

Green fruit visitor insects of *Annona senegalensis* Pers. (Magnoliales: Annonaceae) and their potential role in the plant fruiting

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Abstract

Annona senegalensis Pers. (Annonaceae) is a wild shrub used multipurposely in food and traditional medicine by local people in African tropical countries. During its fruiting period, especially at the green fruit stage, this plant harbors an important community of insects such as the planthopper *Hilda undata* (Tettigometridae) and various ant species (Formicidae). This study aimed at understanding the interactions between ants, *Hilda undata* and the plant *Annona senegalensis*. Green fruit insect visitors of *A. senegalensis* were collected randomly from 30 green fruits of 30 plants per site, at four sites across the Sudanian and Sudano-Sahelian climatic zones of Burkina Faso during the green fruit stage of the plant, from June to July. The behavior of green fruit visiting insects was observed in the field. In addition, ants and *Hilda undata* were collected and their whole body sugars were analyzed. Ants were the most recorded visiting insect group, representing up to 95% of the insects observed on the fruit. The orders of insects recorded were Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera and the most frequently encountered species were *Trichomyrmex abyssinicus*, *Crematogaster* sp (Formicidae), *Messor galla* (Formicidae), *Brachyponera sennaarensis* (Formicidae) and *H. undata*. Behavioural observations showed that ants consume extrafloral nectar from green fruits and honeydew from *H. undata*. The ants would protect the green fruits from their pests and *H. undata* from its natural enemies. This study is the first report highlighting mutualistic interactions between *A. senegalensis*, ants and *H. undata*.

Introduction

Plants are subject to multiple selection pressures related to plant pests and strong environmental constraints (Lebon 2014). Interactions between plants and phytophagous insects involve essentially host plants, pests and auxiliary insects (Pouvreau 1996). These interactions can be summarized around four main positive effects for both partners namely: i) pollination, ii) seed dissemination, iii) nutrition and iv) protection. Insect and plant diversification are closely related because the selective pressure of insects on plants has largely contributed to the diversification of plant lineages (Nicole 2002; Nel 2002; Sauvion et al. 2013). The nature of the age-old coexistence between insects and plants is mostly oriented towards mutualism, so the “partners” have become interdependent (Harry et al. 1994; Male 2011).

Evolution has selected mechanisms allowing phytophagous insects to synchronize their emergence to a period when the host plant is available, and to disperse in space and/or time if the resource becomes scarce or lacking (Sauvion et al. 2013). The phenology of plant hosts would exert selection pressure on insect developmental cycles and their reproductive periods (Sauvion et al. 2013). The effect of insect action on the plant is not always opposed to its development, but is often beneficial to the plant (Baker 1978). This is the case of ants (Hymenoptera: Formicidae) and Angiosperms which are ubiquitous and maintain a wide range of interactions where we usually find a beneficial result for both partners (Male 2011). Beattie (1985) have shown that plants may benefit from interacting with ants in basically five ways: (1) the ants protect the plant against herbivores; (2) the ants promote the growth and survival of the host plant by pruning foliage of neighboring plants; (3) the ants feed the plant with essential nutrients; (4) the ants disperse seeds and fruits; and (5) the ants pollinate the plant. Ants are also natural

enemies of many phytophagous insects and they can be the protectors of plants visited by these phytophagous insects (Orivel & Dejean 1999; Huignard 2013; Diamé et al. 2015). On the contrary, ants have extensive symbiotic relationships with certain groups of sap sucking Hemiptera, which indirectly affects plants (Cherix 1986; Delabie 2001; Guenard 2007; Sauvion et al. 2013). Ant-plant interactions could have positive or negative direct and/or indirect effects on plants and further research is needed to determine the balance that led to this coexistence.

In order to better understand plant-ant relationships, we focused on a shrub from tropical regions of Africa, *Annona senegalensis* (Magnoliales: Annonaceae). This plant is widely encountered in some regions of Burkina Faso (Millogo-Rasolodimby et al. 1996). The leaves, flowers, stem and roots are used by local people and exploited in several areas of use, mainly in traditional medicine and food. Its fruits are highly valued by these people as a major part of their diet (Maydell 1983; Arbonnier 2009; Zouré et al. 2021). In the Sudanian and Sudano-Sahelian climatic zones of Burkina Faso, the plant harbors a wide range of insect species during its fruiting season, particularly ants and the planthopper *Hilda undata* (Hemiptera: Tettigometridae) on its green fruits (Dao et al. 2022). Ants could feed on extrafloral nectar, *H. undata* honeydew or both, and could breed and defend the phytophagous planthopper or protect the plant against carpophagous insects. However, there is no information on the type of relationship that these insects have with this plant.

The aim of this study is to understand the role of ants and planthopper and the implication of the *Annona senegalensis*-ant relationship for the plant and its ecosystem. Specifically, we combined insect capture to behavioral observations and biochemical analysis of individual insects to determine the changes on carbohydrate content depending on their diet. The interaction these insects have with the plant and the role of ants in the fruiting process of the plant and planthopper visiting have been evaluated.

Materials And Methods

Study area

This study was conducted in four sites across the Sudanian and Sudano-Sahelian climatic zones of Burkina Faso, where *Annona senegalensis* is abundant. In the Sudanian zone, data were collected in two sites: the Dindèrèso classified forest (N 11°12'38", W 004°24'43") and Dafra (N 11°07'18", W 004°14'50") wastelands, located in the North-West and in the South of Bobo-Dioulasso. In the Sudano-Sahelian zone, the sites of Toroba classified forest (N 12°29'14", W 003°15'35") and the site of the Koussiri wastelands (N 12°33'09", W 003°15'47") located in the east side of Dédougou, were chosen.

The climate is tropical with two seasons in both study zones: the dry season from October to April, and the rainy one from May to September. Average annual rainfall varies from 800 to 1100 mm and average temperatures range from 25–30°C in the Sudanian zone compared to the Sudano-Sahelian zone where average annual rainfall varies from 500 to 900 mm and average annual temperature, from 25–32°C. The vegetation is composed of steppes, mosaic of woodlands, savannahs, and gallery forests.

