

ORIGINAL ARTICLE

Drought stress influences foraging preference of a solitary bee on two wildflowers

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- **Background and Aims** Pollinators provide critical ecosystem services, maintaining biodiversity and benefiting global food production. However, plants, pollinators and their mutualistic interactions can be affected by drought, which has increased in severity and frequency under climate change. Using two annual, insect-pollinated wildflowers (*Phacelia campanularia* and *Nemophila menziesii*), we asked how drought impacts floral traits and foraging preferences of a solitary bee (*Osmia lignaria*) and explored potential implications for plant reproduction.
- **Methods** In greenhouses, we subjected plants experimentally to drought to induce water stress, as verified by leaf water potential. To assess the impact of drought on floral traits, we measured flower size, floral display size, nectar volume and nectar sugar concentration. To explore how drought-induced effects on floral traits affected bee foraging preferences, we performed choice trials. Individual female bees were placed into foraging arenas with two conspecific plants, one droughted and one non-droughted, and were allowed to forage freely.
- **Key Results** We determined that *P. campanularia* is more drought tolerant than *N. menziesii*, based on measures of turgor loss point, and confirmed that droughted plants were more drought stressed than non-droughted plants. For droughted plants of both species, the floral display size was reduced and the flowers were smaller and produced less, more-concentrated nectar. We found that bees preferred non-droughted flowers of *N. menziesii*. However, bee preference for non-droughted *P. campanularia* flowers depended on the time of day and was detected only in the afternoon.
- **Conclusions** Our findings indicate that bees prefer to visit non-droughted flowers, probably reducing pollination success for drought-stressed plants. Lack of preference for non-droughted *P. campanularia* flowers in the morning might reflect the higher drought tolerance of this species. This work highlights the potentially intersecting, short-term physiological and pollinator behavioural responses to drought and suggests that such responses might reshape plant–pollinator interactions, ultimately reducing reproductive output for less drought-tolerant wildflowers.

Key words: bees, climate change, drought, flower size, foraging behaviour, nectar, *Nemophila menziesii*, *Osmia lignaria*, plant–pollinator interactions, pollination, pollinator preference, *Phacelia campanularia*.

INTRODUCTION

To predict the ecological consequences of global climate change, it is crucial to understand how changes in abiotic context affect interactions between species, particularly those interactions that provide essential ecosystem services (Kattenberg *et al.*, 1996; Cubasch *et al.*, 2001; Gilman *et al.*, 2010). In many terrestrial environments, climatic change is linked to reduced precipitation, or meteorological drought, and reduced water availability in soils, or soil moisture drought (Orlowsky and Seneviratne, 2013). Some regions might experience a 20 % increase in drought frequency by the year 2100 (Prudhomme *et al.*, 2013; Spinoni *et al.*, 2014), and the duration of droughts is expected to increase with global warming, owing, in part, to

increased evaporation of soil moisture with higher atmospheric temperatures (IPCC, 2014; Naumann *et al.*, 2018). Frequent, prolonged droughts can influence the incidence, strength and direction of species interactions by altering abundances, phenologies and traits of populations (Forrest, 2015; Phillips *et al.*, 2018; Kuppler *et al.*, 2021). Among species interactions impacted by drought, plant–pollinator mutualisms are important for the reproduction of most flowering plant species and for the human food supply (Klein *et al.*, 2007; Ollerton *et al.*, 2011).

Most immediately, drought can impact interactions between plants and pollinating insects by inducing water stress and altering floral traits (Brunet and Van Etten, 2019; Descamps *et al.*, 2021b; Kuppler and Kotowska, 2021). Drought can lead to both

short-term structural changes and physiological changes to floral signals and rewards that impact the ability of plants to attract pollinating insects (Bartlett *et al.*, 2014; Descamps *et al.*, 2021b; Kuppler and Kotowska, 2021). Structurally, plants exposed to drought tend to produce fewer and smaller flowers (Carroll *et al.*, 2001; Phillips *et al.*, 2018; Rering *et al.*, 2020). These structural changes, in combination with altered production of volatile organic compounds (Glenny *et al.*, 2018; Jaworski *et al.*, 2022), might, in turn, reduce attractiveness of drought-stressed plants to pollinators (Conner and Rush, 1996; Descamps *et al.*, 2018). Drought has also consistently been shown to decrease the volume of nectar produced by flowers and reduce the rate of visitation by pollinating insects (Carroll *et al.*, 2001; Phillips *et al.*, 2018; Brunet and Van Etten, 2019; Wilson Rankin *et al.*, 2020; Rering *et al.*, 2020). The impact of drought on overall plant function can be mediated by drought tolerance, or the ability to withstand drought (Bartlett *et al.*, 2012, 2014).

Drought-driven changes to floral traits might cause changes in pollinator behaviour that can ultimately affect the persistence of both plants and pollinators (Filazzola *et al.*, 2021). Changes in floral resource availability and quality have important implications for pollinator visitation and pollination success. For example, flowers of *Polemonium viscosum* (Polemoniaceae) that were 4–6 mm smaller in diameter were visited ~30 % less frequently by their primary pollinator, queen bumble bees, resulting in a 50 % reduction in seed set compared with larger flowers (Galen and Newport, 1987). In another example, corolla size, nectar volume and pollinator visitation peaked at intermediate water availability, while seed set increased linearly with increasing water availability (Descamps *et al.*, 2018). Nectar and pollen availability also vary over the course of a day, and the timing of peak nectar and pollen production varies among species (Herrera, 1990; Barp *et al.*, 2011).

Insect pollination can facilitate outcrossing by transferring pollen between plants, and the rate of outcrossing conferred by insect pollinators can be influenced by the type and abundance of pollinators, floral display size and plant population size (Horovitz and Harding, 1972; Fishbein and Venable, 1996; Herlihy and Eckert, 2004; Brunet and Sweet, 2006). This outcrossing helps to maintain the genetic variation within plant populations that is critical to plant adaptation in response to global climate change (Leimu *et al.*, 2010). Changes in floral traits might also alter pollinator preferences for co-flowering species, potentially favouring drought-tolerant species (Sargent and Ackerly, 2008). Drought-driven changes in floral traits might therefore modify which pollinators interact with which plant species (Burkle and Alarcón, 2011; CaraDonna *et al.*, 2017). If novel interactions are more generalized, as has been shown to occur with drought (Morozumi *et al.*, 2022), rates of heterospecific pollen transfer might increase and further reduce seed set (Moraes and Traveset, 2008; Ashman and Arceo-Gómez, 2013; Arceo-Gómez *et al.*, 2016).

