

Quantifying Diversity and Phenology of Cereal Leaf Beetles *Oulema* Spp. (Chrysomelidae: Criocerinae) in Several Cereal Fields of Semi-Arid Zone, Algeria

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Cover Page Footnote

The author is grateful to all volunteers who contributed to sampling during study period, and thanks referees who performed the first version of the manuscript.

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QUANTIFYING DIVERSITY AND PHENOLOGY OF CEREAL LEAF BEETLES *OULEMA* SPP. (CHRYSOMELIDAE: CRIOCERINAE) IN SEVERAL CEREAL FIELDS OF SEMI-ARID ZONE, ALGERIA

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ABSTRACT

The present study treated the taxonomic diversity of the cereal leaf beetle (*Oulema* spp.) community subservient to several cereal fields (barley, durum wheat and oats) in Eastern Algeria. From April to June, which is the appearance period of these insects, the leaf beetle fauna was weekly sampled using two trapping techniques: entomological net and sight hunting method. Spatiotemporal alpha and beta diversity of this community were evaluated by several ecological indices: Shannon index, Simpson index and equitability. The multivariate statistic test (GLM) was used to assess the variation of the different diversity parameters. Catches totaled 1144 individuals belonging to the genus *Oulema* and four species: *Oulema melanopus*, *Oulema cyanella*, *Oulema duftscmidi* and *Oulema gallaeciana*. The highest abundance of the leaf beetles was recorded in oats with 569 individuals (49.73%), followed by barley (390 individuals, 34.09%) and durum wheat (185 individuals, 16.17%), whereas the most speciose field was barley (4 species), followed by oats (3 species) and durum wheat (2 species). The most abundant species was *O. melanopus* with 746 individuals (65.20% of the total). The spectrum of occurrence ranked constant species first, followed by common and very accidental species. GLMs demonstrated that diversity of cereal leaf beetles *Oulema* spp is highly related to cereal field type.

Keywords: Cereal fields, diversity alpha, diversity beta, Eastern Algeria, *Oulema* spp.

INTRODUCTION

Often called leaf beetles and with about 19 subfamilies and 37 000 known species, Chrysomelidae is considered as a very diverse family among insects that feed on plants (Ekiz et al., 2013). Specifically, according to Bezděk and Schmitt (2017), the subfamily Criocerinae contains 1500 species divided into 22 genera in the world, of which about 200 are found in the Palaearctic countries. The larger part of the species lives in subtropical and tropical regions, and belongs to five genera: *Crioceris*, *Lilioceris*, *Lema*, *Oulema*, and *Neolema* (Matsumura et al., 2014). The genus *Oulema* comprises 800 described species,

a variety of them are serious cereal pests like *O. gallaeciana* (Ulrich et al, 2004) and *O. melanopus*, which is the most dangerous (VencI and Leschen, 2014). Indeed, the larval and the adult forms of this taxonomic group prefer living and feeding on leaves. Hence, a lot of species attract many researcher's attention from different specialities for their feature as phytophagous (Booth et al., 1990) and their aptitude to cause considerable damage to some crops, especially cereals (Weloso, 1973). In Algeria, products of cereals have an important position in food system and in economy of the country; this characteristic is clearly perceived through all the phases of the sector (Djermoun, 2009). The great fluctuation of yields and production of cereals are in large

proportion the result of abiotic constraints such as thermal and water stress (Derbal et al., 2015), and biotics such as pests (Belahcene et al., 2015).

The Algerian entomofauna restricted to cereal fields is not well assessed. In fact, few works have been conducted in this context, e.g: Madaci (1991) in El khroub, Kellil (2010) in Setif, Bakroune et al., (2020) in Biskra, Amokrane et al., (2020) in Oum El Bouaghi. As far as the genus *Oulema* is concerned, it remains poorly studied, despite the potential danger that some of its species represent for cereal production. According to Sahraoui et al. (2001), the species *O. melanopus* is the main cereal pest in Algeria. To our knowledge, the notable works dedicated to this genus are Rouag et al., (2012) and Kellil et al., (2018), the first evaluated the infestation by *Oulema spp.* on six varieties of durum wheat in arid conditions of Setif, and the second studied the population dynamics of *O. melanopus* in different durum wheat varieties of the high plains of Eastern Algeria.

