Exploring the interaction between the capitate glandular trichomes of sunflower anthers and arthropods

Mariana Paola Mazzei[1], **Leonardo** Galetto[2], **David** Balaban[3], **Graciela María** Nestares[3,4], **Ana Claudia** Ochogavía[3,5]

[1]*Dipartimento di Scienze e Tecnologie Agro-Alimentari (DISTAL), Università di Bologna, Italy*
[2]*Instituto Multidisciplinario de Biología Vegetal, IMBIV-CONICET-Universidad Nacional de Córdoba, Argentina*
[3]*Instituto de Investigaciones en Ciencias Agrarias de Rosario, IICAR-CONICET-UNR, Santa Fe, Argentina*
[4]*Cátedra Genética, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Santa Fe, Argentina*
[5]*Cátedra Química Orgánica, Facultad de Ciencias Agrarias, Universidad Nacional de Rosario, Santa Fe, Argentina*

Abstract

Different floral traits mediate interactions between plants and their visitors. Capitates Glandular Trichomes (CGT) can attract or repel visitors, particularly arthropods. In *Helianthus annuus* L., CGTs are located at the distal ends of the anther appendages. This study aimed to determine whether the presence of CGTs influences the behaviour of diurnal floral visitors under field conditions by comparing genotypes with high and low CGT density per anther, while maintaining similar phenotypic characteristics. The analysis accounted for three random variables -floral developmental stages (R5.3, R5.6, and R6), daytime ranges (morning, midday, and afternoon), and two flowering seasons- to better understand variations in CGT mediated floral arthropod interactions. Key recorded species included *Astylus atromaculatus* (Blanchard) (Coleoptera Melyridae), *Apis mellifera* L. (Hymenoptera Apidae), and species from the Thomisidae family representing herbivores, pollinators, and insect-predators, respectively. The genotype with a high CGT density received fewer herbivore visits and more pollinator visits compared with the genotype with a low CGT density. Predators did not exhibit genotype-related differences but reduced bee presence by 20% probably due to Thomisidae spiders preying on bees. Furthermore, the inclusion of random variables enhanced the models. Herbivore abundance varied significantly between flowering seasons, while pollinator abundance fluctuated across the daytime ranges. Both herbivores and pollinators also showed variations in abundance across the reproductive stages, likely influenced by the proportion of disc floret phenophases and the CGTs' secretory stage. This study underscores the importance of CGTs as a key morphological feature with potential implications for plant reproduction, justifying broader, more extensive investigations. It also emphasizes the necessity of considering multiple factors in plant-arthropod interactions research.

Key words: capitate glandular trichomes, *Helianthus annuus* L., floral visitors, sunflower-arthropods interaction, sunflower reproductive development.

Introduction

Floral traits such as colour, shape, scent and nutritional rewards are linked to the attraction of a wide range of floral visitors (Junker and Blüthgen, 2010). These visitors can be categorized into pollinators (e.g. bees, butterflies, hoverflies), herbivores (e.g., sucking insects, beetles, butterfly larvae) and predators (e.g., spiders, certain wasps, beetles). Pollinators feed on nectar and pollen contributing to the pollination and the production of fruits and seeds (IPBES, 2016). Herbivores consume nectar, pollen and floral reproductive structures like stamens and pistils, often causing damage (Wäckers *et al*., 2007). Predators can be found in the floral structures to prey on other insects. This can be beneficial for pest control when they consume herbivores like ladybugs (Coccinellidae), or detrimental to plant reproduction when they target pollinators, such as in the case of crab spiders (Ings and Chittka, 2009). These visitors interact within the flower, affecting each other's behaviour and abundance (Mazzei *et al*., 2020), especially in large, flat, multi-flowered structures like sunflower heads (Chamer *et al*., 2020).

Asteraceae inflorescences show a wide variation in their colour, number per capitulum, and nectar traits across species (e.g., Torres and Galetto, 2002; 2007; 2011), leading to differences in floral visitor attraction (Torres and Galetto, 2008). Various morphological features of reproductive organs might be involved in plant-floral visitor communication. Trichomes, epidermal structures widespread on both vegetative and reproductive organs, mediate interactions between plant and floral visitor community. They act as metabolic factories synthesizing and secreting large amounts of chemicals, such as terpenoids and flavonoids (Gershenzon *et al*., 1992; De Moraes *et al*., 1998; Duke *et al*., 2000; Huchelmann *et al*., 2017). These chemicals are frequently associated with protection against insects and pathogens (De Moraes *et al*., 1998; Huchelmann *et al*., 2017).

Currently, glandular trichomes (GTs) in crops and their wild related varieties are being studied to enhance ecological interactions between plants and insects, aiming to reduce agrochemical use in agriculture (Li *et al*., 2024). The most studied aspect is their repellent effect on herbivores. For instance, allelochemicals produced by GTs in tomato leaves and stems have been studied as resistance traits that affect herbivore settlement and growth, such as whiteflies, white spiders, and leaf miners (Riahi *et al*., 2023; Salazar-Mendoza *et al*., 2023). Conversely, research on their attractiveness to pollinators is still in its early stages, as observed in Salvia species (Giuliani *et al*., 2018). Nonetheless, there are studies on the attractive effect of many floral volatiles (Dötterl and Gershenzon, 2023).

In sunflower, Capitate Glandular Trichomes (CGTs) are found on the leaf surface and at the distal ends of apical anther appendages. The maturation of CGTs on apical anther appendages is associated with disc floret maturation, as the secretory phases of these glands are correlated with disc floret phenophases (Göpfert *et al*., 2005; Amrehn *et al*., 2014). Sunflower capitula exhibit a typical centripetal pattern of maturation by spiral phyllotaxis, with the peripheral florets being the first to open (Hladun and Alder, 2009). The anthesis of the disc florets occurs between R5 and R6 stages according to the Schneiter and Miller (1981) phenological scale, where R5.3 and R5.6 represent inflorescences with 30% and 60% of the disc florets in anthesis, respectively. The phenological progression of disc floret includes four phenophases: floret buds (E1), anthesis with anther dehiscence (E2), style elongation and stigma receptivity (E3), and post-pollination stage (E4) (Miller, 1987; Menéndez *et al*., 2022). Most sunflower capitula at different reproductive stages contain at least two disc flower phenophases within the receptacle.

