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Research

Negative and sex-specific effects of drought on flower production, resources and pollinator visitation, but not on floral scent in monoecious *Cucurbita pepo*

Monica Barman[1,2], **Raimund Tenhaken**[1] and **Stefan Dötterl**[1]

[1]Department of Environment and Biodiversity, Paris Lodron University of Salzburg, Hellbrunnerstrasse 34, Salzburg, 5020, Austria; [2]Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Theodor-Echtermeyer-Weg 1, Großbeeren, 14979, Germany

Authors for correspondence:
Monica Barman
Email: barman@igzev.de

Stefan Dötterl
Email: stefan.doetterl@plus.ac.at

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Summary

- The globally changing climatic condition is increasing the incidences of drought in several parts of the world. This is predicted and already shown to not only impact plant growth and flower development, but also plant–pollinator interactions and the pollination success of entomophilous plants. However, there is a large gap in our understanding of how drought affects the different flowers and pollen transfer among flowers in sexually polymorphic species.
- Here, we evaluated in monoecious Styrian oil pumpkin, and separately for female and male flowers, the responses of drought stress on flower production, petal size, nectar, floral scent and visitation by bumblebee pollinators.
- Drought stress adversely affected all floral traits studied, except floral scent. Although both flower sexes were adversely affected by drought stress, the effects were more severe on female flowers, with most of the female flowers even aborted before opening. The drought had negative effects on floral visitation by the pollinators, which generally preferred female flowers.
- Overall, our study highlights that the two flower sexes of a monoecious plant species are differently affected by drought stress and calls for further investigations to better understand the cues used by the pollinators to discriminate against male flowers and against flowers of drought-stressed plants.

Introduction

Changes in climate are predicted to increase the occurrences of drought events globally and lead to adverse effects on terrestrial ecosystems, including agriculture/crop productions (IPCC, 2022; NDMC, 2024). Drought limits the photosynthetic performance in plants, thereby impacting their health and growth (Mafakheri *et al.*, 2010; Pilon *et al.*, 2015; Li *et al.*, 2020), and also affects the processes of pollination and sexual reproduction (Brown *et al.*, 2016; Rering *et al.*, 2020). Most naturally occurring as well as many crop plants depend on insect pollinators for successful sexual reproduction (Klein *et al.*, 2007; Ollerton *et al.*, 2011; Potts *et al.*, 2016), and reward their pollinators with floral resources including pollen and/or nectar (Shuttleworth & Johnson, 2009). Previous studies showed that drought impacts pollination of such plants, either by directly affecting the availability of pollinators (Brown *et al.*, 2016; Soroye *et al.*, 2020), or by indirectly altering floral traits that mediate the interactions with the pollinators (Phillips *et al.*, 2018; Rering *et al.*, 2020; Kuppler *et al.*, 2021).

Flower production, reward (e.g. composition and amount of nectar) as well as visual (e.g. flower size) and olfactory (floral scent) traits of flowers were found to be negatively affected by drought stress (Carroll *et al.*, 2001; Burkle & Runyon, 2016; Waser & Price, 2016; Glenny *et al.*, 2018; Kuppler *et al.*, 2021), and all these changes can alter the foraging behaviour of pollinators, potentially affecting the reproductive success of the plants (Phillips *et al.*, 2018; Rering *et al.*, 2020; Kuppler *et al.*, 2021). Available data also show that effects of drought are not homogenous but differently affect both floral traits and pollinator visits among plant species (Burkle & Runyon, 2016; Campbell *et al.*, 2019; Dötterl & Gershenzon, 2023; Höfer *et al.*, 2023).

Most of the studies on the effects of drought stress on floral traits have been conducted on plant species bearing hermaphrodite flowers (Burkle & Runyon, 2016; Glenny *et al.*, 2018; Rering *et al.*, 2020; Kuppler *et al.*, 2021; Höfer *et al.*, 2023). They found that, apart from adverse effects on the visual and olfactory floral traits, resource allocation during the development of such flowers is disturbed (Teixido & Valladares, 2019; Arathi & Smith, 2023). Plant species with hermaphroditic flowers were shown to channel their resources for the development of female floral parts at the cost of male floral parts (Teixido & Valladares, 2019). By contrast, there is very limited knowledge about drought effects on resource allocation, floral rewards and

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advertisement, attractiveness to pollinators, pollen transfer between the sexes and reproduction in sexually polymorphic plant species. Indeed, while it is known from a single dioecious species (*Silene latifolia*, Caryophyllaceae) that drought results in a reduction in the number and the size of flowers in both sexes (Delph, 2019), it is unknown whether different flowers respond similar or differently in species, where different flower sexes are occurring on the same plant individual (e.g. monoecious species).

The Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.; Cucurbitaceae) is an agriculturally important entomophilous and monoecious plant species. It is highly valued for its seeds, used for the production of unique quality oil that has a dark green colour and viscous consistency with mild nutty aroma (Fuchs & Müller, 2004; Fruhwirth & Hermetter, 2008). For high seed production, it is essential, as is true for many other entomophilous plants with unisexual flowers, that both female and male flowers are produced and attractive to their pollinators for making pollen transfer between the flower (plant) sexes possible. In the past, the Styrian oil pumpkin has faced severe losses (up to 50%), and climatic factors, especially drought, have been considered responsible for these losses (Fruhwirth & Hermetter, 2007).

In the present study, we have studied how drought affects female and male flower production, flower traits and pollinator interactions in the Styrian oil pumpkin. Specifically, we asked, are there sex-specific effects of drought on: floral bud formation and development of flowers; floral phenotypic traits including petal size, composition and quantity of nectar resources and scents; and pollinator visitation patterns? We predicted that drought reduces the production of floral buds, negatively affect petal size, nectar resources as well as scents of the flowers, and also the number of pollinator visits. Moreover, as it is known that energy costs are higher for the development of female than male flowers (Bazzaz *et al.*, 2000; Gao *et al.*, 2021), we expected that the effects of drought are more pronounced in female than male flowers. Alternatively, plants might channel their resources for the development of female flowers, as observed for female flower parts in hermaphrodite flowers (described in the previous section, Teixido & Valladares, 2019).

Materials and Methods

Plant material and growth conditions

Seeds of Styrian oil pumpkin (*Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb.) were obtained from Saatzucht Gleisdorf Gesellschaft m.b.H, Austria. In 2022 and 2023, 20 seeds each were sown in a soil mixture containing 1 : 1 (v/v) compost and mole soil. Fifteen days after sowing, the seedlings were transferred to pots (20 cm height and 25 cm diameter) containing a 3 : 1 (v/v) ratio of steamed compost and steamed soil with a mixture of sheep's wool fertilizer, and Oscorna®, Hackaphos® green and Osmocote® as long-term fertilizers. The plants were randomly assigned to drought stress (n = 10 yr^{-1}) and control (n = 10 yr^{-1}) conditions and were maintained in the glasshouse of the Botanical Garden of the Paris Lodron University of Salzburg, between April and July each year (temperature maintained inside the glasshouse in April–July 2022 and 2023 was 21.7°C ± 4.5°C and 21.1°C ± 4.6°C, respectively; mean ± SD). In 2022, one of originally 10 control plants died before flowering. Plants cultivated in 2022 were used for studies related to vegetative and floral traits, whereas plants cultivated in 2023 were used for pollinator visitation studies. Control plants were watered daily until water drained through the holes in the bottom of the pots. Plants grown under drought conditions were watered every third day following Rering *et al.* (2020), from the seedling stage onwards, again until water drained through the holes at the bottom of the pots. To check whether the reduced watering caused drought conditions, we recorded the soil moisture content and the leaf stomatal conductance. Soil moisture content was recorded for 10 consecutive days during the peak flowering period (7th and 8th week after germination) from a randomly selected control and drought-stressed plant. Leaf stomatal conductance was monitored once a week (1 d before watering of drought-treated plants; 6th to 10th week after germination) during the peak flowering period for both control (n = 8–9) and drought-stressed (n = 8–10) plants (see details in Supporting Information Methods S1). Both measures indicated that drought-stressed, but not control plants, were under severe drought stress (Fig. S1a,b).

First flowering in both treated and control plants was observed on the 6th week after germination, followed by high flower production at least till the 10th week, after which flower production decreased. All data related to floral traits were recorded during the 8th week (one of the peak flowering weeks for both male and female flowers, see Table S1) after germination, with all the different flower traits (petal size, flower weight, nectar and scent; to be described later) collected from one randomly selected male and one randomly selected female flower per plant individual. The female and male flowers of the Styrian oil pumpkin are, as is true for other cucurbits (Collison & Martin, 1979; Vidal *et al.*, 2006; Schaefer & Renner, 2011), very short-lived and only bloom for a few hours in the morning on a single day, in our experiment between *c.* 05:00 h and 10:00 h.

