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Morphology, development and reproduction of *Atractomorpha acutipennis* (Guérin-Méneville, 1844) (Orthoptera: Pyrgomorphidae)

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Abstract: For a better knowledge of *Atractomorpha acutipennis* bio-ecology and to facilitate its identification, we studied the morphology, development and reproduction of this grasshopper on *Manihot esculenta* under laboratory conditions. Five hundred and fifty-one first nymphal stages obtained in the laboratory were reared in cages. Some body parts allowed a clear identification of different nymphal instars. Post-embryonic development passed through six stages (males) and seven stages (females). Mean nymphal development time was 17.14 ± 0.62, 12.91 ± 0.62, 13.45 ± 0.69, 13.80 ± 0.68, 15.23 ± 0.55 days respectively in males nymphal instars 1 to 5 and 16.18 ± 0.54, 13.13 ± 0.59, 12.49 ± 0.42, 13.19 ± 0.58, 14.58 ± 0.61, 16.57 ± 0.68 days respectively, in females nymphal instars 1 to 6. Adult females deposited an average of 3.67 ± 2 egg pods each comprising 30.77 ± 10.5 eggs. First mating was observed 18 ± 15.42 days after the last moult. Oviposition occured on average 19.33± 5.33 days after the first mating. This study provides important information about the biology of *A. acutipennis*, which could help in developing control methods against this grasshopper in southern Cameroon.

Key words: Grasshopper; nymphal development instars; *Manihot esculenta*.

Introduction

Grasshoppers are an important taxon in food chains (Badenhausser 2012). Because of their trophic importance, they are receiving increasing attention among environmentalists and environmental managers. They are important preys for many birds, reptiles, spiders and other insectivorous animals (Barataud 2005). Grasshoppers are highly dependent on vegetation, making them increasingly recognized biological indicators of anthropogenic

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disturbance and community structure (Badenhausser 2012). These insects play a very important role in the cycling of organic matter; they promote the growth of plants from their easily assimilated droppings (Blummer and Diemer 1996; Barataud 2005). However, they are also common insects of grassy vegetation from which they attack crops, thus contributing to the maintenance of starvation (Mestre 1988).

Atractomorpha acutipennis (Guérin-Méneville, 1844) (Orthoptera: Pyrgomorphidae) is a pest which attacks leaves of plants such as cotton, rice, sweet potatoes, tobacco, alfalfa, market gardening, *Amaranthus*, Poaceae (Launois-Luong and Lecoq 1989) and *Ricinodendron heudelotii* (Alene et al. 2004). It is a phytophilous, hygrophilous herbaceous insect that tolerates a wide range of rainfall conditions (Launois-Luong and Lecoq 1989). This grasshopper is found throughout the southern part of the African continent. It has been reported on the West African coast bordering the Indian Ocean, in Madagascar around the Nossi-Bé region (Ottes 1994) and in several West African countries, East, South (Paraïso et al. 2012) and Center including Benin, Guinea-Bissau, Mali, Liberia, Niger, Nigeria, Cameroon, Senegal, Sierra Leone, Chad and Togo (Mestre and Chiffaud 2006). In nature, there are three known subspecies: *A. acutipennis acutipennis* (Guérin-Méneville, 1844) in Madagascar; *A. acutipennis brevis* (Uvarov, 1938) in South-West Asia and in the North-East African region; *A. acutipennis gerstaeckeri* (Bolívar, 1884) in sub-saharan Africa (Banerjee and Kevan 1960) and Madagascar (Roy 2003). *Atractomorpha acutipennis* is a continuous breeding insect in the Sahel, with 2 to 3 generations per year; eggs, nymphs and adult can be found all the year round, adults being reproductively active during the dry season (Braud et al. 2014). Apart from the reports of the presence of the species, no study exists on the characteristics of this species in the forest zone (development, reproduction and pest status). However, with the current pace of landscape changes, the pest status of *A. acutipennis* could increase because of the adoption of new cultivated crops varieties (Launois 1996). Those changes are observed amongst other pyrgomorpidae as *Zonocerus variegatus* (Linnaeus, 1758) (Kekeunou 2007). It is therefore important to conduct a research study for the control of this species in its area of depredation. However, the development of effective control strategies requires a good identification and a good knowledge of the bio-ecology of this species. Hence, the objectives of this work were to: (1) evaluate the number of post-embryonic stages and durations of each nymphal stage of *A. acutipennis*; (2) study its reproductive behavior; (3) establish the morphological characteristics of the different post-embryonic developmental stages, and (4) establish an identification key for the different nymphal instars. This work will contribute to better understanding the biology of *A. acutipennis,* which could be useful for developing long-term control strategies against this grasshopper in the forest zone.

Material and methods

Sampling site of Atractomorpha acutipennis *adults reared for first nymph instar production*

Sampling was done in Nkolbisson, a borough of Yaoundé town (3°27'- 4°10'N 11°32'-11°49'E), located in the humid forest zone of Southern Cameroon. The Yaoundé city is characterized by alternating hills and swampy lowlands (Fomekong et al. 2008). The geological substratum of this city is covered by sandy-clay alluvia in the thalwegs and by lateritic soils that can be exploited by wells and boreholes on the hillsides (Kuitcha et al. 2008). The vegetation belonging to the so-called semi-deciduous forest is very degraded because of anthropogenic activities. Yaoundé is under the influence of an equatorial climate

of Guinean type with four seasons: a long dry season (from mid-November to mid-March); a short rainy season (from mid-March to the end of June); a short dry season (July to August) and a long rainy season (September to mid-November) (Olivry 1986). Temperatures range from 22 to 29°C (Santoir and Bopda 1995). Captures were conducted in a herbaceous vegetation dominated by the presence of Poaceae (*Panicum maximum* and *Axonopus compressus*), Cannaceae (*Canna indica*), Acanthaceae (*Asystasia gangetica*), Aderaceae (*Aspilia africana*), Asteraceae (*Acanthospermum hispidum*), Moraceae (*Ficus mucuso*), Fabaceae (*Senna corymbosa*, *Senna obtrisifolia* and *Mimosa pudica*), and Euphorbiaceae (*Manihot esculenta*).

First nymph instar production and monitoring in the laboratory

Two series of adults (about 20 in each series) were captured in the herbaceous vegetation in Nkolbisson with a sweep net and reared in the laboratory of Zoology (Faculty of Science, University of Yaoundé 1) from January to March 2014 (first series) and from July to September 2014 (second series) until the obtainment of the first nymphal instars. Each rearing series was done in two transparent cages (21 cm in diameter and 18 cm in height) provided with a lid mesh (mesh = 1 mm^2) (type a cages).