Sampling Of Insects

Sampling was done on the Dindèrèso classified forest and the Dafra wastelands from June to July 2020. Toroba classified forest and Koussiri wastelands have been sampled from July to August 2020. On each day of sampling, 30 mature plants per site of *Annona senegalensis* bearing green fruits and at least 100 m apart from each other and growing in uncultivated areas were selected randomly. A total of 120 green fruits in the four sites were sampled, and the insects were collected on one fruit chosen randomly per tree.

The capture of insects was done by immersion of the green fruit carrying insects in a labeled vials containing 70% ethanol. Lepidopteran were dried after collection.

Observation Of Insect Behavior On Fruit

Observation was carried out at the Dafra and Dindèrèso sites in the Sudanian climatic zone where ants and *Hilda undata* are found simultaneously and are the most frequent insects on green fruit.

Observations were made on green fruits bearing only ants, and then green fruits bearing both ants and *H. undata*, randomly selected from *Annona senegalensis* plants. Insects were monitored on green fruits for 6 observations with a frequency of 10 days at each site during June and July corresponding to the green fruit period at these two sites. At each observation, 10 green fruits on 10 different plants at a rate of one fruit per plant were randomly selected and interactions were observed and described for approximately 10 min per fruit. Observations were conducted between 7:00 am and 10:00 am following the methods of Tchuenguem et al. (2014), Kouyaté et al. (2016) and Tchindebe et al. (2018). Observations consisted of determining the insect species and their abundance on each green fruits, the position of Formicidae and *H. undata* on the fruits and their behaviors. Two behavioral items were defined: **Feeding on extrafloral nectar**: the ant constantly scours the green fruit, affixing its mouthparts to the fine droplets of extrafloral nectar that it encounters in some places; **Feeding on honeydew**: the ant drums with its antennae on the back of the abdomen of *H. undata* present on the surface of the green fruit, a drop of honeydew is produced by the hemipteran and the ant applies its mouthparts on the drop.

Additionally, 20 fruits from each site were randomly selected and protected with waterproof paper bags (Fig. 1a) to exclude insect access, compared with 20 unprotected fruits, following the method described by Fonta et al. (1985) to demonstrate extrafloral nectar production by green fruits.

Identification Of Insects

The identification of the insects was conducted using taxonomic keys of Chinery (1986), Villiers (1943, 1977), Pihan (1986), Cherix (1986), Stanek (1984) and Bland (1947) or specimens already identified by taxonomic entomologist at the Laboratoire d'Histoire Naturelle (Ouagadougou, Burkina Faso).

Nectar And Insects' Collection

To collect extrafloral nectar from green fruits, 10 green fruits from 10 different randomly selected plants were protected in each site with waterproof paper bags, and then the nectar was collected the next morning and put into 5 glass tubes, using a method adapted from Fonta et al. (1985). We used pipettes to collect the nectar on the protected green fruits. The nectar in the tubes is immediately placed in ice and transferred to a freezer at about 15 minutes time after collection, where it was frozen and preserved to prevent enzymatic degradation of sugars.

Insects from 4 green fruits bearing Formicidae and *H. undata* were collected from plants with an anthill underneath the plant (Fig. 1b), preserved in 4 glass tubes, and placed under the same conditions as the collected nectar.

The nectar and insects were then lyophilized before being taken to a laboratory specialized in HPLC analysis of sugars at Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE Angers, France).

Extraction Of Sugars From Nectar And Insects By Hplc Analysis

Extraction of sugars from extrafloral nectar

Lyophilized extrafloral nectar was weighed and diluted in 80% methanol. After 15 minutes of incubation period (76°C), methanol was evaporated in a speed-vac for 3 hours at 42 °C, after which samples were diluted in 500 µL Milli-Q water. Finally, samples were saved in a final volume of 100 µL of Milli-Q water for HPLC analysis.

Extraction Of Sugars From Insects

Lyophilized insects were individually weighed, crushed and the powder obtained was diluted in 80% methanol for each sample. After 15 minutes of incubation period (76°C), methanol was evaporated in a speed-vac (3 hours at 42 °C) and samples were diluted in 500 µL Milli-Q water. Samples were then centrifuged (16000 rpm; 4 °C; 21 min) to isolate the supernatant containing all soluble sugars, and the water of the supernatant was evaporated in a speed-vac. Finally, samples were saved in a final volume of 100 µL of Milli-Q water for HPLC analysis.

Results

Green fruit insect diversity in the Sudanian and Sudano-Sahelian climatic zones

Five (05) orders and 11 families of 21 insect species (Table 1) were collected from 120 green fruits of *A. senegalensis* in the Sudanian and Sudano-Sahelian climatic zones of Burkina Faso. A significant

difference between insect abundance ($\chi^2 = 383.26$, $df = 3$, $p\text{-value} < 2.2 \cdot 10^{-16}$) and species richness ($\chi^2 = 16.771$, $df = 3$, $p\text{-value} < 7.8 \cdot 10^{-6}$) of the insects in the four study sites has been observed, with the Dafra and Dindèrèso sites in the Sudanian climatic zone being richer in species and more abundant than the Koussiri and Toroba sites in the Sudanian-Sahelian climatic zone.

Insects-*A. senegalensis* relationships

Formicidae-*A. senegalensis* interactions

From the 2042 insects collected on the 120 green fruits in the two climatic zones, 95% were ants, either alone or accompanied by other insect groups. The most frequent ants were *Trichomyrmex abyssinicus*, *Crematogaster* sp., *Messor galla* and *Brachyponera sennaarensis*. These Formicidae species have been observed collecting extrafloral nectar from the fruits as they stick their mandibles to the surface of the green fruits (Fig. 2 a).

When the 40 green fruits were protected from insect access at the two sites in the Sudanian climatic zone (Dafra and Dindèrèso), all produced extrafloral nectar was clear, transparent (similar in clarity to drinking water) viscous and sweet, appearing from the many protrusions of the green fruits and in greater quantities (Fig. 2 b). However, when the fruits are unprotected, it is difficult to observe this quantity on the green fruits.

Ants- *H. undata* interactions

Eight species of Formicidae were collected in the four (4) sites of the two climatic zones. In the Sudanian climate zone, 74% of the green fruits carried both Formicidae and mostly larvae of *H. undata* (Fig. 2 c). In contrast, *H. undata* was completely absent in the Sudano-Sahelian zone.

