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Biological diversity of the entomofauna associated with *Citrullus lanatus* (Cucurbitaceae) flowers and assessment of its impact on yields

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Abstract
Experiments were conducted to study the biodiversity of the floral entomofauna of *Citrullus lanatus* and assess its impact on yields. Two treatments of female flowers were differentiated according to the presence or the absence of protection for insect visits. In the third experiment, a hive bee was placed on the field of watermelon. The insect species richness, the relative abundance, the frequency of visits and guilds were recorded and yields were compared between treatments. Four guilds, including nectarivorous, pollinivorous, fruit-feeders and predators were found on flowers. Bees showed floral constancy during the blooming period. Constant species may be of use in the pollination of Watermelon with the prominent *Apis mellifera adansonii*. Female flowers without bee visitations do not produce fruits. Yields were high in treatment with open-pollinated flowers especially with honeybee management. It is important practice to introduce colonies of honeybee hive on the perimeter of *C. lanatus* fields.

Keywords: *Citrullus lanatus*, flower, entomofauna, *Apis mellifera adansonii*, pollination, yield

1. Introduction

Citrullus lanatus is of African origin [13, 21]. However, this crop is now cultivated especially in tropical, subtropical and arid regions of the world [28]. Flowers of *C. lanatus* are unisexual, with male and female flowers occurring on the same plant [24]; this is the characteristic of many species in the family Cucurbitaceae, making them even more dependent on pollinators, as the probability of self-pollination is extremely low [23]. In 2014, the global production of watermelons was 111 million tons, with China alone accounting for 67% of the total. Secondary producers each with less than 4% of world production included Turkey, Iran, Brazil and Egypt [28]. In Cameroon, the domestic production of edible fruits here is about 38,413 tons annually, only represented 0.04% of the world production [22].

Concern for the conservation of biological diversity for the survival of mankind, is a central point of action by countries and institutions [9]. Pollinators are a key component of global biodiversity, providing vital ecosystem services to crops and wild plants [12]. Their abundance and diversity are known as parameters for enhancing crop production [16] and ensuring the maintenance of plant biodiversity [19]. Nowadays, it is necessary to focus on several species of pollinating insects and to take into account the importance of interactions between natural ecosystems and agroecosystems to safeguard pollinating fauna [18]. The management of insects to crop pollination and beekeeping has ever-since spread to most parts of the world, especially to developed countries where bees are used for commercial pollination of high-value crops [15]. In the rest of the world, most farmers rely on natural or unmanaged pollination service [15]. The inventory of anthophilous insect in each region allows their knowledge in the interest of their conservation and could meet the pollination requirements of a greater number of crops. This research work has a main objective to search for entomological skills that could facilitate rising yields in the production of *C. lanatus*. The following specific objectives were targeted: (1) study of the components of flower-visiting insects of *C. lanatus*; (2) assessment of parameters related to the biological diversity of foragers; (3) estimates of the repercussion of the flower-visiting insects on fruit and seed yields of *C. lanatus*.

2. Materials and methods

The study site was located near the Institute of Agricultural Research for the Development (IRAD) of Nkolbisson in the neighborhood of Yaoundé (3°47-3°36 N; 11°10-11°45 E) in the Centre Region of Cameroon. The climate here is of Guinean type, including four seasons with two differently dry and rainy seasons: the brief rainy season (March to June) is followed by the short dry season (July to August) and the long rainy season (September to November) is followed by the more extended dry season (November to March). The annual rainfall can reach 2000 mm and mean annual temperature is about 25°C.

Experiments were carried out in an area of 2500 m^2 during three seasons: the long dry season from January to March 2010, the short rainy season from March to June 2011 and the long dry season from January to March 2012. Three watermelon seeds of "sugar baby" variety were sown in holes of 20 cm of depth and 10 cm in diameter half-filled with a mixture of ash and soil. These holes were separated by 2 m within and apart in rows. Two weeks after sowing, plantlets were trimmed to one per hole and chemical fertilizer (NPK 20-10-10) was applied around each conserved plant of each hole. The weeding of the plants was manually performed, for it was necessary to maintain weed-free plots. Watering was done daily during the first and third seasons (dry season) with one litre of water measured per hole at the dawn and at the twilight. No insecticide or fungicide treatment was applied during our experiments to preserve the anthophilous insect fauna and to easily identify the different causes of yield losses. At the beginning of the flowering, NPK 20-10-10 was widespread once more in each hole.

