Interaction between a nucleopolyhedrovirus and the braconid parasitoid
*Meteorus pulchricornis* (Hymenoptera: Braconidae) in the larvae of
*Spodoptera litura* (Lepidoptera: Noctuidae)

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Abstract

We investigated the survival and development of the braconid parasitoid *Meteorus pulchricornis* in nucleopolyhedrovirus (NPV)-infected *Spodoptera litura* larvae. The first and second molting stages of the larvae were parasitized by *M. pulchricornis* and were exposed to a 95% lethal concentration (LC95) and 10× the LC95 of *S. litura* NPV (SplitNPV) on the first, third, and fifth day post-parasitization (dpp). Infection of the larvae with SplitNPV was deleterious to the survival and development of *M. pulchricornis*. The survival of *M. pulchricornis* in SplitNPV-infected *S. litura* larvae was dependent on the interval between parasitization and viral infection, as well as on the inoculation dose of SplitNPV; few parasitoid larvae emerged from infected hosts when host larvae were exposed to 10× the LC95 of SplitNPV on 1 and 3 dpp. The fecundity of *M. pulchricornis* that emerged from SplitNPV-infected hosts was not affected, and these parasitoids did not transmit the virus to other hosts. The number of eggs laid by *M. pulchricornis* females was not significantly different between the SplitNPV-infected and non-infected larvae during the first 4 d post-infection (dpi). On the other hand, at 5 dpi, *M. pulchricornis* females laid significantly more eggs in non-infected larvae than in SplitNPV-infected larvae.

Key words: *Spodoptera litura*; *Meteorus pulchricornis*; nucleopolyhedrovirus; interaction; competition

INTRODUCTION

The common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), one of the most important insect pests of agricultural crops, is distributed throughout tropical and temperate Asia, Australia, and the Pacific islands. The host range of this polyphagous insect covers at least 90 species of plants (CABI, 1999). Control of *S. litura* has historically relied on chemical pesticides. However, some chemical pesticides are not effective on older-instar larvae, and *S. litura* larvae have developed resistance to several chemical pesticides (Okada, 1977; Hirose, 1995). Thus, integrated pest management is required to control this pest. Several non-chemical control measures have been attempted in Japan, including the application of entomopathogenic nematodes, fungi, and bacteria, as well as capture using sex pheromones (Oyama et al., 1978; Asayama and Ohoishi, 1980; Kawasaki, 1985; Kondo and Ishibashi, 1986; Asano and Suzuki, 1995).

*S. litura* has been reported to be naturally susceptible to nucleopolyhedrovirus (NPV) (Baculoviridae; Hunter-Fujita et al., 1998). NPV has been developed as a bioinsecticide, and has been used effectively against *S. litura* attacking vegetables, cotton, rice, and peanuts in China, India, and Taiwan (Moscardi, 1999). Furthermore, several natural enemies of *S. litura* have been reported in the field (Yamanaka et al., 1972; CABI, 1999). Therefore, it is possible to induce a multi-natural enemy interaction in *S. litura* larvae when *S. litura* NPV (SplitNPV) is sprayed on agricultural fields.

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However, the consequences of these interactions are not known. Understanding the complex interactions that occur between the virus and natural enemies, especially parasitoids, will be key in assessing their potential risks as biological control agents (Brooks, 1993).

*Meteorus pulchricornis* Wesmael (Hymenoptera: Braconidae), a solitary braconid endoparasitoid, is one of the natural enemies of *S. litura* larvae. *M. pulchricornis* is an important natural enemy of many major insect pests including *Helicoverpa armigera*, *H. assulata*, *S. litura*, *Plutella xylostella*, *Mamestra brassicae*, *Anomis flava*, and *Cystidia couaggaria* (CABI, 1999; Takashino et al., 1998), and is widespread in Europe, Asia, and the United States (Askari et al., 1977; CABI, 1999). In our preliminary experiment, we found that female parasitoids laid eggs in the first- to fourth-instars of *S. litura*, although they preferred the third and early fourth instars. Over their lifetime, which averaged 44 d, female *M. pulchricornis* oviposited 268 eggs in an average of 194 hosts (Fuester et al., 1993).

The aim of this study was to investigate multitrophic interactions between the host, *S. litura*, the parasitoid, *M. pulchricornis*, and the nucleopolyhedrovirus (SpltNPV). We investigated the simultaneous effects of parasitization by *M. pulchricornis* and SpltNPV-infection on the mortality of *S. litura* larvae. The survival and development of the parasitoids in SpltNPV-infected hosts were also examined. Moreover, we describe the ovipositional behavior and possible transmission of SpltNPV by female parasitoids.

