Ovipositional Discrimination by *Euplectrus plathypenae* (Howard) (Hymenoptera:Eulophidae) between Healthy and Nucleopolyhedrovirus - Infected *Spodoptera exigua* and *Spodoptera fugiperda* Larvae (Lepidoptera: Noctuidae)

E. E. STOIANOVA[1], J. C. HERNANDEZ[2] and P. E. CABALLERO[2]
[1] Plant Protection Institute, BG - 2230 Kostinbrod, Bulgaria
[2] Laboratorio de Entomologia Agricola y Patologia de Insectos, Departamento de Produccion Agraria Universidad Publica de Navarra, 31006 Pamplona, Spain

Abstract

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Parasitoid behavior was monitored to investigate the ability of *Euplectrus plathypenae* (Howard) (Hymenoptera: Eulophidae) to distinguish between healthy and infected with nucleopolyhedroviruses *Spodoptera exigua* (Hubner) and *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) larvae. Third and fourth instars (L3 and L4) of both Noctuidae species were used in the present study. When *E. plathypenae* females were given choice between healthy and virus-infected *S. exigua* and *S. frigiperda* hosts, the adult females exhibited marked preference ($P < 0.05$) for the healthy hosts of the both instars of the two species. The ovipositional discrimination study also showed that the correlation between the percent parasitism of healthy and virus-infected L3 larvae was significantly higher than the correlation between parasitized healthy and virus-infected L4 *S. frugiperda* larvae. In *S. exigua* larvae the oposite correlation between the two instars has been observed.

The results proved that *E. plathypenae* females and the multiple-enveloped nucleopolyhedroviruses of S. exigua (SeMNPV-SP2) and *S. frugiperda* (SfMNPV) did not significantly compete for the same type of host larvae and are, generally, compatible. Since the healthy and virus-infected larvae were different in a number of ways, several factors may have contributed to the behavioral discrimination evidenced.

Key words: Euplectrus plathypenae, Spodoptera exigua, Spodoptera frugiperda, discrimination, nucleopolyhedrovirus
Abbreviations: NPV - nuclear polyhedrosis virus, SeMNPV-SP2 - *Spodoptera exigua* multiple-enveloped nucleopolyhedrosis virus (an Spanish genotype 2), SfMNPV -

Spodoptera frugiperda multiple-enveloped nucleopolyhedrosis virus, L3 - third instar larvae, L4 - fourth instar larvae

Introduction

Parasitoids and pathogens of insects are important determinants of their host's population dynamics and structure, as has been shown by a number of theoretical and laboratory studies (Begon et al., 1992; Sait and Thompson, 1994a; Begon et al., 1995; Briggs and Godfray, 1995). The interaction between the host and each of the natural enemies has been examined independently, but these parasites are unlikely to act alone and a complex of interactions might be expected between them (Hochberg et al., 1990).

Infection of invertebrates by a pathogen is often a largely random event, being dependent on chance ingestion by the host. Successful parasitism, however, is determined to a great extent by parasitoid searching behavior and the selection of hosts that are suitable for immature parasitoid development to the adult stage (Vinson, 1976; Vinson and Iwantsch, 1980). Infection with a lethal pathogen may reduce the value of an otherwise suitable host, depending on whether there are costs to the parasitoid associated with attacking these hosts and the resulting competition between parasites. Costs may be in terms of loss of progeny that fail to complete development, reduced adult size, and increased development time or time wasted in handling infected hosts (Irabagon and Brooks, 1974; Beegle and Oatman, 1975; Levin et al., 1981; Caballero, Vargas-Osuna and Santiago-Alvarez, 1990; Hochberg, 1991a).

When larval parasitoids are unable to develop in infected and clearly unsuitable hosts, host selection models obviously predict that the adult female parasitoid should reject these individuals (Godfray 1994).

Versoi and Yendol (1982) established significant differences among the number of parasite-host contacts and percent ovipositional attempts observed under various treatments indicated that the parasites preferred noninfected larvae, making fewer efforts to parasitize virus-infected individuals.

Versoi and Yendol (1982) and Caballero et al. (1991) indeed found that parasitoids exhibited some degree of avoidance of infected hosts, though they did not reject them altogether.

