Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant

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Citation: Rering, C. C., J. G. Franco, K. M. Yeater, and R. E., Mallinger. 2020. Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. Ecosphere 11(9):e03254. 10.1002/ecs2.3254

Abstract. Plant–pollinator interactions are mediated by floral signals and by the quantity and quality of floral rewards. Biotic and abiotic disturbances can influence plant reproductive success through both direct effects on plant performance and indirect effects on pollinator attraction. In this study, we examined the effects of drought on buckwheat (*Fagopyrum esculentum* Moensch), a globally cultivated plant that is prone to drought stress, dependent on insect pollinators for reproduction, and increasingly utilized in on-farm conservation. Between drought-stressed and control plants, we compared: nectar quantity and chemical composition, pollen quantity, floral volatile emissions, visits by both managed and wild pollinators, and plant reproductive success. Drought-stressed plants produced significantly fewer flowers and less nectar per flower, though pollen quantity per flower was unaffected. Nectar from drought-stressed plants had a lower proportion of sucrose relative to total sugars, though overall sugar concentration was unaffected. Significantly fewer bumble bees, honey bees, and flies were recorded on drought-stressed plants. While there was no significant difference in the quantity of total floral volatile emissions, volatile compositions differed, with drought-stressed plants having higher emissions of (Z)-3-hexenol, isobutyraldehyde, 2-methylbutanal, and 3-methylbutanal. Finally, drought stress had negative effects on seed set and total seed mass per plant. Our results show that drought stress can have significant effects on floral traits and pollinator attraction, reducing plant reproductive success, and the nectar resources available to pollinators. Thus, the potential value of this plant in pollinator conservation and as a honey plant may be reduced under drought stress.

Key words: bumble bee; drought stress; floral traits; floral volatiles; honey bee; nectar; pollen; pollination; pollinator.

Received 12 February 2020; revised 25 May 2020; accepted 29 May 2020; final version received 20 July 2020. Corresponding Editor: T’ai Roulston.

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INTRODUCTION

Numerous floral traits mediate plant–pollinator interactions, including visual signals such as flower number, shape, size, and color, olfactory signals such as floral volatiles, and the quantity and composition of floral rewards. Floral traits can vary across plants due to genetic and environmental factors and their interaction (Leiss and Klinkhamer 2005). Previous research has shown
that variation in temperature, water availability, and herbivory affect floral traits (Carroll et al. 2001, Caruso 2006, Halpern et al. 2010), but pollinator responses to these changes and subsequent pollination success have not been as well explored (Scaven and Rafferty 2013). Additionally, how environmental conditions affect the quantity and quality of floral rewards for pollinators, specifically nectar and pollen, remains less well understood. In the context of changing landscapes and climates, variation in floral rewards can have consequences not only for pollinator attraction and plant reproductive success but also for the pollinators that rely on floral resources.

Drought stress has been shown to affect numerous plant vegetative and reproductive traits. In general, drought stress reduces flower number, flower size, and nectar volume, but has shown variable or no effects on nectar sugar concentration or on pollen quantity (Boose 1997, Carroll et al. 2001, Leiss and Klinkhamer 2005, Caruso 2006, Halpern et al. 2010, Waser and Price 2016, Descamps et al. 2018). Along with these traits, drought stress can change plant volatile emissions; however, while extensive research has shown that leaf volatile emissions are affected by water availability, less is known about how floral volatiles respond to drought stress (Campbell et al. 2018). Floral scent serves dual functions in plants, attracting beneficial visitors like pollinators and deterring antagonists such as florivores and nectar robbers (Schiestl et al. 2014). In fact, a single floral volatile may act as both an attractant and deterrent, depending on its emission level and the organism receiving the signal (Galen et al. 2011, Kessler et al. 2019). Pollinators can also learn to associate floral volatiles with reward status and thus use floral volatile blends to select plants (Knauer and Schiestl 2015). While such changes in floral traits could affect pollinator attraction, pollinator responses are not often studied alongside those of the plant. In the few studies that have examined pollinator responses, drought stress had variable effects on pollinator visitation rates despite significant negative effects on floral traits and rewards (Leiss and Klinkhamer 2005, Burkle and Runyon 2016, Descamps et al. 2018). Responses to drought stress thus appear to vary across both plant and pollinator species, as well as across environmental contexts.

