Toxicity of insecticides in *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae), a new strawberry pest in Brazil

ABSTRACT

The strawberry caterpillar, *Duponchelia fovealis* Zeller (Lepidoptera: Crambidae) was recently found in Brazil causing significant losses for this crop. Because it is a recent pest in the country, still, there is no record of insecticides for its control. Thus, this work evaluated the toxicity of different commercial insecticides on *D. fovealis* second instar caterpillars. The insecticides tested were: acetamiprid, cyromazine, thiamethoxam + lambda-cyhalothrin, milbemectin, chlorantraniliprole, lambda-cyhalothrin, thiamethoxam, fenpropathrin, alpha-cypermethrin, chlorfenapyr, indoxacarb, and lambda-cyhalothrin + chlorantraniliprole. The application was carried out by spraying the caterpillars in a Potter tower. Insecticides indoxacarb, lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr provided mortality data of *D. fovealis* caterpillars from 70.9 to 100%, and could be considered as promising for the management of this pest, once duly registered with the Ministry of Agriculture, Livestock and Supply (MAPA).

Keywords: Fragaria x ananassa, strawberry caterpillar, chemical control

1. INTRODUCTION

Duponchelia fovealis Zeller (Lepidoptera: Crambidae) is a microlepidopteran originating in the Mediterranean region and the Canary Islands, which is currently distributed throughout much of Europe, Asia, India, Africa, Canada and the United States [1,2]. *D. fovealis* is a polyphagous pest with a high number of host species (cereals, vegetables, medicinal and ornamental plants) [1,3–5]. In Brazil, the occurrence was reported in 2008 [1,2,5–7]. In the country, the pest was established in strawberry (*Fragaria x ananassa*) crops in the metropolitan regions of Curitiba - PR and Mountainous state of Espírito Santo, where it has caused losses of up to 100% in production [2,8,9]. For this reason, the pest was known as a strawberry caterpillar or crown caterpillar [1,5,9].

Formatted: Font: Italic

Duponchelia fovealis caterpillars pierce the crown region and fruit insertion, weakening the circulation of nutrients [7]. ~~In addition, the main losses are due to the impossibility of commercialization, since the fruits are not adequate for consumption [5,6,9].~~ The attack also occurs in the vegetative phase and the defoliation damages the photosynthetic process and, consequently, reduces the production, which in severe attacks can lead the plants to death [1,2]. **In addition,**The **the** main losses are due to the impossibility of commercialization, since the fruits are not adequate for consumption [5,6,9].

Comment [BJ-A1]: I moved the sentence to the end of the paragraph.

The management of *D. fovealis* in strawberry plantations has been hampered by the fact that it is an exotic pest and there is still no registration of products for control by the Ministry of Livestock and Supply (MAPA) [10]. However, some insecticides such as lambda-cyhalothrin, deltamethrin and chlorfenapyr are being used in emergencies in Portugal and Brazil [11].

The control of *D. fovealis* by application of insecticides with contact action is difficult because the caterpillars remain in the basal part of the plant, where they are protected from spraying by the leaves of the strawberry plants [12,13]. Another characteristic that causes difficulty in the control is the habit of this species not to feed during the last larval instar **larva**; so**e, which makes that the** insecticides with action by**of** ingestion do not have a lethal effect in th**is**ese stages [4]. Due to these difficulties and scarcity of control methods, it is necessary to evaluate the potential of synthetic insecticides in the control of this pest. Thus, the objective of this work was to evaluate the toxicity of different commercial insecticides to *D. fovealis* caterpillars.

2. MATERIALS AND METHODS

2.1 Rearing of *Duponchelia fovealis*

Caterpillars of *D. fovealis* w**W**ere collected in the state of Espirito Santo **catterpilar *D. fovealis*** from**in** areas with strawberry crops (Latitude: 20 ° 07'50 "S, Longitude: 40 ° 58'15"W) **in the state of Espirito Santo**. The caterpillars **, which** were**form** sent to **sector of** the Entomology sector of the Scientific and Technological Development in Pest and Disease Management (Nudemafi)**,** They **where they** were kept in a controlled environment at 25 ± 1°C, 70 ± 10% relative humidity (**U**RH) and 14 hours of photophase (14L:10D). R**The r**earing of the juvenile and adult**s** insects followed the methodology described by Pirovani et al.[8].