The basic knowledge on species and their distribution are of paramount importance for the biodiversity study in a given region (Sarhou et al., 2005), particularly for those living in hard environments like arid and semi-arid areas (Chenchouni, 2012). Consequently, this study, which is the first in Algeria to pay particular attention to the diversity of cereal leaf beetle community (*Oulema spp.*), attempted to collect data on this community in many cereal fields located at semi-arid zones. Many research questions could be asked in this context: (i) under semi-arid climatic conditions, what would be the distribution pattern and the structure of cereal leaf beetle *Oulema spp.* communities in different cereal fields? (ii) does the spatiotemporal variation in species richness and abundance exist?

MATERIALS AND METHODS

Study Area

This survey was carried out in two regions located in Eastern Algeria (Figure 1): Tebessa (latitude 35°20'N, longitude 8°6'E, altitude: 960 m) and Batna (latitude 35,33°N, longitude 6,10°E, altitude: 1036 m). Three cereal fields were selected to be sampled: durum wheat (*Triticum durum* Desf.) and barley (*Hordeum vulgare* L.) in Tebessa, and oats (*Avena sativa* L.) in Batna. The cereal field's areas are at least 7000 m² for each.

Based on meteorological data of the period 2000-2011, provided by the meteorological station of Tebessa (latitude 35,48 °N, longitude 8,13 °E, altitude: 813 m) and the meteorological station of Batna (latitude 35,55 °N, longitude 6,18 °E, altitude: 1052 m), both regions are characterized by a semi-arid climate (De Martonne index: $I_{Tebessa} = 16.30$, $I_{Batna} = 14.17$). Moreover, Gaussen and Bagnouls diagram indicates that the dry period lasted about six months, from April to September in Tebessa, and from May to late October in Batna. Annual rainfall averaged 434 mm in Tebessa and 366.06 mm in Batna. The annual average of mean temperature was 16.62 °C with a maximum in July (27.50 °C) and minimum in January (7.60 °C) in Tebessa, nevertheless, in Batna, the annual average of mean temperature was 15.82 °C, the maximum temperature was recorded in July with 28.79 °C, and the minimum was recorded in January with 4.70 °C.

Sampling and Identification

Fieldwork was performed weekly and regularly in the three cereal fields (12 outputs per field), from April, the date of appearance of the first adult, until late June, the harvest day, during the year 2011.

Since adult leaf beetles are generally found on the upper leaves of wheat, two trapping techniques were adopted: (i) trapping by using an entomological net (35 cm of diameter), (ii) the sight hunting method, in order to complemente the first technique. Every field was divided into 10 plots, in each plot

we gave 50 shots randomly, progressing in the direction of the field length. The sampling session took over three hours from 10:00 to 13:00 AM. In the laboratory, the identification of the collected biological material (adults) was performed by using mainly these keys: Lacordaire (1845) and Perrier (1971).

