

## BIOLOGICAL CONTROL

### Temperature-Dependent Development of the Entomopathogenic Fungus *Nomuraea rileyi* (Farlow) Samson in *Anticarsia gemmatalis* (Hübner) Larvae (Lepidoptera: Noctuidae)

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Desenvolvimento Dependente da Temperatura do Fungo Entomopatogênico *Nomuraea rileyi* (Farlow) Samson em larvas de *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae)

**RESUMO** - A influência da temperatura no desenvolvimento do fungo entomopatogênico *Nomuraea rileyi* (Farlow) Samson foi estudada em larvas no terceiro estágio de desenvolvimento de *Anticarsia gemmatalis* (Hübner), mantidas sob regime de temperatura constante. Um modelo não-linear foi utilizado para descrever as taxas de crescimento vegetativo e reprodutivo do fungo. A temperatura requerida para obter o maior crescimento vegetativo foi estimada em 25,5°C, enquanto os limites mínimo e máximo foram estimados em 11°C e 30°C, respectivamente. A taxa de conidiação foi estável dos 18°C aos 26°C. As temperaturas mínima e máxima estimadas para o desenvolvimento vegetativo coincidem com as condições ambientais de ocorrência natural na Argentina Central. Efeitos não-lineares da temperatura no crescimento vegetativo e reprodutivo, bem como a probabilidade de distribuição *in vivo* de *N. rileyi* são descritos.

**PALAVRAS-CHAVE:** Controle biológico, modelo matemático, dinâmica populacional, inimigo natural

**ABSTRACT** - The influence of temperature on the development rate of the entomopathogenic fungus *Nomuraea rileyi* (Farlow) Samson in *Anticarsia gemmatalis* (Hübner) 3<sup>rd</sup>-instar larvae was studied under constant temperature regimes. A non linear model was used to describe temperature-dependent vegetative and reproductive development rates of the fungus. The temperature required to reach the highest vegetative development rate was estimated to be 25.5°C while lower and upper developmental thresholds were 11°C and 30°C, respectively. The rate of development for conidia release was stable from 18°C to 26°C. The estimated lower and upper thresholds of vegetative development coincide with conditions during the natural epizootics in central Argentina. Non-linear effects of temperature on vegetative and reproductive development rates and probability distributions of *N. rileyi* under *in vivo* conditions are described.

**KEY WORDS:** Biological control, mathematical model, population dynamics, natural enemy

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The mitosporic fungus *Nomuraea rileyi* (Farlow) Samson, represents an important natural control agent of *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), one of the most damaging soybean pests in the American continent (Ignoffo 1981, Pedigo *et al.* 1983, Gazzoni *et al.* 1994, Edelstein 2002). Descriptions of *N. rileyi* epizootics on *A. gemmatalis* larvae populations were carried out by a single empirical equation, based on multiple predictive

variables (Kish & Allen 1978), and a posterior update on a mechanistic platform (Sujii *et al.* 2002).

The importance of temperature on the outcome of entomopathogens infection was stressed by Thomas & Blanford (2003) and its effect on *N. rileyi* development was studied *in vitro* (Fargues *et al.* 1992) and *in vivo* (Getzin 1961, Gardner 1985, Tang and Hou 2001). However, none of the models mentioned above dealt with temperature as a variable driving

the speed of vegetative development and sporulation. As with any poikilothermic organism, temperature represents the most important factor governing the speed of development. This is particularly relevant in temperate climate regions where temperature can have considerable daily fluctuations in one season. The velocity of development of the fungus vegetative and reproductive stages within the larval body determines how fast the host stops feeding and becomes infective, respectively. The infectious incubation period is of central importance for epizootics. Indeed, a short or long incubation period could represent the difference between secondary infections occurring or not in the host population. Thus, temperature, as a physical factor regulating developmental rate of the entomopathogen, has epizootiological consequences. In order to make reliable predictions of *N. rileyi* epizootics in the field, epizootiology models would include temperature. Therefore, the aim of this study was to describe *in vivo* temperature-dependent vegetative and reproductive development rates, median and probability distribution, of *N. rileyi* infecting *A. gemmatalis* larvae. Sporulation process may need other interacting variables such as humidity. However, for the sake of simplicity in understanding the process and performing the bioassays and models, only temperature-dependent development of this stage was studied.

