SOIL TEMPERATURE AND DIAPAUSE MAINTENANCE IN EGGS OF THE SPITTLEBUG, *Deois flavopicta* (HEMIPTERA: CERCOPIDAE)

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ABSTRACT

Diapausing eggs of the neotropical pasture pest, *Deois flavopicta* (Stal) (Hemiptera: Cercopidae), were exposed to low overnight temperatures that simulated field conditions during the dry season (23/12, 23/15 and 23/18ºC day/night), for different periods (0-60 days). After treatment, eggs were kept at 28ºC and contact water (100% humidity) until hatching. A group of diapausing eggs were kept all the time under this last condition as a control treatment. Time for hatching (in degree-days) was reduced with decrease in low overnight temperature and increase of exposure time to these cold shocks, although there was no interaction between the factors. Regression of exposure time to cold shock influencing the expected mean hatching time produced independent equations for temperatures below 18ºC and 15ºC. We constructed a model that simulates the expected proportion of the population hatching after the beginning of rainy season based on regression equations to mean hatching time and associated standard deviation. The simulation generated for the model correlated significantly with nymphal population observed in the field. These results showed that overnight soil temperatures below 18ºC, as occurs in Central and South-eastern Brazil between May and August, shorten the period of diapause, increase quiescent eggs in the soil, and may synchronize the population hatching.

Key words: Insecta, phenology, dormancy, cold treatment, population dynamics.

RESUMO

Temperatura do solo e manutenção da diapausa em ovos da cigarrinha-das-pastagens, *Deois flavopicta* (Hemiptera: Cercopidae)

Foram expostos ovos diapáusicos da praga de pastagens na região neotropical, *Deois flavopicta* (Stal) (Hemiptera: Cercopidae), a baixas temperaturas noturnas, simulando condições de campo durante a estação seca (23/12, 23/15 e 23/18ºC dia/noite), por diferentes períodos (0, 15, 30, 40, 50 e 60 dias). Após o tratamento térmico, os ovos foram mantidos a 28ºC e em água de contato (100% de umidade), até a eclosão das ninfas. Um grupo-controle de ovos diapáusicos foi mantido por todo o período experimental em altas temperaturas e umidade. O tempo para eclosão (em graus/dia) foi reduzido devido ao efeito das baixas temperaturas noturnas e ao aumento no período de exposição ao choque frio, embora não tenha sido observada interação significativa entre esses fatores. A regressão do efeito do período de choque frio no tempo médio para eclosão das ninfas provenientes de ovos diapáusicos produziu equações independentes para as temperaturas abaixo de 18ºC e 15ºC. Com base nas equações de regressão do tempo médio e no desvio-padrão associado, construímos um modelo que simula a proporção esperada da população que eclodirá após o início da estação chuvosa. A simulação gerada pelo modelo correlacionou-se significativamente à população de ninfas observada no campo. Estes resultados mostram que temperaturas noturnas abaixo de 18ºC, como ocorre no Centro-Sul do Brasil entre maio e agosto, abreviam o período da diapausa, acumulam ovos quiescentes no solo e podem sincronizar a eclosão da população.

Palavras-chave: Insecta, fenologia, dormência, choque frio, dinâmica populacional.

INTRODUCTION

In central Brazil the rainy warm season, when grasses develop and reproduce, alternates with the dry cool season, when dormancy prevails. In this landscape, a species-complex of spittlebugs, known as pasture spittlebugs (Homoptera: Cercopidae), is the main pest problem in cultivated pastures (Valério & Koller, 1992; Valério & Nakano, 1987). In the savanna-like areas, known as Cerrado, the main species is *Deois flavopicta* (Stal), 1854 (Botelho & Reis, 1980; Cosenza & Naves, 1980). This species avoids adverse conditions by diapausing in the egg stage. Females vary in the proportions of diapausing and non-diapausing eggs that they lay during the rainy season; moreover the duration of diapause is variable within populations (Fontes *et al.,* 1995; Koller, 1991).

