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DOES SEABIRD GUANO AFFECT PLANT PHYSIOLOGY IN INSULAR ECOSYSTEM?

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Abstract: Superabundance seabirds have witnessed a rapid increase in their home range, occupying most islands worldwide and disturbing the sensitive local biodiversity. While superabundant seabirds have the potential to drastically change the physicochemical properties of the habitat through an excess deposition of excrements the potential consequences of these alterations on plant physiology has received little attention. Here we aim to evaluate the effects of the guano deposition of the yellow-legged gull (*Larus michahellis*) on the leaf physiology of six species of plants (*Urtica urens, Malva arborea, Malva multiflora, Pallenis maritima*, *Coelostephus myconis,* and *Limbarda crithmoides*) found during the breeding season of the bird in six Mediterranean islands in central North Algeria. The different islands had different population sizes of the yellow-legged gull (*Larus michahellis*). We specifically collected fresh leaves that were stained or not by guano to estimate the stomatal density, water content, chlorophylls A-B, and carotenoids. The results showed that there was interspecific variability in the response of the guano stain on the four physiological parameters. Overall, there was a negative linear correlation between all parameters and the percentage cover of the leaf by the guano in all plant species. These results suggest that superabundant seabirds might induce physiological changes in plants that might lower their fitness and affect the insular ecosystem.

Keywords: Seabird, organic matter, stomata, chlorophyll, island.

INTRODUCTION

Several species of seagulls have rapidly colonized new islands worldwide, expanding their home range and affecting the local environment (Mulder et al. 2011). The high dispersal capacity, competitive ability, rapid adaptation to new natural and anthropogenic environments are key attributes that allowed their population growth and range expansion (Vidal et al., 1998). A large number of seagulls have the potential to alter both abiotic and biotic factors of insular systems which are known to be sensitive to environmental change. Some of the detrimental ecological effects may include predation on local fauna, pollution of soils, and disease transmission *(*Swennen *C. 1989*, Rgeas *et al. 2003*, Migura-Garcia *et al.* 2017, Navarro *et al*. 2019). However, large populations of seagulls deposit an excessive amount of guano which may cover plant parts (e.g. leaves, stems, flowers) and affect their physiology. Yet, no extensive studies have been carried out on these potential impacts.

One of the most abundant seagulls in the Mediterranean is the yellow-legged gull (*Larus michahellis*). It occupies most coastal cities and islands of the region and has witnessed rapid population growth (Vidal *et al*., 1998). This species meets the criteria of a superabundant species due to the large population growth and range expansion (Blokpoel 1991). It has already shown signs of ecological nuisance in the region including seabird disturbance and ecosystem integrity (Otero *et al.*, 2015; Borg et al. 1995; Keitt *et al.*, 2004; Oro *et al.*,2005). Thus, the yellow-legged gull is expected to affect the flora through trampling, scraping, collecting material for nest construction, boundary clashes related to gull behavior, and excrement (Sobey and Kenworthy 1979). Here we assess the effect of guano covering the leaves of plants on the physiology of leaves of dominant species in the Mediterranean islands. The presence of the guano on the leaves of plants may affect different physiological processes. (Stomata, Chlorophylls A+B, Carotenoids, Water contained in the leaf)

In this study, we assess the impact of guano deposited on plant leaves on the physiology of six plant species plants (*Urtica urens, Malva arborea, Malva multiflora, Pallenis maritima, Coelostephus myconis,* and *Limbarda crithmoides*) in six islands of central North Algerian coast. We specifically investigate the number of stomata, Chlorophylls A+B, Carotenoids, and water content in the leaf. We hypothesize that when there is more guano stain on the leaf, there would be a higher effect on the physiological parameters. Determining these relationships is key to predict the direct and indirect effects of invasive bird species on plant physiology and insular ecosystems.

MATERIAL AND METHODS

Study Area

The study was carried out in the central North Algerian coast near Bejaïa and Jijel provinces (Fig. 1). We selected six islands (Pisans, Sahel, Cap Cigli [El

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Euch], Grand Cavallo, Cavallo islet, and Petit Cavallo) based on yellow-legged gull breeding and accessibility (Table 1). The latter species was the most abundant bird occupying the sites along with a small population of *Falco perrgrinus, Columba livia, Apus pallidus, Sylvia melanocephala, Fringilla coelebs, Cisticola juncidis, Turdus merula*, and *Luscinia megarhynchos*. The number of breeding pairs of the yellow-legged gull varies between 15 and 193. These islands have a surface of 0.2-1.2 ha and do not exceed 30 m in height, except for Grand Cavallo which reaches 50 m. The distance to the shore varies between 7 and 1250 meters.

Breeding pairs estimation

The yellow-legged gull *(Larus michahellis*). is a colonial bird that mainly breeds in islands, starting in March on the Algerian coast (Moulaï *et al.* 2006; Baaloudj *et al.* 2012; Baaloudj *et al.* 2014). We visited each island 2 to 3 times per week (total of 138 h) from late March to late May during 2017 and 2018. We counted the number of active nests (those including eggs) of the species. Since we checked each corner of the island, we believe that we did not miss any nests.