After hatching, the first nymphal instars produced were introduced individually into the type b cages (transparent cylindrical plastic boxes in polypropylene 13 cm in diameter and 9 cm high). These nymphal instars were monitored from February to July 2014 (first rearing series) and from August 2014 to March 2015 (second rearing series). The cages were labeled with the hatching date, number and sex of the individual. Daily observations consisted of searching the exuvia of each nymph and recording the date of exuviation (moulting). The dead individuals were removed from the cages for later morphological and morphometric studies.

Each rearing cage type (a and b) contained (1) sterilized sand and (2) a dry stem of *Chromolaena odorata* to support individuals. Sand which served as oviposition medium was regularly moistened with tap water until the end of rearing to maintain the humidity level in the cages thereby facilating egg and larval development. In all types of rearing, cages were cleaned every two days and at the same time, fresh leaves of *Manihot esculenta* serving as food were introduced.

Study of reproduction in the laboratory

Fourteen couples of adults, obtained after rearing first nymphal instars in the laboratory, were formed. Each couple was placed for rearing in a type b cage without sand and monitored until the death of the couple. Observations were made every two days and the premating behavior, the date of the copulation, the duration of copulation and the date of egg pod deposit were noted. After reproduction, the deposited egg pod was removed, measured and stored in 70% alcohol for subsequent egg counting. During the rearing, cages were cleaned every two days and at the same time, the fresh leaves of *Manihot esculenta* serving as food were renewed.

***Morphological and morphometric characteristics of* Atractomorpha acutipennis**

Studies of *A. acutipennis* in the laboratory focused on individuals fixed in 70% alcohol. This study was carried out on 55 individuals at stage 1, 61 at stage 2, 59 at stage 3, 52 at stage 4, 48 at stage 5, 30 at stage 6 nymphs and 61 adults (Table 1).

The study consisted of counting the number of antennal articles (Naa), the number of

internal (Nei) and external (Nee) spines using a binocular lens. The measurements and the way of measuring the parts concerned the following parameters chosen with reference to the works of De Grégorio (1987) and Default (2012): the total length of the body (Lt), the length of the cephalic capsule (Lcc), the width of the cephalic capsule (lcc), the pronotum length (Lpr), the thoracic length (Lth), the abdominal length (Labd), the length of the antenna (La), the length of the tegmina (Lel), the membranous wing length (Lai), the lengths of the pro-, meso- and metathoracic thighs (Lcu1, Lcu2 and Lcu3), the lengths of the pro, meso- and metathoracic tibia (Lti1, Lti2 and Lti3), and finally the length and diameter of the eggs (Tables 2 and 3).

Collection of laboratory meteorological data

The room temperature and the humidity of the Zoology laboratory of the University of Yaoundé 1 were recorded three times a day (morning between 8:00 am and 12:00 pm, the afternoon between 12:00 pm and 3:00 pm and the evening between 3:00 pm and 6:00 pm) thanks to a Gottingen thermohygrograph. The laboratory humidity ranged from 61.31% to 95% with an average of 79.31%. The least humid month was January 2015 and the wettest month was August 2015. The temperature ranged between 21.15°C in September and 29.98°C in April with an average of 28.54°C.

Statistical analysis

Database was developed using the Excel version 2010 software. The PAST software (version 3.14.) and SAS 9.1 were used to calculate the averages, the associated standard deviations and the 95% confidence intervals for the different morphometric parameters measured and development time. The normality of the quantitative variables was tested using Shapiro-Wilks or Jarque-Bera tests. In the case of normality, we compared the averages using the ANOVA (followed by the Tukey (HSD) post hoc test) or the unpaired student *t* tests. In the absence of homoscedacity, the Welch test was used instead of the ANOVA test. In this case, the Aspin Welch t' test was used to compare two independent samples. In the absence of normality, these averages were compared using the Kruskall-Wallis (followed by the Wilcoxon post hoc test) or Mann-Whitney-Wilcoxon (MWW) tests. All probabilities were assessed at the 5% threshold.

Results

***Number and duration of post-embryonic development stages of* Atractomorpha acutipennis**

Post-embryonic development of *A. acutipennis* in the laboratory passed through 6 stages for males and 7 for females. Total nymphal development time ranged from 49 to 96 days, with an average of 77.05 ± 0.5 days. This duration had a higher average in female nymphs

Table 1. Number of individuals observed and measured.

Nymphal stages	Stage 1 nymphal	Stage 2 nymphal	Stage 3 nymphal	Stage 4 nymphal	Stage 5 nymphal	Stage 6 nymphal	Adult	Total
Males	25	32	29	24	23	/	31	164
Females	30	29	30	28	25	30	30	202
Total	55	61	59	52	48	30	61	366

Table 2. Measurements of body parts of *Atractomorpha acutipennis* male. Each value represents the average and its confidence interval. Values in parentheses represent the minimum, maximum, and sample size. For each column, the same letters reflect the non-significant differences between the averages. H is the value of the Kruskal-wallis test and F value of the ANOVA test.