Beegle and Oatman (1975) and Levin et al. (1983), however, showed no discrimination at all in the host-pathogen-parasitoid interactions they investigated. This lack of a consistent pattern may have a reason because these studies did not examine variation in parasitoid's perception of a suitable host and its propensity to accept or reject.

Hamm et al. (1983) reported that there was no significant difference in productivity of parasitoids in infected and control larvae.

Escribiano at al. (2000) reported that the larval parasitoid *Campoletis sonorensis* and the egg parasitoid *Chelonus insularis* did not discriminate between virus-contaminated and uncontaminated *Spodoptera frugiperda* eggs.

Hegazi and Abo Abd Allah (2004) showed that the adult *Microplitis rufiventris* females exhibited marked preference for granulosis virus-infected *Spodoptera littoralis* larvae than non-infected larvae.

Here, the host-parasitoid-pathogen in-

teraction is that between the beet army-worm *Spodoptera exigua* (Hubner) (Lepidotera Nocuidae) and the fall army-worm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and the ectoparasitoid *Euplectrus plathypenae* (Howard) (Hymenoptera: Eulophidae) and the nucleopolyhedroviruses of the both Noctuidae species. As in many host-pathogen interactions, host mortality increases with the virus dose, while susceptibility decreases with the larval age (Sait, Begon and Thomson, 1994b).

The aim of this study was to determinate if *E. plathypenae* females could discriminate between virus infected and non-infected *S. exigua* and *S. frugiperda* larvae and to determine whether the outcomes differ with variations in the host age.

Materials and Methods

Insect rearing

The rearing of the beet armyworm *S. exigua* and the fall armyworm *S. frugiperda* was performed at a constant temperature (25 °C), humidity (70 %) and photoperiod (16 h light: 8 h dark), in the insectary facilities of the Universidad Publica de Navarra, Spain. Larval stages of all three species were reared on artificial diet developed by Poitout and Bues (1974). The ectoparasitoid E. plathypeanae was maintained continuously using *S. frugiperda* larvae from the laboratory population.

Viruses

The nucleopolyhedroviruses (NPVs) used in this study were the *S. frugiperda* multiple-enveloped nucleopolyhedrovirus (SfMNPV) originally isolated in Nicara-gua and recently chracterizized (Escribano

et al., 1999) and the wild type of S. exigua multiple-enveloped nucleopolyhedrovirus (SeMNPV-SP2) isolated from NPV-infected *S. exigua* larvae during a viral epi-zootic in vegetable greenhouses in El Ejido, Spain (Caballero et al., 1992). The stocks of viruses were produced in early fourth instar (L4) S. exigua and *Spodotera frugiperda* larvae previously orally inocu-lated with SeMNPV and SfMNPV and reared on artificial diet until death. Virus-killed larvae were triturated and occlusion bodies (OBs) were purificated as Munoz et al. (1998). The OBs were re-suspended in distilled water, counted using a Neubauer Improved chamber (Hawsksley, Lancing, United Kingdom) and stored at 4 °C prior to use.

Host discrimination experiments

The present study consisted of two parts, every one in two variants. In the first, third (L3) and fourth instars (L4) *S.exigua l*arvae were used in the first and second variant respectively. In the first variant 30 newly molted L3 *S. exigua* lar-vae were inoculated by droplet-feeding bioassay (Hugues and Wood, 1981) with SeMNPV-SP2 suspension containing 2.44 x 10^6 OBs/ml which presented LC90 con-centration. In the second variant 30 newly molted L4 *S. exigua* larvae were treated with viral suspension with LC90 2.3 x 10^7. After inoculation, the larvae from the two variants were transferred to a plastic Petri dish (115 mm in diameter, 45 mm in height) (5 larvae in a Petri dish), containing fresh diet. When larvae started molting (over 24 hours after inoculation), they were indi-vidually transferred to a Petri dish con-taining one healthy molting L3 to L4 lar-vae in the first variant and L4 to L5 instar in the second variant and then exposed to one *E. plathypenae female* (with experi-

ence i.e. which previously had parasitized larvae). The infected larvae were marked with a small spot of dark marker to distinguish them from the healthy larvae. Immediately after one of the larvae (healthy or infected) in every Petri dish was parasitized, the parasitoid was removed. The marked infected larvae were checked every day for mortality due to the virus. The experiment was repeated 3 times in the every variant.