### Material and Methods

Third-instar larvae of *A. gemmatalis* and the *N. rileyi* isolate (Nr32) were obtained from the collection at the Laboratorio de Hongos Entomopatógenos (IMYZA, INTA Castelar). The fungal isolate was originated from the Manfredi (Córdoba, Argentina) and isolated from an *A. gemmatalis* larva, with a maximum of two subsequent *in vitro* passages. Culture media contained potato, maltose, yeast and agar (Edelstein et al. 2004).

Ten groups of five 3<sup>rd</sup>-instar larvae, were randomly assigned and dipped in 5 ml 0.01% Tween 80 aqueous suspension with 10<sup>8</sup> conidia/ml and a control suspension. Groups of 50 larvae were placed in individual plastic cages, 3 cm wide, with voile cloth caps, and kept in climatic chambers at constant temperatures of 16, 18, 20, 22, 24, 26, 28, 30 and 34°C (± 0.5°C) for each bioassay. The larvae were daily fed *ad libitum* with a meridic diet. Mortality in each of the treatments was daily recorded, throughout periods of a maximum of 30 days. Survival analysis was performed by Kaplan-Meier statistic and logrank -  $\chi^2$  test (Altmann 1991). Untreated controls were performed to test the survival of the host in response to each thermal condition and the absence of previous infection in the experimental larvae.

It was assumed that vegetative development was complete at the time of death of the host larvae. The mycosed dead larvae (white cadavers) were immediately placed in individual 5 cm plastic petri dishes, with a small piece of soaking cotton and observed through a maximum of 10 days. The time from death to conidia release was recorded at 16, 18, 20, 22, 26 and 28 °C (± 0.5°C). The median of the rates (time<sup>-1</sup>) were calculated for each temperature treatment and the relationship between the developmental rate (in days<sup>-1</sup>) and temperature was described by fitting the following model (Briere et al. 1999):

$$R(T) = a \cdot T (T - T_0) \cdot \sqrt{T_L - T} \quad (1)$$

where R(T) is the developmental rate for a given temperature T, a is a fitting empirical parameter, T<sub>0</sub> is the lower temperature developmental threshold and T<sub>L</sub> is the upper lethal temperature and R(T) = 0 when T is less or equal to T<sub>0</sub> or T is higher or equal to T<sub>L</sub>. The statistical difference from zero of the estimated parameters was evaluated by a t-test.

A non-predetermined function of temperature was fitted to the median reproductive development rates. Least square errors, degrees-of-freedom adjusted coefficient of determination (DOF adj R<sup>2</sup>) and algebraic simplicity were used as fitting selection criteria from a collection of models (Table Curve 2D v2.02, Jandel Scientific - AISN Software, 1994).

Since vegetative development rates at each unit of time, under determined thermal conditions, are fractions of a total physiological time and t<sub>i</sub> are the standardized (by the median at each temperature) developmental rates, its accumulation through time is an estimation of the physiological age t. The absolute frequencies with respect to t<sub>i</sub> were used as a weighting variables in non-linear regression analysis (Curry and Feldman 1987). In order to represent the variability of vegetative and reproductive development rates per cohort, the cumulative relative frequencies of physiological ages (t) for each process was fitted to the following sigmoidal model (Eq. 2):

$$Y = 1 / \left( 1 + \exp \left( - \frac{(\tau_i - a)}{b} \right) \right) \quad (2)$$

where a and b are fitting parameters.

### Results

Survival of infected larvae at the end of the experiments was significantly lower than the mean of the control in all temperatures (P < 0.001;  $\chi^2 = 112.48$ ). However, at 30°C and 34°C a high mortality of uninfected *A. gemmatalis* larvae occurred (Fig. 1) and, consequently, no mycoses were observed. The mean survival of the mycosed larvae was 24% of the total population under all the thermal conditions (Fig. 2). In the range of temperatures between 18°C and 28°C, 50% of the cohort died after 5 to 10 days of infection. The highest mortalities (66 to 90%) were observed between 10 to 16 days. Distinctively, at 16°C, the survival of the infected larvae decreased slowly through time reaching a 41% survival at 26 days and 36% at 29 days after infection.