Without any apparent diapause terminating cues, hatching extends from July through December, with a peak in September/October (Sujii *et al*., 1995, Fontes *et al*., 1995; Koller, 1991). In central Brazil this peak coincides with the transition between dry and rainy seasons.

Dormancy in eggs of *D. flavopicta* has, at least two distinct parts: diapause and post-diapause quiescence (Pires *et al*., 2000). Neither photoperiod (Pires *et al.,* 1991) or temperature and humidity (Pires *et al*., 2000), are apparently used as environment cues for diapause termination in *D. flavopicta*.

On the contrary, Sujii *et al*. (1995) observed that diapausing eggs experiencing an overnight low temperature of 15ºC for > 8 d, during the dry season, terminate dormancy and hatch earlier and within a synchronized period.

The present study examined the effect of low overnight temperatures (duration and intensity of exposure) on the maintenance and termination of diapause in *D. flavopicta*. Initially, the study takes an experimental approach; subsequently, the data are modeled and tested against emergence pattern of first instar in the field. It is our expectation that precise understanding of the phenology of this insect will allow forecasting of first instar populations in the field, and significantly improve the pest management program of this insect.

MATERIAL AND METHODS

Study sites

This work was conducted in the Distrito Federal, Brasília, which is located in the western region of central Brazil (15º40’S, 47º40’W). Field experiments were conducted in pastures of *Brachiaria ruziziensis* Stapf and *B. decumbens* Stapf (Poaceae), in experimental fields of the Brazilian Agricultural Research Organization – Center for Agriculture Research in the Cerrados (Embrapa Cerrados), and the Agriculture High School of Brasília (CAB). Laboratory experiments were carried out at EMBRAPA’s Center for Agriculture Research in Genetic Resources and Biotechnology (Embrapa/Genetic Resources and Biotechnology) at the Biological Control facilities.

The weather in that region is characterized as warm and rainy with dry winters (Garrido *et al*., 1982). Mean annual rainfall is 1,537 mm with 96% of precipitation occurring from September through April.

Rainfall rarely exceeds 30 mm/month during the dry season (May-August). Average monthly temperature shows little variation (19.8 to 23.2ºC) in relation to the diurnal variation which is frequently > 10ºC.

Low temperatures and diapause termination

From February to April, the period during which *D. flavopicta* lays a high percentage of diapausing eggs, adults were collected every week from pastures of *Brachiaria ruziziensis* using sweep-nets. In the laboratory, groups of 25 pairs were kept in oviposition cages. Each cage consisted of a clear plastic cylinder measuring 21 cm length, and 10 cm in diameter. Three of these cylinders were placed in an aluminum tray containing a 1 cm layer of agar (20 g of agar and 0.45 g ZnSO per liter of water). The agar surface was rutted with a knife to form 0.5 cm squares to provide better support and facilitate ovipositor insertion. Seedlings of *Brachiaria decumbens* served as food for the insects in each cage. Plants were kept fresh by immersing their roots in water in a small vial or an Erlenmeyer flask covered with aluminum foil (modified from Valério, 1993; Koller, 1991; Magalhães *et al*., 1987). The top of the cage was covered with nylon net. Eggs were collected from the agar 2 or 3 days after placing the adults in the cage. The eggs were then washed in a 0.5% sodium hypochlorite solution. Groups with 25 randomly selected eggs were placed in Petri dishes lined with a thin layer of moistened cotton and filter paper.

Groups of 4 Petri dishes, forming a total of 100 eggs per treatment, were exposed to temperature regimes of 23/12, 23/15, and 23/18°C (day/night) for periods of 0, 15, 30, 40, 50, and 60 days, in environmental chambers. Day temperature of 23ºC was used because this is the average daytime temperature during the period of May through August when the main proportion of diapausing eggs stays dormant in the field. The photoperiod used during the experiment was 12.5 hours of photophase. All eggs were constantly exposed to moist conditions. A control treatment consisted of 4 Petri dishes with 25 eggs each which were kept constantly under 28ºC temperature and moisture was monitored in order to evaluate hatching distribution of diapausing eggs in the absence of low overnight temperature.

