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MATING BEHAVIOR OF THE PREDATOR *Podisus nigrispinus* (HETEROPTERA: PENTATOMIDAE) UNDER EXPOSURE TO NEEM

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The preservation of natural enemies is one of the basic foundations for integrated pest management. Botanical insecticides have shown low impact on beneficial arthropods in relation to survival. Insecticides studies usually focus on the direct physiological effects of insecticides, whereas relatively little attention is placed on the behavioral response to exposure. A study was conducted to evaluate the effect of the botanical insecticide neem (*Azadirachta indica* A. Juss.; Meliaceae) on the mating behavior of the predatory stinkbug *Podisus nigrispinus* (Heteroptera: Pentatomidae). Unmated 5 to 7 d-old adults, separate by sex, were exposed to azadirachtin per contact on the treated surface. The treatments were composed for: untreated male and female; untreated male and treated female; treated male and untreated female; and treated male and female. Azadirachtin affected the duration of first mating (Wilcoxon test, $\chi^2 = 13.38$, $df = 3$, $p = 0.004$), which resulted in a higher effective average time of mating ($EATM_{50}$) for treatment whose only female was treated with azadirachtin. This finding points to a sublethal effect of azadirachtin on mating behavior of *P. nigrispinus* that may compromise its reproduction.

Key words: Azadirachtin, *Azadirachta indica*, biological insecticide, natural enemy.

The suborder Heteroptera is renowned by its faunal diversity (Mendonça *et al*., 2009) with predators of agricultural and forest pest (Neves *et al*., 2009). *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), a neotropical species, has been found preying different pests in several crops, showing high potential for biological control of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelekiidae), *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae), *Spodoptera* spp. (Lepidoptera: Noctuidae) (De Clercq *et al*., 1998; De Clercq, 2000), *Leptinotarsa decemlineata* (Say) (Tipping *et al*., 1999) in tomatoes and also in some pests found in soybean (*Glycine max* (L.) Merr. crops, cotton *Gossypium hirsutum* L. (Medeiros *et al*., 2000) and eucalyptus (Torres *et al*., 1996). Pest biological control depends strongly of the population growth rate of natural enemies (Holling, 1959). However, the conservation of the agents of biological control in the field may be limited by the use of insecticides (Moura *et al*., 2010). *Podisus nigrispinus* can be exposed to residues of insecticides during its locomotion, body cleaning or even by eating contaminated prey and this may affect its establishment as agent of biological control (Torres *et al*., 2002). The integrated pest management (IPM) establishes the use of insecticides more toxic to pests than to natural enemies (Dhadialla *et al*., 1998) and botanical insecticides have been shown to be selective to beneficial arthropods (Isman, 2006). The azadirachtin is nowadays one of the most important natural insecticide due to a secondary compound produced by the neem tree (*Azadirachta indica* A. Juss.; Meliaceae), which permit a great success in pests control in tropical and temperate zones (Schmutterer, 1990). Its toxicity has been reported to more than 500 insect species (Roy *et al*., 2010), including pests of tomato crop like *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (Kumar and Poehling, 2007) and *Liriomysa sativae* (Blanchard) (Diptera: Agromyzidae) (Hossain and Poehling, 2006). The use of azadirachtin in IPM programs is related mainly to a higher contamination by ingestion rather than by contact (Martinez and van Emden, 2001), which presumably makes it less toxic to natural enemies (Flavia *et al*., 2004). Although there is some information about their bioactivity in certain pests of economic importance (von Elling *et al*., 2002), information about its effects on natural enemies is scarce.

The determination of dose-response curves has been the primary means for assessing the impact of insecticides on beneficial arthropods (Guedes *et al*., 2009). However, the exposure of natural enemies to insecticide may affect its reproductive behavior and, thereafter, the control of insect pests (Desneux *et al*., 2004). Parameters such as duration and frequency of mating behavior have direct influence on the reproductive success of insects (Rodrigues *et al*.,

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2009), and insecticides may cause deviation in the acts of sequential mating of natural enemies (Claver *et al*., 2003). The objective of this study was to investigate the impact and possible consequences of azadirachtin on the mating behavior of the predator *P. nigrispinus*.

