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A RAPID SPECTROPHOTOMETRIC METHOD FOR THE MONITORING OF EMBRYONIC DEVELOPMENT IN TICKS (ACARINA: IXODOIDEA)*

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ABSTRACT

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A rapid spectrophotometric method for the monitoring of embryonic development in *Boophilus decoloratus* (Koch, 1844) is described. The method is based on a quantitative assessment of guanine, the principal end-product of nitrogenous metabolism in ticks, which is gradually built up and stored in the rectal sac during embryonic development of the larvae. A study of the growth of tick embryos under constant temperature conditions and 6 different humidity conditions demonstrated that embryonic development was dependent on the water content of the eggs at the time of oviposition. When eggs lost more than 35% of their initial mass through evaporation, nitrogenous metabolism (as indicated by guanine production) was seriously affected and embryos died.

INTRODUCTION

Biological and ecological work on ticks has shown that physical conditions of the environment have a profound influence on the successful development and hatch of eggs (Legg, 1930; Arthur, 1951; Hitchcock, 1955; Sonenshine & Tigner, 1969). Although earlier workers demonstrated that eggs would not hatch under certain humidity conditions they were unable to assess the degree of embryonic growth that had taken place in these partially-developed eggs. It is hoped that the information provided in this paper will help to overcome this problem.

Guanine has been shown to be the principal component of the excreta of certain spiders, scorpions, amblypygids, uropygids and solifugids (Schmidt, Liss & Thannhauser, 1955; Rao & Gopalakrishnareddy, 1962; Horne, 1969) while Schulze (1955), Kitaoka (1961), Balashov (1968) and Hamdy (1972) have reported guanine as the chief ingredient of tick excrement. As Vischer & Chargaff (1948) were able to detect quantitatively minute amounts of purines and pyrimidines spectrophotometrically it was decided that guanine production by developing tick embryos might prove a useful characteristic in the production of a sensitive method for the monitoring of embryonic growth. The subject species was *Boophilus decoloratus* (Koch, 1844).

MATERIALS AND METHODS

To ascertain that the excrement of *B. decoloratus* is indeed guanine, samples of pure guanine, deposited larval excrement and egg homogenate (in this instance eggs which were near to hatching) were studied both chromatographically and spectrophotometrically. In each instance the samples were homogenized for 1 min in a glass tissue grinder together with 5 ml 0,1 N H_2SO_4, as Vischer & Chargaff (1948) demonstrated that guanine is only slightly soluble at neutrality. Using Whatman's No. 1 chromatographic filter paper and a solvent system of 95% ethanol: 0,4 N NaOH (3:1), the Rf values for the 2 possible sources of guanine were compared with the guanine standard. Paper chromatographs studied under ultra violet (UV) light show guanine as a black spot (Oser, 1965). The dissolved samples were also centrifuged for 5 min at 10 000 RPM and spectra between 220 and 320 nanometers (nm) recorded against a 0,1 N H_2SO_4 blank.

The methods used in the ensuing work on egg development were as follows: Large numbers of freshly laid *B. decoloratus* eggs were placed in 6 chambers containing atmospheres of 50, 60, 70, 80, 90 and 100% Relative Humidity (RH) respectively. All these humidity values were produced with KOH solutions as described by Peterson (1953). All 6 humidity chambers were placed in an incubator held at a constant temperature of 26 °C and complete darkness. Three batches, each of 100 eggs, were removed from each humidity chamber at 3-day intervals, homogenized in 5 ml 0,1 N H_2SO_4, centrifuged and examined spectrophotometrically. Curves of percentage light transmission were obtained by means of a pen recorder coupled with the spectrophotometer and, to facilitate comparison of the curves, the differences between the transmissions at 246 nm and 320 nm were calculated. A wavelength of 246 nm was selected as one of the reference points as this appeared to be the wavelength at which peak absorption (or minimum transmission) of light for guanine took place. Vischer & Chargaff (1948) give 249 nm as this maximum and the difference might be due to calibration error. The second reference point (320 nm) was selected on the basis of convenience only. In addition to the eggs used in the spectrophotometric investigation, 4 small glass tubes containing eggs, sealed at both ends with fine nylon mesh, were placed in each humidity chamber. These vials were mass-measured at 5-day intervals to assess the amount of water lost or gained through evaporation or absorption up to the end of the incubation period, when the percentage hatch was calculated. Preliminary experiments (Londt, unpublished data) have shown that the nylon mesh used did not absorb significant amounts of water vapour when placed in high humidities.

