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First record and some aspects of the bio-ecology of *Hilda patruelis* Stål (Hemiptera: Tettigometridae) on *Vernonia amygdalina* Delile (Asteraceae) in Southern Cameroon

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ABSTRACT

The bug Hilda patruelis *Stål is an ant-tended hemipteran, recorded on several non-related plant species such as* Arachis hypogea *L.,* Phaseolus vulgaris *L,* Hibiscus rosa-sinensis *L,* Ficus sur *Forsskal in Afrotropical region. For the first time, the bug is recorded in Cameroon on a new host plant,* Vernonia amygdalina *Delile. The present study carried out from December 2012 to August 2013 at Yaoundé, aimed to provide data on the biology and ecology of* H. patruelis*. For this purpose, natural enemies and ants associated with the bug were monitored, bug colonies were characterized according to seasons; and finally, its life history was established under field and laboratory conditions. As results, five ant species were recorded:* Pheidole megacephala *(F).,* Myrmicaria opaciventris *Emery,* Crematogaster castanea *Smith,* Camponotus *sp. and* Tetramorium simillimum *Smith. Among them the more frequent was* P. megacephala*. Despite the presence of ants,* H. patruelis*'s eggs were frequently parasitized by the wasp,* Psyllechthrus oophagus *Ghesquière. The mean number of bug colonies in the field was greater during the wet period than during the dry, with a highly significant variation. Accordingly, the number of individuals showed the same trend. The fecundity of the females of this bug was positively correlated with the number of males present; thus the number of eggs was lower in polygyny and in monogamy and higher in polyandry. By contrast, female's life span decreased drastically in polyandry. The whole duration of the development of* H. patruelis*, including five immature stages ranged from 54 days in field conditions to 55 days in laboratory conditions.*

Keywords: New host plant, Cameroon, Bitter-leaf shrub, Ant-tending, Fecundity, *Hilda patruelis*, Live span, Pests.

INTRODUCTION

Plant-hoppers (Hemiptera: Fulgoromorpha) constitute a cosmopolitan group of insects, feeding mainly on the phloem tissues of woody or herbaceous plants [1, 2], but sometimes on fungi, mosses, horsetails, or ferns [1, 3]. Usually, they are highly polyphagous, feeding on numerous taxa of unrelated plants or sometimes strictly monophagous, feeding on only a single host plant species. Members of the family Tettigometridae are of concern to agriculture because several species are recognized as pests on various crop plants [4]; some of them are regarded as potential biological control agents of weeds [5]. As sap-sucking insects, they excrete a great amount of honeydew, attracting tending ants and other honeydew feeders which compete for this potentially high value resource [6, 7, 8, 9]; they may also transmit various plants pathogens. The species *Hilda patruelis* Stål, also called groundnut hopper [10], was formerly known to be restricted to sub-Saharan Africa [11, 12, 10, 13], but it has recently been introduced in the USA [2]. In the Sub-Saharan Africa, the species is recorded in several countries including Congo, Malawi, Nigeria, Soudan, Kenya, Tanzania, South Africa, Rhodesia, Zambia, Zimbabwe etc. on several non-related host plants, such as the groundnut or *Arachis hypogea* L. (Fabaceae) [12, 10, 14, 15, 16], the common beans or *Phaseolus vulgaris* L. [17], *Crotalaria grahamiana* Wight & Arn. (Fabaceae) [18, 19], *Hibiscus rosa-sinensis* L. (Malvaceae) and *Ficus sur* Forsskal (Moraceae) [20].

The groundnut hopper, *H. patruelis*, is known to be involved in mutualistic interactions with several ant species. In this interaction, the ants protect the bug from predation and environmental constraints and in turn benefit from the honeydew it excretes. Commonly encountered ant species include *Anoplolepis* spp. and *Pheidole* spp. [11, 13].

For the first time, *H. patruelis* is recorded in Cameroon. We encountered it feeding on *Vernonia amygdalina* (Delile) (Asteraceae), a new host plant, and on *Ficus vallis-choudae* Delile. It is supposed, with other hemipterans such as psyllids, aphids, scale insects, to be potentially harmful for the multipurpose shrub *V. amygdalina* in case of population outbreak. Consequently, their populations are to be invigilated since they might rapidly determine outbreaks in certain environmental conditions such as optimal weather, monoculture systems, etc.