For each experiment, 120 plants were labelled at random and split into two treatments: the unrestricted-visit treatment (treatment 1), which included 100 plants tagged at random with open-pollinated flowers that were freely exposed to the foraging activity of anthophilous insects. In no-visit treatment (treatment 2) made of 20 plants tagged at random, pistillated flowers were isolated from insects using gauze bags (5x10 cm) to prevent anthophilous insect visitations. Isolation of female flower buds was done the day before their opening; emerged petals were allowed to accurately detect the imminent blossoming of the correspondent flowers the following day, and the bags were removed a day after anthesis. One honeybee colony was placed along the edge of the field at the flowers' anthesis of watermelon in the third experiment.

Direct observations on treatment 1 flowers were made daily on natural population of insects present in the experimental field during the blooming period according to the usual protocol [2, 3, 4]. Insect identification was made using the collections available in the entomology lab of the Institute of Agricultural Research for Development of Nkolbisson (Yaoundé, Cameroon) and specialized identification keys of [8]. Bee identification was done by Pr. Tchuenguem Fohouo Fernand-Nestor and Dr. Pauly Alain.

The following parameters were registered: the relative abundance of each insect species, the number of guilds, which determines the functional diversity [7], and the seasonal frequency of forager visits. Comparisons were done between treatments based on the fruit set rate [(the number of fruits developed/the number of female flowers) x 100], the rate of ripe fruits [(number of ripe fruits/number of female flowers) x 100], the abortion rate [(100% – the rate of ripe fruits)] and the average number of mature seeds per fruit.

Data were analyzed through descriptive statistics and the normal-theory statistical analysis was used on continuous variables by applying the standard analysis of variance (ANOVA). Statistical analyses were facilitated using SAS 6.03.

3. Results

Table 1 shows the components and the relative abundance of the entomofauna associated with *C. lanatus* flowers in Nkolbisson (Yaoundé, Cameroon). This table emphasizes seven insect orders. Hymenoptera with 97.48% of the total visits was the numerically predominant order and the most species-rich, instead of the other orders which represented only 2.52% of the total insect visits. Twenty families were found during our investigations with Apidae (90.83% of visits) the prominent. Overall, 21,498 visits of 37 flower-visiting insect species were recorded on 32,939 *C. lanatus* flowers for three seasons. Among these insect species, the African Honeybee *Apis mellifera adansonii* was predominant with 69.86% of the total number of the visits.

For our field observations and based on the feeding strategies of the insects recorded, four guilds associated with *C. lanatus* flowers were found (table 2). The guild of pollinivorous included mainly pollen grains collectors especially bee species. The corbiculate bee species actively collected large amounts of pollen grains to provision their offspring. The guild of nectarivorous included insects which were seen extending their proboscises to the nectary for nectar harvesting. Moreover, bee species appeared as the most efficient and numerically important. Flower feeders included insect species which ate flower petals with *Zonocerus variegatus* as the most impressive of them. The flies of the genus *Dacus* were the main fruit feeders with respect to the feeding behavior of their larvae which were responsible for high fruit decaying. Lastly, predation behavior was expressed by the capture or the attempted capture of certain flower visitors by predators such as *Mantis religiosa* and *Polistes* sp. Globally, pollinivorous and nectarivorous insects were numerically predominant, representing up to 97% of the number of visits of individuals collected on *C. lanatus* flowers.

The distribution of flower-visiting insect species according to their daily regularity is reported in table 3. Three groups of anthophilous insects were found on watermelon flowers: constant species that include only bees, accessory species and accidental species.

From table 4, the fruit set rate was 67.3%, 59.8% and 78.4% in treatment 1 and 0 in treatment 2 in 2010, 2011 and 2012 respectively. The abortion rate was optimal (100%) in treatment 2; abortion also occurs in treatment 1 with 73.5% in 2010, 87.9% in 2011 and 59.9% in 2012. The mean number of seeds per fruit was important in treatment 1 especially with bee management and 0 in each treatment 2. Consequently, the influence of insects mainly bees was positive and decisive for *C. lanatus* production.