Sunflower capitula are highly attractive to various floral visitors, both diurnal and nocturnal, due to their large size, UV reflectance patterns, high pollen and nectar production, and aroma code (Pham-Delegue *et al*., 1990; Seiler, 1997; Torreta *et al*., 2009; Todesco *et al*., 2022). Sunflowers genotypes can vary the quality of these attractants (Etievant *et al*., 1984; Pham-Delègue *et al*., 1990; Vear *et al*., 1990). In fact, cultivated sunflower varieties exhibit a higher number of CGTs on their apical anther appendages compared with the wild type *Helianthus annuus* L. (Prasifka et al., 2015). It has been suggested that CGTs may positively influence reproduction by repelling herbivorous insects and/or attracting pollinators (Junker and Blüthgen, 2010).

Nevertheless, the influence of trichomes on arthropod groups in sunflower has been understudied, and there is a limited information on arthropod behaviour under field conditions; most studies have been carried out under controlled laboratory conditions (Rogers *et al*., 1987; Duke *et al*., 2000; Prasifka *et al*., 2015; Chamer *et al*., 2020). The aim of this study was to experimentally evaluate whether the presence of anther-CGTs (comparing genotypes with high and low CGT densities per anther but with similar phenotypic characteristics) affects the behaviour of diurnal floral visitors (pollinators, herbivores and predators) under field conditions. We focused on diurnal visitors, as stigmas are highly receptive during the day, particularly around midday (Torreta *et al*., 2009). Furthermore, floral developmental stages (R5.3, R5.6, and R6), daytime range (morning, midday, and afternoon), and two flowering seasons, were considered as random variables in the data analysis to better understand the variations in CGTs-floral visitor interactions. We hypothesize that sunflower genotypes with a high density of anther's CGTs repel more herbivores and attract more pollinators than genotypes with a low density of CGTs, disregarding the reproductive stage of the capitulum, the daytime range, and the flowering season. Additionally, we expect the predator presence will decrease the abundance of the other arthropods on the capitula, regardless of CGT density, reproductive stage, the daytime range, and the flowering season.

Materials and methods

Plant material and growth conditions

Two sunflower inbred lines, P2015-1a and Rf975, were used in this experimental study. P2015-1a characterized by a low density of CGTs in its anthers (low-CGT), and Rf975 characterized by a high density of CGTs in its anthers (high-CGT) were developed and provided by the Sunflower Germplasm Bank of the Estación Experimental Agropecuaria (EEA) of the Instituto Nacional de Tecnología Agropecuaria (INTA) Pergamino, Argentina (supplemental material figure S1).

Ninety plants of each genotype were grown in two plots separated by 15 m in the Experimental Field Station of Universidad Nacional de Rosario, Santa Fe, Argentina (33°1'S 60°53'W). No pesticides were applied to sunflowers during the experiment to avoid effects on the behaviour of the floral visitors. Plants were watered by drip irrigation and weeds were manually controlled. Lateral inflorescences were cut to obtain one capitulum per plant in both genotypes to standardize experimental conditions for the interactions with insect-floral visitors. The experiment was repeated in two flowering seasons: 2021/2022 and 2022/2023.

Both lines bloom simultaneously. Several phenotypic traits that could influence insect attraction were measured including capitulum size (analysed using Student's t-test), plant height (analysed using Student's t-test), UV light reflectance (UV irradiated capitula, photographed in a dark chamber), and the number of pollen grains per floret (following the protocol described by Loste *et al*., 2018). Additionally, we quantified the number of disc florets at each phenophase (E1, E2, E3, E4; supplemental material figure S2) using an image analysis method (Ochogavía 2022) (figure S2) to evaluate putative differences between genotypes in the composition of disc floret phenophases. The experimental design was randomized with three replications, each replication consisting of one capitulum. Floret number per capitulum was analysed at each reproductive stage (R5.3; R5.6; and R6) using a two-way analysis of variance (ANOVA) with an F-test. Means were separated using Tukey's Honestly Significant Difference (HSD) test, with a significance level of $\alpha = 0.05$.

Floral arthropod assemblage

The abundance of floral visitors was surveyed at three daytime ranges (9:00-10:00, 12:00-13:00, and 17:00-18:00) throughout the flowering season (16-14 days per season, avoiding rainy days). Records of floral visitors (abundance and richness) were obtained by simultaneously observing four capitula for 10 minutes. Each day, three records were collected for each daytime range (morning, midday, and afternoon) and genotype (P2015-1a and Rf975) totalling 30 minutes of observation per genotype per day, while also registering the reproductive stage of the capitula (R5.3, R5.6, and R6; supplemental material figure S2).

Arthropods were collected, photographed, and classified using taxonomic keys (Morrone and Coscarón, 1998; Michener, 2007; Claps *et al*., 2008; Dalmazzo *et al*., 2020). The arthropods were categorized into three functional groups (table 1): Herbivores, Pollinators and Predators.

Table 1. Diurnal floral visitors observed on inflorescences of the two sunflower genotypes during the evaluated seasons.

Group	Order	Stage	Family	Species	Req.	Visits low-CGT	high-CGT
Herbivores	Coleoptera	adult	Melyridae	*Astylus atromaculatus* (Blanchard)	abef	1478	624
	Diptera	adult	Agromyzidae	*Melanogromyza minimoides* Spencer	df	92	100
	Coleoptera	adult	Chrysomelidae	*Diabrotica speciosa* (Germar)	abef	23	55
	Diptera	adult			cdf	23	76
	Coleoptera	adult	Chrysomelidae	*Spintherophyta* sp.	e	29	30
	Hemiptera	adult			e	14	15
	Hymenoptera	adult	Formicidae		e	17	4
	Coleoptera	adult	Chrysomelidae		e	2	17
	Hemiptera	adult	Cicadellidae	*Empoasca kraemeri* Ross et Moore	e	4	5
	Coleoptera	adult	Curculionidae		e	1	0
Pollinators	Hymenoptera	adult	Apidae	*Apis mellifera* L.	abcd	711	1069
	Hymenoptera	adult	Apidae	*Melissodes* sp.	abcd	78	57
	Hymenoptera	adult	Halictidae		abcd	94	51
	Hymenoptera	adult	Apidae	*Bombus pauloensis* Friese	abcd	44	14
	Lepidoptera	adult	Hesperiidae		abcd	7	5
	Diptera	adult	Syrphidae		acd	3	6
	Hymenoptera	adult	Megachilidae		abcd	2	0
	Hymenoptera	adult	Vespidae	*Polybia occidentalis* Olivier	acd	1	0
	Hymenoptera	adult	Vespidae	*Brachygastra* sp.	acd	1	0
Predators	Araneae	-	Thomisidae		g	34	51
	Araneae	-	Salticidae		g	12	11
	Coleoptera	adult	Coccinellidae	*Eriopis connexa* (Germar)	g	7	6
	Coleoptera	larva	Coccinellidae	*Harmonia axyridis* (Pallas)	g	5	1
	Hemiptera	adult	Reduviidae	*Cosmoclopius nigroannulatus* Stal	g	0	6

