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

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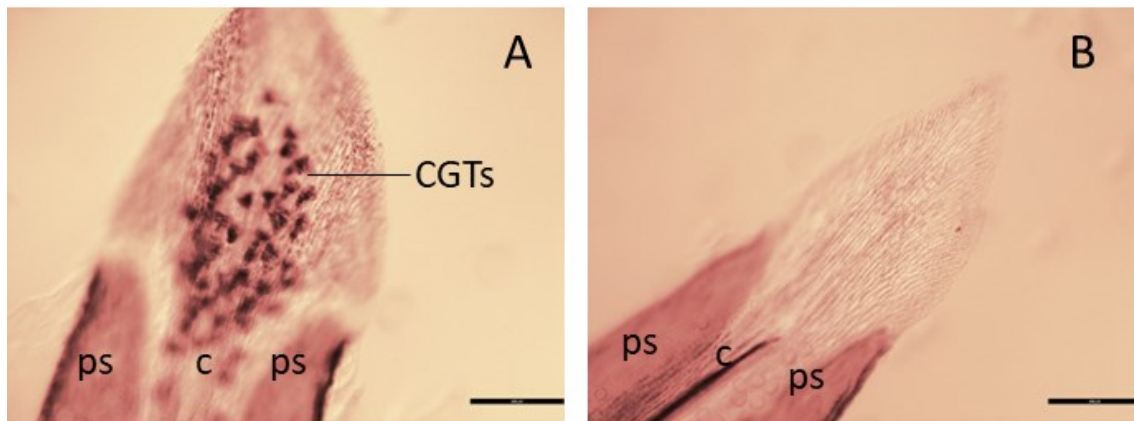
Index of supplemental materials:

Figure S1. Images of distal sections of sunflower anthers, obtained by Differential Interference Contrast (DIC) from clarified E3 disc flowers. **A.** Rf975 genotype. A high density of Capitate Glandular Trichomes (CGTs) is visualized in the distal end of apical anther appendages, on the consecutive tissue (c) (high-CGT genotype). **B.** P2015-1a with no CGTs in the distal end of apical anther appendages (low-CGT genotype). ps: pollen sac. Bars: 100 µm

Figure S2. Representative reproductive stages of the P2015-1a sunflower capitulum (R5.3, R5.6, and R6) and phenological scales of disc flowers (E1-E4). The reproductive stages are based on the scale described by Schneiter and Miller (1981). The R5 stage is the start of the anthesis of disc flowers, initiating from the peripheral region of the receptacle. This stage is sub-divided into sub-stages depending on the percentage of the capitulum that is at anthesis. For example, if 50% of the capitulum has achieved anthesis, the phenological stage of the capitulum is R5.5. The phases of tubulous flowers at different phenological stages are: E1 (flower buds are visualized), E2 (anthers emerge and a dark structure can be visualized over the corolla), E3 (two yellow stigmatic branches emerge and the pollen release), E4 (senescence is initiated and the stigmatic branches wilt and curve); and their proportions vary among each Schneiter and Miller's sub-stages.