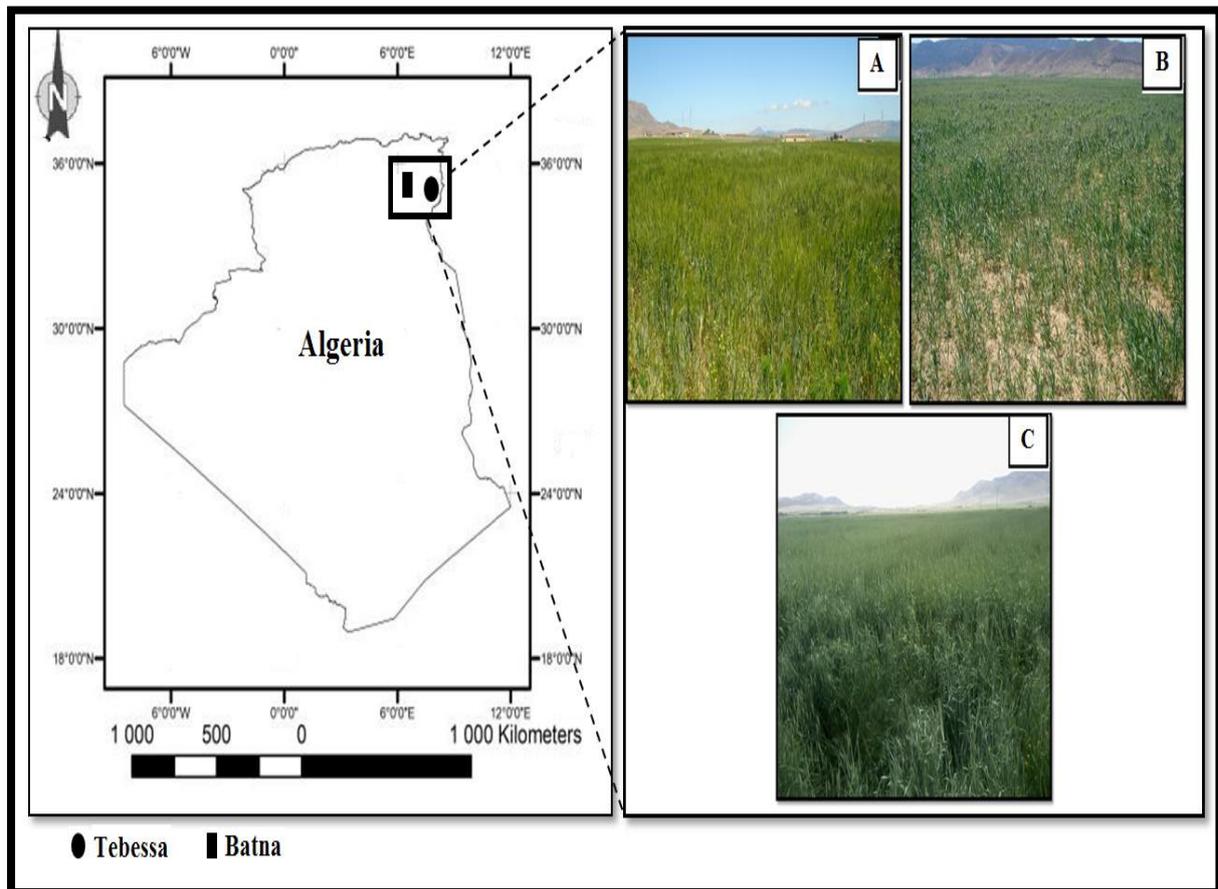


Figure 1: Geographical location of Tebessa and Batna (left), and the cereal fields (right). (A) barley, (B) durum wheat, (C) Oats.

Data Analysis

The diversity of the collected leaf beetle community was evaluated using different parameters: (i) relative abundance (RA) which is the number of individuals of a species / the total number of individuals (N), (ii) average of abundance (AA) represents the number of specimens of a given species (Magurran, 2004). Occurrence frequency (Occ) which is the number of times the species was detected on the number of all samples. So, four species categories were determined: very

accidental species (Vac), with occurrence < 10 %, accidental species (Acc) with occurrences varying between 10–25 %, common species (Cmt) are found in 25–50 % of samples, and constant species (Cst) which detected in 50% or more of the total samples (Bigot and Bodot, 1973).

By the use of different non-parametric indices, which are calculated per field and per month, alpha diversity were evaluated: (i) number of species (S) that comprises the population present on a study site and at a given time, (ii) Shannon index (H'): $H' = - \sum p_i \ln p_i$, where p_i is

the proportion of the number of individuals of the species i (n_i) on the total number of individuals (N), (iii) Simpson index ($1 - D$): $D = \sum n_i (n_i - 1) / N (N - 1)$, (iv) equitability (E): $E = H' / H'_{\max}$, $H'_{\max} = \ln S$. These indices were computed by Past (Hammer et al., 2001).

The estimation of species richness of this community was carried out with EstimateS software (Colwell, 2013), by calculating the non-parametric asymptotic estimators (Chenchouni, 2017): (i) Jackknife 1 estimator ($S_{\text{Jack 1}}$): $S_{\text{Jack 1}} = S + t_1 (v - 1) / v$, (ii) Chao 1 estimator ($S_{\text{Chao 1}}$): $S_{\text{Chao 1}} = S + [t_1 (t_1 - 1)] / 2 (t_2 + 1)$, with v : total number of samples, S : empirical species richness, t_1 : the frequency of species that found in only one sample, t_2 : species that found in only two samples. The estimator values were given as mean \pm SD (standard deviation).

The beta diversity of leaf beetle taxocenoses was compared between the cereal fields. Several qualitative (Jaccard and Sørensen) and quantitative (estimated Chao-Jaccard, Morisita-Horn and Bray-Curtis) indices were computed. Similarity values were obtained by using EstimateS software (Colwell, 2013).

Pearson correlation tests were applied between diversity parameters of leaf beetle communities for the three cereal fields and for the three months of the study by Past software. In addition, the spatiotemporal variation of diversity parameters (N , S and H') was tested by the multivariate statistic test GLM (generalized linear model) in R version 3.6.1 (R Core Team, 2016).