Populations of insect pollinators might also be negatively affected by the effects of drought on flowering plants. Lower availability of resources such as pollen and nectar can diminish the reproductive output of insect pollinators, especially bees that rely on these floral resources to provision their offspring (Vaudo *et al.*, 2015; Wilson Rankin *et al.*, 2020). For example, bumble bee foragers fed nectar reflecting the sugar content of

optimally watered flowers of *Trifolium willdenovii* (Fabaceae) lived 60 % longer than those fed nectar reflecting the sugar content of droughted flowers (Wilson Rankin *et al.*, 2020). However, many pollinators, including honeybees and solitary bees, can learn which species and individuals of plants provide the most resources, enabling a shift in behaviour in response to resource limitation (Amaya-Márquez *et al.*, 2008; Simcock *et al.*, 2014, 2018). Some bees, including solitary megachilid bees, can retain learned information for ≤ 3 h; thus, exposure to flowers in the morning can affect foraging preferences in the afternoon (Amaya-Márquez *et al.*, 2008).

Although the influence of drought on plant–pollinator interactions is a topic of growing concern (Waser and Price, 2016; Gallagher and Campbell, 2017; Descamps *et al.*, 2021b), no studies have focused on the foraging responses of solitary bees to drought-induced changes in the floral traits of annual plants. Yet, climate change might have pronounced effects on the population dynamics and persistence of annual plants and solitary insect pollinators (Fitter and Fitter, 2002; Biesmeijer *et al.*, 2006), making it important to understand their physiological and behavioural responses to drought. Crucially, the impacts of drought might be more pronounced for annual plants, because perennial plants are able to store resources from previous years to jump-start growth in drought conditions (Gallagher and Campbell, 2017). Furthermore, the life-history strategies and more specialized diets of solitary bees might mean that they are more vulnerable to climatic shifts than social bees (Radmacher and Stroh, 2011; Fliszkiewicz *et al.*, 2012; Menzel and Feldmeyer, 2021).

Here, we used two annual wildflower species and one solitary bee species that pollinates them to test how drought stress affects floral traits and, in turn, pollinator preference and visitation. We subjected plants experimentally to water limitation in greenhouses and asked: (1) how does drought stress affect floral traits, such as flower size and nectar volume; (2) do bees exhibit preferences for the flowers of droughted vs. non-droughted plants; and (3) are pollinator preferences mediated by plant drought tolerance or time of day?

MATERIALS AND METHODS

Study species

Our focal plant species were *Phacelia campanularia* (Boraginaceae) and *Nemophila menziesii* (Boraginaceae). These annual, spring-blooming species are native to Southern California: *P. campanularia* occurs in deserts of CA and AZ, and *N. menziesii* occurs throughout the western USA (USDA Plants Database, 2023). These species (and/or congeners) are self-compatible, but their floral morphologies and developmental timing promote outcrossing [through physical separation of anthers and stigmas (herkogamy) and temporal separation of their maturation (dichogamy)], and within-flower selfing in the absence of pollinators (autonomous autogamy) results in low seed set (Gillett, 1961; Cruden, 1972). *Nemophila menziesii* is gynodioecious (has female plants and hermaphroditic plants with perfect flowers), and female plants produce sterile pollen (Cruden, 1972; McCall, 2008). Hermaphroditic *N. menziesii* flowers are protandrous: anthers mature shortly after the flower opens, then stigmas become receptive 2 days later (Cruden, 1972).

We chose these species in part because we expected that they would differ in drought tolerance, because the range of *P. campanularia* includes the Mojave and Sonoran deserts.

The focal insect species used in choice trials was *Osmia lignaria* (Megachilidae), a solitary, cavity-nesting bee that is native to western North America (Williams and Tepedino, 2003; Haider et al., 2014) and is known to visit flowers of both of our focal plant species in natural communities (Boyle et al., 2020) and to pollinate them effectively in experimental settings (de Manincor et al., 2023). Furthermore, *O. lignaria* is an important commercial pollinator of spring-blooming orchard crops, increasing the importance of understanding its response to changes in floral traits (Torchio and Asensio, 1985; Peterson and Artz, 2014; Pitts-Singer et al., 2018).

Plant preparation

We germinated seeds of *P. campanularia* and *N. menziesii* in peat pellets in greenhouses in Riverside, CA, USA in 2021 and 2022. In 2021, our goals were to measure the drought tolerances of our focal species and to test whether drought affected their floral traits. In 2022, we again quantified the effects of drought on floral traits, but our main goal was to determine whether bees preferred to visit non-droughted flowers. Seeds were purchased from a local nursery (Theodore Payne Foundation) and were sourced from San Diego County, CA, USA. Once seedlings had two true leaves, we planted them in 1-L pots filled with a 1:1 mix of potting soil (Sunshine Professional Mix 1, SunGro, Los Angeles, CA, USA) and coarse sand. Plants were watered with 125 mL every 2 days for 4 weeks, then randomly assigned to droughted and non-droughted treatments. In 2021, non-droughted plants were watered with 125 mL every 2 days and droughted plants were watered with 125 mL only when the soil moisture of a randomly selected subset of droughted plants was <10 % volumetric water content. In 2022, non-droughted plants were watered with 125 mL daily and droughted plants were watered with 125 mL every 2 days to reflect the approximate watering scheme of 2021 and reduce overall plant mortality. Throughout the experiment, both droughted and non-droughted plants were watered at the same time of day.

