BIOLOGICAL CONTROL

Temperature and Relative Humidity Requirements for Conidiogenesis of *Beauveria bassiana* (Deuteromycetes: Moniliaceae)

DANIEL R. SOSA-GÓMEZ[1] AND SÉRGIO B. ALVES[2]

[1]Embrapa Soja, Caixa postal, 231, 86001-970, Londrina, Paraná, Brazil.
[2]Departamento de Entomologia, ESALQ/USP, Caixa postal, 9, 13400-970, Piracicaba, São Paulo, Brazil.

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Requerimentos de Temperatura e Umidade Relativa para a Conidiogênese de *Beauveria bassiana* (Deuteromiceto: Moniliaceae)

RESUMO - A fase reprodutiva dos fungos entomopatogênicos é dependente da quantidade de água em torno do local onde se encontram. Neste trabalho realizaram-se ensaios para quantificar a formação de conídios de *Beauveria bassiana* (Bals.) Vuill. sobre cadáveres de *Diatraea saccharalis* F. (Lepidoptera: Pyralidae), *Nezara viridula* (L.) e *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) mantidos em diferentes níveis de umidade relativa (UR) (75%, 80%, 85%, 90% e 100%) associadas com temperaturas de 22°C, 26°C, 30°C e 34°C durante cinco dias. Os isolados formaram conídios entre 75% e 100% de UR. A conidiogênese foi incipiente a 75% de UR sobre larvas de *D. saccharalis*, mas não se manifestou sobre os percevejos *N. viridula* e *P. guildinii*. Foram determinadas as equações que explicam a conidiogênese dos isolados ARSEF 933 e ARSEF 2515 em um período de dez dias, em condições ideais de umidade. O número de conídios formados foi função da UR, temperatura, isolado de fungo, espécie hospedeira, fase do hospedeiro e tempo.

PALAVRAS-CHAVE: Insecta, *Nezara viridula, Diatraea saccharalis, Piezodorus guildinii*, inóculo, fungos entomopatogênicos.

ABSTRACT - Assays were conducted to assess the number of *Beauveria bassiana* (Bals.) Vuill. conidia on *Diatraea saccharalis* F. (Lepidoptera: Pyralidae), *Nezara viridula* (L.) and *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae) corpses maintained at different levels of relative humidity (RH) (75%, 80%, 85%, 90% and 100%) and temperatures (22°C, 26°C, 30°C and 34°C) during five days. The isolates produced conidia when exposed to RH from 75% to 100%. Conidiogenesis was incipient at 75% RH on *D. saccharalis* larvae, but did not occur on *N. viridula* and *P. guildinii*. In ideal conditions of RH and during 10 days, mathematical equations were developed to estimate the number of conidia produced by isolates ARSEF 933 and ARSEF 2515. Conidia number were shown to be dependant on RH, temperature, fungal isolate, host species, host stage, and time.

KEY WORDS: Insecta, *Nezara viridula, Diatraea saccharalis, Piezodorus guildinii*, inoculum, entomopathogenic fungi.

Considering the sequence of events in the biology of an entomopathogenic deuteromycete fungi, the following phases can be emphasized in successful colonization: 1) conidiogenesis; 2) conidial release; 3) dissemination; 4) attachment on the host tegument; 5) induction of germination; 6) differentiation of germinative tube; 7) appressorial formation or not; 8) penetration; 9) inner growth; and 10) extrusion and new conidiogenesis. During conidiogenesis, the relative humidity of the external environment has a decisive influence on the process. High prevalence of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. (Deuteromycetes: Moniliaceae) on populations of several species of coleoptera [*Aracanthus* sp. (Coleoptera: Curculionidae), *Cerotoma arcuata* (Olivier), *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae)] and Plusiinae [*Diachrysia orichalcea* Fabr., *Chrysodeixis acuta* Wlk., *Plusia signata* Fabr. (Lepidoptera: Noctuidae)] has been observed (Sosa-Gomez & Moscardi 1994, Sharma 1995). The initiation and duration of these epizootic is due to the virulence of the fungus for such species and the amount of conidia produced. Few studies have concentrated on conidia quantification of deuteromycete fungi on cadavers at different temperatures and humidities (Ramoska 1984, Fernandes *et al*. 1989). The interactions between number of conidia produced and different hosts have not been reported. Determination of temperature and relative humidity (RH) requirements would allow the definition of places and periods with a higher probability of epizootic occurrence. These data would also provide an input to the development of epizootiological models. The objective of the present work was to study the influence of environmental conditions on conidial production of *B. bassiana* on different hosts.