The individual vegetative development rates ranged from 0.03 to 0.20 days<sup>-1</sup>, averaging 0.12 days<sup>-1</sup> with medians per temperature ranging from 0.05 to 0.17 days<sup>-1</sup>. According to the fitted model temperature accounted for 89% (F-ratio<sub>(2,4)</sub> = 15.884; P < 0.05) of the median vegetative developmental rates variance (Fig. 3). The expected optimum temperature - 25.5°C - required for the highest vegetative development rate, 0.16 days<sup>-1</sup>, was estimated by finding the zero first derivative of the fitted model. The lower and upper temperature thresholds for vegetative development were estimated to be at 10.9 ± 2.11°C and 30.1 ± 0.98°C, respectively. Equation 2 provided a good description of the cumulative frequency distribution of death (a = 0.94 ± 0.003; b = 0.08 ± 0.003; R<sup>2</sup>

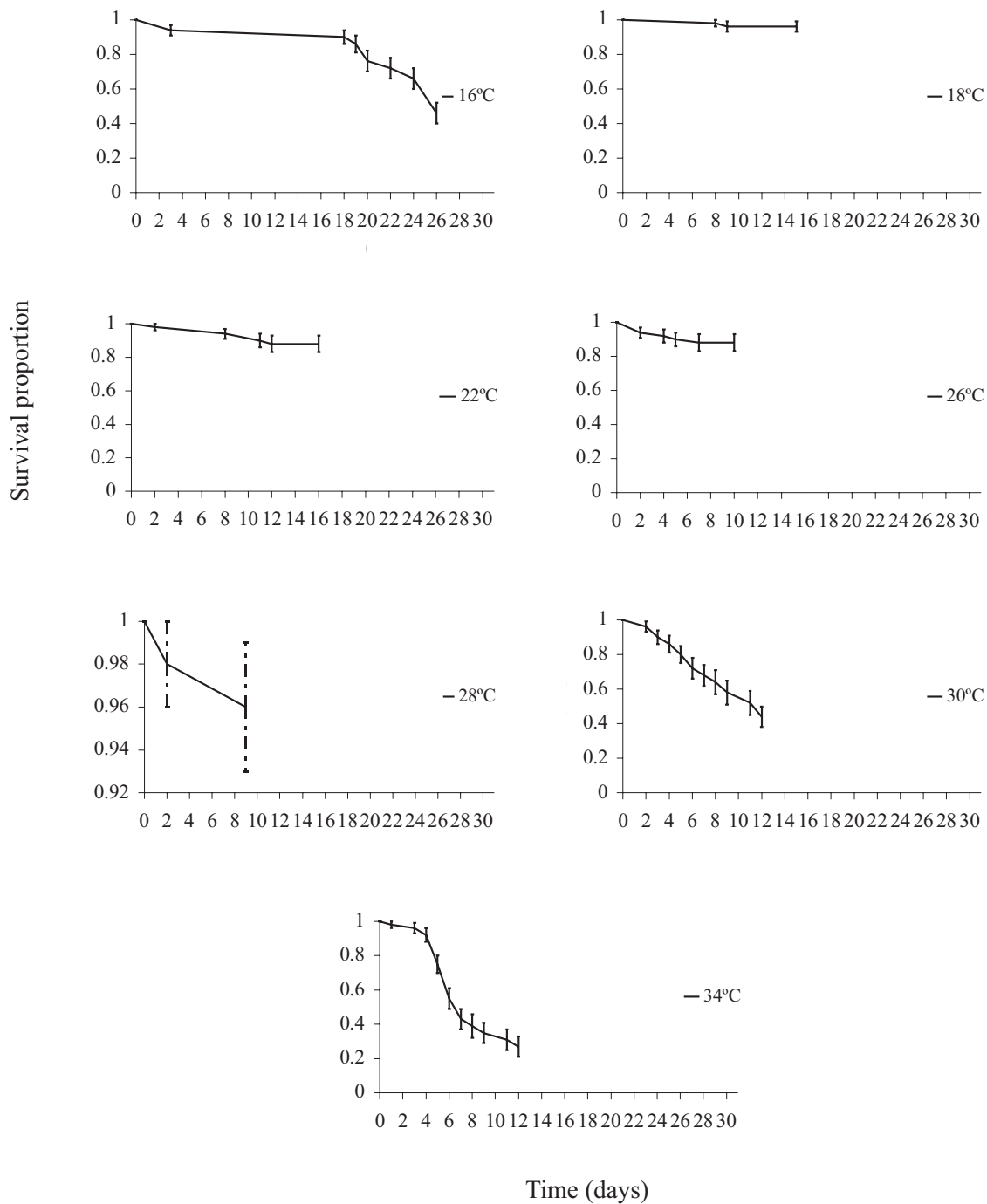


Figure 1. Survival of *A. gemmatalis* uninfected 3<sup>rd</sup>-instar larvae at several constant temperatures (°C) (Kaplan-Meier estimator ± standard error).

= 0.99, F-ratio<sub>(1,18)</sub> = 2423; P < 0.001) (Fig. 4).

The effect of temperature on the variation of reproductive development rates was explained by the following function:

$$R_{Esp} = a \cdot \exp\left(-0.5 \cdot \left(\frac{|T - b|}{c}\right)^d\right) \quad (3)$$

where *T* represented temperature and *a* (0.32 ± 0.012), *b* (22.72 ± 24.44), *c* (5.14 ± 11.899) and *d* (19.31 ± 1717.92) were fitting constants (Fig. 3). No conidia release was recorded at 16°C but, above this limiting condition, this phase required from 3.19 ± 0.19 days at 22°C to 6.4 ± 0.87 days at 28°C. Extrapolating from Eq. 3, the upper threshold temperature for reproductive development is approximately 30°C. The cumulative frequency distribution of conidia

