Impact of host exposure time on mass-rearing of *Psyttalia concolor* (Hymenoptera Braconidae) on *Ceratitis capitata* (Diptera Tephritidae)

Augusto Loni  
Dipartimento di Coltivazione e Difesa delle Specie Legnose “G. Scaramuzzi”, Università di Pisa, Italy

Abstract

The efficiency of the *Psyttalia concolor* (Szépligeti) - *Ceratitis capitata* (Wiedemann) mass-rearing system was investigated. A series of experimental parameters were fixed in order to reproduce in a small-scale, the mass-rearing conditions. Four different parasitization time exposure values were set to study the impacts of these parameters on the parasitization levels and on the parasitoid fitness. Larvae superparasitized with two eggs seemed to represent the most efficient strategy for parasitoid development, whereas the clutch of 4 or more eggs resulted in a disadvantage for both the host and the parasitoid. Egg distribution patterns were analyzed and further justified with the Poisson distribution. A random egg distribution seemed the strategy adopted by the parasitoid during the 20 minutes host-parasitoid exposure time only. The significances of such kind of egg distribution are discussed.

Key words: *Psyttalia concolor*, *Ceratitis capitata*, mass-rearing, parasitoid-host relationship, superparasitism, egg distribution patterns, Hymenoptera, Braconidae.

Introduction

 Parasitoid mass-rearing is one of the most fundamental elements in biological control programs, particularly for those based on the inundative or inoculative release methods. It also represents a very useful context for studies on parasitoid behaviour and relationship with the host. *Psyttalia concolor* (Szépligeti) is a koinobiont endophagous parasitoid of *Bactrocera oleae* (Rossi) and other fruit flies as *Capparymyia savastani* (Martelli), *Carpomya incompleta* (Becker) and *Ceratitis capitata* (Wiedemann) (Feron, 1952; Biliotti and Delanoue, 1959; Monastero, 1969). This species was used in recent years in biological control programs against the olive fruit-fly *Bactrocera oleae* (Rossi) both by inundative than inoculative methods (Raspi and Loni, 1994). The programs were implemented by various research institutes involved in establishing a mass-rearing production of this parasitoid with *Ceratitis capitata* as host.

One of the difficulties most frequently encountered in the mass production units was the low percentage of parasitoid emergence (at most 40%) compared to the very high parasitization rate, which often reached 100% of host larvae (Genduso, 1968; Greany *et al*., 1976; Loni, 1997; Raspi and Loni, 1994; Peri *et al*., 1994).

Abiotic factors, such as unsuitable temperatures and relative humidity, are reported as possible mortality causes as well as microbial contamination by fungi belonging to the Genera *Aspergillus* and *Serratia* (Genduso, 1968). These factors though crucial, do not offer a sole explanation for the extent of this phenomenon. Other mortality causes could be found in the physiological changes induced in the host immune-defensive system by the parasitization.

Self and conspecific superparasitism is frequently observed in the *P. concolor* - *C. capitata* system (Canale, 1998). This condition produces a high level of competition among the supernumerary number of parasitoid larvae in the same host (Vinson and Iwantsch, 1980; Potting *et al*., 1997; Ueno, 1997), in particular in the case of *P. concolor*, where only one parasitoid will complete its development. Clearly, under mass-rearing conditions no direct control over this phenomenon can be achieved.

A possible strategy resides in the management of a range of technical factors influencing this phenomenon. Parasitoid population density, parasitoid/host ratio and time of host exposure to female parasitoids, are the factors most strongly linked to the level of parasitization, and to the successful of parasitoid development.

Fixed a set of such factors, the analysis of the data regarding the parasitoids emergence results and the egg distribution patterns, offers a useful information channel for explaining the intimate host-parasitoid relationship (Waage, 1986; Bautista *et al*., 1998). On the basis of these considerations, a small scale experiment reproducing laboratory mass rearing conditions was conducted to decode the optimal host-parasitoid exposure time.

The latter parameter was chosen because it provides an easy tool to manage factors influencing parasitization level and parasitoid fitness.