Nymphal Stages	Lt	Lcc	lcc	Lth	Labd	Lpr	La	Lel	Lai	Lcu1	Lcu2	Lcu3	Lti1	Lti2	Lti3	Na	Nee	Nei
L1	5.06±0.10	1.80±0.04	0.94±0.02	1.14±0.03	2.38±0.07	0.81±0.01	1.44±0.04	/	/	0.85±0.01	0.94±0.01	2.31±0.03	0.74±0.01	0.83±0.01	2.09±0.02	6±0	10.76± 0.09	10.92± 0.06
	(4.1-6.2)	(1.2-2)	(0.8-1)	(0.9-1.5)	(2-3.2)	(0.6-0.9)	(1-1.8)			(0.7-1)	(0.8-1.1)	(2-2.5)	(0.6-0.9)	(0.7-0.9)	(1.9-2.2)	(6-6)	(10-11)	(10-11)
	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**			(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**	(25) **a**
L2	8.18± 0.12	2.6±0.06	1.00±0.01	1.78±0.04	3.83±0.08	1.33±0.05	1.91±0.02	/	/	1.11±0.02	1.23±0.02	3.35±0.03	0.98±0.01	1.07±0.01	3.07±0.02	6±0	10.47±0.09	10.75±0.08
	(7-9.8)	(2-3)	(0.9-1.1)	(1.2-2.1)	(3-5.3)	(1.2-3)	(1.7-2.1)			(1-1.3)	(1-1.4)	(3-3.8)	(0.9-1.2)	(0.9-1.3)	(2.9-3.3)	(6-6)	(10-11)	(10-11)
	(32) **b**	(32) **b**	(32) **b**	(32) **b**	(32) **b**	(32) **b**	(32) **b**			(32) **b**	(32) **b**	(32) **b**	(32) **b**	(32) **b**	(32) **b**	(32) **a**	(32) **a**	(32) **a**
L3	10.26±0.20	3.46±0.06	1.25±0.02	2.15±0.05	4.66±0.13	1.84±0.04	2.61±0.06	/	/	1.68±0.03	1.73±0.03	4.69±0.06	1.37±0.03	1.44±0.03	4.17±0.06	6±0	9.82±0.11	10.03±0.09
	(7.9-12.1)	(3-4)	(1-1.4)	(1.73-3)	(3.2-6)	(1.1-2)	(1.9-3)			(1.4-1.9)	(1.4-2)	(4-5.3)	(1-1.7)	(1.2-1.8)	(3.5-4.9)	(6-6)	(8-11)	(9-11)
	(29) **c**	(29) **c**	(29) **c**	(29) **c**	(29) **c**	(29) **c**	(29) **c**			(29) **c**	(29) **c**	(29) **c**	(29) **c**	(29) **c**	(29) **c**	(29) **a**	(29) **b**	(29) **b**
L4	13.29±0.30	4.26±0.07	1.59±0.07	3.08±0.08	6.09±0.21	2.59±0.07	3.68±0.07	/	/	2.11±0.03	2.25±0.04	6.03±0.10	1.8±0.04	1.94±0.04	5.37±0.11	8.13±0.35	10.04±0.15	10±0.12
	(10-16)	(3.5-5)	(1.3-2.9)	(2.2-4.1)	(4.1-8)	(2-3.3)	(2.9-4.2)			(1.9-2.5)	(2-2.7)	(4.9-6.9)	(1.52.5)	(1.3-2.2)	(4.1-6.5)	(6-11)	(8-11)	(9-11)
	(24) **d**	(24) **d**	(24) **d**	(24) **d**	(24) **d**	(24) **d**	(24) **d**			(24) **d**	(24) **d**	(24) **d**	(24) **d**	(24) **d**	(24) **d**	(24) **b**	(24) **b**	(24) **b**
L5	13.4±0.43	4.52±0.12	1.80±0.08	3.56±0.15	5.75±0.21	2.94±0.09	4.14±0.15	2.97±0.24	3.43±0.30	2.42±0.08	2.54±0.09	6.91±0.20	1.99±0.07	2.36±0.19	6.30±0.17	11±0.11	10.22±0.11	10.17±0.17
	(10.2-17.5)	(3-5.6)	(1.2-2.8)	(2.5-5)	(4.5-8.2)	(2.3-3.8)	(3-5.5)	(1.5-5)	(1.3-5.5)	(2-3)	(2-3.3)	(5.8-9)	(1.5-2.7)	(1.7-6.4)	(5.1-8)	(10-12)	(9-11)	(9-11)
	(23) **d**	(23) **d**	(23) **d**	(23) **d**	(23) **d**	(23) **d**	(23) **e**	(23) **a**	(22) **a**	(23) **d**	(23) **e**	(23) **d**	(23) **d**	(23) **e**	(23) **e**	(23) **c**	(23) **b**	(23) **a**
Adults	18.67±0.25	5.91±0.07	1.97±0.04	5.02±0.08	8.19±0.18	3.96±0.06	6.67±0.12	16.93±0.21	15.09±0.19	3.43±0.07	3.79±0.07	9.63±0.11	3.02±0.04	3.13±0.04	8.47±0.16	11.84±0.15	10.74±0.10	10.77±0.10
	(16.5-21.4)	(4.7-6.9)	(1.6-2.7)	(4-5.9)	(6.2-10.2)	(3-4.5)	(5-8.5)	(14.5-18.9)	(12.3-17)	(2.7-4.2)	(3.2-4.9)	(8.1-10.9)	(2.5-3.5)	(2.5-3.7)	(4.89.8)	(11-13)	(10-12)	(10-12)
	(31) **e**	(31) **e**	(31) **e**	(31) **e**	(31) **e**	(31)	(31) **f**	(31) **b**	(31) **b**	(31) **e**	(31) **f**	(31) **e**	(31) **f**	(31) **f**	(31) **f**	(31) **d**	(31) **a**	(31) **a**
Test	**F**=430.2	**H**=157.9	**H**= 150.4	**H**= 155.3	**H**= 147.0	**H**= 157.2	**F**= 574.3	**H**=89.7	**H**=89.7	**H**= 160.9	**F**= 518.9	**H**= 161.1	**H**=159.5	**H**= 187.9	**H**= 174.4	**H**=199.2	**H**=70.3	**H**=135.8
P-values	**P**<0.0001	**P**<0.0001	P<0.0001	P<0.0001	**P**<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	**P**<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Lt: total length of the body, Lcc : length of the cephalic capsule, lcc : width of the cephalic capsule, Lth : length of the thorax, Labd : length of the abdomen, Lpr: length of pronotum, La : length of the antenna, Lel : length of the elytra, Lai : length of the hind wing, Lcu1, Lcu2, Lcu3 : length of the femur, length of median femur, length of anterior femur Lti1, Lti2 et Lti3 : length of the posterior tibia, length of the median tibia, length of the anterior tibia, Na : Number of antennal articles, Nee and Nei: Number of external and internal spines.

Table 3. Measurements of body parts of *Atractomorpha acutipennis* female. Each value represents the average and its confidence interval. The values in the parenthesis represent the minimum, the maximum, and the size of the sample. For each column, the same letters reflect the non-significant differences between the averages. H is the value of the Kruskal-wallis test and Fvalue of the ANOVA test.

