

E-ISSN: 2320-7078 P-ISSN: 2349-6800 www.entomoljournal.com UF7S 2020: 8(3): 1462-146

JEZS 2020; 8(3): 1462-1467 © 2020 JEZS Received: 07-03-2020 Accepted: 09-04-2020

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Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com



Influence of ants on colonies of *Diaphorina* enderleini Klimaszewski, 1964 (Hemiptera: Psyllidae) and *Hilda patruelis* Stål, 1855 (Hemiptera: Tettigometridae) living on *Vernonia* amygdalina Dellile in Yaoundé

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Abstract

Diaphorina enderleini and Hilda patruelis are the 2 most important hemipteran pests of Vernonia anygdalina Dellile. These pests develop several tri-trophic associations with ants and host plant called Trophobiosis. In order to master their bio-ecology and protect their host plant, a study was carried out to determine the effect of ants in the growth and survival of the colonies of these hemipterans. Results showed that, in the absence of ants, the colonies of hemipterans were bound to disappear with *D.* enderleini exhibiting an average life expectancy of 1.50 ± 0.50 days and *H. patruelis* 2.50 ± 0.75 days. However in the presence of ants, 5 species of ants (*Pheidole megacephala*, Myrmicaria opaciventris, Camponotus sp, Crematogaster castanea and Tetramorium simillimum) where found to live in association with these hemipterans with *D. enderleini* exhibiting an average life expectancy of 59.10 ± 8.93 days whereas that of *H. patruelis* was 61.13 ± 9.75 days. This study proved that ants are indispensable for the development and survival of these hemipterans.

Keywords: Diaphorina enderleini, Hilda patruelis, ants, interactions, trophobiosis, ndolè

Introduction

Vernonia amygdalina ^[1] is popularly known in Cameroon as « ndolè ». It is cultured mainly for its comestible leaves. In the Africa Caribbean and Pacific countries, it is considered as a culinary heritage of Cameroon ^[2]. *V. amygdalina* also have exceptional therapeutic virtues ^[3]: it is used in the treatment of numerous pathologies notably diabetes, jaundice, intestinal worms etc. ^[4]. Due to its high consumption, marketing of « ndolè » has known a remarkable boom in the local markets and beyond the country's borders ^[5]. Consequently, its production has become a significant source of income in various market gardens in urban and peri-urban areas in Cameroon.

This plant is prone to attacks by various insects with the main 2 being Hemipterans: *Diaphorina enderleini* (Klimaszewski, 1964) and *Hilda patruelis* (Stål, 1855) respectively of the families Psyllidae and Tettigometridae which live in association with ants. The association plant-Hemipteran-ant is a biotic interaction of a tri-trophic type having a mutualistic character. Hemipterans feed on the sap of plants. After having assimilated the nutrients they need, especially the nitrogenous substances, the surplus carbohydrates is excreted in the form of a highly concentrated liquid called "honeydew" which are liked by ants. The ants in turn protect the hemipteran from potential natural enemies ^[6], sometimes transporting them from one nutrition site to another. Being generalists, the ants will supplement their protein diet with prey they catch on the host plants of hemipterans, thus protecting the plant against other predators. This type of interaction called "trophobiosis" has been subject to several studies all over the world ^[6]. In hemipterans, trophobiosis is exhibited by the sub-orders Sternorrhyncha, Cicadomorpha and Fulgoromorpha ^[6, 7, 8, 9] and in Heteropterans, the families Coreidae, Pentatomidae and Plataspida ^[7, 8, 10, 11, 12].

Despite the agronomic potential of *V. amygdalina*, there is paucity of information on the bioecology of *D. enderleini* and *H. patruelis* and also the impact of ants on the development of these 2 hemipterans. Studies have been done on the entomofauna of *Vernonia* in Ivory Coast^[5]

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and Nigeria ^[4]. However, ^[13] revealed certain aspects of the biology of *D. enderleini* including the description of adults, larvae, host plant and also of *H. patruelis* ^[14]. These authors demonstrated trophobiotic relations between this psylli and various ant species ^[13].