The Asteraceous shrub, *V. amygdalina* (fig. 1), commonly known as bitter-leaf shrub, is a perennial plant of about 5 m in high, native to tropical Africa and tropical Asia [21, 22, 23] . In Africa, it is encountered in Western, Central and Southern regions where it naturally colonizes secondary forests and cultivated lands [23, 24]. It is also planted by farmers in gardens and as fences around houses [23, 24]. For the cultivation purposes, it is generally proliferated by shoot planting [25]. This multipurpose shrub is used as a vegetable in West Africa, [26, 27] or as a medicinal plant in all its distribution range [21, 28, 22, 23, 29]. Its insecticidal or insect repellant properties have been successfully assessed by various studies [30, 26, 31]. These properties may be related to the presence of lactone, oxalic acid and hydrocyanic acid (HCN) in its essential oil [30]. Others have tested its fodder potentials [32]. In Cameroon, the “ndolè” meal made by mixing leaves of *V. amygdalina* with peanuts paste is regarded as a culinary heritage; then the demand has considerably increased on local and progressively in international markets; thus, its production has noticeably been boosted in various production basins [27].

Damages caused by various pest insects might constitute a severe constraint to the production of this vegetable and appear as a main cause of low yields and poor product quality (Personal Observation). The groundnut-hopper *H. patruelis* is one of these pests. It feeds on *V. amygdalina* by sucking sap from stems, leaves veins and peduncles. Considering the socio-economic importance of the shrub, it appears necessary to invigilate outbreaks of the bug. The aim of this study is to gather biological and ecological data that could serve in the conception of integrated pest management programs in limitation of *H. patruelis* populations. For this purpose, the variations of the number, the size as well as the distribution of the bug’s colonies were assessed according to seasons; also, some aspects of its life history under both field and laboratory conditions was determined; finally, interactions with ants on the plant were described.

Figure 1. A young shrub of *Vernonia Amygdalina* Delile

MATERIALS AND METHODS

Study site and period.

Field observations and sample collection were carried out in an experimental garden set up in the campus of the “University of Yaoundé I” (UYI) (3°51’28.9”N, 11°29’52.2”E, 729 m asl.) and biological studies conducted in the Laboratory of Zoology of the same institution. Yaoundé is situated on the Cameroonian Southern Plateau, a geographic region characterized by a humid tropical climate with bimodal rainfall regime: In Yaoundé, the climate is a transitional subequatorial type, highly influenced by the geomorphology of the area which is dominated by small hills of about 1000-1200 m asl. In fact, with a mean rainfall of 1500 mm/year, the “Yaoundean microclimate” is characterized by a short dry season usually hidden by a high moisture rate [33]. Comparatively to the rest of the Centre Region, where the average temperature is 25°C, the one of Yaoundé is slightly low (23°C). The vegetation of the site derived from a semi-deciduous forest landscape, highly disturbed by anthropic activities. It is made of grasslands dominated by *Imperata cylindrica* (L.) (Poaceae) and *Chromoleana odorata* (L.) (Asteraceae) surrounding buildings.

Observations and experiments were conducted during three different periods; the first one lasted from December 2012 to March 2013, the second in May 2013 and the third from July to August 2013.

Experimental design

Ants and entomophagous insects encountered with *Hilda patruelis* Stål on *Vernonia amygdalina* Delile were monitored by weekly examination of the plant. Some insect determinations were done *in situ* when possible. Otherwise, adult insects were caught and brought to the laboratory where they were preserved in 70 % ethanol for further identifications. Immatures and mummified eggs of *H. patruelis* (fig. 4) were collected and reared up to emergence of bugs or parasitoids. They were then preserved in 70 % ethanol. Identifications of these insects were done using the following keys: Villier [34], Goureau [35], Couilloud [36], Delvare & Aberlenc [37], Anonyme [38]. Ants were identified using the keys of Hölldobler [39], Bolton [40] and Taylor (http:/www.antbase.org/, April, 06th, 2015). These determinations were later confirmed by comparing specimens to the Voucher collection of the Royal Museum for Central Africa (RMCA) at Tervuren (Belgium).

During the monitoring, the number, size and spatial distribution of bug clusters were register on a daily bases. Analysis of their variations in relation to some main climatic factors allowed evaluating the influence of climatic variation on their fitness.

