



# Morphology and biology of *Noorda blitealis* (Lepidoptera: Crambidae) immature instar for a biological control perspective

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## Abstract

*Moringa oleifera* (Capparales: Moringaceae) is a plant grown for its roots, stems and leaves. However, the leaves are attacked by insect pests including larvae of *Noorda blitealis* (Lepidoptera: Crambidae). These larvae cause a significant reduction in leaf yield and loss of income for growers. This study was conducted to determine some morphological and ecological aspects of larvae, using mass-reared larvae. Parameters such as larva length, body coloration, number of instars and their duration, damages caused on Moringa leaves, and larvae survival rate were collected. Results showed that *N. blitealis* larvae passed into 5 instars before pupating. The first instar larva averaged  $2.4 \pm 0.8$  mm in length and was light green. At the 5th instar, the larvae reach  $10.8 \pm 0.4$  mm and appear reddish. The mean duration of egg incubation was  $3.00 \pm 0.35$  days, that of larval instars and chrysalis were respectively,  $10.61 \pm 2.28$  days and  $9.78 \pm 0.42$  days. The Kruskal-Wallis test showed that the leaf attack rate depends on the larva's age (p-value < 0.0001). Larvae aged between 4 and 7 days attacked the greatest number of leaflets. Larvae survival rate was 86.67%, for 1st instar and 100% for the other instars. Knowledge of larvae's biological and morphological characters is necessary to identify the pest in the early stages of infestation and develop control methods based on using bioinsecticides could rapidly be developed.

**Keywords** *Moringa* · Insect pest · *Noorda Blitealis* · Crop protection · Biology

## Introduction

The insect pests are one of the most common constraints for Moringa cultivation (Halilou et al. 2022; Kabré et al. 2020; Kant et al. 2017; Yusuf and Yusuf 2014; Ojiako et al. 2012). According to Ke et al. (2016), more than 40 main pest species were found on the *Moringa* and each species can cause damage to different parts of the plant. Among these insect pests budworm (*N. moringae*), hairy caterpillar (*Eupterote mollifera*), bark caterpillar (*Indaberla tetraonis*), pod fly

(*Gitona distigma*), and leaf caterpillar (*N. blitealis*) cause major agricultural losses (Suman et al. 2024).

*N. blitealis* (Lepidoptera: Crambidae) is a small moth whose larvae are phytophagous and feed on *Moringa* leaves. Generally, the larvae remain on a thin silken web on the underside of the leaf and feed on the leaflets, resulting in the drying of the leaves into a papery structure (Ravindra et al. 2016). In cases of heavy infestation, the larvae can cause 100% defoliation of trees (Bedane et al. 2013; Satti et al. 2013). The larvae can also destroy the apical bud and make a gallery in the plant's central cylinder, which dries (Kant et al. 2017). The silken webs of larvae asphyxiate the leaves and eventually die (Dao et al. 2015).

*N. blitealis* is a small moth whose larvae are phytophagous and feed on *Moringa* leaves. Generally, the larvae remain on a thin silken web on the underside of the leaf and feed on the leaflets, resulting in the drying of the leaves into a papery structure (Ravindra et al. 2016). In cases of heavy infestation, the larvae can cause 100% defoliation of whole trees (Bedane et al. 2013; Satti et al. 2013). The larvae can also destroy the apical bud and make a gallery in the plant's central cylinder, which dries (Kant et al. 2017). Because of

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this damage, *N. blitealis* is an important barrier to *Moringa* cultivation (Ke et al. 2016).

To control these insect pests, particularly *N. blitealis* larvae, *Moringa* growers use bioinsecticides (Kabr e et al. 2022), synthetic chemical insecticides (Halilou et al. 2022; Ratnadass et al. 2011), and in some cases silvicultural practices such as pruning infested plants (Kabr e et al. 2022). These control methods are used singly or in combination, but the results are below growers' expectations. Furthermore, synthetic chemical insecticides present health risks for users, consumers, and the environment (Devine and Furlong 2007), and can lead to resistance phenomena in insect pests (Chaturvedi and Chaturvedi 2007; Srinivas et al. 2004).

In Burkina Faso, the extent of leaf damage has forced some growers who earned additional income from this crop to abandon it. However, *Moringa* is a plant that is being popularized by institutions to fight malnutrition among children and women (PNDES-II 2021) and poverty in rural areas by selling different parts of the tree. Given the socio-economic importance of *Moringa* for the population (Fadeyi et al. 2023; Tanga 2022), it is important to implement management strategies against *N. blitealis*.

