Short Communication

MISCONCEIVED EXPERIMENTS MAY YIELD VALUABLE INSIGHTS: SIMULATED FLORIVORY HAS UNPREDICTABLE CONSEQUENCES FOR PLANT REPRODUCTION IN *KNAUTIA ARVENSIS* (CAPRIFOLIACEAE)

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Abstract—An experimental manipulation of ray florets in *Knautia arvensis* (Caprifoliaceae) carried out in 2001 originally yielded results that were hard to reconcile with a hypothesis about floral attraction. Reframed as a test of florivory the archived data reveal that cutting ray florets left pollinator visitation and seed set essentially unchanged, but altered plant resource economics. Nectar concentration (and consequently available sugar after accounting for volume) declined under the large cutting treatment, while individual seed weight was consistently lower in the cut inflorescences. Together, these patterns suggest that simulated florivory can leave pollination largely intact yet reduce nectar quality and seed provisioning, producing lighter seeds without reducing seed set. More broadly, the study shows how re-examining archived "inconclusive" experiments through alternative perspectives can generate new ecological insights beyond those originally sought.

Keywords—Bumblebees, butterflies, Lepidoptera, hoverflies, pollinators, pollination, reproductive allocation

INTRODUCTION

> "There's almost literally no such thing as an 'unusual' or 'weird' empirical result in ecology…So shouldn't our preexisting expectation about the effect of *x* on *y* always be ¯_(ツ)_/¯ ?" (Fox 2026)

Experimental pollination ecology designed to test hypotheses about the functional significance of floral traits has a long and venerable history (Kessler & Baldwin 2011). Manipulation of flower and inflorescence phenotypes (as opposed to using natural variation in floral form, e.g. Alexandersson & Johnson 2002; Sletvold et al. 2016) can be challenging, but, if done successfully, may provide insights into the adaptive, non-adaptive and ecological functions of flower traits (e.g. Larue et al. 2016; Lamborn & Ollerton 2000; Ollerton et al. 2007; Pyke et al. 2024; Wilson 1995). Sometimes, however, manipulation of flower traits produces results that are difficult to reconcile with the hypotheses being tested, resulting in a form of "file drawer effect", whereby inconclusive or counterintuitive data are not published (Rosenthal 1979; Csada et al. 1996; Bauchau 1997). Such biases can distort our understanding of floral adaptation and pollination mechanisms, underscoring the importance of transparent reporting and replication in experimental pollination studies.

In 2001 the first author conducted a set of experiments designed to test the attractive role of the specialised ray florets found around the margins of capitulate inflorescences (flower heads) of species from families such as Apiaceae, Asteraceae, and Caprifoliaceae. The aim was to investigate the role played by these specialized florets in attracting the diverse pollinators of these usually ecologically generalized plants. Specifically, we wanted to understand whether the ray florets function only in relation to particular subsets of pollinators. Different pollinator groups vary markedly in their sensory capabilities, foraging behaviour, and modes of flower handling, and may therefore differ in how they

perceive and respond to floral traits such as enlarged or visually conspicuous ray florets.

The study species, *Knautia arvensis* (L.) Coult. (Field Scabious - Caprifoliaceae), is a common Eurasian grassland species that is equally well pollinated by three different insect groups: day-flying Lepidoptera, bumblebees (*Bombus* spp.) and hoverflies (Syrphidae) (Ollerton et al. 2025). These insects differ in body size, proboscis morphology, visual acuity and foraging strategies. This diversity of effective pollinators makes *K. arvensis* an ideal system in which to test whether ray florets serve a generalised attractive function across pollinator groups, or whether their role is more nuanced and contingent on the identity and behaviour of pollinators.

Once the results were analysed, however, it became clear that the data raised more questions than answers and could not be easily interpreted in respect of the original aims of the experiment. The data were archived and largely ignored, but not forgotten. With hindsight, and upon reading recent published work on florivory (e.g. Boaventura et al. 2022; Cárdenas-Ramos & Mandujan 2025), the results are interpretable if one considers the study as an experiment in the impact of artificial florivory on plant fitness rather than its original aim of trying to understand the attractive role of specialised florets. We therefore present the data in this light as a small contribution to understanding how florivores can impact plant reproduction in subtle and unexpected ways.

MATERIALS AND METHODS

Field observations and experimental manipulations were conducted during the peak flowering time of *Knautia arvensis*, in August 2001 at the Quarry Field site of Bradlaugh Fields, Northampton, central England (Ordnance Survey grid reference SP 765639; 52.26962° N, 0.87999° W). This is a mesotrophic grassland with calcareous floristic elements, overlying a long-defunct, back-filled Jurassic (Great Oolite) limestone quarry. The site is managed by the Wildlife Trust for Bedfordshire, Cambridgeshire & Northamptonshire.

INFLORESCENCE MANIPULATIONS

In *Knautia arvensis*, inflorescence diameter is 43.4 ± 3.8 mm (mean ± SD), comprising 23.7 ± 5.7 ray florets and 58.9 ± 13.6 disc florets (one inflorescence from each of n = 28 plants – see Fig. 1 and the Supplementary Information for these and all other data). At the start of the experiment, one young, unopened inflorescence on each of 99 randomly chosen plants was tagged and numbered and covered with a mesh bag to prevent insect visitation. As the inflorescences opened, they were randomly assigned, in equal number, to one of three treatments: Control (no manipulation except handling); Small cut (the outermost one or two millimetres of each ray floret removed); Large cut (all of the outermost ray floret corolla tissue removed) – see Fig. 1. The vagaries of weather and dog walkers meant that not all of the 99 tagged inflorescences survived to the end of the experiment, so variable sample sizes are presented throughout the analyses.