Plant traits

With our 2022 plant cohort, we quantified drought tolerance as leaf turgor loss point (π_{up}), for which more negative values indicate greater drought tolerance. Turgor loss point is the value of water potential at which leaves lose turgor, a metric useful for comparing drought tolerance among species (Bartlett et al., 2012). To determine the turgor loss point of each species, we collected three leaf samples from three plants and measured leaf osmotic potential using a vapour pressure osmometer (Wescor Vapro model 5600; Logan, UT, USA) set on 'delay' mode until measurements equilibrated for at least five steps (Bartlett et al., 2012). Values for π_{up} were calculated using the linear regression equation provided by Bartlett et al. (2012). To quantify plant water status, at 0700 and 1300 h we measured leaf water potential from five randomly selected plants of each species per treatment. We used a pressure chamber (model 1000; PMS Instrument Company, Albany, OR, USA) to determine the leaf

water potential of these leaves as the balancing pressure, for which more negative values indicate reduced tissue hydration (Rodríguez-Domínguez et al., 2022).

For both of our 2021 and 2022 cohorts, we recorded the day of first flowering and monitored plants every 2 days until flowering ceased. We measured floral traits daily on a subset of five droughted and five non-droughted plants of each species in 2021, and a subset of five to ten droughted and five to ten non-droughted plants of each species in 2022. We counted the open flowers on these plants and measured floral traits for one or two flowers per plant, which were chosen randomly and marked with paper tags on strings to prevent repeated measurement. We measured flower size, including measurements at the widest diameter of each flower, the diameter at a right angle to this measurement and the corolla length measured parallel to the petiole from the base of the corolla to the longest petal, the nectar volume and nectar sugar concentration. We used flower size measurements to calculate the landing area of flowers as the product of the two diameter measures (Knauer and Schiestl, 2015). Nectar volume was measured using microcapillary tubes (Microcaps; Drummond Scientific Co., Broomall, PA, USA), and nectar concentration was measured using hand-held optical refractometers (Eclipse Optical Refractometer; Bellingham + Stanley, Nottingham, UK). In 2022, we measured nectar volume and concentration twice per day (morning and afternoon) for the same flowers, to quantify the nectar that was refilled in flowers before their use in afternoon choice trials. To calculate the caloric value of nectar on a per-flower basis, we used the following equation: $y = 0.00226 + 0.00937x + 0.0000585x^2$, where x is the percentage sugar concentration and y the grams of sugars in 1 μ L of nectar (Bolten et al., 1979; Dafni et al., 2005; Lange et al., 2017). We then multiplied y by the total volume of nectar (in microlitres), then by four to convert to total calories, because sugar has 4 calories g^{-1} (Dafni et al., 2005).

Pollinator preference

To measure pollinator preference via choice trials, we used female *O. lignaria* (Mountain West Mason Bees, UT, USA). In April 2022, diapausing adult bees in cocoons were stored at 4 °C, then warmed to 20 °C over 24 h to trigger emergence. All bees were used in choice trials within 14 h post-emergence to attempt to standardize starvation time. For each choice trial, we placed one emerged bee inside each mesh foraging arena (5.8 m³; EVEN Naturals, Boston, MA, USA; or 4.6 m³; BugDorm, Taichung, Taiwan) at 0800 h. The number of trials performed per day ranged from four to 14, depending on the number of bees that emerged. We selected plants from our 2022 cohorts that had been flowering for 14–21 days, because this represented the point at which plants were producing more than five flowers on average. We then measured traits and enclosed all but five flowers per plant in mesh drawstring bags to standardize the number of flowers accessible to bees and to control for differences in floral display size. For morning choice trials, we placed one droughted and one non-droughted plant of the same species into a foraging arena, randomizing the cardinal directions of plants in the cage, then observed the bee for 10 min, recording the time spent foraging for nectar or pollen on flowers of droughted and non-droughted plants. We defined foraging

for nectar as bees using their proboscis to probe and extract nectar for ≥ 1 s. We defined foraging for pollen as bees using their legs to attach pollen to the scopae located on the underside of their abdomens. Before the 10 min observation period, bees were moved to the floor of the foraging arena to standardize their starting position. Plants remained in the arenas, except for a 10 min interval in the afternoon when they were removed in order that a second set of floral trait measurements could be made. We then performed a second afternoon 10 min observation of the same bee in a foraging arena with the same plants. We performed a total of 155 successful choice trials, wherein bees foraged on flowers at some time during the observation period, using 90 naive female *O. lignaria*. We performed 28 morning and 27 afternoon trials on *N. menziesii* and 53 morning and 47 afternoon trials on *P. campanularia*.

Data analysis

All analyses were performed in R (R Core Team, 2023). To compare the turgor loss point between species, we used a linear model, with log-transformed turgor loss point values as the response variable and plant species as the predictor variable. Aside from the analysis of turgor loss point, all analyses were conducted separately for each plant species. Baseline models for all other plant traits included watering treatment and, when applicable, time of day (morning vs. afternoon) and the interaction between treatment and time of day as predictors. We performed model selection using likelihood ratio tests (R package lmerTest; Zeileis and Hothorn, 2002). For all analyses for which baseline models included multiple predictors, we report the best-fitting models in Table 1. When the best-fitting model included the interaction between treatment and time of day, we used estimated marginal means calculated using the interaction of predictors with the functions *emmeans* and *contrast* to compute the contrasts among predictors (R package emmeans; Lenth, 2023). Outliers were hidden in figures to improve readability by setting *outlier.shape = NA* in the package *ggplot2* (Wickham, 2016).

To compare the water potential between droughted and non-droughted plants, we used linear mixed-effects models (LMMs), with water potential as the response variable and plant identity as a random effect because plant water potential was measured once in the morning and again in the afternoon. Any water potential measurements that were below the turgor loss point were interpreted as measurements on wilted leaves for that species and indicate severe, potentially life-threatening drought stress (Tyree and Hammel, 1972).

To test for differences in the number of flowers, we used generalized linear mixed-effects models (GLMMs), with flower counts per plant per day, modelled with Poisson error distribution, as the response (R package glmmTMB; Brooks et al., 2017). For flower size, we fitted LMMs using flower landing area and corolla length as response variables and plant identity as a random effect, because multiple flowers were measured per plant. For nectar volume and calories, we fitted LMMs using log+1-transformed nectar volume and log+1-transformed nectar calories as response variables because logarithmic transformation improved the normality of the model residuals and because adding one allowed us to logarithmically transform the zeros in our data. For nectar concentration, we used beta

regressions with a logit-link function because concentration varied between zero and one. Across all nectar analyses, we used plant identity as a random effect.