Materials and Methods

Insect resources

* C. capitata and *P. concolor* were reared following the basic methods described by Raspi and Loni (1994). Parasitoids for the experiment are all taken from a sample of contemporaneously parasitized host larvae. They were supplied with water and a mixture of honey and pollen as a food resource, inside cylindrically shaped plexiglas cages and maintained in laboratory conditions at a temperature of 25 ± 1 °C, relative humidity 60 ± 5% and a 12L:12D photoperiod. Under these conditions about 18 days are necessary to complete the total development egg-adult (Loni, 1997).
Experimental units
7-day-old females, after the mating behaviour was observed in the mass rearing units, were collected and introduced into the experimental units consisting of cylindrically shaped 20 litre (diameter 25 cm; length 40 cm) plexiglas cages, similar to those used for the mass-rearing. 60 naïve females and 20 males were used in order to maintain a sex-ratio similar to that present in the mass rearing cages (Raspi and Loni, 1994). 120 mature *C. capitata* larvae (late 3rd instar) of the same cohort were used to provide a parasitoid/host (p/h) ratio of 1:2. Larvae were exposed to parasitization inside a nylon net bag, tightly fixed to the surface of a foam-rubber cylindrical support. The larvae were uniformly distributed inside the bag to ensure they were equally available for female parasitization.

Experimental procedure
The trial was conducted under the same laboratory conditions as the mass-rearing. Four different exposure time values were tested, starting from 20 minutes and increasing by 20-minute interval up to 80 minutes (i.e. 20, 40, 60, 80 min exposure). After each parasitization exposure a 1-hour rest was adopted with the goal to reduce a possible effect of time- or egg-limitation (van Alphen and Jervis, 1996) on the pro-synovicenic *P. concolor* females (Genduso, 1969; Quicke, 1997). Each treatment was replicated three times. A sub-sample of 100 larvae was collected from each parasitization unit and stored in little cages under controlled conditions (25 °C and 69-70% RH) until the emergence of the parasitoid or the fly was complete. This period varied from 25 days (Loni, 1997). The sex-ratio males/females of the emerged parasitoids was registered. Three samples of 100 mature *C. capitata* larvae, from the same cohort used for the experiment, were used as controls and were stored in little cages under the same conditions mentioned above. Their development is the criteria used for the subsequent estimation of natural mortality.

The remaining 20 larvae, of each treatment and replicate parasitization were immediately stored in ethanol 30% for later dissection under a stereoscopic binocular in Rüngen’s solution (Canale, 1998). These larvae were examined for egg number and then subdivided into percentage based on the number of the egg found. The most appropriate class in terms of parasitoid emergence was recorded. From this sub-sample were also calculated the percentage values of parasitized and super-parasitized larvae.

Statistical analysis
Percent emergence of *P. concolor* and *C. capitata* and dead puparia were calculated. Data obtained were analysed by the Friedman two-way analysis of variance by ranks for dependent data; significantly different rank sums were separated at 0.05 probability level by the multiple comparison between groups test, using the critical *z* values for *c* multiple comparisons (Siegel and Castellan, 1988; Conti et al., 1997). Data of the first time exposure of 20 minutes were excluded from the statistical analysis, because of the naïve status of the females not comparable with the experienced females of the other treatments. Egg distribution of the 20–larvae sub sample was compared with the theoretical values of Poisson random distribution by using Chi-square analysis (Sokal and Rolf, 1981; van Lenteren et al., 1978).

Results

Emergence results
*P. concolor* emergence was optimal (38.66%) after 40 minutes of exposure. Significantly differences resulted in the comparison between 40 and 80 minute treatments (P ≤ 0.05, df = 2, F1 = 6). Non significantly different emergence values were obtained in the 40-60 and 60-80 minute exposure comparisons (table 1).

The secondary sex ratio showed an ever more females-biased value with the increasing of the time exposure (figure 1).

Around two thirds of the larval hosts of *C. capitata* were killed during the 20-minute exposure interval. In the other treatments, the emergence results were not significantly different and ranged to less than 10% (*P* > 0.05, df = 2, *F1* = 4,5) (table 1). An exponential linear function could be traced to describe the phenomenon (figure 2a).