**MATERIALS AND METHODS**

**Insects.** *S. litura* were originally collected from a soybean field at the Tokyo University of Agriculture and Technology, Tokyo, Japan, in 2000, and were continuously maintained in the laboratory. Larvae were reared in plastic containers on an artificial diet, as described by Kawasaki (1991). Twenty pairs of adults were transferred to individual paper bags (8×15×20 cm) containing a 10% sugar solution for food. The egg masses were decontaminated with 10% formalin solution for 15 min and were then washed carefully in tap water.

*M. pulchricornis* originated from parasitized *S. litura* larvae that were collected from soybean fields in Kagawa Prefecture, Japan, in 2001 and were continuously maintained on *S. litura* larvae in our laboratory. *M. pulchricornis* used in the experiments were a thelytokous strain; unfertilized females that produce all-female broods. Sixty to 100 adult parasitoids were reared in a transparent plastic box (8.5 cm height×15 cm diameter) and were fed honey. Five- to eight-d-old females were used for the experiments. Colonies were maintained and the experiments were conducted at 25°C and using a 16L:8D photoperiod.

**Virus.** *S. litura* nucleopolyhedrovirus (SpltNPV) was originated from the diseased larvae of *S. litura* collected in Fukuyama, Hiroshima Prefecture, Japan (Takatsuka et al., 2003), and isolated as an individual genotypic variant (SpltNPV-C1) by the in vivo cloning method developed by Smith and Crook (1988). SpltNPV was propagated in *S. litura* larvae and viral occlusion bodies (OBs) were purified as described previously (Okuno et al., 2003). The concentration of OBs in the stock suspension was determined using a Thoma hemocytometer under phase-contrast microscopy. The stock suspension was kept at 4°C until use.

**Survival of parasitoids in virus-infected hosts.** *S. litura* first-instars beginning to molt, as determined by head capsule slippage, were individually exposed to 5- to 8-d-old *M. pulchricornis* females in a 30-ml cup for 15 h. After parasitization, a subset of these parasitized larvae that had already molted to the second instar was exposed to the LC95 of SpltNPV (1.1×10^7 OBs/ml) or 10× the LC95 of SpltNPV by a modified droplet feeding method (Hughes and Wood, 1981, 1986). The droplets of SpltNPV OBs suspension contained 5% sucrose and 1% red food coloring (Kyoritsu Food Co., Ltd., Tokyo). The LC95 was previously determined by probit analysis. After inoculation, each larva was given a piece of artificial diet. Other groups of these parasitized larvae were dosed with the LC95 (4.1×10^7 and 4.2×10^7 OBs/ml for third and fourth instars, respectively) or 10× the LC95 of SpltNPV after molting to the third (3 d post-parasitization, dpp) and fourth (5 dpp) instars. Additionally, we focused our attention on elucidating the effects of SpltNPV on *M. pulchricornis* with the timing of parasitization. *S. litura* second-instars beginning to molt were individually exposed to 5- to 8-d-old female *M. pulchricornis* in a 30-ml cup for 15 h. After parasitization, a subset of these parasitized larvae that had already molted to the third
instar was immediately exposed to the LC95 of SpltNPV (4.1×10^7 OBs/ml) or 10× the LC95 of SpltNPV, as above. After inoculation, each larva was given a piece of artificial diet. Other groups of these parasitized larvae were exposed to the LC95 (4.2×10^7 OBs/ml for fourth and fifth instars) or 10× the LC95 of SpltNPV after molting to the fourth (3 dpp) and fifth (5 dpp) instars.

As a means of cross-checking, parasitized larvae from the various instars were inoculated with sterilized distilled water containing 5% sucrose and 1% red food coloring. Non-parasitized larvae of the second (P0V2), third (P0V3), fourth (P0V4) and fifth (P0V5) instars were inoculated with SpltNPV as described above for use as virus-infection controls. Mortality due to SpltNPV and/or parasitoids, emergence of parasitoid larvae from hosts, and cocoon formation was assessed daily. Mortality due to parasitoids was determined by the emergence of parasitoid larvae. The dead host larvae were dissected to examine the presence of parasitoid and/or virus occlusion bodies. Mortality due to SpltNPV was confirmed by examining the tissues of dead insects for OBs under phase-contrast microscopy. The dead host larva with no parasitoid or virus occlusion body were excluded from data. Each treatment was replicated three times with 35 larvae.