The objective of the present work was to study the role of ants in the growth and maintenance of colonies of these 2 hemipterans in the field and the laboratory. For so doing, a censor on the fauna of ants associated to *Vernonia* was realized in order to determine those who interact with *D. enderleini* and *H. patruelis* and a study on the interaction between the ants and the 2 hemipterans carried out. Finally, a study of the seasonal variation (wet and dry months) of abundance of the different ants and the 2 studied hemipterans censored on the plant was realized.

Materials and Methods Study site

The study was 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, and 729m asl.). The vegetation of the zone is dominated by Imperata cylindrica, an herbaceous plant of the Poaceae family. The vegetation of the site derived from a semi-deciduous forest landscape is highly disturbed by anthropogenic activities. The climate is of a transitional subequatorial type, a characteristic specific to Yaoundé [15]. In fact, Yaoundé is a basin surrounded by several hills which confers a microclimate of the Yaoundean type ^[15]. Compared to the Centre region where mean temperature is 25°C, that of Yaoundé due to its relief is slightly lower (23°C). The hottest month are February and March (mean temperature of 28°C) and the coldest are July and August (mean temperature of 23,5°C) ^[15]. The mean rainfall is 1500 mm/year ^[15]. The climate is cup-up into 4 seasons: long dry season from November to February, a short dry season from July to Mid-August, a long rainy season from Mid-August to Mid-November and a short rainy season from March to June. This cut-up of seasons is however very disturbed today due to climate change.

Collection and Identification of specimen

Collection of ants and hemipterans were carried out during the wet (March-August) and dry (November-February) months. Observations were done twice a week with each plant of *Vernonia* well scrutinized. For each morphotype, a sample was collected to make up the reference collection.

Identifications of the hemipterans were done with the aid of keys and guides of several authors ^[16, 17, 18, 19]. The identification was completed at the Royal Museum for Central Africa (RMCA) at Tervuren (Belgium) by comparing with the species of the reference collection. Identification of ants was done with the aid of some dichotomous keys of ^[8, 20].

Experimental setup

In the field, the experimental plot comprised 14 planks of 4m long and 60cm wide separated by furrows of 1m wide. Each plank carried 6 plants distanced by 0,80m. The influence of the presence of ants on the development of the colonies of *D. enderleini* and *H. patruelis* was studied on basis of evaluation of the interactions. For each species, 10 colonies consisting of 30-120 individuals (larvae and adults) were isolated using gauze capes and three interactions realized: (i) without ants: here, the colony was put in place naturally and the ants were removed simply with the aid of a fine bristled brush without disorganizing the structure; (ii) adults hemiptera were captured in fields and bagged, creating a new colony without ants; (iii) colonies with ants: Hemiptera and the various species of ants present (Figure 1) were bagged.

In the laboratory, evolution of hemipteran colony size with respect to presence or not of ants was observed. With an aid of rearing boxes, hemipterans were isolated and gradually, ants were added until saturation of the milieu. This was to be able to evaluate the survival of these hemipterans in a controlled environment.



Fig 1: Hemipteran colony benefiting from the presence of ants (Voula Valteri Audrey, 2013)

Data analysis

The relative abundance of the different species collected was calculated. The seasonal variation in abundance of the main identified species was tested using the Generalized Linear Model (GLM). This procedure includes a linear regression analysis followed by an Analysis of Variances (ANOVA). The correction of Poisson was thus applied for counting data. Pairwise comparisons were performed using the Tukey HSD test corrected by the Bonféronni sequential procedure. All these analyses were carried out using software R (Version

3.0.2, 2013). The results were appreciated at 5% confidence level.