SCHNEITER A. A., MILLER J. F., 1981.- Description of sunflower growth stages 1.- *Crop Science*, 21: 901-903. https://doi.org/10.2135/cropsci1981.0011183X002100060024x
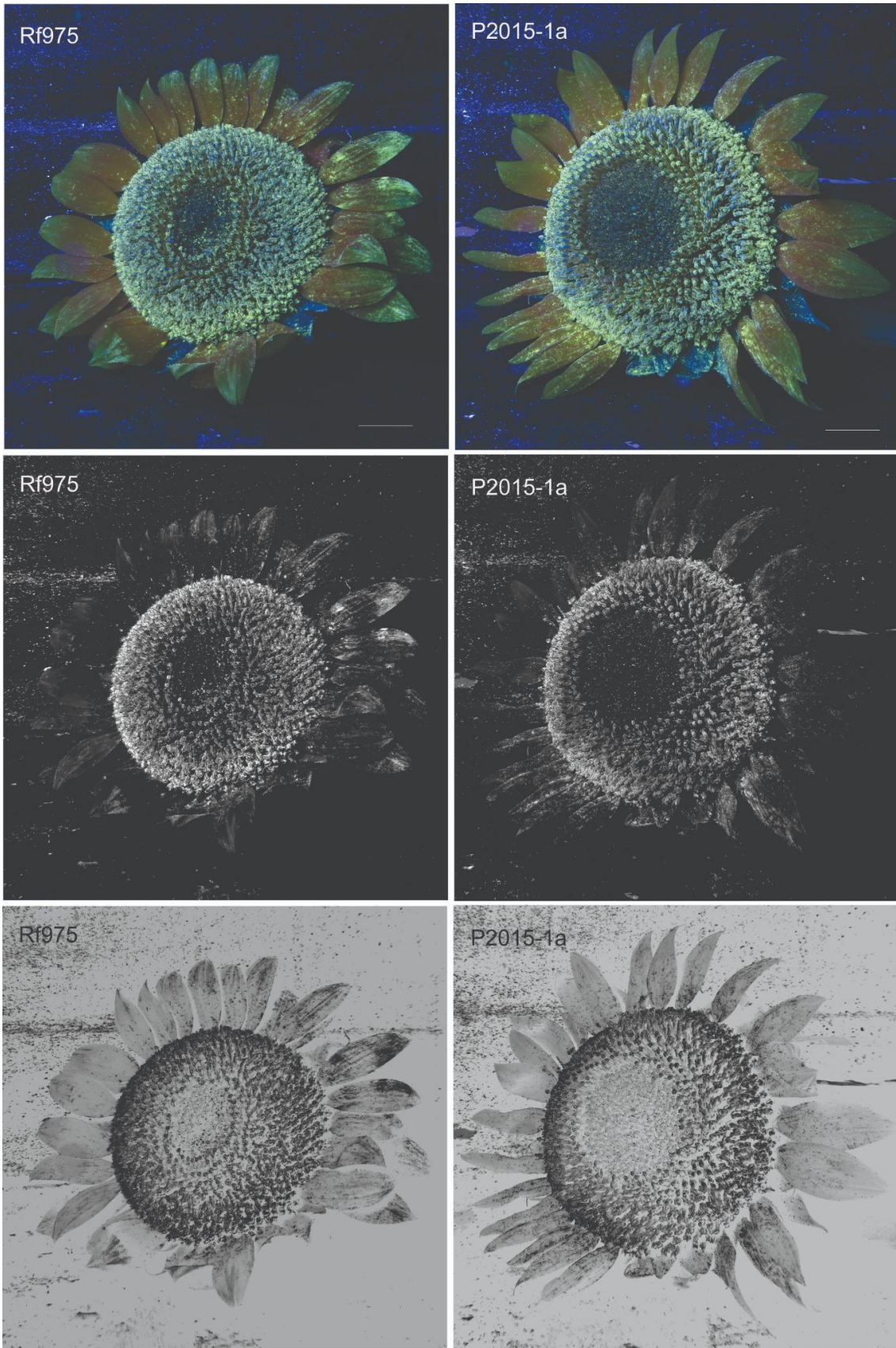
Figure S3. Representative *Helianthus annuus* capitula of Rf975 and P2015-1a genotypes photographed during the R5.6 developmental stage (Schneiter and Miller, 1981) under UV light. The lower images show UV-pigmented patterns in ligulate flowers. No differences were observed between genotypes, and both produced similar amounts of pollen. Scale: 2 cm.
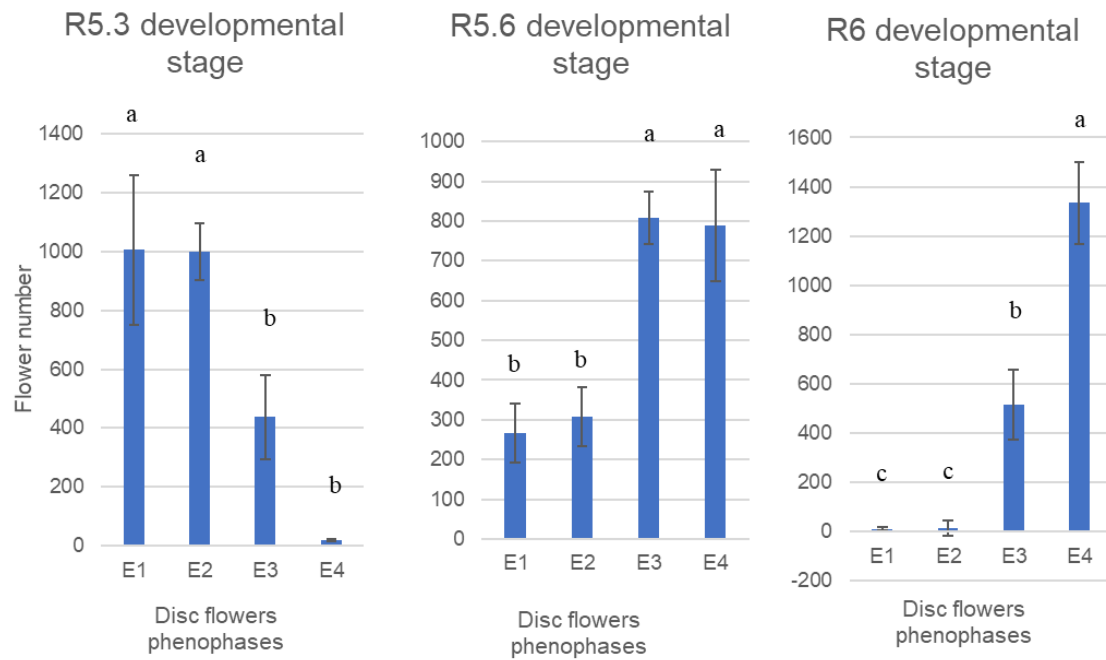
Figure S4. Estimation of the number of disc flowers (fn) of each phenophase (E1, E2, E3, and E4), at different developmental stages of the sunflower capitula (R5.3, R5.6, and R6). The number of disc flowers at each phenophase and each reproductive stage (Schneiter and Miller, 1981) was estimated by image analysis in 24 capitula of each genotype, applying the protocol described by Ochogavía (2022). The experimental design was randomized with three replications and each replication consisted of one capitulum. Flower number per capitulum (FN) was analyzed at each capitula`s developmental stage independently (R5.3; R5.6; and R6) by two-way analysis of variance to evaluate phenophase and genotype effects. The normality of the empirical distribution of each variable was assessed by the Shapiro and Wilk's W statistic test. Homogeneity of variance was evaluated using the Levene's test. Statistical analyses were performed using agricolae (de Mendiburu, 2023) and car (Fox and Weisberg, 2019) packages of R software (R Core Team, 2022). ANOVA analysis by F-test showed a significant effect of the phenophases (E1, E2, E3, and E4) 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). Means were analyzed by Tukey's Honestly Significant Difference (HSD) test at alpha = 0.05. During R5.3, when 30% of disc flowers were opened in the capitulum, most of them were at E1 and E2 (figure S2). During R5.6, most disc flowers were at E3 and E4 in similar proportions and differing significantly from the E1 and E2 phenophases. Finally, during R6, the E4 phenophase was the most abundant and was significantly different respect to the other phenophases. Phenophase means and standard errors of fn are presented, common letters indicate that there were no significant differences among phenophases.