**Virus transmission and fecundity of parasitoids emerging from SpltNPV-infected hosts.**

This study was carried out to examine the possibility of virus transmission by parasitoids that had emerged from virus-infected hosts. Newly molted second-instars of *S. litura* were parasitized by 8-d-old female parasitoids for 15 h in a 150-ml plastic box. Parasitized larvae were individually reared on an artificial diet in a 30-ml cup. Three days after parasitization, parasitized larvae were exposed to the LC95 of SpltNPV and reared as described above. Control parasitized larvae were exposed to sterilized distilled water containing 5% sucrose and 1% red food coloring. Female parasitoids that had laid eggs into SpltNPV-infected larvae were each transferred to another 150-ml plastic cup and allowed to oviposit into 10 non-infected second-instars for 24 h. These parasitized larvae were individually transferred to 30-ml cups with fresh diet and observed daily for virus infection and emergence of parasitoids. Dead hosts and hosts from which parasitoids emerged were examined of the presence of OBs using phase-contrast microscopy.

**Discrimination of the parasitoid between virus-infected and non-infected hosts.**

Newly molted second-instars of *S. litura* were dosed with the LC95 of SpltNPV and reared as described above. Control larvae were exposed to sterilized distilled water containing 5% sucrose and 1% red food coloring. Eight-day-old female parasitoids were individually exposed to 10 SpltNPV-infected and 10 control larvae at 2, 3, 4, or 5 dpi in a 150-ml plastic box for 7 h. The control larvae were marked on the head with a red maker to distinguish them from the SpltNPV-infected larvae. After exposure to the parasitoids, larvae were dissected to check for parasitoid eggs. Twelve parasitoids were used in each treatment.

**Data analysis.** Frequency data were arcsine transformed before analysis. Frequency data and the period of lethal infection were analyzed with a one-way analysis of variance (ANOVA); means were compared using Tukey’s honestly significant difference (HSD) test. Fecundity and discrimination of the parasitoids between virus-infected and non-infected hosts were analyzed by Student’s *t* test. All statistical analyses were conducted using JMP Version 3 (SAS Institute Inc., 1997).
RESULTS

Survival of parasitoids in SpltNPV-infected hosts

The percentages of larval emergence, pupation, and adult eclosion of *M. pulchricornis* from SpltNPV-infected hosts were significantly lower than those from non-infected hosts when the first molting stages of *S. litura* larvae were parasitized and exposed to SpltNPV at the second (P1V2), third (P1V3), or fourth (P1V4) instars, regardless of the inoculation dose (Tukey’s test, *p*<0.05; Fig. 1). The timing of viral treatment did not affect the percentages of larval emergence and adult eclosion of *M. pulchricornis* when parasitized *S. litura* larvae were dosed with the LC95 of SpltNPV (Tukey’s test, *p*>0.05). However, the percentages of pupation of *M. pulchricornis* from hosts inoculated with the LC95 of SpltNPV at the fourth instar were significantly higher than those from hosts inoculated with the LC95 of SpltNPV at the second instar. On the other hand, the percentages of larval emergence of *M. pulchricornis* from hosts inoculated with 10× the LC95 of SpltNPV at the fourth instar were significantly higher than those from hosts inoculated with 10× the LC95 of SpltNPV at the second and third instars (Tukey’s test, *p*<0.05). The percentages of pupation of *M. pulchricornis* from hosts inoculated with 10× the LC95 of SpltNPV at the fourth instar were significantly higher than those from hosts inoculated with 10× the LC95 of SpltNPV at the second instar (Tukey’s test, *p*<0.05). The timing of viral treatment did not affect the percentages of adult eclosion of *M. pulchricornis* when parasitized *S. litura* larvae were dosed with 10× the LC95 of SpltNPV (Tukey’s test, *p*>0.05). The inoculation dose of SpltNPV had no effect on the percentages of larval emergence, pupation, or adult eclosion of the wasps, regardless of the timing of viral treatment (ANOVA, *p*>0.05).

