# Development of *Rhodnius prolixus* (Hemiptera: Reduviidae) under Constant and Cyclic Conditions of Temperature and Humidity

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*Development of* Rhodnius prolixus *after eclosion until the adult stage was studied at constant temperatures (T), 15, 20, 25, 28, 35°C, and relative humidities (RH), 75, 86 and 97%, and fluctuating (16/8 hr) temperatures, T I/II, 15/28°C, 20/25°C, 25/28°C and 25/35°C, and relative humidities, RH I/II, 86/75% and 97/75%. Eclosion or molting were not observed at 15°C and 86 or 97% RH, respectively. At 35°C and 75% RH only few insects molted. By alternating T I/II, 15/28°C and 25/35°C, insects developed at high frequency. Cumulating the average lengths of the interphases within independent groups for each instar,* R. prolixus *reached the adult stage most rapidly (86.7 days) and at highest frequency per instar (mean: 91.8%) at 28°C and 75% RH. Under fluctuating T I/II, development was completed within 100 days or less at 25/28°C and 25/35°C with high rates of hatch and molting. Development was slowest at fluctuating TI/II, 15/28°C and 20/25°C (>185 days), and at constant 20°C (>300 days). Mortality was higher at constant 97% RH or fluctuating RH I, 97%, than at constant or fluctuating 86% RH. Refeeding was minimal at optimal conditions of T and RH for development. The most refeeding was observed at a constant 35°C.*

Key words: *Rhodnius prolixus* - development - temperature - humidity

*Rhodnius prolixus* is considered to be one of the most serious vectors of Chagas disease. All five instars including adults are obligatory hematophagous insects and potential vectors of *Trypanosoma cruzi*. *R. prolixus* is unable to molt without feeding. Nymphs need to feed at least once *ad repletionem* to attain the following stage. Moreover, abiotic factors such as temperature and humidity affect insect development. Under laboratory conditions, the temperature range for eclosion and molting of *R. prolixus* was reported to be between 16-34°C (Gomez-Nuñez & Fernandez 1963). No development was observed at 15°C and 35°C (Galliard 1935, Okasha 1964). Development is generally studied at constant temperatures between 25 and 28°C and about 70% humidity or unspecified conditions of ambient temperature and humidity. However, temperature and humidity in insect habitats may differ considerably and vary according to circadian and seasonal patterns. *R. prolixus* is found mainly in Colombia and Venezuela from 0 to 2,600 m above sea-level, in regions with annual median temperatures from 11 to 29°C and 250 to 2,000 mm annual precipitation (Carcavallo et al. 1977, 1978, Lent & Wygodzinsky 1979). There is little information on the linked impact of cyclic conditions of temperature and humidity on development of Chagas disease vectors. Pippin (1970) showed that development of several triatomine species at unspecified fluctuating temperatures between 18 and 28°C and humidities from 15 up to 65%, was prolonged compared to 25°C and 65% humidity. More detailed investigations on *R. prolixus* biology in conditions reflecting the insect's natural environment are required to improve control methods. Recently Luz et al. (1998) reported an influence of fluctuating temperature and humidity on starved *R. prolixus*. In the present study, development of *R. prolixus* was examined at constant and fluctuating temperatures and humidities within a range of 15 and 35°C, and 75 and 97%.

## MATERIALS AND METHODS

*Insect rearing - R. prolixus* was reared at 28 ± 1°C and 75 ± 5% humidity with a photoperiod of

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12/12 hr. Once a week they were fed heparin-ammended (5 000 I.U./ml) sheep's blood using a simple apparatus (Luz 1994). The insect colony originated from an unknown laboratory culture in São Paulo, Brazil, and was maintained for more than 30 years at the Institute of Tropical Medicine, Tübingen, Germany.