MATERIALS AND METHODS

Insects and exposure

The predator *P. nigrispinus* was obtained from the Laboratory of Biological Control of Insects of the Federal University of Viçosa (Viçosa, Minas Gerais, Brazil) and raised with pupae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) according to the methodology of Lemos *et al*. (2003).

The commercial formulation of azadirachtin (AzaMax, General Hydroponics, Sebastopol, California, USA), available for use in agriculture in Brazil and used in tomatoes crops, was used in the maximum dose recommended for tomato: 0.03 g ai L^{-1}. The insecticide was diluted in deionized water and 1 mL of solution was applied evenly on a filter paper with 9 cm of diameter (Whatman N° 1), reaching 0.47 μg ai cm^{-2} of concentration. The same procedure was made for the control treatment in which filter paper was previously moistened with 1 mL of deoinized water. Both untreated and treated papers were dried in a laminar flow of gases before use and placed in a 9 cm Petri dish covered with Teflon to prevent insects from escaping.

Unmated adults, 5 to 7 d of age, and separate by sex, were exposed to dry residues of azadirachtin on the treated surface at 26 ± 2 ºC, 70% RH, and 12:12 h photoperiod.

Mating behavior assay

The survivor adults of *P. nigrispinus* exposed to azadirachtin and also deionized water were used in pairs and placed in a Petri dish with a sterile filter paper disk on the bottom and Teflon on its inner wall. The treatments were composed for: untreated male and female; untreated male and treated female; treated male and untreated female; and treated male and female. Ten replicates were realized per treatment. The mating behavior was recorded with a Digital video camera recorder. The mating duration, latency (time preceding mating) and frequency were assessed from the continuous record of behavior. The tests were conducted at a temperature of 26 ± 2 ºC, 70 ± 5% RH and 24:0 h photoperiod.

Statistical analysis

The experimental design was completely randomized. The outcomes of duration and latency of first mating were subjected to the non-parametric LIFETEST to obtain curves of probability and the effective average time of mating ($EATM_{50}$) by Kaplan-Meier estimators. Data of frequency of mating were subjected to non-parametric test Kruskal-Wallis (SAS Institute, 2008).

RESULTS AND DISCUSSION

Survival analysis indicated significant differences in the duration of first mating between treatments (Wilcoxon test, χ^2 = 13.38, df = 3, p = 0.004) (Figure 1), which resulted in a higher effective average time of mating ($EATM_{50}$) for only female treated with azadirachtin (Figure 2). The latency curves of first mating were not significantly different between treatments (Wilcoxon test, χ^2 = 0.7166, df = 3, p = 0.869), as well as the effective average premating time ($EAPT_{50}$) with values of 9.41 (CI = 1.32 to 17.00), 6.66 (CI = 2.72 to 24.17), 4:51 (CI = 1.93 to 19.57) and 12.18 (CI = 2.42 to 57.55) for treatments with untreated male and female, untreated male and treated female, treated male and untreated female and treated male and female, respectively. The frequency of mating did not differ significantly, with values from 1.4 to untreated male and female, untreated male and treated female and treated male and untreated female and 1.5 to treated male and female (Kruskal-Wallis test, χ^2 = 0.55, df = 3, p = 0.91). Although selectivity of insecticides