Measurement of floral bud and flower production, plant and petal sizes, and flower weight

To determine whether drought stress had effects on plant and flower growth, data were recorded on plant shoot length (shoot base touching the soil till the tip of the utmost terminal leaves), floral bud and flower production as well as on petal size (measuring the length of a randomly selected petal starting from the receptacle to the tip of the petal by using a scale ruler) and flower fresh weight (g). Data on floral bud and flower production were recorded from all plant individuals (control, n = 9; drought, n = 10). Data on petal size and floral weight were obtained between 07:00 h and 08:00 h from fully bloomed flowers (male: n = 9–10 per treatment; female: control, n = 8–9; drought, n = 3). Data on plant shoot length were recorded both at the beginning and the end of the flowering period from a randomly selected subset of plant individuals (n = 5–7 per treatment and time point).

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Nectar collection and sugar analysis

To evaluate whether drought stress had an impact on the quantity and quality of nectar resources, we collected nectar in the morning from fully bloomed flowers (male: n = 9–10 per treatment; female: control, n = 9; drought, n = 3), between 07:00 h and 08:00 h, by using 10-µl microcapillaries (Blaubrand®; BRAND GMBH + CO KG, Wertheim, Germany) as given in Dellinger *et al.* (2019). We were able to quantitatively collect the nectar from *in situ* female flowers by capillary forces. In males, however, some nectar remained in the edges of the nectary. To also obtain this nectar, we carefully excised the filaments to expose the nectary and actively collected the remaining nectar by using disposable rubber bulbs attached to the microcapillaries. We determined the volume and transferred the collected nectar to 1.5-ml microcentrifuge tubes, which were immediately frozen with liquid nitrogen and stored at −80°C until analysis of the sugar composition.

The composition of nectar sugars was analysed using a high-performance liquid chromatography (HPLC) system (Dötterl *et al.*, 2014; Dellinger *et al.*, 2019). Therefore, 10 µl of collected nectar was diluted (1 : 20, v/v) with double-distilled water. This diluted sample was briefly spun to precipitate down any pollen particles potentially present in the nectar. The nectar mixture was further diluted to 1 : 1000 with double-distilled water and analysed using an ICS300 HPLC (Dionex, Vienna, Austria), where sugars were separated using anion-exchange chromatography coupled with pulsed amperometric detection. The nectar sugars were separated on a CarboPac PA1 column (2 × 250-mm separation columns and 2 × 50-mm guard columns) following an isocratic separation with 80 mM NaOH and a flow rate of 0.25 ml min^{-1}. Calibration curves of authentic standards (glucose, fructose and sucrose) were prepared for quantification of each compound. The total concentration of nectar sugar was calculated by adding up the concentrations of glucose, fructose and sucrose.

Collection and analysis of floral scents

The emitted scents from Styrian oil pumpkin flowers were collected via dynamic headspace (Dötterl *et al.*, 2006; Rachersberger *et al.*, 2019) in the morning from fully bloomed flowers (male: n = 9–10 per treatment; female: control, n = 8; drought, n = 3), between 07:00 h and 07:30 h. Briefly, a single bloomed flower was bagged using polyethylene oven bags (Toppits®, Minden, Germany; dimensions: 15 cm × 15 cm) for 5 min. The bag was then cut at one top corner, and an adsorbent tube was inserted. Adsorbent tubes were made up of quartz glass tubes (length of 25 mm and inner diameter of 2 mm), filled with 1.5 mg of Tenax-TA (mesh 60–80; Supelco, Merck KGaA, Darmstadt, Germany) and 1.5 mg of Carbotrap B (mesh 20–40; Supelco). This mixture was secured with silanized glass wools (Supelco) in the tubes. The scents were trapped in the adsorbent tubes for 5 min with the help of a membrane pump (G12/01 EB; Rietschle Thomas Inc., Puchheim, Germany) by maintaining a flow rate of 200 ml min^{-1}. This sampling time resulted in strong samples (Fig. S2), and preliminary analyses showed that increasing the scent trapping time to, for example 10 min, resulted in a saturation of the mass spectrometer. Scent samples were also collected simultaneously from ambient air and leaves, which served as negative controls. After trapping the VOCs, the adsorbents were transferred to 1.5 ml screw neck glass vials and stored at −20°C until analysis.

The scents collected in the adsorbent tubes were analysed using an automated TD (thermal desorption) system (TD-20, Shimadzu, Japan) coupled to a GC-MS (QP2010 Ultra EI, Shimadzu, Japan) equipped with a ZB-5 fused silica column (5% phenyl polysiloxane; length of 60 m, inner diameter of 0.25 mm, film thickness of 0.25 µm), as described previously in Gfrerer *et al.* (2021) and Rupp *et al.* (2021). The compounds were identified using libraries available for mass spectral and retention index data: NIST 11, Wiley 9, Essential oils, FFNSC 2 and Adams 2007. Moreover, several compounds were authenticated by comparison of mass spectra and retention index data with standard compounds available in the Plant Ecology Lab of Salzburg University. We built an own library containing information on mass spectra and retention indices of the detected pumpkin floral scents. This pumpkin floral library was used for the (semi)automatic detection and integration of the floral scents in all samples. Total scent amounts in ng per flower were calculated upon adding peak areas of all detected floral scents and converting obtained peak area (TIC) in equivalence to ng from known amounts of standard compounds.

Behavioural assays with bumblebee pollinators

To understand whether drought influenced the visitation of flowers by bumblebee pollinators, we performed behavioural assays with *Bombus terrestris* (obtained from Biobest® Belgium NV) in a flight cage (4 m width × 8 m length × 2.20 m height; Rachersberger *et al.*, 2019) built in the Botanical Garden of the University of Salzburg. Bees were individually marked on their thorax with a numbered, coloured sticker (Bienenzucht Profi, Schöneck, Germany). Some of the bees lost their marking, but 33 of them retained their marks throughout the experimental period. During nonobservational times, the bees were allowed to forage naturally for floral resources on nonexperimental *Reseda* plants available in the flight cage. During experiments performed in June/July 2023 for 2 h in the mornings (06:00 h–08:00 h) just after the pumpkin flowers bloomed on days with clear sky and bright sun, however, the *Reseda* plants were covered with a net to prevent the bees from foraging on these plants, but to rather visit pumpkin flowers. On each experimental day, we introduced four individuals each of regularly watered and drought-stressed pumpkin plants at the same time. The plants were placed at distance of 50 cm from each other, whereby the plant individuals were randomly assigned to the different positions in the flight cage. For all available male (control, n = 54; drought, n = 48) and female (control, n = 19; drought, n = 5) flowers that bloomed within a total of 10 observation days (total of 20 observation hours), the number of bee visits was recorded by three same persons simultaneously, whereas each day at least 20 bees were found to be active inside the flight cage during the observation time. For the 33 bees marked throughout the experiment, we additionally recorded

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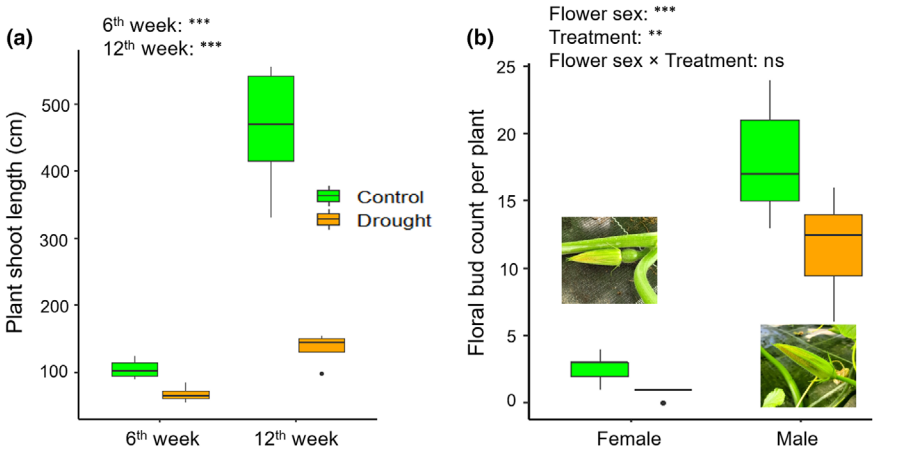

(c)