*Bioassays* - Newly deposited eggs (<24 hr) and newly emerged and *ad repletionem*-fed insects of all five instar nymphs (N1 to N5) were used for the tests. Eggs and first instars were kept in small glass tubes (40x20 mm) and other instars were kept in Petri dishes with round openings (70 mm) in both, top and bottom. Openings in both containers were closed by gauze. Four Petri dishes were superimposed with a distance of 10 mm between each dish. In each assay, four replicates of 20 individuals each were used. Insects were kept in temperature adjusted closed incubators and provided with a constant humidity-regulated airflow (720 l/hr). Vapour pressure was regulated by saturated solutions of salt. The following salts (1 kg/0.5 l distilled $H_2O$) were used to provide specific realtive humidities: NaCl (75%), KCl (86%) and $K_2SO_4$ (97%) (Winston & Bates 1960). Temperature and humidity inside incubators were monitored constantly. Changing temperatures and humidities were obtained by transferring insects between incubators. Insects were allowed to feed once a week during the assay as mentioned above. Eclosion, molting and mortality were recorded daily. Assays were run for 60 days in each instar tested.

*Temperature and humidity* - Assays were done at constant and fluctuating temperature (T) and relative humidity (RH). Fluctuating temperatures, T I/II, and humidities, RH I/II, were performed at a 16/8 hr rhythm. Five temperature regimes, 15, 20, 25, 28 and 35°C, and three humidity regimes, 75, 86 and 97%, were tested in four combinations of T I/II, 15/28°C, 20/25°C, 25/28°C and 25/35°C, and two combinations of RH I/II, 86/75% and 97/75%. At constant temperatures the following humidities were tested: 15°C and 20°C, 86 and 97%, 25°C, 75, 86 and 97% and 28 and 35°C, 75%. Photoperiod was 16/8 hr at constant temperature and humidity. For fluctuating conditions, darkness corresponded to T/RH I (16 hr) and lightness to T/RH II (8 hr).

*Data analysis* - Angular transformed percentages of cumulative eclosion, molting and mortality were analyzed by ANOVA (analysis of variance) and means compared by the Student-Newman-Keuls test. Length of interphases and rates of refeeding were calculated by frequency tables (SAS Institute Inc. 1989).

## RESULTS

Cumulative rates of eclosion, molting and mortality of *R. prolixus* 60 days after incubation at different regimes of constant and fluctuating temperatures and humidities are shown in Figs 1, 2, 5, 6. Regimes tested proved to have a highly significant influence on rate of molting of *R. prolixus* ($F_{16,306}$ = 244.4), rate of refeeding ($F_{16,255}$ = 50.3) and on mortality ($F_{16,255}$ = 73.3). Moreover, there was a highly significant effect of the instar on molting ($F_{5,306}$ = 115.0), rate of refeeding ($F_{4,255}$ = 28.1) and mortality ($F_{4,255}$ = 84.4). The period between the first blood meal *ad repletionem* and molting increased significantly with the instar ($F_{5,245}$ = 770.0) and at lower constant or alternating temperatures, T I, 20 and 15°C, tested ($F_{13,245}$ = 589.6).

*Constant temperatures and humidities* - At constant 15°C and 86 and 97% RH, eclosion and molting did not take place within 60 days (Fig. 1). Most instars which had been transferred to 28°C and 75% RH after 60 days at 15°C, molted within a few days. However, a few individuals died during molting, unable to shed the exuvia. Eclosion was not observed in eggs stored for 60 days at 15°C after transfer to 28°C and 75% RH. Eggs were not rose-coloured and transparent, as directly after oviposi-

