Desenvolvimento ontogenético do parasitoide Nasonia vitripennis (Hymenoptera: Pteromalidae) utilizando Chrysomya putoria (Diptera: Calliphoridae) como hospedeiro

Ontogenetic development of the parasitoid Nasonia vitripennis (Hymenoptera: Pteromalidae) using pupae of Chrysomya putoria (Diptera: Calliphoridae) as host

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RESUMO
O objetivo do estudo foi avaliar o desenvolvimento ontogenético de *Nasonia vitripennis* (Hymenoptera) usando como hospedeiro pupas de *Chrysomya putoria* (Diptera). Foi estudada a relação de três hospedeiros para uma fêmea parasitóide com os tempos de 24, 48 e 72 horas de exposição ao parasitismo, totalizando 20 replicações por tempo de exposição. O desenvolvimento ontogenético do parasitóide foi prolongado quando o tempo de exposição ao parasitismo foi estendido. A menor média de desenvolvimento do parasitóide foi observada com 24 horas de exposição (13,65 dias) e a mais longa em 72 horas (14,15 dias). Foi observada uma variação entre o início e o final de emergência de parasitoides adultos entre os tratamentos. O pico de emergência ocorreu 14 dias após o início da exposição após o tempo de exposição em todos os tratamentos. A produtividade de parasitoides por pupa foi influenciada pelo tempo de exposição, onde o maior rendimento ocorreu após 72 horas de exposição. A produção tendeu a uma queda com a redução do tempo de exposição. O número de pupas viáveis sofreu um aumento conforme o tempo de exposição foi prolongado.

Palavras-chave: Controle biológico, interação parasitóide-hospedeiro, vespa parasitóide.

INTRODUCTION
The biological control is a safe alternative to the use of insecticides that may harm the environment contaminating the soil, food, atmosphere and water resources. In addition, it enables the
development of resistant insect populations, promoting a selective pressure, leading the fittest to survive and produce resistant offspring (Brisola-Marccondes, 2001).

Several species of microhymenoptera parasitoids, especially those belonging to Pteromalidae family, are commercialized and widely used to control flies such as the house fly *Musca domestica* Linnaeus, 1758 and the stable flies *Stomoxys calcitrans* (Linnaeus, 1758) (Diptera: Muscidae) in poultry houses and stables dairy cattle (Mandeville *et al.*, 1990; Morgan *et al.*, 1991). Among the Pteromalidae family, *Nasonia vitripennis* (Walker, 1836) stands out as a cosmopolitan parasitoid, able to parasitize more than 68 species of cyclorrhapha flies, among other arthropods used as hosts (Whiting, 1967). Among the cyclorrhapha families, Caliphoridae and Sarcophagidae are the most sensitive hosts, while Muscidae is the least one. This natural preference of *N. vitripennis* for Sarcophagidae and Calliphoridae pupae is related with the size, as larger pupae can optimize the parasitoid reproductive rate (Ullyett, 1950; Cardoso and Milward-de-Azevedo, 1995). The post-embryonic development time of *N. vitripennis* ranges from 13 to 20 days, varying according to the host species, the host parasitoid density or the environmental conditions (Cardoso and Milward-de-Azevedo, 1995; Mello *et al.*, 2010; Barbosa *et al.*, 2008; Barbosa *et al.*, 2010).

Most of the biological control techniques used in Brazil are imported from other countries and not always are adequate to the reality of tropical countries (Neves *et al.*, 2005). Aiming to support the implementation of biological control of flies of economic importance, many laboratory experiments on the parasitism action of *N. vitripennis* on pupae of *Chrysomya* and *Cochliomyia* Townsend, 1915 species were conducted in Brazil (Cardoso and Milward-de-Azevedo, 1996; Barbosa *et al.*, 2008; Barbosa *et al.*, 2010, Mello *et al.*, 2010). However, for a better understanding of the biology of *N. vitripennis* and its use in biological control, there is a need to increase the knowledge about the time of development of this parasitoid in different host species, using different exposure times to parasitism.