2.2 Insecticides used

The insecticides and dosages, in g active ingredient (a.i.)/L, tested were: acetamiprid (Saurus®, Iharabras S.A. Chemical Industries) – 0.08; cyromazine (Trigard® 750 WP, Syngenta Proteção de Cultivos Ltda) – 0.1125; methoxyfenozide (Intrepid® 240 SC, Dow Agrosciences Industries Ltda) – 0.12; thiamethoxam + lambda-cyhalothrin (Platinum Neo®, Syngenta Proteção de Cultivos Ltda) – 0.10575 + 0.0795; milbemectin (Milbeknock®, Iharabras S.A. Chemical Industries) - 0,02; chlorantraniliprole (Premio®, Du Pont of Brazil S.A) – 0.0368; lambda-cyhalothrin (Karate Zeon® 50 CS, Syngenta Proteção de Cultivos Ltda) – 0.04; deltamethrin (Decis® 25 EC, Bayer CropScience Ltda) – 0.0125; thiamethoxam (Actara® 250 WG, Syngenta Proteção de Cultivos Ltda) – 0.75; fenpropathrin (Danimen® 300 EC, Sumitomo Chemical of Brazil Representations Ltda) – 0.195; alpha-cypermethrin (Fastac® 100, Basf S.A) – 0.01; chlorfenapyr (Pirate®, Basf S.A) – 0.24; indoxacarb (Rumo® WG, Du Pont of Brazil S.A) – 0.048 and lambda-cyhalothrin + chlorantraniliprole (Ampligo®, Syngenta Proteção de Cultivos Ltda) – 0.025 + 0.05. The highest doses recorded in the package leaflets of each product were used and as a control treatment distilled water was used**.**

2.3 Toxicity Bioassay

For the bioassay, foliar discs of strawberry of the variety "Tudla" (ø = 4 cm) **hygienised** cleaned in 5% (v / v) sodium hypochlorite solution and washed immediately under running water. For the removal of excess water, the leaf discs were kept on paper towel, then transferred to Petri dishes (9.5 x 1.5 cm), lined with filter paper. After this procedure, five second instar caterpillars were transferred to the leaf discs and in the sequence were sprayed on Potter's Tower®. The second instar larvae of *D. fovealis* were selected for use in the bioassay because **due to the fact that** only from this instar and onward it is possible to handle without causing mortality. For the calculation of the dilutions the application volume of 300 L/ha was adopted. The equipment was set at a pressure of 15 lb/in², using 18 mL of each treatment, measured with 2 mL automatic **pipettor**pipette. The upper part of the Petri

Comment [BJ-A2]: Or do you mean «sterilized»? This word, hygienised, does not seem to occur in English. I can't find it.

Comment [BJ-A3]: Plastic or glass Petri dishes?

dish (6 mL), the abaxial face (6 mL) and the adaxial leaf discs (6 mL) were sprayed. After drying, the Petri dishes were closed and transferred to an air-conditioned room set at a temperature of 25 ± 1ºC, relative humidity of 70 ± 10% and photophase of 12h.

The bioassay was installed in a completely randomized design with 14 treatments (insecticides) with five replicates (25 caterpillars / replicate) for each treatment. The evaluation of larval mortality was performed after 48, 72 and 96 h after spraying. The accumulated mortality data in 96h were corrected by Abbott's formula [14], transformed into $\sqrt{x+1}$ and submitted to analysis of variance [15]. Mortality averages were grouped by the Scott-knott test ($P \leq .05$) using the software R version 3.3.3 [16] using the ExpDes.pt package [17]. The Henderson and Tilton formula [18] was used to calculate control efficiency.

2.4 Concentration-Mortality Curves of Insecticides

From the results of the previous experiment the concentration-mortality curves of caterpillars were established for the insecticides that caused higher mortality to the caterpillars. The spraying and evaluation procedure were the same as in the previous bioassay. The concentrations used were based on preliminary tests aiming at a mortality between 0 and 100%, being equidistantly spaced by means of logarithmic transformation [15]. Based on **the** previous results, insecticides (concentration range, mg a.i./L) were selected for this test: chlorfenapyr (25.0-250.0); indoxacarb (0.025-1.0) and; lambda-cyhalothrin + chlorantraniliprole (10.0-2,250.0).

