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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Effect of Temperature, Air Relative Humidity and Water Presence on Some Biological Parameters of *Clavigralla tomentosicollis* Stål (Hemiptera:Coreidae), the Pod Sucking Bug of Cowpea

¹C. Dabiré, ¹M.N. Ba, ²A. Sanon and ³K. Foua-Bi

¹Institut de l'Environnement et des Recherches Agricoles, CREAM de Kamboinsé,
01 BP 476, Ouagadougou 01, Burkina Faso

²UFR Sciences de la vie et de la terre, Université de Ouagadougou, Burkina Faso

³UFR Biosciences, Université de Cocody, Côte d'Ivoire, Burkina Faso

Abstract: A laboratory study was conducted on the influence of temperature, air relative humidity and water presence on some biological parameters of *Clavigralla tomentosicollis* Stål, a major pest of cowpea, *Vigna unguiculata* Walpers, in West Africa. The feeding of the first-instar larvae was also studied. The aim was to provide some indicators on the insect adaptation to hot and dry conditions in Sahelian zone. Results show that increased temperature reduced embryo and larvae development length and female fertility and lifespan. Temperature fluctuations had little effect on egg viability to the extent of hatching limit around 38°C. A full larval development and adult reproductive activity were possible at a constant temperature of 40°C. Increased air relative humidity (80%) improved larval survival especially under high temperatures. The presence of water was necessary for first-instar larvae development. The insect showed good adaptation to Sahelian hot and dry environment, which enables it to go through the long dry season.

Key words: Larval survival, fertility, hatching, lifespan, embryo development length, adaptation, hot and dry environment

INTRODUCTION

The complex of bugs that attacks cowpea pods in Africa includes a species more subservient to the crop and widely disseminated: *Clavigralla tomentosicollis* Stål^[1-3]. This species is responsible for major yield losses on cowpea and could reduce the grain germinating capacity^[4-6]. Data are available on the influence of temperature and air relative humidity on the development of bugs feeding on cowpea (*Vigna unguiculata* WALP.) and *Cajanus cajan* M. pods^[7].

However, these studies were conducted in humid zone and related only to development length and larval mortality, leaving aside adult reproductive activity. Even, the effect of hygrometric variation on the biology was not accounted for. All studies indicated that low temperature delays growth whereas high temperature speeds it up to the extent of 40°C at which no larva can survive^[8].

There is a controversy on the feeding of *C. tomentosicollis* first-instar larvae. Another study reported that they do not feed and that they develop to

second larval stage with only a water-soaked cotton buffer^[9]. In the Sahel zone, where water becomes scarce in dry season and hygrometric conditions very poor, how does the first-instar larva survive? An experiment was carried out to check the assumption.

Burkina Faso is a Sahelian country with a semi-arid tropical climate characterized by poor rainfall and a dry season lasting 7-8 months. In this dry season, air relative humidity is very poor (15%) and temperature could reach 42°C under shelter and 50°C in the sun. In natural conditions, all the insect development stages may be subject to heat and hygrometric extremes. It is, hence, important to know how the insect secures its survival in these conditions and under which form the first-instar larva gets the necessary water for its development. Hence, the study on the influence of temperature, air relative humidity and water presence on some multiplication parameters of *C. tomentosicollis* aims at understanding the insect behavior in the Sahelian environment in view of predicting infestation risks and developing adapted control methods.

MATERIALS AND METHODS

Studies were conducted in a controlled environment using a Fi-totron 600 H (FISONS Environmental Equipment, Loughborough, England) type incubator with an automatic temperature, air relative humidity and photoperiod adjustment. All experiments were submitted to a constant photoperiod of 12 h of daylight and 12 h of dark. All the insect development stages in the study were derived from a laboratory mass rearing. The fresh pods necessary for bug nutrition were taken in continuous crops of the sensitive cowpea variety, KN1. Studied biological parameters included length of embryo and larval development, egg fertility and adult lifespan. Temperature choice was guided by past studies and focused on high temperatures and Sahelian conditions in a range of 24 to 42°C. Tested relative humidity corresponded to dry season (15%) and humid season (80%) extremes in Burkina Faso. These extremes are referred to as poor and high air relative humidity.

