Peruvian Agricultural Research
ISSNe 2706-9397
Homepage: http://revistas.unjfsc.edu.pe/index.php/PeruvianAgriculturalResearch

Received: January 12, 2023 / Accepted: April 14, 2023

Artículo original

Biological cycle and comparative study of bioinsecticide efficiency on the *Heliothis virescens* (Lepidoptera:Noctuidae) borer in blueberry cultivation

Ciclo biológico y estudio comparativo de la eficacia de bioinsectisidas sobre el barrenador *Heliothis virescens* (Lepidoptera: Noctuidae) en el cultuvo de arándano

R.E. Velázquez-Ochoa[1,*] & J. P. Quiliche-Durán[2]

https://doi.org/10.51431/par.v1i1.809

Abstract

Objectives: To determine the biological cycle of *H. virescens* and the comparative efficiency of bioinsecticides of *Bacillus thuringiensis* (Bt) (native strain) with respect to Bt (Kurstaki strain) and nuclear polyhedrosis virus (NPV), on fifth instar larvae. *Methodology*: Postures and larvae of *H. virescens* were collected from agricultural farms in Huaral (Lima) and reared in the laboratory. The Bt and NPV strains were applied to fifth instar larvae under field conditions, evaluating larval mortality at 72 hours using Tukey's test with the Infostat software. *Results*: It was found that the life cycle of *H. virescens* was 77.78 ± 9.14 days with complete metamorphosis. Larval mortality was statistically similar ($p > 0.05$) with native Bt and Bt Kurstaki treatments, and NPV application outperformed ($p < 0.05$) both Bt treatments in fifth instar larval mortality. *Conclusion*: The life cycle of *Heliothis virescens* in blueberries has a complete metamorphosis with six larval stages ranging from 59-88 days and better control of fifth-instar larvae was obtained with NPV.

Keywords: *Bacillus thuringiensis* Kurstaki; fruit bollworm; nuclear polyhedrosis virus; Tukey test; *Vaccinium corymbosum* var. Biloxi.

Resumen

Objetivos: Determinar el ciclo biológico de *H. virescens* y la eficacia comparativa de los bioinsecticidas *Bacillus thuringiensis* (Bt) (cepa nativa) respecto a Bt (cepa Kurstaki) y del virus de la poliedrosis nuclear (NPV), sobre larvas de quinto estadio. *Metodología*: Posturas y larvas de *H. virescens* fueron recolectadas de fincas agrícolas de Huaral (Lima) y criadas en laboratorio. Las cepas Bt y NPV fueron aplicadas a larvas de quinto estadío en condiciones de campo, evaluando la mortalidad larval a las 72 horas mediante la prueba de Tukey con el software Infostat. *Resultados*: Se encontró que el ciclo de vida de *H. virescens* fue de 77,78 ± 9,14 días con una metamorfosis completa. La mortalidad larvaria fue estadísticamente similar ($p > 0,05$) con los tratamientos Bt nativo y Bt Kurstaki y la aplicación de NPV superó ($p < 0,05$) a ambos tratamientos Bt en mortalidad larvaria en el quinto estadio. *Conclusión*: El ciclo biológico de *Heliothis virescens* en arándanos tiene una metamorfosis completa con seis estadíos larvarios que oscilan entre 59-88 días y se obtuvo un mejor control de larvas de quinto estadio con NPV.

Palabras clave: *Bacillus thuringiensis* Kurstaki; gusano perforador de frutos; prueba de Tukey; virus de la poliedrosis nuclear; *Vaccinium corymbosum* var. Biloxi.

[1] Instituto Nacional de Innovación Agraria-INIA, E.E.A.Donoso, Huaral, Lima Perú.

[2] Escola da Engenharia de Lorena, programa de Pós-Graduação, Universidade São Paulo. Estrada municipal do Campinho, No. 100, Campinho, Lorena/ SP, Brasil.