	Male flowers		Female flowers	
	Buds produced	Flowers developed	Buds produced	Flowers developed
Control	161	161	23	23
Drought	117	117	18	3

(d)



Fig. 1 Plant shoot length 6 (control $n = 7$, drought $n = 7$) and 12 (control $n = 5$, drought $n = 5$) wk after germination (a), female and male floral bud counts per plant (b; control $n = 9$, drought $n = 10$), and the number of female and male flower buds that developed into flowers from control ($n = 9$) and drought-stressed ($n = 10$) *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb. plants (c). A control female flower bud (left) that developed finally into a flower and a drought female flower bud (right) that died and did not develop into a flower (d). In the boxplots, thick black horizontal lines represent the medians, boxes indicate the interquartile ranges, whiskers indicate the minimum/maximum ranges without outliers, and black dots represent outliers. In (a), data were analysed using *t*-tests; in (b), data were analysed using generalized linear mixed model (GLMM) tests; the levels of significance are given as: $P > 0.05$ is nonsignificant (ns); **, $P < 0.01$; ***, $P < 0.001$.

their recurrent visits to specific male (control, $n = 29$; drought, $n = 25$) and female (control, $n = 8$; drought $n = 2$) flowers. We cannot exclude that bumblebees visiting the flowers have left chemical or electrostatic footprints, affecting future visits by other bees (Saleh *et al.*, 2007; Clarke *et al.*, 2017). However, such potential effects are expected to be similar between the flower sexes and treatments and, thus, unlikely have affected our comparisons among treatments and flower sexes. Furthermore, such effects would also occur in a natural setting.

Statistical analyses

Statistical analyses on univariate plant traits and flower visits of pollinators were performed using R programming language (v.4.2.2; R Core Team, 2021) in the RSTUDIO platform (RSTUDIO 2021.09.2 + 382 'Ghost Orchid' Release). Multivariate scent compositions were analysed in PRIMER 6 (Clarke & Gorley, 2006). The variations in the phenotypic traits in control and treated plants were represented via boxplots using 'GGPLOT2' package (v.3.4.0). In these plots, thick black horizontal lines represent the median, boxes indicate the interquartile range, whiskers indicate the minimum/maximum range without outliers, and black dots represent outliers. The homogeneity of variance for the studied univariate traits was checked using Levene's test; traits showing nonhomogenous distribution were transformed logarithmically to reach homogeneity of variances. *t*-tests were used on two different time points during plant growth to evaluate the effect of drought on plant shoot length. Generalized linear mixed model (GLMM) tests were performed using 'GLMMTMB' package (v.1.1.7) to assess the effects of treatment, floral sex as well as their interaction (sex × treatment) on flower weight, corolla length, nectar volume and scent amount using Gaussian distribution, whereas Poisson distribution was considered for floral bud count. In these analyses, treatment (control and drought) and floral sex were used as categorical factors, and plant individual as a random factor. Since female flowers of drought-treated plants did not produce any nectar (to be described later), one-way ANOVA followed by Tukey's HSD was used to check whether nectar sugar concentration differed among the available male and female flower samples. To evaluate the effects of drought on floral scent composition, we performed PERMANOVA (10 000 permutations) considering treatment and flower sex as fixed factors and plant individual as a random factor. We also carried out nonmetric multidimensional scaling (NMDS) to visualize similarities and dissimilarities in scent composition among the scent samples. Both these analyses were based on semi-quantitative Bray–Curtis similarities calculated on square root transformed relative scent data. The effect of drought on the number of bumblebee visits per flower and the number of visits by a specific bee individual to a specific flower were evaluated via GLMM considering Poisson distribution, with plant and bee individuals used as random factors. To determine the statistical significance for model parameters, we used ANOVA function (CAR package, v.3.1-2). To check whether drought affected the likelihood for female and male flowers to receive at least one bumblebee visit, Pearson's chi-squared tests were performed.

Results

Plant shoot length

The vegetative growth (shoot length) of Styrian oil pumpkin plants was negatively affected by drought both at the start (6th week; median values: control, 102 cm; drought, 65 cm) and the end (12th week; 470 cm vs 144 cm) of the flowering period (Fig. 1a; Dataset S1; *t*-test: 6th week: $T = 5.95$, $P < 0.001$; 12th week: $T = 9.70$, $P < 0.001$).

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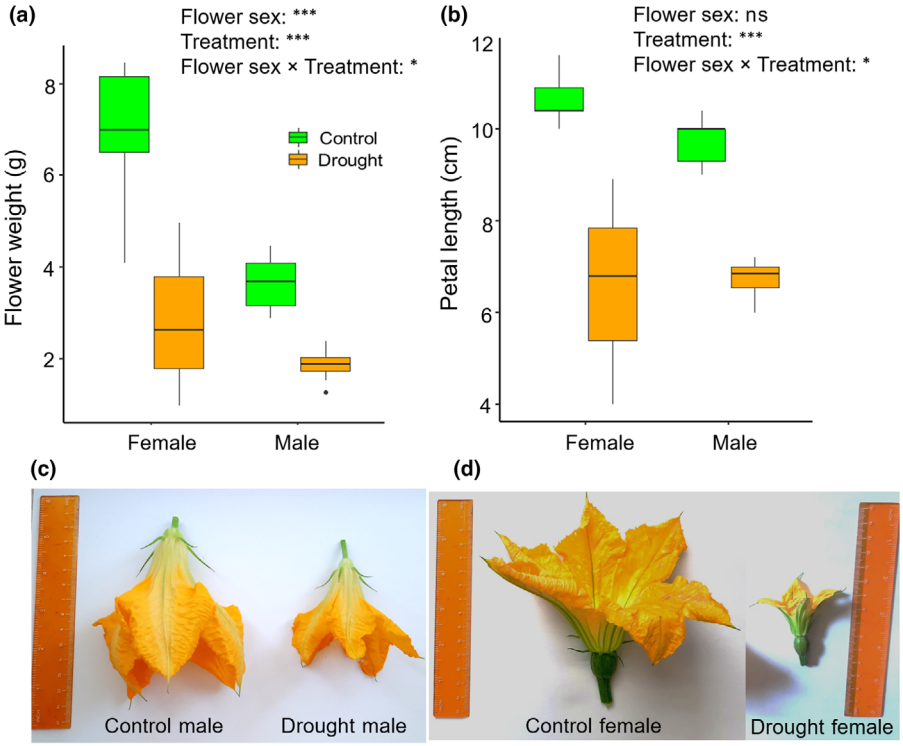

Fig. 2 Fresh weight (a) and petal length (b) of female (control $n = 9$, drought $n = 3$) and male (control $n = 9$, drought $n = 10$) flowers of *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb. plants. Photographs showing the petal length difference between male (c) and female flowers (d) of control and drought-stressed plants. In the boxplots, thick black horizontal lines represent the medians, boxes indicate the interquartile ranges, whiskers indicate the minimum/maximum ranges without outliers, and black dots represent outliers. Data were analysed using generalized linear mixed model (GLMM) tests and the levels of significance are given as: $P > 0.05$ is nonsignificant (ns); *, $P < 0.05$; ***, $P < 0.001$.

Female and male flower bud formation and flower production

Throughout the flowering period, plants produced a higher number of male (more than five folds higher) than female flower buds, and drought resulted in a similar reduced production of buds in both sexes (reduced by *c.* 1.4-fold and threefold in male and female buds, respectively) when compared to control plants (Fig. 1b; Dataset S2; GLMM: flower sex: $\chi^2 = 76.20$, $P < 0.001$; treatment: $\chi^2 = 7.04$, $P < 0.01$; sex × treatment: $\chi^2 = 2.26$, $P = 0.13$; see also Table S2). All male buds developed into flowers irrespective of the treatment (Fig. 1c), whereas this was only true for female flower buds in the control plants. In the drought-stressed plants, only a small proportion of produced female buds developed into flowers (Fig. 1c) as most female buds aborted 1–2 d before blooming, with the appearance of brownish-yellow coloration starting 6–8 d before that (Fig. 1d).

Floral weight and corolla length

The weight was higher in female than male flowers and drought negatively affected the weight in both sexes, but to higher extent in females (Fig. 2a; Dataset S3; GLMM: flower sex: $\chi^2 = 31.34$, $P < 0.001$; treatment: $\chi^2 = 35.81$, $P < 0.001$; sex × treatment: $\chi^2 = 4.25$, $P < 0.05$). The petal length was overall similar in female and male flowers, but similar to flower weight, negatively affected by drought and again to higher extent in females (Fig. 2b–d; GLMM: flower sex: $\chi^2 = 3.39$, $P = 0.06$; treatment: $\chi^2 = 52$, $P < 0.001$; sex × treatment: $\chi^2 = 4.60$, $P < 0.05$).

