Larval competition between *Aphidius colemani* and *Lysiphlebus testaceipes* after multiparasitism of the host *Aphis gossypii*

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**Abstract**

The parasitoids *Aphidius colemani* Viereck and *Lysiphlebus testaceipes* (Cresson) (Hymenoptera Braconidae and Aphidiinae) play an important role in both natural and applied biological control of *Aphis gossypii* Glover (Rhynchota Aphididae). The use of both parasitoid species in biological control of *A. gossypii* may expose them to interspecific competition. Larval competition was evaluated to determine the existence of intrinsic superiority of either *A. colemani* or *L. testaceipes* after multiparasitism of *A. gossypii*. Nymphs of the 2nd instar of *A. gossypii* parasitized by both parasitoid species were used to test the outcome of interspecific larval competition. Thirty nine adults of *A. colemani* and 72 adults of *L. testaceipes* emerged from multiparasitized aphids. It appears that the parasitoid *L. testaceipes* was superior in larval competition ($\chi^2_{IGL} = 15.46, P \leq 0.01$) to *A. colemani* in *A. gossypii*. The intrinsic superiority of *L. testaceipes* may cause the displacement of *A. colemani* and, therefore, the simultaneous use of both parasitoids in biological control of *A. gossypii* should be carefully analyzed.

**Key words:** Intrinsic competition, parasitoid, Braconidae, Aphidiinae, Aphididae.

**Introduction**

Competition among parasitoids can occur extrinsically or intrinsically. Extrinsic competition involves host population exploration by a female parasitoid through effective host localisation and parasitism. Intrinsic competition occurs among larvae after multiparasitism within the host (Zwolfer, 1971). In solitary parasitoids only one adult emerges per host. All other parasitoids are eliminated by competition and usually this occurs during the parasitoid’s larval stages (Vinson and Iwantsch, 1980). According to the counter-balance competition model, one of the parasitoid species in competition will be the winner in intrinsic competition and may exclude the other parasitoid species (Zwolfer, 1971; Force, 1972; Briggs, 1993).

Using one or several species of natural enemies for biological control of a given pest is a controversial issue among biological control scientists. According to Briggs (1993), the use of the most effective parasitoid species only gives better control of the host population compared to releasing several parasitoid species. However, it is extremely difficult to determine the best candidate from a parasitoid assemblage (Ehler, 1990). Thus, the advantages of finding the most effective parasitoid species and using it alone, or to use multiple species introductions should be investigated (Hagvar, 1989; Briggs, 1993; Heinz and Nelson, 1996; Collier et al., 2002).

The subfamily Aphidiinae is composed of solitary parasitoids of aphids (Hagen and Bosh, 1968). *Aphidius colemani* Viereck and *Lysiphlebus testaceipes* (Cresson) are the dominant species occurring in South America; both have a broad host range and parasitize various aphid species (Starý and Cermeli, 1989; Sampaio et al. 2005a). Vökl and Stadler (1991) did not find intrinsic superiority with larval competition between *A. colemani* and *L. testaceipes* and recommended the simultaneous use of these parasitoids in biological control programs. Van Steenis (1993) pointed out that the use of *A. colemani* should be preferred over *L. testaceipes* for biological control of *Aphis gossypii* Glover in protected crops, since *Myzus persicae* (Sulzer) could also be attacked by that parasitoid. We evaluated larval competition between *A. colemani* and *L. testaceipes* after multiparasitism of the host *A. gossypii*, (1) to determine the occurrence of possible intrinsic superiority between these two aphid parasitoids and (2) to evaluate the best release programme for aphid biological control in protected cultivation, i.e. the release of one or two parasitoid species.

**Materials and methods**

**Aphid rearing**

Cotton aphids, *A. gossypii* were collected on cucumber plants (*Cucumis sativus L.*), and reared on cucumber cv. ‘Caipira’ plants in the laboratory. About 30 to 50 adult female *A. gossypii* were removed from the rearing and placed into a Petri dish (15 cm diameter) containing a cucumber leaf disc on a layer of 1% water-agar, and kept in a climatic chamber at 25 ± 1 °C and 70 ± 10% RH. Twenty four hours later the adult females were removed, and nymphs of 48 hours old were used in the experiments.
A. colemani and L. testaceipes rearing

The parasitoid A. colemani was obtained from mumified Aphis spiraeola Patch collected on Citrus sinensis (L.) on August 21, 2002. L. testaceipes was obtained from A. gossypii on cotton (Gossypium hirsutum L.) plants in the field on August 23, 2002. Both parasitoid species were collected at the campus of the Federal University of Lavras, in Lavras (21°14’43” south latitude and 44°59’59” west longitude), Minas Gerais State, Brazil. The parasitoids were reared for 16 generations on Schizaphis graminum (Rondani) on sorghum (Sorghum bicolor L.) at 19-25 °C, 12h photophase and 75 ± 15% RH under laboratory conditions.