Req. = Requirements: a) have the size and pilosity to carry pollen, b) carry pollen on their bodies (we observed this feature in the field), c) fly frequently among capitula (remained on the flower shorter than the full observation period - 10 minutes), d) feed from nectar and pollen without damaging the flower structures such as stigmas and anthers, e) feed on nectar or pollen or other floral structures damaging floral structures, f) and/or do not fly frequently between capitula, g) the predatory arthropods that occupy flowers as hunting platforms.

The criteria for assigning floral visitors to the functional groups were: a) possessing the size and pilosity necessary to carry pollen, b) carrying pollen on their bodies, c) flying frequently among capitula (remaining on the flower for less than 10 minutes), d) feeding on nectar and pollen without damaging the floral structures, e) feeding on nectar or pollen or other floral structures while damaging floral structures (such as beetles; Van den Berg *et al.*, 2008), f) remaining on the flower longer than 10 minutes, and g) using flowers as hunting platforms. Arthropods that met at least three of the requirements from 'a' to 'd' were classified as Pollinators; those that met the requirements in 'e' or 'f' were classified as Herbivores, and those that met the 'g' requirement were classified as Predators.

Statistical analysis of arthropod abundance

All analyses were performed using R statistical software version 4.2.1 (R Core Team, 2022). The effects of genotype on floral visitor behaviour were examined using Generalized Linear Mixed Models (GLMM) with genotype as the fixed variable comprising two levels: high-CGTs and low-CGTs and the abundance of each arthropod group as the response variable. Abundances (count data) were modelled using the Negative Binomial distribution, which proved to be more suitable than the Poisson distribution. This was confirmed through a graphical comparison of both fitted distributions using the fitdistrplus package (Delignette-Muller and Dutang,

2015) and the analysis of residuals. The variables daytime range (with levels: morning, midday, and afternoon), reproductive stage (with levels: R5.3, R5.6, and R6), and flowering season (with levels: 2021 and 2022) were used as random variables with independent (crossed) random intercepts for each level of each variable. Furthermore, to investigate potential interactions among arthropod groups on the flowers, we also conducted GLMMs. We used the presence of one arthropod group (either herbivores or pollinators) as the response variable with a Binomial distribution, while taking into account the predator presence as the fixed variable. This was because 81% of predator visits had a value of 1 per capitulum per ten minutes. The random variables were the same as in the previous GLM involving independent random intercepts for each level of daytime range, reproductive stage and flowering season.

GLMMs were performed utilizing the glmer.nb function (for Negative Binomial Distribution) and the glmer function (for Binomial Distribution) from the lme4 package (Bates *et al.*, 2015), fitted through maximum likelihood estimation. The significance of including the fixed effect in each model was assessed using a chi-square test (likelihood ratio test) between two GLMM: one with the fixed variable and another without the fixed variable (null model) (supplemental material table S1). Plots of the best-fitted models were created using the ggeffects (Lüdecke, 2018) and ggplot2 (Wickham, 2016) R packages.

Results

Phenotypic studies of the plant genotypes

The two sunflower inbred lines exhibited comparable biometric and anatomical variables, including main capitulum sizes (mean ± SE: 13.66 cm ± 0.72 and 13.40 cm ± 0.49 for P2015-1a and Rf975, respectively; t = −0.299, d.f. = 17, P = 0.384), plant height (98.11 cm ± 3 and 99.9 cm ± 2.2 for P2015-1a and Rf975, respectively; t = 0.47; d.f. = 17, P = 0.642). The capitula UV reflectance was compared between genotypes but no differences were detected in the ligulate florets UV pigmentation pattern (supplemental material figure S3). Additionally, no differences in the number of pollen grains per floret were detected between genotypes (25806 ± 1217 and 26411 ± 949 for Rf975 and P2015-1a, respectively).

The disc floret number of each phenophase was compared between genotypes at different capitula developmental stages. No significant effect of the genotype was detected at R5.3 (ANOVA: F = 0.17, d.f. = 1, P = 0.765), R 5.6 (ANOVA: F = 0.29, d.f. = 1, P = 0.5888) and R6 developmental stage (ANOVA: F = 0.21, d.f. = 1, P = 0.6432). However, a significant effect of phenophases on the floret number per capitulum was detected in both genotypes at R5.3 (ANOVA: F = 18.04, d.f. = 3, P = 2.411×10^{-06}), R5.6 (ANOVA: F = 17.52, d.f. = 3, P = 1.62×10^{-06}) and R6 developmental stage (ANOVA: F = 39.06, d.f. = 3, P = 5.825×10^{-10}). No significant effect of the genotype was detected at R5.3 (ANOVA: F = 0.17, d.f. = 1, P = 0.765) R 5.6 (ANOVA: F = 0.29, d.f. = 1, P = 0.5888) and R6 developmental stage (ANOVA: F = 0.21, d.f. = 1, P = 0.6432).

Floral insect assemblage

A total of 4,885 arthropods were recorded on the capitulum during 30 days across two flowering seasons. Twenty-four diurnal floral visitors that interacted with the crop were identified (table 1) and then classified into 6 orders, 15 families, and 13 genera. Ultimately, the floral visitors were categorized into three functional groups: 9 as pollinators, 10 as herbivores, and 5 as predators (table 1). The richness of flower-visiting arthropods varied between sunflower genotypes: 23 floral visitors in the low-CGT genotype and 19 in the high-CGT one, although these differences were primarily among less abundant species (fewer than 2 individuals) (table 1). Herbivores were the most abundant arthropod group. The main species recorded for the different functional groups were: *Astylus atromaculatus* (Blanchard) (Coleoptera Melyridae), *Apis mellifera* L. (Hymenoptera Apidae), and Thomisidae family for herbivores, pollinators and predators, respectively (figure 1, table 1).