Figure 1: Examples of the experimental manipulation of *Knautia arvensis* inflorescences, showing a Control inflorescence (left), an inflorescence with a Small cut (centre), and an inflorescence with a Large cut (right). The positions of disc and ray florets are marked on the Control inflorescence.

Following the manipulations, insect visitors to these inflorescences were observed during 15 minute census periods on warm, still, sunny days, for a total of 1,050 minutes. Each visitor was identified to the level of broad functional/taxonomic group according to the main pollinators (bumblebees, day-flying Lepidoptera, and hoverflies – Ollerton et al. 2025). The amount of time of each insect remained on an inflorescence (hereafter residence time) was also recorded in seconds. After this the inflorescences were again bagged with fine mesh for a period of 24 hours to allow nectar to accumulate. Nectar volume in a sample of florets was then measured the following morning between 09:00 and 11:00, using 1 microlitre disposable glass microcapillary tubes and the concentration of the nectar measured using a Bellingham & Stanley hand-held refractometer. In addition, we calculated sugar mass following the standard procedure (e.g. Carrión-Tacuri et al. 2012, Pyke et al. 2020) using the formula: $Y = 0.00226 + 0.00937 * X + 0.0000585 * X^2$ where X is nectar sugar concentration, expressing it as sugar mass per unit volume using Y*V (where V = volume of nectar).

Following the observation period, each inflorescence was allowed to set seed. Seed heads were harvested as they matured and returned to the laboratory where the number and proportion of seeds set per inflorescence was counted, noting that each flower can only produce a maximum of one seed. A sample of seeds from each inflorescence was then allowed to air dry and mean seed weight was determined using a sensitive electronic balance.

Data analysis

We analysed pollinator visitation counts within and between treatments with a single negative-binomial mixed model (log link) of the form Count ~ Treatment × Group + (1|Inflorescence), which simultaneously tests differences between treatments within each pollinator group and between pollinator groups within each treatment. Inflorescence identity was included as a random effect to account for repeated observations (multiple pollinator visits) recorded on the same inflorescence. Overdispersion motivated the negative-binomial mean–variance structure; the dispersion parameter (θ) was initialized from a preliminary Negative Binomial GLM and used in the mixed model. Models were fitted by penalized quasi-likelihood using glmmPQL from MASS (Venables & Ripley, 2002) with the nlme backend (Pinheiro & Bates, 2000). From the fitted model we obtained estimated mean visitation rates with 95% confidence intervals for each Treatment × Group combination. We then conducted pairwise contrasts among pollinator groups within treatments and among treatments within pollinator groups, reporting incidence rate ratios with 95% confidence intervals. False discovery rate was controlled within each set of contrasts using the Benjamini–Hochberg procedure. Because observation durations were equal, no offset was included.

Residence time (the number of seconds insects remained on inflorescences) was analysed with a single generalized linear model assuming a Gamma error distribution and log link: Time ~ Treatment × Pollinator. The interaction allows simultaneous inference on differences between pollinator groups within each treatment and differences between treatments within each pollinator group. A Gamma mean–variance structure was chosen a priori for positive, right-skewed durations; no zeros were present, so a two-part model was unnecessary. We obtained population-level estimated mean residence times and 95% Wald confidence intervals for all Treatment × Pollinator cells by predicting on the link scale and back-transforming. Model terms were assessed with likelihood-ratio χ^2 tests from nested model comparisons (e.g., testing the Treatment × Pollinator interaction against an additive model). To address our focal questions, we computed pairwise Wald contrasts as log-ratios of means, reported as mean-time ratios (exp[contrast]) with 95% CIs, and controlled multiplicity within each family of comparisons using the Benjamini–Hochberg false-discovery-rate adjustment.

Nectar traits were analysed separately by response type. Nectar volume and sugar mass were modelled using Gamma generalized linear models with a log link, and nectar concentration was analysed as a proportion using a fractional logit (quasi-binomial, logit link). For each model, the effect of Treatment was assessed using omnibus tests (likelihood-ratio χ^2 tests for Gamma models; quasi-likelihood F-tests for the fractional

logit). Model-based treatment means with 95% confidence intervals were obtained by back-transforming predictions from the link scale. Pairwise treatment contrasts were reported as ratios of means (volume and sugar mass) or odds ratios (concentration), with false discovery rate controlled using the Benjamini–Hochberg procedure. Analyses used available cases; sugar mass was calculated only where concentration was measured. No offsets were required because measurements were made per inflorescence under comparable conditions. To investigate the conundrum of why nectar sugar mass appeared unchanged across treatments while concentration differed (see Results), we first characterised concentration missingness by treatment and as a function of nectar volume. We tabulated missingness rates per treatment and compared volume distributions between records with concentration present vs absent; we also summarised missingness by volume quartile to detect volume-dependent nonresponse. We then modelled sugar mass (mg) using a Gamma GLM with log link, including Treatment and log(Volume) as predictors: Sugar ~ Treatment + log(Volume). This controls for the strong positive scaling of sugar with volume and yields volume-adjusted treatment effects; inference on the treatment term used likelihood-ratio χ^2 tests from nested model comparisons. For interpretability, we report adjusted mean sugar mass and 95% Wald CIs at a common reference volume (the geometric mean of observed volumes). Because sugar mass is proportional to volume × sucrose proportion × density, and density is (approximately) constant given a concentration, we also ran two sensitivity analyses using a proxy measure of sugar content (sugar*) defined as sugar* = volume × proportion (proportion = concentration/100): (i) an observed-only analysis on rows with measured concentration, and (ii) analyses after single imputation of missing concentration either by treatment-specific means or by logistic regression on Treatment + log(Volume) with inverse-logit predictions constrained to (0,1). Each sensitivity model used a Gamma(log) GLM with the same treatment structure and LRTs for the treatment effect; results were compared to the volume-adjusted sugar mass model to assess the impact of informative missingness.