RESULTS

Abundance and Occurrence Frequency

The total dataset consisted of 1144 individuals, belonging to one genus *Oulema* and four species: *Oulema melanopus* (Linné, 1758), *Oulema cyanella* (Linné, 1758), *Oulema duftscmidi* (Redtenbacher, 1874) and

Oulema gallaeciana (Heyden, 1870). Variations in values of number of individuals and number of species between cereal fields and months are displayed in Figure 2. The abundance of the cereal leaf beetles was higher in oats with 569 individuals (49.73%), followed by barley (390 individuals, 34.09%) and durum wheat (185 individuals, 16.17%). Furthermore, May was the month with most abundance (686 individuals, 59.96%), followed by April (286 individuals, 25%) and June (190 individuals, 16.60%). Specifically, *O. melanopus* was the most abundant species in the three cereal fields: barley (242 individuals, RA = 62.05%), durum wheat (142 individuals, RA = 76.75%), and oats (362 individuals, RA = 63.62%). Regarding the occurrence frequency, only *O. duftscmidi* was very accidental in barley field, the rest of species was constant. However, in durum wheat and oats, all species were constant except *O. cyanella* which was a common species (Table 1).

Diversity Parameters

Generally, barley was the richest cereal field with $S = 4$ and $N = 390$, followed by oats ($S = 3$, $N = 569$) and durum wheat ($S = 2$, $N = 185$). In contrast, the months of the study period had the same species richness value ($S = 3$).

Fluctuations in diversity parameters values between the three cereal fields are shown in table 2. Values of Shannon index and Simpson index in barley ($H' = 0.94 \pm 0.11$, $1 - D = 0.54 \pm 0.04$) and oats ($H' = 0.88$, $1 - D = 0.52 \pm 0.03$) were higher than those noted in durum wheat ($H' = 0.54 \pm 0.18$, $1 - D = 0.35 \pm 0.07$). The equitability value ranged from 0.68 ± 0.04 in barley to 0.80 ± 0.04 in oats. The month of May recorded the highest values of diversity indices ($H' = 0.94 \pm 0.04$, $1 - D = 0.55 \pm 0.03$, $E = 0.85 \pm 0.04$), while the lowest values were observed during June ($H' = 0.63 \pm 0.11$, $1 - D = 0.34 \pm 0.09$, $E = 0.57 \pm 0.10$).

Table 1: List, abundance and occurrence of leaf beetle species captured in the cereal fields, Eastern Algeria.

	Species	<i>O. melanopus</i>	<i>O. cyanella</i>	<i>O. duftscmidi</i>	<i>O. gallaeciana</i>
Barley	AA	242	62	84	2
	RA (%)	62.05	15.89	21.53	0.51
	Occ (%)	92.30	69.23	7.69	84.61
	Scale	Cst	Cst	Vac	Cst
Durum wheat	AA	142	43	0	0
	RA (%)	76.75	23.24	0	0
	Occ (%)	64.28	42.85	0	0
	Scale	Cst	Cmt	/	/
Oats	AA	362	138	0	69
	RA (%)	63.62	24.25	0	12.12
	Occ (%)	100	100	0	66.66
	Scale	Cst	Cst	/	Cst

AA, average abundance; RA, relative abundance (%); Occ, occurrence frequency (%); Vac, very accidental species; Cmt, common species; Cst, constant species.