The fixed variables significantly influenced herbivore and pollinator abundance (GLMM Negative Binomial, χ^2 = 33.759, d.f. = 1, P = 6.238×10^{-9} and GLMM Negative Binomial, χ^2 = 4.244, d.f. = 1, P = 0.039 respectively) (supplemental material table S1). The genotype with a high density of CGT per anther showed lower herbivore abundance (GLMM Negative Binomial, mean [CI 95%]: 2.90 [1.19, 7.04] *vs* 1.86 [0.76, 4.53]; Z = 5.938, d.f. = 617, P = 2.88×10^{-9}) and higher values of pollinator abundance (GLMM Negative Binomial, mean [CI 95%]: 2.14 [1.20, 3.82] *vs* 1.94 [1.09, 3.44]; Z =-2.067, d.f. = 742, P = 0.038, respectively) (figure 2). Capitula from the high-CGT genotype had 1.04 fewer herbivore individuals and 0.20 more pollinator individuals per 10 minutes compared to the low-CGT genotype (figure 2). The presence of a high density of CGT did not show trends with predator abundance (GLMM Negative Binomial, Z = −0.397, d.f. = 118, P = 0.691) (figure 2).

Interestingly, the presence of predators in the capitulum significantly decreased pollinator abundance by 21% (GLMM Binomial, Z = −3.952, d.f. = 1277, P = 7.76×10^{-5}) but did not significantly affect herbivore presence (GLMM Binomial, Z = −0.435, d.f. = 1277, P = 0.664) (figure 3).

The random variable that accounted for the most variation in herbivores was the flowering season (2021 and 2022) (figure 4, supplemental material table S2), with the 2021 season exhibiting the highest herbivore abundance (figure 4C). For pollinators, the random variable that explained the greatest variation was the daytime range (morning, midday, and afternoon) (figure 4, supplemental material table S3), with the highest pollinator abundance in the morning and the lowest in the afternoon (figure 4D). Additionally, the capitula developmental stage

Figure 1. The most abundant sunflower floral visitors in each arthropod group. **A.** *A. atromaculatus* (herbivore). **B.** *A. mellifera* (pollinator). The arrow highlights the pollen carried on the bee's pollen basket. **C.** *Thomisidae* sp. (cryptic visitor) with a prey (Diptera) (see arrow). Bars: 1 cm.
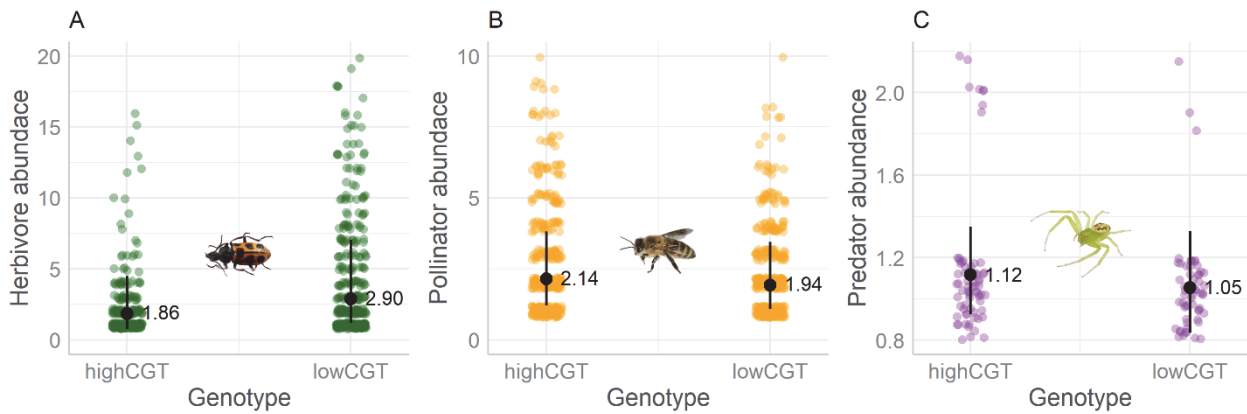
Figure 2. The abundance of flower visitors per capitulum in 10 minutes predicted by the generalized linear mixed models with negative binomial distribution. The means are represented by black points (the predicted value is labelled next to the point) and the lines represent the 95% confidence intervals. **A.** Values for herbivore abundance. **B.** Values for pollinator abundance. **C.** Values for predator abundance. The genotype with a high density of capitate glandular trichomes reduces significantly the herbivore abundance (GLMM Negative Binomial, $Z = 5.938$, d.f. $= 617$, $P = 2.88 \times 10^{-9}$) and increases the pollinator abundance (GLMM Negative Binomial, $Z = -2.067$, d.f. $= 742$, $P = 0.038$). Abbreviations: CGT: capitate glandular trichomes, highCGT: Rf975 genotype with a high density of CGTs in its anthers, lowCGT: P2015-1a genotype with a low density of CGTs in its anthers.
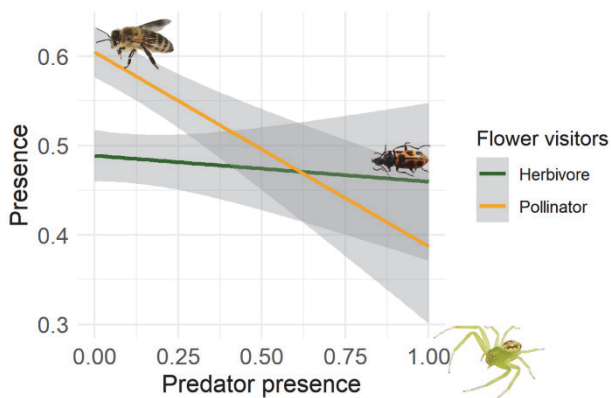

Figure 3. The probability of the presence of the herbivore or the pollinator groups per capitulum in 10 minutes predicted by the model with the increase of the probability of the predator presence as the fixed variable. The presence of pollinators decreases significantly in the predator presence (GLMM Binomial, $Z = -3.952$, d.f. $= 1277$, $P = 7.76 \times 10^{-5}$).

was the second random variable affecting both more abundant groups (herbivores and pollinators). Herbivore abundance increased at the R6 reproductive stage in the low-CGT genotype, while pollinator abundance increased at the R5.3 reproductive stage in the high-CGT genotype (figure 4). In other words, the pollinator abundance decreased with the number of disc florets at postanthesis (E4 phenophase, supplemental material figure S4) in both genotypes, raising the minimum of pollinators at R6. Both R5.3 and R6 stages had a similar number of E3 disc florets (anthesis), but the R6 capitula had twice the number of E4 disc florets (supplemental material figure S4). Predator abundance was not significantly affected by the random variables.