This study aims to determine the duration of the post-embryonic development, parasitism, sex ratio and the productivity of parasitoid per pupae of *N. vitripennis* using *C. putoria* as host.

### 2 MATERIALS AND METHODS

This study was conducted in the Laboratório de Estudo de Dípteros (LED) of Universidade Federal do Estado do Rio de Janeiro (UNIRIO) and in the Laboratório de Diptera of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), both located in Rio de Janeiro city, Brazil.

The experiment was carried out in a climatic chamber at 27°C day/25°C night, with 60 ± 10% RH and 14 hours of photophase beginning at 06:00 AM. The parasitoid-host relationship studied was three hosts per 1 female parasitoid and the exposure times used were 24 hours (treatment 1), 48 hours.
(treatment 2) and 72 hours (treatment 3). A control treatment was conducted with the same batch of pupae used in the others treatments. These pupae were not exposed to the parasitoid, and were used to verify the natural mortality rate of *C. putoria*. The relation host/parasitoid was repeated twenty times for each treatment, including the control treatment, totaling 240 pupae of *C. putoria* and 80 females of *N. vitripennis*.

To test the biological parameters, we used nulliparous females of *N. vitripennis* belonging to the second laboratory generation. As hosts we used pupae of *C. putoria* belonging to the 11th laboratory generation and with up to 24 hours age. The body mass of the hosts was recorded in lots of five larvae in an analytical balance, totaling 60 larvae with a mean body mass of 0.775 mg (+ 0.0053) per treatment.

For each exposure time, three pupae of *C. putoria* were exposed to one female of *N. vitripennis* and were allocated in test tubes with 5 ml of capacity, sealed with nylon fabric, totaling 20 replications per treatment. In each tube, a honey drop was offered to the female parasitoid and the nylon fabric used for seal the tube was moistened with water to promote the hydration of the wasps. After the parasitism exposure time, females of *N. vitripennis* were removed from the tubes and the host pupae were individualized in test tubes with a capacity of 5 ml. The insects were observed daily, at the same time, until the 30th day of the experiment to record the emergence of the adults.

The adults emerged from the host pupae were sexed and counted according to the sexual dimorphism, where the males have shorter wings than the females. We observed the parasitism rates, the average length of the post-embryonic development, the parasitoid productivity per host and the sex ratio. We considered the productivity per host, the number of adult parasitoids emerged per host pupae, while the parasitism rate was the number of pupae of *C. putoria* with parasitoids emergency. The sex ratio was defined, according to Silveira-Neto *et al.* (1976), by the ratio between the number of females and the total number of emerged parasitoids.

For the data analysis and preparation of the graphics, we used the software Microsoft® Excel 2010. To test the significance of the results, the Analysis of variance (ANOVA) and Tukey test were used with the software BioEstat® 5.3 (Magalhães and Lima, 2001).

3 RESULTS

The post-embryonic development duration for females and males of *N. vitripennis* in pupae of *C. putoria*, using different exposure times to the host (24, 48 and 72 hours), varied significantly by analysis of variance (ANOVA) followed by the Tukey post-test at 5% significance level. The shortest development time mean duration was observed in the exposure time of 24 hours (13.65 days) and the greatest in the 72 hours exposure time (14.15 days) (Table 1).
Analyzing the developmental time average of separately female and males, we found a significant difference in both sexes. In both sexes, the development time mean was shorter with 24 hours of exposure time to the parasitism. As the exposure time increased, we observed an increase of the development time average (Table 1).

