INTRODUCTION

The main way to reduce the wide gap between the costs of biocidal agents (within an IPM program) and those of acaricides, is to develop economical rearing and transport systems that would allow growers to take advantage of efficient natural enemies. The conversion of a tritrophic mass-rearing system (plant-herbivore-predator) into a ditrophic one (without the plant) could reduce production costs by more than 90% (estimate of Van Houten and Bolckmans, 2004). The data available for this phytoseid (Castagnoli et al., 2007). Stored food mites were chosen because another astigmatid species, the dust mite Dermatophagoides farinæ Hughes, proved to be an optimal food for N. californicus (Castagnoli and Simonî, 1999b). However, this mite is less suitable for commercial mass-rearing because it is a major cause of allergic reactions in humans. In addition to assuring an acceptable increase in the predator population, a good alternative prey should be cosmopolitan, easy and economical to mass-rear and to have a low capacity to sensitize humans. Our initial screening showed that Lepidoglyphus destructor (Schrank) was the best of three selected candidates with the necessary attributes, whereas Acarus siro (L.) was the worst. The former prey enabled us to establish long-term mass-rearing, but with A. siro the culture of N. californicus collapsed (Castagnoli et al., 2007).

The aim of the present study was to evaluate the biological and demographic parameters of N. californicus reared on A. siro and on L. destructor, in particular during the first three consecutive generations on each tested prey. We also compared these values with those obtained after numerous generations, when predator adaptation had presumably occurred.

MATERIALS AND METHODS

MITÉ SOURCE AND STOCK CULTURES

Neoseiulus californicus, derived from a strain collected on strawberry near Florence, was reared for at least two years on this phytoseid (Castagnoli et al., 2007). Stored food mites were chosen because another astigmatid species, the dust mite Dermatophagoides farinæ Hughes, proved to be an optimal food for N. californicus (Castagnoli and Simonî, 1999b). However, this mite is less suitable for commercial mass-rearing because it is a major cause of allergic reactions in humans. In addition to assuring an acceptable increase in the predator population, a good alternative prey should be cosmopolitan, easy and economical to mass-rear and to have a low capacity to sensitize humans. Our initial screening showed that Lepidoglyphus destructor (Schrank) was the best of three selected candidates with the necessary attributes, whereas Acarus siro (L.) was the worst. The former prey enabled us to establish long-term mass-rearing, but with A. siro the culture of N. californicus collapsed (Castagnoli et al., 2007).

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Quercus ilex pollen at about 25°C, RH>80% and a 16L:8D-hour photoperiod. Acarus siro was collected from a dairy, L. destructor from stored cereals.

Mass-rearing of the two stored food mites was established using the following diet:
- 2/3 of a commercially prepared product for weaning baby rabbits;
- 1/3 of de-bittered dried brewer’s yeast;
- 1 g of animal fat/100 g of the former diet.

This food must be kept under conditions of 18°C and 80-85% RH for 10 days prior to its use. The stored food mites were reared at 18±2°C in large Petri dishes kept in a larger box with water.

**Experimental Procedures**

The rearing unit (RU) used was a plastic arena (diameter about 4.5 cm) surrounded by wet cotton and partially covered, in order to maintain high humidity (fig. I).

Young ovipositing females of N. californicus were randomly collected from the pollen mass-rearing and transferred to the RUs. All stages of A. siro or of L. destructor were daily added to the arenas, to ensure the availability of prey in excess of possible phytoseiid consumption.

The eggs laid by N. californicus during the first 2 days were removed. The eggs laid in the following days were collected daily; some were placed in new RU in small groups of 8-15 eggs, in order to calculate mortality rates and determine the sex ratio; others in groups of 35-40 eggs, were isolated to calculate the development and the egg-to-egg times. Single newly emerged virgin females were confined with a mature male and each couple was observed for ten days to record oviposition and female longevity. The resulting coeval eggs were collected and used to start the second generation on the selected prey: the procedure used for the first generation was repeated for the ensuing generations. Some differences in the rearing procedure were introduced in order to obtain the strain adapted for A. siro. The nth generation of N. californicus reared on L. destructor was the result of continuous mass-rearing at 25±1°C, RH>80% and a 16L:8D-hour photoperiod, and the observations were carried out after about 18 months. However, to maintain the culture on A. siro after the initial three generations at 25±1°C, it was necessary to lower the temperature to 22±2°C to limit the escape of the stored food mites and hindered the development of fungi in the culture, which might have led to the collapse of the system. Furthermore, the A. siro-adapted strain of N. californicus was obtained from specimens that came from mass-rearing on Q. ilex pollen, were transferred and adapted to L. destructor for some generations, and then reared on A. siro for five-six months. Observations were made daily.