The infection of hosts with SpltNPV also significantly reduced the percentages of larval emer-
gence, pupation, and adult eclosion of *M. pulchricornis* when the second molting stages of *S. litura* larvae were parasitized and exposed to SpltNPV at the third (P2V3), fourth (P2V4), or fifth (P2V5) instar, regardless of the inoculation dose (Tukey’s test, *p*<0.05) (Fig. 2). The timing of viral treatment significantly affected the percentages of larval emergence and pupation of *M. pulchricornis* (emergence rate, *F*=8.4, *p*<0.05; pupation rate, *F*=7.4, *p*<0.05), but did not affect the percentage of adult eclosion (*F*=1.8, *p*>0.05) when parasitized *S. litura* larvae were dosed with the LC$_{95}$ of SpltNPV. The timing of viral treatment significantly affected the percentages of larval emergence, pupation, and adult eclosion of *M. pulchricornis* when parasitized *S. litura* larvae were dosed with 10× the LC$_{95}$ of SpltNPV (emergence rate, *F*=13.1, *p*<0.01; pupation rate, *F*=7.6, *p*<0.05; eclosion rate, *F*=26.7, *p*<0.01). The percentages of larval emergence, pupation, and adult eclosion of parasitoids were highest in P2V5, followed by P2V4 and P2V3 regardless of the viral treatment, but no significant differences were observed between P2V4 and P2V5. The percentages of larval emergence, pupation, and adult eclosion of *M. pulchricornis* from hosts inoculated with the LC$_{95}$ of SpltNPV were higher than those from hosts inoculated with 10× the LC$_{95}$ of SpltNPV regardless of the timing of viral treatment, but a significant difference was observed only in the percentage of adult eclosion of *M. pulchricornis* from hosts inoculated with SpltNPV at the third instar (*F*=66.3, *p*<0.01).

Percent cumulative mortality from parasitization alone and SpltNPV prevalence between parasitized and non-parasitized hosts were determined from our data (Figs. 3 and 4). Parasitoids emerged from uninfected host larvae between 8 and 12 d after parasitization. The period of lethal infection in the SpltNPV-infected larvae varied between 3 and 10 d. The mean period of lethal infection was 7.0 d for the second instars, 5.7 d for the third instars, 6.6 d for the fourth instars and 6.7 d for the fifth instars when larvae were infected with the LC$_{95}$ of Splt-

![Fig. 3. Percent cumulative mortalities from parasitization alone and SpltNPV prevalence between parasitized and non-parasitized Spodoptera litura larvae. The first molting stages of S. litura larvae were parasitized by Meteorus pulchricornis and exposed to the LC$_{95}$ or 10× the LC$_{95}$ of SpltNPV in the second (P1V2), third (P1V3), or fourth (P1V4) instars. Closed and open circles indicate cumulative mortalities caused by the emergence of parasitoid larvae in non-infected (P1V0) and SpltNPV-infected hosts, respectively. Closed and open triangles indicate cumulative mortalities caused by SpltNPV in non-parasitized (P0V2, P0V3, P0V4) and parasitized hosts, respectively. Arrows indicate the timing of SpltNPV inoculation.](image)
NPV and differed significantly with larval age ($F=36.1$, $p<0.001$). The mean period of lethal infection was 6.6 d for the second instars, 5.6 d for the third instars, 6.1 d for the fourth instars and 6.0 d for the fifth instars when larvae were infected with $10^{5}$ the LC$_{95}$ of SpltNPV and differed significantly with larval age ($F=22.0$, $p<0.001$). The period of lethal infection of the third-instars was significantly shorter than that of the second-, fourth-, and fifth-instars regardless of the inoculation dose (Tukey’s test, $p<0.05$). The periods of lethal infection of $S$. litura larvae infected with $10^{5}$ the LC$_{95}$ of SpltNPV were significantly shorter than those of larvae infected with the LC$_{95}$ of SpltNPV regardless of the inoculation timing (second instar, $F=6.9$, $p<0.01$; third instar, $F=16.4$, $p<0.01$; fourth instar, $F=4.6$, $p<0.05$; fifth instar, $F=18.3$, $p<0.01$). The parasitized larvae were more quickly killed by infecting with SpltNPV than non-parasitized larvae, but significant differences were observed between P0V2 and P1V2 (LC$_{95}$, $F=24.5$, $p<0.001$; $10 \times$ LC$_{95}$, $F=20.6$, $p<0.001$), P0V4 and P1V4 (LC$_{95}$, $F=41.8$, $p<0.001$; $10 \times$ LC$_{95}$, $F=41.0$, $p<0.001$), and P0V5 and P2V5 (LC$_{95}$, $F=4.8$, $p<0.05$; $10 \times$ LC$_{95}$, $F=10.2$, $p<0.01$) regardless of the inoculation dose.

