Interaction between MbMNPV and the braconid parasitoid Habrobracon hebetor (Hym., Braconidae) on larvae of beet armyworm, Spodoptera exigua (Lep., Noctuidae)

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SHORT COMMUNICATION

Interaction between *MbMNPV* and the braconid parasitoid *Habrobracon hebetor* (Hym., Braconidae) on larvae of beet armyworm, *Spodoptera exigua* (Lep., Noctuidae)

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The survival of a braconid parasitoid *Habrobracon hebetor* was investigated on nucleopolyhedrovirus (NPV)-infected *Spodoptera exigua* larvae. The second-instar larvae were exposed to 30, 51.4 and 180 PIB/mm² of *Mamestra brassicae* NPV (*MbMNPV*) as under-LD₅₀, LD₅₀ and over-LD₅₀ values, respectively. They were accessible to be parasitized by *H. hebetor* after 24, 48 and 72 h post-treatment. Infection of the larvae with *MbNPV* was deleterious to the survival and parasitism of *H. hebetor*. The survival of *H. hebetor* in *MbNPV*-infected *S. exigua* larvae was dependent on the interval between viral infection and parasitization, as well as on the treatment dose of *MbMNPV*; very few adults of parasitoid emerged from infected hosts when host larvae were exposed to 180 PIB/mm² of *MbNPV* on 72-h interval treatment. The inoculation dose of *MbNPV* and the timing of parasitoid release had significant effect on the development of *H. hebetor* on virus-infected hosts. Field applications of virus for biocontrol of *S. exigua* may lead to substantial mortality of immature parasitoids.

**Keywords:** *Spodoptera exigua; Habrobracon hebetor; MbMNPV; parasitism; interaction*

*Habrobracon hebetor* Say. (Hym., Braconidae), a cosmopolitan gregarious larval ectoparasitoid, is an important natural enemy of many insect pests including the beet armyworm, *Spodoptera exigua*. Preliminary experiments showed that female parasitoids laid eggs on all larval stages of *S. exigua*, although they preferred the last instars (Azimi, Baimani and Hallaj-dezfouli 1995). The aim of this study was to investigate interactions between the *Mamestra brassicae* nucleopolyhedrovirus (*MbMNPV*), the parasitoid *H. hebetor* and their host, *S. exigua*. We investigated survival of the parasitoids in *MbMNPV*-infected hosts. Moreover, we evaluated the effect of time duration between virus infection and wasp parasitization of host larvae on survival of parasitoids and hosts.

*Spodoptera exigua* were originally collected from a sugar beet field in Mashhad, Iran and were continuously maintained on sugar beet leaves in the laboratory. The egg masses were surface sterilized with 0.2% sodium hypochlorite solution.
H. hebetor fertilized females reared on S. exigua, were obtained from the Research Center for Agriculture and Natural resources of Mashhad. Colonies were fed with a 20% honey solution. The experiments were carried out at 25 ± 2°C, 60 ± 5% RH and 16 h L:8 h D photoperiod.

Mamestra brassicae nucleopolyhedrovirus (MbMNPV) originally from Mamestrin® (Calliope SA, France) with two passages on S. exigua larvae was produced in third- and fourth-instar S. exigua larvae which were individually maintained in plastic cups (3 x 5.5 cm, height x diameter) containing a sugar beet leaf-disc inoculated with MbMNPV suspension. Virus-killed larvae were homogenized in sterile distilled water and filtered through double-layered cheesecloth. The filtrate was suspended in 0.1% (w/v) sodium dodecyl sulfate (SDS) and centrifuged at 1000 rpm for 10 min. The supernatant was then centrifuged at 3200 rpm for 10 min to pellet the virus. The pellet containing the PIBs was resuspended in SDS and centrifuged again at 4000 rpm for 12 min. The pellet was resuspended in SDS and PIB concentrations were quantified after 10-fold dilutions using a haemocytometer. The quantified MbMNPV stock suspension was stored at 4°C until use.

Newly molted second instars of S. exigua were individually exposed to MbMNPV-inoculated sugar beet leaf-discs (18 mm²) in plastic cups for 24 h. The average leaf area consumed in 24 h by a second-instar larva was previously measured. Ten microliters of each of 30, 51.4 and 180 PIB/mm² of virus inoculum (as less, equal to and higher than LD₅₀ values, respectively unpublished data), were applied on the leaf-disc using a micropipette and spread uniformly with a sterile 6 mm glass rod. The virus LD₅₀ for second-instar larvae of S. exigua was previously determined through the preliminary bioassays. Beet armyworm larvae were released individually onto the virus treated leaf-discs for 24 h. Thereafter, 12 treated larvae were transferred at random to untreated leaf-discs in clear plastic containers (7 x 12 cm, height x diameter). In total, 36 plastic containers were set up on the basis of a factorial experiment in a completely randomized design, virus dose as factor A and post-inoculation time as factor B. At different times post inoculation, one 3–5-day-old fertilized female H. hebetor was transferred to each container. One replicate was considered without parasitoid for control. Parasitoids were removed after 24 h. Containers were maintained until emergence of parasitoids and moths. Data regarding numbers of emerged moths and parasitoids were used in statistical analysis.

In another set of experiments, 12 newly molted second-instar larvae of S. exigua were first exposed to one 3–5-day-old fertilized female H. hebetor in a clear container (7 x 12 cm, height x diameter) for 24 h; then 24, 48 and 72 h after parasitization, larvae of each container were individually transferred to virus treated leaf-discs in plastic cups (3 x 5.5 cm, height x diameter). None of these parasitized larvae fed on MbMNPV treated leaf-discs.