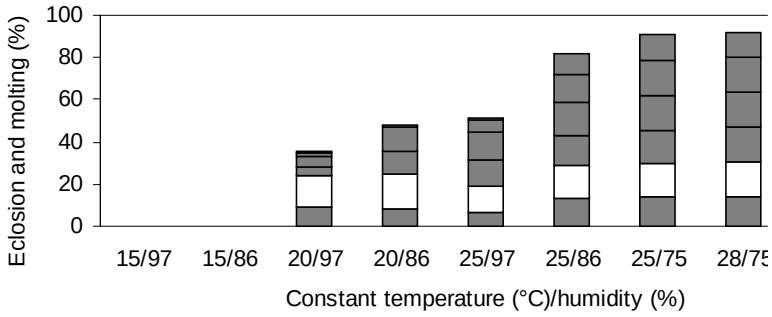


Fig. 1: cumulative eclosion and molting (%) of independent groups of *Rhodnius prolixus*, eggs and nymphs (N1-N5), after exposure at constant temperatures, 15, 20, 25, 28, 35°C, and humidities, 75, 86, 97%, for 60 days.

tion, but opaque, dark-coloured and most with a wrinkled shell.

At 35°C and 75% RH, eclosion was not observed. Only 0.6% of the first, 15.7% of the second and 0.3% of the forth instars had molted (Fig. 1). After 60 days of exposure there was almost complete mortality (Fig. 2). However, significantly more second instars survived than other instars ($F_{4,15} = 7.5$).

Between 20 and 28°C, eclosion and molting were frequent with the highest rates occuring at 25 and 28°C (Fig. 1). Fewer insects hatched and molted after 60 days at 20°C, compared to 25 and 28°C. At different humidities, significantly more insects hatched and molted at 25°C, when kept at 75 and 86% RH than at 97% RH. This was also observed with first, second and third instars that were kept at 20°C and 86 and 97% RH. Mortality of all instars was lowest at 25 and 28°C and 75% RH (Fig. 2). Mortality also increased with both humidity and instar number, showing highest rates at 97% RH in the fifth instar. At 20°C and 86 and 97% RH respectively, molting was significantly higher at 86% RH in second ($F_{4,15} = 20.8$) and third instars ($F_{4,15} = 62.6$) and more insects died after 60 days incubation at 97% RH compared to 86% RH.

Cumulating the average lengths of the embryonal and nymphal interphases within individual groups, *R. prolixus* developed most rapidly (86.7 days) at 28°C and 75% RH (Fig. 3). At 20°C and 86% or 97% RH, cumulated interphases were greater than 300 days. Most fifth instars had not molted within 60 days at 20°C (Fig. 3).

At 28°C and 75% RH insects did not refeed until molting except the fourth (0.8 out of 20 individuals) and fifth instars (2 out of 20 individuals) (Fig. 4). Refeeding increased between 25°C (2.2 out of 20 individuals) and 20°C (2.5 out of 20 individuals) at all the humidities tested. Refed individuals showed a delayed molting compared to individuals which did not refeed. Refeeding of nymphs increased at 15°C (5.3 out of 20 individuals) with a significantly higher rate at 86% RH (6.7 out of 20 individuals) than at 97% RH (3.8 out of 20 individuals). Refeeding was highest at 35°C with a rate of 16.9 out of 20 individuals (Fig. 4).

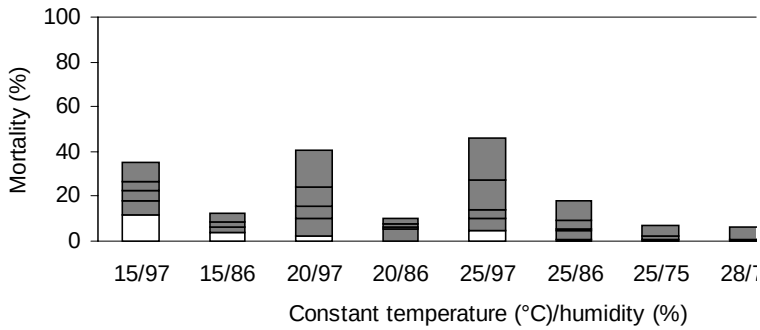


Fig. 2: cumulative mortality (%) of independent groups of *Rhodnius prolixus* nymphs (N1-N5), after exposure at constant temperatures, 15, 20, 25, 28, 35°C, and humidities, 75, 86, 97%, for 60 days.