Six mated females of each parasitoid species from the stock rearing were released in a Petri dish with about 100 2nd instar A. gossypii nymphs placed on a cucumber leaf disc on a layer of 1% water-agar. The parasitoids were allowed to parasitize the aphids for a period of 2h. Thereafter, the aphids were kept in a climatic chamber at 22 ± 1 °C, 70 ± 10% RH and 12h photophase, until emergence of adult parasitoids.

Competition between A. colemani and L. testaceipes larvae

To obtain parasitized aphids, 24-48h old mated females of A. colemani and L. testaceipes and 2nd instar A. gossypii nymphs were used. The parasitized aphids, both after single or multiple parasitism, were kept in Petri dishes (5-cm) containing a cucumber leaf disc (4-cm) on a layer of 1% water-agar.

After multiparasitism, the aphids were kept in groups of 7-11 individuals until mummy formation. Four days after oviposition by the parasitoids, the aphids were transferred to a new Petri dish (5-cm) with a new leaf disc. The formed mummies were individually put into glass tubes (100 x 8mm) and observed daily until emergence of the parasitoids. The parasitoids were identified to evaluate the number of adult of each species that emerged from multiparasitized hosts.

Single parasitism of the host

One A. colemani or L. testaceipes female was released in a Petri dish with a cucumber leaf disc and six 2nd instar A. gossypii nymphs. Parasitism was observed under a stereo microscope and each host was allowed to be attacked only once by the parasitoid female. Nymphs that had been attacked by parasitoids on the thorax or abdomen were used in the experiments. Hosts were discarded when oviposition occurred in other body parts or when they were only probed with the ovipositor and refused for oviposition by the parasitoid, according to the methodology proposed by Giri et al. (1982). After all nymphs were attacked by the parasitoid, six other nymphs were added in the arena until each female had parasitized 14-18 aphids. Six L. testaceipes and seven A. colemani females were used to obtain a total of 108 single parasitized aphids per parasitoid species. About 4-5 attacked aphids per parasitoid female were allowed to develop in order to evaluate the presence of the parasitoid larvae in its interior (see section “Percentage effective single and multiparasitism”).

Multiple parasitism of the host

About 70% of the aphids attacked by one parasitoid female (11-14 individuals) were offered in groups of six hosts to one female of the other parasitoid species. The following oviposition sequences were used to obtain multiparasitized hosts: one oviposition by A. colemani with a subsequent one by L. testaceipes; or one oviposition by L. testaceipes with a subsequent one by A. colemani. Seven L. testaceipes and six A. colemani females were used to obtain 83 multiparasitized hosts for each oviposition sequence. The interval between interspecific ovipositions in the same host did not exceed three hours. Twenty five hosts from each oviposition sequence were dissected to determine the presence of larvae of both parasitoid species in the same host. All other multiparasitized hosts (58 individuals per oviposition sequence) were allowed to develop until adult parasitoid emergence.

Percentage effective single and multiparasitism

To evaluate the percentage of parasitoid attacks resulting in oviposition, 25 aphids that were attacked only once were dissected under a stereo microscope three days after attack. The percentage of hosts with a parasitoid larva was determined for the two parasitoid species, which we consider as the percentage effective ovipositions. According to the methodology proposed by Völlki and Stadler (1991), this percentage effective ovipositions was used to estimate the number of emerging parasitoid adults for the two species after multiparasitism. The calculation of the estimate was base on the assumption that neither of the two parasitoid species was superior in elimination and, thus, that elimination would be at random.

The number of emerging parasitoids emerging from a multiparasitized host can be estimated as follows: (1) The number of L. testaceipes is $CN_{Lt} = N_{Lt}*(EO_{Lt}/100)$, where $CN_{Lt}$ is the corrected number of L. testaceipes individuals that developed after multiple parasitism of the host; $N_{Lt}$ is the number of emerged L. testaceipes; and $EO_{Lt}$ is the percentage effective ovipositions by A. colemani. (2) The number of A. colemani is $CN_{Ac} = N_{Ac}*(EO_{Ac}/100)$. The estimated number of parasitoids that should be found for one parasitoid species after single parasitism of the host, if no selective elimination occurs, can be obtained by multiplying the number of all the emerged parasitoid adults of both species by the percentage effective oviposition of that species. Thus, the expected number of L. testaceipes individuals ($EN_{Lt}$) after single parasitism is $[EN_{Lt} = (N_{Lt} + N_{Ac})*(EO_{Lt}/100)]$, where is $N_{Lt}$ is the number of emerged L. testaceipes, $N_{Ac}$ is the number of emerged A. colemani, and $EO_{Lt}$ is the percentage effective ovipositions by L. testaceipes. The expected number ($EN_{Ac}$) of A. colemani individuals after single parasitism is $[EN_{Ac} = (N_{Lt} + N_{Ac})*(EO_{Ac}/100)]$.