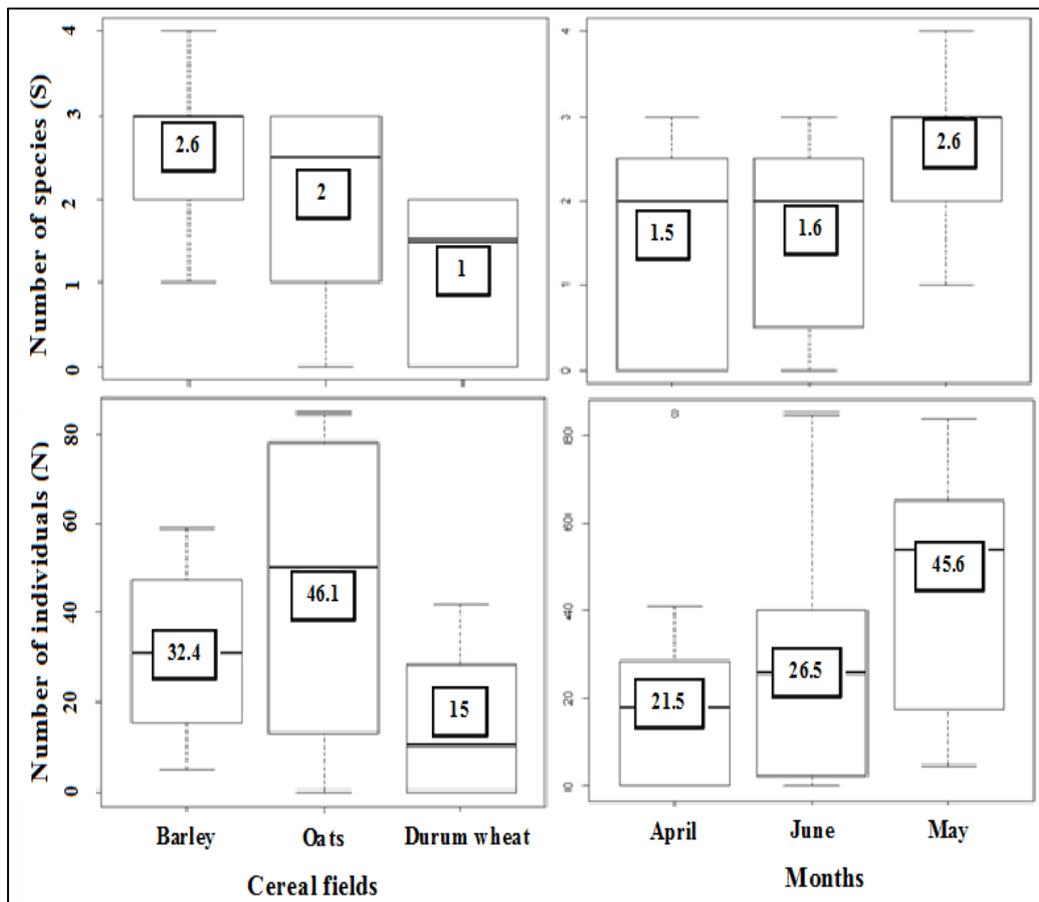


Figure 2: Abundance and species richness of the leaf beetle communities following cereal fields and months, Eastern Algeria. Boxed values indicate the mean of observed values.

Table 2: Spatiotemporal variation of diversity parameters of leaf beetle communities in several cereal fields, Eastern Algeria.

Diversity parameters	Cereal fields			Study period		
	Durum wheat	Barley	Oats	April	May	June
Abundance (N)	185	390	569	268	686	190
Species richness (S)	2	4	3	3	3	3
Shannon index ($H' \pm SD$)	0.54 ± 0.18	0.94 ± 0.11	0.88	0.82 ± 0.07	0.94 ± 0.04	0.63 ± 0.11
Simpson index ($1-D \pm SD$)	0.35 ± 0.07	0.54 ± 0.04	0.52 ± 0.03	0.47 ± 0.05	0.55 ± 0.03	0.34 ± 0.09
Equitabilité ($E \pm SD$)	0.78 ± 0.11	0.68 ± 0.04	0.80 ± 0.04	0.74 ± 0.07	0.85 ± 0.04	0.57 ± 0.10

The multivariate statistic test GLM indicated that the variation of diversity parameters was significant and different according to cereal fields (Table 3). In durum wheat, a significant decrease was observed for the individual number and the species richness ($P < 0.001$). In oats, the number of individuals increased significantly ($P < 0.001$), whereas, the H' value had a significant decrease ($P < 0.001$). During months, the diversity parameters did not differ ($P > 0.05$).

For the entire study period, the relationship between diversity parameters showed only one significantly positive correlation between Simpson index ($1 - D$) and equitability (E) at $P < 0.05$. The same, except for Simpson index ($1 - D$), which showed a significant correlation with Shannon index (H') at $P < 0.05$, no significant correlation was observed with the other diversity parameters between cereal fields ($P > 0.05$) (Figure 3).

Table 3: Summaries of generalized linear models (GLMs) testing the spatiotemporal variation of diversity parameters of leaf beetles, Eastern Algeria.