* Autor para correspondencia: rvelasquez@inia.gob.pe

Introduction

In recent years, people's habits around the world have been changing with healthier trends, mainly due to the increase in diseases and conditions such as obesity and cancer; for this reason, the consumption of highbush blueberries (*Vaccinium corymbosum* L.) is essential in diets, because the high content of antioxidants, fibre, vitamin C and low caloric intake; blueberries have one of the highest concentrations of iron of the temperate fruits (Michalska & Lysiak, 2015; Romo et al., 2019). *Vaccinium* species are unique compared to many of the world's fruit crops in that, most of the harvesting and culture of species for food occurred in the geographic regions of their origin (Curutchet et al., 2019). This suggests that insects involved in many of the ecosystem services for these berries are native species, that have a shared co-evolutionary history (Jones et al., 2014).

In addition, blueberry production presents high profitability for farmers and has a commercial opportunity that allows exporting the product within international competition (Romo et al., 2019). Despite the enormous export potential that blueberry production has, it represents an alternative to product diversification in the Lima region (Ramos et al., 2018). Unfortunately, one of the limiting factors of crop production is insect pests, highlighting *Heliothis virescens*, whose larvae feed on leaves, young shoots, flowers, and fruits (Fulcher et al., 2015). In this regard, Fulcher et al. (2015) indicate that it can cause damage to up to 40% of the total plant population.

Heliothis virescens (L.) (Lepidoptera: Noctuidae) is a polyphagous pest that has the ability to feed on more than 100 plant species (Blanco et al., 2008). Sullca et al. (2019) mention that this is the main eating pest of foliage of the highbush blueberry in Peru conditions. The current markets are highly demanding, which forces producers to obtain crops with high yields and high-quality standards, free of damage by pests, as well as toxic pesticide residues which restrict their access to the world market, for exceeding the maximum residue limits (Tarkanyi et al., 2019) and affecting bee pollination (Jones et al., 2014).

For a few years has emphasized the use of bioinsecticides because they are biodegradable and target-specific products; entomopathogenic organisms that include fungi, protozoa, nematodes, viruses, and some groups of bacteria. Within this latter group, *Bacillus thuringiensis* (Bt) is one of the most important species frequently used to produce bio-insecticides (Chakroun et al., 2016). Presents the peculiarity of producing crystal-type inclusions in their sporulation phase. Active δ-endotoxins have been discovered against Lepidoptera (butterflies), Coleoptera (beetles), Diptera (flies), Hymenoptera (ants), mites and against other invertebrates such as nematodes, flatworms, and protozoa (Sullca et al., 2019). The advantages of Bt are that it is safe for humans, domestic animals, flora, and fauna. Meanwhile, *H. virescens* is a significant pest that has the ability to feed many plant species, and the extensive use of Bt crops or Bt sprays has already led to the evolution of resistance to insects in the field for some species of Lepidoptera and Coleoptera (Pickett et al., 2017).

The nuclear polyhedrosis virus (VPN) is part of the baculoviruses that infect numerous species of insects, mainly Lepidoptera (Nawaz y Ghramh, 2019; Teakle et al., 1985). These offer great potential to be used within Integrated Pest Management, due to their high specificity to certain pests, increased virulence, compatibility with other control tactics, ease of production, stability in storage, safety (harmless to man and other animals) and the advantage of not affecting the natural balance of the agroecosystem (Kumar & Singh, 2015; Kumar, 2012). The production of viruses in live host insects is currently the most widely used method to obtain material that can be used in viral insecticides (Lasa et al., 2008; Badii & Abreu, 2006).

In the case of *H. virescens* control, Romero et al. (2012) reported that the treatments that showed the best effect were Indoxacarb (Avaunt 150 SC) and *Streptomyces scabies* (Agryben 5 SG), with 100% control in the field. Romeu & Veitía (2012) used the essential oil of *Cymbopogon nardus and Coriandrum sativum*, turning out to be inhibitors of feeding in third instar larvae of *H. virescens* in 90 and 60% respectively.

