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Prior experiences of endoparasitoids affect their ability to discriminate NPV-infected from non-infested caterpillars

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ABSTRACT

Nucleopolyhedrovirus (NPV) and parasitic wasps are two important biological control agents of lepidoptera caterpillars, and the compatibility of parasitism and NPV infection remains a complicated process. In this study, we examined the effects of different learning experiences by the endoparasitoid *Microplitis pallidipes* on their ability to discriminate NPV-infected from healthy caterpillars (larvae of beet armyworm, *Spodoptera exigua*). Compared to the parasitoids with no prior host contact (control), parasitoids with oviposition experience with healthy caterpillars had shorter searching time, shorter attacking time, a greater number of attacks, higher parasitism rate and greater comprehensive discrimination ability to both non-infected and NPV-infected caterpillars. They also had a higher percentage of first attacks on non-infected caterpillars than NPV-infected caterpillars, and these differences were more obvious with increasing time post-virus-inoculation. However, the values of these six host-discrimination indicators on NPV-infected caterpillars were not significant between the control parasitoids and parasitoids with oviposition experience on NPV-infected caterpillars from hours 24 to 96 after virus inoculation. Compared to parasitoids with oviposition experience on healthy caterpillars, parasitoids with oviposition experience on NPV-infected caterpillars generally had longer searching time, longer attacking time, fewer attacks, lower parasitism rate, and lower comprehensive discrimination ability to both non-infected and NPV-infected caterpillars, and a lower percentage of first attacks on non-infected vs. NPV-infected caterpillars. These results suggest that experience with healthy hosts helps parasitoids distinguish NPV-infected caterpillars from healthy caterpillars, but that experience with NPV-infected caterpillars did not convey the same discrimination ability.

1. Introduction

Parasitic wasps are at the top of the tritrophic system of plants, insect herbivores and their natural enemies, and they are important in the biological control of insect herbivores in both natural and agricultural ecosystems (Wei et al., 2013). When they forage for herbivores, food, and mates in complex environments, these wasps always depend on several stimuli, such as visual (D'Adamo et al., 2000; Benelli and Canale, 2012) and olfactory cues (Krisag and Georgee, 2007; Ichiki et al., 2011). Learning behavior is a general characteristic of parasitoids, helping them adapt to complex natural environments (Vet and Groenewold, 1990; Giunti et al., 2015; Taylor et al., 2016), and this ability allows parasitoids to efficiently respond to chemical cues from plants and herbivores to find hosts (Teder and Tammaru, 2002; Ponzio et al., 2016) and reactto varying environmental conditions (Peri et al., 2006; Maunsell et al., 2015).

The prior experiences of both larval and adult parasitic wasps affect both host searching and discrimination (Olson et al., 2003; Giunti et al., 2016; Lentzronning and Kester, 2013; Masry et al., 2018). Some studies have indicated that parasitoid orientation or searching responses to host herbivores can be modified through experience before adult emergence (Bjorksten and Hoffmann, 1998; Bayram et al., 2010). Several studies report that adult learning of parasitic wasps, such as *Apanteles melanoscelus* (Versoi and Yendol, 1982) and *Microplitis rufiventris*, affect the parasitoids' ability to discriminate NPV-infected herbivores from healthy herbivores.

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Host discrimination of parasitoids plays a key role in the parasitoids' control of herbivores and enhancing parasitoid fecundity (Canale and Benelli, 2012; Benelli et al., 2013; Jiang et al., 2014a). In the process of discriminating between NPV-infected and healthy caterpillars, parasitoids may use searching, attacking or other behaviors to detect and reject infected hosts before oviposition (Stoianova, et al., 2007), maximizing their fecundity by avoiding ovipositing or simply laying fewer eggs in NPV-infected caterpillars (Nakai et al., 2005; Jiang et al., 2014a,b). A number of studies have found that immature parasitoids (ones in the egg or larval stages) in NPV-infected caterpillars may die before their host caterpillars die from the virus infection (Kaya, 1970; Okuno et al., 2002), while other studies have reported that parasitoids are able to finish their development before the host caterpillars die from virus infection (Hochberg, 1991; Jiang et al., 2014a,b).

Beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), is a well-known pest of vegetables (Jiang et al., 2018; Wan et al., 2018) while *Microplitis pallidipes* (Hymenoptera: Braconidae) is an important solitary endoparasitoid of the 1st to 4th instar *S. exigua* larvae in China (Wan et al., 2015a, 2017b; Jiang et al., 2018). Beet armyworm caterpillars stop growing at the late 4th instar and fail to complete their life cycle if parasitized earlier by *M. pallidipes* in the 2nd host instar (Zeng et al., 2010).

Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) is another important biological control agent of beet armyworm, able to cause epizootics in beet armyworm populations (Jiang et al., 2011; Stoianova et al., 2012). *Microplitis pallidipes* can complete their development in beet armyworm caterpillars that are infected with a low virus dosage if there is sufficient time before the beet armyworm caterpillars die from the virus, with parasitoid survival rates up to 68.9% in larvae infected with a virus dosage of 1.71×10^4 OB·mL^{-1} (Jiang et al., 2018; Wan et al., 2015b, 2017b). Parasitoids without any prior learning experience can discriminate between infected and healthy beet armyworm caterpillars (Jiang et al., 2011), but the effects of different types or degrees of learning experience on this discrimination is not clear. In this study, we examined the effects of prior learning experience on the ability of adult *M. pallidipes* to effectively discriminate between NPV-infected and healthy beet armyworm caterpillars in order to provide a better basis for combining these two biological control agents.