RESULTS AND DISCUSSION

The Rf values obtained in the qualitative chromatographic comparison of pure guanine, larval excreta and egg homogenate (Table 1) are highly suggestive of compatibility between samples as regards their purine content. The values obtained for egg homogenate were slightly lower than those for pure guanine and larval excrement. This homogenate, however, contained numerous other organic substances which may have caused these differences.

* This investigation was undertaken at the Tick Research Unit, Rhodes University, Grahamstown

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TABLE 1 Rf values obtained chromatographically for pure guanine, *Boophilus decoloratus* larval excreta and egg homogenate using an ethanol: sodium hydroxide solvent system

Replicate No.	Rf values		
	Pure guanine	Larval excreta	Egg homogenate
1.....	0,46	0,49	0,44
2.....	0,49	0,46	0,44
3.....	0,48	0,46	0,43
4.....	0,46	0,47	0,43
5.....	0,48	0,46	0,43
6.....	0,47	0,48	0,44
Means.....	0,47	0,47	0,44

Transmission spectra for the 3 different samples (Fig. 1) were very similar. Each possessed an absorption maximum in the region of 246 nm, which strongly supports the contention that all 3 samples contained the same purine. It is appreciated that, by using the simple method outlined above, it cannot be assumed that guanine alone is being assayed. The technique as a means of monitoring embryonic development is, however, in no way invalidated.

The results of the spectrophotometric study of tick eggs are presented in Fig. 2, 3, 4 and 5. Fig. 2, 3 and 4 represent the progressive change in form of the transmission spectra throughout the incubation period of the eggs held at 100, 70 and 50% RH respectively. In each instance the mean difference in percentage transmission at 246 nm and 320 nm, for each successive sampling day, has been indicated in the figures. In Fig. 5 this information is summarised and data collected at the other 3 humidity levels included. The ever-increasing differences in percentage transmission for embryos growing in 80, 90 and 100% RH reflect the continuous build-up of guanine in the rectal sacs of the developing larvae. Unfortunately the last readings (24th day) for eggs held at 100% RH were spoilt. Growth of embryos held at 50, 60 and 70% RH demonstrated a marked fall off in guanine production after approximately Day 6 of the incubation period of the eggs. Guanine production appeared to almost cease in embryos held at 50% RH.

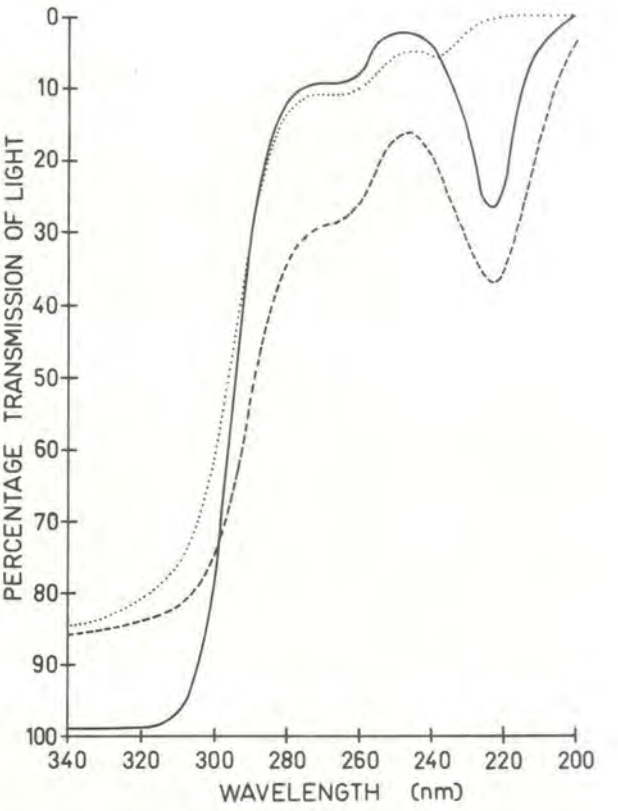

FIG. 1 The form of spectrophotometric transmission curves obtained for samples of pure guanine (———), *Boophilus decoloratus* larval excrement (— — —) and egg homogenate (. . . . .). The concentration of guanine in each instance was unknown