The fruit set rate, the abortion rate and the average number of mature seeds per fruit from treatments 1 vary with the season associated with the experimental design. Differences of the fruit set rate (F = 7.72; df = 2, 297; P < 0.05), the abortion rate (F = 10.82; df = 2, 297; P < 0.05) and the average number of mature seeds per fruit (F = 13.17; df = 2, 297; P < 0.05) were significant between the three cropping seasons. Overall, yields were increased considerably in the third season within treatment 1 which benefited from honeybee management.

Table 1: Biological diversity and relative abundance of flower-visiting insects of *Citrullus lanatus* in Nkolbisson

Order	Family	Insect species	n	P (%)
Hymenoptera	Apidae	*Apis mellifera adansonii*	15018	69.86
		Meliponula bocandei	2636	12.26
		Meliponula nebulata	1026	4.77
		Dactylurina staudingeri	488	2.27
		Xylocopa olivacea	265	1.23
		Hypotrigona gribodoi	91	0.42
		Xylocopa torrida	4	0.02
	Total Apidae		19528	90.83
	Halictidae	*Halictus* sp. 1	452	2.11
		Halictus sp. 2	430	2.00
		Crocisaspidia chandleri	277	1.30
		Thrinchostoma wessmannii	29	0.13
	Total Halictidae		1188	5.54
	Formicidae	*Camponotus brutus*	81	0.38
		Camponotus flavomarginatus	56	0.26
		Camponotus acvapimensis	32	0.15
		Myrmicaria opaciventris	31	0.14
	Total Formicidae		200	0.93
	Vespidae	*Polistes* sp.	11	0.05
		Belonogaster juncea	2	0.01
	Total Vespidae		13	0.06
	Sphecidae	*Philanthus triangulum*	25	0.12
Total Hymenoptera			20954	97.48
Diptera	Syrphidae	*Paragus borbonicus*	68	0.31
		(1 sp.)	12	0.06
	Total Syrphidae		80	0.37
	Muscidae	*Musca domestica*	51	0.24
	Tepritidae	*Dacus* spp.	43	0.20
	Calliphoridae *Calliphora* sp.		3	0.01
Total Diptera			177	0.82
Coleoptera	Coccinellidae	*Cheilomenes lunata*	54	0.25
	Lagriidae	*Lagria villosa*	40	0.19
	Chrysomelida	*Monolepta intermedia*	37	0.17
		Asbecesta transversa	34	0.16
		Aulacaphora foveicollis	11	0.05
Total Chrysomelidae			82	0.38
Total Coleoptera			176	0.82
Lepidoptera	Acraeidae	*Acraea acerata*	36	0.17
	Cassidae	*Leuzera pyrina*	33	0.15
	Nymphalidae	(1 sp.)	12	0.06
		(1 sp.)	4	0.02
Total Nymphalidae			16	0.08
	Amateidae	*Oecromia* sp.	7	0.03
Total Lepidoptera			92	0.43
Orthoptera	Pyrgomorphidae *Zonocerus variegatus*		48	0.22
	Acrididae	(1 sp.)	22	0.10
Total Orthoptera			70	0.32
Hemiptera	Alydidae	*Riptortus dentipes*	15	0.07
Dictyoptera	Mantidae	*Mantis religiosa*	14	0.06
Total général			21498	100

Legend: n: cumulated number of visits of an insect during the three seasons; P: percentage of visits = (n/21498) x 100

Table 2: Functional groups associated with *Citrullus lanatus* flowers in Nkolbisson