Discussion and conclusions

Our results suggest that CGTs on sunflower anthers may have a repellent effect on herbivores under field conditions. In addition, for the first time, we propose a potential attractive effect on bee abundance, a notion previously hinted at in some studies (Giuliani *et al.*, 2018), which justifies the need for broader, more extensive investigations into the effects on arthropods.

Leaf glandular trichomes have long been associated with resistance to herbivores, such as beetles in potatoes (Flanders *et al.*, 1992), aphids in tomatoes, Hemiptera in alfalfa (Ranger and Hower, 2001), and Lepidoptera larvae in Cucurbitaceae (Kaur and Kariyat, 2023). While the study of trichomes and their role in herbivore deterrence have gained attention in recent years, research on floral trichomes and their potential for attracting pollinators remains relatively limited. Some studies have hinted that floral trichomes might produce fragrances that attract pollinators (Giuliani *et al.*, 2018; Stpiczyńska *et al.*, 2018). However, comprehensive research measuring the differential abundance between genotypes with high and low trichome density in flowers is lacking. Furthermore, chemical analysis of the volatile compounds emitted by sunflower CGTs is necessary to relate them to known attractants for pollinators, such as terpenoids or fatty acid-derived compounds, as has been detected in other species (Grajales-Conesa *et al.*, 2011; Huchelmann *et al.*, 2017).

Previous research on sunflowers has demonstrated the harmful effects of different compounds emitted by CGTs on Lepidoptera larvae, specifically *Homoeosoma electellum* Hulst (Lepidoptera Pyralidae), following the ingestion of these compounds by butterflies under controlled laboratory conditions (Prasifka, 2014). However, no differences were found in seed damage caused by lepidopteran larvae -*Cochylis hospes* Walsingham (Lepidoptera Tortricidae)- between genotypes with high and low CGT
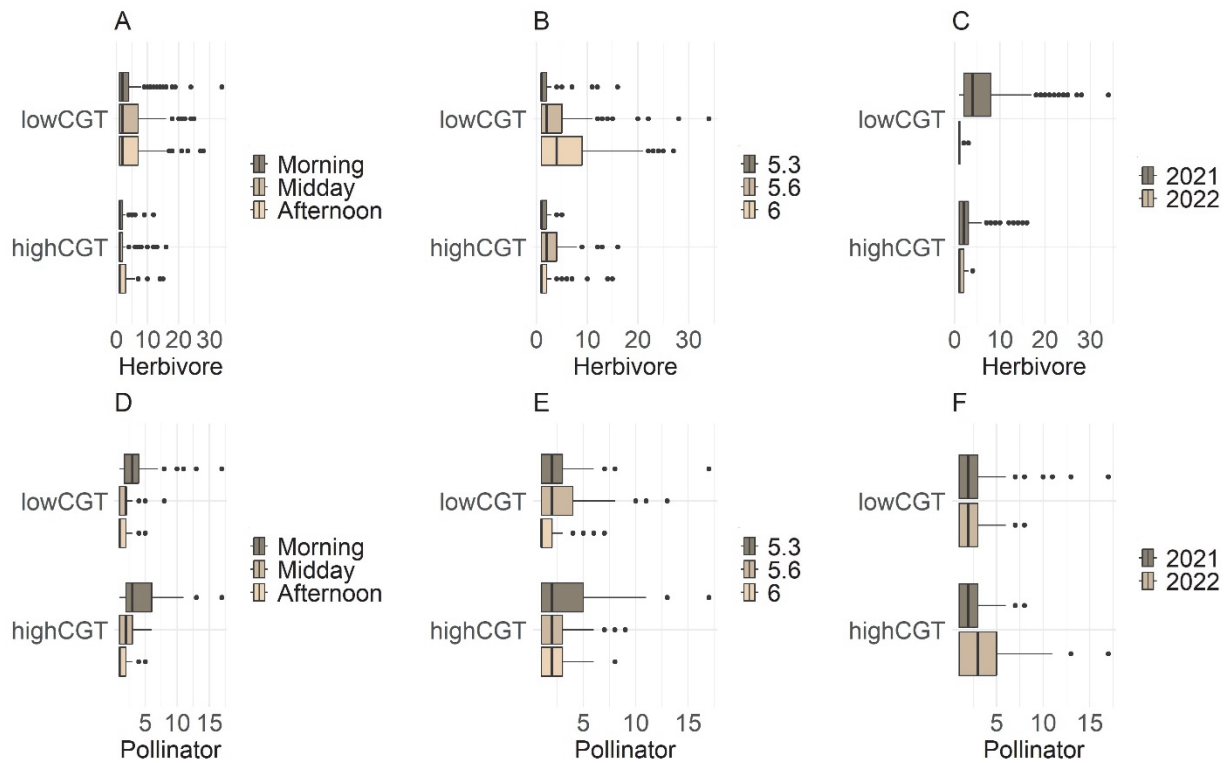
Figure 4. The random variables in the herbivore abundance models (**A**, **B**, **C**) and pollinator abundance models (**D**, **E**, **F**) per genotype (refer to supplemental material table S2, table S3 and figure S4). These variables include the reproductive stage (with levels: 5.3, 5.6, 6), the daytime range (with levels: morning, midday, and afternoon) and flowering season (with levels: year 2021, year 2022).

