Article

Microhabitat preference and biology of *Holconia insignis* (Thorell, 1870) (Araneae:Sparassidae) in banana plantation agroecosystem

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Abstract
The abundance of *Holconia insignis* in banana plantations in Cameroon is a matter of concern. However, to date there have been no published studies that specifically synthetize information on the seasonal abundance and biology of *H. insignis* in banana plantation agroecosystem. In this study we made intensive samplings to study the seasonal response of *H. Insignis* across different banana plant stratain a banana agroecosystem in Cameroon while the biology of *H. insignis* was studied in the laboratory. Our results demonstrated that seasonal patterns of *H. insignis* differed among strata.Female *H. insignis* attached her egg sacin which she oviposited 238.3 ± 4.2 eggs in loosepeudostems and banana bunches. The female guarded the egg sac until hatching. Females required longer development time than males. Our results underscore the importance of ecology and biology of *H. insignis*that facilitate the development of appropriate management for *H. insignis.*

Keywords *Holconia insignis*; banana; huntsman spider; cocoon; spiderlings; microhabitat; biology.

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1 Introduction

Huntsman spiders are members of the family Sparassidae with over 1,200 different species distributed in warm climates throughout the world (Isbister and Hirst, 2003; Moradmand and Jaeger, 2011; Zhang et al., 2019; Jäger, 2020). Among different subfamilies distributed in the old world, *Heteropoda venatoria, Delenacan cerides* are more intensively, investigated (Rowel et al., 1995; Jäger et al., 2006; Yipet al., 2009; Ntonifor et al., 2012; Agnarsson and Rayor, 2013; Ewunkem et al., 2016; Jones and Rayor, 2017; Huang et al., 2017; Salvatierra and Almeida, 2017; Ding et al., 2020). The female spins a silken pad onto which she lays several hundred blue-green eggs into a tough web sac (McKeown, 1968). *H. venatoria* are known to carry her cocoon around her jaws under her body for weeks without feeding (Edwards, 2003; Ewunkem et al., 2016). Under laboratory conditions male and female require 305 and 391 days respectively to complete development (Ewunkem et al., 2016). *Delena cancerides* are social spiders living in proximity colonies (Beavis et al., 2007; Auletta and Rayor, 2011). A colony may consist of more than 300 individuals living in proximity under bark of dead Acacia and loose banana pseudostems where they feed communally (Rowell and Avilés, 1995; Ewunkem et al., Manuscript in prep). After mating, the female produces an egg sac in which she lays about 70 eggs and guards over it (Ewunkem et al., Manuscript in Prep). After incubation, the eggs hatch into spiderlingsand females required a significantly longer time to develop (339 days) compared to the males (218days) (Ewunkem et al., Manuscript in Prep).

Holconia is a genus of South Pacific huntsman spiders. The genus contains 9 species found in Australia which include, *Holconia colberti, Holconia flindersi, Holconia hirsute, Holconia immanis, Holconia insignis, Holconia murrayensis, Holconia neglecta, Holconia nigrigularis* and *Holconia westralia* (Dunlop et al., 2011). However, biological, and ecological knowledge of species of holconia are poorly investigated and sometimes very limited. *H. immanis* and *H. insignis* are the only two specieswhich have been studied (Jäger et al., 2006). *H. immanis* are found primarily on the trunks of trees and branches. Adults *H. immanis*live for about 4-16 months (Henle, 1993). Females *H. insignis* are known to produce egg sac without mating (Lake, 1986). During incubation, the female cease feeding and guarded the egg sac for about for several weeks (Lake, 1986).

Cameroon is one of the major producers of bananas in the world and the number one producers of bananas in Afro-Caribbean countries (Olumba and Onunka, 2020). The consistent presence of *H. Insignis* in banana plantations Cameroon is a course for concern because some species of huntsman spiders manage to survive in bunches of bananas in grocery stores despite precautions and visual inspections. Every year there are several news reports of huntsman spiders lurking in bunch of bananas in grocery stores scaring handlers and consumers of bananas (Edward, 2003). Insights intodevelopmental biology and the seasonal distribution of *H. insignis* in various micro habitatsin the banana plantation will potentially forecast the species vulnerability to environmental change or human activities, and as it provides where the populations occur along with their habitat requirement.