The accumulated caterpillar mortality data were submitted to probit analysis [15,19], using the ecotoxicology package [20]. The toxicity ratios (TR) were obtained by means of the quotients between the LC_{50} of the insecticide that showed the lowest toxicity and the LC_{50}s of the other treatments [15].

3. RESULTS

3.1 Toxicity of insecticides

The mortality of *D. fovealis* varied between the insecticides studied (F = 5.404, df = 14, 32, $P < .001$) (Table 1). The insecticides**,** lambda-cyhalothrin, milbemectin, cyromazine, thiamethoxam, methoxyfenozide and deltamethrin did not cause mortality and therefore were not considered in the analysis**analyzes** (Table 1). Among the other insecticide mortality ranged from 17 to 100%. The insecticides chlorfenapir, indoxacarb and lambda-cyhalothrin + chlorantraniliprole were the ones that caused higher mortalities, being statistically similar among them and superior to the other insecticides tested. Thus, these insecticides were the most efficient in controlling *D. fovealis* (Table 1).

The insecticides alpha-cypermethrin, chlorantraniliprole, acetamiprid, thiamethoxam + lambda-cyhalothrin and fenpropatrin caused mortality of less than 41%.

Table 1. Mortality of second instar caterpillars of *Duponchelia fovealis* treated with different insecticides and control efficiency. Temp .: 25 ± 1 ° C, RH 70 ± 10% and 12h of photophase

Active ingredient*	Corrected Mortality [1,2]	CE[3]
Alfacipermethrin	40.6 ± 6.46 b	40.0
Indoxacarbe	70.9 ± 12.47 a	73.3
Chlorantraniliprole	34.6 ± 12.36 b	35.0
Acetamiprid	24.5 ± 10.75 b	33.3
Lambda-cyhalothrin + Chlorantraniliprole	80.0 ± 12.93 a	80.0
Thiamethoxam + Lambda-cyhalothrin	24.5 ± 10.75 b	33.3
Phenpropathic	16.9 ± 6.23 b	20.0
Chlorfenapyr	100.0 ± 0.00 a	100.0
F	5.404	
P	<.001	

[1] Means followed by the same letter in the column do not differ from each other by the Scott-Knott test ($P \leq .05$).
[2] Data transformed by $\sqrt{x+1}$.
[3] Efficiency of control [Henderson and Tilton equation (1955)].
* Products that did not cause mortality were omitted and did not participate in the analysis.

3.2 Concentration-Mortality Curves of Insecticides

The mortality data of *D. fovealis* treated with **the** different concentrations of the three insecticides selected were adjusted to the Probit model ($P > .05$). The LC_{50} values for chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole were 64, 0.08 and 30 (mg a.i./L), respectively (Figure 1, Table 2). Based on these results, the lowest LC_{50} was observed for **the** indoxacarb **insecticide**, followed by lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr. The toxicity ratio of indoxacarb is **to be** 800 times more toxic than chlorfenapyr.