density were observed in field conditions (Prasifka and Hulke, 2020). Unlike the findings of Prasifka and Hulke (2020), our study did not detect lepidopteran larvae on sunflower inflorescences during the flowering period, as they are not common floral herbivores in the region (Casuso *et al.*, 2017). The novelty of our approach was analysing the entire arthropod community interacting with sunflower inflorescences throughout the complete flowering period, which included potential interactions among arthropod species. General trends related to anther-CGT density and arthropod abundance may also be influenced by other variables, such as flowering seasons, daytime range, and reproductive stages. For instance, the herbivore abundance showed variability across seasons, underscoring the importance of conducting ecological studies over multiple years and considering these variations for a better understanding of the relationships between flowers and animal visitors. Inter-seasonal variations might be attributed to different biotic or abiotic factors. Biotic factors, related to the herbivore population or larval survival, can influence beetle populations directly. Abiotic environmental factors, like temperature and moisture, can also play a role, particularly in affecting beetle populations without significantly impacting bee populations (Birch, 1953). Pollinators' abundance was also affected by random factors, primarily by the time of the day. Bees typically have a peak activity in the morning, as has reported Polatto *et al.* (2014). In the case of sunflower, the increased bee abundance during the morning in both genotypes could be directly related to the

availability of pollen and nectar, which are accessible until the early hours before noon (Grandinetti, 2022). The reproductive stage of the capitulum was the second random factor contributing to the explained variability for herbivores and pollinators. Pollinator abundance tended to rise during the early reproductive stages, while herbivore abundance increased in advanced stages. The pollinators behaviour may be explained by the "unpredictable rewards hypothesis" where pollinators might seek to avoid reward variability within the same plant (or capitulum, in this case), potentially leading to reduced visitation rates (Kacelnik and Bateson, 1996; Grandinetti, 2022). In herbivores, the feeding habits of *A. atromeculatus*, particularly their consumption of various floral parts beyond pollen, may be more pronounced in advanced reproductive stages (Van den Berg *et al.*, 2008). *Melanogromyza minimoides* Spencer (Diptera Agromyzidae), another key herbivore, tends to become more abundant in later developmental stages, likely linked to its reproductive cycle that involves egg-laying among flowers and larval feeding on developing fruits (Zerbino, 2001).

The presence of spiders on plants has been associated with glandular trichomes functioning as an anti-herbivore defence mechanism. In such cases, the presence of more predators and fewer herbivores is typically observed when trichomes are present (Romero *et al.*, 2008). This trend was slightly observed in our study as well; however, the predator presence was significantly associated with a lower pollinator presence rather than the herbivore presence. The most abundant registered predator was the

crab spider (Thomisidae), which primarily feeds on pollinators such as bees and certain flies (see figure 1), rather than beetles (Morse, 1981). It has been reported that *A. mellifera* perceives danger in flowers inhabited by spiders and avoids visiting them (Dukas and Morse, 2003; Gonzálvez *et al.*, 2013).

Since trichomes are a heritable trait and can be eventually used in plant classification (Spring, 2000), our findings support the role attributed to sunflower anther CGTs by previous researchers (Göpfert *et al.*, 2005; Amrehn *et al.*, 2014; Prasifka and Bazzalo, 2016), as sunflower anther CGTs may deter herbivorous arthropods under field conditions. Additionally, we propose a potentially attractive effect on bee abundance, a concept previously suggested by some studies (Giuliani *et al.*, 2018). It is desirable to expand research into morpho-anatomical variations beyond just plant-arthropod interactions. Such research could encompass the comparison of genotype variations in CGT density across wider spatial scales, with the combination of different genotypic lines in large plots, and the scrutiny of interactions with diverse arthropod groups under varying environmental conditions. Understanding these complex interactions could inform novel crop breeding approaches to improve yield and pest management.

Acknowledgements

References

AMREHN E., HELLER A., SPRING O., 2014.- Capitate glandular trichomes of *Helianthus annuus* (Asteraceae): ultrastructure and cytological development.- *Protoplasma*, 251: 161-167.

BATES D., MÄCHLER M., BOLKER B., WALKER S., 2015.- Fitting Linear Mixed-Effects Models using lme4.- *Journal of Statistical Software*, 67 (1): 1-48.

BIRCH L. C., 1953.- Experimental background to the study of the distribution and abundance of insects: I. the influence of temperature, moisture and food on the innate capacity for increase of three grain beetles.- *Ecology*, 34: 698-711.

CASUSO M., SIMON C., CAVALIERI J., PÉREZ G., 2017.- *Guia practica para la identificacion de plagas del cultivo de giraso.*- Instituto Nacional de Tecnología Agropecuaria, Las breñas, Chaco, Argentina.

CHAMER A. M., MEDAN D., MONTALDO N. H., MANTESE A. I., DEVOTO M., 2020.- Visitantes florales del girasol (*Helianthus annuus*) y su vegetación acompañante en la Pampa Interior.- *Ecología Austral*, 30: 228-238.

CLAPS L., ROIG S., DEBANDI G., 2008.- *Biodiversidad de artrópodos argentinos volumen II, 1a ed adaptada.*- Universidad Nacional de Tucumán, San Miguel Tucumán, Argentina.

DALMAZZO M., VAQUERO R. A., GONZÁLES ALSIMA A. R., DEBANDI G., 2020.- Hymenoptera: Halictidae, In: *Artrópodos argentinos volumen 4* (ROIG-JUŃENT S., CLAPS L. E., MORRONE J. J., Eds).- Universidad Nacional de Tucumán, Tucumán, Argentina.

DELIGNETTE-MULLER M. L., DUTANG C., 2015.- Fitdistrplus: an R package for fitting distributions.- *Journal of Statistical Software*, 64: 1-34.

DE MORAES C. M., LEWIS W. J., PARÉ P. W., ALBORN H. T., TUMLINSON J. H., 1998.- Herbivore-infested plants selectively attract parasitoids.- *Nature*, 393: 570-573.

DÖTTERL S., GERSHENZON J., 2023.- Chemistry, biosynthesis and biology of floral volatiles: roles in pollination and other functions.- *Natural Product Reports*, 40: 1901-1937.

DUKAS R., MORSE D. H., 2003.- Crab spiders affect flower visitation by bees.- *Oikos*, 101: 157-163.

DUKE S., CANEL C., RIMANDO A., TELLE M. R., DUKE M.V., PAUL R. N., 2000.- Current and potential exploitation of plant glandular trichome productivity.- *Advances in Botanical Research*, 31: 121-151.

ETIEVANT P. X., AZAR M., PHAM-DELEGUE M. H., MASSON C. J., 1984.- Isolation and identification of volatile constituents of sunflowers (*Helianthus annuus* L.).- *Journal of Agricultural and Food Chemistry*, 32: 503-509.

