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## VIRULENCE OF ENTOMOPATHOGENIC NEMATODE TO PUPAE AND ADULTS OF *Drosophila suzukii* IN LABORATORY

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**ABSTRACT:** *Drosophila suzukii* is considered one of the most important pests of fruit farming. Due to its rapid expansion, control alternatives of this fly should be investigated. The use of entomopathogenic nematodes (EPNs) represents an important tool in the control. This study aimed to evaluate the virulence of EPNs isolates in pupae and the repercussion in adults of *D. suzukii* in laboratory. The experiment was conducted in a completely randomized design with five treatments and five replicates. Each plot consisted of a Petri dish lined with two sheets of filter paper. The isolates *Heterorhabditis amazonensis* IBCBn 24, *Heterorhabditis indica* IBCBn 05, *Steinernema carpocapsae* IBCBn 02 and *Steinernema feltiae* IBCBn 47 were inoculated into 2 mL at the concentration of 1,000 infective juveniles IJs/mL. The control treatment consisted of 2 mL distilled water. After inoculation, five pupae of *D. suzukii* were placed in the Petri dishes, which were then sealed and stored in a BOD climate chamber at  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH in the dark. Assessments were performed daily until the emergence of adults. Dead pupae and adults were dissected for the observation and quantification of IJs. The isolates, *H. indica* IBCBn 05, *H. amazonensis* IBCBn 24, *S. carpocapsae* IBCBn 02 and *S. feltiae* IBCBn 47, infected and made unviable 35.0, 16.0, 13.0 and 43.0% in pupae and 47.0, 80.0, 84.0 and 57.0 % in adults of *D. suzukii*. *H. indica* IBCBn 05 obtained the highest number of IJs produced in pupae and adults, 35.0 and 125.0.

**Keywords:** invasive fly; biological control; infective juveniles

## VIRULÊNCIA DE NEMATOIDES ENTOMOPATOGÊNICOS EM PUPAS E ADULTOS DE *Drosophila suzukii* EM LABORATÓRIO.

**RESUMO:** *Drosophila suzukii* é considerada uma das pragas mais importantes da fruticultura. Devido à sua rápida expansão, alternativas de controle desta mosca devem ser investigadas. O uso de nematoides entomopatogênicos (NEPs) representa uma ferramenta

importante no controle. Este estudo teve como objetivo avaliar a virulência de isolados de NEPs em pupas e a repercussão em adultos de *D. suzukii* em laboratório. O experimento foi conduzido em delineamento inteiramente casualizado com cinco tratamentos e cinco repetições. Cada parcela consistiu em uma placa de Petri revestida com duas folhas de papel de filtro. Os isolados *Heterorhabditis amazonensis* IBCBn 24, *Heterorhabditis indica* IBCBn 05, *Steinernema carpocapsae* IBCBn 02 e *Steinernema feltiae* IBCBn 47 foram inoculados em 2 mL na concentração de 1000 juvenis infectantes JIs/mL. O tratamento de controle consistiu em 2 mL de água destilada. Após a inoculação, cinco pupas de *D. suzukii* foram colocadas nas placas de Petri, que foram então seladas e armazenadas em uma câmara climatizada B.O.D a  $26 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  de HR no escuro. As avaliações foram realizadas diariamente até o surgimento de adultos. As pupas mortas e os adultos foram dissecados para a observação e quantificação dos JIs. Os isolados, *H. indica* IBCBn 05, *H. amazonensis* IBCBn 24, *S. carpocapsae* IBCBn 02 e *S. feltiae* IBCBn 47, infectaram e tornaram inviáveis 35,0, 16,0, 13,0 e 43,0% em pupas e 47,0, 80,0, 84,0 e 57,0% em adultos de *D. suzukii*. *H. indica* IBCBn 05 obteve o maior número de JIs produzidos em pupas e adultos, 35,0 e 125,0.

*Palavras-chave:* mosca invasiva; controle biológico; juvenil infectante

## INTRODUCTION

The spotted-wing drosophila (SWD) *Drosophila suzukii* (MATSUMURA, 1931) is a polyphagous, multivoltine pest, originating in Japan and endemic to Southeast Asia (BERRY, 2012), expanding to North America and Europe in 2008 (HUSER, 2011; CINI et al., 2012) and in South America in 2013 (DEPRÁ et al., 2014) with potential for further invasions in Africa and Oceania (DOS SANTOS et al., 2017).

SWD is a quarantine pest with wide dissemination, mainly through the commercialization of small fruit such as mulberry (*Rubus* spp.) and strawberry (*Fragaria vesca*) and through the use of wild fruit as breeding sites. Females of *D. suzukii* pierce the surface of the fruit for egg laying, opening the door for infestation of other opportunists such as bacteria, fungi and yeasts. After the larvae feed on the pulp of fruit, they depreciate the quality of the fruit, and when infested from holes they lead to rotting (VILELA; MORI, 2014; RENKEMA et al., 2016).