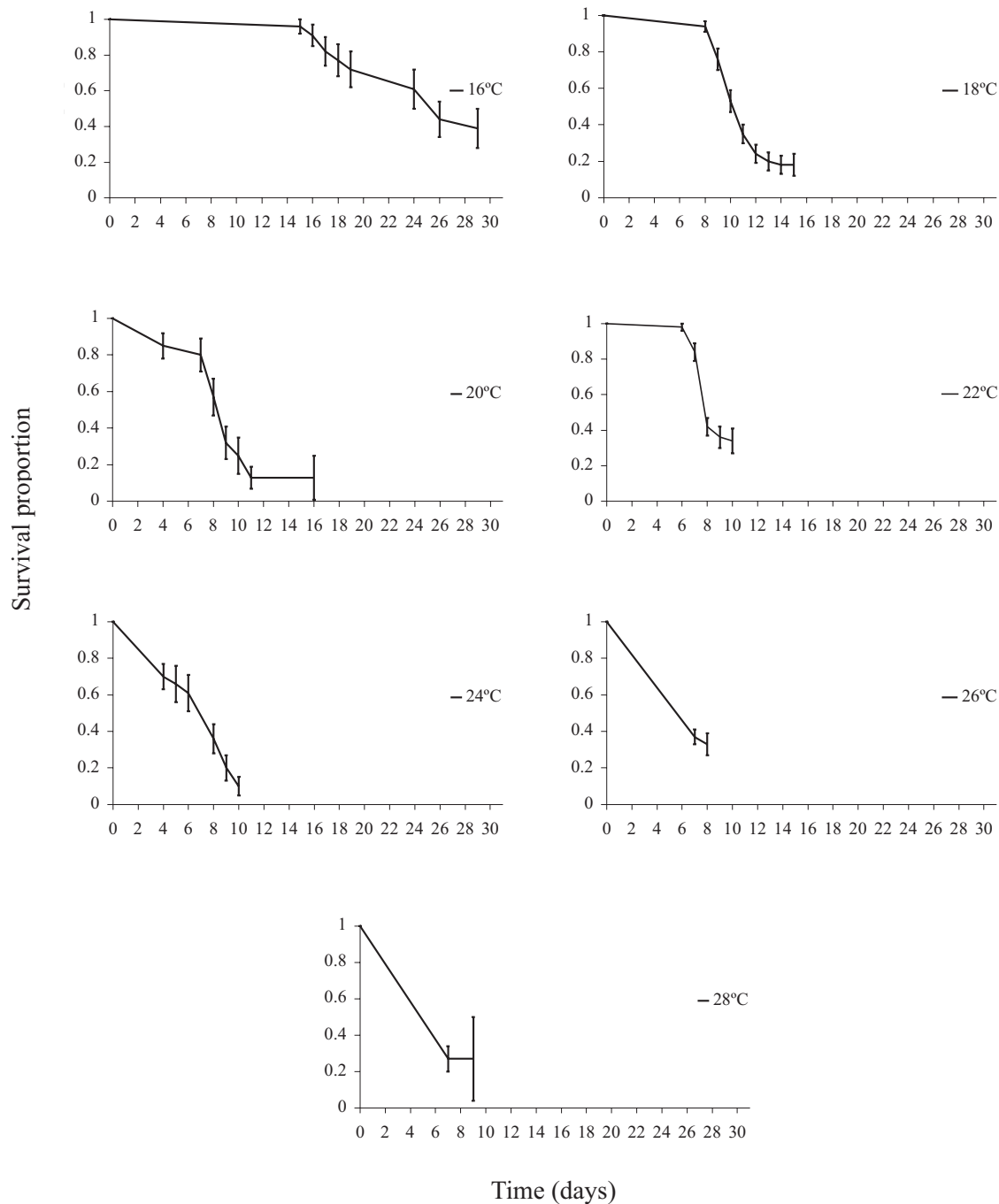


Figure 2. Survival of *A. gemmatalis* 3<sup>rd</sup>-instar larvae infected by *N. rileyi* at several constant temperatures (°C) (Kaplan-Meier estimator  $\pm$  standard error).

release was significantly represented by Eq. 2 ( $a = 0.89 \pm 0.007$ ,  $b = 0.17 \pm 0.008$ ;  $R^2 = 0.99$ , F-ratio<sub>(1,18)</sub> = 1793.71;  $P < 0.001$ ) (Fig. 4).

### Discussion

Because moisture was not a limiting factor during the observations, our study helps to understand only the thermal

effect on sporulation. However, the sporulation process is clearly also dependent on humidity (Sujii *et al.* 2002). Thus, future efforts should be focused on testing possible interactions of both temperature and moisture effects. Besides, initial inoculum burden in the field may affect the infection probability making the system less predictable.

Secondary transmissions of entomopathogenic fungi are determined both by the infection processes and conidia