2 Materials and Methods

2.1 Study areas

The study sites were Tiko Banana Project in Tiko and Buea for field and laboratory studies, respectively. Buea is 15.8 km (9.8 mile) from Tiko. Tiko is characterized by high temperature (21.1°C) and rainfall (2975 mm) annually while Bueahas an average temperature of 20°C and an annual rainfall of 2625 mm.

2.2 Sampling program and techniques

To study the seasonal variation of *H.insignis,* the banana plantation was visited twice weekly in the morning (08:30-10.30 hrs) during the dry and rainy seasons.The sampling units consisted of 100 pseudostems which were randomly selected and a 2.0 m circumference around each sampled pseudostem. Each pseudostem was stratified into 3 sections based on heights from the ground:

- Stratum A= ≤ 1 m from the base of the plant
- Stratum B= >1≤2.5 m from section
- Stratum C=>2.5 m to the leaves and bunches

Through direct or *in situ* counts, the number of *H. insignis* different insect species, lizards, geckoes, frogs and toads on each pseudostem and anywhere within the 2.0 m circumference were recorded on data forms.

2.3 Laboratory rearing of *Holconia insignis*

Ten adults *H.insignis were* captured with hands and sometimes nets from their hideouts during every farm visit. The captured spiders were then transported to the laboratory in nylon mesh bags (10 cm wide and 25 cm high) provided with a 30.0 cm string to tie the opening or in plastic specimen cups measuring 7.2 cm deep 6 cm wide around the brim. Each cup was covered with a lid that had several tiny holes on it to aerate the containers. In the laboratory the spiders were released into mesh cages (31 cm × 31 cm × 36 cm). The spiders were kept at mean ambient temperatures of 20 ± 3°C and relative humidity of 70 ± 10% and fed *ad libitum* with insects *Musca domestica* (Insecta: Diptera: Muscidae) feral moths, crickets, cockroaches, and grasshoppers. The insects were attracted as described by Ewunkem et al. (2016).

2.4 Incubation period and developmental durations of *Holconia insignis*

Twentyegg sacswere collected in their natural habitat in the field and put in a specimen cup described above. The females were often seen guarding their egg sacs. The eggs sacs were kept at ambient laboratory conditions and observed for hatching. Ten other cocoons were torn open with aid of forceps and the eggs therein counted.After emergence, each individual neonate was transferred meticulously into a specimen cup to study the developmental durations. The total number of 30 spiderlings were reared in separate containers. During the study, each neonate was fed *ad libitum* with *Musca domestica* (Insecta: Diptera: Muscidae). About 5-6months later each neonate was fed with 8 bigger houseflies per spiderlings per day. The developmental duration after each molt was recorded and the length of the various body parts measured. The specimen cups were cleaned immediately after the spiders had fed, to shun ants which fed on the insect left over.

2.5 Data analysis

Data on vertical distribution, seasonal changes in field distribution and total developmental periods of male and female were analyzed for significance with *t*-test ($p<0.05$) using GraphPad Prism Version 8.

3 Results

3.1 Habitats of *Holconia insignis* in the banana ecosystem

During the study which lasted for about a year 72 adults female *Holconia insignis were* generally found guarding their egg sacs in dark areas such as banana leaf petioles and under loose barks and banana bunches (Fig. 1). In these habitats the spiders typically had a face-down vertical orientation.

Fig. 1 *Holconia insignis* adult female guarding an egg sac in banana bunch.

3.2 Seasonal Occurrence of *Holconia insignis* in various habitats

Holconia insignis displayed the highest abundance between June and November and peaked again between December and March (Fig. 2). The abundance of *H. insignis* in the agroecosystem demonstrated a significant relationship with rainfall (Fig. 2). We also observed a trend towards higher insect fauna and lower insect fauna abundance during dry and the raining seasonrespectively (data not shown).