Sampling

The used plant material is composed of six island species (*Urtica urens, Malva arborea, Malva multiflora, Pallenis maritima, Coelostephus myconis*, and *Limbarda crithmoides*). 220 fresh leaves (110 with guano stain and 110 without stain) were sampled among the most abundant species in order to have a representative sample on each site. They were immediately placed in plastic bags and then in a cooler for temporary storage in order to assess the water content, stomatal density, chlorophyll ab, and carotenoids.

Pigment determination

Total chlorophyll and carotenoid content were determined using 200 mg FW of leaf material ground in a mortar in the presence of 10 ml of acetone 80 % (v/v). The mixture was centrifuged at 4,500g for 10 min. The absorbance of the extract was read at 663, 647, and 470 nm using HACH LANGE DE3900 spectrophotometer; and pigments concentrations were calculated according to formulas to Lichtenthaler (1987) and expressed on a fresh weight basis (mg g^{-1} of FW).

$$\text{Chlorophyll } a\ (mg\ g^{-1}\ \text{of FW}) = 12.25\ A_{663} + 2.79\ A_{647}$$

$$\text{Chlorophyll } b\ (mg\ g^{-1}\ \text{of FW}) = 21.50\ A_{647} + 5.10\ A_{663}$$

$$\textit{Total chlorophyll}\ (mg\ g^{-1}\ \text{of FW}) = 7.15\ A_{663} + 18.71\ A_{647}$$

$$\textit{Total carotenoids}\ (mg\ g^{-1}\ \text{of FW}) = \frac{1000\ A_{647} - 1.82\ Chl\ a\ -\ 85.02\ Chl\ b}{198}$$

Water content

The leaf cut at the base of the limbus is weighed immediately, which represents the fresh weight (FW), then it is placed in an oven set at 80 °C for 48 hours to obtain its dry weight (DW)

The measurement of the water content is calculated by the following formula:

$$Water\ content\ (\%) = \frac{FW - DW}{FW} \times 100$$

Stomatal density

The density of the stomata was determined using the epidermal imprint method (Jones, 1983). The imprints were obtained by applying a layer of transparent nail varnish to the upper surface of a mature leaf of each species. Once dried, this layer of varnish is removed using adhesive tape and placed on a slide. The observations were made using a Microscope (USB 250x) with HD Camera and connected to a computer. The image displayed on the computer screen is analyzed by ImageJ software. The density of the stomata (Ds) was determined by counting their number per mm² of leaf area.

All statistical analyses were performed with R 3.5.2 (R Development Core Team 2019). We calculated the R^2 of the fixed effects of the model using the 'r. squared GLMM' function of the MuMIn package (Barton 2019) Data are expressed as mean ± SD. A probability level <0.05 has been considered significant.