Ants have not been observed attacking planthopper. Ants come and crowd around the colonies of *H. undata* larvae, using their antennae to drum on them (Fig. 2 d). As a result, the larvae remain motionless when an ant individual comes near them. After about 1 to 2 minutes of palpation of a larva by an ant, the larva can produce honeydew at the end of its abdomen which is collected by ant, thus continuing its path to another larva or onto the green fruit.

In addition, eggs of *H. undata* are often found on young green fruit (Fig. 2 e).

Sugar composition in nectar and insects

Sugar spectrum of extrafloral nectar and insects

Glucose, fructose, raffinose, sucrose and an unidentified sugar (close to maltose) named unidentified 1, were found in the *A. senegalensis* extrafloral nectar. Overall, sugar concentration in extrafloral nectar (Fig.

3) showed that the unidentified sugar is worth 75% of the total sugars, followed by glucose (14%) and sucrose (9%). Raffinose and fructose are in very small concentrations in the nectar (1%).

Fructose, glucose, raffinose, sorbitol, saccharose, unidentified 2 (sugar close to fructose), unidentified 3 (close to maltose) and unidentified 4 (close to sucrose) were sugars found in the analyzed ants and the planthopper individuals. The sugars identified are the same but with different concentrations depending on the insects. Fructose, glucose and unidentified 3 are the major sugars in ants and the planthoppers (Fig. 3).

Sugars concentration of insects

Differences were observed between total sugar concentration of insects and nectar. Besides, *H. undata* presented higher concentration of glucose (Wilcoxon test, $p = 7.4 \cdot 10^{-4}$), sorbitol (Wilcoxon test, $p = 9.7 \cdot 10^{-6}$), unidentified 2 (Wilcoxon test, $p = 4.6 \cdot 10^{-3}$), unidentified 3, (Wilcoxon test, $p = 1.4 \cdot 10^{-3}$) and unidentified 4 (Wilcoxon test, $p = 0.04$) compared to ants (Table 2).

In contrast, there was no significant difference between Formicidae and *H. undata* for sugars such as fructose, raffinose and sucrose.

Discussion

Insect-*A. senegalensis* relationships

The climatic conditions of the two climatic zones are different. Indeed, rainfall is slightly higher in the Sudanian climatic zone while temperature is slightly higher in the Sudano-Sahelian climatic zone. Insects are particularly sensitive to abiotic factors of temperature and hygrometry (Sauvion et al. 2013). This could explain the distribution of insects, with a considerable decrease in the species richness and abundance of insects in the Sudano-Sahelian climate zone compared to the Sudanian zone. The Sudanian zone could provide more favorable living conditions established for insect nutrition, reproduction and development than the Sudano-Sahelian zone.

Green fruits of *A. senegalensis* are strongly associated with ants in the Sudanian and Sudano-Sahelian climatic zones of Burkina Faso. It is very rare to see a green fruit of this plant that is not associated with ants. Ants are very common and abundant on green fruits independent to the presence or absence of planthopper. Indeed, the presence of extrafloral nectar on the fruits could be a leading factor that drives their interaction. This study is the first highlighting the extrafloral nectar production of the green fruits of this plant as food source for ants. Heil and Mckey (2003) showed that some plants, through nectar glands located outside the flowers (here on the green fruits) secrete extrafloral nectar that attracted ants. Extrafloral production is involved in protection and not in pollination (Bentley 1977). Ants can protect green fruits from attacks by small pests as they are known to be the most important predators of arthropods (Novotny 1999; Floren et al. 2002). Some plants and ants have developed reciprocal specializations and are in specific and often obligate interactions, such as *Crematogaster mimosae*

Santschi, which protects the leaves of *Acacia depranlobium* Harms ex Sjostedt from vegetarian vertebrates in Kenya (Huignard 2013).

Ceratitis cosyra is a fruit fly attacking *A. senegalensis* fruits (Issa 2017). It infests green fruits in which it lays eggs and the damage is observed on fruits at the maturity or before the maturity (De Souza et al. 2016). The presence of ants, known as formidable predators, could exclude fruit pests. *Ceratitis cosyra* is not recorded on green fruit in the presence of ants. All terricolous species ants are predatory (Orivel & Dejean 1999). Diamé et al. (2015) showed the effectiveness of the weaver ant, *Oecophylla longinoda* Fabricius, in controlling *Batrocera dorsalis* Hendel infestations of mango trees and confirmed that the presence of *Oecophylla longinoda* on a tree contributes to a greatly reduction in the level of mango pest infestation on that tree. Anthills of terricolous ant species were observed underneath *A. senegalensis*, corroborating the hypothesis that the plant maintains protective mutualistic relationships with ants, attracting more protective ant species for better protection. The presence of nectar on a plant can lead ants to establish their nests near it, this is beneficial for the plant because soils from ants nests contain higher standing stocks of soil nutrients (Wagner & Nicklen 2010).

Formicidae-*H. undata* relationships

This is the first report of observations suggesting a protective mutualistic relationship between Formicidae and *H. undata* where *H. undata* produces honeydew on which ant species such as *Messor galla*, *Brachyponera sennaarensis* and *Trichomyr mex abyssinicus* feed. Host-plant selection by insects for oviposition is critical for the success of their offspring (Robert 1986; Atiama-Nurbel 2014). *H. undata* chooses the green fruit to lay eggs. This is more important for it with low mobility larval stages. Larvae from the eggs of this insect are thought to feed on the sap of green fruit to develop. They filter large quantities of sap, poor in amino acids and rich in sugars and water, to concentrate the amino acids, and reject the excess water and sugars (90%) through the anus: this is the honeydew, which is very appreciated by ants (Lenoir 2011; Sauvion et al. 2013). The relationship between ants and hemiptera, called trophobiosis (Cherix 1986; Delabie 2001), allows ants to protect hemiptera that lack adequate protection (Guenard 2007), especially at the rather critical and vulnerable nymphal life stage of *H. undata*. Hemipterans could be protected by ants from predators such as ladybugs and some parasites (Lenoir 2011). The presence of ants ensures around the hemipteran colony, a kind of close guard against any aggressor (Sauvion et al. 2013) and avoids interspecific competition by other non-mymecophilic homoptera species (Verheggen 2009; Laamari 2011). However, the association between ants and Hemiptera is a facultative association since the two species can live separately (Lehouck et al. 2004).