Considering that outbreaks of *D. suzukii* can threaten the future of fruit farming causing damages to the production and export of fruits in Brazil, as it has been already observed in other countries, it is fundamental to know alternatives of control within the context of Integrated Pest Management (DEPRÁ et al., 2014).

The use of entomopathogenic nematodes (EPNs) of the families

Steinernematidae and Heterorhabditidae is a promising tool in the biological control (BRIDA et al., 2018) strategies of this insect due to the mutual association with bacteria of the genera *Xenorhabdus* (Thomas and Poinar) and *Photorhabdus* (Boemare, Louis and Kuhl), respectively (POINAR; GREWAL, 2012; BRIDA et al., 2017).

Infective juveniles (IJs) enter the host through natural openings or through the cuticle and release the bacterium into the haemocoel (LEWIS et al., 2006; FUJIMOTO et al., 2007), where they reproduce and kill the host by septicemia within 24 to 48 hours (CICHE; ENSING, 2003; MEKETE et al., 2005), making the environment favorable for the development and reproduction of nematodes (KUCHARSKA et al., 2015),

Most of the published studies using EPN isolates against fruit flies were performed in immature stages of Tephritidae (LINDEGREN; VAIL, 1986; ATTALA et al., 2002; SILVA et al., 2010; FOELKEL et al., 2016), being still scarce the works with *D. suzukii*. The nematode species *Steinernema kraussei* (Steiner, Travassos), *Steinernema feltiae* (Filipjev) and *Steinernema carpocapsae* (Weiser) were tested for control of *D. suzukii* larvae, but showed low mortality rates under laboratory conditions except *Heterorhabditis bacteriophora* (Poinar), which has shown mortality rates around 95.00% against larvae of *D. suzukii* (CUTHBERTSON et al., 2014; WOLTZ et al., 2015; CUTHBERTSON; AUDSLEY 2016).

Considering the scarce information on the virulence of EPNs in pupae of *D. suzukii*, and that the pupal phase of this fly can occur in the soil (GREWAL, 2000), the use of entomopathogenic nematodes becomes an important method to be used in the control of this pest. In this way, this study aimed to evaluate the virulence of different entomopathogenic nematode isolates against *D. suzukii* pupae and their repercussion in adults in laboratory.

## MATERIAL AND METHODS

The isolates *Heterorhabditis amazonensis* (Andaló, Nguyen & Moino) IBCBn 24, *Heterorhabditis indica* (Poinar, Karunakar & David) IBCBn 05, *Steinernema*

*carpocapsae* IBCBn 02 and *Steinernema feltiae* IBCBn 47 were obtained from the Entomopathogenic Nematode Collection “Oldemar Cardim Abreu” of the Biological Institute of São Paulo, State of São Paulo, Brazil. The experiment was conducted in a completely randomized design at the Federal University of Pelotas, State of Rio Grande do Sul, Brazil. Pupae of *D. suzukii* were obtained from a rearing kept on artificial diet maintained according to the methodology described by Dalton et al. (2011) in a BOD climate chamber at 22°C, 70 ± 10% RH, 12h photoperiod.

The infective juveniles (IJs) of *H. amazonensis* IBCBn 24, *H. indica* IBCBn 05, *S. carpocapsae* IBCBn 02 and *S. feltiae* IBCBn 47 were multiplied separately in five larvae of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) (fourth to fifth Instar) per Petri dish (9 cm in diameter) lined with two sheets of filter paper moistened with a nematode suspension at the concentration of 500 IJs/cm<sup>2</sup>, providing 100 IJs/larvae. After inoculation of the IJs and release of the larvae, Petri dishes were capped and sealed with PVC transparent film and later stored in a BOD climate chamber at 26 ± 1°C, 70 ± 10% RH in the dark (WOODRING; KAYA, 1988).

After three days, the dead larvae of *G. mellonella* were transferred to White traps (WHITE, 1927) and stored in a BOD climate chamber at 25 ± 1°C, 70 ± 10% RH. The IJs that left the cadavers of the *G. mellonella* larvae were collected in distilled water (1 cm deep) in Erlenmeyer flasks kept in a BOD climate chamber at 18 ± 1°C, 70 ± 10% RH and used two days after collection. We evaluated the virulence of the isolates *H. indica* IBCBn 05, *H. amazonensis* IBCBn 24, *S. carpocapsae* IBCBn 02 and *S. feltiae* IBCBn 47 to pupae of *D. suzukii*.