To determine whether bees exhibited a preference for non-droughted flowers in morning or afternoon choice trials, we calculated preference as the percentage of time that bees spent foraging on non-droughted flowers out of their total time spent foraging within a single observation period. We fitted LMMs with this preference metric as the response variable, centred around zero such that no preference was equal to zero, preference for non-droughted plants was positive, and preference for droughted plants was negative. For these LMMs, we designated time of day as the predictor variable, bee identity as a random effect, and the intercept was forced through zero. We forced the intercept through zero to determine whether time spent on non-droughted flowers was significantly different from zero, which indicated no preference. To determine whether preference differed between morning and afternoon choice trials, we fitted LMMs with bee preference as the response variable, time of day as the predictor variable and bee identity as a random effect.

RESULTS

Plant traits

Turgor loss point differed between our study species ($F_{1,4} = 20.07$, $P = 0.01$), with *N. menziesii* showing less negative and relatively drought-intolerant values [turgor loss point: -0.68 ± 0.10 MPa (mean \pm s.d.)] and *P. campanularia* showing more negative and relatively drought-tolerant values (-1.20 ± 0.13 MPa; Supplementary Data Fig. S1). Droughted *N. menziesii* and *P. campanularia* plants had significantly lower leaf water potential in morning measurements than non-droughted plants and similar water potential in afternoon measurements (Table 1; Supplementary Data Fig. S2). In *P. campanularia*, 33.8 and 16.4 % of water potential measurements on droughted and non-droughted plants, respectively, were below the turgor loss point. In *N. menziesii*, 63.8 and 45.0 % of water potential measurements on droughted and non-droughted plants, respectively, were below the turgor loss point.

In 2022, droughted plants of both *N. menziesii* and *P. campanularia* had 61 and 75 % fewer flowers, respectively, than non-droughted plants (Table 1; Fig. 1). Droughted *N. menziesii* and *P. campanularia* produced flowers with 26 and 30 % shorter corollas (Table 1; Fig. 2) and 55 and 39 % smaller landing areas, respectively (Table 1; Supplementary Data Fig. S3). Similar patterns were found in 2021 (Supplementary Data Table S1; Figs S3, S4).

Watering treatment, time of day and their interaction had significant effects on the nectar volume of *N. menziesii* flowers (Table 1; Fig. 3). In *P. campanularia*, only the interaction of watering treatment and time of day had a significant effect on nectar volume (Table 1; Fig. 3). In *N. menziesii* only, droughted plants produced 75 % less morning nectar than non-droughted plants ($t_{152} = -8.46$, $P < 0.0001$; Fig. 3). Droughted *N. menziesii* and *P. campanularia* produced 89 and 61 % less afternoon nectar refill, respectively, than non-droughted plants ($t_{152} = -2.79$, $P < 0.01$ and $t_{279} = -4.37$, $P < 0.001$, respectively; Fig. 3).

TABLE 1. Best-fitting model results for plant traits and pollinator preference.

Response	Distribution	Species	Predictor	Estimate	s.e.	z	P-value	n
Water potential	Gaussian	<i>N.m.</i>	Watering treatment	-0.08	0.03	-3.23	0.001**	106
		<i>P.c.</i>	Watering treatment	-0.23	0.07	-3.36	0.0008***	166
			Time of day	-0.16	0.07	-2.41	0.02*	
			Watering treatment × time of day	0.19	0.09	2.07	0.04*	
Number of flowers	Poisson	<i>N.m.</i>	Watering treatment	-0.95	0.05	-19.85	<0.00001***	68
		<i>P.c.</i>	Watering treatment	-1.37	0.04	-38.37	<0.00001***	153
Flower landing area	Gaussian	<i>N.m.</i>	Watering treatment	-379.15	36.41	-10.41	<0.00001***	80
		<i>P.c.</i>	Watering treatment	-179.48	16.66	-10.77	<0.00001***	151
Corolla length		<i>N.m.</i>	Watering treatment	-1.62	0.45	-3.59	0.0003***	80
		<i>P.c.</i>	Watering treatment	-4.26	0.51	-8.41	<0.00001***	151
Nectar volume		<i>N.m.</i>	Watering treatment	-0.21	0.03	-8.46	<0.00001***	158
			Time of day	-0.21	0.02	-8.63	<0.00001***	
			Watering treatment × time of day	0.14	0.03	4.15	0.00003***	
		<i>P.c.</i>	Watering treatment	0.13	0.11	1.16	0.25	285
			Time of day	-0.02	0.11	-0.15	0.89	
			Watering treatment × time of day	-0.62	0.15	-4.10	0.00004***	
Nectar concentration	Beta with logit link	<i>N.m.</i>	Watering treatment	0.14	0.15	0.97	0.33	95
			Time of day	-0.57	0.17	-3.38	0.0007***	
			Watering treatment × time of day	0.57	0.29	1.99	0.047*	
		<i>P.c.</i>	Watering treatment	0.31	0.12	2.67	0.008**	187
			Time of day	-1.40	0.13	-10.69	<0.00001***	
			Watering treatment × time of day	0.75	0.25	2.97	0.003**	
Nectar calories	Gaussian	<i>N.m.</i>	Watering treatment	-0.53	0.05	-11.59	<0.00001***	146
			Time of day	-0.47	0.05	-9.66	<0.00001***	
			Watering treatment × time of day	0.34	0.06	5.28	<0.00001***	
		<i>P.c.</i>	Watering treatment	-0.48	0.14	-3.49	0.0005***	256
			Time of day	0.33	0.14	2.35	0.02*	
			Watering treatment × time of day	-0.76	0.20	-3.70	0.0002***	
Bee preference		<i>N.m.</i> , AM [†]	Time of day	0.25	0.05	4.69	<0.00001***	28
		<i>N.m.</i> , PM [†]	Time of day	0.27	0.06	4.92	<0.00001***	27
		<i>P.c.</i> , AM [†]	Time of day	0.07	0.04	1.71	0.09	53
		<i>P.c.</i> , PM [†]	Time of day	0.27	0.05	5.79	<0.00001***	47

Abbreviations: *N.m.*, *Nemophila menziesii*; *P.c.*, *Phacelia campanularia*.