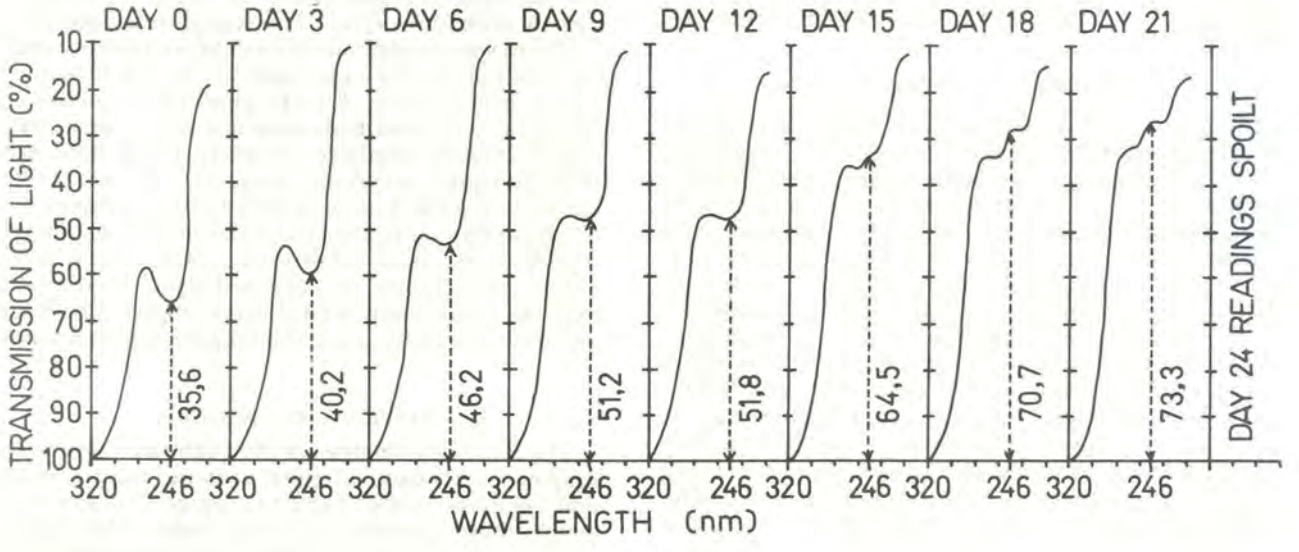

FIG. 2 The change in form of spectrophotometric transmission curves produced, at 3-day intervals, by egg homogenates during the incubation of *Boophilus decoloratus* eggs held at 26 °C and 100% RH. All the curves have been adjusted so that transmission at 320 nm equalled 100% for comparative purposes

