmutterer H. 1985. Which insect pests can be controlled by application of neem seed kernel extracts under field conditions? Journal of Applied Entomology 100: 468-475.

Simmon Ascher KR. 1993. Nonconventional insecticidal effects of pesticides available from the Neem tree, *Azadirachta indica*. Archives of Insect Biochemistry and Physiology 22: 433-449.

Singh S. 2003. Effects of aqueous extract of neem seed kernel and azadirachtin on the fecundity, fertility and post-embryonic development of the melonfly, *Bactrocera cucurbitae* and the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Journal of Applied Entomology 127: 540-547.

Smagghe G, Deknopper J, Meeus I, Mommaerts V. 2013. Dietary chlorantraniliprole suppresses reproduction in worker bumblebees. Pest Management Science 69: 787-791.

Stern VM, Smith RF, Van Den Bosch R, Hagen KS. 1959. The integrated control concept. Hilgardia 29: 81-101.

Tomé HVV, Martins JC, Correa AS, Galdino TVS, Picanço MC, Guedes RNC. 2013. Azadirachtin avoidance by larvae and adult females of the tomato leafminer *Tuta absoluta*. Crop Protection 46: 63-69.

Wang X, Li X, Shen A, Wu Y. 2010. Baseline susceptibility of the diamondback moth (Lepidoptera: Plutellidae) to chlorantraniliprole in China. Journal of Economic Entomology 103(3): 843-848.

Zappalá L, Biondi A, Alma A, Al-Jboory IJ, Arnò J, Bayram A, Chailleux A, El-Arnaouty A, Gerling D, Guenaoui Y, Shaltiel-Harpaz L, Siscaro G, Stavrinides M, Tavella L, Aznar RV, Urbaneja A, Desneux N. 2013. Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. Journal of Pest Science 86: 635-647.