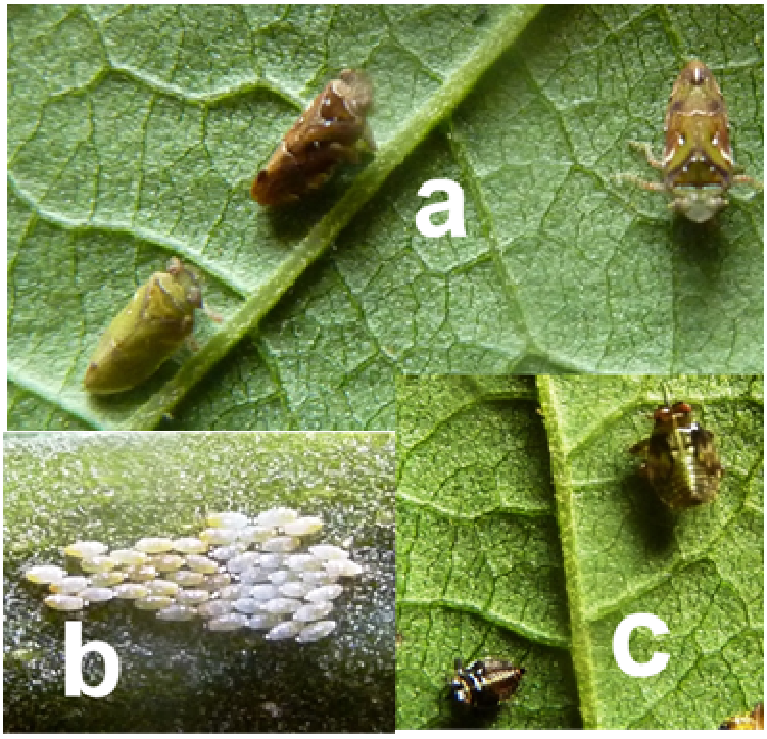

Figure 2. *Hilda patruelis* Stål. (A) adults, (B) eggs, (C) larvae

Figure 3. Gauze cape isolating a colony of *Hilda patruelis*.

Figure 4. Parasitized eggs of *Hilda patruelis*

The environmental constraints, related to weather, predators and parasitoids lead *H. patruelis* to develop different interactions with various ants' species. These partners are attracted by the high nutritive potential honeydew secreted by the bug. These associations were assessed according to their occurrence in the field.

In the laboratory, the insects breeding was conducted in plastic boxes bearing a pipe channel for the breathing requirements. Each box was provided with a small twig supporting *H. patruelis* individuals.

The life cycle of the bug was studied simultaneously in the garden and in the laboratory. Parameters assessed were the duration of immature stages (eggs and nymphs) and the life span of females. In the garden, pairs of *H. patruelis* (males and females) (fig. 2 A) were isolated on a non-infested leaf by a fine mesh (0.5 mm) cape (fig. 3) for 24 hours. Then, adults were removed and eggs (fig. 2 B) laid by the females followed up to the emergence of adults (fig. 2 C), constituting "individuals of the first generation" (F1). In the laboratory, pairs of adults, collected from the garden, were isolated in the up-cited plastic boxes previously provided with a fresh uninfected leaf. This leaf was replaced daily. Eggs laid by females were followed up until the emergence of adults, constituting also the first generation (F1). Similarly, experiments were simultaneously conducted both in the garden and in the laboratory using adults of the first generation, until the emergence of adults constituting the second generation (F2).

Female fecundity and life span were assessed by observing insects reared both in the garden and in the laboratory. To achieve this, two trials were set up. The trial 1, using the F1 generation adults, consisted exclusively in monogamous pairs of bugs (1 male + 1 female). The trial 2 using the F2 generation adults consisted in three models of matting systems only in the garden: (i) the monogamy (1 male + 1 female), (ii) the polyandry (2 males + 1 female) and (iii) the polygyny (2 females and 1 male). Then the egg laying process was observed daily up to the death of females. In the laboratory, the following up of each set of bugs was done in Petri dishes which was daily provided with fresh leaves of *V. amygdalina* as food resource and laying substrate. In the garden, groups involved in each matting system were isolated on a leaf using a fine gauze cape for 24 hours and then, moved to another leaf. The former was cut and brought to laboratory in order to count the number of eggs laid under a stereomicroscope. A total of 240 set of individuals (30 for every matting system model in field and in the laboratory) were thus followed. Eggs were followed and after hatching, larvae were reared until emergence of F2 adults. Finally, the sex-ratio was computed and the female longevity estimated. These important biological parameters could contribute to a better understanding of some aspects of pest population dynamics.