We analysed seed production outcomes separately by data type. Seed number per inflorescence (counts, overdispersed) was modelled with a negative-binomial GLM with log link (Total_seeds ~ Treatment; dispersion parameter θ estimated from the data). Seed set (proportion of ovules setting seed) was analysed as a fractional-logit model using a quasi-binomial family with logit link (prop = Percentage_seed_set/100 ~ Treatment), providing robust standard errors and an omnibus F-test for the treatment effect. Individual seed weight (mg), a positive right-skewed variable, was analysed with a Gamma GLM with log link (Seed_weight ~ Treatment). For each model, we obtained model-based treatment means and 95% Wald confidence intervals by predicting on the link scale and back-transforming to the data scale; planned pairwise contrasts were expressed as incidence-rate ratios (counts), odds ratios (seed set), or ratios of means (weight), with Benjamini–Hochberg adjustment where multiple comparisons were reported. Analyses were conducted in RStudio/Posit (RStudio Team 2025), using glm from stats in the base R (R Core Team 2025) unless otherwise noted. All figures were produced with ggplot2 (Wickham 2016).

Results

Pollinator visitation within and between treatments

Using the single negative binomial mixed model, pollinator groups differed within each treatment, driven by butterflies being consistently lower (Table 1, Fig. 2). Within Control, bumblebees visited far more than butterflies (BB vs BF: IRR = 3.71, 95% CI 1.76–7.80, *P*adj = 0.0025), but did not differ from hoverflies (BB vs HF: IRR = 1.05, 0.59–1.86, *P*adj = 0.869); butterflies were lower than hoverflies (BF vs HF: IRR = 0.28, 0.13–0.60, *P*adj = 0.0025). The same pattern held in Small cut (BB vs BF: IRR = 7.03, 2.82–17.52, *P*adj = 0.00016; BB vs HF: IRR = 0.68, 0.40–1.17, *P*adj = 0.167; BF vs HF: IRR = 0.097, 0.039–0.238, *P*adj = 1.4×10^{-5}) and in Large cut (BB vs BF: IRR = 4.74, 1.85–12.17, *P*adj = 0.0062; BB vs HF: IRR = 1.15, 0.58–2.24, *P*adj = 0.693; BF vs HF: IRR = 0.24, 0.093–0.626, *P*adj = 0.0075).

By contrast, treatment effects within each group were not detected after FDR correction (all *P*adj ≥ 0.22) (Table 1, Fig. 2). For example, within bumblebees: Control vs Small cut IRR = 1.12 (0.58–

Table 1: Values for the observed pollinator behaviour variables for the experimental treatments of *Knautia arvensis* inflorescence. Each value is the mean ±SD (n), with the sample size being number of individuals.

	Control	Small cut	Large cut
Bumblebees (visits per 15 minutes)	1.4 ± 1.1 (24)	1.3 ± 1.4 (26)	1.4 ± 2.2 (20)
Day-flying Lepidoptera (visits per 15 minutes)	0.4 ± 0.7 (24)	0.2 ± 0.7 (26)	0.3 ± 0.6 (20)
Hoverflies (visits per 15 minutes)	1.4 ± 1.5 (24)	2.0 ± 2.1 (26)	1.1 ± 1.5 (20)
Bumblebees (time on inflorescence, seconds)	17.6 ± 25.9 (33)	21.6 ± 22.9 (32)	12.7 ± 9.8 (19)
Day-flying Lepidoptera (time on inflorescence, seconds)	36.0 ± 28.4 (9)	14.0 ± 7.0 (4)	11.6 ± 5.3 (5)
Hoverflies (time on inflorescence, seconds)	26.1 ± 39.6 (31)	23.2 ± 22.2 (41)	20.3 ± 11.4 (21)