Results and Discussion

Diversity and seasonal variation in specific abundance of *D. enderleini* and *H. patruelis* with respect to season

Specific diversity variations in terms of absolute and relative abundance respectively of ants censored and the 2 hemipterans with respect to seasonal months (wet and dry) are presented in Table 1. At the end of the censor, 5 species of Hymenoptera were inventoried: *Pheidole megacephala* (Fabricius, 1793), *Myrmicaria opaciventris* (Florel, 1909), *Camponotus sp, Crematogaster castanea* (Schmidt, 1858), and *Tetramorium simillimum* (mith, 1851) (Table 1). *T. simillimum* and *Camponotus sp* were the only ones who presented no significant difference ($p < 10^{-3}$) with respect to seasonal months.

These ant species belong to the family of Formicidae and are associated to the 2 studied Hemipterans. These results differ from those of Aléné *et al.* ^[13] who illustrated the implication of only 4 of the aforementioned ant species in association with *D. enderleini* on the same host plant in Yaoundé. This difference could be attributed to the method of sampling which was periodically for Aléné *et al.* ^[13] and spread out during the course of the year (rainy and dry seasons) for the

present study. In the same light, Dejean et al. [11] also observed 4 ant species living in association with Euphyonarthex phyllostoma (Homoptera: Tettigometridae) on groundnut plants. In the presence of *H. patruelis*, Bohlen^[21] and NRI ^[21] observed the presence of 2 species: *Pheidole spp*. Formicidae) and (Hymenoptera: Anoplolepis spp. (Hymenoptera: Formicidae) while Weaving [23] observed 3 species: P. megacephala, C. castanea and Camponotus sp. These observations contradict the present results and could be linked to the difference in host plant as these authors worked on groundnut. Further studies are required to confirm this hypothesis. In general, ants of the genera Crematogaster and Pheidole were observed in the colonies of the 2 hemipterans. These ant species play an important role in the development of larvae and the stability of the 2 hemipterans.

Table 1: Diversity and seasonal variation in specific abundance of ants associated with Diaphorina enderleini and Hilda patruelis

Order	Smaailaa	Wet months		Dry months		F test
Order	Species	AA	RA	AA	RA	F test
	Camponotus sp	140	0,08	875	0,50	F _{1,99} =0,014; p=10 ⁻³ S
Hymenoptera	Crematogaster castanea	7760	4,22	5935	3,37	F _{1,99} =1,053; p=0,307, NS
	Myrmicaria opaciventris	9980	5,43	8275	4,70	F _{1,99} =0,532; p=0,467 NS
	Pheidole megacephala	79290	43,16	78785	44,79	F _{1,99} =0,164; p=0,686 NS
	Tetramorium simillimum	15	0,01	125	0,07	$F_{1,99}=0,002; p=10^{-3} S$
Hamintana	Diaphorina enderleini	48740	20,56	48115	0,50 3,37 4,70 44,79	F _{1,99} =0,090; p=0,764 NS
Hemiptera	Hilda patruelis	37770	26,53	33781	27,35	F _{1,99} =1,81; p=0,18 NS

AA: Absolute abundance; RA; Relative abundance

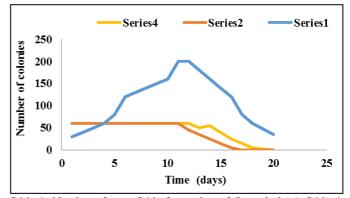
Influence of ants on the survival of the colonies of *Diaphorina enderleini* and *Hilda patruelis*

D. enderleini in association with ants showed an average life expectancy of 59.10 ± 8.93 days (Min. = 32, Max. = 71) while *H. patruelis* showed an average life expectancy of 61.13 ± 9.75 days (Min. = 35, Max. = 77). Colonies of ant-deprived hemipterans, had average life expectancies of 1.50 ± 0.50 days (Min. = 1, Max. = 2) and 2.50 ± 0.75 days (Min. = 2, Max. = 3) respectively for *D. enderleini* and *H. patruelis*.