Effect of temperature on embryo development length and egg fertility: Fresh eggs were deposited into petri dishes at the rate of one set of laying per dish and incubated at constant temperatures of 24, 25, 27, 28, 30, 35, 38 and 40°C under a 15% relative humidity. For each temperature, the petri dishes containing the laid eggs were grouped in 4 lots of 75 eggs, each lot representing a replication. At hatching, the incubation length corresponding to embryo development and the number of hatched eggs were recorded. The incubation length is the period between laying and hatching and egg fertility corresponds to the ratio of hatched eggs.

Effect of temporary exposure of eggs to the highest limit temperature of 38°C on the length of embryo development and egg fertility: This study was conducted at a temperature of 38°C, which is the maximal temperature at which the eggs remain viable. Eggs laid by females submitted to a temperature of 38°C were kept at this temperature for 12, 24, 36 or 48 h, then they were withdrawn and placed on draining board in order to record hatching. For each period of exposure, lots of 75 eggs were made and placed in one Petri dish, each. Each lot represented a replication and four replications were composed. The study was conducted under poor air relative humidity.

Effect of exposure at 40°C of different-aged eggs on embryo viability and hatched larvae survival: This study was conducted at a temperature of 40°C, the temperature at which eggs are no more viable. The eggs aged 24, 48 and 72 h were incubated at 40°C. Four lots of 75 eggs of each age category were placed in a petri dish each to

represent a replication. The air relative humidity conditions were 15 and 80%. After hatching, larvae were monitored for 24 h and mortality rates recorded.

Effect of temperature on development length and larval survival: Larval development was studied at 24, 25, 27, 30, 35, 38, 40, 41 and 42°C under a 15% air relative humidity. First-instar larvae obtained from rearing were distributed individually into 4 lots of 25 petri dishes, each lot representing a replication. Larvae were provided daily with ten day-old fresh pods at filling stage of the sensitive cowpea variety, KN1. The length of larval development corresponds to the period between hatching of eggs and fledgling.

Influence of temperature on reproduction parameters and female lifespan: The study was carried out at 25, 30, 35 and 40°C under a 15% air relative humidity. Mass-rearing newly hatched bugs were distributed by couple into the petri dishes. Four lots of 15 dishes were constituted, each representing a replication. These adults were fed on cowpea pods at filling stage in the same way as the larvae. Every 12 h and until female death, hatched eggs were retrieved and recorded. The duration of pre-oviposition is the period between female emergence and the beginning of hatching. When the female dies, the lifespan and pre-oviposition length were calculated.

Effect of air relative humidity on larval development, female fertility and longevity: These parameters were studied in poor (15%) and high (80%) air relative humidity conditions under optimal (30°C) and extreme (40°C) temperatures. The methodology was the same as for the studies on the influence of temperature.

Effect of water as feeding source on first-instar larvae survival: Study conditions were: air relative humidity of 15 or 80%, first-instar larvae submitted to an optimal temperature of 30°C and an extreme of 40°C. They were distributed individually just after hatching into 4 lots of 10 petri dishes. Each lot represented a replication. Three treatments were conducted: (I) control for which larvae were kept in the dishes without any feeding source, (ii) treatment with soaked cotton where water is available to larvae using the soaked cotton, (iii) the last treatment where a fresh cowpea pod at filling stage is available to larvae. Larval evolution was monitored until first exuviation.

Statistical Analysis: All the data were submitted to Analysis of Variance (ANOVA) using SAS^[10] software. Where the ANOVA probability was significant, means were separated using the Student Newman Keuls Multiple Comparison Test. Means were regarded as different when the test provided discrimination at a level of 5%.