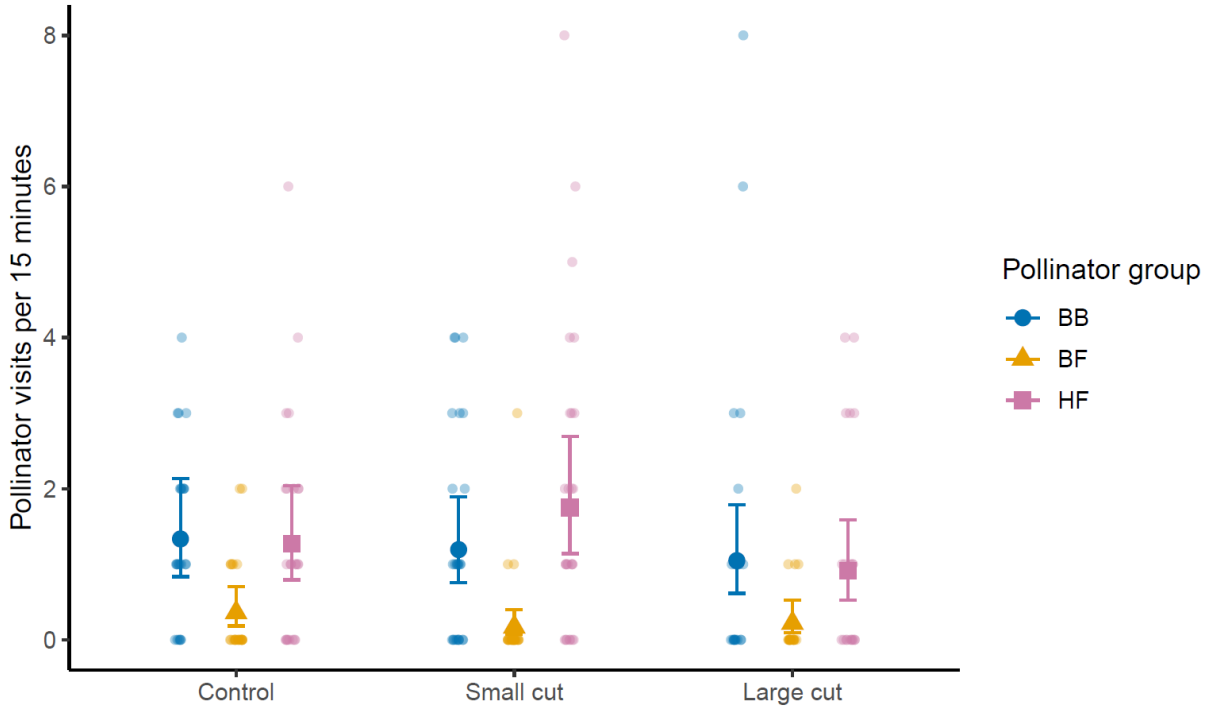

Figure 2: The number of visits per 15 minutes to the experimental *Knautia arvensis* inflorescences (defined on the x axis) by the different groups of pollinators: BB = bumblebees; BF = day-flying Lepidoptera; HF = hoverflies. Data points are raw observations with model-estimated means and bars showing 95% CIs.

2.15, *P*adj = 0.74) and Control vs Large cut IRR = 1.27 (0.63–2.59, *P*adj = 0.74); within butterflies: Control vs Small cut IRR = 2.12 (0.72–6.28, *P*adj = 0.54); within hoverflies: Small cut vs Large cut IRR = 1.91 (0.95–3.85, *P*adj = 0.22).

Overall, pollinator groups differed in their visitation rates (BF ≪ BB ≈ HF) but cutting treatments did not measurably change those rates (Table 1, Fig. 2).

Using a single Gamma GLM (log link) for residence time, we found no clear differences among pollinator groups within any treatment and no treatment effects within any pollinator group after FDR correction (Table 1, Fig. 3). Within Control, mean-time ratios (group A ÷ group B) were: BB vs BF 0.49 (95% CI 0.21–1.12, *P*adj = 0.24), BB vs HF 0.68 (0.39–1.17, *P*adj = 0.24), and BF vs HF 1.38 (0.60–3.17, *P*adj = 0.45). In Small cut, BB vs BF 1.54 (0.48–4.93, *P*adj = 0.70), BB vs HF 0.93 (0.55–1.56, *P*adj = 0.78), BF vs HF 0.60 (0.19–1.90, *P*adj = 0.70). In Large cut, BB vs BF 1.09 (0.36–3.29, *P*adj = 0.87), BB vs HF 0.63 (0.31–1.25, *P*adj = 0.47), BF vs