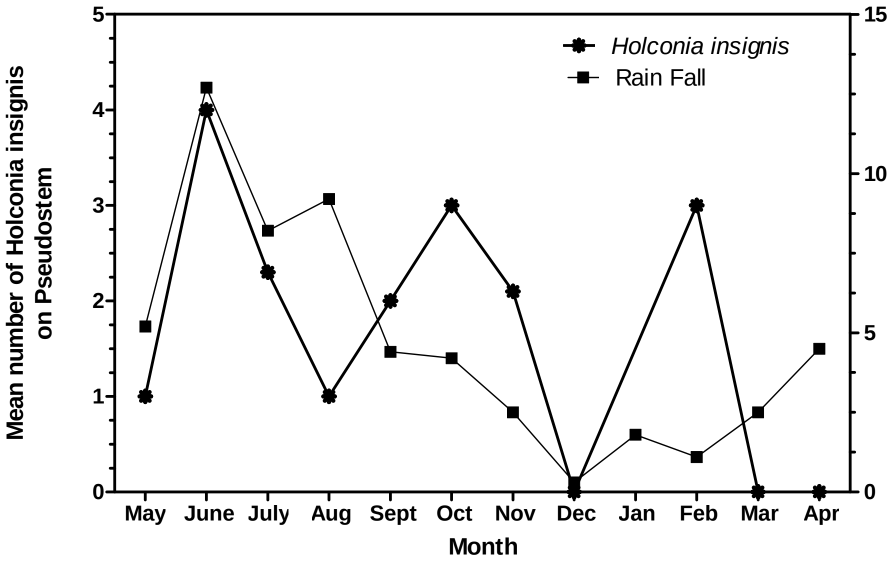

Fig. 2 Seasonal variation in mean number of *Holconia insignis* in banana agroecosystem in Tiko, Cameroon.

3.3 Vertical distribution of *Holconia insignis*

Rainfall affected the vertical distribution of *H. insignis* on pseudostems. During the dry season a significantly (p<0.05) high abundance of *H.insignis* was observed under loose barks at stratum A ≤ 1m from the base of the plant while during the rain the relative abundance of the spiders was significantly (p<0.05) higher at stratum C= from upper limit of C to the leaves (Fig. 3).

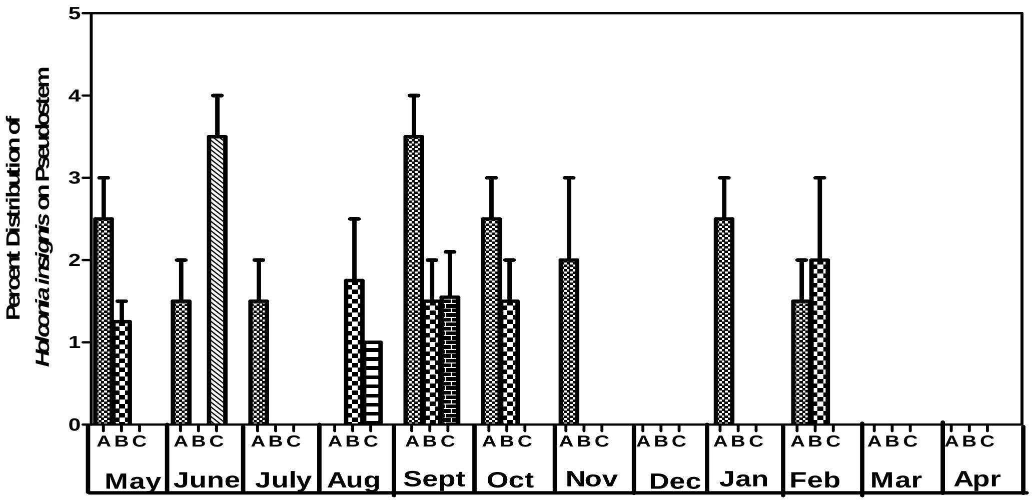

Fig. 3 Seasonal changes in the vertical distribution of *Holconia insignis* density (mean ± SE) on banana pseudostems in banana agroecosystem,Tiko, Cameroon.Each pseudostem was stratified into 3 sections based on heights from the ground: A= ≤ 1m from the base of the plant; B= ≥ 1.5m from A; C= from upper limit of C to the leaves.