RESULTS

Effect of temperature on embryo development length and egg fertility: Embryo development significantly varied in relation with temperatures ($p < 0.05$). It was inversely proportional to temperature increase with a mean range of 12.6 days at 24°C to 2.5 at 38°C (Fig. 1). The hatching rate was uniform between 24 and 35°C and higher at 98%. It decreased to 30% from 38°C and above this temperature, no embryo could survive (Fig. 1).

Effect of temporary exposure of eggs to the highest limit temperature of 38°C on embryo development length and egg fertility: The length of embryo development was inversely proportional to the increase of egg exposure time at the temperature of 38°C (Table 1). The hatching rate decreased with an increase in egg exposure time at 38°C from 98% for a 12 h exposure to 39% for 48 h.

Effect of exposure at 40°C of different-aged eggs on embryo viability and hatched larvae survival: Twenty four hour aged eggs continuously exposed to 40°C did not hatch whatever the air relative humidity. However, 20% of 48 h aged eggs exposed to that temperature hatched whatever the air relative humidity, but no larvae survived. Only 72 h aged eggs exposed to 40°C achieved a hatching rate of more than 92% with 22% of larval survival (Table 2).

Effect of temperature on development length and larval survival: Temperature had a significant influence on total development length and larval survival ($p < 0.05$). Increasing temperature between 24 and 40°C shortened larval cycle length by about 9 days (Fig. 2). Beyond 41°C, larval development could not be completed and the larvae died at the third stage of their development. Optimal temperatures for larval survival ranged between 25 and 35°C with a rate of more than 50% (Fig. 2).

Influence of temperature on reproduction parameters and female lifespan: *C. tomentosicollis* females expressed a reproductive activity at temperatures comprised between 25 and 40°C. The most important egg laying was observed at 30°C with a total of 218 eggs. More than 50% of the females submitted to a constant temperature of 40°C still lay eggs. This situation was, however, the less favorable to egg laying since it gave only a mean of 43 eggs. Temperature, also, significantly influenced the length of the pre-oviposition period ($p < 0.05$). The shortest pre-oviposition period was recorded at 30°C and the longest at 25°C (Table 3). Finally, the increase in temperatures significantly reduced female longevity (Table 3).

Table 1: Length of embryo development and fertility of *C. tomentosicollis* eggs in function of their time of exposure to a temperature of 38°C

Exposure time (hours)	Mean length ± SD of embryo development (days)	Mean proportion ± SD of fertile eggs (%)
12	3.44±0.16a	98.14±4.02a
24	3.00±0.00b	90.46±11.70a
36	2.96±0.13b	62.16±15.78b
48	2.66±0.24c	39.86±16.13c

Means±SD followed by the same alphabetical letter within a column are not significantly different at the probability level of 5%, using the SNK test

Table 2: Influence of humidity and exposure of various-aged-eggs to 40°C on the fertility of eggs and the survival of first-instar larvae

Age of eggs exposed to 40°C	15% relative humidity		80% relative humidity	
	Rate of fertile eggs (%)	Survival rate of 1st instar larvae (%)	Rate of fertile eggs (%)	Survival rate of 1st instar larvae (%)
24 h	0	0	0	0
48 h	20.62±16.02b	0	24.73±22.82b	0
72 h	92.00±10.41a	9.50±12.13	92.12±12.17a	22.5±14.9

Means±SD followed by the same alphabetical letter within a column are not significantly different at the probability level of 5%, using SNK test

Table 3: Influence of various temperatures on fertility, pre-oviposition length and longevity of *C. tomentosicollis* females

Temp. (°C)	Mean rate of fertile female (%)	Mean length (±SD) of pre-oviposition (days)	Mean number (±SD) of eggs laid by female	Mean longevity (±SD) of females (days)
25	63.64	14.96±10.51a	158.37±113.10b	47.69±6.71a
30	83.33	5.04±1.10c	218.04±86.00a	39.12±6.22b
35	57.41	9.34±7.34b	56.55±75.03cd	23.71±4.86c
40	68.42	7.13±4.49bc	43.00±51.71d	24.6±4.95c