The experimental design was completely randomized, with five treatments and five replicates, each with five pupae of *D. suzukii* (five days old) per Petri dish (9 cm) lined with two sheets of filter paper. Two mL of the aqueous suspension of the isolate was inoculated separately, at a dosage equivalent to 1,000 IJs/insect, making available 166.66 IJs/cm<sup>2</sup> per dish. For the control, the Petri dishes containing filter paper were moistened with two mL of distilled water (without nematode). Petri dishes were capped and sealed with PVC transparent film and stored in a BOD climate chamber at 26 ± 1°C and 70 ± 10% RH in the dark.

The evaluation of insect mortality was based on the number of adults emerged

in Petri dishes. After the emergence period (17 days), was counted the number of emerged adults and dead pupae, these were rinsed in tap water and kept individually in Petri dishes (5 cm) about moistened filter paper until dissected. Pupae and adults were dissected for confirmation of the cause of death and the number of IJs was quantified under a microscope.

The data for mortality and virulence of pupae and adults were submitted to analysis of variance (Anova) and the means compared by Scott-Knott test at 5% of propability through the program Sisvar 5.6 (FERREIRA, 2011).

## RESULTS

The general mortality rate of pupae and adults of *H. indica* IBCBn 05 isolate was lower 80.0% ( $p = 0.0139$ ) differing statistically when compared to the other EPNs isolates 96.0, 96.0 and 100.0% (Table 1).

Table 1. General mortality (%), mortality (Mort.) and number of juveniles (N°IJs) of pupae and adult of *Drosophila suzukii* (Diptera: Drosophilidae), by *Heterorhabditis indica* IBCBn 05, *Steinernema carpocapsae* IBCBn 02, *Steinernema feltiae* IBCBn 47 and *Heterorhabditis amazonensis* IBCBn 24.

Tabela 1. Mortalidade geral (%), mortalidade (Mort.) e número de juvenis (N° Jis) em pupas e adultos de *Drosophila suzukii* (Diptera: Drosophilidae) de *Heterorhabditis indica* IBCBn 05, *Steinernema carpocapsae* IBCBn 02, *Steinernema feltiae* IBCBn 47 e *Heterorhabditis amazonensis* IBCBn 24.

Isolate	General (%)	Pupae		Adults	
		Mort ± SD	N° IJs ± SD	Mort ± SD	N° IJs ± SD
<i>H. indica</i>	80.0±1.30b	33.0±11.78a	35.0±28.12a	47.0±18.25b	125.0±70.96a
<i>S. carpocapsae</i>	96.0±0.45a	16.0±28,86a	4.4±6.98b	80.0±27,38a	33.2±10.94b
<i>S. feltiae</i>	96.0±0.45a	13.0±18.25a	0.4±0.89b	84.0±23,56a	10.8±23.57b
<i>H. amazonensis</i>	100.0±0.00a	43.0± 19.00a	30.0±28.16a	57.0± 9.00b	76.0±43.69a
Control	0±0.00c	0±0.00b	-	0±0.00c	-
CV%	12.9	76.5	115.7	33.5	68.8
P	0.0139	0.1059	0.0316	0.0524	0.0029

Means followed by distinct letters, low in the columns indicate significant differences between treatments by the Scott-Knott test at 5% probability.

The repercussion of nematodes in adults was considered satisfactory, as the numbers of dead pupae among the isolates were lower 13.0 to 43.0% ( $p = 0.1059$ ) when compared to the number of dead adults 47.0 to 84.0% ( $p = 0.0524$ ).

The nematode IJs, *H. indica* IBCBn 05 and *H. amazonensis* IBCBn 24, presented the highest mortality in pupae with 33.0 and 43.0% not statistically differing and the isolates *S. carpocapsae* IBCBn 02 and *S. feltiae* IBCBn 47, mortality of 80.0 and 84.0% ( $p = 0.0524$ ) of adults of *D. suzukii*, respectively.

*Heterorhabditis indica* IBCBn 05 and *H. amazonensis* IBCBn 24 presented the highest number of IJs, 35.0 and 30.0 ( $p = 0.0316$ ) in cadavers of pupae with no significant difference between these three isolates and in adults 125.0 and 76.0 IJs ( $p = 0.0029$ ) respectively. The numbers minors of IJs production found in the cadavers was of *S. carpocapsae* IBCBn 47 and *S. feltiae* IBCBn 02 isolate with 4.40 and 0.40 IJs in pupae and 33.2 and 10.8 ( $p = 0.0029$ ) IJs in adults (Figure 1).