After having completed the cold treatments, eggs were transferred to another incubator and kept at a constant temperature of 28 ± 2ºC, and in moist conditions until hatching. Eggs were monitored every other day, and hatched nymphs and their respective chorions were counted and removed from the dishes. Eggs that changed color (became white or grayish) or shape (became flattened) were considered to have died and were removed.

The time from the beginning of cold treatment, June 1st, until hatching was converted into degree-days above a lower thermal threshold of 13.7°C for development, as determined by Sujii *et al*. (1995). This procedure allowed for correction of temporal effect of treatments, thus comparisons among the results were possible. The effect of low overnight temperature and exposure time on the average time for egg hatching was evaluated by a factorial analysis of variance. This test was followed by Student Newman Keuls Test to compare mean values (Kuo *et al*., 1992). Nondiapausing eggs were eliminated from the experiment because they do not survive the adverse conditions of the dry season (Fontes *et al*., 1995).

Data modelling

Average time for egg hatching in the laboratory was modeled through linear regression to simulate occurrence of first generation nymphs in the field. The standard deviation associated with average hatching time was regressed against the duration low temperature exposure. Because the egg hatching pattern fit normal distribution, we could use the average hatching time and its standard deviation to calculate the standardized Z variable (Snedcor & Cochran, 1980).

It was then possible to estimate a theoretical curve for the accumulated frequency of egg hatching in degree-days above 13.7ºC (the lower developmental threshold), based on periods of egg exposure to low temperatures.

Weather conditions were monitored in the field during the dry season. These data were used with the simulation model to produce a curve of expected accumulated egg hatching in the next year's first generation.

This curve was compared with the empirical curve of egg hatching observed in a field population that year. The weather information was obtained from the weather station of Embrapa Cerrados, c.a. 6 km distant from the pastures where the population was surveyed. The population of first instars was monitored weekly. Each sample, consisting of 25 cm², was randomly thrown a hundred times in the area. The observed and expected curves for egg hatching time were compared with those of the Kolmogorov-Smirnov test for two samples (Wilkinson, 1990).

All statistical analyses were performed with software from Systat Inc. (Wilkinson, 1990) and Jandel Scientific (Kuo *et al*., 1992).

RESULTS

The factorial variance analysis showed that the main effect of all factors, and low overnight temperature and exposure period, are significant and reduced the time hatching, although there was no interaction between the factors (Table 1). Comparisons of night temperature effects revealed that the average hatching time decreased progressively from control treatment, 18 and 15ºC, and became stable from 15 to 12ºC (Fig. 1). Plotting the average times for egg hatching related with period of exposure to low overnight temperature of 18ºC allowed adjustment of the equation (Fig. 2a)

$$HT = 1{,}128 - 8.61 \times D_{18} + 0.0008 \times (D_{18})^3$$

where:

HT = time required for hatching (degree-days);

D_{18} = number of days during which soil temperature at night ≤ 18°C.

The same procedure was done to adjust a curve to fit the average hatching time for diapausing eggs exposed to overnight temperature of 15 and 12ºC (Fig. 2b), resulting the follow equation:

$$HT = 1{,}115.79 - 12.51 \times D_{15} + 0.0013 \times (D_{15})^3$$

where:

HT = time required for hatching (degree-days);

D_{15} = number of days during which soil temperature at night was ≤ 15°C.

The two equations above were combined in a function to simulate the expected average hatching time for diapausic eggs of *D. flavopicta* exposed to any period of low overnight temperature.