3.4 Biology of *Holconia insigna*

During the whole period of study, mating, constructions of cocoon and oviposition behaviour were not observed. A female made flattened egg sac or cocoon 21± 1.8 mm in diameter which contained 238.3 ± 4.2 eggs (Table 1) were generally constructed at night. The initially white cocoon gradually changed to a greyish colour 12 days after construction. Throughout the period that the female spider guarded her cocoon, she did not feed, and movement was restricted. After hatching the spiderlings (222 ± 1.6)(Table 1) stayed within the egg sac or cocoonfor 5 days. Thereafter, the spiderlings emerged through a series of holes along the circumference of the cocoon and produced silk strands.

Table 1 Mean diameter (mm ± SE) of cocoon, number of eggs and spiderlings per cocoon of the huntsman spiders.

Diameter of cocoons±SE (range) N=20	Number of eggs±SE (range)	Number of spiderlings±SE (range).
21± 1.8	238.3± 4.2	222 ± 1.6
(20-22)	(215-230)	(216-230)

Values in parentheses represent ranges.

After the emergence of the spiderlings the female still guarded her cocoon for10 days. The total number of 30 spiderlings (Fig. 4) were reared in separate containers as alluded to in the materials and methods. Three males (Fig. 5) and 14 female (Fig. 1) attained maturity (Table 2). After the penultimate moult, the reproductive organs became well developed. In adult males, the tips of the pedipalps were greatly enlarged and the epigynum of the female was conspicuous. Females needed a significantly longer (p<0.05)(347.3±0.3 d) development time than males (256 ±0.3 d) (Table 2). The various body parts increase after every moult (data not shown). The appendages of the males were not significantly (p<0.05) longer than the female's (Table 3).

Fig. 4 Juvenile *H. Insignis.*

Fig. 5 Adult male *H.insignis.*

Table 2 Mean developmental duration (Days ± SE) of various instars of *Holconia insigna.*

	Developmental duration (Days ± SE)	
Instars	Male: n = 3	Female: n = 14
L_1	32.2±0.2	24.1±0.4
	(22 – 35)	(25 – 32)
L_2	23±0.3	29.6±0.4
	(20 – 32)	(18 – 33)
L_3	18±0.4	29.6±0.6
	(21 – 35)	(18 – 42)
L_4	42±0.1	42.3±0.2
	(21.3 – 36)	(19 – 30)
L_5	23±0.2	22±0.3
	(22 – 39)	(26 – 42)
L_6	17±0.4	20.2±0.4
	(23 – 40)	(28 – 45)
L_7	36±0.3	39±0.3
	(22 – 41)	(23.2 – 32)
L_8	29±0.2	35.1±0.2
	(23 – 43)	(25 – 40)
L_9	36±0.2	42.1±0.3
L10	-	(30-45)
L11	-	30.2±0.2
		(35-50)
		34.1±0.2
Total	256±0.3	347.3±0.3

Values in parentheses represent range.

Table 3 Mean length (mm ± SE) of various body parts of adult *Holconia insigna*.

Body Part	Male: n=3	Female: n=10
Pedipalps	8.5±0.12	9.4 ± 0.32
	(7.1-9.2)	(9.0 – 10.2)
1st Walking leg	32.5±0.23	29.6 ± 0.73
	(30.2-33.1)	(28.1 – 33.2)
2nd Walking leg	40.3±0.24	35.1 ± 0.65
	(36.1-42.2)	(31.1- 37.2)
3rd Walking leg	32.2±0.35	26.2± 0.68
	(32.1-35.2)	(24.1 – 30.2)
4th Walking leg	28.6±0.32	26.7 ± 0.58
	(28.1-33.2)	(23.9 – 30.1)
Cephalothorax	5.2±0.21	6.0 ± 0.37
	(5.0-7.1)	(5.2 – 7.2)
Abdomen	5.6±0.31	6.6 ± 0.32
	(5.1-7.2)	(6.1 – 7.3)

Values in parentheses represent ranges.