Nymphal Stages	Lt	Lcc	lcc	Lth	Labd	Lpr	La	Lel	Lai	Lcu1	Lcu2	Lcu3	Lti1	Lti2	Lti3	Na	Nee	Nei
L1	5.18± 0.11	2.1±0.04	0.86±0.03	1.17±0.04	2.20±0.04	0.87±0.03	1.47±0.04	/	/	0.88±0.04	0.89±0.05	2.54±0.07	0.76±0.02	0.84±0.03	2.36±0.07	6±0	10.6±0.09	10.9±0.06
	(4.2-6.8)	(1.7-2.8)	(0.7-1.5)	(0.9-1.8)	(1.9-2.8)	(0.7-1.5)	(1.1-2.1)			(0.7-1.6)	(0.05-1.8)	(2.1-3.9)	(0.6-1.2)	(0.7-1.5)	(1.9-3.9)	(6-6)	(10-11)	(10-11)
	(30) **a**	(30) **a**	(30) **a**	(30) **a**	(29) **a**	(30) **a**	(30) **a**			(30) **a**	(30) **a**	(30) **a**	(30) **a**	(30) **a**	(30) **a**	(30) **a**	(30) **a**	(30) **a**
L2	8.50±0.15	2.70±0.06	1.02±0.01	1.88±0.04	3.98±0.12	1.29±0.02	1.91±0.02	/	/	1.14±0.02	1.36±0.08	3.38±0.10	1.01±0.02	1.2±0.07	3.12±0.05	6±0	10.62±0.09	10.86±0.07
	(7-10)	(2-3.1)	(0.9-1.2)	(1.5-2)	(3-5.1)	(1.2-1.7)	(1.5-2.2)			(1-1.5)	(1-3.6)	(0.9-4.5)	(0.9-1.2)	(1-3.1)	(2.6-4.2)	(6-6)	(10-11)	(10-11)
	(29) **b**	(29) **b**	(29) **b**	(29) **b**	(29) **b**	(29) **b**	(29) **b**			(29) **b**	(29) **b**	(29) **b**	(29) **b**	(29) **b**	(29) **b**	(29) **a**	(29) **a**	(29) **a**
L3	10.09±0.21	3.52±0.06	1.31±0.03	2.36±0.06	4.60±0.13	1.89±0.03	2.9±0.17	/	/	1.74±0.04	1.90±0.03	4.75±0.09	1.46±0.03	1.57±0.04	4.30±0.08	6±0	9.97±0.09	9.9±0.1
	(8-12.2)	(3-4.1)	(1-1.6)	(2-3)	(3.8-6.3)	(1.4-2.1)	(2-7.7)			(1-2.1)	(1.4-2.3)	(3.5-6)	(1.1-1.8)	(1.2-2.1)	(3-5.8)	(6-6)	(9-11)	(9-11)
	(30) **c**	(30) **c**	(30) **c**	(30) **c**	(30) **c**	(30) **c**	(30) **c**			(30) **c**	(30) **c**	(30) **c**	(30) **c**	(30) **c**	(30) **c**	(30) **a**	(30) **b**	(30) **b**
L4	13.65±0.30	4.29±0.07	1.50±0.04	3.13±0.05	6.34±0.21	2.31±0.03	3.29±0.05	/	/	2.08±0.03	2.23±0.04	6.07±0.08	1.84±0.02	1.89±0.04	5.27±0.07	7.75±0.25	9.86±0.12	9.71±0.10
	(10-16)	(3.5-5)	(1.3-2.9)	(2.2-4.1)	(4.1-8)	(2-3.3)	(2.9-4.2)			(1.9-2.5)	(2-2.7)	(4.9-6.9)	(1.5-2.5)	(1.3-2.2)	(4.1-6.5)	(6-11)	(8-11)	(9-11)
	(28) **d**	(28) **d**	(28) **d**	(28) **d**	(28) **d**	(28) **d**	(28) **c**			(28) **d**	(28) **d**	(28) **d**	(28) **d**	(28) **d**	(28) **d**	(28) **b**	(28) **b**	(28) **b**
L5	17.95±0.55	5.94±0.39	2.08±0.13	4.63±0.17	7.68±0.34	3.97±0.14	4.45±0.12	3.04±0.20	3.41±0.26	3.05±0.07	3.08±0.08	8.21±0.31	2.73±0.18	2.62±0.08	7.31±0.26	11.24±0.24	10.72±0.11	10.16±0.17
	(11.9-25)	(4.6-15)	(1.5-5)	(1.8-6)	(3.5-10.3)	(2.7-6.3)	(2.9-5.3)	(2.1-6)	(2.5-8)	(2-4)	(2.2-4)	(2.4-10.9)	(1.9-6.9)	(2-3.2)	(2.2-9.4)	(6-12)	(911)	(9-11)
	(25) **e**	(25) **e**	(25) **e**	(25) **e**	(25) **e**	(25) **e**	(25) **d**	(24) **a**	(25) **a**	(25) **e**	(25) **e**	(25) **e**	(25) **e**	(25) **e**	(25) **e**	(25) **c**	(25) **a**	(25) **b**
L6	21.66±0.40	6.70±0.08	2.52±0.05	5.82±0.12	9.67±0.27	4.99±0.06	5.50±0.08	6.00±0.09	7.09±0.12	3.47±0.07	3.96±0.05	10.4±0.13	3.12±0.05	3.28±0.06	9.24±0.12	11.43±0.14	10.93±0.15	10.8±0.16
	(18.2-25)	(5.9-7.5)	(2-3)	(5-7)	(7.8-13.7)	(4.4-5.9)	(4.56.3)	(5-7.3)	(6-8)	(2.9-4)	(3.2-4.6)	(9-12)	(2.5-4.1)	(2.9-4)	(7.9-11)	(10-12)	(10-13)	(9-14)
	(30)**f**	(30) **d**	(30) **f**	(30) **f**	(30) **f**	(30) **f**	(30) **e**	(30) **b**	(30) **b**	(30) **f**	(30) **f**	(30) **f**	(30) **f**	(30) **f**	(30) **f**	(30) **c**	(30) **a**	(30) **a**
Adults	27.38±0.56	7.85±0.09	3.01±0.07	7.58±0.23	12.91±0.37	6.24±0.08	6.63±0.11	24.56±0.46	20.85±0.39	4.37±0.09	4.89±0.06	13.13±0.17	3.90±0.05	4.22±0.09	11.78±0.13	12.47±0.11	10.77±0.14	10.97±0.13
	(20.2-32.5)	(6.6-8.9)	(2.1-3.7)	(2.5-9.5)	(9-17.9)	(5.1-7)	(5.6-8)	(17.5-30.2)	(13.7-25)	(3.6-5.5)	(4.2-5.5)	(10.9-15)	(3.2-4.3)	(3.3-5.7)	(10-13.2)	(12-14)	(9-12)	(10-13)
	(30) **g**	(30) **e**	(30) **g**	(30) **g**	(30) **g**	(30) **g**	(30) **f**	(30) **c**	(30) **c**	(30) **g**	(30) **g**	(30) **g**	(30) **g**	(30) **g**	(30) **g**	(30) **d**	(30) **b**	(30) **a**
Tests	**F**= 524.6	**H**= 192.1	**H**= 184.9	**H**= 187.6	**H**= 182.2	**H**= 194.2	**F**= 364.6	**H**=121.6	**H**=120.2	**H**= 190.6	**H**= 190.5	**F**=722.3	**H**= 192.8	**H**= 187.9	**H**= 189.9	**H**=178.4	**H**=82.5	**H**=139.2
P-values	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Lt: total length of the body, Lcc : length of the cephalic capsule, lcc : width of the cephalic capsule, Lth : length of the thorax, Labd : length of the abdomen, Lpr: length of pronotum, La : length of the antenna, Lel : length of the elytra, Lai : length of the hind wing, Lcu1, Lcu2, Lcu3 : length of the femur, length of median femur, length of anterior femur Lti1, Lti2 and Lti3 : length of the posterior tibia, length of the median tibia, length of the anterior tibia, Na : Number of antennal articles, Nee and Nei: Number of external and internal spines.