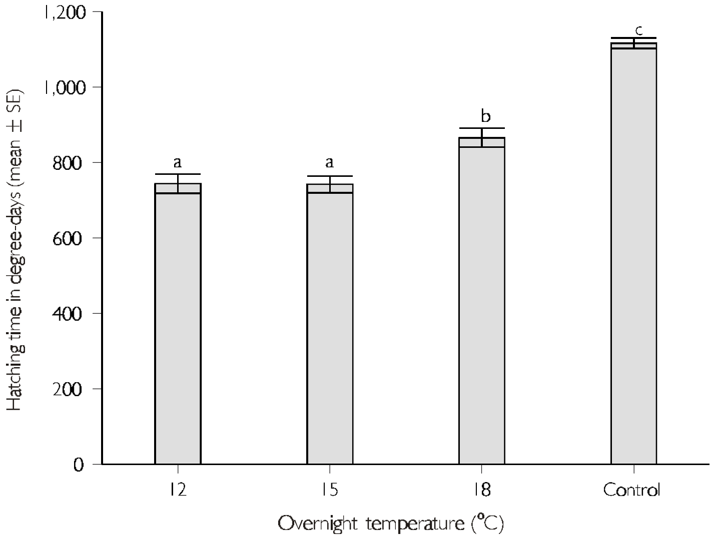

Fig. 1 — Effect of low overnight temperature on the mean hatching time of diapausing eggs of spittlebug, *Deois flavopicta*. SE = standart error.

TABLE 1

Factorial analysis of variance of mean hatching time of the spittlebug *Deois flavopicta*'s diapausic eggs (average of four replicates).

Source	df	SS	MS	F	P
Treatments	15	1,234,422	82,294	51.18	< 0.001
Control *vs*. others	1	414,710	414,710	26.67	< 0.001
Night temperature (NT)	2	200,529	100,264	61.17	< 0.001
Exposure period (EP)	4	597,863	149,465	89.70	< 0.001
NT × EP	8	21,318	2,664	1.60	0.152
Error	60				

Note: df = degrees of freedon; SS = sum os squares; MS mean square; F = fratio; P = probability.