The second part of the study was conducted as the method described above. Instead of *S. exigua* larvae were used L3 and L4 S. frugiperda larvae and SfMNPV. The LC90 virus concentration used for inoculation of L3 larvae was 3, 32 x 107 OBs/ml and 6, 83 x 108 OBs/ml for the L4 larvae.

Data analysis

Data were subjected to analysis of variance for determination of differences between mean values. For mean separation student's t-test was applied.

Results and Discussion

In the first part of the study when *C. insularis* females were given a choice between healthy and infected with SeMNPV-SP2 *S. exigua* larvae, highly significant (P<0.05) differences in female acceptance between these two types of hosts were found. In the first variant the percent ovipositional attempts in the healthy L3 larvae was 20.67 ± 0.06 % and was significantly greater than the virus-infected L3 larvae - 13.13 ± 0.12 %. The same dependence of the mean percent parasitism - 71.1 ± 0.02 % for the healthy and 28.9 ± 0.1 % for the virus-infected larvae - has been established in the sec-

ond variant where L4 *S. exigua* larvae were used (Figure 1).

In the first variant of the second part of the study the mean percentage of parasitism by *E. plathypenae* differed significantly (P < 0.05) when were offered L3 healthy (88.9 ± 0.01 %) and infected (11.1 ± 0.11 %) *S. frugiperda* larvae. In the second variant the mean percent of parasitism of healthy L4 *S. frugiperda* larvae was 66.7 ± 0.01 % and those of the virus-infected larvae - 33.3 ± 0.03 % (Figure 2).

In the ovipositional choice study, significant differences were also found in the mean percent of parasitism of healthy and virus-infected larvae between the different instars of the *S. frigiperda* larvae. The percent of parasitism of the healthy L3 *S. frigiperda* larvae was 88.9 ± 0.01 %, that significantly differed form the parasitism of the healthy L4 larvae- 66.7 ± 0.01 %.
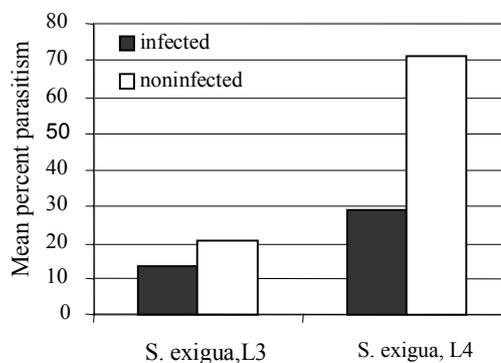
Fig. 1. Percentages of parasitism of third (L3) and fourth (L4) virus-infected and healthy *S. exigua* larvae exposed simultaneously to individual *E. plathypenae* females. Significant differences between parasitism of noninfected and infected larvae were observed at the two instars
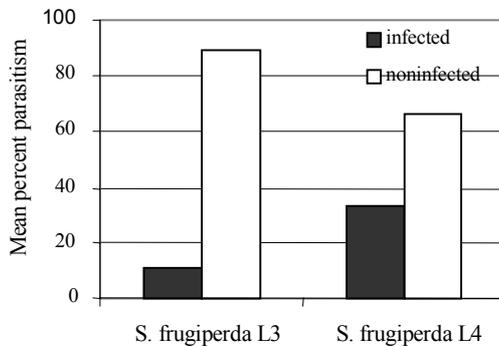
Fig. 2. Percentages of parasitism of third (L3) and fourth (L4) virus-infected and healthy S. frugiperda larvae exposed simultaneously to individual E. plathypenae females. Significant differences between parasitism of non-infected and infected larvae were observed at the two instars

The percent parasitized infected L3 and L4 *S. frigiperda* larvae was 11.01 % ± 0.11 % and 33.3 ± 0.03 % respectively (Figure 2). In *S. exigua* larvae the opposite correlation has been observed. The parasitism of healthy L3 *S. exigua* larvae was 20.67 ± 0.06 % compared with the healthy L4 - 71.1 ± 0.02 %. The percent parasitized virus-infected L3 *S. exigua* larvae was 13.13 ± 0.12 % and those of the virus-infected L4 larvae - 28.9 ± 0.1 % (Figure 1).