Figure 1. Deformed pupae of *Drosophila suzukii* (Diptera: Drosophilidae) infected with *Heterorhabditis indica* IBCBn 05 (A); adult entomopathogenic nematodes after desiccation of the head of the adult fly (B); Infective juveniles recovered from the head and abdomen (C). Origination: Andressa Lima de Brida.

Figura 1. Pupas deformadas de *Drosophila suzukii* (Diptera: Drosophilidae) infectadas com *Heterorhabditis indica* IBCBn 05 (A); adulto de nematoides entomopatogênicos após a dissecação da cabeça da mosca adulta (B); Juvenis infectantes recuperados da cabeça e do abdome (C). Fonte: Andressa L. de Brida.

## DISCUSSION



*Drosophila suzukii* is considered a threat to fruit farming, so control methods should be used, once previous studies have achieved low efficiency of EPNs in control of *D. suzukii* (CUTHBERTSON et al., 2014; WOLTZ et al., 2015; CUTHBERTSON; AUDSLEY 2016).

Although there are few studies on the use of EPN for control of *D. suzukii*, the present study shows that the EPNs isolates evaluated are efficient in causing mortality of *D. suzukii* pupae, however there are some controversies about the efficiency of these agents as reported by some researchers. Cuthbertson et al. (2014) tested the efficiency of the association of fungi and EPNs and reported that *S. carpocapsae*, *S. kraussei* and *S. feltiae* (10,000/IJs mL) were not efficient, although the biological agents (fungi and nematodes) caused a reduction in the population of *D. suzukii*, they are not able to control or eradicate the population.

The low infection (0-2%) was also verified when evaluating the virulence of *H. bacteriophora*, *S. carpocapsae* and *S. feltiae* to larvae of *D. suzukii* on blueberries (*Vaccinium myrtillus*), these species did not affect the survival of the insect (WOLTZ et al. 2015), however, the results obtained by Cuthbertson; Audsley (2016) show that the nematodes *S. carpocapsae*, *S. feltiae*, *S. kraussei* and *H. bacteriophora* (10,000/IJs/mL) were efficient in reducing the number of emergent adults of *D. suzukii* on blueberries after suspension of fruit in a solution containing these microorganisms; *H. bacteriophora* proved to be the best of the four nematodes evaluated, with 95.00% mortality in larvae.

The genus *Drosophila* shows to be susceptible to entomopathogenic nematodes (AREFIN et al., 2014). The IJs of *H. bacteriophora* at concentrations of 10 and 1000 IJs/larvae caused mortality of 50.00% and 74.00% after 48 h, they penetrated through the cuticle or natural openings of larvae of *Drosophila melanogaster* Meigen larvae (HALLEM, 2007) in turn, axenic *Heterorhabditis* strain OregonR caused mortality in *D. melanogaster* in three days after infection with rates of 50 to 100 IJs/insect, with the repercussion of IJs in adults over a period of 24-48 hours (CASTILLO, 2011).

The presence of IJs of EPNs in adults of *D. suzukii* intensified the ability of the insect to survive infection during the pupal phase for all isolates evaluated. According

to Castillo et al. (2011), insects present a system of detection and recognition of pathogens, which when infected stimulate the production of defense cells (hemocytes), which act by phagocytizing the invader and forming an encapsulation over the same and induces efficient cellular and humoral responses. Humoral responses produce genes encoding antimicrobial peptides, which act in association with the cellular system, and this mediated by phagocytic cells, produce the encapsulation and inhibition or destruction of the pathogen (STRAND, 2008).

In *D. melanogaster*, it was found that the production of these genes starts within 12 hours after infection by *Heterorhabditis* (nematodes carrying *Photorhabdus*) suggesting that there is an immune response triggered against this complex of bacteria (MALLON et al., 2003; HALLEM, 2007). In *D. suzukii* after emergence, it appears that some of the adults overcame the immune system activity against the bacteria of the genera *Xenorhabdus* and *Photorhabdus* during the pupal phase, since bacteria can secrete antibiotic molecules that inhibit the action of the melanization enzyme produced by the host allowing their survival during the phagocytosis process (VALLET-GELY et al., 2008), although in the current study the adults of *D. suzukii* presented mortality two days after emergence.

## CONCLUSION

The species tested in this study, *S. carpocapsae* IBCBn 02, *S. feltiae* IBCBn 47 and the isolates never tested before *H. indica* IBCBn 05 and *H. amazonensis* IBCBn 24, were promising as to mortality and the ability to multiply in newly emerged adults of *D. suzukii*. Given the results obtained for mortality rates, virulence and repercussion in adults, this work opens the door to further studies to elucidate responses on the mechanisms that the symbiotic bacteria of the respective nematodes evaluated prevent or suppress in the activation of immune pathways that *D. suzukii* may present, besides the identification of molecules responsible for the detection of nematodes.



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