Pestic. Phytomed. (Belgrade), 25(2), 2010, 133-137
Pestic. fitomed. (Beograd), 25(2), 2010, 133-137

UDC: 578.84:632.937.3
Scientific paper * Naučni rad
DOI: 10.2298/PIF1002133S

Transmission of Different Nucleopolyhedroviruses by Two Ectoparasitoids – *Bracon hebetor Say* (Hymenoptera: Braconidae) and *Euplectrus plathypenae* (Howard) (Hymenoptera: Eulophidae)

Emanouela E. Stoianova[1] and Nikolay A. Balevski[2]

Plant Protection Institute,35 Panayot Volov str., Kostinbrod 2230, Bulgaria (vorakul@abv.bg[1], nbalevski@abv.bg[2])

Received: December 3, 2009
Accepted: April 4, 2010

SUMMARY

The transmission of nucleopolyhedroviruses (NPVs) of *Autographa gamma* (AgNPV), *Mamestra brassicae* (MbNPV), *Lacanobia oleraceae* (LoNPV), *Helicoverpa armigera* (HaNPV) and *Xantia c-nigrum* (XnNPV) to their relevant larvae by the ectoparasitoid *Bracon hebetor* and the transmission of the multiple-enveloped NPVs of *Spodoptera exigua* (SeMNPV) and *Spodoptera frugiperda* (SfMNPV) by the ectoparasitoid *Euplectrus plathypenae* was examined. Two methods of contamination of the both parasitoids (exposure to infected hosts and total body surface) and two subsequent transmissions of the viruses by *Bracon hebetor* to healthy hosts were tested. The results showed that both parasitoids were capable to be mechanical vectors of the tested NPVs. Every *Bracon hebetor* female was able to transmit subsequently twice the virus in 27% to 52.2% of the five Noctuidae species by preliminary exposing to infected larvae. The second method of contamination (applying virus suspension to the total body surface of the parasitoid) was also efficient causing virus infection in between 29.4% and 54.15% of the larvae.

The parasitoid *E. plathypenae* transmited the virus from infected to noninfected larvae in 20% and 25.57% of the *S. frugiperda* and *S. exigua* larvae, and 6.43% and 11.10%, respectively of them died from the virus infection. The same observation was established by the second method of contamination – respectively 33.33% and 40% infection and between 13.23% and 16.67% mortality.

The mortality of all tested larvae exposed to virus contaminated parasitoids was higher when the parasitoid entire body surface had been artificially contaminated with the virus than when the parasitoid itself was previously allowed to oviposit the larvae.

Keywords: Hymenoptera; Lepidoptera; Nucleopolyhedroviruses; Transmission

INTRODUCTION

Certain hymenopterous parasitoids can transmit mechanically entomopathogenic viruses to lepidopterous larvae. Female parasitoids that have become contaminated with virus during oviposition in an infected host may subsequently infect other hosts during oviposition. Another possible route of pathogen transmission is the artificial contamination of the parasitoid body surfaces leading to viral infection of the host (Raimo et al., 1977; Hochberg, 1991; Young and Yearian, 1990). Information on transmission from infected to noninfected hosts would provide insight on the role of parasitoids in dispersal of baculoviruses within or between natural host populations. In addition, dispersal of the viruses by artificially contaminated parasitoids may be a means of an artificial introduction of the viruses into host populations in pest management programs. Dissemination of insect viruses by parasitic insects especially parasitic hymenoptera has been suggested by several authors. For example McCutchen et al. (1996) reported virus transmission by *Microplitis croceipess* (Cresson) from 3 wild type viruses with 4.0, 10.6, and 8.3% infection of the uninfected larvae. Five percent and three percent of *Heliothis virescens* Fabricius larvae exposed to female *Cotesia marginiventris* Cresson that developed in a wild type virus died from virus infection. Raimo et al. (1977) demonstrated intrahaemocoelic inoculation of *Lymantria dispar* Linnaeus NPV by *Apanteles melanoscelus* Ratzeburg when the parasitoid oviposited in noninfected larvae after oviposition in NPV-infected larvae. Adults of *Campoletis sonorensis* Cameron transmitted heliothis NPV to as many as 40% of larvae of *H. virescens* in which they oviposited after ovipositoin in NPV-infected larvae (Irabagon and Brooks, 1974). Levin et al. (1979) reported a higher frequency (81% to 95%) of transmission of the GV of *Pieris rapae* Linnaeus by the parasitoid *Apanteles glomeratus* Linnaeus.

The aims of this study was to determine the potential role of the ectoparasitoids *Bracon hebetor* Say to transmit the nucleopolyhedroviruses of *Autographa gamma* Linnaeus, *Lacanobia oleraceae* Linnaeus, *Helicoverpa armigera* Hübner and *Xantia c-nigrum* Linnaeus and *Euplectrus plathypenae* Howard to be a vector of NPVs of *Spodoptera exigua* Hübner and *Spodoptera frugiperda* J.E. Smith.