Material and Methods

The isolates of *B. bassiana* used were ARSEF 2515, obtained from *D. speciosa* during a period of high prevalence of the disease at Tucumán, Argentina, and ARSEF 933, isolated from *Tibraca limbativentris* Stal (Heteroptera: Pentatomidae) at Goiânia, Goiás state, Brazil. *Diatraea saccharalis* F. (Lepidoptera: Pyralidae) larvae of the same size were used in the assays. The stink bugs *Nezara viridula* (L.) (Heteroptera: Pentatomidae) and *Piezodorus guildinii* (Westwood) (Heteroptera: Pentatomidae) were obtained from field collections. Sulfuric acid solutions or saturated solutions of salts were used to regulate the RH (Winston & Bates 1960, Teixeira Alves 1986). In all cases, determination of the number of conidia per insect was made using a hemocytometer. The conidia of *B. bassiana* produced on the insect cadavers were removed by scraping the insect, submersed in a 0.01% Tween solution, with a rough short hair paintbrush, and counted.

Conidiogenesis Patterns Through Time. Conidia production on cadavers of *D. saccharalis* was studied at 26°C and 100% RH. Conidia collected from potato dextrose agar and yeast extract plates were suspended in a 0.01% Tween 80 solution and the conidial concentration was adjusted to 3×10^7 conidia per ml. Larvae were dipped into suspensions of both strains for 3 seconds and on the same day of death, eighty larvae (20 ± 1mm of length) per strain, without fungal extrusions, were selected and placed in moist chambers at 100% RH. To assess the number of conidia per cadaver, four individuals (four replicates) were sampled at intervals of 24 hours until the 10th day. The equations were calculated and data were analyzed using the Statistical

Analysis System (SAS Institute, 1985).

Conidiogenesis on Sugar Cane Borer at Different Levels of RH and Temperature. To obtain the desired number (64 specimens) on the same day, 250 *D. saccharalis* larvae were inoculated as previously mentioned. Four days after inoculation, groups of four dead individuals with signs of fungal infection (pink and mummified) were transferred to each combination of temperature and RH. The insects were kept in hermetic chambers under different temperature and RH regimes (22°C, 26°C, 30°C and 34°C and 70%, 75% and 90%). Each individual was considered as one replicate. The evaluations of conidial number were made after five days.

Conidiogenesis on Stink Bugs. In this assay, five nymphs and six adult specimens of *P. guildinii* and 20 of *N. viridula* were used to determine the number of conidia produced at each combination of temperature and RH. The inoculation was performed by powdering dry conidia on stink bugs. Data were analyzed by ANOVA and means compared using the Tukey test.

Results and Discussion

Mycelial growth became obvious on the larvae 24h after death. It is possible that some conidia found 24h after death were those which adhered at the time of inoculation. Rapid increase of spore production occurred between the third and seventh day. Maximum spore production occurred after the seventh day. Conidiogenesis of ARSEF 2515 and ARSEF 933 at 100% RH was described by logistic equations (Fig. 1). The number of conidia of *B. bassiana* produced on *D. saccharalis* was significantly different between isolates at 100% RH, with ARSEF 2515 producing more conidia than ARSEF 933 (t test, $P \leq 0.01$).

Sporulation on *D. saccharalis* was directly proportional to RH, and the lowest limit was 75%. At this RH both isolates produced conidia sparsely when the temperature was 22°C or 26°C, and no conidia were produced at 30°C or 34°C (Fig. 2). At 85% RH conidiogenesis occurred at 22°C, 26°C and 30°C with both isolates, but the conidia number was also low. At 90% RH the optimal temperature for ARSEF 2515 was 26°C, followed by 22°C and 30°C, although at the latter temperature conidia formation was strongly reduced. Fernandes *et al*. (1989), studying conidiogenesis of *B. bassiana* on *Cerotoma arcuata* Oliv. (Coleoptera: Chrysomelidae), observed absence of conidia at 30°C and 89% RH. ARSEF 933 and ARSEF 2515 showed a similar trend at 100%RH, conidia production increased at 26°C and dropped at 22°C being lower at 30°C. At 34°C no conidia formation was observed at any RH on *D. saccharalis* and stink bugs (Fig. 2, Table 1).