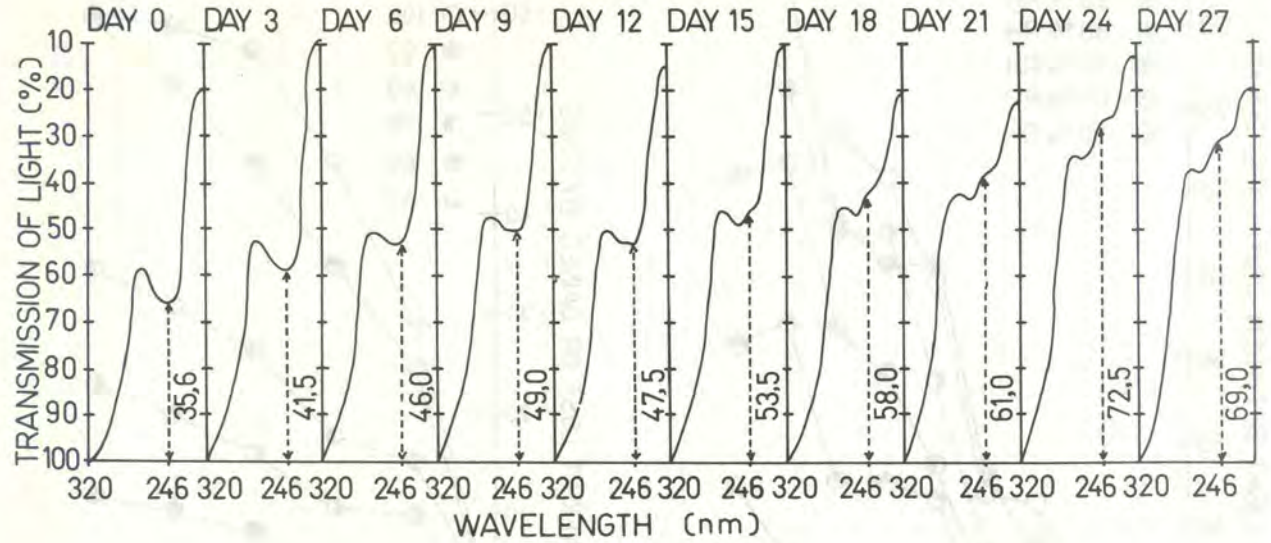

FIG. 3 The change in form of spectrophotometric transmission curves produced, at 3-day intervals, by egg homogenates during the incubation of *Boophilus decoloratus* eggs held at 26 °C and 70% RH. All the curves have been adjusted so that transmission at 320 nm equalled 100% for comparative purposes

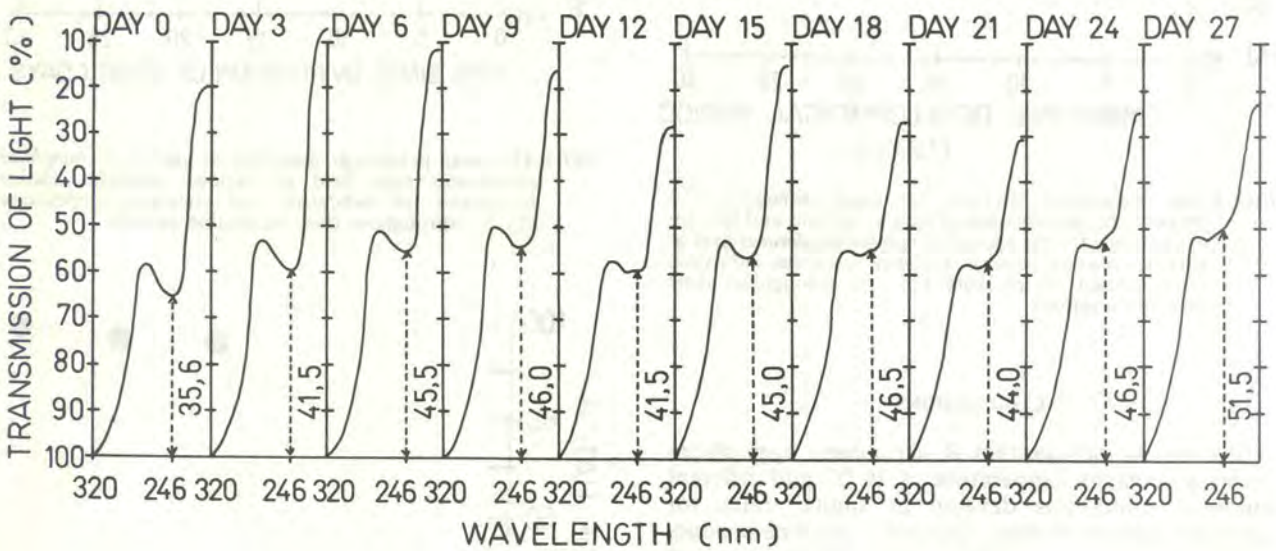

FIG. 4 The change in form of spectrophotometric transmission curves produced, at 3-day intervals, by egg homogenates during the incubation of *Boophilus decoloratus* eggs held at 26 °C and 50% RH. All the curves have been adjusted so that transmission at 320 nm equalled 100% for comparative purposes

The mean percentage mass lost or gained by batches of eggs held at the 6 different humidity levels throughout their period of incubation is shown in Fig. 6 while the mean percentage hatch of the same egg batches is graphically illustrated in Fig. 7. Eggs kept at 100% RH gained approximately 15% of their initial mass during the first 10 days of incubation and then gradually lost mass during the remaining period before hatching. Eggs held at less than 100% RH lost mass, presumably due to evaporation of water, in a manner directly related to the particular humidity used. No hatch took place at either 50 or 60% RH; the hatch at 70% RH was about 37% while eggs held at 80% RH or more gave hatches of over 90%.