The objective of the research was to describe the life cycle of the fruit borer in blueberries and to establish, preliminarily, the effect of three bioinsecticides on *H. virescens* larvae under controlled conditions.

Methodology

Study area

The research was carried out at the Instituto Nacional de Innovación Agraria (INIA) Experimental Station (EEA) ¨Donoso¨ at Huaral (Lima), (11°52' S 77°23' W, 180 masl), with average temperatures between 18 and 27º C and 58 to 96 % relative humidity. The insect breeding was carried out under laboratory conditions and the samples were obtained in experimental fields of blueberries *Vaccinium corymbosum* cv. Biloxi under bed system, having a planting density of 0.50 m between plants and 2.00 m. between lines, during the phenological stage of the vegetative growth and fruiting of the blueberry, in the summertime.

Determination of the life cycle of H. virescens

The experimental material consisting of *H. virescens* postures and larvae was collected from Agrícola Santa Azul S.R.L, Agrocosta e Inkas Berries. Flasks containing the samples were placed in cages, feeding them with tender blueberry sprouts and cotton dipped in water to maintain high humidity conditions. Then, the material was transported to Laboratorio de Entomología at Instituto Nacional de Innovación Agraria, Huaral.

The insect breeding began with 20 eggs of *Heliothis virescens* under laboratory conditions and the newly hatched larvae were individually located in Petri dishes and fed daily with blueberry sprouts. Throughout the larval stage were conditioned in cages and blueberry sprouts were placed in bottles with water, to facilitate oviposition. Once the population was uniform, the evaluation of the larval stages began, which consisted of the number of days for each stage of development, as well as the dates of oviposition, pre-oviposition periods, oviposition, as well as the total number of oviposition. After the adults emerged, females were evaluated on a daily basis, counting the number of eggs per female.

Biological control assay of H. virescens

It was used one native strain of *B. thuringiensis* that was isolated, characterized and quantified at a concentration of 4.68 x 10^8 CFU mL L^{-1}, at Laboratorio de Biotecnología de la Producción at the University José Faustino Sánchez Carrión (Huacho, Lima). A commercial strain of *B. thuringiensis var.* Kurstaki had obtained from a commercial product 6.4% PM. Nuclear polyhedrosis virus (NPV), was extracted from a commercial product; these products were prepared and applied as directed by the manufacturer.

The experimental phase was carried out under controlled conditions, which consisted of collecting larvae from the fifth stage of *Heliothis virescens* and covering the experimental material with an anti-aphid mesh of the size of 20 x 50 cm for each individual, with blueberry sprouts. The treatments were applied with a manual sprayer at the indicated doses using a sample of 25 larvae for each treatment and three replications each (Bt Kurstaki, native Bt, and VPN), plus the control without application. Larvae were counted 72 hours after the treatment application and expressed in mortality percentage. Means were analyzed by the Tukey test multiple comparisons ($P = 0.95$), with the statistical program Infostat.

Results and Discussion

Determination of the life cycle of H. virescens

H. virescens eggs presented a hemispherical shape with a length of 0.5-0.8 mm as indicated by Méndez (2003), with numerous radial striations. At the beginning of oviposition, they were white, turning orange and finally greyish brown. The eggs had an incubation period of 3-4 days, values similar to those obtained in other reports, such as 3 days, 2-3 days (Méndez, 2003), and 4.59 days (Pérez & Moraima, 2012).

The larvae presented variable colouration, dark green to yellowish-green with longitudinally ordered white dots, with three dark lines on the back. Six larval stages were observed, which were differentiated by body length. The duration of the complete larval stage was from 28 to 54 days (table 1). The larval stage I (6-8 days), larval II (5-7 days), larval III (4-7 days), larval IV (3-7 days), larval V (3-7 days), larval VI (7-14 days) corroborating the results of Méndez (2003), who indicates that each stage lasts approximately between 2-4 days.