FLANDERS K. L., HAWKES J. G., RADCLIFFE E. B., LAUER F. I., 1992.- Insect resistance in potatoes: sources, evolutionary relationships, morphological and chemical defenses, and ecogeographical associations.- *Euphytica*, 61: 83-111.

GERSHENZON J., MCCASKILL D., RAJAONARIVONY J. I. M., ALBORN H. T., TUMLINSON J. H., 1992.- Isolation of secretory cells from plant glandular trichomes and their use in biosynthetic studies of monoterpenes and other gland products.- *Analytical Biochemistry*, 200: 130-138.

GIULIANI C., ASCRIZZI R., LUPI D., TASSERA G., SANTAGOSTINI L., GIOVANETTI M., FLAMINI G., FICO G., 2018.- *Salvia verticillata*: linking glandular trichomes, volatiles and pollinators.- *Phytochemistry*, 155: 53-60.

GONZÁLVEZ F. G., SANTAMARÍA L., CORLETT R. T., RODRÍGUEZ-GIRONÉS M. A., 2013.- Flowers attract weaver ants that deter less effective pollinators.- *Journal of Ecology*, 101: 78-85.

GÖPFERT J., HEIL N., CONRAD J., SPRING O., 2005.- Cytological development and sesquiterpene lactone secretion in capitate glandular trichomes of sunflower.- *Plant Biology*, 7: 148-155.

GRAJALES-CONESA J., MELÉNDEZ-RAMÍREZ V., CRUZ-LÓPEZ L., 2011.- Floral scents and their interaction with insect pollinators.- *Revista Mexicana de Biodiversidad*, 82: 1356-1367.

GRANDINETTI G., 2022.- Incidencia de las recompensas florales en la producción de semilla híbrida de girasol. 147 p., *Doctoral thesis*, Universidad Nacional del Sur, Bahía Blanca, Argentina. [online] URL: https://repositoriodigital.uns.edu.ar/handle/123456789/6157

HLADUN KRISTEN R., ALDER L. S., 2009.- Influence of leaf herbivory, root herbivory, and pollination on plant performance in *Cucurbita moschata.*- *Ecological Entomology*, 34: 144-152.

HUCHELMANN A., BOUTRY M., HACHEZ C., 2017.- Plant glandular trichomes: natural cell factories of high biotechnological interest.- *Plant Physiology*, 175: 6-22.

INGS T. C., CHITTKA L., 2009.- Predator crypsis enhances behaviourally mediated indirect effects on plants by altering bumblebee foraging preferences.- *Proceedings of the Royal Society B: Biological Sciences*, 276: 2031-2036.

IPBES, 2016.- *The assessment report of the intergovernmental science-policy platform on biodiversity and ecosystem services on pollinators, pollination and food production.*- The Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany. [online] URL: https://files.ipbes.net/ipbes-web-prod-public-files/downloads/pdf/2016.methodological_assessment_report_scenarios_models.pdf

JUNKER R. R., BLÜTHGEN N., 2010.- Floral scents repel facultative flower visitors, but attract obligate ones.- *Annals of Botany*, 105: 777-782.

KACELNIKK A., BATESON M., 1996.- Risky theories - the effects of variance on foraging decisions.- *American Zoologist*, 36: 402-434.

KAUR I., KARIYAT R., 2023.- Trichomes mediate plant-herbivore interactions in two Cucurbitaceae species through pre- and post-ingestive ways.- *Journal of Pest Science*, 96: 1077-1089.

LI J., HU H., FU H., LI J., ZENG T., LI J., WANG M., JONGSMA M. A., WANG C., 2024.- Exploring the co-operativity of secretory structures for defense and pollination in flowering plants.- *Planta*, 259: 41.

LOSTE N., NESTARES G. M., FELITTI S. A., OCHOGAVÍA A. C., 2022.- Gene expression profiling by cDNA-AFLP reveals potential candidate genes for imazapyr-induced male sterility in sunflower.- *Crop Science*, 62: 301-314.

LÜDECKE D., 2018.- ggeffects: tidy data frames of marginal effects from regression models.- *Journal of Open Source Software*, 3 (26): 772.

MAZZEI M. P., VESPRINI J. L., GALETTO L., 2020.- Visitantes florales no polinizadores en plantas del género *Cucurbita* y su relación con la presencia de abejas polinizadoras.- *Acta Agronómica*, 69: 256-265.

MENÉNDEZ A., BIANCHI M. B., PICARDI L. A., NESTARES G. M., OCHOGAVÍA A. C., 2022.- Developmental variability in advanced megagametogenesis and embryogenesis in cultivars of sunflower.- *Crop Science*, 62: 1024-1036.

MICHENER C. D., 2007.- *The bees of the world, 2nd ed.*- The Johns Hopkins University Press, Baltimore, USA.

MILLER J. F., 1987.- Sunflower, pp. 626-668. In: *Principles of cultivar development, volume 2* (FEHR W., Ed.).- Macmillan, New York, USA.

MORRONE J. J., COSCARÓN S., 1998.- *Biodiversidad de artrópodos argentinos una perspectiva biotaxonómica*.- Ediciones Sur, La Plata, Argentina.

MORSE D. H., 1981.- Prey capture by the crab spider *Misumena vatia* (Clerck) (Thomisidae) on three common native flowers.- *American Midland Naturalist*, 105: 358.

OCHOGAVÍA A. C., 2022.- Quantifying the reproductive progression of sunflower using FIJI (Image J).- *MethodsX*, 9: 101879.

PHAM-DELEGUE M. H., ETIEVANT P., GUICHARD E., MARILLEAU R., DOUAULT P., CHAUFFAILLE J., MASSON C., 1990.- Chemicals involved in honeybee-sunflower relationship.- *Journal of Chemical Ecology*, 16: 3053-3065.

POLATTO L. P., CHAUD-NETTO J., ALVES-JUNIOR V. V., 2014.- Influence of abiotic factors and floral resource availability on daily foraging activity of bees.- *Journal of Insect Behaviour*, 27: 593-612.

PRASIFKA J. R., 2014.- Variation in the number of capitate glandular trichomes in wild and cultivated sunflower germplasm and its potential for use in host plant resistance.- *Plant Genetic Resources*, 13: 68-74.

PRASIFKA J. R., BAZZALO M. E., 2016.- Susceptibility of sunflower inbreds to *Melanagromyza minimoides* in Argentina and potential association with plant resistance traits.- *International Journal of Pest Management*, 62: 105-110.