**Data Analysis**

The egg-to-egg development time and the fecundity of N. californicus fed on each prey, and the duration of every generation were compared by one-way ANOVA and the Tukey test (SPSS, 1994). The $\chi^2$-test was used to reveal differences in survivorship and sex ratio. The data about the development time, survival and fecundity of each test situation were used to calculate the $r_G$, or intrinsic rate over a generation time interval (G, i.e. egg-to-first offspring egg time). In phytoseiid mites, $r_G$ represents about 91% of $r_0$ (ABOU-SETTA and CHILDERS, 1991). The $r_G$ was determined using the HULTING et al. (1990) program, based on the method of BIRCH (1948) and Jackknife procedures, which, given the variance and standard error of the calculated values, allowed statistical comparisons. The Student Newman Keuls sequential test (SNEDECOR and COCHRAN, 1989) was used to evaluate differences in the $r_G$ values.

Only when fed on L. destructor N. californicus maintained a constant rate of population increase for a very long time after adaptation (CASTAGNOLI et al., 2007). Therefore, for this adapted phytoseiid strain, we calculated the stable age distribution which provides the percentage of individuals of each age class in the population (LOTKA, 1922; ANDREWARTHA and BIRCH, 1949).

**RESULTS**

**Biological Parameters of N. Californicus During the First Three Generations and After Adaptation to A. Siro**

Juvenile mortality and daily oviposition rates did not significantly differ during the three first generations, although they increased slightly in the third. The best results for egg-to-egg time and escape rate occurred in the second generation; in fact, these rates increased by 38% and about 16%, respectively, in the third generation. The percentage of females was unexpectedly very low (35.05%) in the second generation, but they increased slightly in the third. The best results for egg-to-egg time and escape rate occurred in the second generation; in fact, these rates increased by 38% and about 16%, respectively, in the third generation. The percentage of females was unexpectedly very low (35.05%) in the second generation, which is the lowest rate recorded for N. californicus in all situations that allowed a population increase. Therefore, for this adapted phytoseiid strain, we calculated the stable age distribution which provides the percentage of individuals of each age class in the population (LOTKA, 1922; ANDREWARTHA and BIRCH, 1949).

In the nth generation (after 5-6 months), when the strain was already adapted, the sex ratio shifted to a value similar to
the initial one. The egg-to-egg time showed the same pattern. However, this parameter is highly affected by temperature, which was lower than in the first generations and may have prolonged development time. The escape rate decreased significantly, whereas the daily oviposition showed a significant increase. The $r_G$ declined dramatically during the second generation, increased in the third generation, and at the nth generation this value was much better than in the previous generations, despite the lower temperature (Table 2).

**Table 1** – Biological parameters of *N. californicus* feeding on *A. siro* in the first three generations and after adaptation (nth generation). Means followed by the same letter within the same row are not significantly different ($\chi^2$ or Tuckey test, $P = 0.05$).

<table>
<thead>
<tr>
<th>Generations</th>
<th>$25^\circ$C</th>
<th>$22^\circ$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>egg-to-egg time (days)</td>
<td>10.83±0.96ab</td>
<td>9.01±0.90c</td>
</tr>
<tr>
<td>n = 24</td>
<td>n = 11</td>
<td>n = 11</td>
</tr>
<tr>
<td>juvenile mortality (%)</td>
<td>1.86a</td>
<td>2.02a</td>
</tr>
<tr>
<td>n = 108</td>
<td>n = 99</td>
<td>n = 46</td>
</tr>
<tr>
<td>sex ratio (females %)</td>
<td>62.26a</td>
<td>35.05b</td>
</tr>
<tr>
<td>n = 106</td>
<td>n = 97</td>
<td>n = 46</td>
</tr>
<tr>
<td>escape rate (%)</td>
<td>79.73a</td>
<td>66.55b</td>
</tr>
<tr>
<td>n = 523</td>
<td>n = 290</td>
<td>n = 200</td>
</tr>
<tr>
<td>daily oviposition (10-day period)</td>
<td>0.93±0.36a</td>
<td>0.99±0.37a</td>
</tr>
<tr>
<td>n = 18</td>
<td>n = 21</td>
<td>n = 16</td>
</tr>
</tbody>
</table>

The biological parameters of *N. californicus* during the first three generations and after adaptation to *L. destructor* are summarized in Table 1. Juvenile mortality was very low and constant in all generations. The escape rate increased with the generations, being significantly higher at the nth than at the first and the second (Table 3). The same held true for the sex ratio and, surprisingly, the worst value was at the nth generation. Egg-to-egg times were better at the first and nth generations, daily oviposition rates at the first and at the third. The $r_G$ was therefore significantly higher at the first generation than at the others. The worst values were obtained in the second and third generations and, at the nth generation (after 18 months), the values were slightly higher than at the second but never approached the values recorded at the first generation. By the nth generation the age distribution of *N. californicus* consisted mostly of eggs and immatures, which constituted more than 2/3 of the entire population, indicating a rapidly growing population.