Plant identity was used as a random effect in all models with plant traits as the responses, and bee identity was used as a random effect in the models with bee preference as the response. *P*-values of less than 0.05 are in bold.

P* < 0.05, *P* < 0.01, ****P* < 0.001.

[†]Time of day was used as a predictor in these models, but standard error, *z*-score, *P*-values and sample sizes are shown separately for each time of day.

Time of day and its interaction with watering treatment had a significant effect on nectar concentration in *N. menziesii* (Table 1; Fig. 4). In *P. campanularia*, watering treatment, time of day and their interaction had a significant effect on nectar concentration (Table 1; Fig. 4). In *P. campanularia*, morning and afternoon nectar sugar concentration was 14 and 118 % higher, respectively, in droughted plants ($t_{181} = 2.86$, $P < 0.01$ and $t_{181} = 4.67$, $P < 0.001$; Fig. 4). In *N. menziesii*, refill nectar was 38 % more concentrated in droughted than in non-droughted plants ($t_{89} = 2.86$, $P < 0.01$; Fig. 4).

In both *N. menziesii* and *P. campanularia*, watering treatment, time of day and their interaction significantly impacted the calories available in nectar (Table 1; Fig. 5). In *N. menziesii*,

droughted plants produced 71 % fewer nectar calories in their morning nectar ($t_{140} = -9.66$, $P < 0.001$; Fig. 5). In *P. campanularia*, droughted plants produced 57 % fewer calories in their afternoon nectar ($t_{250} = -2.73$, $P < 0.01$; Fig. 5). Similar patterns in nectar volume, concentration and calories were found in 2021 (Supplementary Data Table S1; Figs S5–S7).

Pollinator preference

When presented with droughted and non-droughted flowers of *N. menziesii*, bees spent significantly more of their total time foraging on flowers of non-droughted plants in both the morning and afternoon, spending 75 and 77 % of their time on

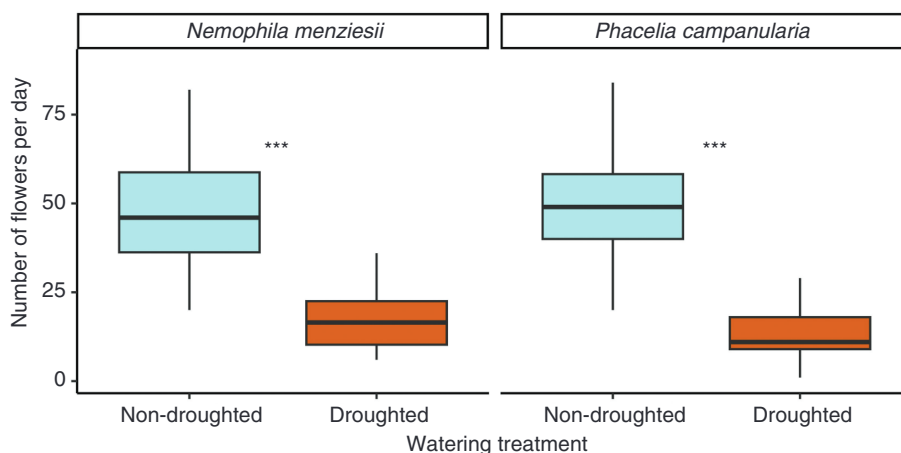


FIG. 1. Number of open flowers per plant on a given day for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions. Asterisks indicate significance level (***) $P < 0.001$. Outliers were hidden in the figure to improve its readability. Boxes display the median as the centre line, first and third quartiles as the lower and upper hinges, and 1.5 times the interquartile range as the whiskers.

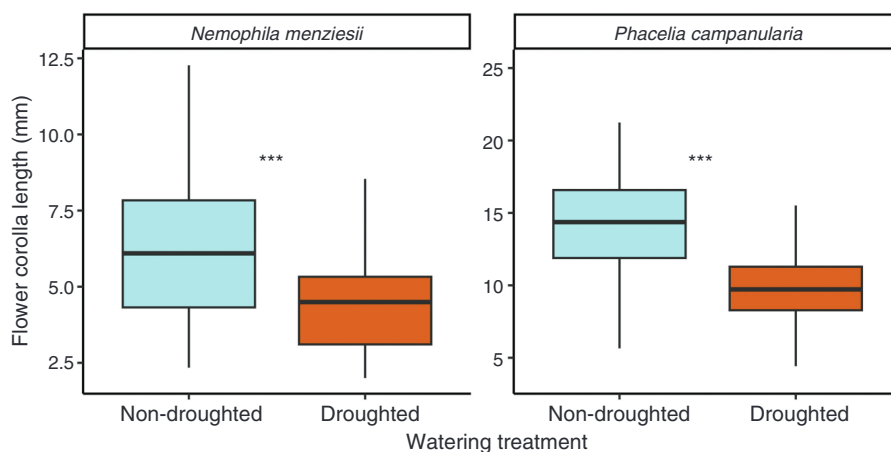


FIG. 2. Corolla length for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions. Asterisks indicate significance level (***) $P < 0.001$. Outliers were hidden in the figure to improve its readability. Boxes display the median as the centre line, first and third quartiles as the lower and upper hinges, and 1.5 times the interquartile range as the whiskers.