MATERIAL AND METHODS

Two methods of contaminating female parasitoids with NPVs were tested: (1) exposing the parasitoid to infected larvae (transmission type A) and (2) applying virus suspension to the total body surface of the parasitoid (transmission type B).

Bioassays with *E. plathypenae*

(1) Twenty newly molted third and four instar *S. frugiperda* and larvae were fed with an aqueous suspension containing 2.44 x 10^6 and 2.3 x 10^7 OBs ml^{-1}, in a solution of 10% sucrose and 0.01% Flourella blue dye. These concentrations represented LC_{90} values for the third and fourth instar larvae, respectively (Escribiano et al., 1999). *S. exigua* larvae were fed with a viral suspension with LC_{90} – 3.32 x 10^7 and 6.83 x 10^8 OBs ml^{-1} (for the third and fourth instar, respectively (Muñoz et al.,1999). After virus inoculation larvae were transferred to petri dishes containing fresh diet. When the larvae started molting, they were offered to the parasitoid females. Immediately after they had been parasitized, the parasitoids were removed and offered healthy premolting respectively second to third instar and third to fourth instar *S. exigua*. The experiment was repeated three times. The larvae were checked for the presence of OBs.

(2) Fifty *E. plathypenae* parasitoids were put in petri dishes preliminarily smeared with viral suspension for over 30 min. Then the parasitoids were transferred to healthy third instar *S. exigua* and *S. frugiperda*. The larvae were checked daily for virus-mortality and the larvae died by the parasitoid were checked for the presence of OBs.

The control group of larvae and parasitoids fed with virus-free solution of 10% sucrose and 0.01% Flourella blue dye. Every bioassay was repeated 3 times.

Bioassays with *Bracon hebetor*

The bioassays were similar but the species used were *A. gamma*, *M. brassicae, H. armigera*, *X. c-nigrum* and *L. oleraceae* and their homologous NPVs (AgNPV,

HaNPV, XcNPV and LoNPV, respectively) with suspensions with lethal concentration LC_{90} defined by Величкова-Кожухарова (1984) and in the bioassay transmission type A every parasitoid female was consecutively transferred to two healthy larvae of every mentioned species.

Analysis of data

Data in all tests were transformed to arcsine and analyzed by analysis of variance (ANOVA).

RESULTS

Our study showed that both parasitoids *B. hebetor* and *E. plathypenae* transmitted different viruses virus to noninfected larvae. Every female of *B. hebetor* was able to transmit the virus twice from infected to noninfected larvae between 27% to 52,2% of the five tested species. The second method of contamination (applying virus suspension to the total body surface of the parasitoid) was also efficient causing virus infection from 29.4% to 54.15% of the larvae (Figure 1).

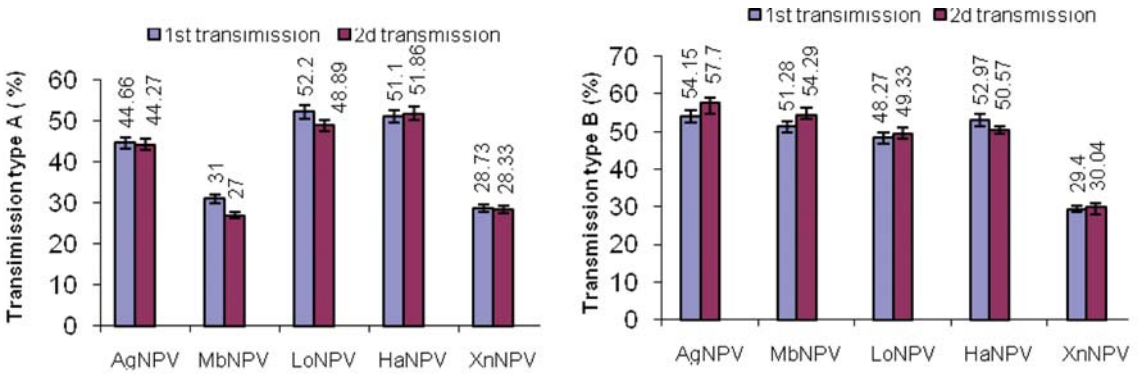

Figure 1. Transmission (type A and B) of various viruses by the parasitoid *B. hebetor* to their homologous Noctuidae larvae

The parasitoid *E. plathypenae* transmits the virus from infected to noninfected larvae in 25.57% of the larvae and 11.10% of the larvae died from the virus infection. The same observation was established in the second method of contamination – 40% infection of the noninfected larvae from which 16.67% mortality. Similar results in *S. frugiperda* larvae were observed (Table 1).