(83.07 ± 1.14 days) than in male nymphs (71.54 ± 1.48 days) (t '= 6.11, p <0.0001). However, when we considered each nymphal instar, the developmental time was longer in male nymphal stages 1 and 5 than in females (Table 4).

Mean nymphal development time (in days) was 17.14 ± 0.62, 12.91 ± 0.62, 13.45 ± 0.69, 13.80 ± 0.68, 15.23 ± 0.55 respectively in males of nymphal stages 1 to 5, and 16.18 ± 0.54, 13.13 ± 0.59, 12.49 ± 0.42, 13.19 ± 0.58, 14.58 ± 0.61, 16.57 ± 0.68 respectively in females of nymphal stages 1 to 6 (Table 4).

In each sex, the differences in development time were very significant between the nymphal developmental stages (p <0.0001): the duration of development was longer in stages 1 and 5 male nymphs as well as in stages 1, 5 and 6 females nymphs (Table 4).

Morphology of developmental stages of Atractomorpha acutipennis

Egg pods

Egg pods of *A. acutipennis* are whitish in colour. Their lengths ranged from 9 to 14 mm (average 10 ± 0.33 mm, n=16), while their diameters were comprised between 5 and 10.1 mm (average 6.63 ± 0.29 mm, n=16). The number of egg pods per female varied from 2 to 5 (average 3.67 ± 2 egg pods).

Eggs

The number of eggs per egg pod varied from 19 to 42 (on average 30.77 ± 10.5 eggs, n=480). The egg is light yellow in colour. It has an elongated shape, slightly curved with rounded ends; eggs measured between 3.9 and 5 mm in length (on average 4.08 ± 0.23 mm) and between 0.7 and 1.1 mm in diameter (on average 0.99 ± 0.02 mm).

Common characteristics of nymphs

The body, usually green, is covered with many small white granules. The head is green, conical, with a parabolic fastigium and round apex. The width of the inter-ocular space is shorter than the vertex base. The vertex is tapered and concave at its base. At the level of the face, there is the presence of a frontal suture with a low depression; on either sides of this frontal suture, there is the presence of two sub-ocular sutures, clearly visible and reaching the base of the mouthparts. The mouthparts have a green colour,

Table 4. Variation in the nymphal development time of *Atractomorpha acutipennis* in the laboratory. Values represent averages and their confidence limits. Values in brackets indicate the minimum, maximum, and number of individuals. H is the value of the Kruskal-wallis test.

Sexes	Stage 1 nymphals	Stage 2 nymphals	Stage 3 nymphals	Stage 4 nymphals	Stage 5 nymphals	Stage 6 nymphals	H-values	P-values
Male	17.14±0.62 (5-28) **a**(65) **A**	12.91±0.62 (6-26) **b**(64) **A**	13.45±0.69 (4-28) **b c**(60) **A**	13.80±0.68 (3-26) **b c**(56) **A**	15.23±0.55 (5-24) **a**(47) **A**	/	38.3	<0.0001
Female	16.18±0.54 (10-29) **a**(60) **A**	13.13±0.59 (6-26) **b**(60) **B**	12.49±0.42 (7-22) **b**(59 **B**	13.19±0.58 (4-22) **b**(54) **B**	14.58±0.61 (7-23) **ac**(50) **A**	16.57±0.68 (7-25) **ac**(42)	46.79	<0.0001
Mann-Whitney U test	P=0.001	P=0.32	P=0.23	P=0.51	P=0.04	/	/	P=0.05

Lowercase letters indicate a horizontal comparison and capital letters indicate comparison of both sexes (Mann-Whitney U test).

with the exception of the clypeus which has a black end. The pronotum is cylindrical, tectiform, slightly convex at the superior margin and concave at the inferior border. The median carina of the pronotum is clearly visible while the two lateral carinas are not very distinct. The eyes have one or more longitudinal bands or streaks. The hind tibia has external and internal spines. The open mesosternal space is much wider than long, with rounded lobes. The abdomen has 11 segments dorsally and 8 ventral. The non-segmented circles are located behind the epiproct and the paraproct (Figures 1 and 2)

Distinctive characters of the nymph: identification key for nymphal instars of A. acutipennis

The distinctive characters of the nymphs of *A. acutipennis* (Figure 3) are summarized in the following dichotomous keys:

Keys of males

1. Visible and measurable wing buds (also called pterotheca); subgenital plate not indented and not reaching half of the paraproct 2
- Reduced wing buds, difficult to measure; subgenital plate indented 4
2. V-shaped pterotheca, main axis pointing downwards; mesothoracic pterotheca covering the anterior part of metathoracic pterotheca . . . . . Stage 3 nymph
- Triangular-shaped pterotheca, main axis directed upwards; overturning of the pterotheca, metathoracic wing buds covering the mesothoracic wing buds 3
3. Pterotheca not turned . . . . . Stage 4 nymph
- Pterotheca turned . . . . . Stage 5 nymph
4. Visible meta-pterotheca with 3-4 visible veins; pterotheca reaching the base of the 1st abdominal segment; subgenital plate slightly indented, not reaching half of the paraprocts . . . . . Stage 2 nymph

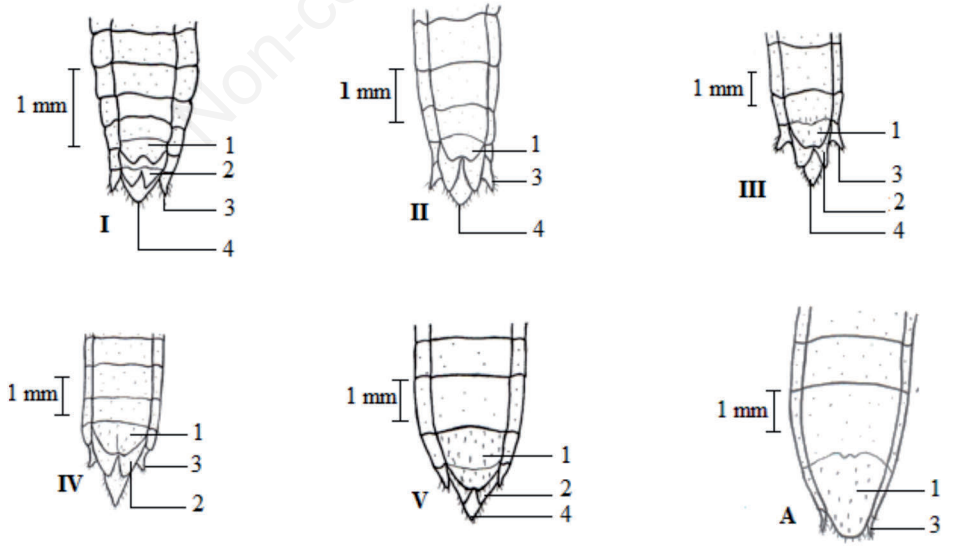

Figure 1. Morphological variation of male subgenital plaque during postembryonic development in *A. acutipennis* (ventral view). 1: subgenital plaque, 2: paraproctes, 3: cercus, 4: epiproct. I-V: nymphal stages, A: adult.