Drought stress may directly affect plant reproductive success through changes in plant physiology, but also through indirect effects on pollinator attraction and pollination services. Water limitation may reduce plant investment in reproduction, reducing flower number, viable pollen production, and seed number, or it may increase flower and fruit abortion rates (Guilioni et al. 2003, Akhalkatsi and Lösch 2005, Alqudah et al. 2011, Jorgensen and Arathi 2013). Additionally, seed viability may be reduced during periods of drought stress even when seed number is unaffected (Akhalkatsi and Lösch 2005). But changes in floral traits that reduce pollinator attraction can also influence plant pollination success, compounding the more direct effects of drought stress on plant reproduction (Alqudah et al. 2011). For animal-pollinated plants, effects of drought stress on seed set may thus be due to changes in plant resource allocation, pollinator attraction, and/or their interaction, though this has not been well documented.

Common buckwheat (Fagopyrum esculentum Moensch; buckwheat) is a global and economically important plant grown for a variety of purposes (Campbell 1997, Björkman and Shail 2013, Mallinger et al. 2019). Buckwheat flowers are visited by a large diversity of pollinators, and the plant is relatively dependent on animal pollinators for adequate seed set though wind can also transfer pollen over short distances (Carreck and Williams 2002, Jacquemart et al. 2007, Taki et al. 2009, 2010, Bartomeus et al. 2014, Campbell et al. 2016). Increasingly, it is being incorporated into conservation plantings for beneficial insects including pollinators as it provides both nectar and pollen resources (Carreck and Williams 2002, Pontin et al. 2006, Razze et al. 2016, Mallinger et al. 2019), and it is also widely used as a honey crop (Alekseyeva and Bureyko 2000). Since it is typically not irrigated nor is it considered drought-tolerant, it may be vulnerable to drought conditions (Myers and Meinke 1994). Thus, the impacts of water availability on buckwheat plant–pollinator interactions may have significant economic and ecological consequences, though this has not been previously examined.

In this study, we experimentally imposed drought stress on buckwheat plants and measured a suite of floral traits including visual and
olfactory signals, and floral reward quantity and quality. We furthermore measured pollinator attraction and seed set to elucidate direct and potential indirect effects of drought stress on plant reproductive success. Our study expands our understanding of how environmental disturbances affect plant–pollinator interactions with implications for both the plant and the pollinator. Crucially, we examine not just impacts on visual signals, but on the less well-studied olfactory signals, and on both nectar and pollen floral rewards. We furthermore document how these changes in floral traits can cascade to pollinator attraction and plant reproductive success.

**METHODS**

**Study design**

We planted buckwheat in 352 cone-tainers (SC10 model, 1.5” diameter by 8.25” deep, Greenhouse Megastore, Danville, Illinois, USA) at a seeding rate of two seeds per cone-tainer and in two locations: Gainesville, Florida and Mandan, North Dakota (29.64° N, −82.36° W and 46.82° N, −100.88° W, respectively) in May 2018. We used Sun Gro Horticulture Basic Mix 4 growing media (Sun Gro Horticulture, Agawam, Massachusetts, USA) mixed with Osmocote fertilizer (The Scotts Company, Marysville, Ohio, USA) at a rate of 1 tablespoon per 2 gallons soil and with 1 standard-sized cotton ball at the bottom of each cone-tainer to avoid soil loss. Cone-tainers were arranged in four rows of four within a 98-cone capacity tray (Greenhouse Megastore) and then placed on greenhouse benches under ambient light. Regulated by an evaporative cooling system, nighttime temperatures were generally maintained between 10° C and 16° C and daytime temperatures between 18° C and 24° C; however, daytime temperatures occasionally reached as high as 38° C. After germination, we thinned to one buckwheat plant per cone-tainer and added one heaping teaspoon of pea gravel to the soil surface to prevent excessive evaporation. Plants were watered daily for the first three weeks until the first sign of flower bud formation.

Approximately three weeks after planting, at the first sign of flower bud formation, we began our watering treatments. Within each tray, every other cone-tainer was assigned to control or drought treatment for a total of 176 cone-tainers per treatment per location. Control plants were watered at least once per day as needed and drought treatment plants every three days, both to the point of saturation. All data were collected on the third day of drought stress prior to watering when treated plants were maximally stressed, beginning on the 9th and 12th day after treatment initiation in Florida and North Dakota, respectively, and repeated for up to five sampling dates thereafter over a period of one month. Exact sampling dates varied between locations and were distributed over a period of one month post-treatment initiation, due to inclement weather that affected our ability to conduct outdoor observations. However, data were always collected on the third day of drought stress according to our watering regime in both locations.

Buckwheat flowers are small (6 mm diameter), white-pink in color, and organized in racemes, each composed of several cymes or whorls of flowers (Campbell 1997, Halbrecq and Ledent 2001, Jacquemart et al. 2007). The axillary ends, as well as the main stem, form a terminal cluster of several inflorescences (Cawoy et al. 2008, 2009). Flowering is profuse and lasts for a long period (several hundred flowers over 2–3 months), with individual flowers typically open for less than 1 d (Quinet et al. 2004, Jacquemart et al. 2007). Growing buckwheat in cone-tainers in greenhouses may reduce the total number of inflorescences produced as compared to field-grown plants (Halbrecq and Ledent 2001), though our plants did produce many flowers over a long bloom period.