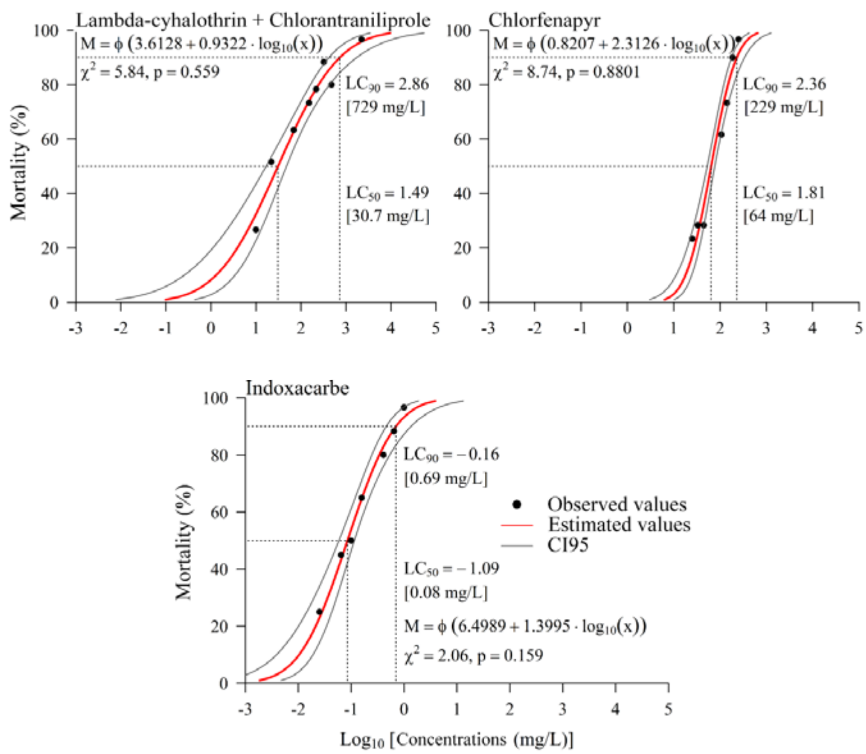

Figure 1. Concentration-mortality curves of chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole on second instar caterpillars of *Duponchelia fovealis*. Temp.: 25 ± 1°C, RH 70 ± 10% and 12h of photophase.

Table 2. Response of the concentration-mortality curves of chlorfenapyr, indoxacarb and lambda-cyhalothrin + chlorantraniliprole on second instar caterpillars of *Duponchelia fovealis*. Temp.: 25 ± 1°C, RH 70 ± 10% and 12h of photophase

Active ingredient	n[1]	LC_{50} (CI a 95%)[2] * (mg a.i./L)		TR_{50}[3]
Chlorfenapyr	420	64 (52-77)	c	-
Lambda-cyhalothrin + chlorantraniliprole	480	30.7 (16.7-47.5)	b	2.13
Indoxacarbe	420	0.08 (0.07-0.1)	a	800

[1] Number of insects used in the test;
[2] Confidence interval of the LC_{50} at 95% probability;
[3] Toxicity ratio;
* LC_{50}s accompanied by the same letter do not differ from each other based on the confidence interval at 95% probability.

4. DISCUSSION

The absence or reducedtion of mortality of *Duponchelia. foveali*s by the insecticides , lambda-cyhalothrin, milbemectin, cyromazine, thiamethoxam, methoxyfenozide and deltamethrin, alpha-cypermethrin, chlorantraniliprole, acetamiprid, thiamethoxam + lambda-cyhalothrin and fenpropatrin may be related **to the fact of** the low concentrations tested **are insufficient to cause mortality**, or this species may have **has** some degree of resistance/tolerance to the active ingredients.

Among the insecticides tested, chlorfenapyr, which caused higher mortality in *D. foveali*s caterpillars, is a de-coupling of oxidative phosphorylation through the interruption of the H^+ proton gradient, acting on the respiratory metabolism of the insect [21]. This insecticide presented the highest slope of the concentration-mortality curve among the three products (Figure 1). High line slope values indicate that small variations in insecticide concentration promote greater responses in target pest mortality. Thus, small amount of the active principle is effective in the mortality of this pest species **of pest** in less time of exposure as it **. Thus, as** occurs with *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) after 24h exposure [22].

The insecticide indoxacarb is used in the control of different **species of insects of the order** Lepidoptera species; for example**,** **such as** damage by *Neoleucinoides elegantalis* (Guenée) (Lepidoptera: Crambidae) has an 80% **damage** reduction effectiveness [23]. In addition, indoxacarb has low toxicity to non-target organisms [24], which makes this insecticide an option to be used in association**s** with other management tactics, such as biological control.

Comment [BJ-A4]: Spelling is Neoleucinodes elegantalis (Guenée)

Indoxacarb**e** acts after **the** bioactivation in the activity of enzymes present in the insect and that result in the formation of the metabolite JT333 (N-decarbomethoxyllated) that causes the blockade of the sodium channels. The metabolism of indoxacarb in JT333 causes small seizures that **to** paralyze the insects, followed by death [25]. Activities such as feeding and oviposition are interrupted rapidly after exposure to the product, whereas death may take 4 to 72 hours [21], which can be proven **here** in the present study with **for** *D. foveali*s.

The insecticide based on lambda-cyhalothrin + chlorantraniliprole is available as **composes** a ready-to-use mixture with activity at two sites of action. The active principle lambda-cyhalothrin belongs to the chemical group of pyrethroids and**s** they are known as modulators of the sodium channels of **the** nerve cells of the peripheral nervous system, **thus acting on the sodium channels**, prolonging the influx and reducing the threshold of the action potential of the membrane, causing immediate paralysis and mortality, which is called a knock-down effect [25].

