Influence of starvation and blood meal-induced moult on the susceptibility of nymphs of *Rhodnius prolixus* Stål (Hem., Triatominae) to *Beauveria bassiana* (Bals.) Vuill. infection

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Abstract: The influence of starvation and nutritional status on the time–mortality response of nymphs of *Rhodnius prolixus* Stål (Hem., Reduviidae) contaminated with *Beauveria bassiana* (Bals.) Vuillemin (Deuteromycotina, Hyphomycetes) was determined. Because of the induction of the moulting process by the blood meal, first-instar nymphs were less susceptible on day 12, when they were in pre-moult. Starvation did not affect nymph susceptibility to *B. bassiana* up to a period of 1 month. There was no significant difference in time–mortality responses between unfed nymphs and blood-engorged nymphs. This information is discussed for microbial control of vectors with respect to the importance of periods of starvation in triatomines under natural conditions.

1 Introduction

*Rhodnius prolixus* Stål (Hem., Reduviidae) is the most serious vector of Chagas disease in northern Latin America (Lent and Wygodzinsky, 1979; Schofield et al., 1987). Control of triatomines in peri-domestic areas with synthetic insecticides is difficult due to their low effectiveness in open areas (Dias, 1987). Because of the detrimental impact of synthetic insecticides on the environment and the risk of developing resistance, the role of entomopathogenic microorganisms in pest and vector control is expanding. Hyphomycetous fungi, which invade hosts via the integument, are good potential candidates for microbial control of sucking insects (Lacey and Goettel, 1995). *Beauveria bassiana* (Bals.) Vuill. has been reported to be highly active against *R. prolixus* and other triatomine species under both laboratory (Moura-Costa, 1978; Sherlock and Guttin, 1982; Messias et al., 1986; Romãña and Fargues, 1987; Fargues and Luz, 1998, 2000; Luz and Fargues, 1998, 1999; Luz et al., 1998b, 1999b; Lecuona et al., 2001) and field conditions (Romãña, 1992; Luz et al., 1994, 1999c). Interactions between entomogenous fungi and their hosts are usually influenced by environmental factors. Temperature and humidity are known to affect the dynamics of the disease and that of the fungal recycling on the insect cadavers (Fargues and Luz, 1998, 2000; Luz and Fargues, 1998, 1999; Luz et al., 1999b). The moulting process induced by feeding or a prolonged starvation may affect insect resistance to pathogenic microorganisms and, therefore, have an impact on the biological control of the insect (Watanabe, 1987). The effect of starvation and feeding on the fungus infection of nymphs was therefore evaluated here.

2 Materials and methods

2.1 Fungus culture

The fungal isolate, *B. bassiana* INRA 297, isolated in 1971 from an unidentified heteropteran insect in Poland, was selected because of its high virulence for *R. prolixus* (Romãña and Fargues, 1987). This isolate was previously host-passaged on first-instar nymphs of *R. prolixus* at the beginning of these experiments. Conidia were harvested from 3-week-old sporulating cultures, grown at 25±1°C on agar slants of semi-synthetic medium (0.36 g KH₂PO₄, 1.42 g Na₂HPO₄·12H₂O, 0.6 g MgSO₄·7H₂O, 1 g KCl, 0.7 g NH₄NO₃, 10 g glucose, 5 g yeast extract, 18 g agar and 1000 ml H₂O). Inocula were suspended in 10 ml of sterile distilled water by shaking (700 oscillations per minute) in 45-ml flasks containing five to six dozen glass beads (3 mm in diameter) for 5 min. Conidial germination tests, carried out on nutrient agar under humidity-saturated atmosphere at 25±1°C in darkness (Luz and Fargues, 1997), showed high germination rates (>98%) after 24-h incubation. Suspensions were then adjusted to defined doses based on haemacytometer counts.

2.2 Insect rearing

Experiments were carried out on a Brazilian trypanosome-free strain of *R. prolixus* obtained from the Institute of Tropical Medicine of the University of Tübingen. Insects were held at 28±1°C and 75±5% RH, and a photoperiod
of 12 : 12 (L : D) h. Once a week, they were fed heparin-amended (5000 IU/ml) sheep blood using an apparatus (Romáñá, 1992) derived from that of Nicolle (1941). It consisted of a bell-shaped glass chamber containing 50 ml of blood and a 6.5-cm-diameter opening covered with paraffin. Blood was kept at 37°C by circulating thermo-regulated water through the double-shell wall of the glass chamber. The glass chamber was placed in inverted position, with the paraffin over the cage containing the insects. This cage consisted of a glass container (8 cm high and 16.5 cm wide) with a wooden platform inside containing 0.5-cm-diameter holes through which the insects could access the glass chamber containing the blood. The cage was covered with a fine mesh gauze to keep the insects in. Fifty nymphs or 20 adults were introduced in the cage for feeding periods of 60 min.