Variables	GLMs			
	<i>Est.</i>	<i>SE.</i>	<i>z-value</i>	<i>P</i>
N				
Intercept	3.444	0.076	45.272	<0.001**
Months	0.005	0.008	0.613	0.540
Oats	0.353	5.345	5.345	<0.001**
Durum wheat	-0.765	-8.503	-8.503	<0.001**
S				
Intercept	0.965	0.286	3.364	<0.001**
Months	0.002	0.034	0.069	0.944
Oats	-0.287	0.270	-1.065	0.286
Durum wheat	-0.826	0.320	-2.580	<0.001**
H'				
Intercept	4.222	5.321	79.342	<0.001**
Months	-6.604	6.144	-1.075	0.282
Oats	-1.782	5.291	-3.369	<0.001**
Durum wheat	1.462	5.051	0.001	0.999

Est.: estimate, *SE.*: standard error, *P*: p-value, **: significant p-value.

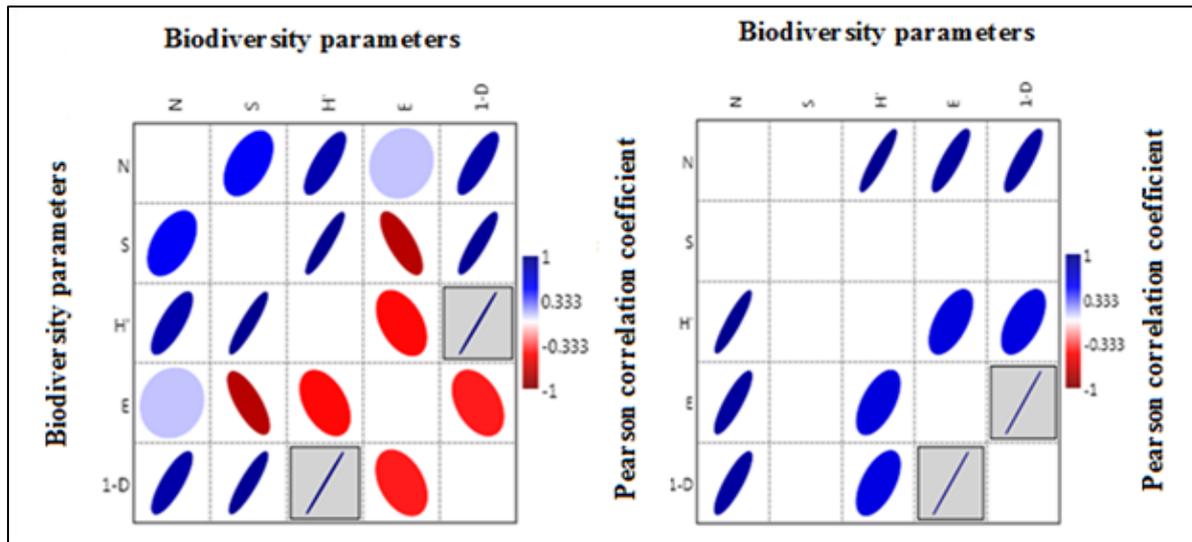


Figure 3: Correlograms displaying correlations of diversity parameters between cereal fields (left) and between months of the study period (right). Pearson correlation tests are given as correlation coefficients (shown by colour and intensity of shading). p values < 0.05 are boxed.

Species Richness Estimation

The non-parametric estimator (Chao 1) revealed that the estimated values of species richness for the different cereal fields were mostly close to the observed values of species richness, with a maximum difference of one species in the case of durum wheat ($S_{obs} = 2 \pm 0.33$, $S_{Chao1} = 3.32 \pm 0.34$) and barley ($S_{obs} = 3$, $S_{Chao1} = 4 \pm 0.37$). Likewise, the non-parametric estimator (Jack 1) indicated that the estimated species richness values and the observed species richness values were barely different in durum wheat ($S_{obs} = 2 \pm 0.33$, $S_{Jack1} = 3.32$), barley ($S_{obs} = 3$, $S_{Jack1} = 4.64 \pm 0.35$) and oats ($S_{obs} = 3$, $S_{Jack1} = 4$) (Table 4).

Similarity Analysis

For all the quantitative and qualitative indices, similarity gave generally values > 39%, except for Bray-Curtis index (< 10%). The lowest similarities were observed between barley and oats, while the highest similarities marked durum wheat-barley and durum

wheat-oats with values ranging from 75% to 99.7% (Table 5).