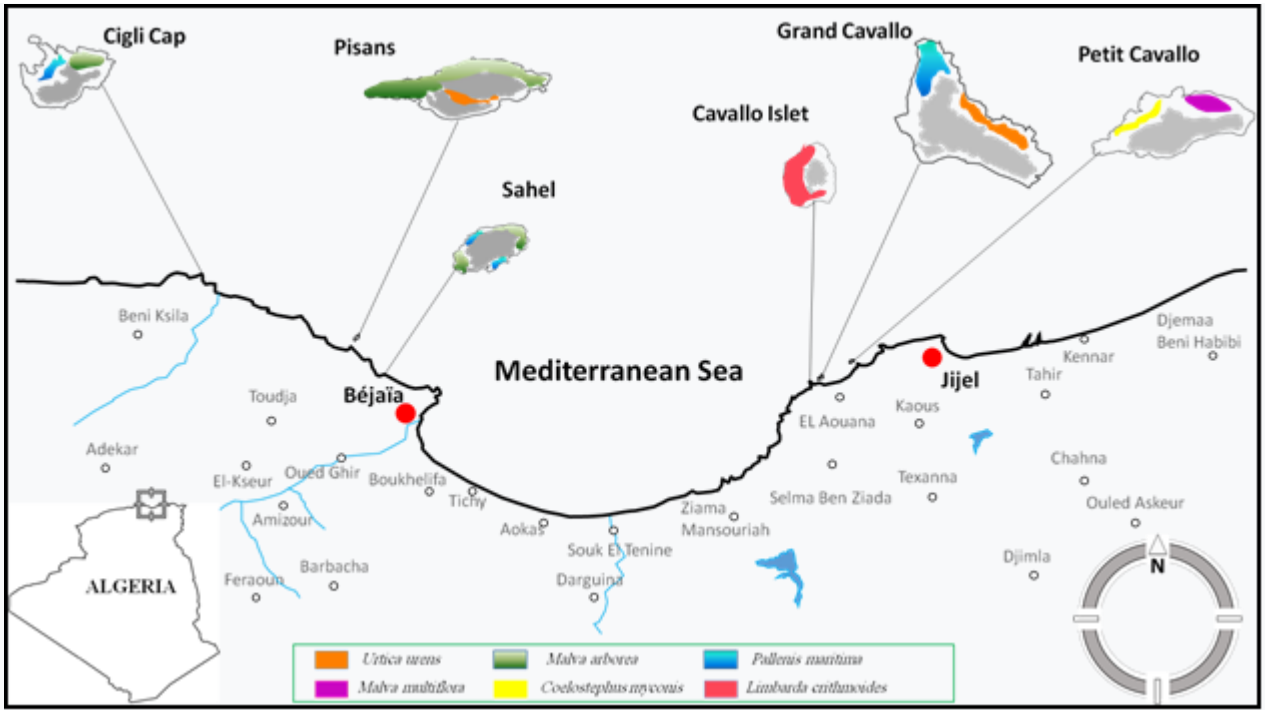

Fig. 1 Distribution map of *Urtica urens, Malva arborea, Pallenis maritima, Malva multiflora, Coelostephus myconis,* and *Limbarda crithmoides* across major islands sites in central North Algeria

Table 1.

Geographic coordinates and physical characteristics of the studied Mediterranean islands in central North Algeria

Province	Name	Coordinates N	Coordinates E	Area (ha)	Distance to the continent (m)	Height (m)	Colony size	plant species
Bejaïa	Pisans	36°49'30"	4°59'50"	1.2	1250	30	139	02
	Sahel	36°47'38"	5° 1'23"	0.2	7	15	15	02
	Cap Cigli	36°53'34"	4°47'20"	0.8	120	20	193	02
Jijel	Grand Cavallo	36°47'7"	5°36'29"	6	950	50	302	02
	Petit Cavallo	36°48'5"	5°39'1"	4	750	10	91	02
	Cavallo Islet	36°46'42"	5°36'2"	0.15	50	30	39	01

Table 2.

Average ± SD of five phenotypic traits in six plant species living in an insular ecosystem

Species		Mean	Count	Range	SD
Guano stain coverage	*Urtica urens*	,17	40	,51	,19
	Malva arborea	,08	60	,24	,09
	Pallenis maritima	,22	60	,76	,24
	Malva multiflora	,05	20	,17	,06
	Coelostephus myconis	,15	20	,42	,16
	Limbarda crithmoides	,19	20	,46	,20
Stomatal density (mm^2)	*Urtica urens*	28	40	38	11
	Malva arborea	210	60	334	95
	Pallenis maritima	94	60	135	49
	Malva multiflora	199	20	376	90
	Coelostephus myconis	129	20	170	50
	Limbarda crithmoides	65	20	75	27
Cholorophyll A+B (mg/g FW)	*Urtica urens*	,15	40	,20	,05
	Malva arborea	,05	60	,07	,02
	Pallenis maritima	,05	60	,10	,02
	Malva multiflora	,04	20	,05	,01
	Coelostephus myconis	,10	20	,08	,02
	Limbarda crithmoides	,33	20	,56	,15

Carotenoides (mg/g)	*Urtica urens*	,22	40	,20	,06
	Malva arborea	,09	60	,22	,03
	Pallenis maritima	,13	60	,73	,12
	Malva multiflora	,07	20	,08	,02
	Coelostephus myconis	,16	20	,12	,03
	Limbarda crithmoides	,58	20	1,37	,45
Water content (%)	*Urtica urens*	75,02	40	23,32	6,29
	Malva arborea	79,17	60	27,23	8,50
	Pallenis maritima	84,24	60	21,25	7,09
	Malva multiflora	83,12	20	20,80	6,85
	Coelostephus myconis	76,62	20	21,25	6,51
	Limbarda crithmoides	92,70	20	11,52	4,70

RESULTS

Stomatal density

There was a significant difference in stomata among species ($F_{5.208}$=92.259; df=5; P < 0.0001). The number of stomata declined significantly with the percentage of guano coverage in all six plant species ($F_{1,\ 208}$=167.87; P < 0.0001). There was a strong interaction between species and percent guano coverage revealing that the magnitude of the decline varied among species ($F_{5,\ 208}$= 23.988; df=5; P < 0.0001). *Malva multiflora* and *Malva arborea* showed the strongest response to percent guano coverage (N=220; R^2 = 0.795) (Fig. 2). The four other species *Urtica urens*, *Coelostephus myconis*, *Limbarda crithmoides*, and *Pallenis maritime*, exhibited relatively similar rates of decline (Table 3).

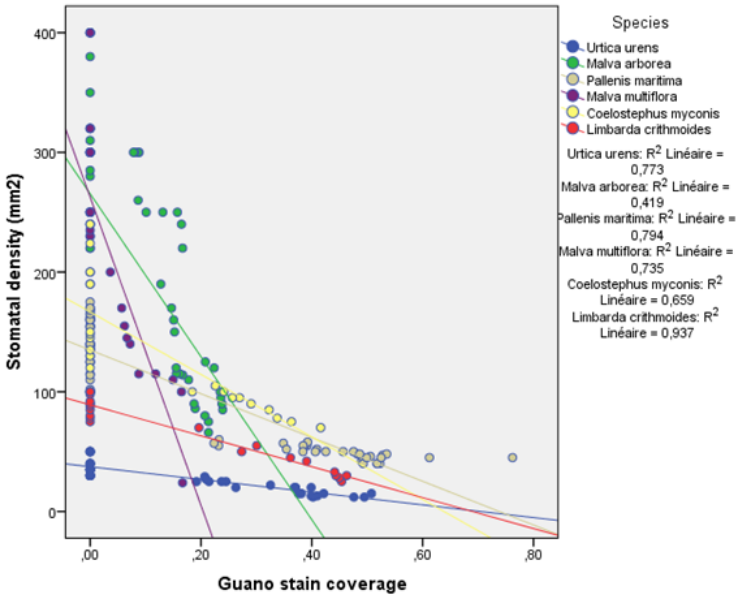

Fig. 2 The relationship between stomata number and guano stain coverage in six plants species in insular ecosystem