Nectar

Female flowers produced more nectar than male flowers and drought negatively affected the nectar production in both sexes, but to higher extent in females (Fig. 3a; GLMM: flower sex: $\chi^2 = 28.21$, $P < 0.001$; treatment: $\chi^2 = 2007.18$, $P < 0.001$; sex × treatment: $\chi^2 = 1444.79$, $P < 0.001$). Compared with control flowers, the nectar volume in drought-stressed male flowers was reduced by *c.* 75%, whereas female flowers of drought-stressed plants did not produce any nectar.

The nectar sugar concentration was highest in control female flowers, and lower and in a similar range in control and drought-stressed male flowers (Fig. 3b; ANOVA: $F = 13.23$; $P < 0.001$). As no nectar was produced in the female flowers of drought-stressed plants, it was not possible to evaluate their nectar sugar concentration. We identified fructose, glucose and sucrose as nectar sugars in all samples available for measurements. Independent of flower sex and treatment, the nectar was dominated by sucrose (median: control male 66.58%, control female 72.45%, drought male 61.36%) and contained smaller percentages of glucose (median: control male 16.95%, control female 14.51%, drought male 19.61%) and fructose (median: control male 16.47%, control female 13.04%, drought male 19.02%; Fig. 3c).

Floral volatiles

Female and male flowers of control and drought-stressed plants emitted similar amounts of scent (Fig. 4a; GLMM: flower sex: $\chi^2 = 0.38$, $P = 0.54$; treatment: $\chi^2 = 0.02$, $P = 0.88$; sex ×

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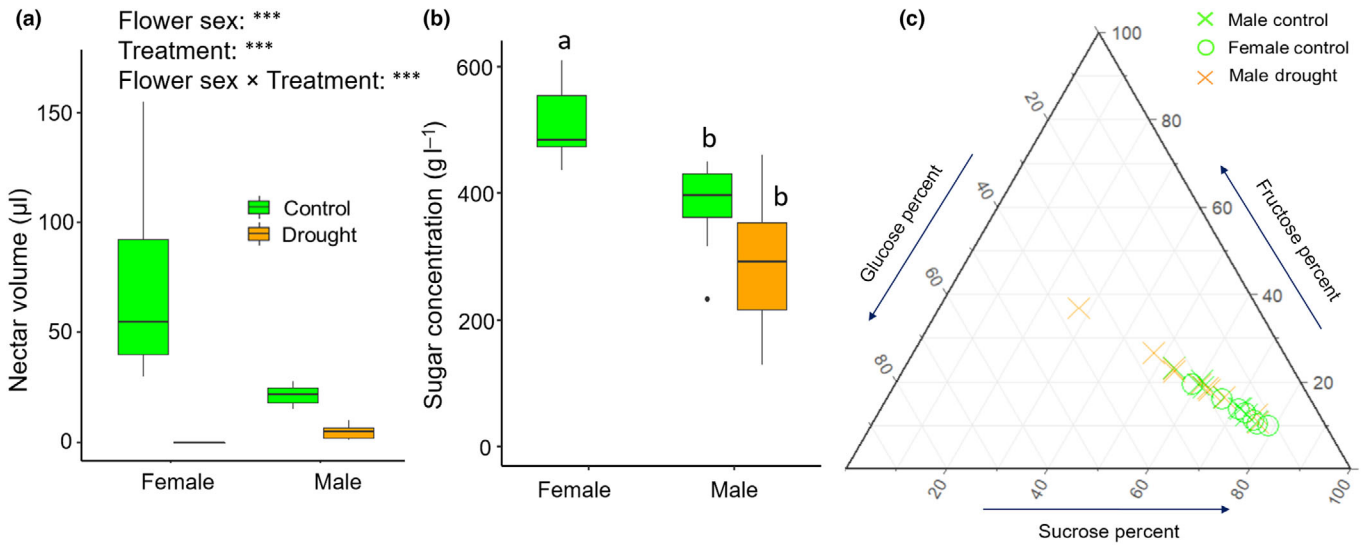

Fig. 3 Nectar volume (a) and sugar concentration (b) of female (control $n = 9$, drought $n = 3$) and male (control $n = 9$, drought $n = 10$) flowers of *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb. plants. Ternary plot representing the percentage of the nectar sugars glucose, fructose and sucrose obtained from flowers of control and drought-stressed plants (c). As female flowers of drought-stressed plants did not produce nectar, it was not possible to obtain data for the sugar concentration and composition of these flowers. In the boxplots, thick black horizontal lines represent the medians, boxes indicate the interquartile ranges, whiskers indicate the minimum/maximum ranges without outliers, and black dots represent outliers. In (a), data were analysed using generalized linear mixed model (GLMM) tests and the levels of significance are given as: ***, $P < 0.001$; in (b), data were analysed using one-way ANOVA followed by Tukey's HSD and different letters indicate significant differences.

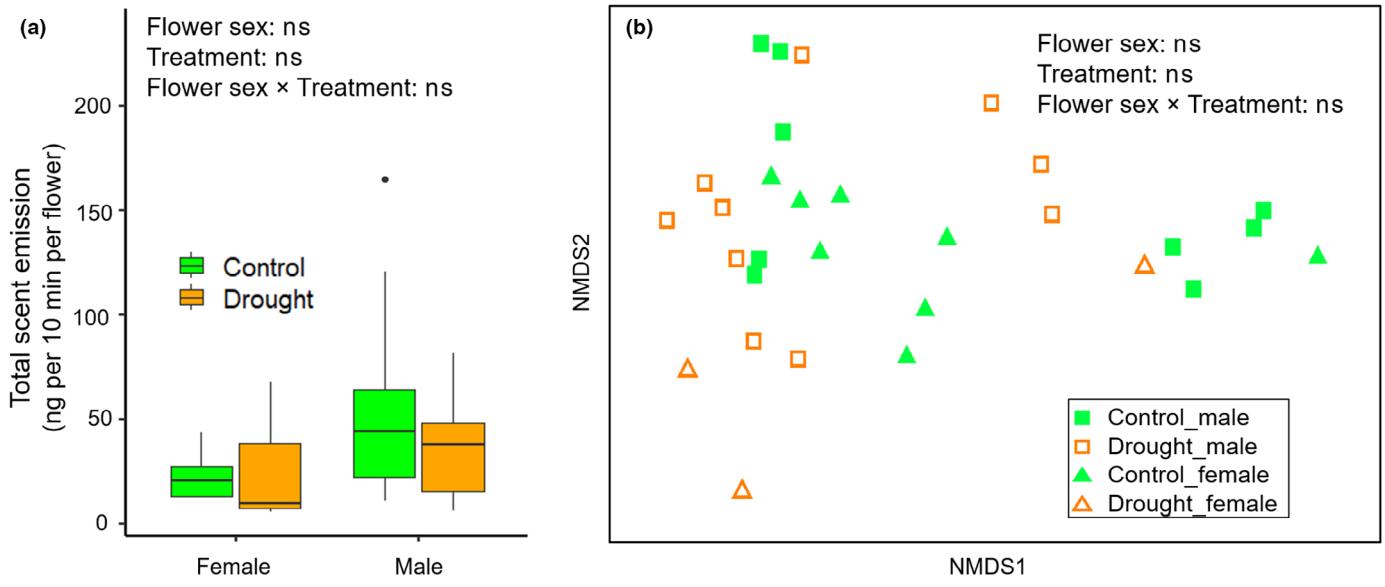

Fig. 4 Total amount of scent emitted from female (control $n = 8$, drought $n = 3$) and male (control $n = 9$, drought $n = 10$) flowers of *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb. plants (a). Nonmetric multidimensional scaling (NMDS) based on semi-quantitative Bray–Curtis similarities calculated on square root transformed relative scent amounts among the scent samples (b). In the boxplot, thick black horizontal lines represent the medians, boxes indicate the interquartile ranges, whiskers indicate the minimum/maximum ranges without outliers, and black dots represent outliers. In (a), data were analysed using generalized linear mixed model (GLMM) tests; in (b), data were analysed using PERMANOVA; ns, nonsignificant differences ($P > 0.05$).

treatment: $\chi^2 = 0.003$, $P = 0.95$), and a total of 33 different flower volatile compounds were detected in the scent samples. Most of these compounds were present in the scent bouquet of both male and female flowers of both control and drought-stressed plants, with the exception of few minor compounds not emitted from either of the floral sexes (Fig. S2). The scent profile was dominated by aromatic compounds (19 compounds), followed by monoterpenes (5), irregular terpenes (3), nitrogen bearing compounds (3) and other compounds (3). The relative scent composition did not differ between sexes and