When the second molting stage of larvae was parasitized by $M$. pulchricornis and subsequently inoculated with SpltNPV, the average time for emergence of parasitoid larvae from non-infected hosts had no significant difference with that from SpltNPV-infected hosts regardless of the inoculation dose (ANOVA, $p>0.05$). When the first molting stage of larvae was parasitized and subsequently inoculated with SpltNPV at the fourth instar, the average time for emergence of parasitoid from non-infected hosts was shorter than that from SpltNPV-infected hosts regardless of the inoculation dose (LC$_{95}$, $F=23.0$, $p<0.001$; $10 \times$ LC$_{95}$, $F=9.2$, $p<0.01$).
Virus transmission and fecundity of parasitoids emerging from SpltNPV-infected hosts

No occlusion body formation was observed in the 332 *S. litura* larvae parasitized by female parasitoids that had emerged from SpltNPV-infected hosts. During the first 3 d after eclosion, the mean numbers of hosts parasitized by wasps developing in SpltNPV-infected hosts did not differ from those parasitized by wasps developing in non-infected hosts (d 0, *t* = 0.14, *p* > 0.05; d 1, *t* = 0.24, *p* > 0.05; d 2, *t* = 0.35, *p* > 0.05; d 3, *t* = 1.59, *p* > 0.05; Fig. 5). On d 4 and 5 after eclosion, however, the mean numbers of hosts parasitized by wasps developing in SpltNPV-infected hosts were lower than those parasitized by wasps developing in non-infected hosts (d 4, *t* = 2.52, *p* < 0.05; d 5, *t* = 2.31, *p* < 0.05). Overall, the mean total numbers of hosts parasitized by wasps developing in SpltNPV-infected hosts over 6 d did not differ from those parasitized by wasps developing in non-infected hosts (*t* = 1.46, *p* > 0.05).

Virus transmission by parasitoids that had previously parasitized SpltNPV-infected hosts

No OBs of SpltNPV were found in the 233 host larvae parasitized by wasps that had previously parasitized SpltNPV-infected hosts.

Discrimination by female parasitoids between SpltNPV-infected and non-infected hosts

The mean number of eggs laid by *M. pulchricornis* females differed between SpltNPV-infected and non-infected larvae at 5 dpi (*t* = 2.69, *p* < 0.05), but not at 3 and 4 dpi (3 dpi, *t* = 0.49, *p* > 0.05; 4 dpi, *t* = 1.92, *p* > 0.05) (Fig. 6).

DISCUSSION

Our data demonstrated that the infection of *S. litura* larvae with SpltNPV was deleterious to the development and survival of *M. pulchricornis*. The effect of SpltNPV-infection on the survival of parasitoids was dependent on the time between parasitization and SpltNPV-inoculation. When *S. litura* larvae were parasitized at the first molting stages and exposed to 10× the LC95 of SpltNPV at the second, third, or fourth instars, the emergence rates of parasitoid larvae from hosts were 0, 1.5, and 18.9%, respectively (Fig 1B). Thus, earlier virus-inoculation after parasitization causes a lower rate of larval emergence and survival of parasitoids. One of the possible explanations for this is premature host death (i.e., the host dies from the viral infection before development of the parasitoid is completed) (Brooks, 1993). When *S. litura* second-instars were inoculated with SpltNPV, virus-
induced death of hosts occurred from 3 to 9 dpi. Meanwhile, *M. pulchricornis* needed 8 to 12 d to complete development in the host (Fig. 3). Therefore, the larvae of *M. pulchricornis* did not have enough time to complete development in the hosts when SpltNPV was inoculated on 1 or 3 dpp. However, parasitoids had more time to complete development in hosts inoculated with SpltNPV at the fourth instar (5 dpp). The premature death of parasitoids in virus-infected hosts for various combinations of larval parasitoids and viruses has been reported by several authors (Laigo and Tamashiro, 1966; Laigo and Paschke, 1968; Irabagon and Brooks, 1974; Beegle and Oatman, 1975; Levin et al., 1981; Eller et al., 1988; Easwaramoorthy and Jayaraj, 1989; Hochberg, 1991; Kyei-Poku et al., 1999). As reviewed by Brooks (1993), premature host death is the most common consequence of a host-parasitoid-virus interaction.