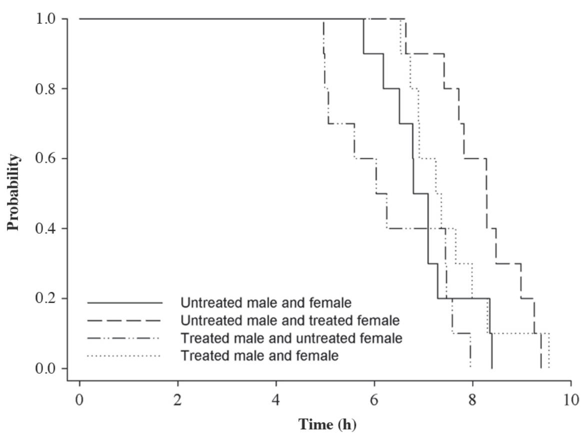
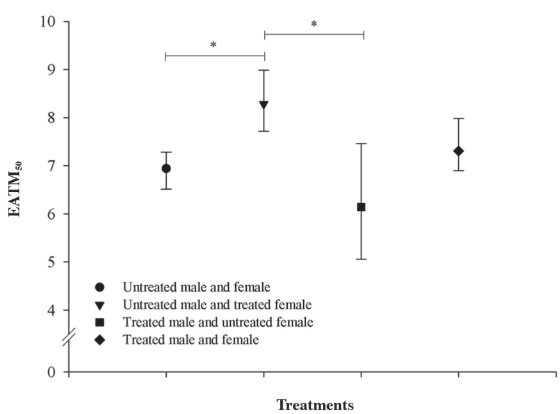


Figure 1. Estimates of the probability of the duration of first mating of *Podisus nigrispinus* exposed to azadirachtin.



*Indicates difference (p < 0.05) between treatments covered by a longitudinal bar.

Figure 2. Effective average time of the first mating ($EATM_{50}$) of *Podisus nigrispinus* exposed to azadirachtin.

to natural enemies is often determined with bioassays of mortality (Preetha *et al*., 2010), they can also be involved in a variety of morphological, physiological, reproductive, and behavioral effects (Cordeiro *et al*., 2010). In agreement with this work, which was observed that exposure of the predator *P. nigrispinus* azadirachtin interferes with the mating behavior. *P. nigrispinus* mating are multiple and long and the prolonged period of mating is associated with the appropriate transfer of seminal material to females (Rodrigues *et al*., 2009).

Females of *P. nigrispinus* exposed to azadirachtin required a higher period of mating than unexposed ones. In situations where the organisms are exposed to some insecticide, biochemistry and physiological responses can be expected. One of this responses expected here is the detoxification or metabolism of the insecticide by enzymes.

The metabolism or detoxification is a process that allows the insect modify or detoxifying the pesticide at a rate sufficient enough to prevent the action at the target site (Fukuto and Mallipudi, 1983). The degradation of the insecticide can occur by several processes in which the metabolic product is converted into a non-toxic form or even eliminated quickly from the insect body (Beckel *et al*., 2006).

Nevertheless, this process requires energy and the resources to physiological processes are relocated to detoxification. Once females and males did not eat during this essay, the lack of energy could not be replaced. Thus, it can be inferred that females need more energy for reproduction, which can be from nutritional compounds of the male's secretion. Although the effect on reproduction cannot be completed, the longer duration of mating can increase the reproductive success in *P. nigrispinus* which can be associated with increased transfer of nutritional compounds and seminal material during mating (Tram and Wolfner, 1999). Treated male had a reduced time of mating when treated alone. Thus, they may suffer a reduced sperm production and short-term transfer (Rodrigues *et al*., 2009).

Studies with *Schistocerca gregaria* (Forskal) (Orthoptera: acrididae) was shown a blockage of cell division prior to metaphase (Linton *et al*., 1997). Metaphase is the stage of cell division at which microtubules form the spindle apparatus prior to the physical separation of homologous pairs of chromosomes to opposite at this stage in cell division suggest that cell microtubular events may have been affected by azadirachtin (Mordue and Nisbet, 2000) and thus, affecting the formation of sperm.