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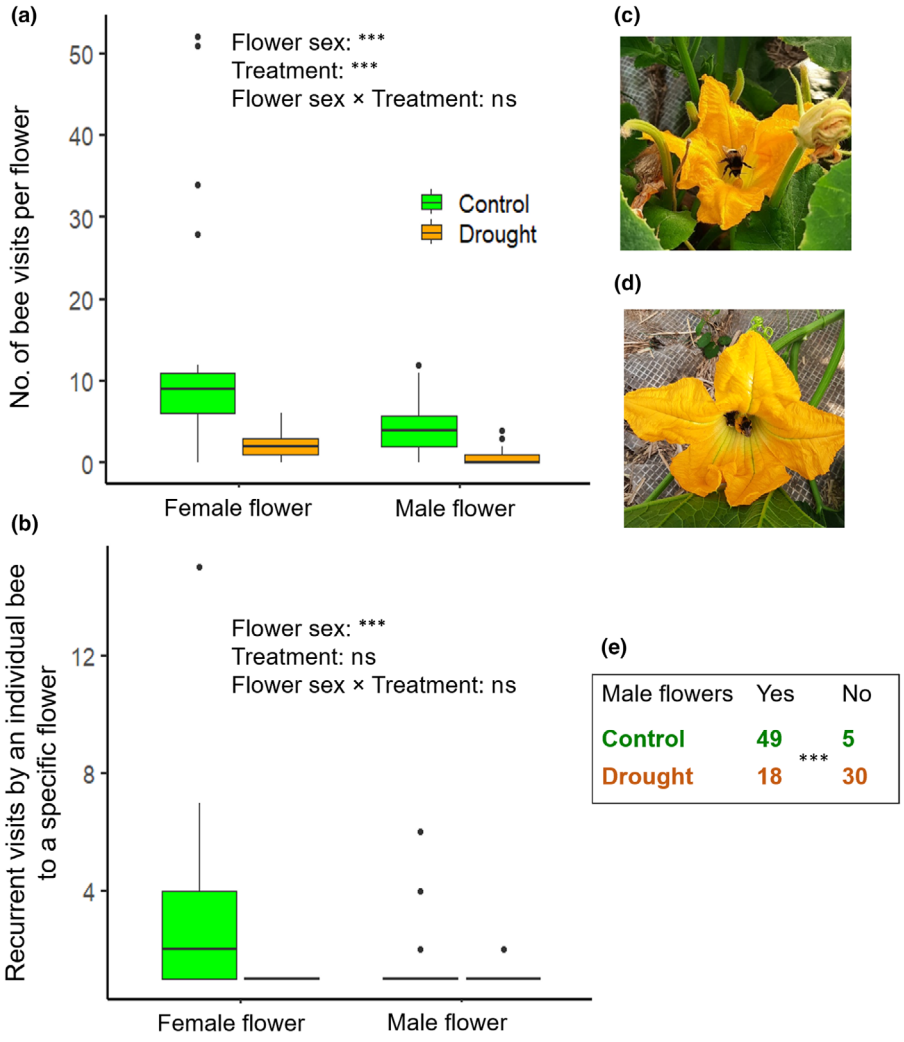

Fig. 5 Number of *Bombus terrestris* visits per female (control $n = 19$, drought $n = 5$) and male (control $n = 54$, drought $n = 48$) flower of the *Cucurbita pepo* L. subsp. *pepo* var. *styriaca* Greb. (a). Recurrent visit count of individually marked bee ($n = 33$) to specific female (control $n = 8$, drought $n = 2$) and male (control $n = 29$, drought $n = 25$) flowers (b). Photographs showing the bumblebee visits to male (c) and female (d) flowers of experimental nonstressed plants. Likelihood of pollinator visit to male flowers of control and drought-stressed plants (e). In the boxplots, thick black horizontal lines represent the medians, boxes indicate the interquartile ranges, whiskers indicate the minimum/maximum ranges without outliers, and black dots represent outliers. Data were analysed using generalized linear mixed model (GLMM) tests and the levels of significance are given as: $P > 0.05$ is nonsignificant (ns); ***, $P < 0.0001$.

treatments (PERMANOVA: flower sex: $P = 0.19$; treatment: $P = 0.74$; flower sex × treatment: $P = 0.19$; Fig. 4b), with the aromatic compound 1,4-dimethoxybenzene being typically the most abundant compound followed by compounds such as benzyl alcohol, *p*-anisaldehyde, linalool and phenylacetonitrile (Fig. S2; Table S3; for absolute peak area, see Dataset S4).

Pollinator visits

In male flowers, the likelihood of getting at least one pollinator visit was higher in control (91%) than drought-stressed (38%) plants (Fig. 5e; Pearson's chi-squared test: $\chi^2 = 31.96$, $P < 0.001$), whereas in females, where only a small number of flowers was available from especially drought plants, the likelihood of getting at least one pollinator visit did not significantly differ between flowers of control (95%) and drought (80%) plants (Pearson's chi-squared test: $\chi^2 = 1.12$, $P = 0.29$). Drought stress had similar negative effects on the number of visits per female and male flower, whereas female flowers received generally more visits than male flowers (Fig. 5a; Dataset S5; GLMM: treatment: $\chi^2 = 15.38$, $P < 0.001$; flower sex: $\chi^2 = 136.99$, $P < 0.001$; flower sex × treatment: $\chi^2 = 0.66$, $P = 0.42$). Independent of treatment, individual bees visited specific female flowers more often than specific male flowers (Fig. 5b; Dataset S6; GLMM: treatment: $\chi^2 = 0.80$, $P = 0.37$; flower sex: $\chi^2 = 27.37$, $P < 0.001$; flower sex × treatment: $\chi^2 = 0.49$, $P = 0.48$).

Discussion

Our findings show that drought stress negatively affects plant growth, floral bud formation, petal size, nectar resources and pollinator visits in Styrian oil pumpkin plants. Although both flower sexes were negatively affected by drought stress, the effects were more severe on female flowers, and most of the female flowers were even aborted before opening. In contrast to all other plant and flower traits studied, the floral scent emission was unaffected by drought. Moreover, the severe losses reported previously in the yield of Styrian oil pumpkin under drought stress (Fruhwirth & Hermetter, 2007) may be due to negative effects on the formation of female floral buds (Fig. 1b), as well as due to negative effects on floral phenotypic traits (Figs 2, 3), leading to reduced pollinator visits (Fig. 5a).

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Sex effects in nonstressed plants

Consistently with previous studies on cucurbits and other unisexual flower-bearing plants, we found that nonstressed plants produced a higher number of male than female flowers (Fig. 1; Raju & Ezradanam, 2002; Huang *et al.*, 2006; Vidal *et al.*, 2006; Allesh *et al.*, 2019; Revanasidda, 2019) and female flowers were heavier than male flowers (Fig. 2; Gehring *et al.*, 2004; Sowndhararajan *et al.*, 2020). Similarly, female flowers produced more nectar (Fig. 3), likely due to a larger nectary in female than male *Cucurbita* flowers (Nepi & Pacini, 1993), and a more concentrated nectar (Nepi *et al.*, 2001). These differences in nectar traits between the sexes explain, why female flowers were preferred by bumblebees (Fig. 5) that visited the flowers exclusively for their nectar (see also Nepi & Pacini, 1993). Other bee pollinators, such as *Peponapis pruinosa*, a species specialized on pollen of *Cucurbita* species (Hurd *et al.*, 1974; Cane *et al.*, 2011; Barman *et al.*, 2023), might prefer male flowers (Tepedino, 1981; Phillips & Gardiner, 2015) and, thus, behave similar as other bee pollinators of other monoecious plant species, where pollen is the main reward (Kawagoe & Suzuki, 2002; Raju & Ezradanam, 2002) or male flowers are larger than female ones (Huang *et al.*, 2006). In contrast to other studies, which found that male flowers are often more strongly scented and have another scent composition than female flowers as a result of sexual selection (Svensson *et al.*, 2010; see also Ashman, 2009), the two flower sexes in the Styrian oil pumpkin released the same amount of scent and had the same scent composition (Fig. 4). This might assure that pollinators have difficulties in discriminating between the flower sexes from distance (Grison-Pigé *et al.*, 2001; Brandt *et al.*, 2020), and that pollen collecting bee species not only visit male but also female flowers and nectar collecting pollinators also visit male flowers at least to some extent, making pollen transfer between the sexes possible.