***A. senegalensis*-Formicidae-*H. undata* interaction**

The tritrophic interaction between plant-ants-*H. undata* seems to be well established. The observation of *H. undata* eggs only on young green fruits shows that this species targets this fruit stage to lay eggs. Once the eggs are laid, they hatch at the same time that the ants visit the green fruit, so the ants visit both the green fruit and the larvae to feed on the extrafloral nectar and honeydew that are rich in sugars. The eggs are deposited on young green fruits, and the larvae from these eggs are not predated by ants that

constantly visit the green fruits, but the larvae are appreciated by the ants, confirms the hypothesis that *H. undata* seeks adequate protection from the ants. The best way to attract ants that have good memory skills is to provide them an abundant food, and ants are very fond of liquids rich in sugars (Huignard 2013). But mutualism is only maintained when the costs of maintaining the association are low and the benefits are high for both partners (Bristow 1991). The plant spontaneously produces the extrafloral nectar in small quantities during the rainy period when it receives sufficient water, and the honeydew that is produced as a waste product from the filtration of excess sap by *H. undata* could be in lower cost to the plant and *H. undata* respectively. The ants receive a sugar-rich food and can offer an adequate protection service to these "two partners" to ensure the survival of their offspring if the planthopper is not a danger to the plant. If ants breed and protect a planthopper, the balance between the plant protection and the pest protection could be positive, negative, or null for the plant. Future studies could focus on the actual contributions of the ants and highlight their role in the mandatory protection and survival of the plant and *H. undata*.

The extrafloral nectar of green fruits and the honeydew of *H. undata* are mostly composed of different sugars as glucose, fructose unidentified 1 and unidentified 3 that could be important parts of the ants' diet. The sugars structure of the ant species immediately collected on green fruits is very similar to those of *H. undata* and extrafloral nectar. These sugars could be sought-after nutrients in the ant diet, that would lead them to be very interested in nectar and honeydew. Our results are comparable to those of Luquet et al. (2021) who also found mostly fructose and glucose, but also sucrose in HPLC results of extrafloral nectar of faba bean *Vicia* spp. and honeydew of aphids and their parasitoids *Aphidius* spp. Extrafloral nectar and honeydew are important for the diet of tree ant species and of some terricolous species (Dejean et al. 1997; Kenne et al. 1999). Styrsky & Eubanks (2007) showed that aggressive ant species provide greater protection to honeydew-producing hemipterans and can be expected to have greater direct and indirect effects on community structure and host plant fitness. Wood ants are the natural enemies of many mites, spiders, hoverflies, staphylinids and other beetles (Gösswald 2012). If these arthropod groups or any of them prey on green fruit and/or *H. undata* larvae, then the latter would be "safe" if they are visited continuously by predatory ant species. Since ants are formidable predators, their mere presence may be enough to deter many plant pests, and they maintain their food source in the best possible condition (Verheggen 2009), which may mean that the plant and the hemiptera attract ants for adequate protection of their offspring.

Conclusion

The results of this study are the first contribution to the study of tritrophic interactions between *A. senegalensis*, ants and *H. undata*. Ants feed both on the nectar of green fruits and on the honeydew produced by *H. undata*. The supply of food rich in sugars, which would constitute an important part of the ants' diet, could keep ants on the green fruits. *H. undata* seems to thrive with ant species that do not repel or predate them either. It is still important to define these types of interactions between plant and insects to improve the understanding of the plant-insect relationships and their consequences for a better exploitation. The results of this study could be exploited in a process of plant protection or the

implementation of an effective biological control method based on the use of natural enemies of plant pests.

Declarations

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Author contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by ZAD, RR, APC, PS and BJ. AO and OG supervised. The first draft of the manuscript was written by ZAD and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

- Arbonnier M (2009) Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. Editions Quae, Paris.
- Atiama-Nurbel T (2014) Réponses des femelles de *Batrocera cucurbitae* (Diptera, Tephritidae) aux composés volatils de fruits-hôtes. Thèse de doctorat, Université de La Réunion.
- Baker HG (1978) Non-sugar chemical constituents of nectar, *Apidologie*, Springer Verlag 8: 349–356.
- Beattie AJ (1985) The Evolutionary Ecology Ant-Plant Mutualisms. Cambridge University Press, Cambridge.
- Bentley BL (1977) Extrafloral nectaries and protection by pugnacious bodyguards. *Ann Rev Ecol Syst* 8: 407-428. <https://doi.org/10.1146/annurev.es.08.110177.002203>

Bland RG (1947) How to know the insects? The key Nature series. Wm C. Company, Iowa.

Boheman CH (1860) Coleoptera Caffraria annis 1838–1845 a J.A.Wahlberg collecta. Öfversigt Kongliga Svenska Vetenskapsakademiens Förhandlingar 17: 107–120.

Bristow CM (1991) Why are so few aphids ant-tended? In: C.R. Huxley & D.F. Cutler (eds), Ant-plant interactions. Oxford University Press, Oxford.

Cherix D (1986) Les fourmis des bois. Editions Payot, Lausanne.

Chinery M (1986) Le multiguide nature des insectes en couleurs. Editions Bordas, Paris.

Cresson ET (1872) Hymenoptera Texana. Trans Am Entomol Soc 4: 153–292.

Dao ZA, Romba R, Traoré M, Birba S, Zouré AB, Ouédraogo I, Ouédraogo A, Gnankiné O (2022) Diversity and role of entomological fauna associated with *Annona senegalensis* Pers. (Magnoliales: Annonaceae) in Burkina Faso, West Africa. *Environmental Entomology* 51: 989–997. <https://doi.org/10.1093/ee/nvac040>.