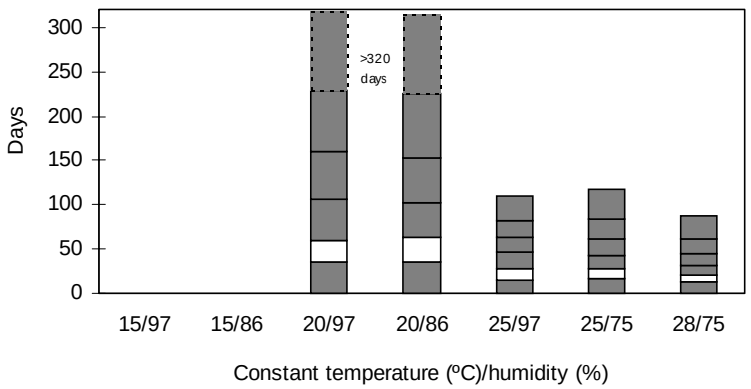


Fig. 3: cumulative average lengths of interphases (days) for independent groups of *Rhodnius prolixus*, eggs and nymphs (N1-N5), after exposure at constant temperature, 15, 20, 25, 28°C, and humidity, 75, 86 and 97%.

*Fluctuating temperatures and humidities* - Alternating T I, 15°C, with T II, 28°C, at 16/8 hr, high rates of eclosion and molting within all instars were observed (Fig. 5). However, molting of insects after 60 days was reduced at fluctuating 15/28°C when compared to constant 28°C and 75% RH, except for third instars which all molted under fluctuating conditions at RH I, 86%. At 15/28°C, more first, second and third instars molted to the next stage at RH I, 86%, compared to RH I, 97%. This was found to be inverted for eclosion of first instars and molting of fourth and fifth instars to the next stage. Mortality within first and fifth instars was higher than in other instars (Fig. 6).

At fluctuating T I/II, 25/35°C, higher rates of eclosion and molting were observed at both RH I, 97 and 86%, than at constant 25°C and RH I, 86 and 97%. Results of molting and mortality at fluctuating 25/35°C showed no significant difference between RH I, 97 and 86%. Cumulative mortality after 60 days increased with instar (Fig. 6).

Highest rates of eclosion and molting were observed at T I/II, 20/25°C and 25/28°C. Values were higher at fluctuating temperatures than at constant 20 and 25°C respectively at both RH I, 86 and 97%, and were not significantly different from the results found at constant 25 and 28°C respectively. No differences were observed between RH I, 97 and 86%, at these temperature regimes (Fig. 5).

Cumulated average lengths of the embryonal and nymphal interphases within independent groups gave results of 209 days at 15/28°C at RH I, 97%, and in 185.4 days at RH II, 86% (Fig. 7). At 20/25°C results were similar with 194.5 days at RH I, 97%, and 187.1 days at RH I, 86%. These figures were all different from results found at the constant temperatures of 20, 28 and 25°C. At T I/II, 25/28°C and 25/35°C, and RH I, 86%, the cumulated average lengths of the interphases were distinctly shorter and varied between 92.9 at 25/35°C and 100 days at 25/28°C.

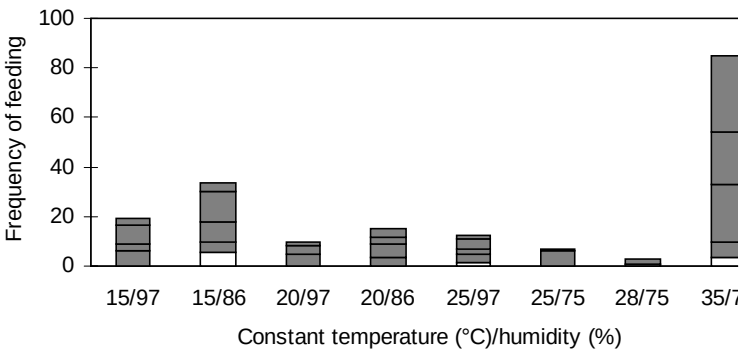


Fig. 4: cumulative average rates of refeeding for independent groups of *Rhodnius prolixus*, nymphs (N1-N5), exposed to constant temperature, 15, 20, 25, 28, 35°C, and humidity, 75, 86 and 97%, for 60 days or until molting after a first blood meal *ad repletionem*.