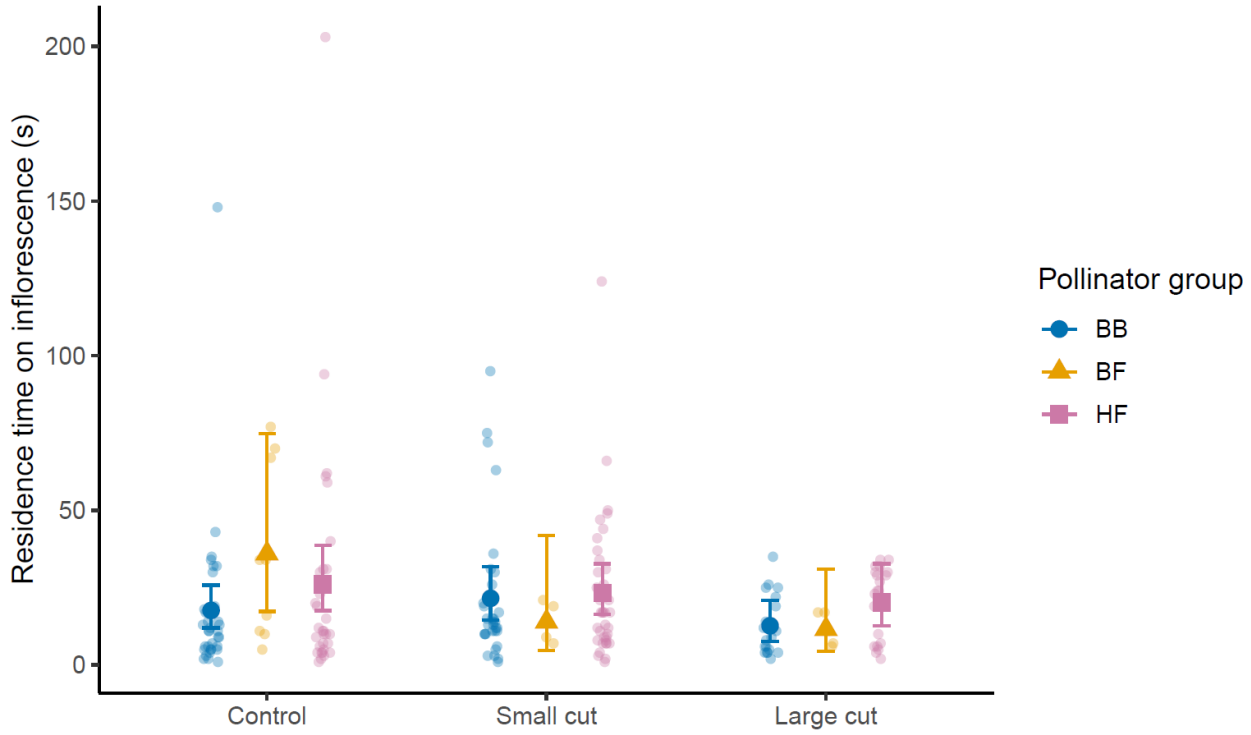

Figure 3: Residence times (in seconds) of pollinating insects on the experimental *Knautia arvensis* inflorescences, with treatments defined on the x axis. BB = bumblebees; BF = day-flying Lepidoptera; HF = hoverflies. Data points are raw observations with model-estimated means and bars showing 95% CIs.

HF 0.57 (0.19–1.70, *P*adj = 0.47). Treatment comparisons within groups likewise showed no reliable effects (all *P*adj ≥ 0.21); e.g., for bumblebees, Control vs Small cut 0.82 (0.47–1.41, *P*adj = 0.47) and Control vs Large cut 1.39 (0.74–2.62, *P*adj = 0.46); for butterflies, Control vs Large cut 3.10 (0.91–10.55, *P*adj = 0.21); for hoverflies, Small cut vs Large cut 1.14 (0.64–2.06, *P*adj = 0.66).

In short, residence times are statistically indistinguishable across pollinator groups and cutting treatments in this experiment (Table 1, Fig. 3).

Nectar characteristics

Across the three nectar traits, there were treatment effects for concentration and sugar mass, but not volume (Table 2, Fig. 4). For nectar volume, a Gamma GLM (log link) showed no overall treatment effect (LRT $\chi^2(2) = 0.036$, $P = 0.958$). Model-based mean volumes (µL; 95% CI) were similar: Control 0.386 (0.316–0.470), Small cut 0.372 (0.313–0.442), Large cut 0.385 (0.298–0.497). Pairwise ratios of mean volume were close to 1 and not significant after BH adjustment (e.g., Control vs Small cut ratio 1.04, 0.80–1.35; *P*adj = 0.985). For nectar concentration, a fractional logit model (quasi-binomial, logit link) detected a clear treatment effect ($F(2,75) = 26.12$, $P = 2.5\times10^{-9}$). Estimated mean concentrations were: Control 46.7% (44.1–49.3), Small cut 44.3% (42.4–46.3), and Large cut 32.1% (29.1–35.3). Large cut was substantially lower than both Control (odds ratio 1.85 favouring Control, 1.55–2.20; *P*adj ≈ 3.2×10^{-11}) and Small cut (OR 1.68, 1.43–1.98; *P*adj ≈ 6.4×10^{-10}), whereas Control and Small cut did not differ (OR 1.10, 0.96–1.25; $P = 0.157$). For sugar mass, the Gamma GLM indicated no overall effect ($\chi^2(2) = 0.129$, $P = 0.727$), with similar means (mg): Control 0.183 (0.152–0.219), Small cut 0.190 (0.165–0.218), Large cut 0.170 (0.134–0.215); pairwise mean ratios again straddled 1 (e.g., Control vs Large cut 1.08, 0.80–1.45; *P*adj = 0.749).

The fact that nectar concentration was affected by the treatments, but sugar mass was not, is a statistical artefact. Nectar concentration data were unevenly missing across treatments (Control 42.5%, Small cut 22.6%, Large cut 41.7% missing), and the missingness was volume-dependent: in Control, the records without concentration tended to have larger volumes (mean 0.463 µL vs 0.329 µL

Table 2: Values for the measured nectar and reproductive variables for the experimental treatments of *Knautia arvensis* inflorescences. Each value is the mean ± SD (n), with the sample size as indicated.