On the other hand, chlorantraniliprole is an insecticide modulator of rianodine receptors, which regulate the release of calcium in the sarcoplasmic reticulum of muscle cells, thus affecting the muscular contraction of insects [21,26]. These insecticides require a longer time to cause insects to die [27]. However, the association between lambda-cyhalothrin and chlorantraniliprole, in the insecticide tested, promoted mortality similar to those of indoxacarb and chlorfenapyr at the same time exposure period.

Although the LC_{50} of lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr in *D. foveali*s is superior, when compared to that of indoxacarb, these insecticides are promising for the integrated management of the pest, since they can be used in rotation of mechanisms of action, thus minimizing the risk of selection of insecticide-resistant individuals.

5. CONCLUSION

Of the insecticides tested, indoxacarb, lambda-cyhalothrin + chlorantraniliprole and chlorfenapyr were the most effective for the control of *Duponchelia. fovealis*, the first being the one with the highest toxicity (<LC_{50}) and whih can be used in *D. fovealis* integrated management programs.

REFERENCES

[1] M.A.C. Zawadneak, H.R. Vidal, B. Santos, Lagarta-da-coroa, *Duponchelia fovealis* (Lepidoptera: Crambidae), in: E. Vilela, R.A. Zucchi (Eds.), Pragas Introduzidas Insetos e Ácaros, FEALQ, Piracicaba (Brazil), 2015: pp. 216–231.

[2] M.A.C. Zawadneak, R.B. Gonçalves, I.C. Pimentel, J.M. Schuber, B. Santos, A.S. Poltronieri, M.A. Solis, First record of *Duponchelia fovealis* (Lepidoptera: Crambidae) in South America, Idesia (Arica). 34 (2016) 91–95. doi:10.4067/S0718-34292016000300011.

[3] C.P. Bonsignore, V. Vacante, *Duponchelia fovealis* Zeller. Une nuova emergenza per la fragola?, Prot. Delle Colt. 3 (2010) 40–43. https://www.researchgate.net/profile/C_Bonsignore/publication/273153213_Duponchelia_fovealis_Zeller_A_new_emergency_for_strawberry/links/54fade590cf20b0d2cb84a51/Duponchelia-fovealis-Zeller-A-new-emergency-for-strawberry.pdf.

[4] M.C. Franco, M.C. Baptista, *Duponchelia fovealis* Zeller - nova praga em Portugal, Frutas, Legum. e Flores. 110 (2010) 34–35.

[5] J.P.P. Paes, V.D. Pirovani, D. Pratissoli, Lagarta do morangueiro, in: D. Pratissoli (Ed.), Pragas Emergentes No Estado Do Espírito St., Unicopy, Alegre - ES, 2015: pp. 88–95.

[6] V.D. Pirovani, D. Pratissoli, J.R. Carvalho, L.P. Dalvi, Manejo de Pragas para Cultura do Morangueiro: Sem Resíduo de Agrotóxicos, SÉRIE TÉCNICA. (2015). doi:10.13140/RG.2.1.3987.7364.

[7] M.A.C. Zawadneak, R.B. Gonçalves, A.M. Bischoff, Duponchelia fovealis: nova praga em morangueiro no Brasil, UFPR, Curitiba - PR, 2014. http://www.bio.ufpr.br/portal/pimo/wp-content/uploads/sites/11/2014/08/Duponchelia-fovealis-UFPR_digital.pdf.

[8] V.D. Pirovani, D. Pratissoli, M.O. Tibúrcio, J.R. de Carvalho, A.P. Damascena, L.V. Faria, Trichogramma galloi and Trichogramma pretiosum for the management of Duponchelia fovealis (Lepidoptera: Crambidae) in strawberry plants, Pesqui. Agropecuária Bras. 52 (2017) 690–693. doi:10.1590/s0100-204x2017000800015.

[9] J.P.P. Paes, V.L.S. Lima, D. Pratissoli, J.R. Carvalho, V.D. Pirovani, R.C.O.F. Bueno, Thermal requirements, development and number of generations of Duponchelia fovealis (Zeller) (Lepidoptera: Crambidae), An. Acad. Bras. Cienc. 90 (2018) 2447–2457. doi:10.1590/0001-3765201820160891.