Drought effects

Our finding of reduced vegetative and reproductive plant growth and reduced nectar reward production in drought-stressed plants is consistent with results obtained on other plant species (vegetative: Nonami *et al.*, 1997; Rering *et al.*, 2020; Kuppler *et al.*, 2021; Broberg *et al.*, 2023; reproductive: Höfer *et al.*, 2023; reward: Carroll *et al.*, 2001; Phillips *et al.*, 2018). It is also in agreement with our findings of reduced gas exchange and transpiration rates (Fig. S1b; see also Miyashita *et al.*, 2005; Centritto *et al.*, 2009; Ors *et al.*, 2016; Liu *et al.*, 2018) that compromise photosynthetic efficiency and carbon assimilation, thereby negatively affecting vegetative plant biomass production (Lawlor & Cornic, 2002; Eziz *et al.*, 2017; Yang *et al.*, 2019), flower production and size (Bazzaz *et al.*, 2000; Lambrecht & Dawson, 2007; Centritto *et al.*, 2009; Eziz *et al.*, 2017) and nectar production (Carroll *et al.*, 2001; Phillips *et al.*, 2018).

It was interesting to observe that the negative effects of drought were more pronounced in female flowers. Thus, drought increased the sexual dimorphism recorded in nonstressed plants. The drastic reduction in flower production and weight as well as the stop in producing any nectar in female flowers could be attributed to the purpose of preserving the plant's energy resources and making survival in stressful conditions possible (Delph, 2019). The plant seems to translocate the resources away from female flowers that otherwise need more resources than male flowers to being produced in cucurbits (Gao *et al.*, 2021; see also Fig. 2) as well as in other plants with unisexual flowers (Bazzaz, 1979; Bazzaz *et al.*, 2000; Juvany & Munné-Bosch, 2015). Even more energy is needed to develop, following successful pollination, fruits from the female flowers (Primack, 1987; Avila-Sakar *et al.*, 2001). Overall, the male fitness function requires less energy compared with the female fitness function, making it reasonable to invest relatively less in the female function at stressful conditions (Peruzzi *et al.*, 2012; Gao *et al.*, 2021).

Our observation that, in comparison with all other traits measured, the floral olfactory traits were not negatively affected by drought stress was surprising; however, in accordance with previous studies on some other flowering species, which also found that floral scent emissions were unaffected by drought (Rering *et al.*, 2020; Höfer *et al.*, 2023) or even found that floral scent emissions were increased under drought stress (Burkle & Runyon, 2016; Glenny *et al.*, 2018; Campbell *et al.*, 2019), although other floral traits (e.g. size) were severely affected. The maintenance or even increase in floral volatile formation might help drought-stressed plants to keep being attractive to pollinators despite decreased visual display sizes. Available literature data generally suggest that the floral olfactory display of plants is less sensitive to drought when compared to other stressors, such as temperature (Sagae *et al.*, 2008; Hu *et al.*, 2013; Fu *et al.*, 2017; Cordeiro & Dötterl, 2023). The reason might be that temperature more directly acts on the enzymes involved in the biosynthesis of floral scent compound (Cna'ani *et al.*, 2015; Cheng *et al.*, 2016; Barman & Mitra, 2021; Dötterl & Gershenzon, 2023).

Despite the unchanged scent emission, both female and male flowers of drought-stressed plants received less pollinator visits than flowers of control plants, which is consistent with results obtained in other studies that tested for effects of drought stress on floral traits and pollinator visitation (Burkle & Runyon, 2016; Glenny *et al.*, 2018; Rering *et al.*, 2020; Höfer *et al.*, 2021). This suggest that the discrimination against flowers of drought-stressed plants is mediated by their compromised floral visual traits. Indeed, visual floral traits, such as flower size, are well known to affect the attraction of bumblebee pollinators (Ishii, 2006; Kuppler *et al.*, 2021) and other pollinators (Burkle & Runyon, 2016; Rowe *et al.*, 2020). However, we cannot exclude those other sensory cues potentially affected by drought stress but not considered in our study, such as floral colour, or humidity and carbon dioxide within the floral microenvironment, influenced the behaviour of the bumblebee pollinators. Humidity and carbon dioxide were shown to correlate with nectar availability and pollinator attraction in other plants (von Arx *et al.*, 2012; von Arx, 2013; Dahake *et al.*, 2022; Dötterl & Gershenzon, 2023). These cues also might have been used in the present study by the bumblebee pollinators to assess nectar

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availability and to preferably visit female flowers (Fig. 5; Nepi & Pacini, 1993).

Conclusions

We for the first time studied the effects of reduced soil water availability on major floral traits of unisexual flowers and its impacts on pollinator attraction in a monoecious plant species. Our findings that drought stress negatively affects plant growth and flowers of both sexes (with stronger effects on female flowers and their production) as well as pollinator visitation show that more severe drought events predicted for the future by climate change scenarios will have negative effects on the sexual reproduction of the Styrian oil pumpkin. Future studies on other monoecious plant species will show whether drought-induced changes in flower sex ratio and negative effects of this stressor on floral traits and pollinator visitation are more widespread among crop and wild plants, with potential negative effects on crop production and ecosystems. The observation that drought negatively affects all traits studied, except floral volatiles in Styrian oil pumpkin, is intriguing and calls for future mechanistic studies to better understand how plants manage to emit the same amount of scent from much smaller flowers. Furthermore, our data ask for more investigations on tracking the floral visitor behaviour and understanding the exact cues used by the pollinators to find the flowers and discriminate against male and drought-stressed flowers.

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Competing interests

None declared.

Author contributions

MB and SD drafted the research idea and, together with RT acquired the funding. MB collected all and analysed most of the data. SD supported the statistical analyses. RT analysed the nectar samples. MB wrote the original draft manuscript. SD and RT validated the data, and edited and reviewed the manuscript. All authors contributed to and approved the final version of the manuscript.

ORCID

Monica Barman https://orcid.org/0000-0003-1406-4809
Stefan Dötterl https://orcid.org/0000-0001-5228-1332
Raimund Tenhaken https://orcid.org/0000-0002-6764-4826

Data availability

All study data are available as Dataset S1–S6.

References

Allesh SP, Varma S, Reshmi KS, Aswathi K, Megha PP, Jasna TV, Nikhila Reshmi MV, Sibisha VC. 2019. Effect of flower sex ratio on fruit set in pumpkin (*Cucurbita maxima*). *Scientia Horticulturae* **246**: 1005–1008.

Arathi HS, Smith TJ. 2023. Drought and temperature stresses impact pollen production and autonomous selfing in a California wildflower, *Collinsia heterophylla*. *Ecology and Evolution* **13**: e10324.

von Arx M. 2013. Floral humidity and other indicators of energy rewards in pollination biology. *Communicative & Integrative Biology* **6**: e22750.

von Arx M, Goyret J, Davidowitz G, Raguso RA. 2012. Floral humidity as a reliable sensory cue for profitability assessment by nectar-foraging hawkmoths. *Proceedings of the National Academy of Sciences, USA* **109**: 9471–9476.

Ashman TL. 2009. Sniffing out patterns of sexual dimorphism in floral scent. *Functional Ecology* **23**: 852–862.

Avila-Sakar G, Krupnick GA, Stephenson AG. 2001. Growth and resource allocation in *Cucurbita pepo* ssp. *texana*: effects of fruit removal. *International Journal of Plant Sciences* **162**: 1089–1095.

Barman M, Mitra A. 2021. Floral maturation and changing air temperatures influence scent volatiles biosynthesis and emission in *Jasminum auriculatum* Vahl. *Environmental and Experimental Botany* **181**: 104296.

Barman M, Tenhaken R, Dötterl S. 2023. A review on floral scents and pigments in cucurbits: their biosynthesis and role in flower visitor interactions. *Scientia Horticulturae* **322**: 112402.

Bazzaz FA. 1979. The physiological ecology of plant succession. *Annual Review of Ecology and Systematics* **10**: 351–371.

Bazzaz FA, Ackerly DD, Reekie EG. 2000. *Reproductive allocation in plants*. Wallingford, UK: CABI.

Brandt K, Machado IC, Navarro DMAF, Dötterl S, Ayasse M, Milet-Pinheiro P. 2020. Sexual dimorphism in floral scents of the neotropical orchid *Catasetum arietinum* and its possible ecological and evolutionary significance. *AoB Plants* **12**: plaa030.

Broberg MC, Hayes F, Harmens H, Uddling J, Mills G, Pleijel H. 2023. Effects of ozone, drought and heat stress on wheat yield and grain quality. *Agriculture, Ecosystems & Environment* **352**: 108505.