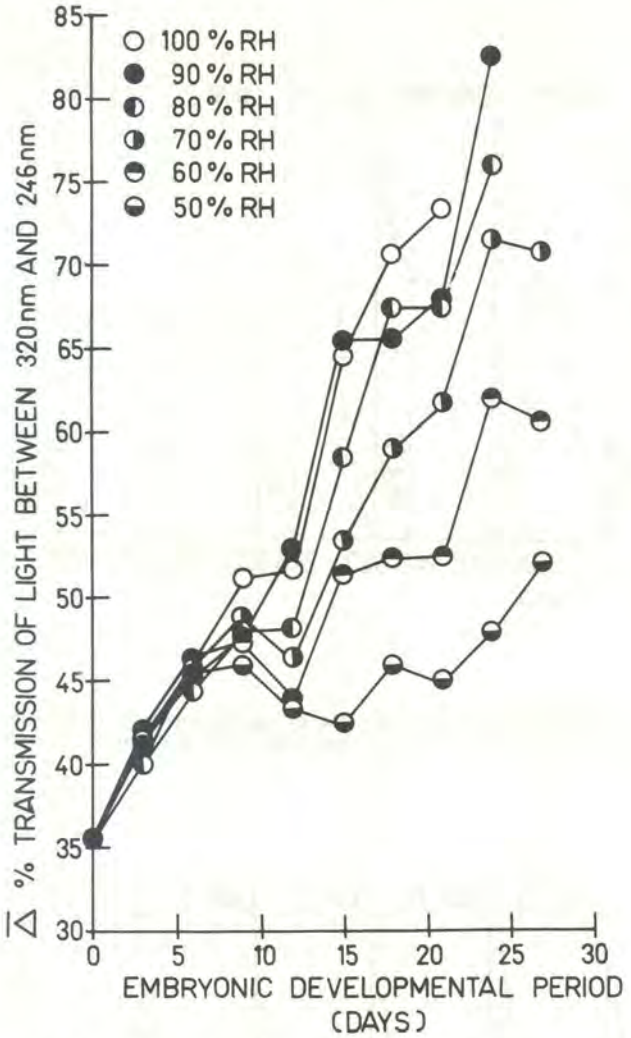

FIG. 5 The relationship between the mean difference ($\bar{\Delta}$) between the transmission of light at 320 nm and 246 nm against time for the eggs of *Boophilus decoloratus* held at various constant relative humidity levels (as indicated) and constant temperature (26 °C) throughout their incubation periods

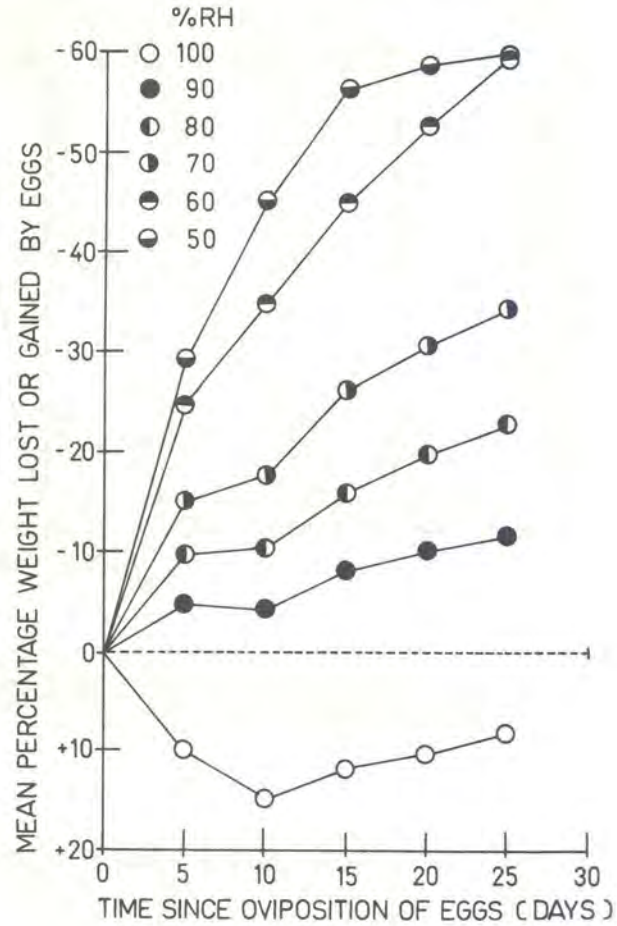

FIG. 6 The mean percentage mass lost or gained by *Boophilus decoloratus* eggs held at various constant relative humidities (as indicated) and constant temperature (26 °C) throughout their incubation periods