De Souza EO, Anato FM, Bokonon-Ganta AH, Agboto A, Zohounmegan E (2016) Adaptation et élevage au laboratoire de *Ceratitis cosyra* (Walker) et *Ceratitis anonae* (Graham) [(Diptera: Tephritidae)] sur *Irvingia gabonensis*, *Carica papaya* et *Musa* sp. International Journal of Biological and Chemical Sciences, 10: 1717–1732. <https://doi.org/10.4314/ijbcs.v10i4.22>.

Dejean A, Djieto-Lordon C, Durand JL (1997) Ant mosaic in oil palm plantations of the South-west Province of Cameroon: impact on a leaf miner chrysomelid beetle, J Econ Entomol 90: 1092–1096. <https://doi.org/10.1093/jee/90.5.1092>

Delabie JHC (2001) Les trophobioses entre Formicidae et Hemiptera Sternorrhyncha et Auchenorrhyncha. Neotrop Entomol 30 : 501–516. <http://dx.doi.org/10.1590/S1519-566X2001000400001>

Diamé L, Grechi I, Rey JY, Sané CAB, Diatta P, Vayssières JF, Yasmine A, Bon HD, Diarra K (2015) Influence of *Oecophylla longinoda* Latreille, 1802 (Hymenoptera: Formicidae) on mango infestation by *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) in relation to senegalese orchard design and management practices. African Entomology 23 : 294–305. <https://doi.org/10.4001/003.023.0207>.

Dramé AY, Adamou S, Saminou DDE, Garba M (2021) Evaluation de la faune entomologique associée à *Balanites aegyptiaca* (L.) Del. dans le sud-ouest du Niger. International Journal of Advanced Research 9: 597–605. <http://dx.doi.org/10.21474/IJAR01/12333>

Fabricius JC (1775) Systema entomologiae, sistens insectorum classes, ordines, genera, species adiectis synonymis, locis, descriptionibus, observationibus. Flensburgi et Lipsiae. Flensburg and Leipzig, Korte.

Fabricius JC (1782) *Species insectorum exhibentes eorum differentias specificas, synonyma, auctorum loca natalia, metamorphosin adiectis observationibus, descriptionibus*. Hamburgi et Kilonii, Bohn.

Fabricius JC (1794) *Entomologia systematica emendata et aucta. Secundum classes, ordines, genera, species adjectis synonymis, locis, observationibus, descriptionibus*. Proft, Hafniae.

Fabricius JC (1798) *Supplementum entomologiae systematicae*. Proft and Storch, Copenhagen.

Floren A, Biun A, Lisenmair KE (2002) Arboreal ants as key predators in tropical lowland rainforest trees. *Oecologia* 131 : 137-144. <https://doi.org/10.1007/s00442-002-0874-z>

Fonta C, Marilleau R, Masson C, Yvette, FB (1985) Rôle des nectars de tournesol dans le comportement des insectes pollinisateurs et analyse qualitative et quantitative des éléments glucidiques de ces sécrétions. *Acta OEcologica/OEcologia Applicata* 6: 175-186.

Forel A (1894) Abessinische und andere afrikanische Ameisen, gesammelt von Herrn Ingenieur Alfred Ilg, von Herrn Dr. Liengme, von Herrn Pfarrer Missionar P. Berthoud, Herrn Dr. Arth. Müller. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 9: 64–100.

Gösswald K, (2012) *Die Waldameise – Biologie, Ökologie und forstliche Nutzung*. Wiebelsheim, AULA-Verlag.

Guénard B, Lucas É, Francoeur A (2007) Mutualisme fourmis pucerons et guildes aphidiphage associée: le cas de la prédation furtive. Thèse de Doctorat, Université du Québec à Montréal.

Hahn CW (1835) *Die Arachniden*. C. H. Zeh'sche Buchhandlung, Nürnberg, Zweiter Band.

Harry M, Rasplus JY, Lachaise D (1994) La coévolution plantes-insectes : L'Engrenage de deux enjeux génétiques. *Insectes* 94: 2–5.

Heil M, Mckey D (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annu Rev Ecol Evol Syst* 34 : 425–453. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132410>

Huignard J (2013) Les plantes et les insectes : une lutte permanente. *Les défenses des plantes*. *Insectes* 168 : 3-8.

Issa MH (2017) Études écologiques des mouches des fruits (Diptera : Tephritidae) nuisibles aux cultures fruitières aux Comores. Thèse de doctorat, Université de la Réunion.

Kenne M, Corbara B, Dejean A (1999) Impact des fourmis sur les plantes cultivées en milieu tropical Ant impact on plants grown in the tropics. *Année Biologique* 38: 195–212. [https://doi.org/10.1016/S0003-5017\(00\)87668-0](https://doi.org/10.1016/S0003-5017(00)87668-0)

- Kouyate AM, Hugu J, Kamissoko S, Diawara H (2016) Contribution à l'identification des insectes visiteurs des fleurs de *Detarium microcarpum*. Les cahiers de l'économie rurale 23 : 48-56.
- Laamari M, Tahar CS, Benferhat S, Abbas SB, Merouani H, Ghodbane S, Khenissa N, Stary P (2011) Interactions tritrophiques : plante-puceron-hyménoptère parasitoïde observées en milieux naturels et cultivés de l'Est algérien. Entomologie faunistique – Faunistic Entomology 63 : 115-120. <https://popups.uliege.be/2030-6318/index.php?id=1724>
- Lebon A (2014) La compensation dans les interactions plantes-insectes: modélisation, simulation et expérimentation. Biologie végétale. Thèse de doctorat, Université Montpellier II.
- Lehouck V, Bonte D, Dekoninck W, Maelfait JP (2004) The distribution of ant nests (Hymenoptera, Formicidae) in coastal grey dunes of Flanders (Belgium) and their relationship to myrmecochorous plants. Belgian Journal of Zoology 134 : 89–96.
- Lenoir A (2011) Les plantes et les fourmis. Texte basé sur une conférence Inter-âges à Touraine le 9 décembre 2010, Université François Rabelais, Tours.
- Luquet M, Peñalver-Cruz A, Satour P, Anton S, Cortesero AM, Lavandero B, Jaloux B (2021) Aphid honeydew may be the predominant sugar source for *Aphidius* parasitoids even in nectar-providing intercrops. Biological Control. <https://doi.org/10.1016/j.biocontrol.2021.104596>
- Male PJ (2011) Stabilité évolutive des mutualismes et mécanismes de contrôle : le cas d'une relation plante-fourmis. Thèse de doctorat de l'Université de Toulouse.
- Maydell HJ (1983) Arbres et arbustes du Sahel : leurs caractéristiques et leurs utilisations. Schriftenreihe der GTZ, Eschborn.
- Mayr G (1862) Myrmecologische Studien. Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 12: 649–776.
- Mayr G (1904) Results of the Swedish Zoological Expedition to Egypt and the White Nile. Library of the Royal University of Uppsala.
- Moser J (1908) Beitrag zur Kenntnis der Cetoniden IV. Annales de la Société entomologique de Belgique. 52 : 85–96.
- Nel A (2002) « Les insectes : un succès de l'évolution ». Pour la science 293 : 32-39.
- Nicole MC (2002) Les relations des insectes phytophages avec leurs plantes hôtes, Antennae 9 : 1–5.
- Novotny V, Basset Y, Auga J, Boen W, Dal C, Drozd P, Kasbal M, Isua B, Kutil R, Manumbor M, Molem K (1999) Predation risk for herbivorous insects on tropical vegetation: a search for enemy-free space and time. Australian Journal of Ecology 24 : 477–483.