Data analysis

For the diversity data, daily relative abundances were calculated and their seasonal variation assessed using the Generalized Linear Model (GLM) procedure which involved a linear regression analysis and variance analysis (ANOVA). Then the correction of Poisson was applied for counting data.

The mean numbers of eggs laid were compared between matting system using the ANOVA (GLM proc) test with associated TukeyHSD test corrected by sequential Bonferroni procedure for Pairwise comparisons. The analyses were conducted with R software (Version 3.0.2, 2013) and the results were appreciated at the 5% confidence level.

RESULTS AND DISCUSSION

Entomophagous insects encountered with H. patruelis *on* V. amygdalina

Among entomophagous arthropods associated with *Hilda patruelis* on *Vernonia amygdalina* were polyphagous predators and parasitoids, more specific.

The nine predators species identified included *Latrodectus* sp. (Araneae : Salticidae), *Ozyptila paticola* (Araneae: Thomisidae), *Chrysops* sp. (Nevroptera : Chrysopidae), *Coccinella septempunctata* L. (Coleoptera : Coccinellidae), *Cycloneda* sp. (Coleoptera : Coccinellidae), *Scymnus (Pullus) auritus* (Thunberg) (Coleoptera : Coccinellidae), *Nephus* sp. (Coleoptera : Coccinellidae), *Dicomorpha* sp. (Hymenoptera : Vespidae) *Mantis* sp. (Dictyoptera: Manthidae). Among them, the most abundant were *Latrodectus* sp. with 8690 individuals (42.88 %) and *S. (Pullus) auritus.* With an absolute abundance 5290 individuals, they represented 26.10 % of the whole sample.

The sole parasitoid species recorded on this planthopper, *Psyllechthrus oophagus* Ghesquière (Hymenoptera: Encyrtidae), emerged from the eggs. Its abundance fluctuated according to air moisture, with 110 individuals (1.17 %) collected during the wet period to 1455 individuals (13.40 %) collected during the dry periods ($F_{1.99}$ = 16.85; $P < 10^{-3}$).

Global distribution of H. patruelis *colonies and seasonal abundance of individuals per colony*

In general, the population size varied from one season to another (fig. 5). The mean number of colonies was 24.77 ±

5.05. It decreased from 25.76 ± 4.18 during the wet season to 20.33 ± 7.63 during the dry season, with a significant variation between wet and dry season ($F_{1.99}$ = 56.16; P< 10^{-3}). Similarly, the absolute abundance of individuals decreased from 27990 during the wet period to 20871 during the dry season. Thus the variation was significant between the two seasons ($F_{1.99}$ = 16.85; P < 10^{-3}). As the number of colonies decreased, the abundance of individuals decreased accordingly, so the correlation between the two values in the garden was positive and significant (r = 0.81; P < 10^{-3}).

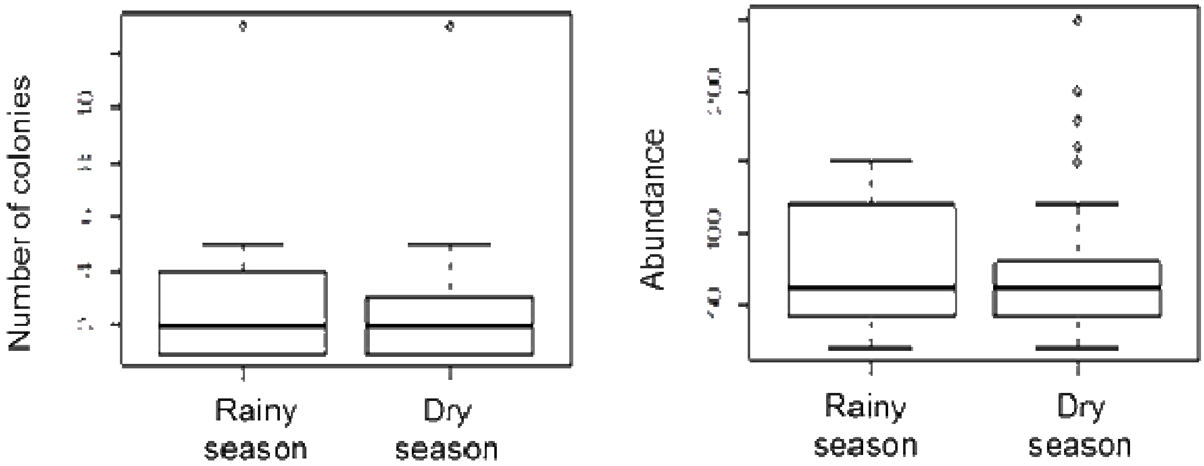

Figure 5. Distribution of *Hilda patruelis* colonies and individual abundance

Occurrence of associations between H. patruelis *and ants*

Five ant species were encountered associated to *H. patruelis* during the present study: *Crematogaster castanea* Smith, *Myrmicaria opaciventris* Emery, *Pheidole megacephala* F., *Camponotus* sp. and *Tetramorium simillimum* (Smith). The most abundant were *P. megacephala, M. opaciventris and C. catanea.* From one season to another, the abundances of these three species did not vary significantly (Table 1).