**Table 2** – $r_G$ and estimated $r_m$ of *N. californicus* on *A. siro* in the different generations.

<table>
<thead>
<tr>
<th>Generations</th>
<th>$r_G$ calculated (day$^{-1}$)</th>
<th>$r_m$ estimated (day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st (at 25°C)</td>
<td>0.123±0.005a</td>
<td>0.135</td>
</tr>
<tr>
<td>2nd (” “ ”)</td>
<td>0.095±0.007b</td>
<td>0.104</td>
</tr>
<tr>
<td>3rd (” “ ”)</td>
<td>0.105±0.005ab</td>
<td>0.115</td>
</tr>
<tr>
<td>nth (at 22°C)</td>
<td>0.164±0.006c</td>
<td>0.180</td>
</tr>
</tbody>
</table>

**DISCUSSION AND CONCLUSIONS**

Generalist phytoseiids rapidly adapt to different conditions (McMurtry and Croft, 1997), and the selection of strains with desired traits is therefore sometimes possible in a relatively short time. When *N. californicus* is shifted from an optimal to a suboptimal food, its development time, survival and fecundity rates are usually quite stable during the first generation, but decline by the second or third. Several additional generations on the new food are necessary for this mite to regain and to equal, or exceed, the performance of the first generation. This was observed on *D. farinae* (CA-
Such behaviour was also observed when the phytoseid preyed on spider mites on non-agreeable host-plants, such as tomato (CASTAGNOLI et al., 1994). In the preliminary screening, when prey was offered for the first time to young females and juveniles of N. californicus that had developed on pollen, both stored food mites tested herein seemed to be possible candidates as alternative food, although they differed greatly in the degree of acceptance by the predator. On A. siro, with respect to L. destructor, the predator’s development time was approximately two days longer, it laid slightly more than a third eggs per female and its escape rate was very high (CASTAGNOLI et al., 2007). When successive generations were fed on A. siro, the classical trend in the parameters was observed: the intrinsic rate of increase was worst at the second generation, but began to increase at the third and was much improved at the nth generation. With L. destructor as alternative prey, the differences in the parameters between the different generations were generally smaller than with A. siro. To sum up, when transferring N. californicus to a new diet the best results were obtained in the first generation, the worst in the second. The third and nth generations showed very similar results, which were significantly different from those of the former generations - better than the second but not as good as the first. A similar trend (i.e. a small decrease in performance) was also observed when N. californicus was reared for successive generations in the laboratory on a ‘friendly’ plant (strawberry) infested with T. urticae (CASTAGNOLI et al., 1999a). In that case, the test conditions were probably immediately acceptable to the phytoseids without any adaptation. The slightly reduced population increase could have been due to inbreeding in a continuous population, derived from a small number of founders. That resulted from the experimental methodology which required a clear count and discrimination of successive founders. That resulted from the experimental methodology which required a clear count and discrimination of successive generations. To avoid inbreeding and to assure high predator efficiency, periodic renewals of the reared population, via the introduction of new predators, is a common practice in long-term mass-rearing.

In conclusion, N. californicus showed a greater population increase on L. destructor than on A. siro, even though an improvement in efficiency due to adaptation was observed with the latter prey. However, the situation was checked for a longer time on L. destructor (18 months) than on A. siro: on this latter, quite good efficiency was maintained for 5-6 months. Then, in fact, the phytoseid rearing on A. siro underwent an irremediable decline within a couple of months (CASTAGNOLI et al., 2007). The estimated r_{e} was considerably higher on L. destructor (0.219 day^{-1}) than on D. farinae (0.143 day^{-1}) (CASTAGNOLI et al., 1999b), the latter being already considered a good alternative prey for long-term mass-rearing. With respect to D. farinae, the development time of N. californicus on L. destructor was shorter by about one day from that on D. farinae and the fecundity was higher. At 25°C, N. californicus performed best on T. urticae, whereas the r_{e} ranged from 0.227 to 0.287 day^{-1} (CASTAGNOLI and SIMONI, 2004), and the r_{e} value on L. destructor was the highest obtained on any other prey. The predominance of non-adult stages in the N. californicus reared for a long time on L. destructor confirmed that this prey ensures the good condition of the reared population. Therefore, as predicted in our preliminary study, L. destructor is confirmed as an optimal candidate for large-scale mass-rearings of N. californicus.

### REFERENCES