The data were subjected to analysis of variance followed by means separation by Fisher’s Least Significant Difference (F-LSD) at software SYSTAT 12.

MbMNPV had a significant effect on the percentages of adult emergence for both the wasps (F₉,₂₆ = 181.9, P < 0.01) and moths (F₂,₂₆ = 0.03, P < 0.05). Adult emergence of H. hebetor from infected hosts treated with 30 PIB/mm² was significantly higher than from infected hosts treated with 51.4 PIB/mm² which in turn was significantly higher than emergence from infected hosts treated with 180 PIB/mm² (F-LSD, P < 0.05; Table 1). The same phenomenon was found in the
emergence of adult moths (Table 1). On the other hand, the timing of parasitoid release had significant effect on adult wasp emergence ($F_{2,26} = 122.5, P < 0.01$) but no significant effect on adult moth emergence ($F_{2,26} = 23.1, P > 0.05$). Emergence of *H. hebetor* adults from infected hosts in the 72 h interval treatment was significantly lower than from the 48 h interval treatment and beyond 72 h, emergences were significantly lower than those from inoculated hosts in the 24 h interval treatment (F-LSD, $P < 0.05$; Table 1).

Our data demonstrated that the infection of *S. exigua* larvae with *MbMNPV* was deleterious to the development and survival of *H. hebetor*. The effect of *MbMNPV*-infection on the survival of parasitoids was dependent on the time between *MbMNPV*-infection and parasitization. When the duration between infection and parasitization was increased, adult emergence of *H. hebetor* significantly decreased.

One of the possible explanations for this is premature death of hosts as has been reported by Nguyen et al. (2005) for the parasitoid *Meteorus pulchricornis* and NPV-infected larvae of *Spodoptera litura* (i.e., the host dies from the viral infection before development of the parasitoid is completed). Our preliminary experiments showed that when *S. exigua* second-instar larvae were inoculated with *MbMNPV*, virus-induced death of hosts occurred between 3 and 9 days, while *H. hebetor* needed 11–13 days to complete development in the host as reported by Attaran (1995). Therefore, we speculate that larvae of *H. hebetor* did not have enough time to complete development in the *MbMNPV*-inoculated hosts. The premature death of parasitoids in virus-infected hosts for various combinations of larval parasitoid and viruses has been reported by several authors (Laigo and Tamashiro 1966; Laigo and Pascke 1968; Irabagon and Brooks 1974; Beegle and Otman 1975; Levin, Laing and Jacques 1981; Hochberg 1991; Kyei-Poku, Nakai and Kunimi 1999; Escribano, Williams, Goulson, Cave and Caballero 2000; Nguyen et al. 2005). As reviewed by Brooks (1993), premature host death is the most common consequence of a host-parasitoid-virus interaction.

In addition to parasitization timing, viral dose also affected survival of the parasitoid. The numbers of emerged adults of *H. hebetor* from *MbMNPV*-infected hosts with doses less than the LD$_{50}$ were significantly higher than those at higher

<table>
<thead>
<tr>
<th>Concentration (PIB/mm$^2$)</th>
<th>Wasp 24 h</th>
<th>Wasp 48 h</th>
<th>Wasp 72 h</th>
<th>Moth 24 h</th>
<th>Moth 48 h</th>
<th>Moth 72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>26.6 (±1.76)</td>
<td>17.7 (±0.66)</td>
<td>8.0 (±0.6)</td>
<td>33.3 (±4.81)</td>
<td>38.8 (±10.1)</td>
<td>36.1 (±2.78)</td>
</tr>
<tr>
<td>51.4</td>
<td>21.0 (±1.15)</td>
<td>11.3 (±0.66)</td>
<td>6.0 (±0.9)</td>
<td>19.4 (±2.78)</td>
<td>13.9 (±5.56)</td>
<td>13.9 (±7.36)</td>
</tr>
<tr>
<td>180</td>
<td>10.6 (±0.88)</td>
<td>5.0 (±0.57)</td>
<td>2.0 (±0.6)</td>
<td>5.5 (±2.78)</td>
<td>8.3 (±4.81)</td>
<td>8.3 (±0.00)</td>
</tr>
</tbody>
</table>

Second-instar larvae were individually exposed to the 30, 514 and 180 PIB/mm$^2$ of *MbMNPV* (as less, equal to and higher than LD$_{50}$ values, respectively) and exposed to 3–5-day-old fertilized female *H. hebetor* at 24, 48 and 72 h after virus inoculation at different treatments. Mean numbers and percentages (±SE) followed by different letters are significantly different (F-LSD, $P < 0.05$) for emergence of wasp and moth adults, respectively. Upper-right and lower-left letters are compared in columns (among different concentrations) and rows (among time durations separately for wasp and moth treatments).
doses. Similarly, Nguyen et al. (2005) reported that when larvae of *Spodoptera litura* inoculated with a 10-fold dose of the LC$_{95}$ of *Spl*NPV, adult emergence of the braconid parasitoid *Meteorus pulchricornis*, was lower than that with the LC$_{95}$ of *Spl*NPV. This suggests that the higher dose shortens the survival time of the host, and consequently parasitoids do not have enough time to complete their development.

In this study, we demonstrated the risk of virus application to survival of *H. hebetor*. However, the timing and dose of the application could limit this risk. Field applications of virus for biocontrol of *S. exigua* may lead to substantial mortality of immature parasitoids, although field experiments have not yet demonstrated such an effect.

References