Table 3.

Uni-Anova Stomatal density by Species with Guano stain coverage

Parameter	B	Std. Error	t	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	89,062	13,471	6,611	0	62,504	115,62
[Species=1]	-51,652	16,436	-3,143	0,002	-84,054	-19,249
[Species=2]	176,402	15,497	11,383	0	145,852	206,953
[Species=3]	45,863	15,546	2,95	0,004	15,214	76,511
[Species=4]	173,572	18,534	9,365	0	137,034	210,11
[Species=5]	77,655	19,06	4,074	0	40,079	115,231
[Species=6]	0[a]	.	.	.	.	.
Guanostaincoverage	-129,13	49,183	-2,626	0,009	-226,091	-32,169

[Species=1] * Guanostaincoverage	76,022	61,612	1,234	0,219	-45,443	197,486
[Species=2] * Guanostaincoverage	-551,68	80,016	-6,895	0	-709,432	-393,94
[Species=3] * Guanostaincoverage	-53,644	54,612	-0,982	0,327	-161,308	54,02
[Species=4] * Guanostaincoverage	-1158,1	173,424	-6,678	0	-1500	-816,21
[Species=5] * Guanostaincoverage	-131,99	80,668	-1,636	0,103	-291,025	27,037
[Species=6] * Guanostaincoverage	0[a]	.	.	.	.	.

Chlorophylls A-B

There was a very highly significant difference in the chlorophyll AB between the different species ($F_{5.208}$= 68.471; df=5; P < 0.0001). The difference in guano stain coverage is significant between the six species ($F_{1.\ 208}$=26.386; P < 0.0001). There was a significant interaction between the six species and the guano stain coverage ($F_{5.\ 208}$= 20.42; df=5; P < 0.001). Noticeably, the two species *Limbarda crithmoides and Urtica urens* show a sharp decline (N=220; R^2 = 0.755) (Fig. 3). The three remaining species (*Malva arborea, Malva multiflora,* and *Coelostephus myconis*) had similar linear trends, but *Pallenis maritima* had a slower decline (Table 4)**.**

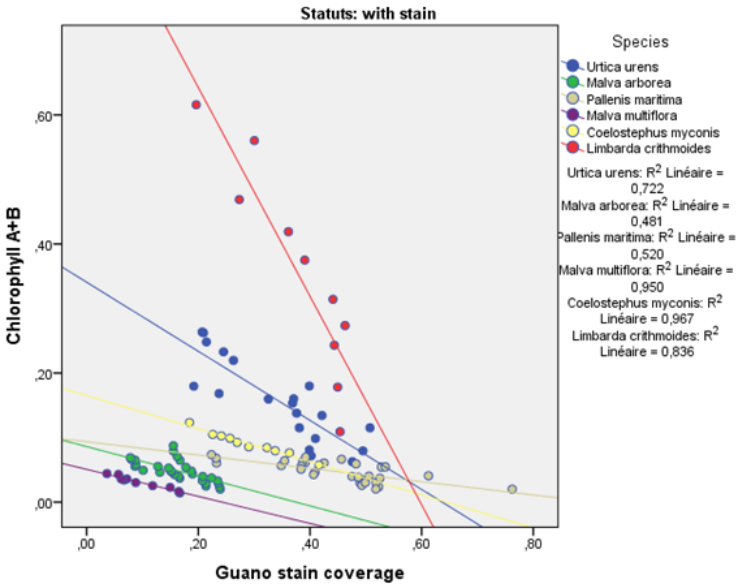

Fig. 3 The relationship between the rate of chlorophyll AB and guano stain coverage in six plant species in an insular ecosystem

Table 4.

Unianova rate of Chlorophyll A+B by Species with Guano stain coverage

Parameter	B	Std. Error	t	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	0,344	0,015	22,611	0	0,314	0,374
[Species=1]	-0,179	0,019	-9,636	0	-0,216	-0,142
[Species=2]	-0,283	0,018	-16,142	0	-0,317	-0,248
[Species=3]	-0,283	0,018	-16,08	0	-0,317	-0,248
[Species=4]	-0,293	0,021	-13,984	0	-0,334	-0,252
[Species=5]	-0,225	0,022	-10,417	0	-0,267	-0,182
[Species=6]	0[a]	.	.	.	.	.
Guanostaincoverage	-0,054	0,056	-0,977	0,33	-0,164	0,055
[Species=1] * Guanostaincoverage	-0,011	0,07	-0,156	0,876	-0,148	0,126
[Species=2] * Guanostaincoverage	-0,039	0,09	-0,43	0,668	-0,217	0,139
[Species=3] * Guanostaincoverage	0,018	0,062	0,293	0,77	-0,104	0,14
[Species=4] * Guanostaincoverage	-0,16	0,196	-0,816	0,415	-0,547	0,227
[Species=5] * Guanostaincoverage	-0,054	0,091	-0,595	0,552	-0,234	0,126
[Species=6] * Guanostaincoverage	0[a]	.	.	.	.	.