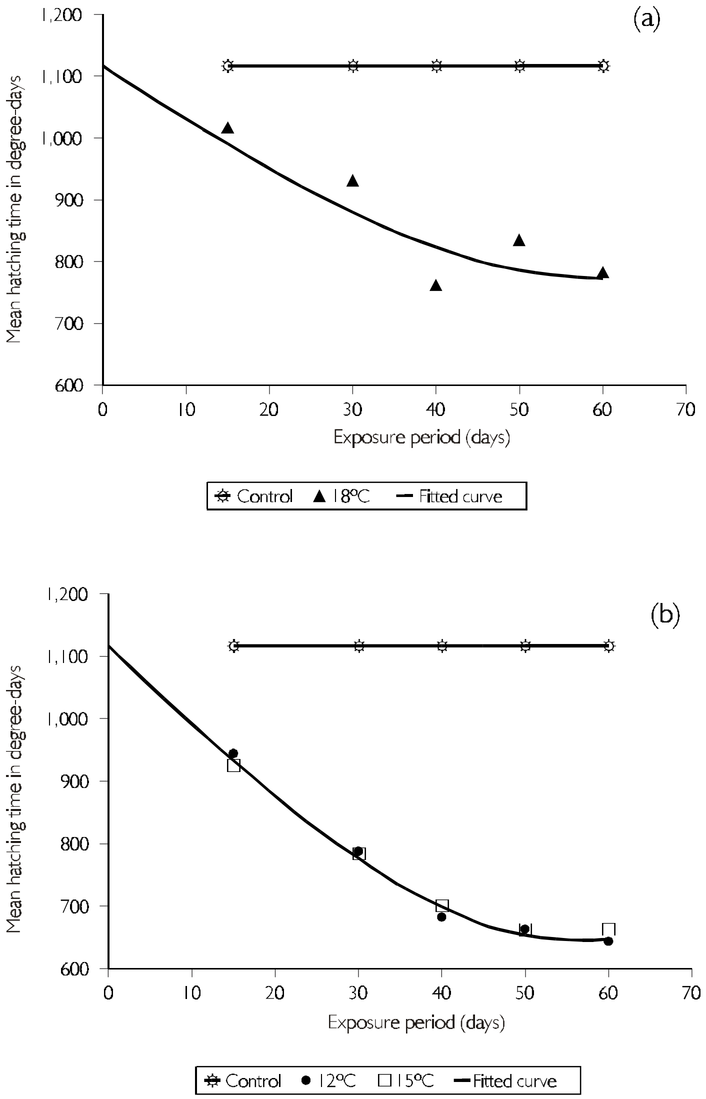

Fig. 2 — Regression anlysis adjusting a curve to model the effect of period of exposure to low overnight temperature on the mean hatching time of *Deois flavopicta*'s diapausing eggs. **a)** cold shock of 18°C; **b)** cold shock of 15°C or below.

Resulting model has a constant obtained from the average hatching time of the control treatment and the slope determining terms mean weighted from both equations:

$$HT = 1{,}116.75 - (8.61 \times D_{18}) - (12.51 \times D_{15}) + [(0.0008 \times (2 \times D^{18})^{3}) + (0.0013 \times (2 \times D_{15})^{3})] \times 2^{-1}$$

where:

HT = time required for hatching (degree-days);

D_{18} = days during which soil temperature at night was ≤ 18°C and >15°C;

D_{15} = days during which soil temperature at night was ≤ 15°C.

A synchrony of hatching time was observed when the diapausic eggs were exposed to the cold treatments for increasing periods. This phenomenon is showed through the decrease of standard deviation of the average hatching time, in Fig. 3. Standard deviations associated with the average time for egg hatching were inversely related to the time of exposure to cold temperatures, in a spatial pattern similar to that of the time taken for egg hatching (Fig. 3). This indicates that low overnight temperature < 18ºC and period of exposure determines not only average time for eggs hatching but also the standard deviation of this mean, and it regulates egg hatching synchrony. The standard deviations were modeled using linear regression, similarly to the average time for egg hatching.

The resulting equation of this analysis was:

$$SD = 195.87 - 1.99 \times D \ (r^2 = 0.885,\ n = 16,\ F = 49.99,\ p < 0.001)$$

where

SD = standard deviation of the mean in degree-days necessary for egg hatching for the equivalent cold treatment;

D = number of days with soil minimum temperature < 18°C.

This model allows simulating the standard deviation of the mean hatching time of postdiapausing eggs for each low overnight temperature exposure.

To predict the occurrence of first instars nymphs of the first generation in the field, we used the number of days and the mean of minimum soil temperatures < 18ºC during the dry season (May through August). Using the monthly average temperature between June 1 and the beginning of the rainy season, with rainfall > 30 mm, it was possible to predict hatching egg distribution through the standardization of the mean and standard deviation with the Z variable of the normal distribution to simulate the accumulated proportion of hatching eggs.

Monitoring the occurrence of first instar nymphs in the beginning of the rainy season, in two separate pasture fields in the Federal District, showed that our simulation using field weather conditions during the dry season and during the transition from dry to rainy season predicted the timing of egg hatching very well. The comparison of observed and simulated curves of accumulated proportion of hatching eggs showed a high degree of agreement when compared with the Kolmogorov-Smirnov test (Fig. 4).