	Control	Small cut	Large cut
Nectar volume (µL, n flowers)	0.39 ± 0.24 (40)	0.37 ± 0.26 (53)	0.39 ± 0.22 (24)
Nectar concentration (%, n flowers)	46.7 ± 4.8 (23)	44.3 ± 7.5 (41)	32.1 ± 4.0 (14)
Sugar mass (mg, n flowers)	0.18 ± 0.06 (23)	0.19 ± 0.10 (41)	0.17 ± 0.07 (14)
Total seeds (n inflorescences)	14.7 ± 12.9 (20)	15.6 ± 12.5 (18)	11.1 ± 13.2 (17)
Seed set (%, n inflorescences)	28.7 ± 24.5 (20)	29.6 ± 23.4 (18)	21.5 ± 25.0 (17)
Seed weight (mg, n seeds)	0.0098 ± 0.0021 (90)	0.0079 ± 0.0022 (101)	0.0077 ± 0.0028 (84)

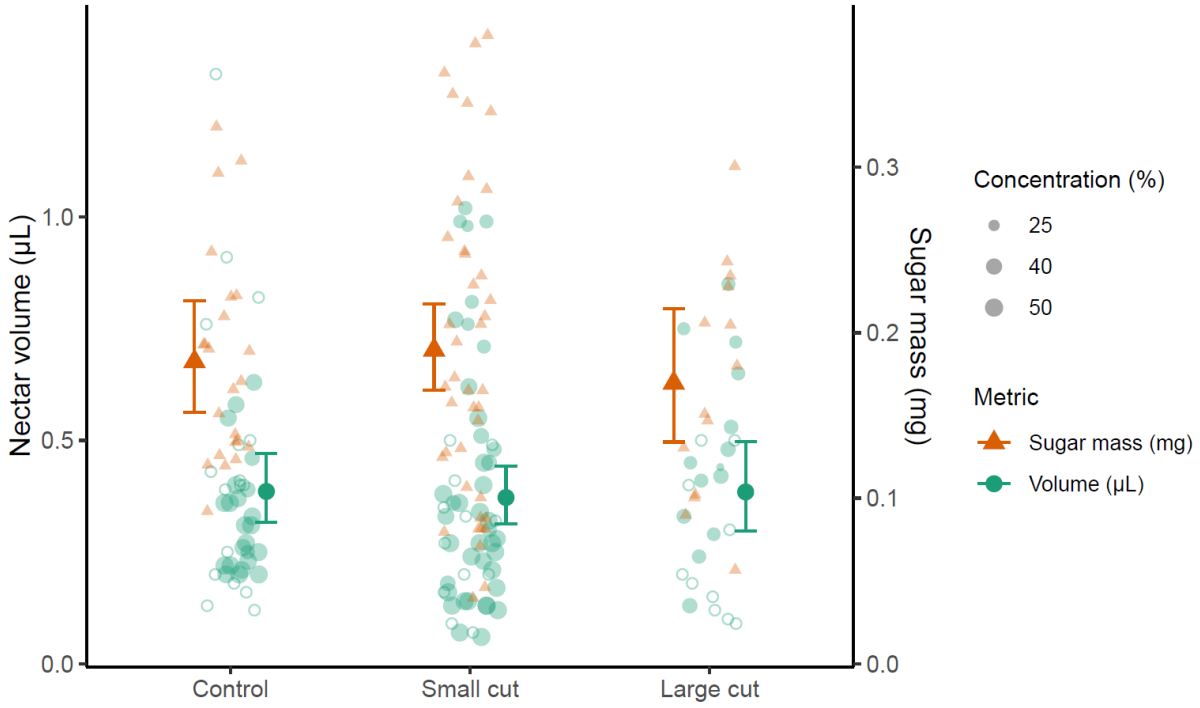

Figure 4: Nectar traits of the experimental *Knautia arvensis* inflorescences (defined on the x axis) by treatment, showing raw observations and model estimates. Points show individual inflorescence measurements; on the left y axis, filled circles are nectar volume (µL) with measured concentration (point size ∝ % concentration), while open circles indicate volume where concentration was missing; on the right y axis, triangles are sugar mass (mg), linearly rescaled to the left axis for plotting. Larger points with 95% confidence bars are Gamma GLM (log link) treatment means (volume and sugar modelled separately). Mean values, standard deviations and sample sizes are shown in Table 2.

when concentration was present), whereas in Large cut the missing cases were smaller (0.254 vs 0.478 µL), and in Small cut they were also smaller (0.283 vs 0.398 µL). Missingness was most common in the lowest (Q1) and highest (Q4) volume quartiles, especially for Control and Large cut. When we analysed sugar mass with a Gamma GLM that adjusted for log(volume), treatment did matter overall (LRT $\chi^2(2) = 1.208$, $P = 1.44\times10^{-13}$): Large cut was markedly lower than Control ($\beta = -0.375 \pm 0.049$, $P = 7.5\times10^{-11}$), whereas Small cut did not differ from Control ($\beta = -0.060 \pm 0.037$, $P = 0.112$); volume was strongly positive ($\beta = 0.795 \pm 0.027$, $P < 2\times10^{-16}$). At a common reference volume

(geometric mean 0.33 μL), adjusted mean sugar masses (mg, 95% CI) were: Control 0.184 (0.173–0.195), Small cut 0.173 (0.166–0.181), Large cut 0.126 (0.117–0.136). Sensitivity analyses supported this pattern: using only observed concentration and modelling sugar* = volume × proportion showed no treatment effect (LRT $P = 0.864$), but imputing concentration for missing rows yielded a significant treatment effect with Large cut lower (mean-imputation LRT $P = 0.036$) and a borderline effect under regression imputation (LRT $P = 0.069$).

Together, these results indicate that the initial "no effect" for sugar mass was masked by informative missingness in concentration and volume variation; after accounting for volume (or imputing plausible concentrations), Large cut flowers contained substantially less sugar, while Small cut was similar to Control (Table 2, Fig. 4).