Table 1. Transmission (type A and B) of the viruses SeMNPV и SfMNPV by the parasitoid *E. plathypenae* to healthy *Spodoptera exigua* and *S. frugiperda* larvae

Host	Transmission type	Number of donor-larvae / donor parasitoids	Number of larvae-recipients	Number of infected larvae-recipients	Transmission of the virus (%)	Mortality (%) of larvae-recipients by virus transmission
S. exigua	A	100	90	23	25.57 ± 5.09	11.10 ± 3.81
	Control	100	90	0	0	0
	B	50	90	40	40.00 ± 3.30	16.67 ± 8.84
	Control	50	90	0	0	0
S. frugiperda	A	100	90	20	20.00 ± 3.30	6.43 ± 3.37
	Control	100	90	0	0	0
	B	50	90	33.3	33.33 ± 3.35	13.23 ± 3.96
	Control	50	90	0	0	0

Donor-larvae – larvae that were artificially infected with virus
Donor parasitoids- parasitoid females that were artifically contaminated with virus
Larvae-recipients- larvae that were infected by parasitoid ovipositoin

DISCUSSION

The present study indicates the significant role that the ectoparasitoids *B. hebetor* and *E. plathypenae* may have in the dissemination of nucleopolyhedroviruses in field populations. The high incidence of mortality of the mentioned Noctuidae larvae by NPVs after oviposition by adult parasitoids that had developed in NPV-deseased larvae indicate the potential role of the parasitoid in the dissemination of NPVs in field populations of Noctuidae larvae. In addition, the finding that the adult parasitoids which oviposited in NPV-infected larvae transmitted the viruses to larvae in which they subsequently oviposited suggested a second mechanism by which parasitism is implicated in the dissemination of this virus.

The both entomophagous *E. plathypenae* and *B. hebetor* are ectoparasitoids that means they oviposited their eggs on the host body. Both species prefer ovipositing larvae in last ages. In our study we tested two ages – third and fourth instar which are relatively susceptible to the virus infection and at the same time prefferred by parasitoid females. A special feature of *B. hebetor* ovipositional behaiviour is that the females stinging the larvae paralize them constantly in the most of the cases. In that meaning our study is aiming not to kill the larvae from viral infection but to use this transmission for subsequent dissemination of the virus.

Our study showed presence of OBs in the larvae-recipients after oviposition with virus-contaminated parasitoid females but not all of them showed virus mortality. The presence of virus infection in the larva's body itself is a potential factor for further dissemination of the virus by vertical or horizontal transmission.

The statistical analysis showed significant differences between the two types of virus transmission. The transmission of the virus by applying the virus suspension to the total body surface of the parasitoid is significantly higher than the transmission from infected to noninfected larvae. That could be explained by the fact the parasitoid could supply additionaly the transmission by parasitizing the larvae and also by contaminating their food with its contaminated body. Our results are analogical of the Young and Yearian (1990) that established that the transmission of the virus by contaminating its body surface *M. croiceps* was higher than transmission from infected to healthy larvae.

The successful transmission by parasitoids is reported by many authors. Lopes et al. (2002) reported that one female *E. viticolee* was able to transmist an iridescence virus in 3.7% of *S. frugiperda* larvae. Irabagon and Brooks (1974) found that the *C. sonorensis* adults could transmist a NPV in 40% of *H. virescens* larvae.

This study did not determine the exact mechanism by which *B. hebetor* and *E. plathypenae* transmitted the virus to their hosts. The experimental techniques and the behaiviour of the parasitoid during oviposition suggested, however, that the virus was transmitted by the ovipositor or by contact with food.

The role of the parasitoids as vectors of insect baculoviruses is very important and has been frequently mentioned and even demonstrated under laboratory conditions (Brooks, 1993). The finding that *E. plathypenae* and *B. hebetor* can transmit NPVs under laboratory conditions suggests a mechanism by which the viruses could be transmitted in field populations of susceptible insects.

REFERENCES

Brooks, W.M.: Host-parasitoid-pathogen interactions. Host-parasitoid-pathogen interactions. In: Parasites and Pathogens of Insects, Vol. 2: Pathogens (Beckage N.E., Thompson S.N., Federici B.A., eds.), Academic Press, San Diego, 1993, pp. 231-272.

Escribano, A., Williams, T., Goulson, D., Cave, R.D., Chapman J.W. and Caballero, P.: Selection of a Nucleopolyhedrovirus for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): structural, genetic, and biological comparison of four isolates from the Americas. Journal of Economic Entomology, 92(5): 1079-1085, 1999.

Hochberg, M.E.: Extra-host interactions between a braconid endoparasitoid, *Apanteles glomeratus*, and a baculovirus for larvae of *Pieris brassicae*. Journal of Animal Ecology, 60: 65-77, 1991.