Statistical analyses

The influence of the oviposition sequence on the number of emerged parasitoids of each species after multiparasitism of the host was evaluated by the G Test of Independence with William’s correction (Sokal and Rohlf, 1995). The comparison of the number of emerged adults of each parasitoid species after multiparasitism of
the host was analyzed by the Contingency Tables Association test (Rosner, 1994), considering as the null hypothesis that both parasitoid species emerged in the same proportion, independently of the oviposition sequence. The statistical analyses of these parameters were done considering the corrected number of parasitoids (CNAc and CNLt).

The impact of the presence of one parasitoid species on the other species due multiparasitism of the host, was evaluated by comparing the number of parasitoids of each species after multiparasitization of the host (NAc and N Lt) with the estimated number of parasitoids of each species after single parasitism of the host (EN Ac and EN Lt). This analysis was done by the Contingency Tables Association test (Rosner, 1994).

Results

The percentages effective ovipositions of A. gossypii from A. colemani (92%) and L. testaceipes (72%) were higher than those observed by Sampaio et al. (2001) for A. colemani (86%) and Bueno et al. (2003) for L. testaceipes (48%). This high number of effective ovipositions was possibly due the fact that we only used hosts that were attacked by the parasitoid in the thorax and abdomen (Giri et al. 1982).

Table 1. Percentage of A. gossypii with parasitoid larvae of A. colemani and L. testaceipes after one or two ovipositions per aphid and percentage effective ovipositions (EO).

<table>
<thead>
<tr>
<th>Oviposition number and sequence</th>
<th>A. gossypii with parasitoid larvae (%)</th>
<th>% Effective Ovipositions (EO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One A. colemani oviposition</td>
<td>No larva 8</td>
<td>1 larva 92</td>
</tr>
<tr>
<td>One L. testaceipes oviposition</td>
<td>28</td>
<td>92</td>
</tr>
<tr>
<td>One A. colemani + one L. testaceipes oviposition</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>One L. testaceipes + one A. colemani oviposition</td>
<td>0</td>
<td>24</td>
</tr>
</tbody>
</table>

* Number of aphids per treatment = 25.

P ≤ 0.01), the number of emerged parasitoids after multiparasitism was much lower compared to the estimated number after single parasitism (table 3).

Discussion

Table 2. Number (N) and corrected number (CN) of emerged adult parasitoids of A. colemani (N Ac and CN Ac) and of L. testaceipes (N Lt and CN Lt) after multiparasitism of A. gossypii.

<table>
<thead>
<tr>
<th>Adult parasitoid obtained</th>
<th>First parasitoid ovipositing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. colemani</td>
<td>L. testaceipes</td>
<td>A. colemani</td>
</tr>
<tr>
<td>N Ac</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>N Lt</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>57</td>
</tr>
<tr>
<td>CN Ac</td>
<td>13 a</td>
<td>15 a</td>
</tr>
<tr>
<td>CN Lt</td>
<td>33 a</td>
<td>33 a</td>
</tr>
</tbody>
</table>

Table 3. Number (N) of emerged parasitoid adults after multiparasitism of A. gossypii and estimated number (EN) after single parasitization by A. colemani and by L. testaceipes. Averages followed by the same capital letter in the column do not differ by the Contingency Table Association test at 1% (χ^2 GL = 15.46).

<table>
<thead>
<tr>
<th>Parasitoid type</th>
<th>A. colemani</th>
<th>L. testaceipes</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (multiparasitism)</td>
<td>39 B</td>
<td>72 A</td>
</tr>
<tr>
<td>EN (single parasitism)</td>
<td>102 A</td>
<td>80 A</td>
</tr>
</tbody>
</table>
The use of an interval of maximally 3 hours between successive ovipositions led to 76% multiparasitized aphids, showing that during this period interspecific discrimination between A. colemani and L. testaceipes did not occur. Völkl and Stadler (1991) found evidence for interspecific discrimination after larval development in A. colemani and L. testaceipes. According to these authors, there was no host discrimination with oviposition intervals of zero to six hours. However, when a longer interval between ovipositions (16 hours) was used, both parasitoid species rejected the aphids previously parasitized by the other species.