The percentage of stink bugs showing sporulation of *B. bassiana* at 90% RH, and the number of conidia produced are presented in Table 1. Conidiogenesis was not observed on stink bugs at 75% RH or lower (data not shown). These results are different from those obtained by Ramoska (1984), who found sporulation on the chinch bug, *Blissus leucopterus* (Say), at 75% RH. Higher sporulation was observed on 5th instar nymphs than on adults of *P. guildinii* (Table 1). *P. guildinii* was the most favorable host for *B. bassiana*, and has previously been shown to demonstrate more susceptibility to *B. bassiana* and *Metarhizium anisopliae* (Metsch.) Sorok. than *N. viridula* (Sosa-Gómez & Moscardi 1998). The percentages of adult insects that showed sporulation were higher at 22°C and 26°C, when compared to the other temperature regimes (Table 1). In general, 4th and 5th instar nymphs of *N. viridula* suffered rapid dehydration and contaminants developed profusely (data not shown). Thus, the conidia production appeared to depend also on host attributes, such as host water content and non-sclerotizated areas of the tegument that allow fungal extrusion, since conidiogenesis of ARSEF 933 occurred on *D. saccharalis* at 75% RH and did not on *P. guildinii*. The best combination for conidiogenesis were 22°C

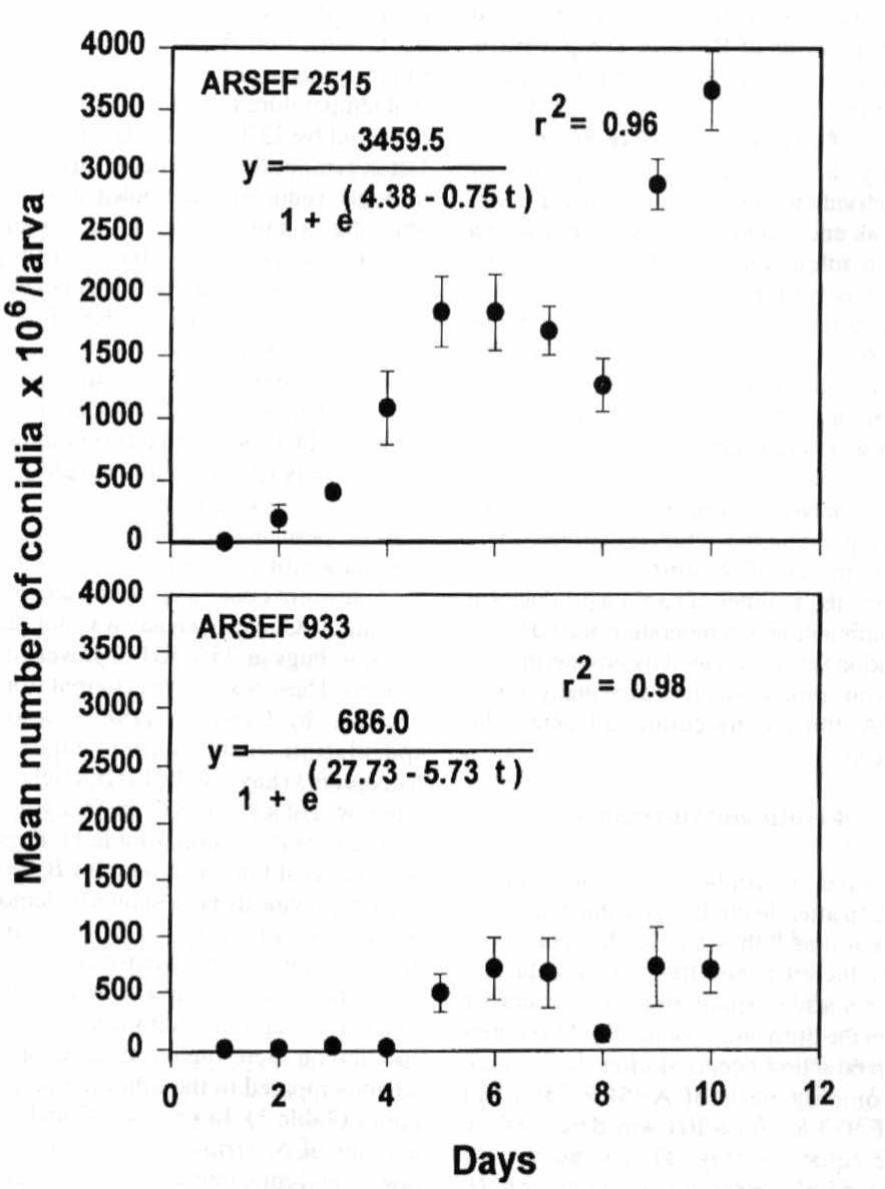

Fig.1. Mean number of conidia of *B. bassiana* (isolates ARSEF 933 and 2515) over ten days on *D. saccharalis* (larvae of 20±1mm of length). Error bars represent standard error of the mean. Temperature: 26±1°C. Relative Humidity 100 %. Where y = theoretical values of cumulative number of conidia formed on larvae of *D. saccharalis* with 20 ± 1mm of length and t = number of days after death.