This study aims to provide a better understanding of the bio-ecology of *N. blitealis* immature instars for a biological control perspective. Specifically, this involves (i) determine some characteristics of *N. blitealis* immature instars (larva length, body coloration, number of instars and their duration); (ii) assess damages caused by *N. blitealis* larvae on *Moringa* leaves and, (iii) assess larvae survival rate.

## Materials and methods

### Experimental site

The experiment was conducted in the Central Agricultural Entomology Laboratory (12°27'21,66 N; 1°32'59,46 W) in Ouagadougou (Burkina Faso). During experimentation, the mean laboratory temperature was  $26 \pm 1$  °C, the relative humidity was  $62 \pm 5\%$  and the photoperiod was 12 h.

### Origin of larvae used for rearing

In the absence of a laboratory strain, mass rearing was carried out using *N. blitealis* larvae collected from *Moringa* plants at the nursery of the Environment and Forestry Department (12°22'49.90 N; 1°30'15.40 W) of the National Centre for Scientific and Technological Research (CNRST). The *Moringa* plants were less than a year old. The larvae collected were transported to the laboratory in 900 ml glass containers containing sand and fresh *Moringa* leaves.

## Rearing

Rearing was first realized in 900 ml glass containers. Fresh *Moringa* leaves were served as food for the larvae. The cut ends of these leaves were wrapped in hydrophilic cotton impregnated with water and covered with pieces of polyethylene. This was done to prevent water loss from the leaves and keep them turgid. Each glass jar was covered with a piece of muslin cloth fastened with a rubber band. One-third part of the jar was filled with moist sand, which provides optimal conditions for pupation. The grown-up larvae pupated in the glass containers. The emerged moths were next released in rearing cages measuring 60 cm x 60 cm x 60 cm. They were fed with a 10% sugar solution suspended inside the cage. Fresh *Moringa* plants were used for egg-laying.

The eggs laid are then collected and stored in petri dishes until they hatch. Newly emerged larvae were used in the present study.

## Data collection

### Characteristics of *N. Blitealis* immature instars

#### Larva length, body coloration and number of larval instars

Larvae length and body color were the morphological characteristics determined in this study. To this end, 15 newly emerged larvae were placed individually in 90 mm diameter Petri dishes and fed daily with fresh *Moringa* leaves. Food in each petri dish was changed daily by bringing fresh *Moringa* leaves from the field. The larvae length was recorded on graph paper after immobilization in the cold. The body color was determined visually with the naked eye. The number of larval instars was determined at pupation by counting the number of larval capsules rejected (Tano et al. 2011).

#### Immature instars duration

The following durations were determined:

- **Egg incubation:** leaves containing eggs were removed and placed in 90 mm Petri dishes and covered with muslin cloth fastened with a rubber band. Observations were made daily. A total of 15 eggs were collected and observed until the larvae emerged.
- **Larva instars** by observing and recording time elapse between the time between two head capsule discharge. These observations were made on 30 larvae that emerged on the same day, using a binocular magnifier Optika brand, coupled to a monitor. The total duration

of larval life was obtained by summing the durations of the five larval instars.

- **pupal instar** by recording the time elapsed between imaginal molting until the apparition of the adult moth in 15 last-stage larvae.

### Assessment of damage caused by *N. Blitealis* larvae on *Moringa* leaves

#### Type of damage according to larval instars

The type of damage caused by each larval stage of *N. blitealis* was observed. This observation was made with the naked eye. It was carried out using the same larvae as those on which the measurements were taken.

#### Quantity of *Moringa* leaflets consumed according to larva age

This parameter was determined according to the protocol described by Dao et al. (2015). The protocol consisted of depositing a newly emerged larva on a potted *Moringa* plant in a 60 cm x 60 cm x 60 cm rearing cage. The number of leaflets on the plant was counted and marked at the start of the test. Daily observations were made until larvae were pupated. During these observations, the attacked leaflets were pulled off. A leaflet was considered attacked when it showed visible signs of attack. The seedling was replaced when all its leaves had been consumed by the larva. A total of 15 replicates were used for this determination.

#### Larvae survival rate

Newly emerged larvae were placed individually in 90 mm-diameter petri dishes. They were fed daily on fresh *Moringa* leaves. The larvae were observed daily in the event of death. The Optika binocular magnifier coupled to a monitor was used to observe larvae and count the number of cephalic capsules present in the petri dish. The number of cephalic capsules has been used to determine the corresponding larval instar (Tano et al. 2011).

#### Data analysis

Analyses were performed using R software 4.4.1. The Shapiro test showed that the data did not follow a normal distribution, so the length of the larvae and the number of leaves consumed for each larval stage were compared respectively using the Kruskal-Wallis test. In addition, a  $\text{Khi}^2$  test was used to compare larval survival rates. All statistical tests were performed at the 5% significance level.