In addition to the timing of inoculation, the viral dose affected the survival rate of the parasitoid. In single SpltNPV-infection treatments, the lethal time of the hosts inoculated with 10× the LC95 of SpltNPV was shorter than that of hosts inoculated with the LC95 of SpltNPV (Figs. 3 and 4). Therefore, as a result, in the dually parasitized and SpltNPV-infected hosts, the emergence rates of parasitoid larvae from the hosts inoculated with 10× the LC95 of SpltNPV were lower than those from the hosts inoculated with the LC95 of SpltNPV. Similarly, Nakai et al. (1997) reported that when the neonate larvae of *Adoxophyes honmai* (Lepidoptera: Tortricidae) parasitized by *Ascosgaster reticulatus* (Hymenoptera: Braconidae) were infected with an entomopoxvirus (EPV), survival of the parasitoid larvae developing in the hosts that were exposed to a high concentration of EPV was lower than for those developing in hosts that were exposed to a low concentration of EPV. This suggests that the higher dose of inoculation shortens the survival time of the host, and parasitoids do not have enough time to complete their development in hosts infected with the higher dose of viruses.

If a female parasitoid that has emerged from a virus-infected host transmits the virus to another host, then the parasitoid may be considered a virus dispersal agent and may increase the effectiveness of the virus. The transmission of viruses by parasitoids can be categorized into two means: mechanical vectors in the translocation of viruses as a result of contaminated body parts contacting host food sources (Irabagon and Brooks, 1974; Beegle and Oatman, 1975; Raimo et al., 1977; Young and Yearian, 1990; Sait et al., 1996), and biological vectors in the translocation of viruses as a result of the direct inoculation of the host via a virus-contaminated ovipositor (Beegle and Oatman, 1975; Levin et al., 1983; Hamm et al., 1985; Caballero et al., 1991). In this study, we examined the possibility of *M. pulchricornis* wasps as mechanical and biological vectors of SpltNPV, but no parasitoid females transmitted an effective dose of SpltNPV to other hosts. This may be partially due to the low susceptibility of *S. litura* larvae to SpltNPV.

Behavioral studies on host selection by parasitoids in relation to viral infections have also been conducted by many workers. Caballero et al. (1991) reported that *Apanteles telengai* (Hymenoptera: Braconidae), *Aleiodes gasteratus* (Hymenoptera: Braconidae), and *Campoletis annulata* (Hymenoptera: Ichneumonidae) parasitized significantly more healthy than the granulovirus-infected larvae of *Agrotis segetum* (Lepidoptera: Noctuidae). Beegle and Oatman (1975) observed that female *Hyposoter exiguae* (Hymenoptera: Ichneumonidae) laid as many eggs in NPV-infected *Trichoplusia ni* (Lepidoptera: Noctuidae) larvae as in non-infected larvae. *Apanteles melanoscels* (Hymenoptera: Braconidae) made significantly fewer ovipositional attempts on or in NPV-infected *Lymnantria dispar* (Lepidoptera: Lymnantriidae) larvae than in non-infected larvae (Versoi and Yendol, 1982). In our study, the number of eggs laid by *M. pulchricornis* females was not significantly different between SpltNPV-infected and non-infected larvae during the first four dpi, and *M. pulchricornis* females laid no more eggs in non-infected than in SpltNPV-infected larvae. On the other hand, at 5 dpi, *M. pulchricornis* females parasitized more non-infected than infected larvae. This suggests that some female *M. pulchricornis* could avoid laying eggs in SpltNPV-infected larvae as the disease advances. As mentioned above, parasitization in SpltNPV-infected hosts was detrimental to the survival of parasitoid progeny. Parasitism would therefore be optimal if *M. pulchricornis* females were able to distinguish between infected and non-infected larvae. The preference of *M. pulchricornis* for ovipositing more eggs in non-infected than infected larvae could be an adaptive advantage,
which has been summarized as recognizing the unsuitability of the infected host for parasitoid development (Laigo and Tamashiro, 1966) or avoiding “wasting eggs” on infected or diseased hosts that are at the point of dying (Versoi and Yendol, 1982).

In this study, we demonstrated the risk of virus application to the survival of *M. pulchricornis*. However, the timing and dose of the application limited the risk. Generally, viral application should be done strictly within a particular time-frame because the susceptibility of insects drastically changes. For example, the LC$_{50}$ of *S. litura* fifth-instar is 50 times higher than that of the second-instar (Okada, 1977). This implies that parasitoid larvae like *M. pulchricornis* that attack younger host stages are inevitably affected by the application of viruses. On the other hand, the risk of virus application should be compared to chemical pesticide application. Chemical insecticides usually kill all stages of insects, both targets and non-targets, immediately upon application in the field, whereas the virus only kills the susceptible stages (typically larvae) of the hosts. More studies are needed to clarify the impact of virus application on field populations of *S. litura* and its natural enemies.

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