Carotenoids

There is a very highly remarkable difference in the carotenoids between the different species (N=220; $F_{5.208}$= 148.69; df=5; P < 0.0001), (Fig. 4). The difference in the effect of the guano stain coverage is very significant between the six species ($F_{1.\ 208}$= 54.475; P < 0.0001). There was an important interaction between the six species and the guano stain coverage ($F_{5.208}$ = 70.33; df=5; P < 0.0001). *Malva arborea* shows a rapid decline compared to the other species which have similar trends (slopes). (Table 5) The model explains a large proportion of variability (R^2 = 0.81).

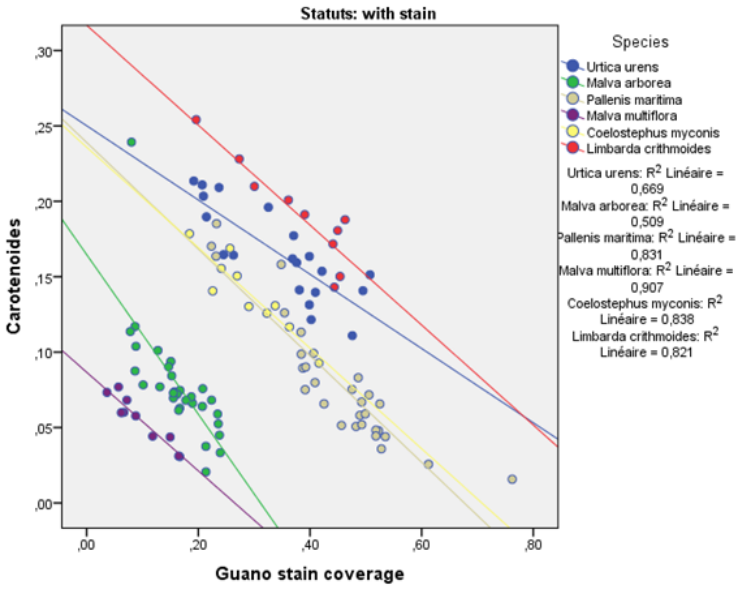

Fig. 4 The relationship between the rate of carotenoids and guano stain coverage in six plant species in an insular ecosystem

Table 5.

Unianova rate of Carotenoids by Species with Guano stain coverage

Parameter	B	Std. Error	t	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	0,94	0,028	33,497	0	0,884	0,995
[Species=1]	-0,673	0,034	-19,656	0	-0,74	-0,605
[Species=2]	-0,831	0,032	-25,766	0	-0,895	-0,768
[Species=3]	-0,754	0,032	-23,29	0	-0,818	-0,69
[Species=4]	-0,857	0,039	-22,198	0	-0,933	-0,781
[Species=5]	-0,763	0,04	-19,237	0	-0,842	-0,685
[Species=6]	0[a]	.	.	.	.	.
Guanostaincoverage	-1,897	0,102	-18,528	0	-2,099	-1,695
[Species=1] * Guanostaincoverage	1,606	0,128	12,521	0	1,353	1,859
[Species=2] * Guanostaincoverage	1,683	0,167	10,105	0	1,355	2,012
[Species=3] * Guanostaincoverage	1,656	0,114	14,565	0	1,432	1,88
[Species=4] * Guanostaincoverage	1,602	0,361	4,436	0	0,89	2,314
[Species=5] * Guanostaincoverage	1,76	0,168	10,477	0	1,429	2,091
[Species=6] * Guanostaincoverage	0[a]	.	.	.	.	.

Water Content

There was a crucial difference in the water content of leaf among species (N=220; $F_{5.208}$= 94.832; df=5; P < 0.0001). Leaf water content declined significantly with the percentage of guano coverage in all six plant species ($F_{1.\ 208}$=816.94; P < 0.0001). There was a strong interaction between species and percent guano coverage, revealing that the magnitude of the decline varied among species ($F_{5.\ 208}$= 70.33; df=5; P < 0.0001). *Malva multiflora* and *Malva arborea* showed

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 31, issue 1, 2021, pp. 11 - 22

the strongest response to the percentage of guano coverage (Fig. 5). The four other species *Urtica urens*, *Coelostephus myconis*, *Limbarda crithmoides*, and *Pallenis maritime*, exhibited relatively similar rates of decline (Table 6). Our model explained 92.5% of the variance.