Brown MJ, Dicks LV, Paxton RJ, Baldock KC, Barron AB, Chauzat MP, Freitas BM, Goulson D, Jepsen S, Kremen C *et al.* 2016. A horizon scan of future threats and opportunities for pollinators and pollination. *PeerJ* **4**: e2249.

Burkle LA, Runyon JB. 2016. Drought and leaf herbivory influence floral volatiles and pollinator attraction. *Global Change Biology* **22**: 1644–1654.

Campbell DR, Sosenski P, Raguso RA. 2019. Phenotypic plasticity of floral volatiles in response to increasing drought stress. *Annals of Botany* **123**: 601–610.

Cane JH, Sampson BJ, Miller SA. 2011. Pollination value of male bees: the specialist bee *Peponapis pruinosa* (Apidae) at summer squash (*Cucurbita pepo*). *Environmental Entomology* **40**: 614–620.

Carroll AB, Pallardy SG, Galen C. 2001. Drought stress, plant water status, and floral trait expression in fireweed, *Epilobium angustifolium* (Onagraceae). *American Journal of Botany* **88**: 438–446.

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Centritto M, Lauteri M, Monteverdi MC, Serraj R. 2009. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. *Journal of Experimental Botany* **60**: 2325–2339.

Cheng S, Fu X, Mei X, Zhou Y, Du B, Watanabe N, Yang Z. 2016. Regulation of biosynthesis and emission of volatile phenylpropanoids/benzenoids in *Petunia* × *hybrida* flowers by multi-factors of circadian clock, light, and temperature. *Plant Physiology and Biochemistry* **107**: 1–8.

Clarke D, Morley E, Robert D. 2017. The bee, the flower, and the electric field: electric ecology and aerial electroreception. *Journal of Comparative Physiology A* **203**: 737–748.

Clarke KR, Gorley RN. 2006. *Primer v.6: user manual/tutorial (plymouth routines in multivariate ecological research)*. Plymouth, UK: PRIMER-E.

Cna'ani A, Mühlemann JK, Ravid J, Masci T, Klempien A, Nguyen TTH, Dudareva N, Pichersky E, Vainstein A. 2015. Petunia × hybrida floral scent production is negatively affected by high-temperature growth conditions. *Plant, Cell & Environment* **38**: 1333–1346.

Collison CH, Martin EC. 1979. Behaviour of honeybees foraging on male and female flowers of *Cucumis sativus*. *Journal of Apicultural Research* **18**: 184–190.

Cordeiro GD, Dötterl S. 2023. Global warming impairs the olfactory floral signaling in strawberry. *BMC Plant Biology* **23**: 549.

Dahake A, Jain P, Vogt CC, Kandalaft W, Stroock AD, Raguso RA. 2022. A signal-like role for floral humidity in a nocturnal pollination system. *Nature Communications* **13**: 7773.

Dellinger AS, Scheer LM, Artuso S, Fernández-Fernández D, Sornoza F, Penneys DS, Tenhaken R, Dötterl S, Schönenberger J. 2019. Bimodal pollination systems in Andean Melastomataceae involving birds, bats, and rodents. *The American Naturalist* **194**: 104–116.

Delph LF. 2019. Water availability drives population divergence and sex-specific responses in a dioecious plant. *American Journal of Botany* **106**: 1346–1355.

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

Dötterl S, Glück U, Jürgens A, Woodring J, Aas G. 2014. Floral reward, advertisement and attractiveness to honey bees in dioecious *Salix caprea*. *PLoS ONE* **9**: e93421.

Dötterl S, Jürgens A, Seifert K, Laube T, Weissbecker B, Schütz S. 2006. Nursery pollination by a moth in *Silene latifolia*: the role of odours in eliciting antennal and behavioural responses. *New Phytologist* **169**: 707–718.

Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J. 2017. Drought effect on plant biomass allocation: a meta-analysis. *Ecology and Evolution* **7**: 11002–11010.

Fruhwirth GO, Hermetter A. 2007. Seeds and oil of the Styrian oil pumpkin: components and biological activities. *European Journal of Lipid Science and Technology* **109**: 1128–1140.

Fruhwirth GO, Hermetter A. 2008. Production technology and characteristics of Styrian pumpkin seed oil. *European Journal of Lipid Science and Technology* **110**: 637–644.

Fu J, Hou D, Zhang C, Bao Z, Zhao H, Hu S. 2017. The emission of the floral scent of four *Osmanthus fragrans* cultivars in response to different temperatures. *Molecules* **22**: 430.

Fuchs R, Müller M. 2004. Pollination problems in Styrian oil pumpkin plants: can bumblebees be an alternative to honeybees? *Phyton* **44**: 155–165.

Gao L, Yu G, Hu F, Li Z, Li W, Peng C. 2021. The patterns of male and female flowers in flowering stage may not be optimal resource allocation for fruit and seed growth. *Plants* **10**: 2819.

Gehring J, Scoby J, Parsons M, Delph L. 2004. Whole-plant investment in nectar is greater for males than pollinated females in the dioecious plant *Silene latifolia*. *Evolutionary Ecology Research* **6**: 1237–1252.

Gfrerer E, Laina D, Gibernau M, Fuchs R, Happ M, Tolasch T, Trutschnig W, Hörger AC, Comes HP, Dötterl S. 2021. Floral scents of a deceptive plant are hyperdiverse and under population-specific phenotypic selection. *Frontiers in Plant Science* **12**: 719092.

Glenny WR, Runyon JB, Burkle LA. 2018. Drought and increased CO_2 alter floral visual and olfactory traits with context-dependent effects on pollinator visitation. *New Phytologist* **220**: 785–798.

Grison-Pigé L, Bessière J-M, Turlings TCJ, Kjellberg F, Roy J, Hossaert-McKey MM. 2001. Limited intersex mimicry of floral odour in *Ficus carica*. *Functional Ecology* **15**: 551–558.

Höfer RJ, Ayasse M, Kuppler J. 2021. Bumblebee behavior on flowers, but not initial attraction, is altered by short-term drought stress. *Frontiers in Plant Science* **11**: 564802.

Höfer RJ, Lindner T, Ayasse M, Kuppler J. 2023. Reduced seed set under water deficit is driven mainly by reduced flower numbers and not by changes in flower visitations and pollination. *Functional Ecology* **37**: 461–471.

Hu Z, Zhang H, Leng P, Zhao J, Wang W, Wang S. 2013. The emission of floral scent from *Lilium* 'siberia' in response to light intensity and temperature. *Acta Physiologiae Plantarum* **35**: 1691–1700.

Huang SQ, Tang LL, Sun JF, Lu Y. 2006. Pollinator response to female and male floral display in a monoecious species and its implications for the evolution of floral dimorphism. *New Phytologist* **171**: 417–424.

Hurd PD, Linsley EG, Michelbacher AD. 1974. *Ecology of the squash and gourd bee, Peponapis pruinosa, on cultivated cucurbits in California (Hymenoptera: Apoidea)*. Washington, DC, USA: Smithsonian Institution Press.

IPCC. 2022. Climate change 2022: impacts, adaptation and vulnerability. In: Pörtner HO, Roberts DC, Tignor MMB, Poloczanska E, Mintenbeck K, Alegría A, Craig M, Langsdorf S, Löschke S, Möller V *et al.*, eds. *Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK & New York, NY, USA: Cambridge University Press.

Ishii HS. 2006. Floral display size influences subsequent plant choice by bumble bees. *Functional Ecology* **20**: 233–238.

Juvany M, Munné-Bosch S. 2015. Sex-related differences in stress tolerance in dioecious plants: a critical appraisal in a physiological context. *Journal of Experimental Botany* **66**: 6083–6092.

Kawagoe T, Suzuki N. 2002. Floral sexual dimorphism and flower choice by pollinators in a nectarless monoecious vine *Akebia quinata* (Lardizabalaceae). *Ecological Research* **17**: 295–303.

Klein A-M, Vaissière BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society B: Biological Sciences* **274**: 303–313.

Kuppler J, Wieland J, Junker RR, Ayasse M. 2021. Drought-induced reduction in flower size and abundance correlates with reduced flower visits by bumble bees. *AoB Plants* **13**: plab001.

Lambrecht S, Dawson T. 2007. Correlated variation of floral and leaf traits along a moisture availability gradient. *Oecologia* **151**: 574–583.

Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell & Environment* **25**: 275–294.