## Results

### Characteristics of *N. Blitealis* immature instars

#### Larvae length, body coloration, number of instars

Based on the number of cephalic capsules rejected, 5 larval instars were determined. Each larval instar differs from the others by the length and the color of its body. The average length of 1st instar was  $2.4 \pm 0.8$  mm. The 2nd instar larva averaged  $4.8 \pm 1.2$  mm in length, reaching an average of  $7.7 \pm 0.6$  mm in the 3rd instar. At the last instar, larvae averaged  $10.8 \pm 0.4$  mm. The Kruskal-Wallis test showed a statistically significant difference between larvae of different stages ( $p < 0.05$ ). Body color changes from light green in 1st instar to red in 5th instar (Fig. 1).

#### Immature instar durations

The incubation period of the eggs was recorded as  $3.00 \pm 0.35$  days. The total larva life was  $10.61 \pm 2.28$  days. The 5th instar was the longest, with an average of  $3.12 \pm 0.64$  days, while the 4th instar was the shortest, with an average duration of  $1.62 \pm 0.51$  days. The chrysalis phase takes on average  $9.78 \pm 0.42$  days. From egg laying to adult emergence was  $23.14 \pm 1.21$  days (Table 1).

### Assessment of damage caused by *N. Blitealis* larvae on *Moringa* leaves

#### Type of damage

Observations showed that the type of damage caused to *M. oleifera* leaflets varied with the larval instars. Larvae of 1st and 2nd instar feed on the underside of *Moringa* leaves. The other instars (3rd, 4th and 5th) gnaw on leaf blades, causing perforations or total loss of leaves (Fig. 2).

#### Quantity of *Moringa* leaflets consumed according to larva age

Results showed that the mean of leaflets attacked varied with larvae age ( $p < 0.0001$ ). Larvae at 4 and 7 days after emergence attacked more leaflets than younger or older larvae (Table 2).

#### Survival rate

Results on larval survival rates showed that *N. blitealis* larvae had a survival rate of over 90% in the laboratory. The first larval instar showed a survival rate of 86.67%, while larvae of other instars showed survival rates of 100% (Fig. 3). The  $\text{Khi}^2$  test showed that there was no statistically significant difference between the survival rates ( $p = 0.81$ ).

**Fig. 1** Characterization of *N. blitealis* larval instars1st instar  
average length: 2.4±0.8 mm  
Color: light green2nd instar larva  
average length: 4.8<sup>b</sup>±1.2 mm  
Color: light green3rd instar  
average length: 7.7±0.6 mm  
Color: dark green4th instar  
average length: 8.9±1.3 mm  
Color: dark green5th instar  
average length: 10.8±0.4mm  
Color: redchrysalis  
average length: 8.77±2.25 mm  
Coloration : metallic red**Table 1** Duration of immature instars

Instars	Mean ± sd* (day)	Minimum -Maximum. (day)
Egg	3.00 ± 0.35	2–4
1str	2.00 ± 0.35	2–3
2nd	1.87 ± 0.35	1–2
3rd	2.00 ± 0.53	1–3
4th	1.62 ± 0.51	1–2
5th	3.12 ± 0.64	3–4
Larva to chrysalis (1st to 5th instar)	10.61 ± 2.28	8–14
Chrysalis	9.78 ± 0.42	9–10
Overall	23.39 ± 3,05	19–27