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Table S1. All the tested and pairwise compared GLMM for each response variable, one with and one without the fixed variable (null model), but both with the same random variables: daytime range (DR), reproductive stage (RS), flowering season (FS). The significance of including the fixed effect was assessed using a chi-square test through the anova function from the stats package (R Core Team, 2022). In bold are the selected models for each response variable with χ^2, d.f. and P values.

Response variable	Number of observations	Error distribution	Fixed variable	Random variables	χ^2	d.f.	P
Herbivore abundance	623	Negative binomial	**Genotype**	**(1|DR)+(1|FS)+(1|RS)**	**33.759**	**1**	**6.238×10⁻⁹**
			-	(1|DR)+(1|FS)+ (1|RS)			
Pollinator abundance	748	Negaive binomial	**Genotype**	**(1|DR)+(1|FS)+(1|RS)**	**4.2445**	**1**	**0.03938**
			-	(1|DR)+(1|FS)+ (1|RS)			
Predator abundance	124	Negative binomial	Genotype	(1|DR)+(1|FS)+ (1|RS)	**0.1161**	**1**	**0.7334**
			-	**(1|DR)+(1|FS)+(1|RS)**			
Herbivore presence	1282	Binomial	Predator presence	(1|DR)+(1|FS)+ (1|RS)	**0.1893**	**1**	**0.6635**
			-	**(1|DR)+(1|FS)+(1|RS)**			
Pollinator presence	1282	Binomial	**Predator presence**	**(1|DR)+(1|FS)+(1|RS)**	**15.962**	**1**	**6.464×10⁻⁵**
			-	(1|DR)+(1|FS)+ (1|RS)			

Table S2. The variance explained by the random variables in the GLMM, with herbivore abundance as the response variable and genotype as the fixed variable. The second column quantifies the variability in the herbivore abundance response variable that is not explained by the fixed variable but is instead attributed to differences between different levels of each random variable. The flowering season random variable had the greatest effect.

Random variable	Variance explained	Standard deviation
Reproductive stage	0.06380	0.2526
Day-time range	0.01269	0.1127
Flowering season	0.35351	0.5946

Table S3. The variance explained by the random variables in the GLMM, with pollinator abundance as the response variable and genotype as the fixed variable. The second column quantifies the variability in the pollinator abundance response variable that is not explained by the fixed variable but is instead attributed to differences between different levels of each random variable. The day-time range random variable had the greatest effect.

Random variable	Variance explained	Standard deviation
Reproductive stage	0.05814	0.2411
Day-time range	0.15842	0.3980
Flowering season	0.02567	0.1602