The most frequent association was *H. patruelis-P. megacephala*, encountered 70 times (representing a relative frequency of 23.41 % in the whole observations); this was followed *H. patruelis-M. opaciventris*, observed 36 times (representing a relative frequency of 12.04%) and *H. patruelis-C. castanea*, encountered 26 times (representing a relative frequency of 8.70%); the less frequent were *H. patruelis-Camponotus* sp. encountered four times (representing a relative frequency of 1.34 %) and *H. patruelis - T. simillium* encountered two times (representing a relative frequency of 0.67 %). Globally, the distribution of occurrences of the associations between this bug and different ant species varied significantly ($F_{1, 99}$ = 0,501; P = 0,003)

Table 1. Diversity and abundances of ant species associated with *Hilda patruelis* on *Vernonia amygdalina* (AA: absolute abundance; AR: relative abundance).

Species	Wet season		Dry season		Total		F test
	AA	RA	AA	RA	AA	RA	
Camponotus sp.	140	0.08%	875	0.63%	1015	0.33%	$F_{1.99}$ = 0.026; P = 0.870 ; NS
Crematogaster castanea Smith	7600	4.46%	1615	1.16%	9215	2.98%	$F_{1.99}$ = 1.053; P = 0.307 NS
Myrmicaria opaciventris Emery, 1909	7820	4.58%	4475	3.22%	12295	3.97%	$F_{1.99}$ = 0.532; P = 0.467 ; NS
Pheidole megacephala F.	64240	37.66%	45975	33.07%	110215	35.60%	$F_{1.99}$ = 0.164; P = 0.686 ; NS
Tetramorium simillimum Smith	0	0.00%	125	0.09%	125	0.04%	$F_{1.99}$ = 0.961 ; P = 0.329; NS

Life cycle

The life cycle of *H. patruelis* comprises an embryonic stage, five larval stages and an imaginal stage. Laboratory observations from eggs laying to emergence of the imagoes allowed determining the mean durations of developmental stages presented in the Table 2. So, the whole pre-imaginal development requires 54.29±6.17 days in the laboratory and 54.60±7.91 days in the garden.

Table 2. Average durations of different stages of the *Hilda patruelis* life cycle.

Developmental stage	Durations (in days)							
	In the laboratory				In the garden			
	Min	Max	Moy	SD	Min	Max	Moy	SD
Egg-L1	7	17	12.41	1.714	9	16	12.72	1.49
L1-L2	5	15	8.17	1.475	5	14	8.36	1.32
L2-L3	7	13	8.31	1.378	5	11	8.12	1.12
L3-L4	5	13	8.22	1.238	2	14	8.35	1.35
L4-L5	6	14	8.29	1.263	6	11	8.31	1.13
L5-imagoes	5	15	8.89	1.777	3	13	8.74	1.52
Pre-imaginal development	35	87	54.29	6.17	30	79	54.60	7.91

Female fecundity and life span

With the F1 generation adults in monogamous matting systems (trial 1), field observations conducted during 43.5 ± 37.48 days (Min=17; Max=70) showed an average of 701.30 ± 17.70 eggs / female (Min = 667; Max = 736) while in the laboratory, observations conducted during 41.00 ± 35.27 days (Min = 12; Max = 70) showed an average of 664.10 ± 30.30 eggs / female (Min = 604; Max = 724). There was no significant difference between these two fecundity rates ($F_{1.99}$ = 1.1; P = 0.32).

With the F2 generation adults in the garden (trial 2), females of *H. Patruelis* in the monogamous matting system laid an average of 692.77 ± 169.49 eggs (Min = 164; Max = 1139) during 45.01 ± 36.77 days (Min = 18; Max = 71). In the polyandrous matting system a female laid an average of 777.17 ± 179.47 eggs (Min = 423; Max = 1089) during 43.02 ± 36.78 days (Min = 16; Max = 69); those in the polygynous matting system laid an average of 604.53 ± 125.19 eggs / female (Min = 305; Max = 947) during 41.00 ± 39.60 days (Min = 12; Max = 69).