Irabagon, T.A. and Brooks, W.M.: Interaction of *Campoletis sonorensis* and a nuclear polyhedrosis virus in larvae of *Heliothis virescens*. Journal of Economic Entomology, 67: 229-231, 1974.

Levin, D.B., Laing, J.E. and Jaques, R.P: Transmission of granulosis virus by *Apanteles glomeratus* to its *host Pieris rapae*. Journal of Invertebrate Pathology, 34(3): 317-318, 1979.

López, M., Rojas, J.C., Vandame, R. and Williams, T.: Parasitoid-mediated transmission of an irridescent virus. Journal of Invertebrate Pathology, 80(3): 160-170, 2002.

McCutchen, B.F., Hermann, R., Heinz, K.M., Parrella, M.P. and Hammock, B.D.: Effects of recombinant baculoviruses on a target endoparasitoid of *Heliothis virescens*. Biological Control, 6: 45-50, 1996.

Muñoz, D., Murillo, R., Krell, P., Vlak, J. and Caballero, P.: Four genotypic variants of a Spodoptera exigua Nucleopolyhedrovirus (Se-SP2) are distinguishable by a

hypervariable genomic region. Virus Research, 59: 61-74, 1999.

Raimo, B., Reardon, R.C. and Podgwaite, J.D.: Vectoring gypsy moth nuclear polyhedrosis virus by *Apanteles melanoscelus*. Entomophaga, 22(2): 207-215, 1977.

Величкова-Кожухарова, М.: Вирусни болести по някои нощенки у нас. Ядрено-полиедрен вирус на залевата нощенка *Mamestra brassicae* L. и възможности за използването му в биологическата борба. Автореферат на дисертация, 1984, 1-20.

Young, S.Y. and Yearian, W.C.: Transmission of nuclear polyhedrosis virus by the parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae) to *Heliothis virescens* (Lepidoptera: Noctuidae) on soybean. Environmental Entomology, 19: 251-256, 1990.

Prenošenje različitih virusa nuklearne poliedroze pomoću dva ektoparazitoida – *Bracon hebetor* Say (Hymenoptera: Braconidae) i *Euplectrus plathypenae* (Howard) (Hymenoptera: Eulophidae)

REZIME

Ispitivano je prenošenje virusa nuklearne poliedroze (NPV) poreklom iz *Autographa gamma* (AgNPV), *Mamestra brassicae* (MbNPV), *Lacanobia oleraceae* (LoNPV), *Helicoverpa armigera* (HaNPV) i *Xantia c-nigrum* (XnNPV) na njihove nezaražene larve pomoću ektoparazitoida *Bracon hebetor.* Takođe je ispitivano prenošenja virusa nuklearne poliedroze sa višestrukom membranom poreklom iz *Spodoptera exigua* (SeMNPV) i *Spodoptera frugiperda* (SfMNPV) pomoću ekotoparazitoida *Euplectrus plathypenae.* Testirane su dve metode kontaminacije oba parazitoida (izlaganje inficiranim domaćinima i ukupne površine tela). Takođe su ispitivana dva uzastopna prenošenja virusa pomoću *Bracon hebetor* na zdrave domaćine. Na osnovu dobijenih rezultata može se zaključiti da su oba parazitoida mehanički prenosioci testiranih NPV. Svaka ženka *Bracon hebetor* je, ukoliko je prethodno bila izlagana inficiranim larvama, bila u stanju da uzastopno dva puta prenese virus testiranih vrsta Noctuidae u iznosu od 27% do 52,2%. Druga metoda kontaminacije (primena suspenzije virusa na celokupnu površinu tela parazitoida) je takođe prouzrokovala virusnu infekciju larvi od 29,4 do 54,15%.

Parazitoid *E. plathypenae* je preneo 20% virusa sa inficiranih na larve *S. frugiperda* koje nisu bile inficirane i 25,57% virusa na neinficirane larve i *S. exigua*.

Pored toga, ustanovljeno je da je 6,43% larvi *S. frugiperda* i 11,10% larvi *S. exigua* uginulo kao posledica infekcije virusom. Ista pojava je zabeležena primenom druge metode kontaminacije prilikom koje je ostvareno 33,33% i 40% infekcije, odnosno između 13,23% i 16,67% smrtnosti.

Smrtnost svih testiranih larvi izloženih virusom kontaminiranim parazitoidima bila je viša kada je celokupna površina tela parazitoida bila veštački zaražena virusom u odnosu na parazitoide kojima je prethodno dozvoljeno da polažu jaja u zaražene larve.

Ključne reči: Hymenoptera; Lepidoptera; nukleopolihedrovirusi; transmisija