Phenology

Regarding the phenology of leaf beetle species in the cereal fields, *O. melanopus* was the species always appeared first, accompanied by *O. gallaeciana* in barley, and *O. cyanella* in oats. In addition, as far as the activity period is concerned, *O. melanopus* and *O. gallaeciana* had a longer activity period, from the 1st week of April to late June in barley, whereas, in oats, the longest activity period was recorded by *O. melanopus* and *O. cyanella* (1st week of April to late May). In durum wheat, both species *O. melanopus* and *O. cyanella* had almost the same activity period, which extended from the 1st week of May to late June. The shortest activity period was registered for *O. duftschmidi* in barley (3rd week of May). It is very important to note that, in durum wheat, the appearance of species was late, as compared to the other cereal fields (Figure 4).

Table 4: Species richness estimates of leaf beetle communities subservient to several cereal field of Eastern Algeria.

Diversity statistics	Cereal fields		
	Durum wheat	Barley	Oats
Number of samples	12	12	12
$S_{est} (\pm SD)$	2 ± 0.33	4	3
Singletons (mean \pm SD)	0.64 ± 0.48	0.38 ± 0.49	0
Doubletons (mean \pm SD)	0.68 ± 0.47	0.29 ± 0.46	0
Uniques (mean \pm SD)	3.32 ± 0.46	1.29 ± 0.46	0
Duplicates (mean \pm SD)	0	2.71 ± 0.46	0
Chao 1 (mean \pm SD)	3.32 ± 0.34	4 ± 0.37	4 ± 0.22
Jack 1 (mean \pm SD)	3.32	4.64 ± 0.35	4

Table 5: Qualitative and quantitative similarities of cereal leaf beetle communities between cereal fields in Eastern Algeria.

Similarity indices	Barley (N=390, S = 4)	Durum wheat (N=185, S = 2)	Oats (N=569, S = 3)
	Durum wheat (N=185, S = 2)	Oats (N=569, S = 3)	Barley (N=390, S = 4)
Classic Jaccard index [%]	75	75	50
Classic Sørensen index [%]	85	85	66
Estimated Chao-Jaccard index [%]	99.7	78.40	49.70
Morisita-Horn index [%]	62	93	39
Bray-Curtis index [%]	3	6.45	3.10

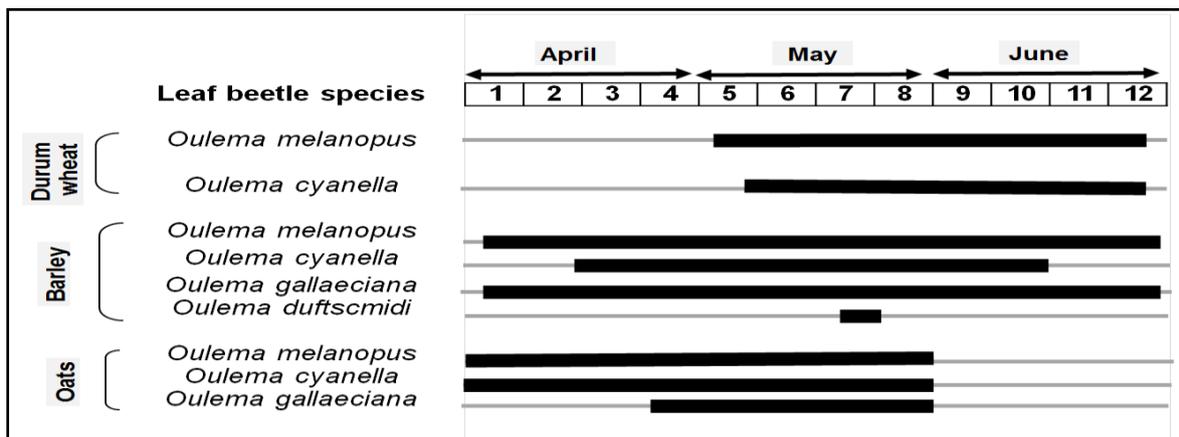


Figure 4: Phenogram of the recorded species in the cereal fields during the study period, thin lines in gray = absence, thick lines in black = presence, numbers from 1 to 12 = output number.

DISCUSSION

The literature reveals few studies on the cereal leaf beetle (*Oulema* spp.) communities subservient to cereal field ecosystems. The present study targets to identify the leaf beetle fauna of the genus *Oulema*, and to determine the variation of the diversity parameters in space and time under semi-arid conditions in Eastern Algeria.