2.3 Standard bioassay procedure

Insects were treated by directly spraying conidial suspensions, titrated at 3 \times 10^5 conidia/cm^2, in a spray tower used to test entomopathogenic fungi under laboratory conditions (Luz and Fargues, 1998). The spray tower, dispensing known volumes of aqueous conidial suspensions at a regulated pressure, provides fine, non-drenching sprays. Ten millilitres of dosage were sprayed onto batches of 20 individuals placed in plastic boxes (105-mm diameter and 80-mm height) on the rotary platform of the apparatus, for 2 min. The insects were then aseptically air-dried for 30 min at 75% RH and 25°C. After drying, contaminated insects were placed in small glass tubes (40 \times 20 mm), covered by gauze and then transferred to test chambers at 25°C, 97% RH, and a photoperiod of 12 : 12 (L : D) h (Fargues and Luz, 1998; 2000; Luz and Fargues, 1998, 1999).

Lots of 20 insects were used per treatment variant and each treatment consisted of four replicates. Three series of bioassays were carried out for studying the influence of feeding on the Beauveria susceptibility of R. prolixus nymphs. A first series consisted of contaminating nymphs at different duration of starvation after hatching, from 4–11 to 32–39 days after hatching. The third series of bioassays consisted of comparing the susceptibility of both unfed and engorged second-instar nymphs to B. bassiana. Contamination of engorged nymphs was done 24 h after the blood meal.

Probabilistic analysis for determination of lethal times LT_50 and LT_90 was conducted on total mortality after Abbott transformation using log-probit analysis software developed by CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement) (CIRAD, 1989). Lethal times were then analysed using either one- or two-way analysis of variance (ANOVA), and the Student–Newman–Keuls (SNK) multiple range test of comparison of means (SAS, 1989); means were considered not statistically different at P > 0.05.

3 Results

All R. prolixus contaminated with B. bassiana were killed at the end of bioassays, 30 days after treatment, independently of their physiological state related to feeding or to starvation (tables 1–3).

The fungus inoculation timing related to that of the blood-feeding affected significantly the susceptibility of R. prolixus nymphs (F_{5,18} = 113.8, P < 0.0001 for LT_{50}; and F_{5,18} = 29.7, P < 0.0001 for LT_{90}). When contamination was applied for the first 7 days after one blood meal, there was no significant difference in LT_{50} of R. prolixus first-instar nymphs, which ranged from 4.7 to 5.7 days or in LT_{90}, which ranged from

### Table 1. Time–mortality response (in days) in first-instar nymphs of Rhodnius prolixus inoculated with Beauveria bassiana at different periods of time after one blood meal

<table>
<thead>
<tr>
<th>Lethal time</th>
<th>First instar</th>
<th>Second instar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>LT_{50}</td>
<td>5.67 ± 0.17 a</td>
<td>5.45 ± 0.43 a</td>
</tr>
<tr>
<td>LT_{90}</td>
<td>8.58 ± 0.81 a</td>
<td>8.44 ± 1.63 a</td>
</tr>
</tbody>
</table>

Means of lethal times 50% and 90% (LT_{50} and LT_{90} ± SD) based on four replicates of 20 insects per replicate. Means followed by different letters are significantly different (P < 0.05) according to the SNK test.

<table>
<thead>
<tr>
<th>Lethal time</th>
<th>Days after the blood meal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4–11</td>
</tr>
</tbody>
</table>

### Table 2. Time–mortality response (in days) in first-instar nymphs of Rhodnius prolixus inoculated with Beauveria bassiana at different duration of starvation after hatching

<table>
<thead>
<tr>
<th>Lethal time</th>
<th>Days of starvation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4–11</td>
</tr>
<tr>
<td>LT_{50}</td>
<td>4.22 ± 0.21 ab</td>
</tr>
<tr>
<td>LT_{90}</td>
<td>5.95 ± 0.60 ab</td>
</tr>
</tbody>
</table>

Means of lethal times 50% and 90% (LT_{50} and LT_{90} ± SD) based on four replicates of 20 insects per replicate. Means followed by different letters are significantly different (P < 0.05) according to the SNK test.
6.5 to 8.6 days (table 1). In contrast, for first-instar nymphs contaminated on day 12 after feeding, the LT values reached 14.7 and 26.7 days, respectively. When treated on day 14, most of test insects consisted of newly moulted second-instar nymphs. Their LT values dropped to 5.4 and 9.8 days, respectively (table 1). No mortality was observed for the control insects.