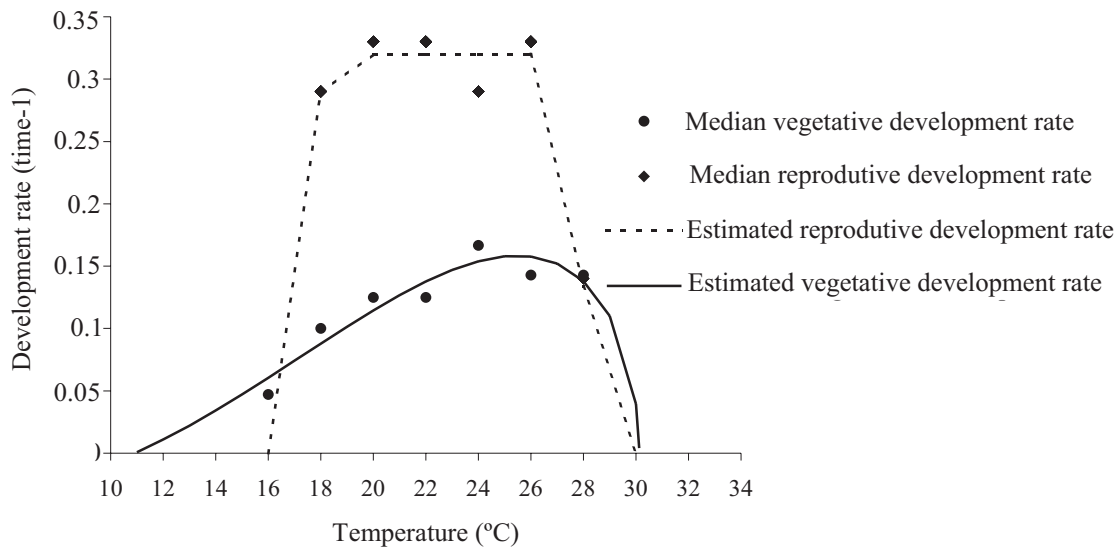


Figure 3. Observed and estimated *in vivo* median development rate of the (a) vegetative and (b) reproductive stage of *N. rileyi* mycosis in 3<sup>rd</sup>-instar *A. gemmatalis* larvae.

release. In the present work it was shown that, although the mycosis incubation, as a fraction of the first process, is strongly affected by temperature, the release of infective bodies is only slightly influenced by thermal conditions. This suggests that a higher number of deaths will happen during temperate days and incubation period is more critical than the sporulation one, at least from the thermal point of view. In contrast, relatively high daily mean temperatures can accelerate development of *A. gemmatalis* larvae. For example, at 28°C this pest develops approximately 10% faster than at 26°C (Johnson *et al.* 1983), while our results show that at the same temperature, increase reproductive development of *N. rileyi* would occur at around half its

optimum rate. In such case, the pathogen is likely to release infective bodies when most of the hosts have already moulted into less susceptible stages. Consequently, it seems that mild temperatures would facilitate secondary transmissions.

The developmental rate of the fungus at relatively high temperature conditions can be estimated from Eq. 3; the vegetative development rate at 32.4°C was estimated as 0.034 day<sup>-1</sup>. However, at this temperature, host death caused by other factors than *N. rileyi* infection was very high, so the fungus development rates were difficult to be recorded. On the basis of previous data (Getzin 1961), personal observations (Edelstein *et al.* 2004) and the present study, the fact that *in vivo* development rates follow a proportional magnitude of

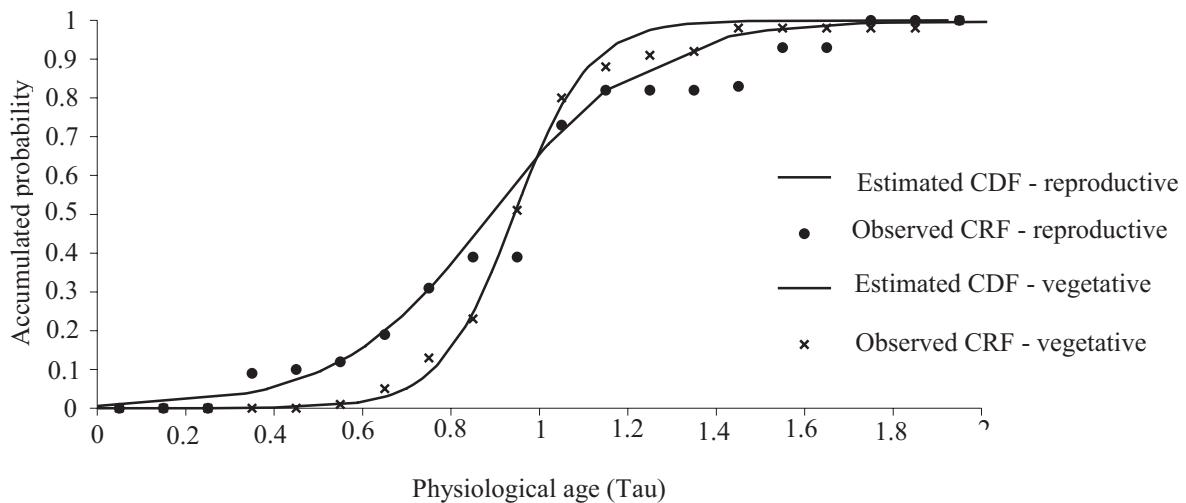


Figure 4. Observed cumulative relative frequencies (ARF) and estimated cumulative distribution functions (CDF) of vegetative and reproductive developmental processes.