Guilds	Family	Insect species	Activities on flowers
Pollinivorous	Apidae	*Apis mellifera adansonii*	Active pollen harvesting and its accumulation on corbiculae of hind legs
		Meliponula nebulata	
		Meliponula bocandei	
		Dactylurina staudingeri	
		Hypotrigona gribodoi	
	Halictidae	*Crocisaspidia chandleri*	
		Thrinchostoma wessmannii	
		Halictus spp.	
	Muscidae	*Musca domestica*	Direct pollen consumption on anthers with mouthparts
	Syrphidae	*Paragus borbonicus*	
Nectarivorous	Apidae	*Apis mellifera adansonii*	1) Nectar gathering ; 2) Passive pollen harvesting through the tegument and its transportation and deposition from flower to flower enabling cross-pollination.
		Meliponula nebulata	
		Meliponula bocandei	
		Dactylurina staudingeri	
		Hypotrigona gribodoi	
	Halictidae	*Crocisaspidia chandleri*	
		Thrinchostoma wessmannii	
		Halictus spp.	
	Formicidae	*Camponotus brutus*	Nectar gathering
		Camponotus flavomarginatus	
		Myrmicaria opaciventris	
	Acraeidae	*Acraea acerata*	
	Lyceanidae	*Virachola anthalus*	
Flower feeders	Chrysomelidae	*Aulacophora foveacollis*	Consumption of floral pieces
		Monolepta intermedia	
		Asbecesta transversa	
	Pyrgomorphidae	*Zonocerus variegatus*	
	Tephritidae	*Dacus* spp.	Egg hatching into the ovary or the young fruit
Predators	Mantidae	*Mantis religiosa*	Capture of other insect species considered as preys
	Vespidae	*Polistes* sp.	

Table 3: Distribution of anthophilous insects according to the seasonal frequency of visits

Insect species	*nj*	*f* (%)	Category of insects
Apis mellifera adansonii	55	91.7	Constant species ($f \geq 50\%$)
Meliponula bocandei	40	66.7	
Meliponula nebulata	36	60.0	
Dactylurina staudingeri	32	53.3	
Xylocopa olivacea	31	51.7	
Acraea acerata	29	48.3	Accessory species ($25\% \leq f < 50\%$)
Philanthus triangulum	27	45.0	
Paragus borbonicus	23	38.3	
Crocisaspidia chandleri	21	35.0	
Thrinchostoma wessmannii	18	30.0	
Camponotus brutus	16	26.7	
Camponotus flavomarginatus	15	25.0	
Dacus spp.	14	23.3	Accidental species ($f < 25\%$)
Calliphora sp.	13	21.7	
Monolepta intermedia	11	18.3	
Asbecesta transversa	11	18.3	
Zonocerus variegatus	11	18.3	
Belenogaster juncea	10	16.7	
Musca domestica	10	16.7	
Philanthus triangulum	8	13.3	
Cheilomenes lunata	5	8.3	
Mantis religiosa	5	8.3	

Legend: $f = (n/60) \times 100$; n = number of days the insect was observed during the 60.

Table 4: Yields of *Citrullus lanatus* as function of treatments and seasons

	Experiment 1		Experiment 2		Experiment 3	
Yield parameters	T_1	T_2	T_1	T_2	T_1	T_2
Number of plants	100	20	100	20	100	20
Number of female flowers	517	258	481	158	496	230
Number of fruit developed	348	0	298	0	389	0
Fruit set rate (%)	67.31	-	59.87	-	78.43	-
Abortion rate (%)	82.2	100	92.52	100	68.55	100
Number of ripe fruits	92	0	36	0	156	0
Percentage of ripe fruits (%)	17.8	0	7.48	0	31.45	0
Mean number of seeds/fruit	359.1 ± 43.3	0	216.7 ± 52.3	0	427.4 ± 83.6	0

Legend: T_1 = Treatment 1; T_2 = Treatment 2

4. Discussion

Species richness and components of the flower-visiting entomofauna of *C. lanatus* vary considerably with biomes. 37 insect species were recorded on the flowers of the crop in Yaoundé (Cameroon). Findings in Sacramento (USA) on the same plant revealed 13 species [18]; those at Yatta (Kenya) 10 species [23] and those near Pune (India) 4 species [5]. Additionally, anthophilous insects of *C. lanatus* listed in the four ecological areas are different from each other with the exception of *Apis*, which is the common genus. Our results confirm other report that Hymenoptera, mainly bees, are the most important foragers for a greatest number of crop plants including *C. lanatus* [17, 18, 31]. Without a doubt, the family Apidae which belongs to this order includes social species which are distinguished particularly by the large size of their colony as well as their ability to recruit several congeners to harvest an attractive source of booty [27]. *Apis mellifera* is known as the main anthophilous insect of other Cucurbit species [1, 2, 4, 11, 14, 23, 26, 29, 31, 32].

Watermelon blossoms are a whole community that interacts with local entomological fauna. Within this community, each group of insects shows a specific link which can be beneficial or detrimental for the production of the host plant. Indeed, many ecologists have found it useful to group insects into guilds to study the ecological interactions between them, their hosts, their natural enemies and the season [30]. Among the trophic groups recorded on *C. lanatus* flowers, nectarivorous, pollinivorous and natural enemies differently influenced yields of watermelon.