- Olivier AG (1789) Entomologie, ou histoire naturelle des insectes, avec leurs caractères génériques et spécifiques, leur description, leur synonymie, et leur figure enluminée. Coléoptères. Baudouin, Paris.
- Orivel J, Dejean A (1999) L'adaptation à la vie arboricole chez les fourmis. *Annee Biologique* 38 : 131–148. [https://doi.org/10.1016/s0003-5017\(00\)87665-5](https://doi.org/10.1016/s0003-5017(00)87665-5).
- Pihan JC (1986) Les insectes, collection verte Masson. Masson et Réalisation Editoriales Pédagogiques, Paris.
- Pouvreau A (1996) Les Coléoptères et les fleurs. *Biologie des espèces. Insectes* 102 : 25-28.
- Preyssler JDE (1791) Beschreibungen und Abbildungen derjenigen Insekten, welche in Sammlungen nicht aufzubewahren sind, dann aller, die noch ganz neu, und solcher, von denen wir noch keine oder doch sehr schlechte Abbildung besitzen. Mayer, Samml. Naturh. Physikal. Aufsätze, Dresden 1: 55–151.
- Reiche I (1850) Ferret and Galinter. Voyage en Abyssinie.
- Robert PC (1986) Les relations plantes-insectes phytophages chez les femelles pondeuses : le rôle des stimuli chimiques et physiques, une mise au point bibliographique. *Agronomie, EDP Sciences* 6 : 127-142.
- Saussure H (1862) Sur divers vespides Asiatiques et Africains du Musée de Leyden. *Stett. ent* 23: 129–207.
- Sauvion N, Calatayud PA, Thiery D, Marion-Poll F (2013) Interactions insectes-plantes. Editions Quae et IRD éditions, Versailles.
- Smith F (1858) Catalogue of hymenopterous insects in the collection of the British Museum. Formicidae, London.
- Stanek VJ (1984) Encyclopédie des insectes Coléoptères. Editions Artia, Prague.
- Styrsky JD, Eubanks MD (2007) Ecological consequences of interactions between ants and honeydew-producing insects. *Biological Sciences* 274: 151–164. <https://doi.org/10.1098%2Frspb.2006.3701>
- Tchindébé G, Douka C, Tope SF, Fohouo FT (2018) Diversité des insectes floricoles et son impact sur les rendements fruitiers et grainiers de *Arachis hypogaea* L. (Fabaceae) à Maroua (Extrême-Nord , Cameroun). *Journal of Applied Biosciences* 129 : 13675–13687. <http://dx.doi.org/10.4314/jab.v129i1.11>
- Tchuenguem FFN, Kingha TBM, Brückner D (2014) Diversité des insectes floricoles et son impact sur les rendements fruitiers et grainiers de *Arachis hypogaea* L. (Fabaceae) à Dang (Ngaoundéré-Cameroun). *International Journal of Biological and chemical Sciences* 3: 983-997. <https://doi.org/10.4314/ijbcs.v8i3.14>

Order	Family	Species	Number of individuals per site			
			Sudanian zone		Sudano-sahelian zone	
			Dafra	Dindèrèso	Koussiri	Toroba
Coleoptera	Chrysomelidae	<i>Aulacophora</i> sp. (Dejean 1835)	0	2	0	0
	Scarabaeidae	<i>Leucocelis nitidula</i> (Olivier 1789)	1	0	0	0
		<i>Leucocelis puncticolis</i> (Moser 1908)	2	0	0	0
		<i>Xeloma maura</i> (Boheman 1860)	1	0	0	0
Diptera	Calliphoridae	<i>Pollenia rudis</i> (Fabricius 1794)	1	1	0	0
	Tephritidae	<i>Ceratitis cosyra</i> (Walker 1849)	1	0	4	0
	Ulidiidae	<i>Physiphora alceae</i> (Preyssler 1791)	0	0	1	0
Hemiptera	Pentatomidae	<i>Staria lunata</i> (Hahn 1835)	0	13	7	0
	Tettigometridae	<i>Hilda undata</i> (Walker 1851)	160	473	0	0
Hymenoptera	Formicidae	<i>Brachyponera sennaarensis</i> (Mayr 1862)	106	14	0	0
		<i>Camponotus maculatus</i> (Fabricius 1782)	22	3	0	0
		<i>Camponotus sericeus</i> (Fabricius 1798)	8	1	0	0
		<i>Crematogaster</i> sp	130	34	0	327
		<i>Messor galla</i> (Mayr 1904)	12	288	0	0
		<i>Monomorium</i> sp.	1	2	3	0
		<i>Pogonomyrmex barbatus</i> (Smith 1858)	7	0	0	0
		<i>Trichomyrmex abyssinicus</i> (Forel 1894)	53	0	191	169
	Vespidae	<i>Polistes bellicosus</i> (Cresson 1972)	1	0	0	0
		<i>Ropalidia capensis</i> (Saussure 1862)	1	0	0	0
Lepidoptera	Lycaenidae	<i>Myrina silenus</i> (Fabricius 1775)	1	0	0	0
	Nymphalidae	<i>Charaxes epijasius</i> (Reiche 1850)	1	0	0	0
Total number of individuals			509	831	206	496
Number of species			18	10	5	2