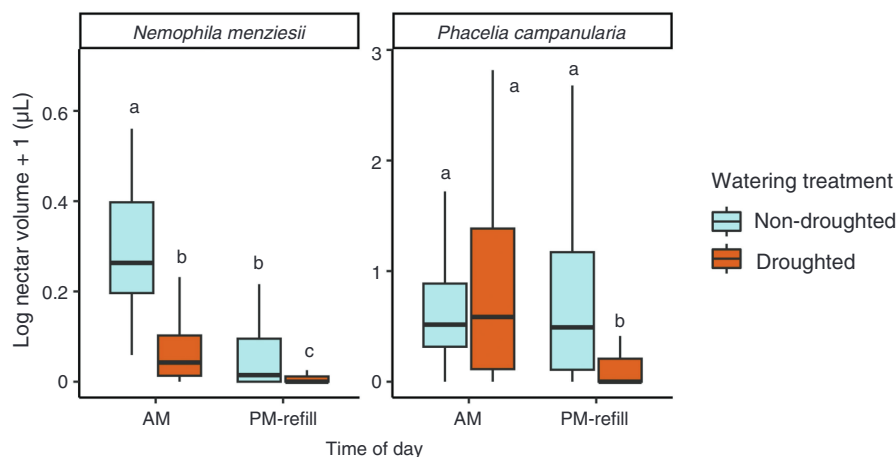


FIG. 3. Log+1-transformed nectar volume per flower by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were hidden in the figure to improve its readability. Boxes display the median as the centre line, first and third quartiles as the lower and upper hinges, and 1.5 times the interquartile range as the whiskers.

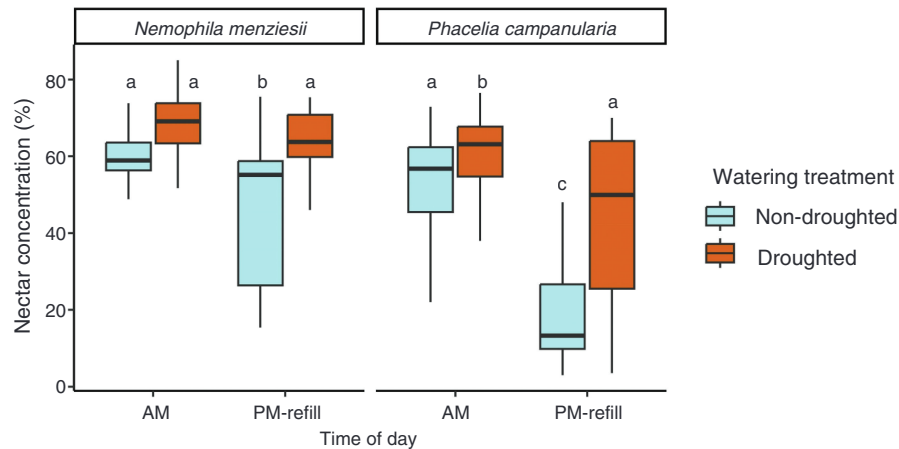


FIG. 4. Nectar sugar concentration by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were hidden in the figure to improve its readability. Boxes display the median as the centre line, first and third quartiles as the lower and upper hinges, and 1.5 times the interquartile range as the whiskers.

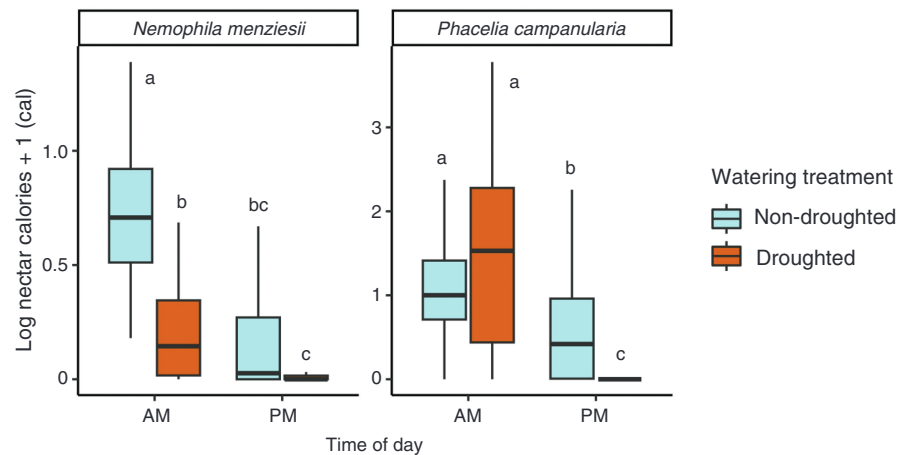


FIG. 5. Log+1-transformed nectar calorie content per flower by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Outliers were hidden in the figure to improve its readability. Boxes display the median as the centre line, first and third quartiles as the lower and upper hinges, and 1.5 times the interquartile range as the whiskers.

non-droughted flowers in the morning and afternoon, respectively (Table 1; Fig. 6). This preference did not differ between morning and afternoon observations ($t_{51} = 0.27$, $P = 0.79$; Fig. 6). However, for *P. campanularia*, preference differed between morning and afternoon: time spent foraging on non-droughted plants increased from 57 % in morning foraging bouts to 77 % in afternoon foraging bouts ($t_6 = 3.37$, $P < 0.01$; Fig. 6). Although there was a marginally significant preference for non-droughted flowers in the morning, the afternoon foraging preference for non-droughted flowers was significantly higher than 50 % (Table 1; Fig. 6).

DISCUSSION

Our results demonstrate that drought can alter floral traits and thereby shape pollinator visitation, because bees preferred to visit flowers on non-droughted plants of both wildflower species. These results indicate that the increased prevalence of droughts associated with climate change might alter the

pollination success of drought-stressed plants and, ultimately, the ecology of their interactions with pollinators.

Plant traits

The differences in the turgor loss point of our focal species are likely to reflect adaptations to different environments (Bartlett et al., 2014). The relatively drought-vulnerable *N. menziesii* is found in regions with higher soil moisture and average annual precipitation, whereas *P. campanularia* is found in more arid regions (Calflora, 2023). For both our focal wildflower species, our drought treatment decreased leaf water potential effectively. Lower water potential in afternoon vs. morning was probably driven by higher transpiration rates in warmer conditions (Chapin, 1995; Carrol et al., 2001).