Means±SD followed by the same alphabetical letter(s) within a column are not significantly different at the probability level of 5%, using SNK test

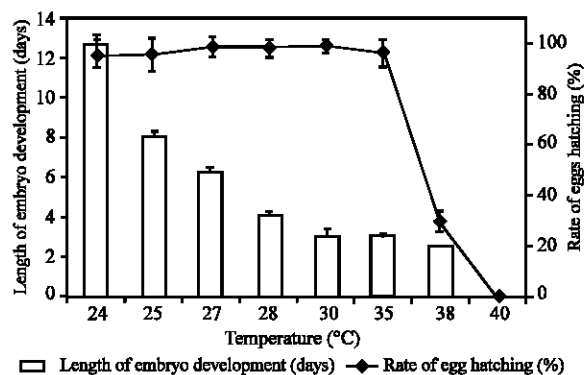


Fig. 1: Length of *C. tomentosicollis* embryo development (mean±SD) and rate of egg hatching under variable temperatures

Effect of air relative humidity on survival and length of larval development: Air relative humidity had significant incidence on larval survival whatever the temperature. High air humidity reduced larval mortality (Table 4). Larval development length was shorter under high air humidity at a temperature of 30°C. However, at 40°C, the increase in air humidity did not have significant influence on larval development length (Table 4).

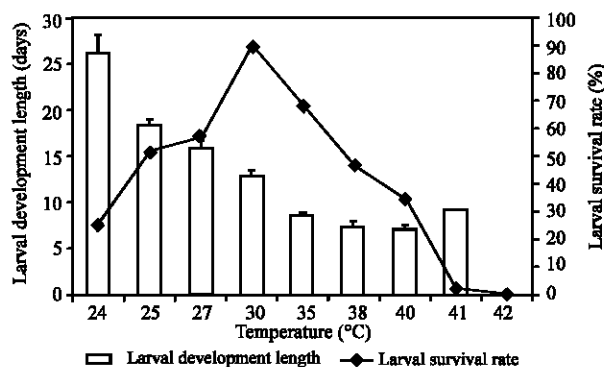


Fig. 2: Length of larval development (mean±SD) and larval survival rate of *C. tomentosicollis* under variable temperatures

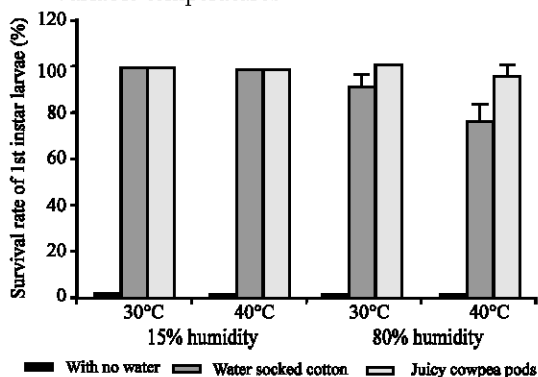


Fig. 3: Survival rate (mean±SD) of *C. tomentosicollis* first-instar larvae in function of water presence as feeding source under variable temperatures and air relative humidity

Effect of air relative humidity on female fertility and longevity: Air relative humidity did not have significant influence ($p>0.05$) on *C. tomentosicollis* reproductive activity, whatever the temperature. Hence, the rate of fertile females, the length of pre-oviposition period and the mean number of eggs laid were not affected by increased air relative humidity (Table 5). Female lifespan was significantly extended by increased air relative humidity at a temperature of 30°C whereas it had no effect at the limit temperature of 40°C (Table 5).

Effect of water as feeding source on the survival of first-instar larvae: The presence of water as feeding source was essential for the survival of first-instar larvae and their development to larval stage 2, whatever the temperature and air humidity (Fig. 3). The larvae died in dishes with no soaked cotton or pods, even in a humid environment.