The percentage of dead puparia is a linear function of the time of exposure, rising from 33.67% after 20 minutes up to 84.66 % after 80 minutes (P ≤ 0.05, df = 2, *F1* = 6) (table 1, figure 2b).

Table 1. Mean percentages of parasitoids and *C. capitata* emergence, mean percentage of dead puparia. Values represented by a different letter, within a column, are significantly different (P ≤ 0.05), values between parenthesis represent the standard deviation. The values regarding the percentages of parasitized and superparasitized larvae are based on the sub-sample of the 20 dissected larvae.

<table>
<thead>
<tr>
<th>Time Exposure</th>
<th><em>P. concolor</em> progeny % (sd)</th>
<th><em>C. capitata</em> progeny % (sd)</th>
<th>Dead puparia % (sd)</th>
<th>Parasitized larvae %</th>
<th>Superparasitized larvae %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>93.4</td>
<td>6.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20'</td>
<td>25 (10.6)</td>
<td>41.33 (13.3)</td>
<td>33.67 (3.1)</td>
<td>38.33</td>
<td>15</td>
</tr>
<tr>
<td>40'</td>
<td>38.66 (4.7) a</td>
<td>7 (1.7) a</td>
<td>54.34 (5.5) a</td>
<td>90 a</td>
<td>76.66 a</td>
</tr>
<tr>
<td>60'</td>
<td>25.33 (4.1) ab</td>
<td>2.33 (0.06) a</td>
<td>72.33 (3.5) ab</td>
<td>83.33 a</td>
<td>71.66 a</td>
</tr>
<tr>
<td>80'</td>
<td>11.67 (2.9) b</td>
<td>2 (0.9) a</td>
<td>86.33 (2.1) b</td>
<td>91.66 a</td>
<td>83.33 a</td>
</tr>
</tbody>
</table>
Table 2. (Sub-sample of 20 host larvae). Egg distribution pattern percentages.

<table>
<thead>
<tr>
<th>Time Exposure</th>
<th>% larvae (0 eggs)</th>
<th>% larvae (1 eggs)</th>
<th>% larvae (2 eggs)</th>
<th>% larvae (3 eggs)</th>
<th>% larvae (4 eggs)</th>
<th>% larvae (5 eggs)</th>
<th>% larvae (6 eggs)</th>
<th>% larvae (7 eggs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20’</td>
<td>61</td>
<td>22</td>
<td>15.33</td>
<td>1.67</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>40’</td>
<td>10</td>
<td>13.33</td>
<td>33.33</td>
<td>13.33</td>
<td>15</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>60’</td>
<td>16.4</td>
<td>11.65</td>
<td>13.46</td>
<td>21.33</td>
<td>25</td>
<td>6.6</td>
<td>6.6</td>
<td>0</td>
</tr>
<tr>
<td>80’</td>
<td>8.3</td>
<td>8.33</td>
<td>10</td>
<td>16.71</td>
<td>15</td>
<td>23.3</td>
<td>13.3</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Table 3. Experimental eggs distribution pattern and the result of its comparison with the theoretical Poisson distribution (p ≤ 0.05, $\chi^2 = 3.08$, df = 3). Theoretical values are written between parenthesis.

<table>
<thead>
<tr>
<th>Time Exposure</th>
<th>Larvae 0 eggs</th>
<th>Larvae 1 eggs</th>
<th>Larvae 2 eggs</th>
<th>Larvae 3 eggs</th>
<th>Larvae &gt; 4 eggs</th>
<th>Goodness to fit Poisson</th>
</tr>
</thead>
<tbody>
<tr>
<td>20’</td>
<td>37 (34.7)</td>
<td>14 (19.07)</td>
<td>8 (5.24)</td>
<td>1 (0.96)</td>
<td>0 (0.13)</td>
<td>YES</td>
</tr>
<tr>
<td>40’</td>
<td>6</td>
<td>8</td>
<td>20</td>
<td>8</td>
<td>18</td>
<td>NO</td>
</tr>
<tr>
<td>60’</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>13</td>
<td>22</td>
<td>NO</td>
</tr>
<tr>
<td>80’</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>10</td>
<td>34</td>
<td>NO</td>
</tr>
</tbody>
</table>

Figure 1. *P. concolor*. Graphic reports the values of the sex ratios males/females, obtained at the four parasitization exposure times tested.