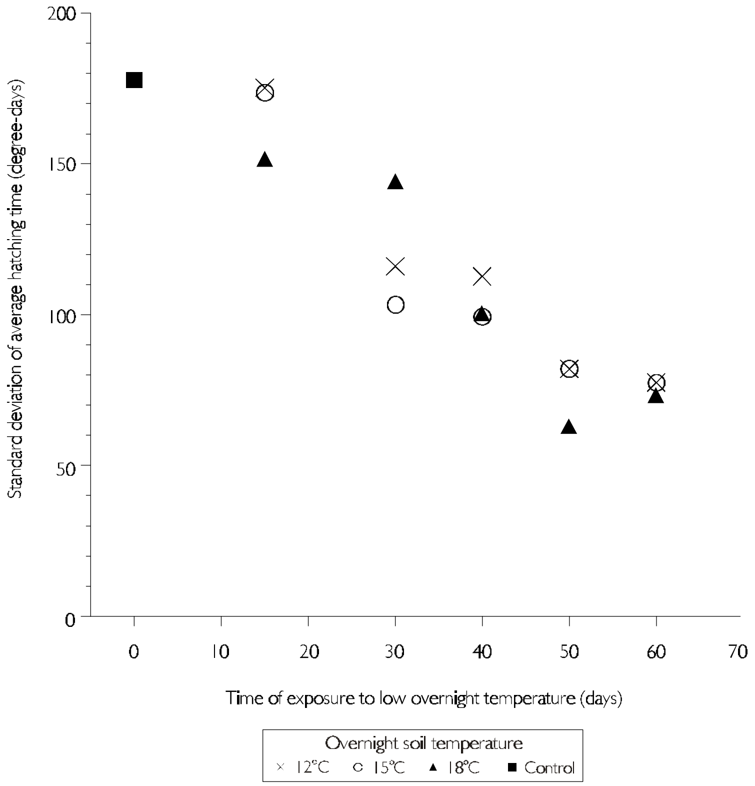

Fig. 3 — Distribution of the standard deviation related to the average hatching time of *Deois flavopicta* eggs exposed to different cold treatments.

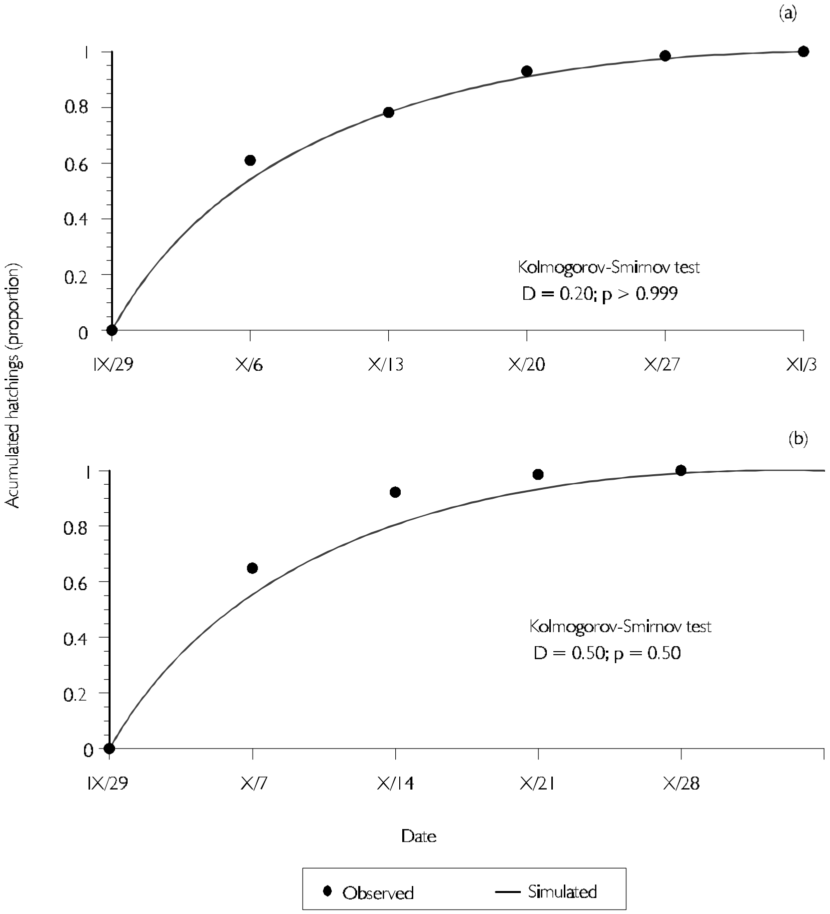

Fig. 4 — Comparison in two areas of the acumulated proportion distribution curves of hatched eggs observed under field conditions and simulated through the model developed for diapause eggs of *Deois flavopicta*. **a)** Embrapa Cerrados; and **b)** CAB.