Seed production

Seed production showed a clear pattern for seed weight but not for seed number (Table 2, Fig. 5). The negative-binomial model for counts found no evidence that cutting altered the number of seeds per inflorescence (overall LRT n.s.; Small cut vs Control IRR ≈ 1.06, $P = 0.892$; Large cut vs Control IRR ≈ 0.76, $P = 0.540$). Overdispersion was present ($\theta = 0.56$), which the model accommodates. By contrast, the Gamma model for individual seed weight indicated significant reductions in both cutting treatments: relative to Control, seeds from Small cut heads were about 20% lighter (ratio = exp(−0.2247) ≈ 0.80, $P = 1.3\times10^{-7}$) and those from Large cut were about 22% lighter (ratio = exp(−0.2497) ≈ 0.78, $P = 2.2\times10^{-8}$). For percentage seed set (proportion of ovules setting seed), the fractional-logit model (quasi-binomial, logit link) found no evidence of a treatment effect (omnibus $F(2,52) \approx 0.59$, $P = 0.56$). Model-based mean seed set (±95% CI) was 28.7% (19.1–40.8) for Control, 29.6% (19.3–42.4) for Small cut, and 21.5% (12.6–34.2) for Large cut. Planned contrasts versus Control gave odds ratios of 1.06 for Small cut (95% CI ≈ 0.44–2.57; $P \approx 0.92$) and 0.76 for Large cut (0.31–1.88; $P \approx 0.37$). Thus, while Large cut shows a non-significant tendency toward lower seed set, the data are consistent with no effect of cutting on the proportion of ovules that mature into seeds.

In summary, cutting did not change fecundity (seed number) but reduced per-seed mass in both cutting treatments (Table 2, Fig. 5).

Discussion

As an experiment to test how ray florets function in species with compound inflorescences, this experiment failed because the manipulations had the consequence of affecting nectar characteristics, which was not the intention of the experiment. Specifically, the amount of sugar in the nectar was reduced by the cutting of the ray florets, though this did not affect either the number of pollinator visits to inflorescences nor the time spent by individual insects on inflorescences. These results in turn explain why seed production (both total number and the proportion of ovules becoming seeds) were not affected by the treatments. There was, however, a strong effect on seed weight, with those produced from the experimentally manipulated inflorescences being around one fifth lighter, on average, than those from the control inflorescences.

It is reported that in other herbaceous species, petal or perianth removal followed by hand pollination can increase seed production, suggesting that producing flowers involves a cost to the plant (Andersson 1999, 2000, 2005, 2006). In our case, we did not remove all the ray florets (just part of the tissue) and did not hand pollinate flowers, therefore, our cutting may not cause resource allocation for flowers and seed production. Instead, we interpret the reduced sugar concentration and seed mass as the plant responding to what was, in effect, an experiment in florivory. The plants responded to the damage to floral tissue by withdrawing resources (principally photosynthate) from those inflorescences. This suggests a trade-off strategy, where plants might reduce investment in compromised flowers to conserve resources for other reproductive functions. This reallocation strategy likely serves as an adaptive mechanism to mitigate the costs of florivory and protect overall reproductive success in a context of resource limitation.

Florivory – the consumption of flower tissues by animals – is a ubiquitous phenomenon in the natural world (Boaventura et al. 2022). However, it remains relatively under-studied compared to other aspects of herbivory and in particular the consequences of flower damage for plant reproduction have until recently not been well explored (McCall & Irwin 2006; Boaventura et al.