Table 2 Statistical tests and their significance on the concentrations of sugars by insect group

Insect sugars	Mean concentration in Formicidae per mg/g dry weight $\pm$ SD	Mean concentration in <i>H. undata</i> per mg/g dry weight $\pm$ SD	Statistical difference (p-value)
Fructose	11.25 $\pm$ 12.84	9.79 $\pm$ 9.4	Wilcoxon test: 0.72
Glucose	6.64 $\pm$ 9.9	17.04 $\pm$ 20.34	Wilcoxon test: 7.4 10^{-4}
Raffinose	0.18 $\pm$ 0.21	1.40 $\pm$ 4.91	Wilcoxon test: 0.9
Sorbitol	0.44 $\pm$ 1	2.9 $\pm$ 1.74	Wilcoxon test: 9.7 10^{-6}
Sucrose	0.41 $\pm$ 0.65	0.35 $\pm$ 0.26	Wilcoxon test: 0.55
Unidentified 2	2.01 $\pm$ 2.01	3.77 $\pm$ 3.03	Wilcoxon test: 4.6 10^{-3}
Unidentified 3	17.69 $\pm$ 22.77	24.60 $\pm$ 15.21	Wilcoxon test: 1.4 10^{-3}
Unidentified 4	1.72 $\pm$ 1.65	2.48 $\pm$ 2.29	Wilcoxon test: 0.04

Figures

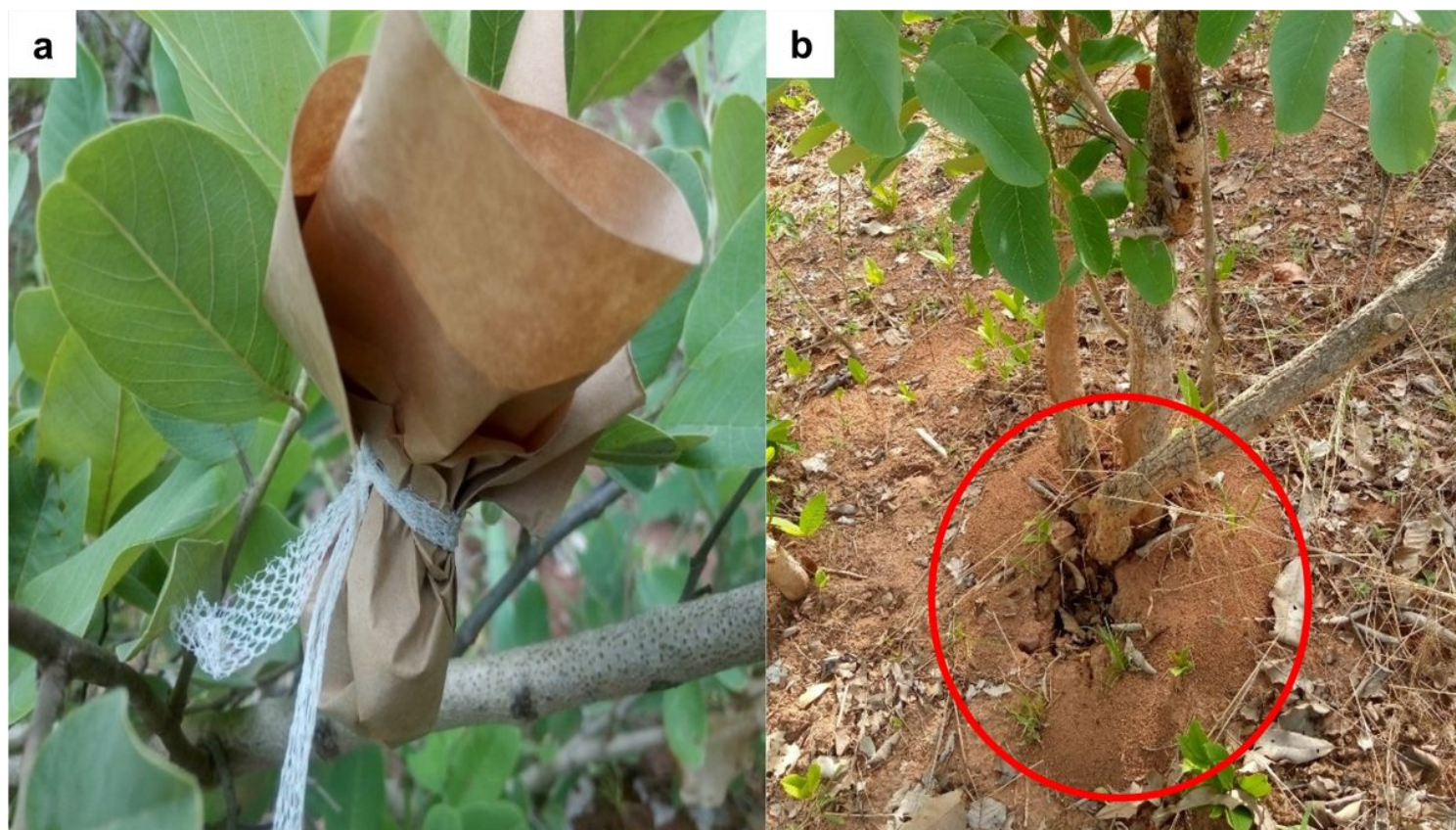


Figure 1

a Protection of the green fruit with waterproof paper bag; **b** Anthill of *Messor galla* at the base of a *A. senegalensis* shrub