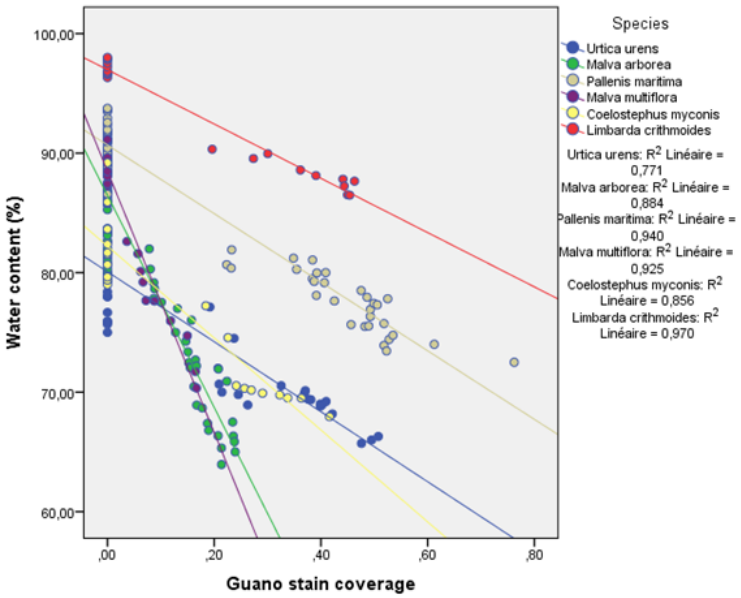

Fig. 5 The relationship between leaf water content and guano stain coverage in six plant species in insular

Table 6.

Unianova water content by Species with Guano stain coverage

Parameter	B	Std. Error	t	P-value.	95% Confidence Interval	
					Lower Bound	Upper Bound
Intercept	96,982	0,747	129,87	0	95,51	98,454
[Species=1]	-16,90	0,911	-18,55	0	-18,702	-15,11
[Species=2]	-10,52	0,859	-12,25	0	-12,216	-8,829
[Species=3]	-6,30	0,862	-7,31	0	-8,002	-4,605
[Species=4]	-8,49	1,027	-8,26	0	-10,52	-6,47
[Species=5]	-14,76	1,057	-13,97	0	-16,85	-12,684
[Species=6]	0[a]	.	.	.	.	.
Guanostaincoverage	-22,71	2,726	-8,33	0	-28,091	-17,341
[Species=1] * Guanostaincoverage	-6,57	3,415	-1,92	0,056	-13,303	0,163
[Species=2] * Guanostaincoverage	-66,00	4,435	-14,88	0	-74,745	-57,257
[Species=3] * Guanostaincoverage	-5,97	3,027	-1,97	0,05	-11,944	-0,008
[Species=4] * Guanostaincoverage	-86,64	9,613	-9,01	0	-105,595	-67,691
[Species=5] * Guanostaincoverage	-15,73	4,472	-3,51	0,001	-24,551	-6,92
[Species=6] * Guanostaincoverage	0[a]	.	.	.	.	.

DISCUSSION

Stomatal density

Near bird nesting sites, the plants, in particular the leaves, are often soiled by the waste of yellow-legged gull (*Larus michahellis*), in this case, the guano. These whitish wastes are often deposited on the leaves and adhered to their surface. Guano hides the stomata which makes them inactive and blocks their physiological roles in gas exchange. In this study, we found a very close relationship between stomatal density and the guano stains on the leaves. In fact, the stomatal density of a leaf is inversely proportional to the importance of the guano stain. The stomatal density on the abaxial surface varies according to the species [24mm² - 400mm²]. There is a significant difference between the 06 studied species in relation to the number of stomata per unit area (N = 220; P <0.0001). Moreover, all species show a decrease in stomatal density depending on the size of the guano stain. However, the reaction is more intense in *Malva arborea* (M_C = 245.9 mm²; M_E = 164.2 mm²) and in *Malva multiflora* (M_C = 271.5 mm²; M_E = 127.4 mm²), indeed, a clear decrease in stomatal density has been recorded in the stained leaves of these species

compared with the controls. These two species are characterized by large foliage and they are dominant by richer soils in nutrients (the case for our study sites). Therefore, they are more exposed to contamination compared to other species. The three species, *Limbarda crithmoides*, *Coleostephus myconis*, and *Pallenis maritima* generally react in the same way but they are less injured as their stomatal densities are reduced with the presence of guano. Nevertheless, the reduction in the number of stomata is significantly smaller in *Urtica urens* (M_C = 36.5 mm²; M_E = 15.65 mm²) and it seems less affected by this constraint. This observed difference in the behavior between the six species can be related to their morphological aspects (size, shape, thickness of the leaf, position, and orientation of the leaves relative to the stem). Also, there are other factors like the distribution of stomata on the leaf, especially on the upper surface, the presence and density of trichomes, and the age of the plant (Damour et al., 2010; Ahmad et al., 2019). In the presence of various abiotic stresses, leaf characteristics such as size, density, and stomatal opening allow better regulation of transpiration and CO2 assimilation for photosynthesis (Zhao et al., 2015). Stomatal pores opening and closing are controlled by two guard cells. When the guard cells are turgid the pores open, whereas when they are flaccid the pores close. (Schroeder et al., 2001). These changes in the state of the guard cells allow rapid adaptation to fluctuating environmental conditions.