Besides the above, the exposure to the insecticide and the likely detoxification process can also affect allocation of energy at the nutritional material, ejaculation and sperm production. Many experimental studies have shown that sperm and ejaculate production costs can be considerable (Dewsbury, 1982; Nakatsumu and Kramer, 1982; Van Voorhies, 1992; Paukku and Kotiaho, 2005).

According to the above, treated female delay the mating expecting receive more seminal material and treated male cannot keep the mating likely due to the reduced sperm or ejaculation formation. When both are treated, it can be seen a compensation in $EATM_{50}$, turning it similar to control treatment. Nevertheless, further studies are needed to evaluate whether sperm formation was appropriate and whether there was an influence on the rate of fertilization and viability of offspring.

The frequency of mating showed no statistical difference between treatments. The occurrence of multiple mating suggests that one or a few mating are not enough for females to obtain enough sperm to maximize the number of offspring in just one reproductive event (Ridley, 1990), in addition to obtaining a greater gain in genetic and nutritional material (Reynolds, 1996).

Our results indicate that couples exposed to azadirachtin may have some changes in reproduction due to a shortened mating period, although mating frequency remained unchanged. This may have implications for IPM to compatibilize between these two techniques of pest control. However, further studies evaluating the reproduction of *P. nigrispinus* and the exposure of insecticide in different stages of development need to be elucidated.

CONCLUSIONS

Many of the recorded effects of insecticides are probably secondary results of poisoning, and their significance is difficult to assess. There is ample evidence to show that synthetic insecticides can produce some effects on insects. This work confirmed that besides synthetic insecticides, also, organic insecticide can cause effects in insects. In this case, couples of *P. nigrispinus* exposed to a single dose of azadirachtin showed behavioral differences regarding the duration of mating. The possible effects, positive or negative, on reproduction have yet to be elucidated.

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Comportamiento de apareamiento del depredador *Podisus nigrispinus* (Heteroptera: Pentatomidae) expuesto al neem. La preservación de los enemigos naturales es la base fundamental para el manejo integrado de las plagas. Los insecticidas botánicos han demostrado un bajo impacto sobre los artrópodos benéficos en relación a la supervivencia. Se desarrolló un estudio para evaluar el efecto del insecticida botánico neem (*Azadirachta indica* A. Juss.; Meliaceae) sobre el comportamiento de apareamiento del chinche depredador *Podisus nigrispinus* (Heteroptera: Pentatomidae). Se expusieron adultos vírgenes de 5-7 días de edad, separados por sexo, a residuos secos de este extracto. Machos y hembras vírgenes entre 5 y 7 d de edad fueron expuestos a la azadiractina, por contacto directo con superficies tratadas. Los tratamientos fueron: machos y hembras no tratados; macho tratado y hembra no tratada; macho no tratado y hembra tratada y macho y hembras tratadas. Los resultados demostraron que la azadiractina afectó la duración de la primera cópula (test de Wilcoxon, $\chi^2 = 13.38$, $df = 3$, $p = 0.004$) lo que se traduce en un alto tiempo medio efectivo de cópula ($EATM_{50}$) en el tratamiento en que sólo la hembra fue tratada con azadiractina. Esta constatación apunta a un efecto subletal de la azadiractina sobre el comportamiento de apareamiento de *P. nigrispinus* que probablemente compromete su reproducción.

Palabras clave: azadiractina, *Azadirachta indica*, enemigo natural, insecticida biológico.

LITERATURE CITED

Beckel, H.S.B., I. Lorini, and S.M.N. Lazzari. 2006. Efeito do sinergista butóxido de piperonila na resistência de *Oryzaephilus surinamensis* (L.) (Coleoptera, Silvanidae) a deltametrina e fenitrotiom. Revista Brasileira de Entomologia 50:110-114.

Claver, M.A., B. Ravichandran, M.M. Khan, and D.P. Ambrose. 2003. Impact of cypermethrin on the functional response, predatory and mating behaviour of a non-target potential biological control agent *Acanthaspis pedestris* (Stal) (Het., Reduviidae). Journal of Applied Entomology 127:18-22.