[10] AGROFIT, Sistema de agrotóxicos Fitossanitários do Ministério da Agricultura, Pecuária e Abastecimento, (2017). http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed January 9, 2017).

[11] J.C. de Souza, R.A. Silva, É.C. da Silveira, F.A. Abreu, M.A. de Toledo, Ocorrência de nova praga nas lavouras de morango no Sul de Minas, Circ. Técnico. (2013) 1–5. http://www.epamig.br/download/circular-tecnica-180/.

[12] S.D. Stocks, A. Hodges, Featured Creatures – European pepper moth – Duponchelia fovealis (Zeller), (2013). http://entnemdept.ufl.edu/creatures/veg/leps/european_pepper_moth.htm (accessed

November 26, 2013).
[13] J.A. Bethke, B.M. Vander, Pest Alert: Duponchelia fovealis, (2013). http://ucanr.org/sites/cetest/files/55177.pdf (accessed June 17, 2013).
[14] W.S. Abbott, A Method of Computing the Effectiveness of an Insecticide, J. Econ. Entomol. 18 (1925) 265–267. doi:10.1093/jee/18.2.265a.
[15] J.R. Carvalho, D. Pratissoli, U.R. Vianna, A.M. Holtz, Análise de probit aplicada a bioensaios com insetos, IFES, Colatina, ES, Brazil, 2017. https://www.researchgate.net/publication/317994149_ANALISE_DE_PROBIT_APLICADA_A_BIOENSAIOS_COM_INSETOS.
[16] R Core Team, R: A language and environment for statistical computing, (2017). https://www.r-project.org.
[17] E.B. Ferreira, P.P. Cavalcanti, D.A. Nogueira, ExpDes.pt: Experimental Designs package (Portuguese), (2013).
[18] C.F. Henderson, E.W. Tilton, Tests with Acaricides against the Brown Wheat Mite12, J. Econ. Entomol. 48 (1955) 157–161. doi:10.1093/jee/48.2.157.
[19] D.J. Finney, Probit analysis, 3rd ed., Cambridge University Press, Cambridge, 1971.
[20] J. Gama, ecotoxicology: Methods for Ecotoxicology, (2015).
[21] P.G. Marçon, Modo de ação de inseticidas e acaricidas-IRAC BR, (2017). http://media.wix.com/ugd/2bed6c_7bae6f13bf0e4e0f9dd38a2bd1ae544e.pdf (accessed April 16, 2017).
[22] J.J. Adamczyk, B.R. Leonard, J.B. Graves, Toxicity of Selected Insecticides to Fall Armyworms (Lepidoptera: Noctuidae) in Laboratory Bioassay Studies, Florida Entomol. 82 (1999) 230. doi:10.2307/3496574.
[23] S. Martinelli, M.A. Montagna, N.C. Picinato, F.M.A. Silva, O.A. Fernandes, Eficácia do indoxacarb para o controle de pragas em hortaliças, Hortic. Bras. 21 (2003) 501–505. doi:10.1590/S0102-05362003000300018.
[24] R.S. Ramos, C.S. Sediyama, E.A. Queiroz, T.L. Costa, J.C. Martins, T.A. Araújo, M.C. Picanço, Toxicity of insecticides to Chrysodeixis includens and their direct and indirect effects on the predator Blaptostethus pallescens, J. Appl. Entomol. 141 (2017) 677–689. doi:10.1111/jen.12382.
[25] S.J. YU, The Toxicology and Biochemistry of Insects, 2nd ed., CRC Press, Boca Raton - Florida, 2014.
[26] T. Masaki, N. Yasokawa, M. Tohnishi, T. Nishimatsu, K. Tsubata, K. Inoue, K. Motoba, T. Hirooka, Flubendiamide, a novel Ca2+ channel modulator, reveals evidence for functional cooperation between Ca2+ pumps and Ca2+ release., Mol. Pharmacol. 69 (2006) 1733–9. doi:10.1124/mol.105.020339.
[27] D.B. Sattelle, D. Cordova, T.R. Cheek, Insect ryanodine receptors: molecular targets for novel pest control chemicals, Invertebr. Neurosci. 8 (2008) 107–119. doi:10.1007/s10158-008-0076-4.