4 Discussion

Bananas are grown widely throughout Cameroon for export and as major staples (Awang, 2016). In early 2004, the Tiko Banana Project received several complaints from the international Market relating to the infestation of their bananas by huntsman Spiders. These spiders were subsequently identified by a senior keeper at Stapley as *Heteropoda venatoria* (Peter, 2004; Ntonifor et al., 2012; Ewunkem et al., 2016). Other species of huntsman spiders such as *Delena cancerides* (Ewunkem et al., manuscript in prep) and *Holconia insigna* also live among banana trees which may show up in banana shipments.No comprehensive studies, however, are available describing the biology and ecology of *H. insigna*. As a result of this study, we identified the suitable habitats and seasonal variation of *H.insigna* in a banana agroecosystem and, explore developmental biology of *H. insigna*.

In the banana agroecosystem *H.insigna* are found living in quiet, undisturbed under loose barks, in banana bunches and leaf petioles.These habitatsprovide a perfect environment for spiders tohide because they are dry, warm, and dark (Ntonifor et al., 2012; Ewunkem et al., 2016; Privet et al., 2020). These microhabitats also serve as safe havens for other species of huntsman spiders due to their flattened bodies (Rowell et al., 1995; Edward, 2009; Nelson et al., 2012; Ewunkem et al., 2019 and Ewunkem et al., manuscript in preparation). The relative abundance of *H. insigna* showed much temporal fluctuation during dry and raining seasons in all the microhabitats. Generally, during the dry season loose barks were the most preferred habitats with maximum abundance of the denizen and they migrated to the bunches for refuge when it rained. Most bunches are covered with blue plastic bag to provide a suitable microclimate for the bananas and *H. insigna*. *H. venatoria* and *D. cancerides* are also known to seek refuge in the bunches during the rainy seasons (Ewunkem et al., 2016; Ewunkem et al., manuscript in preparation).The ability of *H.insigna* to thrive in the banana agroecosystem coupled with its adaptability to microhabitats may help explain the possibility to be exported to international markets.

During this study courtship, mating, construction of an egg sac, and duration of eggs incubation were not observed. Female *H. insigna* produced a white oval egg sac under barks or in banana bunches. She laid ca ~239 eggs in the egg sac guarding it until the spiderlings emerge. Female spiders are known to construct egg sacs in microhabitats characterized by low mean temperature where she provides high temperaturerapid embryonic development andguard the egg sacs against potential predators (Machado and Oliveira, 2002; Goldsbrough et al., 2004; Pike et al., 2012; Pruett et al., 2020). The young growing spiders moltedonce a month. Following every molt,male developed longer legs compared to the females which allow the males to run fasterto reduce likelihood of sexual cannibalism (Moya-Laraño et al., 2008; Alissa and Eileen, 2016; Kuntner and Agnarsson, 2018). However, the developmental duration of females were relative longer compared to male which corroborates with studies on other Sparassids (Ewunkem et al., 2016; Ewunkem et al., manuscript in prep). The frequency of mating and developmental duration may be shorter in the wild because molting frequency of juvenile and adult be influenced by factors such as temperature, season, photoperiod, density, habitat, nutrition, and social interaction (Waddy et al., 1995).

5 Conclusion

Banana cultivation is one of the key aspects of agricultural development in Cameroon. These bananas are grown primarily for export. The presence of the banded huntsman spider, *Holconia insignis* in marketed bananas may scare consumers and can affect market prices resulting in financial losses to the banana industry. The spiderswereseen lurkingin loose psuedostems, and banana bunches due to their flattened bodies. The pattern of fluctuations in *H. insignis* abundance was more predominant in loose psuedostems than the bunches during the raining season compared to the dry season while an opposite trend was observed during the raining season. A female *H. insignis* actively guard her egg sacs for ca~25 days. Late into incubation, the female moistened and gently tear the egg sac to help her spiderlings. Male spiders mature before the females. Future research could usefully document courtship, mating behavior and oviposition of *H. insignis.*

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