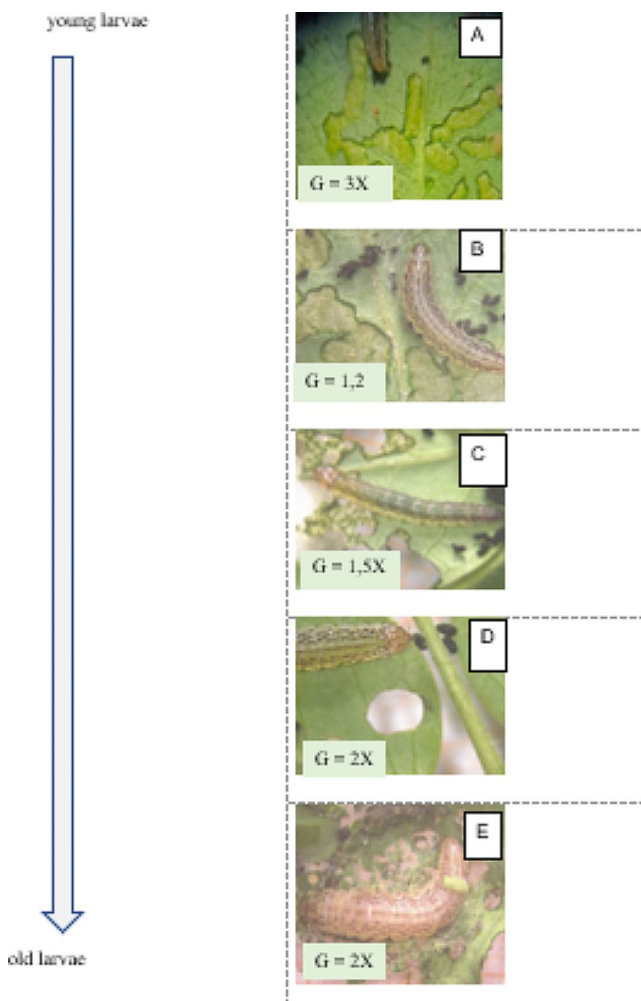
\*sd: standard deviation

## Discussion

### Characteristics of *N. Blitealis* immature instars

The results showed that these larvae, like all holometabolous insects, develop through successive molt. In *N. blitealis*, five (05) larva instars were identified based on the number of head capsules rejected. Similar results were obtained by Ratnadass et al. (2011) who based their analysis

on the observation of larval behavior, and those of Sharjana and Mikunthan (2019) who used the morphometry of the head capsule. Capsule rejection is associated with rejecting the larva's old integument as an exuvium. This process is responsible for the insect's growth and passage from one larval stage to another. The length of the larvae increased rapidly. These larvae grew from less than 2 mm at egg eclosion to more than 10.8 mm at the 5th instar. This rapid development is associated with a variation in larval coloration. The rapid growth of the larvae could be explained by the presence of the food substrate represented by the Moringa leaves, which they actively consume. As they grow, *N. blitealis* larvae change their color from light green to red. The variation in the color could be a homotypy or mimicry mechanism that allows the larvae to be camouflaged from their predators, adapting their coloring to the leaves of the host plant (Vukusic and Chittka 2012). Stage 1–4 larvae are green and resemble the leaves of the host plant, making them easy to camouflage. Stage 5 larvae and pupae are immobile, so red color could be an aposematic color designed to scare off predators. These stages are immobile. Homotypy and mimicry are evolutionary mechanisms that ensure the survival of a species in its battle for existence (Frawal 2018; Shamim et al. 2014). Other authors explain this variation in color for thermoregulatory needs (Umbers et al. 2013), this



**Fig. 2** Type of damage caused by larval instars of *N. blitealis* on *Moringa* leaf (A: 1st instar; B: 2nd instar; C: 3rd instar; D and F: 4th instar; E: 5th instar; G: magnification)

**Table 2** Mean number of leaflets attacked by *N. Blitealis* larvae instars

Days	Mean number of leaflets consumed $\pm$ sd
1st	Less than one
2nd	1.4 $\pm$ 0.51
3rd	2.8 $\pm$ 0.63
4th	4.8 $\pm$ 1.03
5th	5.9 $\pm$ 1.85
6th	6.3 $\pm$ 1.56
7th	6.2 $\pm$ 2.25
8th	3.9 $\pm$ 1.3
9th	1.0 $\pm$ 0.66
10th	Less than one
11th	Chrysalis
12th	Chrysalis
Total	32,8 $\pm$ 10,31
P-value	< 0.0001

being controlled by biotic and abiotic factors (Tanaka and Nishide 2012; Verlinden et al. 2009). From the preceding,