The apparent discrimination by *E. plathypenae* between virus-infected and healthy hosts can be explained with striving of the females parasitoids to select the "higher quality" of host larvae and providing their successful progeny development. The results of this study suggest that *E. plathypeane* females and SeMNPV-SP2 and SfMNPV did not significantly compete for *S. frugiperda* and *S. exigua* larvae respectively and are, generally, compatible. The results are in agreement with

Versoi and Yendol (1982), who mentioned that the parasitoid *Apanteles melanoscelus* is able to discriminate between healthy and virus-infected gypsy month larvae. However, the present results are not comparable with those of Levin et al. (1983), who found that the adult *Apanteles glomeratus* did not exhibit a statistically significant discrimination between GV-infected and non-treated larvae of *Pieris rapae*. Sait et al. (1966) observed that the solitary ichneumonid endoparasitoid *Venturia canescens* (Gravenhorst) examined and probed granulovirus-infected *Plodia interpunctella* (Hubner) larvae to the same degree as healthy larvae, although oviposition behavior was significantly reduced in heavily infected hosts.

Some factors that may have contributed to this behavioral discrimination are: the differences in the chemistry within or on the larval integument; perhaps its physical structure of infected and noninfected larvae; the kairomone associated with the silk deposited by the noninfected larvae (Weseloh, 1977) and the response of the parasitoid to bodily movements of infected larvae.

In this study has also been found significant differences in the percent parasitized healthy L3 larvae in comparison with the healthy L4 *S. frugiperda* larvae (Figures 1 and 2). That results could be explained with the higher susceptibility of the L3 larvae than L4 larvae toward the virus infection and the consequences of early showing of sighs of the disease is a possible reason the female to reject the virus-infected L3 hosts with higher percent than L4 hosts.

Parasitoids have evolved mechanisms for discriminating between low quality hosts because individuals of this kind are com-

monly encountered in the populations in which they forage, and it pays to avoid them (Godfray, 1994). Thus, the results of the present study showed that the parasitoid *E. plathypenae* possesses that adaptive strategy of parasitizing infected hosts providing successful development of its progeny.

Conclusions

• The adult females exhibited marked preference (P < 0.05) for the L3 and L4 healthy *S. exigua* larvae.

• *E. plathypeane* females preferred healthy than infected with SfMNPV L3 and L4 *S. frugiperda*. Larvae.

• The ovipositional discrimination study also shows that the correlation between the percent parasitism of healthy and virus-infected L3 larvae is significantly higher than the correlation between parasitized healthy and virus-infected *S. frugiperda* larvae. In *S. exigua* larvae the opposite correlation between the two instars has been observed.

References

Beegle, C. C. and E. R. Oatman, 1975. Effect of a nuclear polyhedrosis virus on the relationship between *Trichplusia ni* (Lepidoptera: Noctuidae) and the parasite, *Hyposoter exigua* (Hymenoptera: Icheumonidae). *J. Invertebr. Pathol.*, **25**: 59 - 71.

Begon, M., R. G. Bowers, N. Kadianakis and D. E. Hodgkinson, 1992. Disease and community structure: the importance of host self-regulation in a host-host-pathogen model. *Am. Nat.* **139**: 1131-1150.

Begon, M., S. M. Sait and D. J. Thomson, 1995. Persistence of a parasitoid-host system: refuges and generation cycles. Proceeding of the *Royal Society of London B*, **260**: 131-137.

Briggs, C. and H. C. J. Godfray, 1995. The dynamics of insect-pathogen interactions in stage-structured populations. *Am. Nat.*, **145**: 855-887.

Caballero, P., E. Vargas-Osuna and C. Santiago-Alvarez, 1990. Development of *apanteles telengai* (Hym: Braconidae) and *Campoletis annulata* (Hym: Ichneumonidae) in granulosis virus (GV) infected *Agrotis segetum* (Lep: Noctuidae) larvae. *J. App. Entomol.* **110**: 358-364.