2. Materials and methods

2.1. Insect and virus

Both *S. exigua* and *M. pallidipes* originated from laboratory colonies started with insects collected from Chinese cabbage in Zhuanghang town, Fengxian district of Shanghai, China. Colonies were propagated in the laboratory for 7 ~ 9 generations before being used in this experiment. Caterpillars (larval *S. exigua*) were reared in sterile glass containers (10 cm height × 15 cm in dia) on an artificial diet according to Wan et al. (2015b,2017a,b). Groups of 10–12 female parasitoids (1- to 2-day-old virgin females mated for 12 h) were allowed to oviposit in 300–500 2nd-instar caterpillars in a glass rearing container (50 × 40 × 30 cm). Larvae that had been exposed to parasitoids for one day were reared in glass tubes (15 cm length × 2.0 cm dia). Adult parasitoids that emerged from such caterpillars were provided with cotton swabs soaked with 10% honey water (Jiang et al., 2014a,b; Wan et al., 2015a). All insects used in this study were reared and tested in bioclimatic chambers at 28 ± 1.0 °C, 80 ± 5.0% RH, and a 14:10 h (L:D) photoperiod.

Spodoptera exigua multiple nucleopolyhedrovirus (SeMNPV) was also collected from the same cabbage field as the parasitoids and caterpillars, as described above. To harvest virus from NPV-infected caterpillars, field-collected, infected 5th instar caterpillars were homogenized in 0.01 M phosphate buffer solution (PBS) (pH 7.0). The resulting suspension was filtered four times through three layers of cheesecloth, and the filtrates were centrifuged twice for 20 min (900*g*) to remove larval debris. The supernatant was then centrifuged twice for 30 min (10,000*g*) to concentrate the polyhedra. Purified polyhedra were dispersed in PBS and subjected to 50% sucrose gradient centrifugation. The purified occlusion bodies (OBs) were stored at 4 °C until used (Jiang et al., 2018; Wan et al., 2018). For the experiment, purified OBs were diluted 100-fold with distilled water, and the OB concentration was determined using a Thomas hemocytometer under a phase-contrast microscope at 400X magnification to obtain the desired concentration (5.0×10^7 OB·mL^{-1}) (Wan et al., 2015a, 2016).

2.2. Experimental design

Three treatment groups and an untreated control group were established. All parasitic wasps used in the experiment were 1–2 day-old virgin females that had been allowed to mate for 12 h. The control group was comprised of parasitoids with no prior host contact (breeding on healthy caterpillars). The first treatment group was comprised of parasitoids (reared on NPV-infected caterpillars) with no prior host contact; the second treatment group was comprised of parasitoids (reared on healthy caterpillars) with one hour of oviposition experience on 10 healthy caterpillars; and the third treatment group was comprised of parasitoids (reared on healthy caterpillars) with one hour of oviposition experience on 10 NPV-infected caterpillars, 96-h after caterpillars had been exposed to NPV. Each parasitoid in all groups was then simultaneously exposed to one healthy and one NPV-infected host caterpillar in a glass Petri dish for one hour as described by Jiang et al. (2014b) and Wang et al. (2003).

In the first treatment group, where parasitoids had no prior host contact but were reared on NPV-infected caterpillars, these NPV-infected caterpillars were obtained through the following sequence. First, 72 h after 2nd instar caterpillars had been parasitized, caterpillars were individually inoculated with virus using the droplet-feeding method (Hughes and Wood, 1981), in which a 5 μL droplet of virus suspension (5.0×10^7OB·mL^{-1}) was placed on a small amount of diet (a 3 × 3 × 3 mm cube). These caterpillars were allowed to feed on the virus-infected diet cube for 24 h and only caterpillars that consumed all of the diet were used (thus standardizing the size of the viral dose). Caterpillars were then individually transferred to 25 mL tubes containing fresh artificial diet and reared at 28 ± 1.0 °C, 80 ± 5.0% RH and a 14:10 h (L:D) photoperiod to produce parasitoids reared in virus-infected caterpillars, which were collected as cocoons from the bodies of NPV-infected caterpillars and used for the first treatment parasitoids.

In the choice assays where parasitoids were exposed simultaneously to a NPV-infected caterpillar and a healthy caterpillar, we obtained NPV-infected caterpillars by individually inoculating 2nd instar caterpillars using the droplet-feeding method (Hughes and Wood, 1981), as described above. Caterpillars that completely consumed the treated diet within 24 h were considered inoculated and individually transferred into new 25 mL tubes containing fresh artificial diet and reared under the same conditions as above. Control caterpillars (also late 2nd instars) were fed diet treated with 5 μL of sterilized distilled water.

Surviving virus-inoculated caterpillars were used in choice tests 24, 48, 72, 84, and 96 h after inoculation together with a healthy control caterpillar. Infected and control caterpillars were paired for body size and instar in experiments. Differences in the growth and development of NPV-infected and non-infected caterpillars were slight for the first two days after viral inoculation, and on those days all caterpillars were 3rd instars. By 72 h after the start of the experiment, control caterpillars had molted to the 4th instar, followed later by caterpillars in the NPV-infected treatments. By 96 h after the start of the experiment, all caterpillars sampled from the NPV-infected treatment and control groups were 4th instars. Caterpillars at this point were exposed to parasitoids in sterilized glass Petri dishes (9.0 cm dia, 2.0 cm height) in choice tests in an air conditioned room held at 27 °C.

Individual female parasitoids were the unit of experimental replication. For each replicate, one female parasitoid was exposed for