Conclusions

The results indicate that *B. decoloratus* eggs placed under a constant temperature of 26 °C and different humidity conditions develop at similar rates for approximately 6–10 days. Thereafter guanine production by the growing embryos slows down or ceases altogether in environments in which the humidity is less than approximately 70–80% RH. Eggs held at 50 and 60% RH lose about 35 and 30% of their initial mass, respectively, within the first 6–10 days of development and fail to hatch. Eggs in an environment of 70% RH give a hatch of about 37% and lose approximately 33% of their total initial mass during their entire incubation period. It is therefore suggested that successful embryonic development is only indirectly dependent on the prevailing humidity conditions and directly dependent on the water content of the eggs at the time of oviposition. Eggs appear to be able to lose about 35% of their initial mass before serious consequences are experienced. Certainly guanine production, which is here taken as an indicator of metabolism, is drastically affected when mass loss reaches this order of magnitude and eggs fail

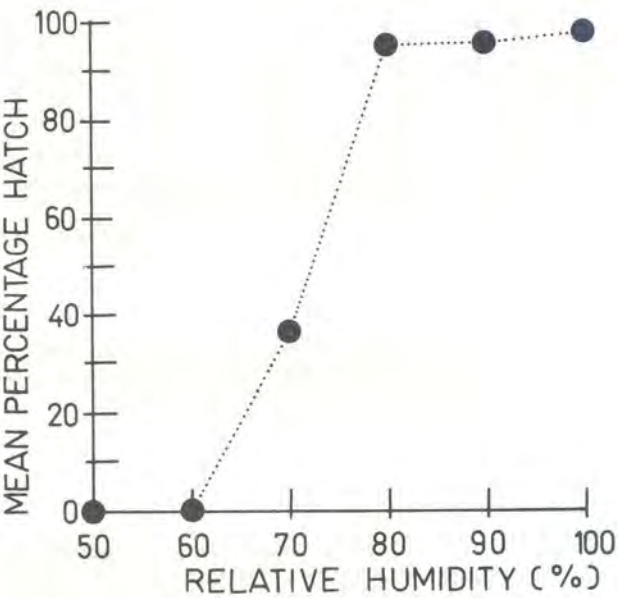

FIG. 7 The mean percentage hatch of *Boophilus decoloratus* eggs held at constant temperature and various constant relative humidity levels

to hatch. Unlike larval ticks, which can actively absorb water vapour from the air (Lees, 1946; Londt & Whitehead, 1972), eggs (i.e. the developing embryos) appear to possess only a very limited capacity for this type of water replenishment (Londt, unpublished data). The microclimatic humidity conditions and the effect of the waterproofing wax layer covering tick eggs on the successful development of tick embryos appear to be of greater importance than was first thought. The spectrophotometric technique outlined in this paper might prove to be a sensitive tool for the future study of these phemomena and their value to the survival of tick species.

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Résumé

J. G. H. LONDT, 1975. *Une méthode spectrophotométrique rapide permettant le repérage de l'évolution embryonnaire chez les tiques (Acarina: Ixodoidea). Onderstepoort J. vet. Res.* 42 (3), 103–108 (1975)

L'auteur rapporte une méthode spectrophotométrique rapide permettant le repérage de l'évolution embryonnaire chez Boophilus decoloratus (*Koch,* 1844). *La méthode s'appuie sur une appréciation quantitative de la guanine, produit final et principal du métabolisme azotique chez la tique. Cette substance étant produite lentement, est mise en dépôt dans le sac rectal lors du développement embryonnaire de la larve. Les résultats de travaux effectués sur la croissance de la larve en milieu de température constante mais de 6 différents niveaux d'humidité, ont montré un rapport positif entre l'évolution embryonnaire et la quantité d'eau dans les oeufs au moment de la ponte de ceux-ci. Une perte de plus de 35% du poids initial par évaporation a suscité une perturbation importante du métabolisme azotique, entrainant la mort des embryons.*

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