Table 1 - Average of the post-embryonic developmental period (in days) of Nasonia vitripennis reared on Chrysomya putoria pupae. Pupae were exposed to parasitism by individual nulliparous females (n = 20 / treatment) for up to 24 hours, in the proportion of one parasitoid per three hosts, under different exposure times (temperature 27°C day / 25°C night, 60 ± 10% relative humidity and 14 hours of photoperiod).

| Host: parasitoid exposure time (hours) | Development time (days) | Females | Males | Total |
|---------------------------------------|-------------------------|---------|-------|-------|
|                                       | X ± dv | IR | X ± dv | IR | X ± dv | IR |
| 24h                                    | 13.07a 0.59 | 13-15 | 13.52a 0.58 | 13-15 | 13.65a 0.59 | 13-15 |
| 48h                                    | 14.04b 0.26 | 14-20 | 14.09b 0.53 | 14-20 | 14.06b 0.37 | 14-20 |
| 72h                                    | 14.19c 0.61 | 14-19 | 14.22c 0.60 | 14-19 | 14.15c 0.59 | 14-19 |

- X = average; dv = standard deviation; IR = range.
- Means followed by the same letter do not differ significantly by analysis of variance followed by Tukey post-test at 5% significance.

The beginning and the end of the adult parasitoids emergence differ between the exposure times used. In treatment 1 (24 hours), males and females begin to emerge at the 13th day until the 15th day after the exposure time. In treatment 3 (72 hours), the emergence began at the 14th day and lasted until 19th, and in treatment 2 (48 hours) began at the 14th day and lasted until the 20th day. In all exposure times used (24, 48 and 72 hours) the emergence peak was reached at the 14th day after the parasitism exposure (Fig. 1). The results for the sex ratio as well as the average and variation range of parasitoids obtained in different exposure times are shown in Table 2.

The average number of parasitoids (males and females) that emerged from C. putoria pupae varied significantly, which was supported by analysis of variance and by the Tukey post-test at the 5% significance level between exposure times of 24 and 48 hours and between 24 and 72 hours.

Among the 48 and 72 hour treatments there were no significant variations. In relation to the emerged females, there were significant variations between treatments of 24 and 48 hours as well as between 24 and 72 hours. There was no variation between the exposure times of 48 and 72 hours. Regarding the average number of the emergence of males, it was observed a significant alteration only among the exposition times of 24 and 72 hours. The major productivity of the parasitoids was observed in 72-hour time, and it tended to decrease with the reduction of the exposure time. The production of parasitoids was significantly lower at 24 hours when compared with the other exposure times.
Figure 1 - Emergence rhythm of *Nasonia vitripennis* males and females reared on pupae of *Chrysomya putoria* and exposed to parasitism for different amounts of time in the proportion of one parasitoid per three hosts (temperature 27°C day / 25°C night, 60 ± 10% relative humidity and 14 hours of photoperiod).

24 hours

| 13 | 14 | 15 |
|----|----|----|
| 27% | 13% | 12% | 5% | 1% |
| Females | Males |
| Emergency days after the host:parasitoid exposure |

48 hours

| 14 | 15 | 20 |
|----|----|----|
| 76% | 20% | 2.5% | 1.2% | 0.1% | 0.2% |
| Females | Males |
| Emergency days after the host:parasitoid exposure |

72 hours

| 14 | 15 | 16 | 17 | 18 | 19 |
|----|----|----|----|----|----|
| 67% | 20% | 8% | 3% | 0.4% | 0.4% | 2% | 0.2% | 0.2% | 0.1% | 0.1% | 0.1% |
| Females | Males |
| Emergency days after the host:parasitoid exposure |

It was observed that in all exposure times analyzed, the number of emerged males was lower than that of females, confirming, therefore, a deviation in sex ratio to more emergences of females. The sex ratio was similar in all three exposure times (24, 48 and 72 hours).
Table 2 - Average of the sex ratio and range of *Nasonia vitripennis* adult parasitoids reared on pupae of *Chrysomya putoria* exposed to parasitism for three different time periods (24, 48 and 72 hours) in the proportion of one parasitoid per three hosts (temperature 27°C day / 25°C night, 60 ± 10% relative humidity and 14 hours of photoperiod).

| Relation Host/Parasitoid | Sex ratio | Average and range of adult parasitoids |
|--------------------------|-----------|---------------------------------------|
|                          |           | Female | Male | Total |
|                          |           | X IR   | X IR | X IR |
| 24h                      | 0,73      | 8,44a  | 1-15 | 3,00a | 1-8 | 11,44a | 1-15 |
| 48h                      | 0,74      | 15,65b | 1-21 | 3,10ab | 1-11 | 18,78b | 1-21 |
| 72h                      | 0,76      | 15,07b | 1-30 | 4,62b | 1-15 | 19,70b | 1-30 |

- *X=* average; *IR=* range.
- Means followed by the same letter do not differ significantly by analysis of variance followed by Tukey post-test at 5% significance.