- Mesothoracic and metathoracic pterotheca very slightly or not visible; subgenital plate deeply indented . . . . . Stage 1 nymph

Keys of females

1. Visible and measurable wing buds (also called pterotheca); ventral valves reaching the anterior base of the 8th abdominal sternite; presence of white granules all over the body 2
- Reduced pterotheca, difficult to measure; ventral valves not reaching the anterior base of the 8th abdominal sternite; absence of white granules all over the body 5
2. V-shaped pterotheca, main axis directed downwards; Mesothoracic pterotheca covering the anterior part of metathoracic pterotheca 3
- Triangular-shaped pterotheca, main axis directed upwards; overturning of the pterotheca, metathoracic drafts covering the mesothoracic drafts 4
3. Nervation on pterotheca less visible (3 to 4 mesothoracic nervures and 7 to 8 metathoracic); ends of the pterotheca reaching 1/3 of the base of the 1st abdominal segment; ventral, presence of three transverse bands at the level of the eyes; ventral valves of triangular shape, acute apex, slightly exceeding the 8th abdominal sternite . . . . . Stage3 nymph

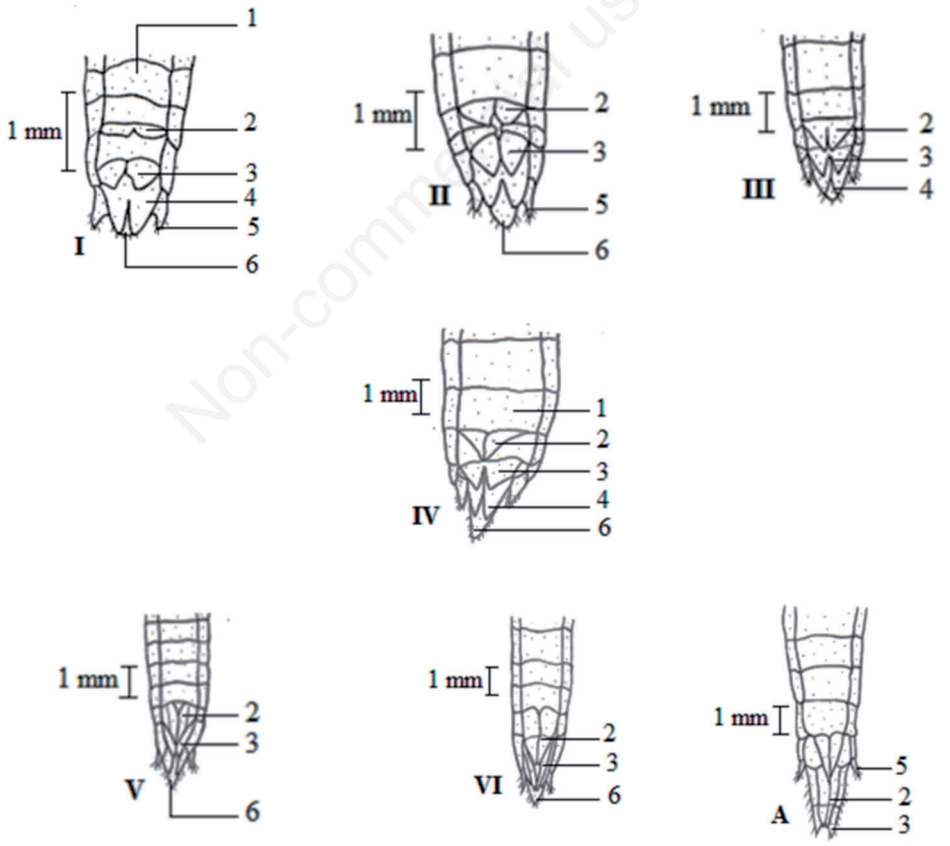

Figure 2. Morphological variation of female external genitalia during postembryonic development in *A. acutipennis* (ventral view). 1: 7th sternite, 2: ventral valve, 3: dorsal valve, 4: paraprocts, 5: cercus, 6: epiproct. I-VI: nymphal stages, A: adult.

- Nervation on the pterotheca more visible (4-5 mesothoracic veins, 8-9 metathoracic veins), extremities of the pterotheca reaching 1/2 of the 1st abdominal segment. Ventral valves reaching the base of the dorsal valves without reaching their half; extremities of the