Figure 2.  
A. *C. capitata*. Emergence percentage values at the four exposure times tested. Exponential equation describing their trend.  
B. *P. concolor* - *C. capitata*. Percentage values relative to the dead pupae at the four exposure times tested. Regression line describing their trend.

The percentage of parasitized larvae, showed a high parasitization level which remained almost stable starting from the 40-minute exposure treatment with no significant differences among the treatments (p > 0.05, df = 2, $F_r = 2$) (table 1).

A similar consideration could be made about the superparasitized larvae percentage, (p > 0.05, df = 2, $F_r = 2.2$) (table 1).

Table 2 reports the egg distribution pattern percentages based on the sub-sample of 20 larvae for each replicates.

Data on comparison with the theoretical Poisson distribution showed a fit only for the shortest treatment, i.e. 20 minutes ($\chi^2 = 3.08$, df = 3) (table 3).

Discussion

Emergence results

The highest parasitoid emergence was produced at the 40-minute-exposure (38.66 %) (table 1). The same treatment produced the highest percentage of larvae containing two eggs (33.33 %) (table 2). These data suggest that larvae superparasitized with two eggs could represent the optimal strategy for the parasitoid development. This could explain the initial rise then the fall of the emergence results with the increase of the time exposure, observing the similar dome-shaped trend of the percentage of host larvae containing two-eggs (figure 3).

It has been argued that a degree of superparasitism may be useful in maximizing host utilization for parasitoids, in particular it could represent a strategy in reducing the host larvae immune defense system and thereby allowing a greater number of parasitoids to complete their development (Puttler, 1959; Puttler, 1967; Streams, 1971; Blumberg and Luck, 1990; Quicke, 1997; Montoya et al., 2000). Virus-like particles have been found in venom glands of *P. concolor* (Jacas et al. 1997). These substances are involved in
avoiding the immune-defense system of the host-larvae, such as the proteic encapsulation of parasitoid eggs, although other possible functions still remain to be clarified (Quicke, 1997). No data are available concerning the amount of venom injected during the oviposition of each egg, and the acquisition of reliable information on this phenomenon is unlikely to be easy. However, an interesting correlation may be hypothesized between a constant amount of venom injected with each egg and the host larvae body-mass, representing a continuously variable parameter. Two synchronous eggs inside a full-grown larva could produce the most appropriate amount of venom, in terms of immune-system avoidance. One egg might be insufficient in the case of “large” third-instar larvae but suitable for the “small” ones (Raspi and Canale, 2000). A similar argument could be put forward in the case of “large” mature larvae superparasitized with three eggs. On the other hand it could be observed that the percentage of parasitoid emergence were consistently higher than the relative percentage of two-egg larvae (figure 3), suggesting that the adult parasitoids also developed from different patterns of parasitized larvae, presumably those with one or three eggs.

Figure 3. P. concolor. Emergence percentage values at the four exposure times tested and the relative percentages values of 2-eggs larvae of the dissected subsample.

The increase in clutch size per host, associated with the longer time exposures, resulted in lower parasitoid emergence rates (tables 1 and 2). This hypothesis is confirmed by the percentage of total dead pupae, which increased progressively with exposure time (table 1).

This kind of result well fitted with a number of recent models showing that maximizing fitness per host may not always be the best strategy for an ovipositing parasitoid since the fitness realized per egg decrease with the number of eggs already present in the host (Waage, 1986). As regards the fitness penalties produced from the increase of exposure time, could be invoke super-parasitism producing competition among supernumerary parasitoid larvae (Vinson and Iwantsch, 1980; Potting et al., 1997). In the case of P. concolor, no evidence of physical attack has been found (Maniglia and Agro, 1994), although this species has mandibulate first instar larvae (Cals-Usciati, 1972). The results of our experiment seems to confirm this observation, as 20-minute exposure, with the maximal percentage of single-egg larvae (absence of larval competition), failed to produce the highest rate of parasitoid emergence. High super-parasitism rates easily could be associated with many causes of mortality, including anoxia, selective starvation as well as an excess of venom-induced changes in host physiology causing the larva to become an unsuitable substrate for parasitoid development (Vinson and Iwantsch, 1980).