Table 4: Survival rate and larval development length of *C. tomentosicollis* in function of air relative humidity

Temperatures (°C)	Air relative humidity (%)	Larval survival rate (%)	Mean length±SD of larval development (days)
30	15	93.39b	12.74±0.85a
	80	93.07a	10.23±1.13b
40	15	34.73b	7.13±0.56a
	80	55.72a	7.02±0.54a

Means±SD followed by the same alphabetical letter within a column are not significantly different at the probability level of 5%, using SNK test

Table 5: Effect of air relative humidity and temperature on reproduction parameters and lifespan of *C. tomentosicollis* females

Temp. (°C)	Air relative humidity (%)	Rate of		Number of eggs laid by female	Female lifespan (day)
		fertile females (%)	Pre-oviposition length (days)		
30	15	83.33	5.04±1.10a	218.04±86.0a	39.12±14.7b
	80	86.67	5.35±1.50a	183.19±78.2a	55.33±18.1a
40	15	68.42	7.13±4.50a	43.00±51.7a	24.6±10.2a
	80	56.60	6.82±3.20a	33.97±31.2a	21.23±8.4a

Means±SD followed by the same alphabetical letter within a column are not significantly different at the probability level of 5%, using SNK test

DISCUSSION

Temperature seems to be a major factor in the insect development. Increased temperatures reduce the length of embryo larval development. The egg is poorly sensitive to temperature variations between 24 and 38°C. Its thick inner shell membrane^[11] may be at the origin of embryo survival at high temperatures. Long exposure of eggs to temperature reduces hatching rate. However, it was shown that if an incubation period escapes this heat, eggs could hatch. In natural conditions, eggs deposited by females on lower parts of leaves^[12] may be a strategy to protect eggs against a long exposure to sun. The behavioral difference between *C. tomentosicollis* larvae and adults in the humid zone of Nigeria and Benin and those in Sahelian zone, could be explained by an adaptation of the insect to the Sahelian environment. In this regard, a total mortality of *C. tomentosicollis* larvae at 40°C has been reported in Nigeria^[11] whereas in our study conditions, we always had a 35% larval survival at 40°C. It was only at 42°C and above that the larvae could not complete their cycle and died at stage 3. The insect adaptation to the Sahelian environment is also demonstrated by the female capacity to lay eggs at a constant temperature of 40°C whereas in Nigeria, from simulations, the limit temperature for laying eggs was estimated to 39°C^[7]. We also noted the capacity of the bug to develop in a dry environment. In fact, the egg laying activity of *C. tomentosicollis* females is not reduced by a decrease in air humidity. Similarly, larval survival remained important under poor air humidity.

The species for which the diversification center might be humid Central Africa^[13] was, hence, adapted to this environment where it used to live for thousands of years.

It eventually adapted itself to high temperatures during its dispersion with cowpea towards the Sahelian zone.

The presence of water as a feeding source is essential for first-instar larvae development. These larvae use the water directly available or suck it from the pod. This conflicts with the observations that first-instar larvae do not feed and only need humidity in the form of water-soaked cotton for molting^[9]. Present study demonstrated that the prevailing humidity, even at saturation (80%), could not enable larval survival and the development to second stage. We could imagine that in rainy season, the insect feeds on rainwater on leaves and in dry season, on juicy pods. This shows again an adaptation of the insect to the Sahelian environment.

Since the first-instar larva depends on water or fresh pods and larval mortality being important at high temperatures, we could foresee that even if hatching occurs, the larvae would hardly survive between March and June. Water and juicy pods are scarce at that period. Adults supporting better this temperature seem more endowed to go through this period of high temperatures and low atmospheric humidity. The female could lay fertilized eggs, but very few larvae would survive after hatching.

This study demonstrated that temperature and air humidity variations are major factors which affect the biology of *C. tomentosicollis*, the cowpea pod-sucking bug. Similarly, the presence of water is essential for the feeding of young larvae. This water could be taken directly by larvae on the soaked cotton or the juicy pods. The insect acquainted itself to increasing temperatures revealing an adaptation to the hot Sahelian environment.

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