Cordeiro, E.M.G., A.S. Corrêa, M. Venzon, and R.N.C. Guedes. 2010. Insecticide survival and behavioral avoidance in the lacewings *Chrysoperla externa* and *Ceraeochrysa cubana*. Chemosphere 81:1352-1357.

De Clercq, P. 2000. Predaceous stinkbugs (Pentatomidae: Asopinae). p. 737-789. *In* Schaefer, C.W., and A.R. Pnizzi (eds.) Heteroptera of economic importance. Cambridge University Press, Cambridge, UK.

De Clercq, P., F. Melevede, I. Mestdagh, K. Vandendurpel, J. Mohaghegh, and D. Degheele. 1998. Predation on the tomato looper *Chrysodeixis chalcites* (Esper) (Lep.: Noctuidae) by *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae). Journal of Applied Entomology 122:93-98.

Desneux, N., M.H. Pham-Delegue, and L. Kaiser. 2004. Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp, *Aphidius ervi*. Pest Management Science 60:381-389.

Dewsbury, D.A. 1982. Ejaculate cost and male choice. The American Naturalist 119:601-610.

Dhadialla, T.S., G.R. Carlson, and D.P. Le. 1998. New insecticides with ecdysteroidal and juvenile hormone activity. Annual Review of Entomology 43:545-569.

Flavia, A.C., D.A. Silva, and S. Martinez. 2004. Effect of neem seed oil aqueous solutions on survival and development of the predator *Cycloneda sanguinea* (L.) (Coleoptera: Coccinellidae). Neotropical Entomology 33:1-9.

Fukuto, T.R., and N.M. Mallipudi. 1983. Supression of metabolic resistance through chemical structure modification. p. 557-578. *In* Georghiou, G.P., and T. Saito (eds.) Pest resistance to pesticides: Challenges and prospects. Plenum Press, New York, USA.

Guedes, R.N.C., L.C. Magalhaes, and L.V. Cosme. 2009. Stimulatory sublethal response of a generalist predator to permethrin: hormesis, hormoligosis, or homeostatic regulation? Journal of Economic Entomology 102:170-176.

Holling, C.S. 1959. Some characteristics of simple types of predation and parasitism. Canadian Entomologist 91:385-398.

Hossain, M.B., and H.M. Poehling. 2006. Effects of a Neem-based insecticide on different immature life stages of the leafminer *Liriomyza sativae* on tomato. Phytoparasitica 34:360-369.

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

Kumar, P., and H-M. Poehling. 2007. Effects of azadirachtin, abamectin, and spinosad on sweetpotato whitefly (Homoptera: Aleyrodidae) on tomato plants under laboratory and greenhouse conditions in the humid tropics. Journal of Economic Entomology 100:411-420.

Lemos, W.P., F.S. Ramalho, J.E. Serrão, and J.C. Zanuncio. 2003. Effects of diet on development of *Podisus nigrispinus* (Dallas) (Het., Pentatomidae), a predator of cotton leafworm. Journal of Applied Entomology 127:389-395.

Linton, Y.M., A.J. Nisbet, and A.J. Mordue (Luntz). 1997. The effects of azadirachtin on the testes of the desert locust, *Schistocerca gregaria* (Forskål). Journal of Insect Physiology 43:1077-1084.

Martinez, S., and H.F. van Emden. 2001. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by Azadirachtin. Neotropical Entomology 30:113-125.

Medeiros, R.S., F.S. Ramalho, W.P. Lemos, and J.C. Zanuncio. 2000. Age-dependent fecundity and life-fertility tables for *Podisus nigrispinus* (Dallas) (Het., Pentatomidae). Journal of Applied Entomology 124:319-324.