Drought stress resulted in the production of fewer and smaller flowers in both our study species, an effect that has been documented in field and greenhouse studies (Gallagher and Campbell, 2017; Descamps et al., 2018; Brunet and Van Etten,

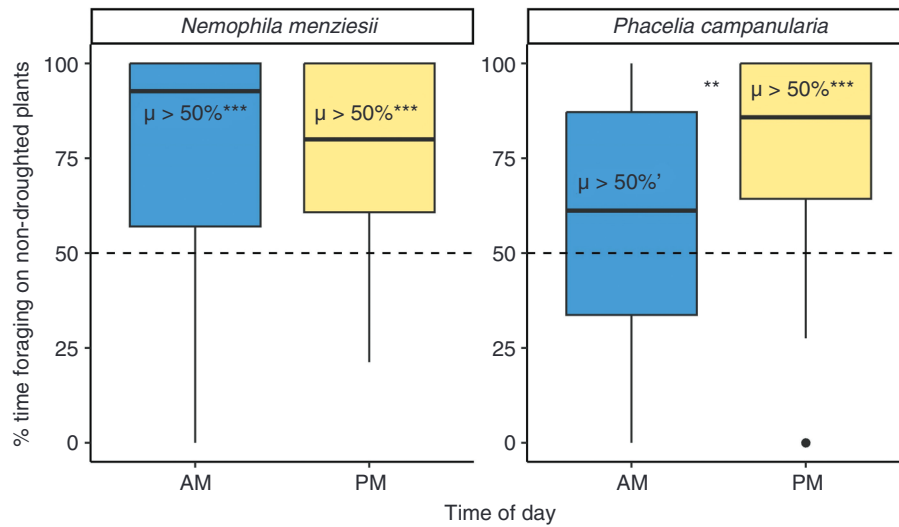


FIG. 6. Percentage of time spent by *Osmia lignaria* foraging on non-droughted *Nemophila menziesii* and *Phacelia campanularia* plants out of total foraging time when presented with both non-droughted and droughted plants of each species in the morning and afternoon. A value of $\mu > 50\%$ and the associated significance level indicates whether bees demonstrated a preference for non-droughted flowers on that plant species at that time of day. Asterisks indicate significance level ($^*P < 0.09$, $^{**}P < 0.01$, $^{***}P < 0.001$). Outliers were hidden in the figure to improve its readability. Boxes display the median as the centre line, first and third quartiles as the lower and upper hinges, and 1.5 times the interquartile range as the whiskers.

2019; Kuppler *et al.*, 2021). Reduced flower production and size under drought are likely to be driven by reduced photosynthetic capacity (Descamps *et al.*, 2021a), which leads to lower availability of photosynthetically derived sugars that can be fixed into reproductive tissues (De Souza *et al.*, 1997). Because flowers have high water costs, producing fewer flowers under drought stress might be adaptive (De la Barrera and Nobel, 2004). In addition, more water is needed to retain turgor pressure in flowers with larger corollas, owing to lower cell wall density (Galen *et al.*, 1999). Flowering can therefore decrease rates of photosynthesis further by inducing stomatal closure in leaves, incurring carbon costs to water-stressed plants (Galen *et al.*, 1999). Thus, a reduction in flower size and number confers benefits to plants that are water stressed (De la Barrera and Nobel, 2004).

In general, droughted plants also produced flowers with less nectar. The often-observed negative effect of drought on nectar volume (Gallagher and Campbell, 2017; Descamps *et al.*, 2018; Brunet and Van Etten, 2019; Rering *et al.*, 2020) is likely to be linked to reduced water availability within the plant (Grant, 2012). Although we found that nectar in droughted flowers tended to be more concentrated in sugars, flowers produced by non-droughted plants generally provided more total calories from nectar. These results are consistent with many studies (Descamps *et al.*, 2018, 2021a; Brunet and Van Etten, 2019; but see Clearwater *et al.*, 2018 and Phillips *et al.*, 2018, who found no effect of drought on nectar production) and were probably driven by simultaneously decreased amounts of water diverted to nectar and decreased sugars produced through photosynthesis in our droughted plants (De Souza *et al.*, 1997; Carroll *et al.*, 2001). Interestingly, morning nectar volume and sugar concentration were not significantly different between droughted and non-droughted flowers of *P. campanularia*, a result that probably reflects higher drought tolerance. Although droughted *P. campanularia* plants had significantly lower leaf

water potential, drought-adapted plants can prioritize water transport to reproductive structures (Sun *et al.*, 2020; Harrison Day *et al.*, 2022). Altogether, the short-term responses we detected in nectar traits are likely to modify pollinator attraction and visitation patterns (Kuppler *et al.*, 2021; Wilson Rankin *et al.*, 2020).

Pollinator preference

Bees preferred non-droughted plants, spending significantly more time in the morning and afternoon foraging on non-droughted flowers of our less drought-tolerant study species, *N. menziesii*. In our more drought-tolerant species, *P. campanularia*, bees showed a significant preference for non-droughted flowers only in afternoon foraging bouts. These findings indicate that female *O. lignaria* tend to visit more nectar-rich flowers preferentially, as has been found for various other pollinators (Best and Bierzychudek, 1982; Cresswell, 1990; Höfer *et al.*, 2021). For example, drought decreased bumble bee foraging time on *Sinapis arvensis* (Brassicaceae), probably owing to a decrease in nectar availability (Höfer *et al.*, 2021).

Nectar is the main source of sugars for bees (Nicolson, 2007), and female *O. lignaria* rely heavily on nectar for their own metabolic needs and for provisioning their offspring (Williams, 1999; Bosch and Kemp, 2004). Although within-species foraging preference by *O. lignaria* has not, to our knowledge, previously been linked to nectar volume and sugar concentration, higher nectar volumes and sugar content are associated with more visits to single plant species by bumble bees (Blarer *et al.*, 2002; Roldan-Serrano and Guerra-Sanz, 2005) and honey bees (Silva and Dean, 2000; Mallinger and Prasifka, 2017). We did not measure sugar composition, but drought can also decrease the ratio of sucrose to other sugars in nectar (Rering *et al.*, 2020), an effect that might also have

influenced bee foraging preferences (Abrahamczyk *et al.*, 2017). Likewise, we did not quantify emissions of floral volatile organic compounds, but these can shape the responses of some pollinators to droughted flowers (Campbell *et al.*, 2019; Rering *et al.*, 2020; Keefover-Ring *et al.*, 2022), and *O. lignaria* females are known to use olfactory cues to locate their nesting sites (Guédot *et al.*, 2006).