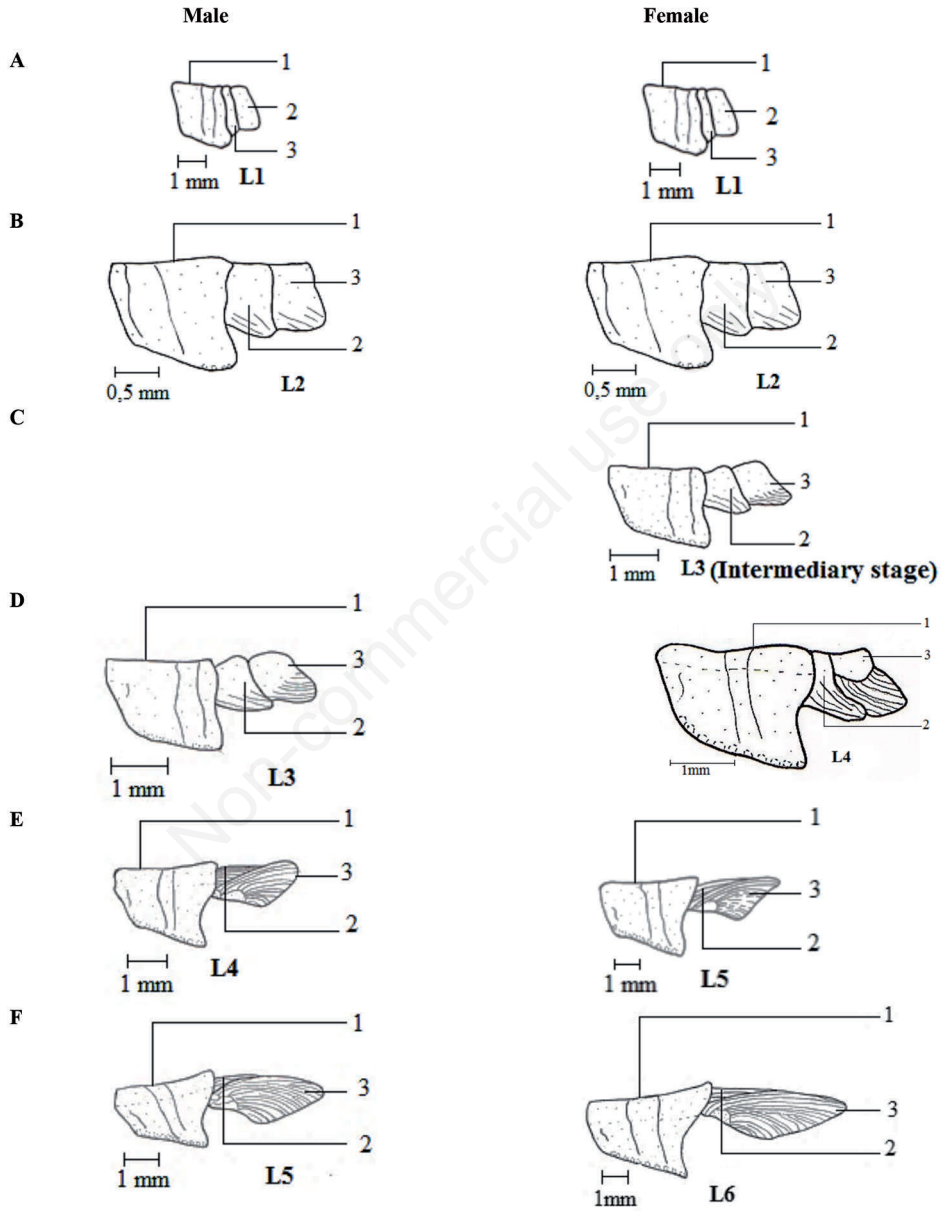

Figure 3. Morphological variations of the pronotum and wing forms of the different stages of post-embryonic development of *A. acutipennis* (side view). 1: pronotum, 2: anterior pteroteca, 3: posterior pterotheca. A: nymph stage 1, B: nymph stage 2, C: intermediary stage (only female), D: nymph stage 3 (only male), E: nymph stage (nymph stage 4 male and nymph stage 5 female), F: nymph stage (nymph stage 5 male and nymph stage 6 female).

dorsal valves almost at the same level as those of the cerci but reaching almost half of the paraproct . . . . . Stage 4 nymph
4. Pterotheca not reaching 2nd abdominal segment; ventral valves up to 3/4 of the dorsal valves; staining similar to that of stage 1 to 4 nymphs in some individuals, with paraprocts slightly exceeding the number; dorsal valves up to half of the paraprocts . Stage 5 nymph
- Pterotheca reaching the base of the 4th abdominal segment; well-developed ventral valves; paraproct and epiproct always visible ventrally . . . . . Stage 6 nymph
5. Visible meta-pterotheca with 3-4 visible veins, pterotheca reaching the base of the 1st abdominal segment; ventral valves significantly more developed and reaching the base of the 8th abdominal sternites . . . . . Stage 2 nymph
- Meta-pterotheca without visible veins very small ventral valves not reaching the base of the 8th abdominal sternite . . . . . Stage 1 nymph

Courtship and mating

Courtship begins 4 to 48 days (average 18 ± 15.42 days) after last moult. The male mounts on the back of the female, when the latter is not receptive, and remains on her back; this gesture can last more than an hour. In some cases, the female, using her hind legs tries to prevent the male from climbing on her. When the female is receptive, the male, thanks to his prothoracic and mesothoracic legs, immobilizes her, the metathoracic legs being free and folded. In this position, the male returns his abdomen 180º below the abdomen of the female, to bring into contact the two genital regions. After a few minutes of friction, copulation starts and can extend over a period of 1 to 5 h (3h 11min on average) if it is not interrupted (by observer during the manipulation).

Oviposition, number of egg pods, number of eggs, number of nymphs deposited by the female

After the first mating, the female took 11 to 30 days (19.33 ± 5.33 days) to deposit the 1st pod which is deposited in a pulsatile manner and contains all the eggs. The number of egg pods per female ranged from 2 to 5 (averaged 3.67 ± 2 egg pods) and the number of eggs per pod varied between 19 and 42 (averaged 30.77 ± 10.5 eggs). The number of nymphs that emerged from each pair was respectively 95, 40, 122 and 121 (mean 94.5 ± 38.42 nymphal).

Discussion

The study showed that the number of instars is constant in each sex in *A. acutipennis* in the humid forest zone, but varies between the two sexes (six in the male and seven in the female), confirming the results of Popov (1989) in the Sahelian zones. This constant number of instar in each sex is a frequent phenomenon amongst the Acrididea. It has been also observed in *Pyrgomorpha vignaudii* (Guérin-Méneville, 1849) (Kekeunou et al. 2015) and *Taphronota ferruginae* (Fabricius, 1781) (Kekeunou et al. 2018) (six instars in both sexes), two pyrgromorphid grasshoppers which share the same habitat with *A. acutipennis* in southern Cameroon, in *Dichroplus maculipennis* (Blanchard, 1851) (six instars in both sexes) and *D. elongatus* Giglio-Tos, 1894 (five instars in both sexes) from Argentina (Mariottini et al. 2011).

The variation in number of instars according to sex were also noted in several species of Acrididae such as *Atractomorpha sinensis* Bolívar, 1905 in Iceland (Sai-Keung 1973), *Epreypocnemis plorans ibandana* (Charpentier, 1825) (Nkwala et al. 2019) in southern

Cameroon, *E. p. meridionalis* Uvarov, 1921 (Hernández and Presa 1984) in Spain, and *E. p. plorans* (Charpentier, 1825) (Schmidt et al. 1996) in Sardinia. However, as in this study Sai-Keung (1973) has observed a constant number of instar in each sex (7 in male and 8 in female of *A. sinensis*) whereas there is a variation in female of *E. plorans ibandana*: 6 or 7 in female and 6 in male (Nkwala et al. 2019).