The highest rates of parasitism were obtained when the host was exposed to the parasitoid for 48 hours (95%) and 72 hours (91.6%). The lowest rate was observed at an exposure time of 24 hours (41.60%). The percentage of non-viable pupae was 8.3% in exposure times of 24 and 48 hours, and 6.66% at time 72 hours. It was observed the emergence of the host flies in treatment 1 (24 hours) in 50% of the exposed pupae. In the control treatment, there was the emergence of 93.3% of flies with 6.66% of unviable pupae (Fig. 2).
Figure 2 - Percentage of pupae parasitized by *Nasonia vitripennis* (followed by parasitoid emergence), non-viable pupae (not available fly adults and/or parasitoids) and fly emergence (*Chrysomya putoria*), in the proportion of one parasitoid per three hosts, and three exposure periods, compared with fly emergence in the control treatment (without exposure to the parasitoid) (temperature 27°C day / 25°C night, 60 ± 10% RH and 14 hours of photoperiod).

4 DISCUSSION

The significant variation in the post-embryonic development of *N. vitripennis* reared in *C. putoria* pupae for 24h, 48h and 72h, observed in this study, was also recorded by Mello *et al.* (2010) using *Chrysomya megacephala* (Fabricius, 1794) as host, and by Barbosa *et al.* (2010) in experiments with pupae of *Cochliomyia macellaria* (Fabricius, 1775). However, Cardoso and Milward-de-Azevedo (1995) did not find significant differences in the developmental time of parasitoids reared from *C. megacephala* after 24 and 48 hours of exposure.

In the 24 hours exposure experiment, it was observed that the average duration of the ontogenetic development of *N. vitripennis* was similar to that found by Schmidt (1986) in a 13-day experiment using *Chrysomya rufifacies* (Macquart, 1843) as host (temperature 27°C+ 1°C, 70 ± 10% RH). In the present study, the duration of parasitoid development was around 14 days, in 48 and 72 hours of exposure, similar to that observed by Mello *et al.* (2010) and Barbosa *et al.* (2010), using *C. megacephala* and *C. macellaria* as hosts, respectively. Barbosa *et al.* (2010) reported that *N. vitripennis* females took longer to develop when the exposure period was longer, which was also observed in males and females in this study. In the results of Cardoso and Milward-de-Azevedo (1995) the development period of *N. vitripennis* was longer when more parasitoids were used per pupa or when a high density of hosts was achieved.

Barbosa *et al.* (2010) did not observe significant variation in the mean development of male and female parasitoids using *C. macellaria* as host (temperature 27°C, UR 60 ± 10%), when exposure times to parasitism were 24, 48 and 72 hours. Mello *et al.* (2010), using *C. megacephala* (27°C, UR
60 ± 10%), obtained significant differences only in the development period of females, differing from the results found in this study, where there was a significant variation in the development period of both males and females, in all exposure times examined.

Several factors can influence the development time of a parasitoid, such as the environmental conditions (photoperiod, temperature and humidity), superparasitism, and the host’s age and size. If the number of immature parasitoids in a host is below the optimum density range for gregarious insects, this can also affect its rate of development (Wylie, 1964; Slansky and Scriber, 1985; Cardoso and Milward-de-Azevedo, 1996; Harvey and Gols, 1998). We believe that these factors may have contributed to the differences in the development of N. vitripennis observed between the present study and the other studies mentioned above.