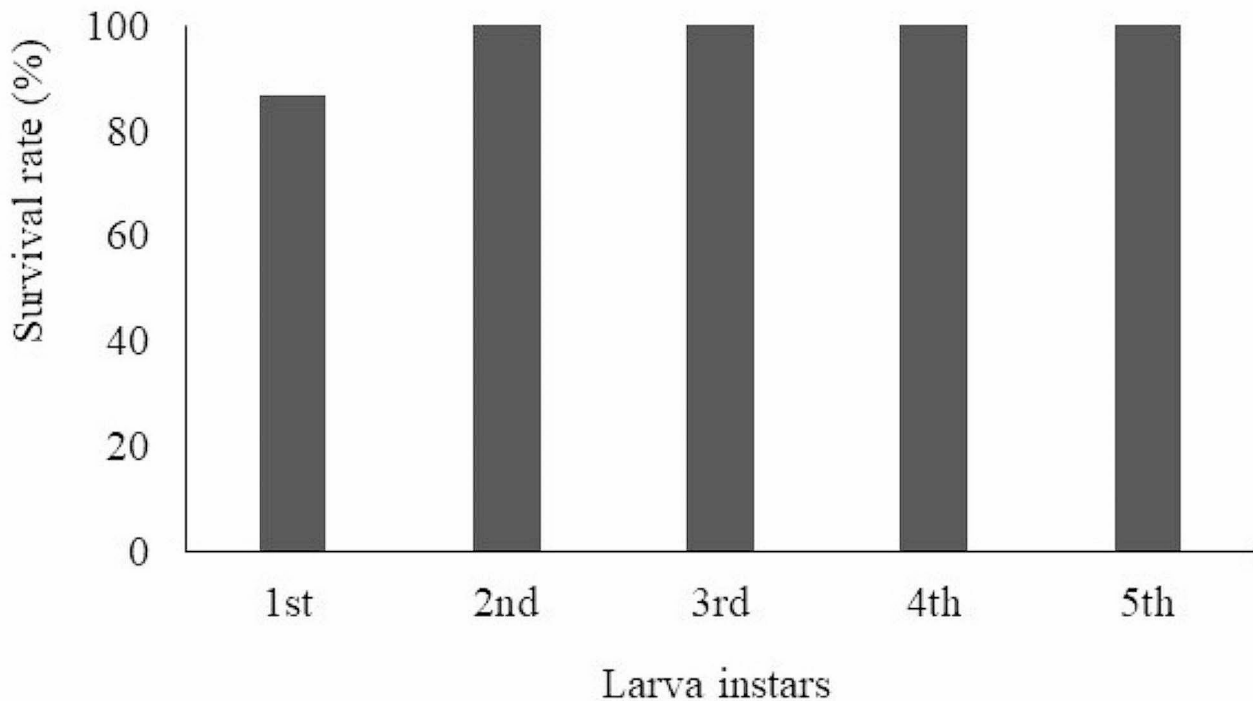
we can say that in *N. blitealis*, the most plausible explanation for the change in body color is homotypy. The lifetime observed from 1st instar to pupation was  $10.61 \pm 2.28$  days. This result corroborates those of Subramoniam and Chitra (2019) who had obtained durations varying between 9 and 12 days under laboratory conditions almost similar to those of our study. In addition, the total lifespan of the immature *N. blitealis* stage is an average of  $23.39 \pm 3,05$  days.

### Damage caused on Moringa leaves

The observation showed that 1st and 2nd instar larvae feed on the underside of *Moringa* leaves. This feeding method makes the leaves chewy and transparent, limiting their photosynthesis ability. The other instars (3rd, 4th and 5th) gnaw on leaf blades, causing perforations or total loss of leaves. Furthermore, observations have shown that larvae of all stages consume *Moringa* leaves but the type of damage varies according to the larva instar considered. Larvae aged between 4 and 7 days are the most voracious on leaves, compared with larvae from other days. Two hypotheses could explain this situation. The first is based on the fact that the buccal pieces of young larvae are not sufficiently developed to cut and crush leaves. The second is that, according to coevolutionary theory, the nutrition of individuals depends on the enzymes they have at their disposal to ensure their proper digestion (Streblor 1980). In this way, the nutrients contained in *Moringa* leaves are digested by the larval stages, which can synthesize the enzymes necessary for their digestion. In addition, this could be explained by the fact that larvae from days 4 to 7 need to store sufficient food as an energy source.

### Survival rate

In this study, larval survival rates ranged from 86.67% for 1st instar larvae to 100% for larvae of other instars. Umbers et al. (2013) obtained larval survival rates of 98.33% in *Spodoptera frugiperda* larvae reared at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  relative humidity, and a 14-hour photophase. This high survival rate is due to the fact that the larvae were reared individually in Petri dishes, thus preventing competition for food. For their survival and normal development, insects consume specific diets (Behmer 2009) which must contain proteins and carbohydrates. These two nutrients provide them with the essential amino acids and energy they need (Wang et al., 2018). In this way, *N. blitealis* larvae find all the nutrients they need for survival and growth in *Moringa oleifera* leaves. The mortality observed in first-day larvae could be explained by the fact that, at this age, larvae are unable to metabolize correctly the toxins or non-nutritive compounds contained in *Moringa* leaves.



**Fig. 3** Survival rate of *N. blitealis* larva

## Conclusion

The present study reports on the biological and morphological aspects of *N. blitealis* larvae on *Moringa oleifera* trees. The results show that all larva stages feed on Moringa plants, causing significant losses and loss of income for growers. Knowledge of the larvae's biological and morphological characteristics could help develop biological control methods capable of limiting its impact on Moringa plantations.

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## Declarations

**Conflict of interest** The authors declare no conflict of interest.

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