The increase in the number of nymphal instars in females of *A. acutipennis* could be explained by differences in the activity rates of moulting glands, corpora allata and corpora cardiaca between the two sexes and possibly due to inadequate rearing conditions (Schultner et al. 2012). Moulting and metamorphosis in hexapods could be affected by the type of diet (Joly 1968; Kekeunou et al. 2010). In fact on *M. esculenta*, the number of nymphal instars in *Zonocerus variegatus* (Linneaus, 1758) varied from 6 to 7: 62.5% passed throught 6 nymphal instars and 7.5% passed through 7 (Kekeunou et al. 2010). Temperature also strongly influenced growth, development and behaviors of grasshoppers (Tu et al. 2012). The tropical grasshopper *Cornops aquaticum* (Bruner, 1906) may have five to seven nymphal instars depending on the host plant, humidity, and its distribution (Adis and Wolfgang 2003; Adis et al. 2004). In general, the number of post-embryonic instars depends on genetic and environmental factors (Hochkirch and Gröning 2008).

This study shows that the nymphal development time in females of *A. acutipennis* is longer than that of males. This result might be linked to the high number of the post-embryonic stages of the females. The same result was obtained by Sai-Keung (1973) in *A. sinensis* in Iceland and Nkwala et al. (2019) in *Eyprepocnemis plorans ibandana* in Southern Cameroon. *Taphronota ferruginae* (Kekeunou et al. 2018) and *Pyrgomorpha vignaudii* (Kekeunou et al. 2015) showed a non significant difference between male and female total developmental time. However, *A. acutipennis* developmental time is longer than that of *A. sinensis* (56.2 days in the male and 59.6 in the female) (Sai-Keung 1973) and shorter than that of *T. ferruginae* (120.6 ± 3.49 days) (Kekeunou et al. 2018), *Eyprepocnemis plorans ibandana* (79.16 ± 0.51 days in males, 89.93 ± 0.58 in six-instar females and 94.96 ± 1.22 in seven-instar females) (Nkwala et al. 2019), and *P. vignaudii* (92.08± 4.36 days in males and 95.50 ±5.66 days in females) (Kekeunou et al. 2015). These results can be explained by the different activities of corpora cardiaca which would facilitate a longer duration of juvenile hormone in the haemolymph of female nymphs (Joly 1968). The duration of nymphal stages seems to be mainly influenced by environmental factors such as temperature and nutritional quality of food (Uvarov 1966; Joern and Gaines 1990; Danner and Joern 2004), this might explain why the development times were longer in stages 1 and 5 nymphs of both sexes.

The dichotomous keys show that several morphological characters are required for a clear differenciation of post-embryonic instars of *A. acutipennis* and it is easy to identify later nymphs (over instar 3) than young one (instars 1-3). In fact, distinguishing nymphal instars generally became easier with advancing development as differences became more pronounced. Differentiation of nymphs based on body size measurements is not always possible because measures of size often overlapped between successive nymphal stages (Schultner al. 2012). There is no significant difference between nymphal instars colour. The dimensions of the body parts of *A. acutipennis* as that of several grasshoppers increased with the age (Schultner al. 2012). In its natural habitat, *A. acutipennis* might be amonst the smallest grasshoppers. In fact, adult body length (18.67) in *A. accutipenis* is relatively small when compared to that of the grasshoppers which share the same habitat with *A. acutipennis*, i.e. *P. vignaudii* (19.92–44.01), *Z. variegatus* (29.39), *T. ferruginae* (34.32), and *E. plorans* (male: 24.33, female: 35.86 and 35.78),.

Courtship and mating behaviors of *A. acutipennis* are similar to those of other Pyrgomorphidae such as *Zonocerus variegatus* (Kekeunou 2007), *Pyrgomorpha vignaudii* (Kekeunou et al. 2015), and *Taphronota ferruginea* (Kekeunou et al. 2018). According to Duranton et al. (1987), this type of courtship and mating is encountered in all Pyrgomorphidae. The study shows that mating begins 18 days after the last moult and it lasts between 1 to 5 hours (on average 3 h 11 min) if it is not interrupted. However, in *P. vignaudii* (Kekeunou et al. 2015) and *T. ferruginea* (Kekeunou et al. 2018), mating began respectively 12 and 42 days and lasts respectively between 3 to 6 hours and 1 to 4 hours. These differences could be explained by the duration of each species to find favorable ecological conditions including temperature, adequate humidity and availability of food (Duranton and Lecoq 1990).

After the 1st mating, the female of *A. acutipennis* took 11 to 30 days (19.33 ± 5.33 days) to deposit the 1st ootheca (egg pod), while in *P. vignaudii, T. ferruginea* and *E. plorans ibandana*, the time after first mating ranged respectively 14 to 34 days (averagely 25.2 ± 4.62 days), 36–57 days (averagely 45.78 ± 4.74 days) and 9 to 70 days (averagely 52.03 ± 5 days) to deposit the first egg pod. These durations after first mating could be explained on one hand by the fact that the reproductive capacity of a population is influenced by a certain number of factors, such as the duration of the pre-oviposition period, the number of females participating in reproduction (Schultner al. 2012) and on the other hand by the use of these periods by the males to help the females to find and select the oviposition sites in peace and ensure the transfer of sperms and seminal fluid into the female reproductive system (Gillott 2005).

From our results, it appears that *A. acutipennis* female deposits two to five egg pods. These results are close to those obtained by Kekeunou (2007) who obtained two egg pods in females of *Zonocerus variegatus*, but differ from those of Kekeunou et al. (2015, 2018) who obtained one egg pod from *T. ferruginea* and nine from *P. vignaudii* females. The number of eggs per egg pod in *A. acutipennis* obtained in the laboratory varies from 19 to 42 (on average 30.77 ± 10.5). This result is close to that of Kekeunou et al. (2018) who obtained 15 to 50 eggs (an average of 30.44 ± 5.06) per egg pod in *T. ferruginea*, but differs from that of Kekeunou et al. (2015) who obtained from 16 to 93 eggs per egg pod in *P. vignaudii* (average 45.31 ± 3.51). In fact, the reproductive capacity of a population is influenced by a number of other factors, such as the length of the pre-oviposition period and the number of females involved in breeding (Whitman 2008; Ackman and Whitman 2008).

Conclusions

In *A. acutipennis* the number of nymphal instars varied between both sexes but is constant in each sex. In general, developmental time is longer in females than in males. The female is bigger than the male only in nymphal instars 5, 6 and the adult. Some body parts allowed a clear identification of different nymphal instars. Adult females deposited an average of 3.67 ± 2 egg pods, each consisting of 30.77 ± 10.5 eggs. Courtship was observed 18 ± 15.42 days after the last moult. Oviposition occured on average 19.33± 5.33 days after the first mating. This study provides important information about the biology of this grasshopper, which could help in developing control methods against *A. acutipennis* in southern Cameroon. However, much is still to be done. We will direct our future work on the construction of the life table of *A. acutipennis.*

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Conflict of interest

No potential conflict of interest was reported by the authors.

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