Female's fecundity then varied significantly according to matting system ($F_{1.\ 99}$ = 5.2; P >10^{-3}). Pairwise comparisons between different matting systems (monogamy/polyandry, polyandrous/ polygyny and monogamy/polygyny) highlighted significant differences [($F_{1.\ 99}$ = 8.9; P = 0.0001), $F_{1.\ 99}$ = 8.9; P >10^{-3}), ($F_{1.99}$ = 8.9; P = P >10^{-3}), respectively)].

The mean longevity of females significantly varied with respect to matting system ($F_{1.\ 99}$ = 13.19; P >10^{-3}), being longer in monogamy with an average of 61.13 ± 9.75 days (Min = 35 ; Max = 77) than in polyandry with 46.17 ± 10.84 days (Min = 28 ; Max = 65) and polygyny with 46,23 ± 16,08 days (Min = 11 ; Max = 65).

Among the natural enemies of *H. patruelis*, predators included mainly coccinelids, which are known to be highly polyphagous. Concerning parasitoids, they were represented by the small egg parasitic wasp, *P. oophagus*. These entomophagous insects, especially the wasp, were also recorded by Weaving [12] in Rhodesia. About *P. oophagus*, it has been described from Congo Belge by Ghesquière [41] as an ovi-parasitoid of *Tryoza erytreae* Del Guercio feeding on *Citrus* spp.; then he recognized his error and studied the wasp's biogeographical and ethological features as an ovi-parasitoid of *H. patruelis* also found on *Citrus* spp. [42].

Among ants species associated with *H. patruelis* on *V. amygdalina*, *P. megacephala*, *M. opaciventris* and *C. castanea* were the most frequent; these ant species are known to be randomly associated with various hemipterans on different non related plant species in tropical regions. For example, Weaving [12], working on groundnut in Rhodesia, recorded *P. megacephala*, *C. castanea* and *Camponotus* sp. associated with this bug while Bohlen [11] and NRI [13] recorded *Pheidole* spp. and *Anoplolepis* spp. Likewise, *P. megacephala*, *Crematogaster* spp. and *Acantholepis capensis* Mayr were mostly recorded tending *H. patruelis* on *F. sur* [20]. This suggests that ant species of the genera *Pheidole* and *Crematogaster* spp. are frequently involved in trophobiotic relationships with *H. patruelis.*

In this survey, the fecundity rate of *H. patruelis* extends from 650 to 770 eggs per female either in laboratory or in the garden during an approximate average of 41.5 days. Weaving [12] obtained a maximal fecundity rate of about 146 eggs per female (T = 23°C, H = 74%) during 44 days. This fecundity rate is weaker than those obtained in our study. The difference may be linked to experimental design or to the host plant which differs from the one studied here. In our results, fecundity rates were higher in polyandry than in monogamy and polygyny. However, the higher live span was recorded in monogamy. This suggests that monogamy ensures the best fitness to this bug. Furthermore, Weaving [12], working exclusively in monogamy, found a life span close to the one in our results.

For the duration of the pre-imaginal development, it extended from 54 days in the laboratory to 55 in the garden. This was longer than the one obtained by Weaving [12], 37 to 42 days. As for the fecundity, these disparities may be linked to differences in environmental conditions, including mainly differences in host plant and matting systems variation in the experimental designs. Further studies are needed to better explain the influence of biological and ecological features on the population dynamic pattern of *H. patruelis*.

CONCLUSION

The present study allowed us to extend the geographical distribution range of *Hilda patruelis* to Cameroon, with a new host plant, *Vernonia amygdalina* Delile. Associated with this bug, five ant species were recorded: *Pheidole megacephala* (F)., *Myrmicaria opaciventris* Emery, *Crematogaster castanea* Smith, *Camponotus* sp. and *Tetramorium simillimum* Smith. Eggs of *Hilda patruelis*were frequently parasitized by the wasp, *Psyllechthrus oophagus* Ghesquière. The wet season was more favorable than the dry for the proliferation of the bug. The fecundity of females was positively correlated with the number of males present. The whole duration of the development of *H. patruelis* ranged from 54 to 55 days. These findings improved knowledge on the biology and the ecology of a polyphagous pest of several tropical crops.

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