DISCUSSION

The present study supports the idea that the interaction between overnight soil temperatures and exposure time produced a thermal cold shock that reduced the expected time for egg hatching and increased hatching synchronization in the spittlebug population.

It is possible that the low temperature accelerated diapause development, but retarded post-diapause development, thus reducing the duration of the egg stage and synchronizing hatching after the eggs experienced warm condition (Pires *et al.*, 2000; Tauber *et al.*, 1986). When this happens, quiescence may be the mechanism through which the eggs maintain their low metabolic rate and drought hardiness after diapause has ended.

Under quiescence *D. flavopicta* eggs can resume embryonic development in response to favorable environmental conditions, thus synchronizing the annual life cycle with plant regrowth and food availability and quality (Fontes *et al.*, 1995).

Observations on the control treatment, when eggs hatched between August and December with a peak in October, revealed that diapausic eggs of *D. flavopicta* exposed to favorable conditions since oviposition terminate diapause spontaneously, without any apparent environmental stimuli. Hatching time distribution occurred within a large range of variability among each population.

Diapausing eggs treated at low temperature hatched steadily faster and synchronized, supporting the above statements.

Eggs of *D. flavopicta* exposed to any low temperature for a period > 40 d discontinued reduction of hatching time and synchronization as expected and tested in this and another study (Sujii *et al.*, 1995). This may indicate that diapause had terminated in all or most eggs by the 40th d of exposure to low temperature.

The beginning of the rainy season means the return of the supply of water in the soil necessary for embryonic development and egg hatching. Water acts as a second factor influencing the end of dormancy of quiescent eggs present in the soil, waiting for favorable conditions to resume development (Fontes *et al.*, 1995; Sujii *et al.*, 1995). Observations that first population peak of spittlebugs only occurs after precipitation that had replaced the humidity soil deficit, in the beginning of rainy season (Reis *et al.*, 1980), supports the idea that soil moisture availability in the transition period of dry to rainy season changes egg hatching pattern and nymph density. Therefore, rains early in the season find a smaller proportion of eggs ready to respond to moisture, the postdiapause stimulus for hatching. Thus first generation eggs are not well synchronized and the population is small and widely distributed temporally. On the other hand, a prolonged dry season probably favor synchronism of hatching in a large proportion of the population, resulting in outbreaks.

The high similarity between simulated and empirical curves from field observations of postdiapause eggs hatching during this study, and observations with other spittlebugs, such as *Aeneolamia occidentalis* Walk and *Prosapia simulans* Walk (Cercopidae) in Mexico showing larger and more synchronized populations in the first generations when rains are abundant and sudden starts in the beginning of the season (Oomen, 1975), support the above proposed hypotheses of interaction of temperature and rain distribution to explain the variation of *D. flavopicta* densities and confirm the importance of moisture as a seasonal cue in the life cycle of insect in tropical and subtropical regions, as proposed by Tauber *et al.* (1998).

The interaction of temperature and soil moisture may be an important seasonal cue instead of a photoperiod in several insects in any region of the world, as had been previously showed with the Colorado Potato Beetle by Tauber *et al.* (1994), and is presented here with *D. flavopicta*.

The construction of a simulator based on the model presented would help predict not only the timing of first generation nymphs in the field, but also the densities of the first generation of annual populations and could be useful in new pest management strategies.

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