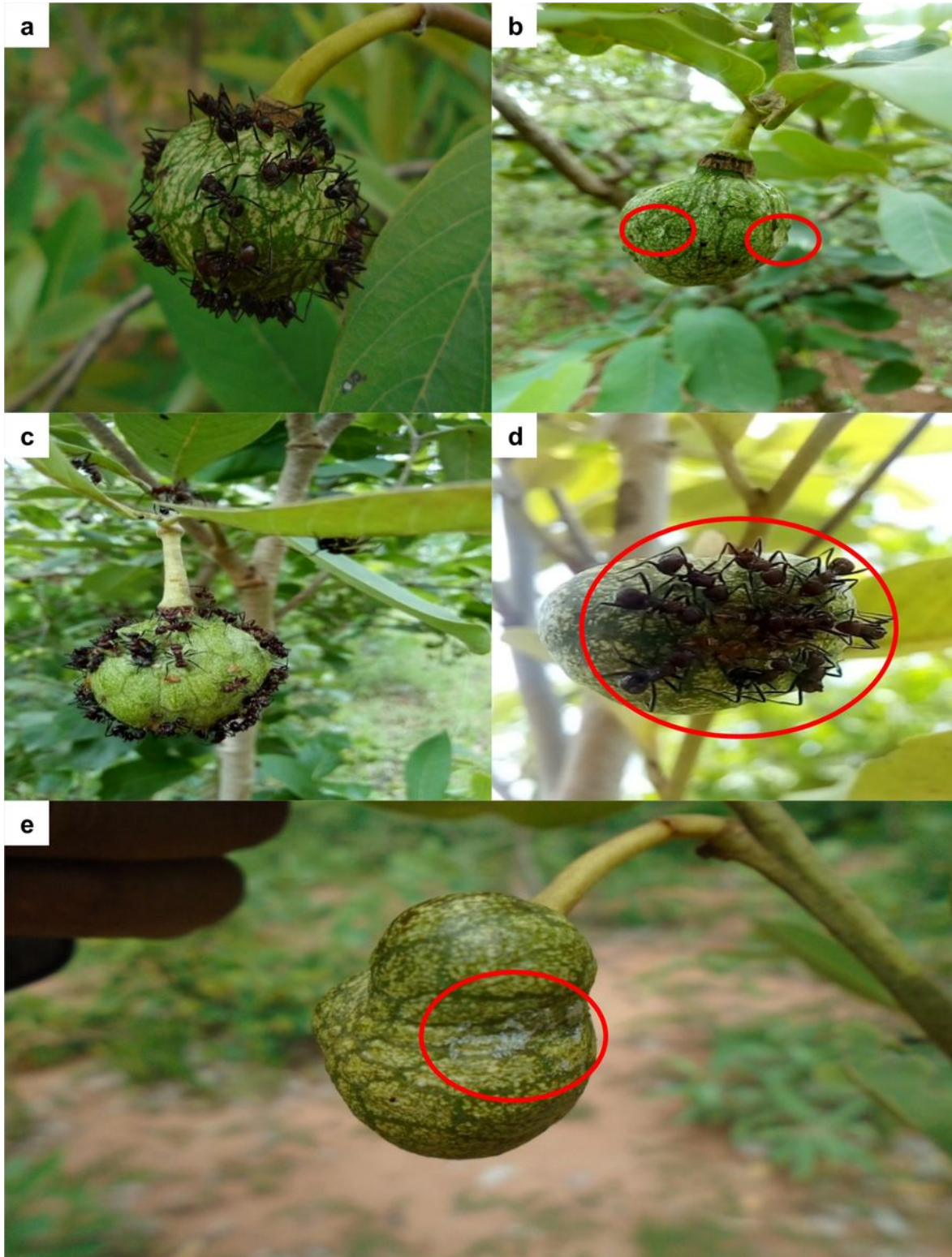


Figure 2

a *Messor galla* searching for nectar droplets on the green fruit; **b** Production of extrafloral nectar by the fruit after protection with waterproof paper bag; **c** Presence of *H. undata* and *Messor galla* larvae on the green fruit; **d** Clustering of *Messor galla* around *H. undata*; **e** Egg deposition by *H. undata* on a young green fruit

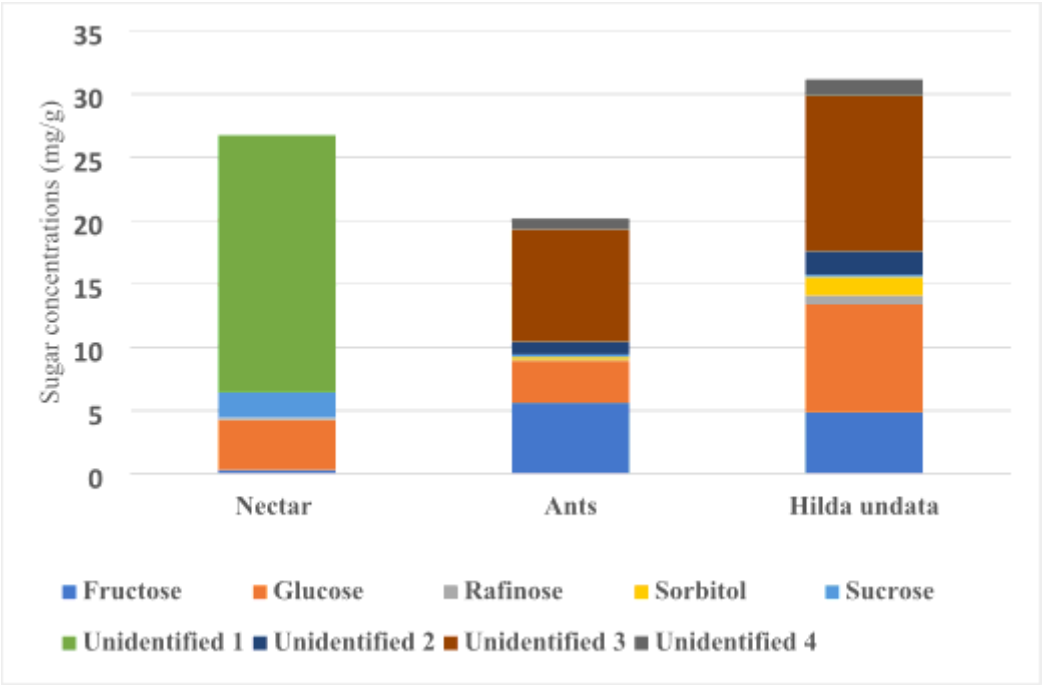


Figure 3

Sugar concentrations in nectar and insects