Mendonça, M.D., C.F. Schwertner, and J. Grazia. 2009. Diversity of Pentatomoidae (Hemiptera) in riparian forests of southern Brazil: taller forests, more bugs. Revista Brasileira de Entomologia 53:121-127.

Mordue (Luntz), A.J. and A.J. Nisbet. 2000. Azadirachtin from the Neem Tree *Azadirachta indica*: Its action against insects. Anais da Sociedade Entomológica do Brasil 29:615-632.

Moura, A.P., G.A. Carvalho, V.F. Moscardini, O. Lasmar, D.T. Rezende, and M.C. Marques. 2010. Selectivity of pesticides used in integrated apple production to the lacewing, *Chrysoperla externa*. Journal of Insect Science 10:1-20.

Nakatsumu, K., and D.L. Kramer. 1982. Is sperm cheap? Limited male fertility and female choice in the lemon tetra (Pisces,Characidae). Science 216:753-755.

Neves, R.C.S., J.B. Torres, and L.M. Vivan. 2009. Reproduction and dispersal of wing-clipped predatory stinkbugs, *Podisus nigrispinus* in cotton fields. Biocontrol 54:9-17.

Paukku, S., and J.S. Kotiaho. 2005. Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. Journal of Insect Physiology 51:1220-1226.

Preetha, G., J. Stanley, S. Suresh, and R. Samiyappan. 2010. Risk assessment of insecticides used in rice on miridbug, *Cyrtorhinus lividipennis* Reuter, the important predator of brown planthopper, *Nilaparvata lugens* (Stal.) Chemosphere 80:498-503.

Reynolds, J.D. 1996. Animal breeding systems. Trends in Ecology & Evolution 11:68-72.

Ridley, M. 1990. The control and frequency of mating in insects. Functional Ecology 4:75-84.

Rodrigues, A.R.S., J.B. Torres, H.A.A. Siqueira, and V.W. Teixeira. 2009. *Podisus nigrispinus* requer cópulas longas para o sucesso reprodutivo. Neotropical Entomology 38:746-753.

Roy, S., G. Gurusubramanian, and A. Mukhopadhyay. 2010. Neem-based integrated approaches for the management of tea mosquito bug, *Helopeltis theivora* Waterhouse (Miridae: Heteroptera) in tea. Journal of Pest Science 83:143-148.

SAS Institute. 2008. SAS Systems for windows: Version 9.2. SAS Institute, Cary, North Carolina, USA.

Schmutterer, H. 1990. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Annual Review of Entomology 35:271-297.

Tipping, P.W., C.A. Holko, A.A. Abdul-Baki, and J.R. Aldrich. 1999. Evaluating *Edovum puttleri* Grissell and *Podisus maculiventris* (Say) for augmentative biological control of Colorado potato beetle in tomatoes. Biological Control 16:35-42.

Torres, J.B., W.S. Evangelista, R. Barras, and R.N.C. Guedes. 2002. Dispersal of *Podisus nigrispinus* (Het., Pentatomidae) nymphs preying on tomato leafminer: effect of predator release time, density and satiation level. Journal of Applied Entomology 126:326-332.

Torres, J.B., J.C. Zanuncio, P.R. Cecon, and W.L. Gasperazzo. 1996. Mortalidade de *Podisus nigrispinus* (Dallas) por parasitóides de ovos em áreas de eucalipto. Anais da Sociedade Entomológica do Brasil 25:463-471.

Tram, U., and M.F. Wolfner. 1999. Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. Genetics 153:837-844.

Van Voorhies, W.A. 1992. Production of sperm reduces nematode lifespan. Nature 360:456-458.

von Elling, K., C. Borgemeister, M. Setamou, and H.M. Poehling. 2002. The effect of NeemAzal-T/S (R), a commercial neem product, on different developmental stages of the common greenhouse whitefly *Trialeurodes vaporariorum* Westwood (Hom., Aleyrodidae). Journal of Applied Entomology 126:40-45.