Flower size also shapes visitation by insect pollinators (Kuppler *et al.*, 2021), and the effects of our drought treatment on flower size might also explain the preference of *O. lignaria* for non-droughted flowers. For example, in flight cage experiments, bumble bees made fewer visits to flowers of an annual wildflower grown in low soil moisture conditions, probably because the bees were less attracted by, and less able to handle, small flowers (Kuppler *et al.*, 2021).

Learning might have influenced the strength of preference for non-droughted flowers in our bees, particularly in the afternoon foraging bouts, when bees had been exposed to flowers for 3–5 h. *O. lignaria* can remember the colour of flowers that provide a greater volume of nectar reward for ≤ 3 h (Amaya-Márquez *et al.*, 2008). Likewise, bumble bees can remember the location of the most profitable flower patches (Cartar, 2004) and can learn to visit plants with fewer flowers in exchange for more nectar per visit (Makino and Sakai, 2007). There is some evidence that as pollinators forage, they might learn to prefer flowers with ‘honest signals’ that accurately reflect their resource availability (Knauer and Schiestl, 2015). Therefore, the bees in our experiment might have learned to associate larger flowers with larger floral rewards, because these traits tended to covary in our experiment.

Consequences for pollination

In this study, we exposed plants to a simulated drought. Although droughts are likely to increase in the southwestern USA with rising temperatures (Seager and Hoerling, 2014), the predicted intensity of drought remains uncertain (Deser *et al.*, 2014). However, our experimental design, which in 2022 reduced total water by 50 %, is likely to represent a realistic decrease in precipitation during drought years. For example, precipitation was 57 % lower in 2014, a year of intense drought, in our study area of Riverside County, CA, USA (12.4 cm in 2014 vs. 21.7 cm annual average; NOAA, 2023). Furthermore, field-based observational studies have found similar reductions in floral abundance and nectar production, in addition to impacts on pollinator behaviour (Thomson, 2016; Waser and Price, 2016). For example, lower floral abundance driven by drought and low spring rainfall might have played a role in lower bumble bee abundance in a Central California coastal scrub community (Thomson, 2016). Thus, the influence of experimental drought on interactions between our focal bee and plants might also extend to a field setting.

Although both our focal plant species are capable of autonomous autogamy, herkogamy and dichogamy limit within-flower selfing, and autonomously autogamous fruits yield few seeds (Gillett, 1961; Cruden, 1972; de Manincor *et al.*, 2023). Thus, adequate seed production is reliant on pollination by animals, which might be disrupted if pollinators are less likely to visit the flowers of droughted populations. However, this study isolated

the interaction of a single bee and plant species. *O. lignaria* can forage ≤ 600 m and thus might have access to a wide array of resources provided by multiple flowering species (Kraemer *et al.*, 2014). Therefore, co-flowering of species with different drought tolerances could play a crucial role in mediating the behaviour of *O. lignaria* in response to climate change-driven shifts in floral traits (Sargent and Ackerly, 2008; Araya *et al.*, 2011). For example, in a greenhouse study, the negative effects of drought on pollinator visitation to *Potentilla recta* (Rosaceae) were 20 % stronger when plants were grown with other species than when *P. recta* individuals were grown alone, possibly owing to an increase in competition among plants (Glenny *et al.*, 2018). This suggests that by ignoring interspecific competition, our study could underestimate the effects of drought on pollinator visitation to droughted plants. Thus, future work investigating whether pollinators favour plants with higher drought tolerance when presented with communities of co-flowering plants would be valuable (Sargent and Ackerly, 2008; Mesgaran *et al.*, 2017; Faust and Iler, 2022).

Drought-induced changes in flower size and floral display might also lead to visitation by different assemblages of pollinators (Thompson, 2001; Gambel and Holway, 2023). Our study isolated the preference of *O. lignaria* for individual flowering species, but it would be useful to investigate whether droughted plants are visited by less effective pollinators, resulting in reduced reproductive output. Furthermore, drought can limit plant reproduction directly by reducing flower display, as documented herein, in addition to reducing pollen viability (Bharucha, 2023) and increasing flower and fruit abortions (Akhalkatsi and Lösch, 2005; Gallagher and Campbell, 2017; Descamps *et al.*, 2018). Although our study did not examine these effects, these potential direct and pollinator-mediated negative impacts could reduce the reproductive success of drought-stressed plants.

CONCLUSIONS

Our findings demonstrate that drought can influence plant–pollinator interactions. Drought-induced reductions in flower size and nectar volume were associated with preferential visitation by bees to larger, non-droughted flowers that contained more nectar, with the strength of these preferences shaped by the drought tolerance of the plants. These experimental results therefore point to the potential for more frequent and sustained drought to alter the attractiveness of annual wildflowers in the short term, thereby decreasing the rate of interactions with pollinators, which might, in turn, alter the population dynamics of both partners.

Drought is likely to co-occur with climate change-driven increases in temperature (Barnabás *et al.*, 2008), and their combined effects will be likely to modify plant traits and plant–pollinator interactions in complex ways (Descamps *et al.*, 2018, 2021b). In a similar greenhouse setting, experimental warming of our focal plant species resulted in reduced visitation by *O. lignaria* and reduced seed set (de Manincor *et al.*, 2023). Work that assesses the interactive effects of multiple abiotic factors on plant–pollinator interactions should lead to more accurate predictions of how climate change will affect pollination services and plant reproduction.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Figure S1: leaf turgor loss point (π_{tp}) of *Nemophila menziesii* and *Phacelia campanularia*. Figure S2: leaf water potential by watering treatment and time of day for *Nemophila menziesii* and *Phacelia campanularia*. Figure S3: flower landing area for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions in 2021 and 2022. Figure S4: flower corolla length for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions in 2021. Figure S5: log+1-transformed nectar volume per flower for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions in 2021. Figure S6: nectar concentration for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions in 2021. Figure S7: log+1-transformed nectar calories for *Nemophila menziesii* and *Phacelia campanularia* grown in non-droughted and droughted conditions in 2021. Table S1: model results for 2021 floral trait measurements.

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