In general, the sampling of this insect group over three months in three different cereal fields (barley, durum wheat and oats) reported four species of the genus *Oulema*: *O. melanopus*, *O. cyanella*, *O. duftschmidi* and *O. gallaeciana*. This result confirms that cereals harbor leaf beetles of the genus *Oulema*. Effectively, in Romania, the prospection of several cereal fields (wheat, barley, triticale, oats and rye) revealed the presence of six species of this genus: *O. melanopus*, *O. gallaeciana*, *O. lichenis*, *O. septentrionis*, *O. rufocyanea* and *O. cyanipennis* (Popov et al., 2005). Likewise, both species *O. melanopus* and

O. gallaeciana are the most represented in wheat and barley crops in the Northern part of Poland (Ulrich et al., 2004). In Turkey, the study carried out by Özdikmen (2011) showed the presence of three species of cereal leaf beetles: *O. duftschmidi*, *O. gallaeciana* and *O. melanopus*.

As far as the number of individuals and the number of species are concerned, the difference is important between the three cereal fields. The GLM test showed a high significant spatial variation ($P < 0.001$). The most abundant cereal fields was oats (569 individuals, 49.73%), whereas, the most speciose was barley (4 species). The lowest species richness and abundance were observed in durum wheat ($N = 185$, $S = 2$). Despite that the occurrence of each species varied from one cereal field to another, most

species were classified constant. The species *O. cyanella* occurred commonly in durum wheat (Occ = 42.85%) and *O. duftschmidi* was very accidental (Occ = 7.69%) in barley. The study conducted by Rouag et al. (2012) provided the same species richness value in durum wheat. The difference in species could be attributed to their different trophic preferences; because barley and durum wheat fields were very close to each other. The floral choice of cereal leaf beetles was the subject of several studies. Price et al. (1980) indicated that barley and oats seem to be more attractive to cereal leaf beetles than wheat. In the same context, Philips et al. (2011) showed that the cereal leaf beetles feed on many species of grasses, and are considered as major enemies of oats, barley and common wheat (*Triticum aestivum* L.), which corroborated to the findings of this study. However, differences in number of individuals could be explained by the practice of cereal monoculture and its intensity, it contributed significantly to the increase in the abundance of cereal leaf beetles (Kher et al., 2011). Furthermore, since they are phytophagous, cereal leaf beetles like leaves, where they lay their eggs and develop the different larval stages. In the case of our study, cultivators irrigated extensively the oats field; because it was intended to feed the animals, hence, the plant size increased remarkably, that is why oats field became a very favorable environment for their reproduction. A study conducted, in Romania, by Bucurean et al., (2012) reported that the intensity of the attack of *O. melanopus* on wheat was low, especially on the pubescent varieties, which are not preferable for the larvae, because they were so difficult to consume (mechanical resistance).

The species *O. melanopus* was the most abundant species in the three cereal fields, and the highest abundance value was recorded in oats with 362 individuals (63.62%). Dandurand (1976) underlined that the preferred host by *O.*

melanopus was oats, which confirmed the results of the present study.

Based on Shannon index and Simpson index, there is an important spatiotemporal variation of the cereal leaf beetle community. In general, the values of diversity indices are low, ranging between 0.34 ± 0.09 and 0.94 ± 0.11 . Barley represents the most diversified cereal field, and May is the most diversified month. Effectively, all species appeared in barley during May according to the phenogram. The low diversity can be attributed, from one side, to local harsh climatic conditions, because Tebessa and Batna belong to semi-arid bioclimatic stage, with a long dry period (April-October), according to Gaussen and Bagnouls diagram of the studied regions. On the other side, our study is interested only in cereals, there is a limitation of the prospected plants, the other plants are not taken into account, and otherwise, a large number of species belonging to the genus *Oulema* could be found. In this context Barbault (1981) reported that the increase in plant diversity leads to an increase in the diversity of phytophagous.

Beta diversity analyzed by quantitative and qualitative similarity indices expressed a high similarity between the three fields (> 50%) in most comparison cases. This enormous similarity would be attributable mainly to the structure and the composition of cereal leaf beetle community subservient to the sampled cereal fields.

In the present study, two trapping techniques were used, which are the entomological net and the sight hunting. Based on the non-parametric estimators values (Chao 1 and Jackknife 1), it is very clear that the difference between the observed species richness and the estimated species richness is neglectible (one species at most), which signifies that the sampling effort was enough and missed species were almost inexistent.

CONCLUSION

In conclusion, this study provided important information on the cereal leaf beetles belonging to genus *Oulema*. This community has an important taxonomic richness, and spatiotemporal variations of alpha and beta diversity were recorded.

CONFLICT OF INTEREST

Author of this article declares that there is no conflict of interest.

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