According to Chow and Mackauer (1986), discrimination of parasitized hosts in afidines occurs among conspecific insects in a short period after their oviposition, through external chemical marking of the host. After oviposition there are physiological and biochemical changes over time in the host due to the development of the parasitoid larva, and this again allows discrimination of parasitized hosts in many species of parasitoids.

Host physiological and biochemical changes may function as internal markers and can sometimes be detected by other parasitoid species, in contrast to external markers, which are species specific (Mackauer, 1990).

In our study, the oviposition sequence did not affect the competition between A. colemani and L. testaceipes larvae, showing that the three hour interval between ovipositions did not benefit either the species ovipositing first, or the species ovipositing last. Völkl and Stadler (1991) found similar results with oviposition intervals from zero to six hours.

The number of emerged adult parasitoids after multiparasitism of A. gossypii was higher for L. testaceipes than for A. colemani. Therefore, we conclude that L. testaceipes was intrinsically superior to A. colemani, which is in disagreement with the results found by Völkl and Stadler (1991), who stated that there is no intrinsic superiority for any of these two parasitoid species. However our results are in agreement with most studies on afidines parasitoid larval competition, where one of the parasitoid species shows intrinsic superiority over the other (Chow and Mackauer, 1984; Chow and Mackauer, 1986; Hofsvang, 1988; Mackauer, 1990; Mcbrien and Mackauer, 1990; Mackauer et al., 1992).

In our study, it was not possible to determine what kind of elimination mechanism had been used by the competing parasitoids after multiparasitism. The use of physical attack by use of mandibles is common in first instar solitary parasitoids (Mackauer, 1990). According to Völkl and Stadler (1991), mandible use was the only mechanism detected in larval competition between A. colemani and L. testaceipes. The parasitoid that has the shortest egg incubation period and emerges first is supposed to have the greatest chance of eliminating the other species (Collier et al. 2002). The egg incubation period of L. testaceipes and A. colemani is not known, but this information could elucidate the mechanisms used in the combat between L. testaceipes and A. colemani.

The number of parasitoids expected after single parasitism of A. gossypii and the number of parasitoids obtained after multiparasitism indicates that L. testaceipes, the superior species in intrinsic competition, has great potential in reducing the population of A. colemani.

According to Sampaio et al. (2005a) there is an inverse correlation between the co-occurrence of A. colemani and L. testaceipes. Those authors found that in periods of high aphid densities both parasitoid species co-exist in aphids of the genera Aphis, Rhopalosiphum and Schizaphis. However, in times of scarcity of hosts, L. testaceipes was found in A. gossypii while A. colemani was not found. The authors pointed at the possibility of competition with L. testaceipes as probable causes for the non-occurrence of A. colemani, but also stated that it might be the effect of high temperatures. In fact, constant temperatures of 28°C are detrimental to both parasitoid species by reducing adult emergence (Toussidou et al., 1999; Rodrigues et al., 2004; Sampaio, 2004). However, there are A. colemani biotypes in Brazil that tolerate constant temperatures up to 28°C without reducing emergence (Sampaio et al., 2005b) So, in this case the effect of high temperatures as a cause for the lack of A. colemani at times of low host density can be discarded.

Conclusions

The results reported here, together with those of Sampaio et al. (2005a), that competition with L. testaceipes is the cause for the change in A. colemani occurrence, support the counter-balance competition model (Zwolfer, 1971; Force, 1972; Briggs, 1993), in which the parasitoid L. testaceipes is winning the intrinsic competition, and thus potentially interferes negatively with the parasitoid A. colemani that is intrinsically inferior.

The combined use of L. testaceipes and A. colemani for A. gossypii control in greenhouses may cause the exclusion of A. colemani. In a situation with a low aphid density, it is expected that superior competitor will remain in the crop and will exclude the poorer competitor. However, the superior competitor may be not the most effective parasitoid for aphid control (Zwolfer, 1971; Force, 1972; Briggs, 1993).

The parasitoid A. colemani presents great potential for control of A. gossypii and M. persicae (van Steenis, 1993; Sampaio et al., 2001), while L. testaceipes presents potential for the control of A. gossypii. L. testaceipes is not suitable for the control of M. persicae (van Steenis, 1993; Bueno et al. 2003; Carnevale et al., 2003; Rodrigues et al. 2004). Thus, the best way of using these parasitoid species for biological control of A. gossypii in crops where M. persicae also is a pest, should be carefully analyzed.

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