PRASIFKA J. R., HULKE B. S., 2020.- Capitate glandular trichomes fail to provide significant resistance to banded sunflower moth (Lepidoptera: Tortricidae).- *Environmental Entomology*, 49: 444-448.

PRASIFKA J. R., SPRING O., CONRAD J., COOK L. W., PALMQUIST D. E., FOLEY M. E., 2015.- Sesquiterpene lactone composition of wild and cultivated sunflowers and biological activity against an insect pest.- *Journal of Agricultural and Food Chemistry*, 63: 4042-4049.

RAHIMI E., BARGHJELVEH S., DONG P., 2021.- Using the Lonsdorf model for estimating habitat loss and fragmentation effects on pollination service.- *Ecological Processes*, 10: 22.

RANGER C. M., HOWER A. A., 2001.- Role of the glandular trichomes in resistance of perennial alfalfa to the potato leafhopper (Homoptera: Cicadellidae).- *Jorunal of Economic Entomology*, 94: 950-957.

R CORE TEAM, 2022.- *R: a language and environment for statistical computing*.- R Foundation for Statistical Computing, Vienna, Austria.

ROGERS C. E., GERSHENZON J., OHNO N., MABRY T. J., STIPANOVIC R. D., KREITNER G. L., 1987.- Terpenes of wild sunflowers (*Helianthus*): an effective mechanism against seed predation by larvae of the sunflower moth, *Homoeosoma electellum* (Lepidoptera: Pyralidae).- *Environmental Entomology*, 16: 586-592.

ROMERO G. Q., SOUZA J. C., VASCONCELLOS-NETO J., 2008.- Anti-herbivore protection by mutualistic spiders and the role of plant glandular trichomes.- *Ecology*, 89: 3105-3115.

SALAZAR-MENDOZA P., MAGALHÃES D. M., LOURENÇÃO A. L., BENTO J. M. S., 2023.- Differential defensive and nutritional traits among cultivated tomato and its wild relatives shape their interactions with a specialist herbivore.- *Planta*, 257: 76.

SCHNEITER A. A., MILLER J. F., 1981.- Description of sunflower growth stages 1.- *Crop Science*, 21: 901-903.

SEILER G. J., 1997.- Anatomy and morphology of sunflower, pp. 100-105. In: *Sunflower technology and production* (SCHNEITER A., Ed.).- American Society of Agronomy, Madison, USA.

SPRING O., 2000.- Chemotaxonomy based on metabolites from glandular trichomes.- *Advances in Botanical Research*, 31: 153-174.

STPICZYŃSKA M., PŁACHNO B. J., DAVIES K. L., 2018.- Nectar and oleiferous trichomes as floral attractants in *Bulbophyllum saltatorium* Lindl. (Orchidaceae).- *Protoplasma*, 255: 565-574.

TODESCO M., BERCOVICH N., KIM A., IMEROVSKI I., OWENS G. L., DORADO RUIZ Ó., HOLALU S. V., MADILAO L. L., JAHANI M., LÉGARÉ J. S., BLACKMAN B. K., RIESEBERG L. H., 2022.- Genetic basis and dual adaptive role of floral pigmentation in sunflowers.- *eLife*, 11: e72072.

TORRES C., GALETTO L., 2002.- Are nectar sugar composition and corolla tube length related to the diversity of insects that visit asteraceae flowers?- *Plant Biology*, 4: 360-366.

TORRES C., GALETTO L., 2007.- Style morphological diversity of some Asteraceae species from Argentina: systematic and functional implications.- *Journal of Plant Research*, 120: 359-364.

TORRES C., GALETTO L., 2008.- Relevance of pollinators in the reproduction of Asteraceae of central Argentina.- *Acta Botánica Venezuelica*, 31: 473-494.

TORRES C., GALETTO L., 2011.- Flowering phenology of co-occurring Asteraceae: a matter of climate, ecological interactions, plant attributes or of evolutionary relationships among species?- *Organisms Diversity & Evolution*, 11: 9-19.

TORRETTA J. P., NAVARRO F., MEDAN D., 2009. -Visitantes florales nocturnos del girasol (*Helianthus annuus*, Asterales: Asteraceae) en la Argentina.- *Revista de la Sociedad Entomológica Argentina*, 68: 339-350.

VAN DEN BERG J., TORTO B., PICKETT J. A., SMART L. E., WADHAMS L. J., WOODCOCK C. M., 2008.- Influence of visual and olfactory cues on field trapping of the pollen beetle, *Astylus atromaculatus* (Col.: Melyridae).- *Journal of Applied Entomology*, 132: 490-496.

VEAR F., PHAM-DELEGUE M., TOURVIEILLE DE LABROUHE D. D., MARILLEAU R., LOUBLIER Y., LE METAYER M., DOUAULT P., PHILIPPON J., 1990.- Genetical studies of nectar and pollen production in sunflower.- *Agronomie*, 10: 219-231.

WÄCKERS F. L., ROMEIS J., VAN RIJN P., 2007.- Nectar and pollen feeding by insect herbivores and implications for multitrophic interactions.- *Annual Review of Entomology*, 52: 301-323.

WICKHAM H., 2016.- *ggplot2: elegant graphics for data analysis*.- Springer, New York, USA.

ZERBINO M. S., 2001.- Mosquita del capítulo del girasol *Melanagromyza minimoides*, nueva plaga.- *Agrociencia*, 5 (1): 90-91.

Authors' addresses: Mariana Paola MAZZEI (corresponding author: marianapaola.mazzei@unibo.it), Department of Agricultural and Food Sciences, *Alma Mater Studiorum* Università di Bologna, viale G. Fanin 42, 40127 Bologna, Italy; Leonardo GALETTO, Instituto Multidisciplinario de Biología Vegetal, IMBIV-CONICET-Universidad Nacional de Córdoba, Vélez Sarsfield 1666, 5016 Córdoba, Argentina; David BALABAN, Graciela María NESTARES, Ana Claudia OCHOGAVÍA, Instituto de Investigaciones en Ciencias Agrarias de Rosario, IICAR-CONICET-UNR, Campo Experimental "J. F. Villarino", Zavalla 2125, Santa Fe, Argentina.

(Supplemental material available at http://www.bulletinofinsectology.org/Suppl/vol77-2024-291-298mazzei-suppl.pdf)