The data obtained in the present study showed that under our experimental conditions, parasitoid females exhibited no limitation in oviposition behaviour. On the other hand, it is also clear that their oviposition rate reached a plateau, where the percentage of parasitized larvae remained at statistically comparable values not exceeding 91.66 % (table 1). From a different perspective, it could be suggested that parasitoid females re-framed ovipositing in certain larvae as shown by the percentage of larvae without eggs (tables 2 and 3). This could represent the result of a discrimination behaviour by which the parasitoid females evaluated such larvae as “poor” or sub-optimal hosts.

Very interesting to note that the sex-ratio of the emerged specimens shifted ever more to female-biased values with the increase of the time exposures. It was showed an high influence of host size on the sex determination in P. concolor, predominating females in large host. This is a condition that increases the fitness of the successive generation enhancing the reproductive capacity of the female progeny (Peri et al., 1994). The overcited causes of mortality as the excess of venom, associated to the high rates of superparasitism, may increase the mortality of the little-sized host containing males.

Egg distribution pattern

Analysis of the egg distribution pattern suggests that P. concolor discriminates between larvae ranked as suitable or unsuitable to host its eggs. This discrimination produced a progressive accumulation of eggs in the same limited group of larvae, not showing the clutch size to level off at the tested conditions (table 2). Comparison with Poisson distribution gave good fit only for the 20 minute exposure time, showing a random egg distribution (table 3). Obviously it should be taken of account of the naïve status of these females, nevertheless the parasitoid females of the other treatments produced a non-random egg distribution, enhancing super-parasitism. These anomalous results are probably due to the forced experimental conditions that induced a progressively increase of the encounter-rate of parasitized larvae, reducing the rarity of this event. Moreover, the limits of Poisson analysis are well discussed in van Alphen and Jervis (1996). Direct observations of parasitoid behaviour are required and the results of this trial cannot be used to support the claim that P. concolor is unable to discriminate between parasitised and unparasitised hosts. However, a random egg distribution pattern could be a useful strategy when the oviposition resource is represented by a patch of numerous gregarious larvae. In the case of fruits infested by C. capitata many larval stages resulting from different oviposition events...
can be found concurrently (Fletcher, 1989). Parasitoid females therefore have to interact with a population of diverse host quality in terms of host larval dimension. In such a situation, the laying of a single egg in a random distribution pattern, could produce the most useful combination of venom dose ad host body mass. Naturally this strategy could involve an elevated wastage of eggs, but its utilisation may be corroborated by the high number of eggs found in the oviducts of P. concolor females (as many as 60 mature eggs) (Genduso, 1969), in comparison to the low efficiency of progeny production in mass-rearing conditions.

This work provided clear evidence that P. concolor shows a very strong tendency to superparasitism under mass-rearing conditions. The fixed experimental, enhanced the stay of the female parasitoid population on a limited host resource, forcing the phenomenon of self and conspecific superparasitism. In this context the best fitness resulted when host larvae were parasitized by two parasitoid eggs, but very high mortality levels were also produced. Mass-rearing will therefore prove more efficient if considerably lower parasitoid/host ratios are maintained. This could represent the condition consenting the parasitoid females to perform as best as possible all the foraging behaviour steps in terms of host manipulation and acceptance, to produce the most appropriate clutch size distribution and simultaneously to reduce the negative effects an excess of superparasitism.

Acknowledgements

I am very grateful to the anonymous referee for the critical review of this manuscript.

References


PUTTLER B., 1959.- Partial immunity of Laphygma exigua (Hübner) to the parasite Hyposerix exigua (Viereck).- Journal of Economic Entomology, 52 (2): 327-329.


Author address: Augusto LONI, (aloni@agr.unipi.it) Dipartimento di Coltivazione e Difesa delle Specie Legnose “G. Scaramuzzi”, Università degli Studi di Pisa, via S. Michele degli Scalzi 2, 56124 Pisa, Italy.

Received April 24, 2003. Accepted November 14, 2003.