Caballero, P., E. Vargas-Osuna and C. Santiago-Alvarez, 1991. Parasitation of granulosis-virus infected and noninfected *Agrotis segetum* larvae and the virus transmission by three hymenopteran parasitoids. *Entomol. Exp. Appl.*, **58**: 55-60.

Caballero, P., D. Zuidema, C. Santiago-Alvarez and J. M. Vlak, 1992. Biochemical and biological characterization of four isolates of *Spodoptera exigua* nuclear polyhedrosis virus, *Biol. Control Sci. and Technol.* **2**: 145-157.

Caballero, P., 1999. Selection of a nucleo-polyhedrovirus for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae): Structural, genetic and biological comparison of four isolates from the Americas, *J. Econ. Entomol.* **92**: 1079-1085.

Escribano, A., T. Williams, D. Goulson, R. D. Cave and P. Caballero, 2000. Parasitoid-pathogen-pest interactions of Chelonus insularis, *Campoletis sonorensis* and a nucleopolyhedrovirus in *Spodoptera frugiperda larvae*, *Biol. Control* **19**: 265-273.

Godfray, H. C. J., 1994. Parasitoids: Behavioral and evolutionary ecology. *Princeton University Press, Princeton, NJ.*

Hegazi, E. M., and L. M. Z. Abo Abd-Allah, 2004. Ovipositional discrimination by

microplitis rufiventris between healthy and granulosis virus-infected larvae. *J. Appl. Ent.* **128** (1): 11-25.

Hochberg, M. E., M. P. Hassel and R. M. May, 1990. The dynamics of host-parasitoid-interaction. *Am. Nat*. **135**: 74-94.

Hochberg, M. E., 1991a. Intra-host interactions between a braconid endoparasitoid, *Apanteles glomeratus*, and a baculovirus for larvae of Pieris brassicae. *J. Anim. Ecol.* **60**: 51-63.

Hughes, P. R. and H. A. Wood, 1981. A synchronous peroral technique for the bioassay of insect viruses. *J. Invertebr. Pathol.* **37**: 154-159.

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

Levin, D. B., J. E. Laing, R. P. Jaques and J. E. Corrigan, 1983. Transmission of the granulosis virus of Pieris rapae (Lepidoptera: Pieridae) by the parasitoid *Apanteles glomeratus* (Hymenoptera: Braconidae). *Environ. Entomol.***12:** 166-170.

Munoz, D., J. I. Castillejo and P. Caballero, 1998. Naturally occurring deletion mutants are parasitic genotypes in a wild-type nucleopolyhedrovirus population of *spodoptera exigua*. *Applied of Environ. Microbiol.* **64** (11): 4372-4377.

Poitout, S. and R. Bues, 1974. Elevage des

chenilles de vingt-huit especes de lepidopteres Noctuidae et de deux especes d'Arctiidae sur milieu artificial simple. Particulares de l'elevage selon les especes, *Ann. Zool. Ecol. Anim*. **6**: 431-441.

Sait, S. M., M. Begon and D. J. Thompson, 1994a. Long term population dynamics of the Indian meal moth, *Plodia interpunctella* , and its granulosis virus. *J. Anim. Ecol*. **63**: 861-870.

Sait, S. M., M. Begon and D. J. Thompson, 1994a. The influence of larval age on the response of *Plodia interpunctella*, to a granulosis virus. *J. Invertebr. Pathol*. **63**: 107-110.

Sait, S. M., R. A. Andreev, M. Begon, D. J. Thompson, J. A. Harvey and R. D. Swain, 1995. Venturia canescens parasitizing *Plodia intepunctella*: host vulnerability - a matter of degree. *Ecol. Entomol*. **20**: 199-201.

Vinson, S. B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology*, **21**: 109-133.

Versoi, P. L. and W. G. Yendol, 1982. Discrimination by the parasite *Apanteles melanoscelus* between healthy and virus-infected gypsy moth larvae. *Environ.Entomol.*, **11**: 42-45.

Vinson, S. B., 1976. Host selection by insect parasitoids. *Annual Review of Entomology*, **21**: 109-133.

Vinson, S. B. and G. F. Iwantsch, 1980. Host suitability for insect parasitoids. *Ann. Rev. Entomol*. **25**: 397-419.