Water Content

In the six studied species, the guano deposits hide part of the leaf surface and therefore reduce the number of functional stomata. This guano coverage thereby causes dehydration in the guard cells which leads to rapid closing of the ostiole and therefore a limitation of transpiration. In fact, this limitation affects the water balance which results in a loss of the water content of the plants. From our results, we found that the leaf water content is quickly reduced in *Malva multiflora* (M_C = 89.08%; M_E = 77.15%) and *Malva arborea* (M_C = 86.41%; M_E = 71.93%) and in a significant manner compared to other 04 species according to the percentage of guano coverage (N = 220; P <0.0001). The accumulation of guanos on the leaves can lead to cellular desiccation. The deposited guanos on the leaves can be corrosive to the cuticle and epidermis due to their alkaline nature and their rich elements of nitrogen and phosphorus. In fact, near bird nesting sites, soils are extremely rich in phosphorus, nitrate, and ammonium (Hutchinson, 1950; Anderson and Polis, 1999; Bouyahmed and Moulai, 2018), which can facilitate the plant growth of certain species, but they prevent the growth of others (Smith, 1978; Wainright*et al.*,1998). The inhibition may be the result of ammonium toxicity, or indirectly via low pH, which inhibits the absorption of certain nutrients (Odasz, 1994). Under certain environmental conditions, dehydration is the first signal that prompts plants to respond to stress (Jia et al., 2002; Matsuo et al., 2009). When the leaves start to dehydrate, plants usually begin to close their stomata. In fact, tissue dehydration occurs when there is an imbalance between the water absorption of the roots and the transpiration of the leaves (Aroca et al., 2001; Jackson et al., 2003). Transpiration is a phenomenon that results in the absorption of water by the roots and its transport through the xylem. When the stomata are closed, little CO2 is absorbed and the transpiration is reduced. By opening and closing the stomata, plants can regulate the amount of water loss, by sacrificing the absorption of CO2, when environmental conditions are unfavorable. Several biotic or abiotic factors modulate stomatal movements. Certain abiotic factors, such as light and high temperatures prompt stomatal opening (Shimazaki et al., 2007; Murata et al., 2015). While others such as drought, high CO2 concentration, darkness, and phytohormones (abscisic acid and ethylene) are known to prompt stomatal closure (Acharya and Assmann, 2009; Kim et al., 2010). The guanos deposition and absorption by the leaves can alter many cellular functions of the plants. Indeed, this constraint can cause the stomata to close, as in the water deficit case (Lassouane *et al.*, 2013). Plants in a dehydration situation respond by promoting the mobilization along with the synthesis of (Abscisic Acid) ABA in vascular leaf tissue. Subsequently, ABA is transported to guard cells to trigger stomatal closure (Seo and Koshiba 2011; Munemasa *et al.*, 2015).

Chlorophyllian pigments

Chlorophyll is the active molecule of the chloroplast which plays an essential role in the photosynthesis of plants. In fact, the chlorophyll content is considered to be an important factor in the determination of the photosynthetic capacity in plants (Anjum et al., 2011). In addition, it is a used factor in detecting the tolerance of species to abiotic stresses (Khayatnezhad et al. 2011). In this study, a large significant difference has been observed in the total leaf chlorophyll levels between the (unstained) plants regulation of the six species (N = 220; P <0.0001). The *Limbarda crithmoides* species has the highest level of chlorophylls compared to other species. Under the effect of the adhesion of guanos to the leaves, a significant decrease has been observed in the total chlorophyll level of all the studied species. In fact, by comparing the six species, the greatest and fastest reduction in total chlorophyll levels is recorded in the two species *Limbarda crithmoides* and *Urtica urens*; respectively estimated at a rate of (0.66) and (0.26). Conversely, the three species *Malva multiflora*, *Malva arborea*, and *Coelostephus myconis* show a slower reduction compared to the two species mentioned before. Whereas the decrease in the total chlorophyll level is relatively slow in *Pallenis maritima* with a maximum level of (0.12). The closure of the stomata causes a limitation of transpiration and leads to an increase in the temperature of the leaves, especially when the light intensity is high (Mafakheri et al., 2010; Arbona et al., 2013). The high leaf temperature disrupts

Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 31, issue 1, 2021, pp. 11 - 22

the synthesis of photosynthetic pigments, damages chloroplast membranes, and reduces the level of chlorophylls and other pigments. In addition to an increased level of abscisic acid that controls stomatal closure (Zandalinas et al., 2016). Various abiotic stresses such as salinity, drought, heat stress, heavy metal toxicity, and air pollution prompt to decrease the photosynthetic pigments, especially chlorophyll. This reduction occurs due to chlorophyll alterations that may be due to chlorophyll degradation or decreased synthesis (Singh and Pandey, 2011; Swelam et al., 2016). The intensity of this effect depends on the species, type of stress, duration of the stress period, and resilience to stress (Watkins et al., 2017). This significant decrease in total chlorophyll levels under the effect of guanos deposition is believed to be mainly due to damage to chloroplasts by reactive oxygen species (ROS). The presence of ROS causes the peroxidation of membrane lipids and leading to the destruction of chlorophyll (Smirnoff, 1993). Murata et al. (2007), suggests that the presence of stress; the light absorbed by the chlorophyll pigments, becomes excessive for the needs of the photosynthetic machinery, hence the production of ROS. Other authors, like Powles (1984) and Krause (1988) also believe that the reduction of photosynthetic pigments in plants may be linked to the photooxidation of pigments resulting from oxidative stress caused by absorbed energy excess. Carotenoids are among the antioxidant molecules that protect plant cells from oxidative damages caused by biotic and abiotic stresses (Pandey et al., 2017). They play an essential role in channeling the harvested light energy into the plant's photosystem and in dissipating excess light energy (McElroy and Kopsell, 2009). They play essential roles in channeling the harvested light energy into the plant's photosystem and in dissipating excess light energy (McElroy and Kopsell, 2009). Besides their structural function in the photosynthetic antenna and reaction center, carotenoids play a crucial role in protecting the photosynthetic system from oxidative damage by scavenging reactive oxygen species (You and Chan, 2015). They also have a key role in the stabilization of biological membranes (Havaux, 1998). Along with the decrease in total chlorophyll contents, our results show a significant decrease in total carotenoid content that was also recorded in all studied species ($N = 220$; $P < 0.0001$). This decrease is more marked in *Malva arborea* with a minimum rate of (0.02), which suggests that in this species, the oxidative damage caused by the accumulation of ROS is higher compared to other species. The four species, *Malva multiflora*, *Coelostephus myconis*, *Pallenis maritima*, and *Limbarda crithmoides*, show a less slow decline. However, the decrease in the level of carotenoids is slower in *Urtica urens*. Indeed, we believe that the small decrease in the carotenoid content in *Urtica urens* explains some efficacy of these compounds in avoiding the photo-inhibition that occurs when light exceeds the photosynthesis level. Carotenoids play very important roles in tolerance to biotic stresses and oxidative damage (Gill and Tuteja 2010; Pandey et al., 2017). However, they are very sensitive to the oxidative destruction of photosynthetic membranes caused by the accumulation of generated ROS during stress. Furthermore, carotenoids are considered as antioxidant defense molecules because they do not only protect the photosynthetic system against photo-oxidative damage but also perform as precursors of abscisic acid which plays an essential role in plant response and tolerance to abiotic stress (Vishwakarma et al. 2017). In the present study, the content of total chlorophyll and carotenoids decreased for all six studied species. The reduction may be related to disruption of pigment synthesis and inhibition of ROS degradation due to conditions prompted by guano deposition. The work of Joshi and Swami (2007) has shown that heavy metals and/or gaseous pollutants cause similar effects. The absorption of pollutants can modify the stomata and the parenchyma characteristics (Qin *et al.*,2014), which can lead to a reduction in the chlorophyll content and therefore in photosynthesis and thus a decrease in the productivity of plants (Doğanlar and Atmaca, 2011; Khosropour *et al.*, 2019). Pollutants can enter the leaf through the stomata or diffuse through the epidermis (Khosropour *et al.*, 2019). In fact, faced with increased pollution, plants can reduce the leaf area and decrease the density of stomata. In addition, the anatomical properties may also be altered, such as increased cuticle thickness and decreased lacunous mesophyll thickness (Pourkhabaz et al. 2010). Anatomical and pigmentary changes will likely combine to reduce the photosynthesis and the growth rate.

CONCLUSION

Our results show the existence of a very close relationship between physiological parameters for the six island species and the deposited guano stain on the leaves. In fact, the guano stain size varies from one species to another because it is determined by the leaf surface which is more important for the two species: *Malva arborea* and *Malva multiflora* that has broad leaves compared to the other species. It is clearly visible in *Limbarda crithmoides*, *Coleostephus myconis*, and *Pallenis maritima* and is very weak for the *Urtica urens* species. The latter has soft stems that reduce deflagration at the impact of feces on the leaves. This observed difference in the behavior between the six species can be linked to their morphological aspect (size, shape, leaf thickness, leaf position, and orientation).

Following our study, we find that the accumulation of Yellow-legged Gull, *Larus michahellis* guano on these island plants will cause leaf dysfunction and subsequently wilting, photo-oxidation, desiccation and possibly asphyxiation of the plant.

In prospect, in order to improve this research, an in-depth dynamic study on the most impacted species *Malva multiflora*, would be interesting, taking into account its growth rate, especially if we know that this plant has a high heritage value in the Mediterranean.

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CONFLICT OF INTEREST

The authors declare no conflict of interest and no financial interest.

ETHICS STATEMENT

No permission was needed to carry out our study, and no bird was manipulated or touched during our sampling.

AUTHOR CONTRIBUTIONS

AAH, SBH and RM designed the study. AAH performed field and laboratory work. AB carried out the statistical analyses and wrote the paper. AB and NL elaborate the discussion. All authors contributed to the revision of the paper and gave final approval for publication.

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