The onset of male and female parasitoid emergence was on the 14th day (Mello et al., 2010; Barbosa et al., 2010) which is similar to the time observed in this study with exposure of 48 and 72 hours. The peaks of parasitoid emergence in the present study are consistent with those found by Gulias-Gomes et al. (2003), in an experiment with C. megacephala at 27°C, UR 65 ± 10%, and also by Mello et al. (2010) and Barbosa et al. (2010). In the latter study, the authors observed that the emergence peaks tended to occur later when the exposure period increased and showed the same trend for males and females, also consistent with the results obtained in this study. The significant difference in total parasitoid productivity with the increase of the exposure time is consistent with the results of Barbosa et al. (2010). However, the results of this study differ from those of Mello et al. (2010) which observed that the total number of parasitoids emerged was not influenced by the different periods of host exposure to female parasitoids. Barbosa et al. (2010) also observed that parasitoid yield was greater in 72 hours and that it fell when exposure time was decreased, coinciding with the results of this study. It is believed that an increase in the number of parasitoids is directly related to an increase in the time the host is exposed to parasitism. This increase allows the females of N. vitripennis more time to lay their eggs. According to Wylie (1965), the female parasitoid tends to lay more eggs in a long contact with the host.

In this experiment, there was a change in the sex ratio of N. vitripennis reared from C. putoria, with more females than males being born, in all exposure times, a result that had been previously obtained by Barbosa et al. (2010). There are some factors that can influence the sex ratio of parasitoid wasps, such as: i) superparasitism, which may lead to a prevalence of males in the offspring (Wylie, 1966); and ii) the use of hosts with more robust pupae, which can favor an increase in the number of female parasitoids (Van Den Assem et al., 1984). It is possible that the latter factor has contributed to the results of this study, since Calliphoridae have larger pupae compared with other flies. In addition to host size, the sex ratio of parasitoids may be influenced by interspecific nutritional
differences of the hosts. The sex of the offspring of *N. vitripennis* can be also regulated and influenced by the mothers’ age and by environmental factors such as photoperiod, temperature and relative humidity. However, it is believed that, in nature, the parasitoids select microhabitats that avoid extreme climate change (Harvey and Gols, 1998).

In our results, the parasitism rate was different of those of Mello *et al.* (2010), using *C. megacephala* as host. These authors observed rates of parasitism similar for all of the different treatments. The 41.60% parasitism after 24 hours was low compared with that obtained by Mello *et al.* (2010) (more than 80%), which employed one parasitoid per host in their experiments. This decrease in parasitism can be the result of the female stress caused by its transfer from the colony to the test tube, or because the host pupa loses its olfactory attractiveness when it is manipulated. Another factor that may have contributed to the low parasitism rate is that the time of 24 hours of exposure may not have been sufficient for the female to explore all three hosts offered. When the exposure times increased to 48 and 72 hours, the parasitism rate increased as observed in other studies (Barbosa *et al.*, 2010; Mello *et al.*, 2010).

Furthermore, in the results of Barbosa *et al.* (2010), there was an increase in the number of non-viable pupae as the exposure time increased and a peak of non-viable pupae after 48 hours. A similar finding was obtained by Cardoso and Milward-de-Azevedo (1995), according to which pupal mortality increased with high host densities and exposure to parasitism of 48 hours compared to 24 hours, coinciding with the results obtained in this study. Mello *et al.* (2010) observed fly emergence in 5% of *C. megacephala* pupae exposed to parasitism for 24 hours, far fewer than in this study (50%), using the same exposure time. These authors found that an increase in exposure time prevents the emergence of flies, as observed in this study after 48 and 72 hours. The latter authors suggest that this deleterious exploratory and feeding effect of *N. vitripennis* females may be caused by disruption of the host’s puparium. This disruption is caused by the insertion of the female ovipositor for the purpose of allowing ingestion of the hosts fluid and recognition of pupae already parasitized, being a strategy to avoid superparasitism.

Based on this study we conclude that the exposure time affected significantly the post-embryonic development, sex ratio and parasitoid productivity. There was a deviation in sex ratio with a greater number of emergencies of females than of males and an increase in the number of non-viable pupae when exposure to parasitism was extended. The parasitoid productivity tended to decrease with the reduction of the exposure time.
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