The drastic decrease of life expectancy of the 2 hemipterans in the absence of ants proved that survival of hemipterans is directly linked to presence of ants. This confirms the hypothesis that survival of these hemipterans is directly linked to the presence of ants given that survival is a useful biological parameter in the understanding of population dynamics in ecology and in the design of pest control strategies. These results corroborates with that of Aléné et al. ^[13] who stated that ants take care of hemipterans through construction of nests with dead leaves debris. During extraction of honeydew, the ants clean the hemipterans thereby improving their health. On the contrary, in the absence of ants, honeydew droplets stick to the anus of the hemipterans which finally kills them by obstruction of the genital track. Moreover, this honeydew could be favourable milieu for the development of certain opportunistic fungi which shortens the life expectancy of these hemipterans. They are at the mercy of predators in the absence of these ants.

The effect of ants on the evolution of the number of colonies of the studied hemipterans as a function of time is illustrated in Figure 2. It showed that despite the increase in the number of ants in the environment, the number of colonies of hemiptera remains constant up to a maximum of 200 ants. This number of hemipteran colonies dropped abruptly with the decrease in the number of ants until disappearance after 20 days. The population of *D. enderleini* disappeared more rapidly and continuously compared to that of *H. patruelis*.

In the laboratory, it was shown that the number of hemipterans increased with increased number of ants up to a certain level. Above this level, the population of hemipterans started disappearing due to the fact that the food source (honeydew) they produce became scarcer and the ants not having enough of it transformed into predators of the hemipterans and cannibalize other ant species leading to interspecific competition for food ^[24]. This phenomenon is rare in nature as they will complete their food ration by capturing other insect predators and parasitoids of these hemipterans and also other destroyers of the host plant ensuring thus the protection of the plant ^[25].



Série 1: Number of ants; Série 2: number of *D. enderleini*; Série 4: number of *H.patruelis*

Fig 2: Evolution of the number of colonies of ants and hemiptera with time

Influence of ant abundance and climate on the distribution of colonies of *Diaphorina enderleini* and *Hilda patruelis* in the field

The influence of ant abundance on the distribution of the colonies and abundance of D. *enderleini* and H. *patruelis* with respect to climate was illustrated in Figure 3. The mean

number of isolated *D. enderleini* colonies was 46.21 ± 9.25 (Min. = 12, Max. = 60). This number was higher in the wet months than the dry months. While the mean number of isolated *H. patruelis* colonies was 24.77 ± 5.05 (Min. = 12, Max. = 36) and was also higher in the wet months than the dry months. These numbers varied significantly (p <10⁻³) among the seasons irrespective of the hemipteran species. As a result, more colonies of *D. enderleini* developed during this experiment and the number was higher during the wet months.

With respect to abundance, *D. enderleini* and *H. patruelis* exhibited significant differences ($p < 2.2e^{-16}$ and $p < 10^{-3}$ respectively) as a function of season (Figure 3).

In the presence of ants, number of colonies and abundance of the studied hemipterans varied significantly ($p < 10^{-3}$). Individuals of *D. enderleini* and *H. patruelis* both demonstrated a positive and significant correlation (r = 0.81; $p < 10^{-3}$) between individual, number of colonies and ants (Figure 3). Thus the more the number of ants increased, the better the colonies of hemipterans were.

These results agrees with the observations of Flatt^[27] who said that an increase number of ants in the colony of Aphids increased five folds the fitness of these hemipterans i.e. improving the reproductive power of Aphides, consequently increasing the number of colonies of the later. In fact, a maximum ratio of 1 hemipteran for 4 ants assures the protection and up keeping of hemipterans, above this ratio, disturbances are observed given that food supply (honevdew) becomes insufficient to meet the nutritive requirements of the ants. Dejean et al. [11] demonstrated how a large sized worker ant (Camponutus brutus) could take care of 5 to 18 larvae of Plaspides stages and explained the reason to be the fact that larvae produce less honeydew than adults. These observations corroborate the present results and illustrates that the presence of ants in any given environment is indispensable for the wellbeing of hemipterans.