Bee species visit watermelon flowers from which they obtain pollen and nectar as food resources. During this, they pollinate female flowers visited. Indeed, bee foragers regularly contacted anthers and carried pollen with which they flew frequently from male flowers of one plant to the stigma of female flowers of the same plant or to that of another watermelon plant. Furthermore, *C. lanatus* is a self and cross-compatible crop in which pollen grains are not windblown. Consequently, bees can increase the chances for geitonogamy and xenogamy on watermelon flowers [2]. Among nectarivorous and pollinivorous species, *A. m. adansonii*, *M. bocandei*, *M. nebulata*, *D. staudingeri* and *X. olivacea* were constantly active on the flowers of *C. lanatus* in full bloom. It was realized that insect floral visits enable fruit set and promote the increase in fruit and seed yields of crops. This is the reason pistillated flowers free from the activity of bees do not produce fruits and seeds. Hence, the foraging activity of bees appears to be the limiting factor in fruit and seed productions of *C. lanatus*. The same observation was made on the other species of Cucurbits [2, 4, 11, 14, 29, 31]. In general, watermelon is a crop that has heavy mellitophilous pollination requirements [18]. It is also neither parthenocarpic nor apomictic under natural conditions. The data show positive effects of the introduction of honeybee pollination in the field of *C. lanatus*. The presence of honeybee colony statistically improved the mean number of fruits per plant, the average number of seeds per fruit and reduced significantly the abortion rate. The increase in these parameters was shown by several authors on Cucurbitaceae species among *C. lanatus* [18] and *Cucumeropsis mannii* [25].

Despite the presence of pollinators and honeybee management in treatments 1, it resulted in important abortion rates as well. Beyond the physiological phenomena peculiar to the crop associated with the lack of pollination by bees, the premature fall of young fruits is also linked to the recrudescence of the Tephritidae species, natural enemies of *C. lanatus* found in our experimental field. It was reported that in West Africa, *Dacus ciliatus* and *Dacus vertebratus* cause losses of fruit yield of *Cucumis melo* by more than 60 % [6, 33]. Findings in Yaoundé, Cameroon indicated that depredations due to insects and microorganisms can cause fruit losses of *C. mannii* exceeding 80 % [10]. So, floral activity by insect pests seriously affects even the precocious fruit yields of *C. lanatus*.

Besides biotic factors, abiotic factors such as heavy precipitations influenced the pollination activity of bees on the host plant. From our field observations, the abortion rate was generally high during the rainy season compared with the dry season. Indeed, wet weather reduced the number of floral visits by bees and represented a threat for fruit set of *C. lanatus.* Our findings corroborated those on the flowers of *Mikania micrantha* in Viti Levu, Fiji [20]. The rainfall has been already documented as an important factor for pollinator visit disruption [2, 20, 27]. Moreover, in the rainy season, *C. lanatus* was experienced an upsurge in fungal diseases, and nematodes which greatly affected the expected fruit yields. Thus, an optimum watermelon yield also requires a good season choice, with off-season and under irrigation cultivation generally favorable.

5. Conclusion

Our investigations on the study of flower-visiting insects of *C. lanatus* revealed that this plant species is primarily mellitophilous. *A. m. adansonii*, *M. bocandei*, *M. nebulata*, *X. olivacea* and *D. staudingeri* were the principal foragers on this crop. Floral visits of these bees on *C. lanatus* were mainly devoted to the collection of nectar and pollen; therefore, this plant species is useful for strengthening the nutritional needs of the bee species concerned for their conservation. Moreover, *C. lanatus* depends on its production to bee floral activity. The absence of pollinating visits of bee species on female flowers resulted in their premature fall. Bee pollination appears as a factor of production in which farmers can increase annual yield of fruits and seeds by installing colonies of bees in the fields of *C. lanatus*. However, the production of this crop remains subject to many yield losses due to the depredating activity of fruit flies. The development of an integrated approach in the process of improving yields of watermelon is necessary; it should promote bee pollination as a key factor for the production of the crop, establish bee conservation policies, and develop methods of integrated protection against fruit flies.

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