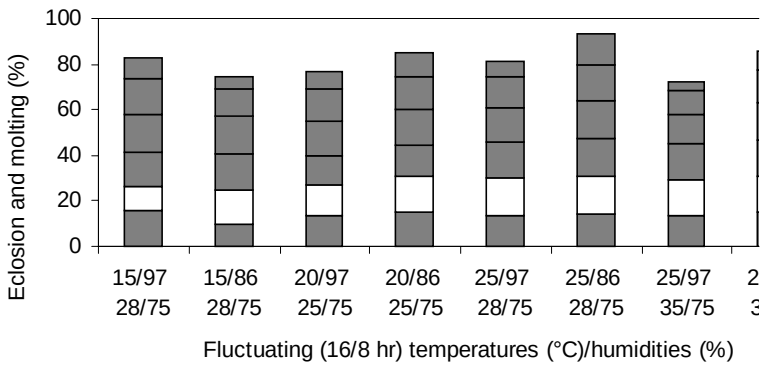


Fig. 5: cumulative eclosion and molting (%) of independent groups of *Rhodnius prolixus*, eggs and nymphs (N1-N5), after exposure at fluctuating temperatures (16/8 hr), T I/II 15/28°C, 20/25°C, 25/28°C, 25/35°C, and humidities RH I/II, 97/75% and 86/97%, for 60 days.

Refeeding was lowest at T I/II, 25/28°C and 20/25°C, with the exception of third instars at T I/II, 20/25°C and RH I, 97% (8 out of 20 individuals). At fluctuating 15/28°C and 25/35°C the mean rate of refeeding increased (3.5 and 2.5 out of 20 individuals respectively), and was significantly higher at RH I, 97% compared to RH I, 86% (Fig. 8).

## DISCUSSION

Development of *R. prolixus* was clearly shown to be dependent on temperature and humidity at both constant and fluctuating conditions. Optimal conditions of temperature and humdity for development were found at 28°C and 75% RH at constant conditions. Constant temperatures at a range

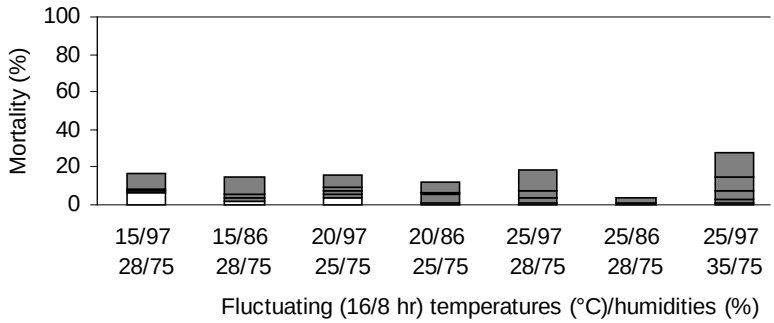


Fig. 6: cumulative mortality (%) of independent groups of *Rhodnius prolixus* nymphs (N1-N5), after exposure at fluctuating temperatures (16/8 hr), T I/II 15/28°C, 20/25°C, 25/28°C, 25/35°C, and humidities, RH I/II 97/75% and 86/75%, for 60 days.