approximately one tenth of *in vitro* development rates can be assumed. If *in vitro* median radial growth rate of *N. rileyi* colonies from the same origin was  $0.33 \text{ mm}\cdot\text{day}^{-1}$  at  $32^\circ\text{C}$  ( $\pm 0.5^\circ\text{C}$ ) (R.E. Lecuona, pers. observ.), then, under *in vivo* conditions the development rate is expected to be  $0.033 \text{ day}^{-1}$ . Temperature lethal effects depend on time of exposure to the extreme thermal condition. Therefore, the effects of daily variations of temperature on development are relative to the amount of hours or days at this condition.

The sporulation process *in vivo* was shown to be as thermal sensitive as *in vitro* to low temperatures. The reproductive development rate was quite constant ( $0.28$  to  $0.33 \text{ day}^{-1}$ ) over the  $18^\circ\text{C}$  to  $26^\circ\text{C}$  range. These temperatures are quite common in temperate regions, like central Argentina where  $17.1^\circ\text{C}$  to  $22.1^\circ\text{C}$  is the range of mean temperatures usually recorded during the February-April period (INTA 2003). During these months *N. rileyi* epizootics happen on *A. gemmatalis* larvae populations at these latitudes. According to the present study, reproductive development should not be affected significantly by temperature, with the exception of extreme thermal conditions. Indeed, at  $28^\circ\text{C}$  reproductive development rate drops to  $0.13 \text{ day}^{-1}$ ; thus, conidia release is expected to last around three to four days, but during a period of above average temperature, the vegetative to reproductive development period could be extended to seven or eight days.

Vegetative and reproductive *N. rileyi* development have similar upper threshold temperatures (ca.  $30^\circ\text{C}$ ), which is slightly higher than Manfredi (Córdoba, Argentina) historical average maximum temperature ( $28.9^\circ\text{C}$ ) in February, the warmest month of the epizootic season (INTA unpubl.). Maximum temperatures would constitute a selection factor for the poikilothermal organisms, such as entomopathogenic fungi. It is possible then that the coincidence in these upper developmental thresholds is a selected trait. This trait allows for developing, killing the host and infecting other individuals throughout the season. According to the present results, minimum temperature is also a limiting condition for the fungus reproductive development and consequently for secondary infections, particularly during March (mean minimum temperature,  $14.49^\circ\text{C}$ ) and April (mean minimum temperature,  $11.04^\circ\text{C}$ ; historical records 1960-2002, INTA unpubl.). At this time, the soybean crop season is finishing and *A. gemmatalis* larvae are about to pupate. Therefore, with few or no hosts available for the fungus and limiting temperature conditions, vegetative cadavers would just persist in the field probably as resistant entities for the next year (Madelin 1963).

Our description of the influence of temperature on *N. rileyi* development rate in infected *A. gemmatalis* larvae improve the predictive capacity of models designed to describe the insect-entomopathogen dynamics in relation to previous models like those by Kish and Allen (1978), Boucias et al. (1984) and Sujii et al. (2002). The assumption of linearity for temperature-dependent development is acceptable within a narrow range of temperatures. In the present study non-linear temperature-dependent development equations are proposed to be applied on epizootiology models similar to the described by Sujii et al. (2002). Consequently,

they will be likely to yield better descriptions of temporal insect disease dynamics. An argument can be done about a more realist approach on fluctuating environmental temperatures or contemplation of microclimatic conditions. However, very realistic models would confront to simplicity in the experimental design to be tested. Moreover, little differences on thermal requirements could be obtained with a variable temperature method (i.e. re-analyzed from Hagstrum & Milliken 1991). Further compilation of the resulting equations from the present study in a general mechanistic model and its validation on the basis of temporal dynamics of the epizootics under field conditions should be done.

It has been argued that the estimation of temperature-development relationships of natural enemies can substantially contribute to the selection of the most appropriate biocontrol agent to be used under different environmental conditions (Perdikis & Lykouressis 2002). To the best of our knowledge, this is the first study on *N. rileyi* in which non-linear effect of temperature on vegetative and reproductive development rates and probability distribution under *in vivo* conditions are described.

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