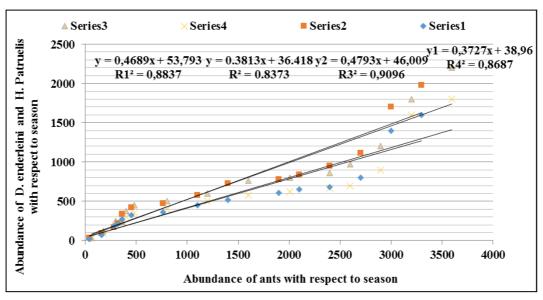


Fig 3: abundance of *D. enderleini* and *H. Patruelis* with respect to ant abundance according to seasons: serie 1: abundance of *H.patruelis* in the wet season, serie 2: abundance of *D. enderleini* in the wet season, serie 3: abundance *H. patruelis* in the dry season, serie 4: abundance de *D. enderleini* in the dry season.

Ant-Hemipteran interactions

The ant-hemipteran associations as a function of season were presented in Table 2. In total, 140 and 159 individuals of both ants and hemipteran exhibited these associations respectively during the wet and dry months. In a particularly hostile environment due to pressure from predators and parasitoids, the two hemipterans; D. enderleini and H. patruelis developed several associations with various species of ants which serve as protective biotic agents. For D. enderleini, the association D. enderleini-P. Megacephala was observed 78 times, followed by D. enderleini-C. castanea 39 times, D. enderleini-M. Opaciventris 38 times, D. enderleini-Camponotus sp. 4 times and D. enderleini-T. Simillimum 2 times. In H. patruelis, the association H. patruelis-P. Megacephala was observed 70 times, that of H. patruelis-M. Opaciventris was observed 36 times, H. patruelis-C. Castanea 26 times; H. patruelis-Camponotus sp. 4 times and H. patruelis-T. Simillimum 2 times.

Generally, the associations ant-Hilda showed significant

differences (F_{1, 99}= 0.501, P= 0.003) when compared to *Diaphorina*-ant associations.

These ants and the studied hemipterans and host plant exhibit mutualistic benefits called "Tri-trophobiosis" [11] where the honevdew produced by the hemipterans serves as a source of food to the ants while the ants protect them from their natural enemies and the host plant provides a habitat. Pheidole spp. abundant in both hemipteran colonies plays an important role in the development of larvae and stability. Buckley [27] revealed right in those days the protective role by P. megacephala, C. castanea, Camponotus floridanus of hemipterans. Weaving ^[23] before then observed C. castanea carrying *H. patruelis* from one point to another during danger. In the same light, Maschwitz et al. [7] as well as Navarrete et al. ^[25] observed P. megacephala and C. floridanus putting D. citri out of danger. Aléné et al. [13] also demonstrated how C. castanea construct shelters using death leaves in order to protect its honeydew against eventual invasion of predators in the colonies of D. enderleini.

Vet months	Dry months		
	Di y montifis	Total	Total Relative abundance (%
20	19	39	13.04
2	2	4	1.34
17	21	38	12.71
34	44	78	26.09
0	2	2	0.67
13	13	26	8.70
2	2	4	1.34
	20 2 17	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

18

36

2

159

36

70

2

299

18

34

0

140

Table 2: Variation of absolute abundance of ant-Hemipteran associations with respect on seasonal variations

Conclusion

The results obtained from the present study showed that 5 species of ants live in association with *D. enderleini* and *H. patruelis*. In the absence of ants, *D. enderleini* colonies could not survive for more than 2 days while *H. patruelis* colonies could not survive for more than 3 days. Thus, these pests develop trophobiotic associations with ants in order to ensure their survival. *P. megacephala* and the other ant species protect the hemipterans against their natural enemies such as parasitoids. The later procure food (honeydew). Further studies are required to evaluate the composition of honeydew and compare it to honey.

H. patruelis-M. opaciventris

H. patruelis-P. megacephala

H. patruelis-T. simillimum

Grand Total

Acknowledgement

Special thanks are addressed to Nkaté Akono Lydie and Urbain D'assise Voula for their material and psychological support as well as their indispensable objective criticism of this work.

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