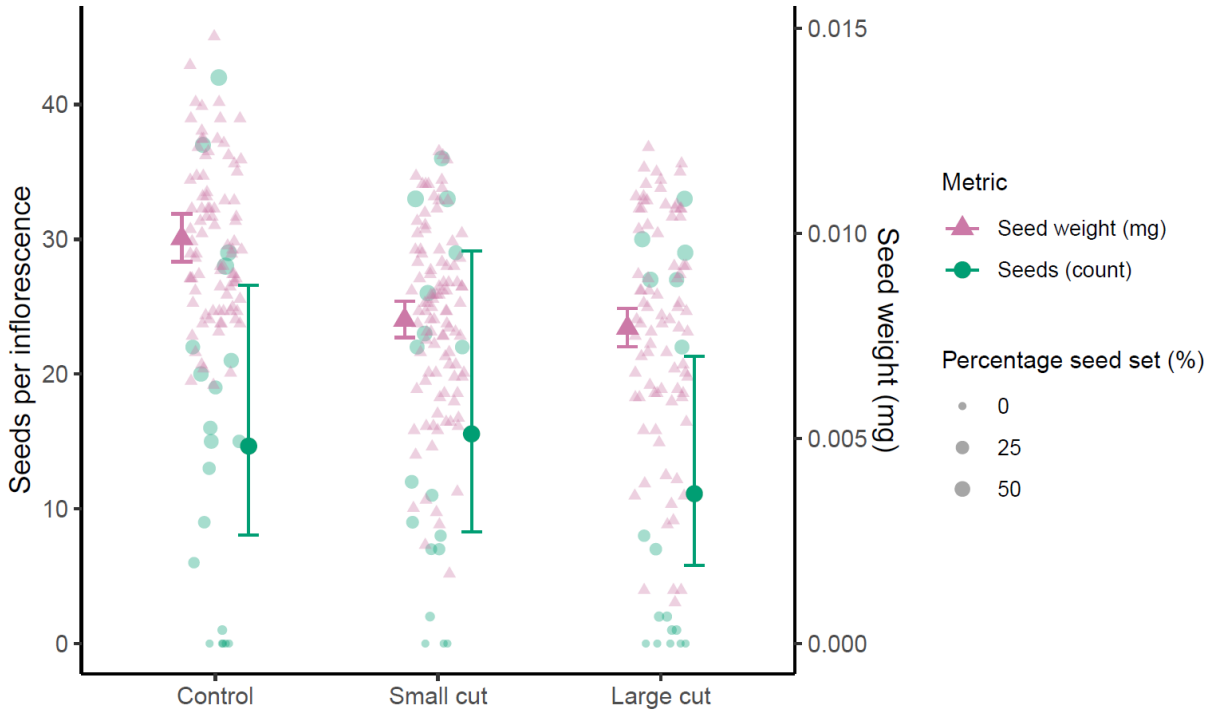

Figure 5: Seed production of the experimental *Knautia arvensis* inflorescences (defined on the x axis) by treatment, showing raw values and model means ± 95% CI. Points show individual observations: circles = seeds per inflorescence (left y-axis), triangles = seed weight (mg) (right y-axis; shown on the left scale via a linear rescaling for plotting only). Point size encodes the percentage seed set for that inflorescence. Large symbols with 95% confidence bars are model-estimated treatment means from a negative-binomial GLM (log link) for seed counts and a Gamma GLM (log link) for seed weight; estimates are back-transformed to the data scale. Seed counts and percentage seed set are measured per inflorescence, whereas seed weight is measured on individual seeds; therefore sample sizes differ across panels within the composite. Mean values, standard deviations and sample sizes are shown in Table 2.

2022). Most experimental florivory studies removing corolla tissue or otherwise simulating floral damage typically report reduced pollinator visitation and lower fruit/seed set, consistent with a causal pathway from altered floral display to diminished pollination services (e.g., Cardel & Koptur 2010; Tsuji & Ohgushi 2018; Cárdenas-Ramos & Mandujan 2025). The magnitude and direction of effects are context-dependent, varying with timing (bud vs. anthesis), damage intensity, and which floral organs are attacked, as well as the broader pollination environment (pollinator-limited vs. saturated). In some systems, tolerance/compensation or indirect interactions can neutralize expected costs (e.g., unchanged seed mass or trade-offs between seed number and size after simulated florivory – Wang et al. 2015), underscoring that fitness outcomes need not be uniformly negative. Nevertheless, a meta-analysis across taxa finds an overall negative effect of herbivores (including florivores) on floral traits, plant attractiveness to pollinators, and reproductive success, while also documenting substantial heterogeneity among studies (Moreira et al. 2019). As our results demonstrate, sometimes the outcomes of florivory can be subtle and unpredictable.

Reduced seed weight may have consequences for plant fitness even when seed number is unchanged, although such effects are context dependent. Seed size in European grasslands can influence seedling growth and establishment, with larger seeds often producing larger seedlings and achieving higher recruitment success (Jakobsson & Eriksson 2000). In addition, damage to developing seeds can reduce seedling vigour or survival even when seeds remain viable, indicating that sub-lethal effects of herbivory may carry through to later life stages (Ollerton & Lack 1996). The reduction in seed weight observed under simulated florivory therefore suggests a shift in resource allocation with potential downstream

effects not captured by seed set alone, consistent with broader evidence that florivory can influence both the quantity and quality of reproductive output (McCall & Irwin 2006). The analyses reported here are exploratory and hypothesis-generating in nature, that we hope will lead to more work on this topic. Although the experiments were originally designed to test the attractive function of ray florets, subsequent literature on florivory suggested an alternative interpretation. Because the study was not prospectively designed to test florivory, we appreciate that we leave ourselves open to the charge of HARKing: Hypothesizing after the results are known (sensu Kerr 1998). However, we would see this more as reframing the experiment (see Fox 2025 for a recent discussion of this) and hopefully our transparent account of the history of these data will allay those charges.

Of course, a mystery remains – what is the function of the ray florets in *Knautia arvensis*? Clearly their influence on pollinator attraction and retention is limited. It is possible that the florets themselves are non-adaptive and do not have a direct role in pollinator attraction beyond the function of the inflorescence itself. This would fit with the fact that inflorescences in the studied population can vary in diameter from about 30mm to 50mm, a difference of 40% that is largely due to the number of flowers per inflorescence rather than the length of the ray florets (Ollerton unpublished data). There are precedents for such non-adaptive explanations of floral traits (Lamborn & Ollerton 2000) but to test this properly requires further work.

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Author contribution

Concept and design JO, data collection JO, data analysis JO & XX, writing JO, XX & ZXR, edits and approval for publication JO, XX & ZXR.

Disclosure statement

No potential conflict of interest was reported by the authors. Author Jeff Ollerton is an Associate Editor of the Journal of Pollination Ecology. Thus, the peer-review process for this article was handled independently by another member of the editorial board.

Generative AI disclosure statement

ChatGPT 5.4 was used to advise on statistical analysis and to help with coding for R analyses.

Data availability statement

Data used to write this article are available as Supplementary Information

Appendices

Additional supporting information may be found in the online version of this article:

Appendix 1. Raw data on Nectar, Seed set, Seed weight, Pollinator visitation, Pollinator residence time, Inflorescence measurements.

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