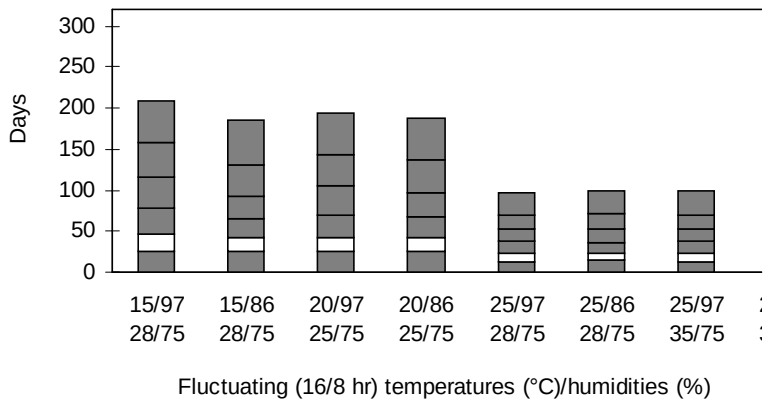


Fig. 7: cumulative average lengths of interphases (days) for independent groups of *Rhodnius prolixus*, eggs and nymphs (N1-N5) after exposure to fluctuating (16/8 hr) temperatures, T I/II 15/28°C, 20/25°C, 25/28°C, 25/35°C, and humidities, RH I/II 97/75% and 86/75%.

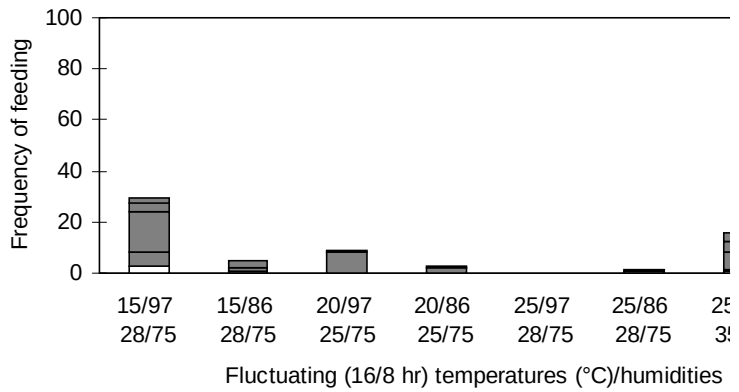


Fig. 8: cumulative average rates of refeeding for independent groups of *Rhodnius prolixus* nymphs (N1-N5) after the first blood meal *ad repletionem* at fluctuating (16/8 hr) temperatures, T I/II, 15/28°C, 20/25°C, 25/28°C, 25/35°C, and humidities, RH I/II, 97/75% and 86/75% until molting.

of 25 to 30°C and humidities between 50 to 80% were also reported by other authors to be favorable for development of *R. prolixus* (Ryckman 1952, Gomez-Nuñez 1964, Lent & Valderrama 1977).

First and fifth instars were more sensitive than other instars, independent of ambient conditions. This was also reported for *T. infestans* by Rabinovich (1972), *T. maculata* by Feliciangeli and Rabinovich (1985) and *Triatoma rubrovaria* by Arguello et al. (1988). Higher sensitivity of these instars may be due to stress of eclosion and molting to the adult stage.

At 15°C, insects were not able to hatch or molt at any stage, although progressive embryogenesis inside eggs was observed. Development within the instars had obviously been initiated, as they molted a few days after being transferred to higher temperatures. However, some individuals were unable to leave the old exuvia and died during molting. As in our studies, Galliard (1935) observed a cessation of development of *R. prolixus* nymphs when kept at 15°C. However, at 16-18°C, *R. prolixus* was shown to be able to molt, but again with high rates of mortality during ecdysis (Buxton 1930). Moreover, embryogenesis was detected at 12°C or higher, but eclosion of first instars only occurred at 16°C or higher (Clark 1935). Elevated refeeding of instars in our studies at 15°C indicated that *R. prolixus* nymphal instars continue, at this temperature, to be active vectors of Chagas disease. Nymphs are able to survive for a long time without molting, when hosts are available, but are also very resistent to starvation at 15°C as shown by Luz et al. (1998).