Li P, Zhu Y, Song X, Song F. 2020. Negative effects of long-term moderate salinity and short-term drought stress on the photosynthetic performance of hybrid *Pennisetum*. *Plant Physiology and Biochemistry* **155**: 93–104.

Liu BB, Li M, Li QM, Cui QQ, Zhang WD, Ai XZ, Bi HG. 2018. Combined effects of elevated CO_2 concentration and drought stress on photosynthetic performance and leaf structure of cucumber (*Cucumis sativus* L.) seedlings. *Photosynthetica* **56**: 942–952.

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik P, Sohrabi Y. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Australian Journal of Crop Science* **4**: 580–585.

Miyashita K, Tanakamaru S, Maitani T, Kimura K. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and Experimental Botany* **53**: 205–214.

NDMC. 2024. *National drought mitigation center: types of drought.* [WWW document] URL https://drought.unl.edu/Education/DroughtIn-depth/TypesofDrought.aspx [accessed 1 February 2024].

Nepi M, Guarnieri M, Pacini E. 2001. Nectar secretion, reabsorption, and sugar composition in male and female flowers of *Cucurbita pepo*. *International Journal of Plant Sciences* **162**: 353–358.

Nepi M, Pacini E. 1993. Pollination, pollen viability and pistil receptivity in *Cucurbita pepo*. *Annals of Botany* **72**: 527–536.

Nonami H, Wu Y, Boyer JS. 1997. Decreased growth-induced water potential (a primary cause of growth inhibition at low water potentials). *Plant Physiology* **114**: 501–509.

Ollerton J, Winfree R, Tarrant S. 2011. How many flowering plants are pollinated by animals? *Oikos* **120**: 321–326.

Ors S, Ekinci M, Yildirim E, Sahin U. 2016. Changes in gas exchange capacity and selected physiological properties of squash seedlings (*Cucurbita pepo* L.)

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under well-watered and drought stress conditions. *Archives of Agronomy and Soil Science* **62**: 1700–1710.

Peruzzi L, Mancuso E, Gargano D. 2012. Males are cheaper, or the extreme consequence of size/age-dependent sex allocation: sexist gender diphasy in *Fritillaria montana* (Liliaceae). *Botanical Journal of the Linnean Society* **168**: 323–333.

Phillips BB, Shaw RF, Holland MJ, Fry EL, Bardgett RD, Bullock JM, Osborne JL. 2018. Drought reduces floral resources for pollinators. *Global Change Biology* **24**: 3226–3235.

Phillips BW, Gardiner MM. 2015. Use of video surveillance to measure the influences of habitat management and landscape composition on pollinator visitation and pollen deposition in pumpkin (*Cucurbita pepo*) agroecosystems. *PeerJ* **3**: e1342.

Pilon C, Oosterhuis DM, Ritchie GL, Paiva ÉAS. 2015. Photosynthetic efficiency and antioxidant activity of cotton under drought stress during early floral bud development. *American Journal of Experimental Agriculture* **9**: 1–13.

Potts SG, Imperatriz-Fonseca V, Ngo HT, Aizen MA, Biesmeijer JC, Breeze TD, Dicks LV, Garibaldi LA, Hill R, Settele J *et al.* 2016. Safeguarding pollinators and their values to human well-being. *Nature* **540**: 220–229.

Primack RB. 1987. Relationships among flowers, fruits, and seeds. *Annual Review of Ecology and Systematics* **18**: 409–430.

R Core Team. 2021. *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

Rachersberger M, Cordeiro GD, Schäffler I, Dötterl S. 2019. Honeybee pollinators use visual and floral scent cues to find apple (*Malus domestica*) flowers. *Journal of Agricultural and Food Chemistry* **67**: 13221–13227.

Raju AJS, Ezradanam V. 2002. Pollination ecology and fruiting behaviour in a monoecious species, *Jatropha curcas* L. (Euphorbiaceae). *Current Science* **83**: 1395–1398.

Rering CC, Franco JG, Yeater KM, Mallinger RE. 2020. Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. *Ecosphere* **11**: e03254.

Revanasidda BVV. 2019. Floral biology and pollination in *Cucumis melo* L., a tropical andromonoecious cucurbit. *Journal of Asia-Pacific Entomology* **22**: 215–225.

Rowe L, Gibson D, Bahlai CA, Gibbs J, Landis DA, Isaacs R. 2020. Flower traits associated with the visitation patterns of bees. *Oecologia* **193**: 511–522.

Rupp T, Oelschlägel B, Rabitsch K, Mahfoud H, Wenke T, Disney RHL, Neinhuis C, Wanke S, Dötterl S. 2021. Flowers of deceptive *Aristolochia microstoma* are pollinated by phorid flies and emit volatiles known from invertebrate carrion. *Frontiers in Ecology and Evolution* **9**: 658441.

Sagae M, Oyama-Okubo N, Ando T, Marchesi E, Nakayama M. 2008. Effect of temperature on the floral scent emission and endogenous volatile profile of *Petunia axillaris*. *Bioscience, Biotechnology, and Biochemistry* **72**: 110–115.

Saleh N, Scott AG, Bryning GP, Chittka L. 2007. Distinguishing signals and cues: bumblebees use general footprints to generate adaptive behaviour at flowers and nest. *Arthropod-Plant Interactions* **1**: 119–127.

Schaefer H, Renner SS. 2011. Cucurbitaceae. In: Kubitzki K, ed. *Flowering plants. Eudicots: sapindales, cucurbitales, myrtaceae.* Berlin, Heidelberg: Springer, 112–174.

Shuttleworth A, Johnson SD. 2009. The importance of scent and nectar filters in a specialized wasp-pollination system. *Functional Ecology* **23**: 931–940.

Soroye P, Newbold T, Kerr J. 2020. Climate change contributes to widespread declines among bumble bees across continents. *Science* **367**: 685–688.

Sowndhararajan K, Kim J-H, Song JE, Kim M, Kim S. 2020. Chemical components of male and female flowers of *Schisandra chinensis*. *Biochemical Systematics and Ecology* **92**: 104121.

Svensson GP, Okamoto T, Kawakita A, Goto R, Kato M. 2010. Chemical ecology of obligate pollination mutualisms: testing the 'private channel' hypothesis in the *Breynia–Epicephala* association. *New Phytologist* **186**: 995–1004.

Teixido AL, Valladares F. 2019. Heat and drought determine flower female allocation in a hermaphroditic Mediterranean plant family. *Plant Biology* **21**: 1024–1030.

Tepedino VJ. 1981. The pollination efficiency of the squash bee (*Peponapis pruinosa*) and the honey bee (*Apis mellifera*) on summer squash (*Cucurbita pepo*). *Journal of the Kansas Entomological Society* **54**: 359–377.

Vidal M, De Jong D, Wien H, Morse R. 2006. Nectar and pollen production in pumpkin (*Cucurbita pepo* L.). *Brazilian Journal of Botany* **29**: 267–273.

Waser NM, Price MV. 2016. Drought, pollen and nectar availability, and pollination success. *Ecology* **97**: 1400–1409.

Yang X, Wang B, Chen L, Li P, Cao C. 2019. The different influences of drought stress at the flowering stage on rice physiological traits, grain yield, and quality. *Scientific Reports* **9**: 3742.

See also the Commentary on this article by Buchmann & Papaj, **244**: 746–748.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Shoot length of Styrian oil pumpkin plants grown under control and drought conditions.

Dataset S2 Male and female bud count per control/drought-stressed Styrian oil pumpkin plant.

Dataset S3 Traits of male and female flowers of Styrian oil pumpkin plants grown under control and drought conditions.

Dataset S4 Peak area (TIC) of scent volatiles emitted from male and female flowers of control and drought-stressed Styrian oil pumpkin plants.

Dataset S5 Number of *Bombus terrestris* visits per female and male flower of control and drought-stressed Styrian oil pumpkin plants.

Dataset S6 Recurrent visit count of individually marked *Bombus terrestris* to specific male and female flowers of Styrian oil pumpkin plants.

Fig. S1 Soil moisture content and leaf stomatal conductance monitored from control and drought-stressed Styrian oil pumpkin plants.

Fig. S2 Representative GC chromatograms showing the most abundant scent compounds emitted from male flowers of control and drought-stressed Styrian oil pumpkin plants.

Methods S1 Soil moisture and leaf stomatal conductance measurements.

Table S1 Floral bud count per Styrian oil pumpkin plant (median value) during the (peak) flowering period.

Table S2 ANOVA table showing the results of generalized linear mixed models (GLMM) for the studied traits.

Table S3 Relative abundance of scent volatiles emitted from female and male flowers of control and drought-stressed Styrian oil pumpkin plants.

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