Pupae showed light brown color with thin spines on the cremaster, length of 15 - 18 mm. The time of the pupal period ranged from 17 - 26 days; various reports suggest values of the pupal period as 8 - 9 days (Pérez & Moraima, 2012) and 10 - 11 days (Méndez, 2003).

Adult females were dark brown and males were light straw-coloured; forewings had three transverse brown bands, and the hind wings were a silver color, with dark margins. The body length of males ranged from 13 - 15 mm and in females from 12 - 24 mm; these data are similar to those of Méndez (2003); the duration in the adult period was 7-35 days.

Table 1

Duration (days) of H. virescens development stages under controlled conditions

Development Stages	Min	Max.	Period (days)
Egg	3	4	3.64 ± 0.49
Larva I	6	8	6.24 ± 0.66
Larva II	5	7	5.23 ± 0.66
Larva III	4	7	4.06 ± 0.83
Larva IV	3	7	3.88 ± 1.22
Larva V	3	7	5.35 ± 1.49
Larva VI	7	14	9.23 ± 2.48
Pupae	17	26	21.05 ± 2.38
Adult female	7	21	15.12 ± 5.79
Adult male	17	35	24.80 ± 6.27
Life cycle	59	88	77.78 ± 9.14

The life cycle of *H. virescens* ranged in conditions of the present study, between 59 - 88 days; Méndez (2003) reported that with a temperature of 27.3 - 29.5 ° C and average relative humidity of 71.1 - 88.5% respectively, the life cycle was 33 - 37 days when the larvae presented five instars, while for those of six instars, was 37 - 44 days, in tobacco leaves. Pérez & Moraima (2012) reported a life cycle of 44.50 ± 1.58 for females and 44.05 ± 3.80 days for males; values of this research do not agree with the authors mentioned due to environmental conditions and to kind of food that was provided during the development of the insect.

The ratio of females to males to females was 6:8, with a higher prevalence of males at 33 %. In the data of *H. virescens* oviposition, females showed a pre-oviposition time of 3 - 7 days before reaching the stage of sexual maturity; Subsequently, the oviposition period varied from 11-23 days, with an average value of 13.6 - 68.5 eggs per day and a total of 313 to 879 eggs per female during their life cycle, a result similar to that reported by Méndez (2003); the post-period oviposition ranged from 2-6 days (Table 2).

Table 2

Oviposition time (days) of H. virescens under controlled conditions

Phases	**Min**	**Max**	**Time (days)**
Pre-oviposition period	3	7	4.6 ± 1.51
Oviposition period	11	23	16.6 ± 5.32
Post oviposition period	2	6	4.0 ± 1.58
Total oviposition	313	879	537.6 ± 276.9
Daily oviposition	13.6	68.5	35.06 ± 21.66

This table shows the oviposition time in days of *Heliothis virescens* under controlled conditions.

Biological control assay of H. virescens

The preliminary results of the application of the treatments to *H. virescens* fifth instar larvae after 72 hours (Table 3), show that the Bt Native and Bt Kurstaki treatments were not significantly different and that the 3 treatments were significantly different from the control without application, and it would be considered that they were effective to decrease insect survival with respect to control. The application of NPV showed the highest level of control in the larvae, with 80% mortality at 72 hours.

Table 3

Larval mortality (%) at 72 hours after the application of bioinsecticides under controlled conditions

Treatment	Mortality (%) +
VPN	80.00 [a]
Bt Native	65.33 [b]
Bt Kurstaki	60.00 [b]
Control	4.00 [c]

Means with different letters are statistically significant according to the Tukey test (p <0.05)

In this regard, Carreras et al. (2009) obtained in the second instar larvae, a mortality of 50% with a concentration of *B. thuringiensis* 2.6 x 10^7 spores mL^{-1} under laboratory conditions, while the present study in conditions of captivity, 60-65% mortality was obtained with Bt strains applied in fifth instar larvae. Romero et al. (2012) in bioassays with the Cry1Ac toxin obtained from *B. thuringiensis*, found that the composition of the diet influences the susceptibility response, considering the criterion correct in our experiment to individually feed the larvae for each source with blueberry.