Time–mortality responses in newly hatched first-instar nymphs did not change with respect to the duration of starvation (table 2). There were variations in LT50, which ranged from 4.0 to 4.6 days ($F_{4,15} = 7.0$, $P = 0.002$), and in LT90, which ranged from 5.6 to 6.5 days ($F_{4,15} = 3.63$, $P = 0.029$). Mortality in control insects did not exceed 5% by the end of the starvation experiments.

There was no effect of the nutritional status on time–mortality responses of second-instar nymphs (table 3). No differences in LT50 or LT90 ($F_{1,6} = 0.004$, $P = 0.95$, and $F_{1,6} = 0.006$, $P = 0.94$, respectively) were observed between unfed nymphs and engorged nymphs. Mortality in control unfed nymphs was nil and that in control-engorged nymphs did not exceed 9% by the end of the starvation experiments.

### Table 3. Time–mortality response (in days) of Rhodnius prolirixus inoculated with Beauveria bassiana as both unfed and engorged second-instar nymphs

<table>
<thead>
<tr>
<th>Lethal time</th>
<th>Unfed nymphs</th>
<th>Engorged nymphs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$LT_{50}$</td>
<td>3.48 ± 0.54</td>
<td>3.50 ± 0.42</td>
</tr>
<tr>
<td>$LT_{90}$</td>
<td>5.39 ± 1.14</td>
<td>5.44 ± 0.58</td>
</tr>
</tbody>
</table>

Means of lethal times 50% and 90% ($LT_{50}$ and $LT_{90} ± SD$) based on four replicates of 20 insects per replicate.

4 Discussion

Because of the induction of the moult process by the blood meal, feeding has a strong effect on the probability of rapid infection by B. bassiana. First-instar nymphs contaminated on day 12 after one blood meal were obviously in pre-moult. The increasing time–mortality response, reaching four times more than the response of young first-instar nymphs in intermoult, revealed an increasing resistance of R. prolirixus nymphs to infection just before moult. In contrast, recently emerged second-instar nymphs were as susceptible to B. bassiana as young first-instar nymphs.

An interaction between fungus infection and insect moult has been observed by numerous authors (FERRON et al., 1991; TANG and CHENG, 1999); however, few of them have investigated the infection process by entomopathogenic hyphomycetes during moult (FARGUES, 1972; ZACHARUK, 1973; FARGUES and VEY, 1974; VEY and FARGUES, 1977). Some larvae of Leptinotarsa decemlineata Say (Col., Chrysomelidae), contaminated with B. bassiana less than 36 h before moult, died without moulting, others succeeded in shedding the infected integument (FARGUES and VEY, 1974), but a large proportion of apparently safe larvae were reinfected during the ecdysis process and died at a later stage (VEY and FARGUES, 1977). When insects died before moulting, the infection-moult interaction also induced a delayed mortality (FARGUES, 1972).

Prolonged starvation of first-instar nymphs of R. prolirixus did not affect their susceptibility to B. bassiana infection. This point is particularly important, as most R. prolirixus specimens collected in silvatic and peridomestic habitats were found to be unfed (PIFANO, 1973). Moreover, as expected, R. prolirixus nymphs are capable of surviving long periods of time without feeding (LUZ et al., 1998a). LI and LIU (1990) reported that there was also no influence of starvation on Beauveria-infection in larvae of Dendrolimus punctatus Walker (Lep., Lasiocampidae).

No significant difference was observed in time–mortality responses between unfed and blood-engorged nymphs, indicating that nutritional status had no effect on probability of infection by B. bassiana. Interestingly, GINDIN et al. (2001) reported that the susceptibility of larvae and nymphs of Rhicephalus sanguineus (Acari, Ixodidae) to one virulent isolate of Metarhizium anisopliae was higher in engorged individuals than in unfed ones. With respect to phytophagous insects, MIETKIEWSKI and MACHOWIECZ–STEFANIK (1993) noted that mortality was lower when larvae of Cydia pomonella Linnaeus (Lep., Tortricidae) and L. decemlineata were starved for 48 and 72 h, respectively, than with continuous feeding.

In spite of its obvious importance, there is lack of information on the relationship between the nutritional status of targeted insects and their susceptibility to the hyphomycetes proposed as biological control agents. Our results suggest that the susceptibility to fungal infection of triatomine insects, such as R. prolirixus, which have long periods of nymphal intermoult phase, is apparently not related to feeding. Moreover, the decrease in nymph susceptibility resulting from the infection-moult interaction was short-term. As such, it might not affect the application strategy of microbial control of triatomine vectors (ROMAÑA, 1992; LUZ et al., 1994) in situations where environmental conditions are favourable for both nymph infection and recycling from fungus-killed cadavers (FARGUES and LUZ, 1998, 2000; LUZ and FARGUES, 1998, 1999; LUZ et al., 1999a).

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