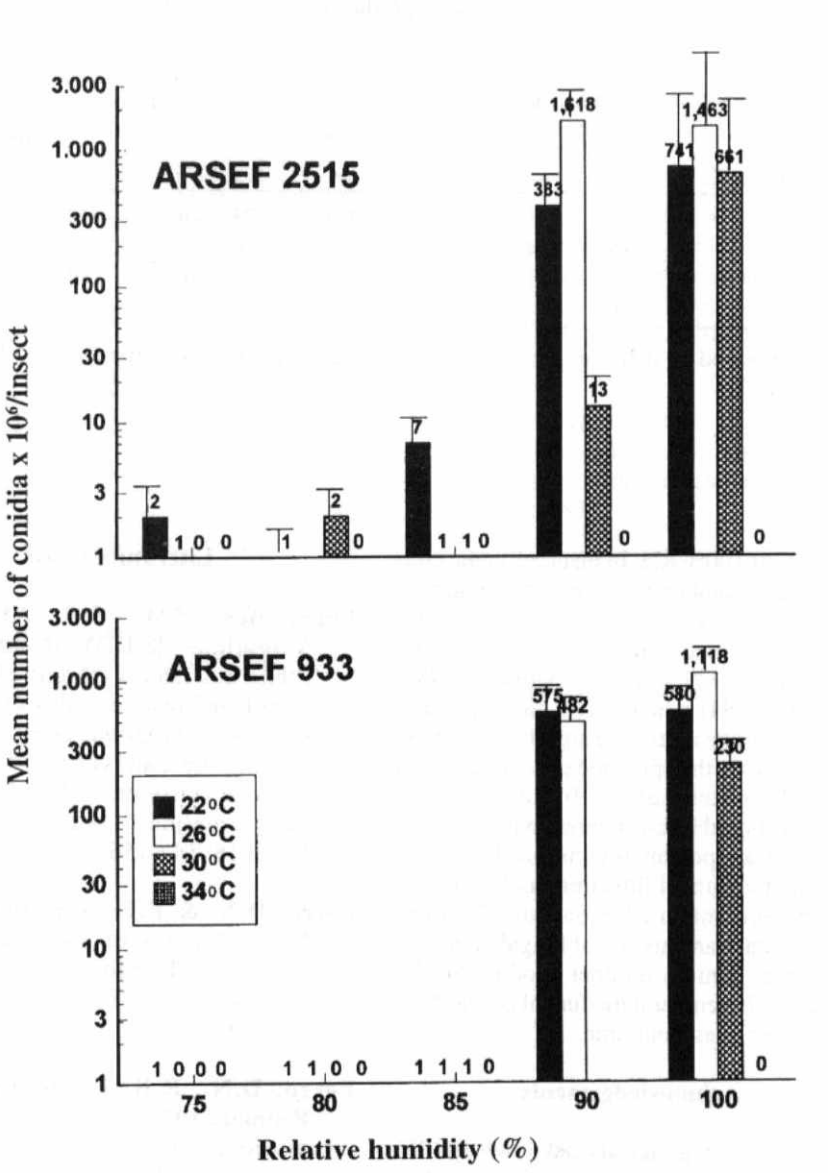

Fig.2. Mean number of conidia of *B. bassiana* (isolates ARSEF 933 and 2515) on *D. saccharalis* (larvae of 20 ± 1mm of length) at different combinations of temperature and relative humidity. Error bars represent standard error of the mean.

Table 1. Percentage of stink bug cadavers showing sporulation of *B. bassiana* (isolate ARSEF 933) and mean number of conidia produced at 90% RH, under different temperature regimes.

	N.viridula		*P. guildinii*			
	4th instar		5th instar		Adults	
Temperature °C	%	n x 10^6	%	n x 10^6	%	n x 10^6
22	90	2.5 b	100	73.6 ab	100	43.2 a
26	85	4.6 a	100	89.1 a	62	16.6 b
30	55	2.9 b	100	26.2 b	50	4.4 b
34	0	-	0	-	0	-

Means followed by different letters are not significantly different at the P ≥ 0.05 level by the Tukey test.

and 26°C at 100% RH. In macroclimatic conditions, this combination is not often reached, although in microclimatic environments, humidity close to saturation is commonly found (Ferro *et al*. 1979, Ferro & Southwick 1984, Ramoska 1984) which might favor production of infective units. The significance of the microclime on the epizootic process has been shown (Sprenkel *et al*. 1979), but another aspect that should be considered in this process is the host component. Insects that die by septicemia after fungal infection, as *N. viridula* nymphs, present an additional disadvantage to horizontal transmission of fungal infection. Therefore, conidia number production depends on RH, temperature, fungal isolate, host insect, host phase and time.

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