Conclusions

The life cycle of *Heliothis virescens* in blueberries has a complete metamorphosis with sixth larval stages ranging from 59-88 days, and fecundity of female adults from 313 to 879 eggs, under controlled conditions. Better control of fifth instar larvae with the nuclear polyhedrosis virus was evidenced compared to native or commercial strains of *Bacillus thuringiensis* in controlled conditions, in Huaral.

Acknowledges

The research project was funded by PNIA 241_PI at the Instituto Nacional de Innovación Agraria (INIA), Donoso Experimental Station, Huaral-Lima, Perú.

References

Badii, M.H. & Abreu, J.L. (2006). Control biológico una forma sustentable de control de plagas. *Daena: International Journal of Good Conscience*. 1(1), 82-89. http://www.spentamexico.org/v1-n1/1(1)%2082-89.pdf

Blanco, C.A.; Teran-Vargas, A.P.; Abel, C.A.; Portilla, M.; Rojas, M.G.; Morales-Ramos, J.A. & Snodgrass, G.L. (2008). Plant host effect on the development of *Heliothis virescens* F. (Lepidoptera: Noctuidae). *Environmental Entomology*, 37, 1538-1547. http://doi.org/10.1603/0046-225X-37.6.1538

Carreras, B.; Rodríguez, D. & Piedra, D. F. (2009). Evaluación de cepas nativas de *Bacillus thuringiensis* Berliner para el control de Heliothis virescens Fabricius en el cultivo del tabaco en Cuba. *Fitosanidad*, 13(4),277-280. http://www.redalyc.org/pdf/2091/209114853009.pdf

Chakroun, M.; Banyuls, N.; Bel, Y.; Escriche, B. & Ferré, J. (2016). Bacterial vegetative insecticidal proteins (Vip) from entomopathogenic bacteria. *Microbiology and Molecular Biology Reviews*, 80, 329–350. http://doi.org/10.1128/MMBR.00060-15

Curutchet, A.; Cozzano, S.; Tárrega, A. & Arcia, P. (2019). Blueberry pomace as a source of antioxidant fibre in cookies: Consumer´s expectations and critical attributes for developing a new product. *Food Science and Technology International 25*, 8, 642-648. http://doi.org/10.1177/1082013219853489

Fulcher, A.; Gauthier, N.; Klingeman, W. & Hale, F. (2015). Blueberry Culture and Pest, Disease, and Abiotic Disorder Management during Nursery Production in the Southeastern U.S.: Review. *Journal of Environmental Horticulture*, *33*(1), 33-47. http://doi.org/10.24266/0738-2898-33.1.33

Jones, M. S.; Vanhanen, H.; Peltola, R. & Drummond, F. (2014). A global review of arthropod-mediated ecosystem-services in Vaccinium berry agroecosystems. *Terrestrial Arthropod Reviews*, 7(1), 41-78. http://www.cabdirect.org/cabdirect/abstract/20143190827

Kumar, S. & Singh, A. (2015). Biopesticides: Present Status and the Future Prospects. *Fertilizers & Pesticides*, 6(2), 1000e129. http://dx.doi.org/10.4172/jbfbp.1000e129

Kumar, S. (2012). Biopesticides: A need for Food and Environmental Safety. *Biofertilizers & Biopesticides*, 3(4), 1000e107. http://www.londom.org/open-acces/biopesticides-a-need-for-food-and-environmental-safety-2155-6202.1000e107.pdf

Lasa, R.; Williams, T. & Caballero, P. (2008). Insecticidal Properties and Microbial Contaminants in a *Spodoptera exigua* Multiple Nucleopolyhedrovirus (Baculoviridae) Formulation Stored at Different Temperatures. *Journal of Economic Entomology*, *101*(1), 42-49. http://doi.org/10.1603/0022-0493(2008)101[42:IPAMCI]2.0.CO;2