Extended interphases at 20°C, particularly in the later stages, elevated rates of mortality and distinct refeeding indicated that 20°C is a suboptimal temperature for the development of *R. prolixus*. High temperature of 35°C was shown to be detrimental for development of *R. prolixus*. This was also reported by Okasha (1964). However, Wigglesworth (1952) found that up to 90% of *R. prolixus* molted to the next instar when held at 34°C. This temperature was also shown to be the upper limit for eclosion of first instars of *R. prolixus* by Clark (1935). Oogenesis in *R. prolixus* females was blocked at this temperature (Okasha et al. 1970). In our study, high rates of refeeding within all instars at 35°C may be related to regulation of temperature by transpiration.

There is no information available about humidity preference of *R. prolixus*, which showed to be more susceptible to extremely high humidity, close to saturation, compared to lower humidities tested at 75 and 86%. *T. infestans*, another important triatomine vector, proved to have no preference for ambient humidity (Wiesinger 1956). *R. prolixus* can be found in very humid regions and humidity in the microenvironment of the habitat can easily reach saturation, as shown by Luz et al. (1994) in Colombia. However, humidity in the natural habitat was not constantly high, but varied with diurnal patterns (Luz et al. 1994). Variation of humidity and adaptation of insects to extremely high or low humidities may explain the higher resistance of insects under natural conditions. Extreme low humidity can interrupt insect development. Clark (1935) reported, that eclosion of *R. prolixus* was blocked at temperatures between 16 and 34°C, when humidity was 20% or less.

*R. prolixus* development was studied for the first time at controlled fluctuating temperatures and humidities. Our results showed that successful molting and lengths of interphases were clearly influenced by temperatures and their periods prevailing during the day and the night and emphasize the importance to investigate more the impact of dynamic temperatures and humidities on the development of vectors. Moreover, low and high temperatures, such as 15 and 35°C, which had been shown to block development of *R. prolixus* during constant exposure, did not impede development at fluctuating patterns of temperature and humidity tested.

Temperature during the night can fall below 15°C in regions with *R. prolixus*. Geographical distribution of the vector will depend not only on low temperatures during the night but also on their duration and on temperatures prevailing during the day. Gorla and Schofield (1989) showed that development of *T. infestans* in the field was influenced more by the average minimal temperatures than by the average temperature. Furthermore, Curto de Casas and Carcavallo (1984) demostrated that the number of days per year with at least 20°C, a temperature which permits normal biological activity of *T. infestans*, is more important for the distribution of this species than the minimal temperature. With more than 60 days of average temperature below 20°C, *T. infestans* will be able to complete only one generation per year (Curto de Casas 1990).

The damaging and irreversible effect of 35°C on the development of *R. prolixus* was demonstrated to be dependent on the length of exposure (Okasha 1970). Our results showed, that an exposure for 8 hr duration at 35°C alternating with 16 hr at 25°C was not harmful to the development of any of the instars. Temperatures of 35°C or even higher can be expected in the natural habitats of triatomine vectors. Insects are able to avoid high temperatures by moving to areas with lower temperatures. Moreover, extreme high temperature in

the habitats does not predominate for extended periods of time during the same day.

Optimal conditions for the development of *R. prolixus* were found at fluctuating T I/II, 25/28°C, 25/35°C and 20/25°C, and RH I/II, 86/75%. In the field, largest populations of *R. prolixus* can be expected at values of fluctuating temperatures between 25 and 35°C and elevated, but not constantly saturated, humidites. Other important limiting factors, such as availability of hosts and habitats have to be considered. However, *R. prolixus* can maintain unnoticed small populations in domestic or peridomestic areas at suboptimal conditions of temperature and humidity. Populations may serve as a reservoir for natural or man-made dissemination to controlled vector-free regions. These areas have to be identified and included in control programmes.

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