Méndez, B. (2003). Aspectos biológicos sobre *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae) en la empresa municipal agropecuaria Antonio Guiteras de la zona norte de la provincia de las tunas. *Revista Fitosanidad*, *7*(3), 21-25. http://www.redalyc.org/pdf/2091/209118166005.pdf

Michalska, A. & Lysiak, G. (2015). Bioactive Compounds of Blueberries: Post-Harvest Factors Influencing the Nutritional Value of Products. *International Journal of Molecular Sciences*, 16(8), 18642-18663. http://doi.org/10.3390/ijms160818642

Nawaz, A. & Ghramh, H. (2019). Comparative bio-efficacy of nuclear polyhedrosis virus (NPV) and Spinosad against American bollworm, *Helicoverpa armiguera* (Hubner). *Revista Brasileira de Entomologia*, *63*(4), 277-282. http://doi.org/10.1016/j.rbe.2019.09.001

Pérez, J. & Moraima, S. (2012). Ciclo de vida y reproducción de Heliothis virescens (F.) (Lepidoptera: Noctuidae) sobre garbanzo. Revista de *Protección Vegetal*, *27*(2), 85-89. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1010-27522012000200003

Pickett, B. R.; Gulzar, A.; Ferré, J. & Wright, D. J. (2017). *Bacillus thuringiensis* Vip3Aa toxin resistance in *Heliothis virescens* (Lepidoptera: Noctuidae). *Applied and Environmental Microbiology*, *83*(9), e03506-16. http://doi.org/10.1128/AEM.03506

Ramos, E.; Espichan, K. & Rodriguez, K. (2018). Blueberry Suppy Chain in Peru: Planning, Integration and Execution. *International Journal of Supply Chain Management*, *7*(2), 1-12. http://www.researchgate.net/publication/325100960_Blueberry_supply:chain.in.Peru_Planning_integration_and_execution

Romero, L; Arévalo, H.; Duarte, W. & Mejía, R. (2012). Susceptibilidad de *Heliothis virescens* (Lepidoptera:noctuidae) a la proteína Cry1Ac incorporada a dietas meridicas. *Rev. U.D.C.A. Actualidad & Divulgación Científica*, *15*(2), 381–389. http://www.cabi.org/isc/FullTextPDF/2013/20133256512.pdf

Romeu, C. & Veitía, R. (2012). Efecto antialimentario de aceites esenciales de plantas aromáticas sobre *Heliothis virescens* y *Spodoptera frugiperda*. *Fitosanidad*, *16*(3), 155-159. http://www.redalyc.org/articulo.oa?id=209126907005

Romo, R.; Dote, J.; Garrido, H.; Araneda, F. & Gil, J.M. (2019). Blueberry consumption and healthy lifestyles in an emerging market. *Spanish Journal of Agricultural Research*, *17*(4), 1-11. http://doi.org/10.5424/sjar/2019174-14195

Sullca, C., Molina, C., Rodríguez, C., & Fernández, T. (2019). Detección de enfermedades y plagas en las hojas de arándanos utilizando técnicas de visión artificial. *Perspectiv@s, 15*,15, 32-39.

Tarkanyi, P.C.; Bordean, D.M.; Catargiu, A. & Poiana, M.A. (2019). Blueberries – Pleading for health. *Journal of Agroalimentary Processes and Technologies*, *25*(4), 187-194. http://www.journal-of-agroalimentary.ro/admin/articole/14765XL31_TarkanyiP.C._2019_25(4)_187-194.pdf

Teakle, R.E.; Jensen, J.M. & Mulder, J.C. (1985). Susceptibility of Heliothis armiger (Lepidoptera:Noctuidae) on Sorghum to Nuclear Polyhedrosis Virus. *Journal of Economic Entomology*, *78*(6), 1373-1378. http://doi.org/10.1093/jee/78.6.1373