**Drought stress and vegetative traits**

Drought stress was confirmed through water potential measurements using a Scholander pressure chamber (Scholander et al. 1965; Model 1505D, PMS Instrument Company, Albany, Oregon, USA) on the most recently expanded leaf with a petiole of at least 0.5 cm in length. Across three sampling dates, pre-dawn leaf water potential measurements were taken on five plants per treatment per location between the hours of 04:30 and 06:00, and mid-day stem water potential measurements were taken on a separate set of five plants between the hours of 12:00 and 14:00 (n = 30 plants per treatment for each of pre-dawn and mid-day measurements). For mid-day measurements, leaves were encased in opaque
reflective bags (Prune bag; PMS Instrument Company) for 30 min prior to placement in the pressure chamber to ensure stomata were closed and water tension in the leaf and stem were at equilibrium. As water potential measurements were destructive, this subset of plants was discarded after data collection. Additionally, to examine the effects of drought stress on plant growth, we recorded plant height on a separate set of 24 plants per treatment per location at treatment initiation and on five dates thereafter over ~30 d, measuring from the top of the cone-tainer to the apical meristem. Height relative growth rate (RGR) was then calculated for each plant using the equation \( \ln(\text{Height}_{t_2} - \ln(\text{Height}_{t_1})/(t_2 - t_1)) \), where \( t_1 \) is the initial measurement date and \( t_2 \) is the final measurement date.

**Floral traits**

**Nectar volume and pollen quantity.**—To examine the effects of drought stress on nectar volume, we quantified nectar from 10 randomly selected flowers within the most recently mature inflorescence(s) on six plants per treatment per location using 0.5-µL microcapillary tubes (Sigma Aldrich Products, St. Louis, Missouri, USA) and repeated with a separate set of plants on each of five sampling dates (\( n = 70 \) plants per treatment). Additionally, in order to examine the chemical composition of nectar, we ejected all collected nectar into a 0.2-mL microcentrifuge tube filled with 70% ethanol. Additional flowers from the same plant were sampled until a minimum of 0.5 µL nectar per plant was obtained, and samples were stored at −80°C.

To quantify pollen production, from each of the same plants sampled for nectar (\( n = 70 \) plants per treatment) we collected five pre-anthesis flower buds with fully developed white petals still in a tight cluster and stored each flower bud individually in a microcentrifuge tube at −80°C until processing. We removed all anthers from the flower bud, used forceps to gently crush anthers within the microcentrifuge tube, and then added 50 µL ethanol lightly stained with basic fuchsin. The tube was subsequently vortexed for 30 s, after which we immediately removed 1 µL of the solution and placed it onto a standard Neubauer hemocytometer (Paul Marienfeld GmbH&Co.KG, Luda-Konigshofen, Germany) in order to count all pollen grains within the 1-µL drop. The number of pollen grains per 1-µL sample was averaged across the five flower buds sampled per plant. Plants sampled for nectar and pollen were discarded after sampling as this process was damaging to the plant.

**Nectar composition.**—From the above-described nectar samples, nectar sugar composition was quantified using an Agilent 1290 liquid chromatograph with a 1260 evaporative light scattering detector (ELSD; Palo Alto, California, USA) and an external calibration curve of fructose, glucose, and sucrose (0.05–1 g/L in 70% ethanol). We investigated the total concentration of sugars as well as the proportion of sucrose relative to total sugars because of honey bees’ noted preference for sucrose-rich nectar (Pham-Delegue et al. 1990, Roldan-Serrano and Guerra-Sanz 2005). Nectar samples were lyophilized to dryness overnight, reconstituted in 200 µL of 70% ethanol, and syringe filtered (0.22 µm, 13 mm, PVDF, Foxx Life Sciences, Salem, New Hampshire, USA). Separation was achieved in a 10 min run time using a ZORBAX carbohydrate analysis column (4.6 × 180 mm, 5 µm) and ZORBAX NH2 analytical guard column (4.6 × 12.5 mm, 5 µm) held at 30°C, and an isocratic elution of 75% acetonitrile and 25% water at 1.4 mL/min. Injection volumes were typically 20 µL but varied between 1 and 20 µL for samples with concentrations outside of the linear range of detection. The ELSD was operated with the following parameters: evaporator temperature, 100°C; nebulizer temperature, 60°C; and N2 gas flow, 1.60 SLM.

**Floral volatiles.**—Additionally, in one study location (Gainesville, Florida, USA), we analyzed floral volatile emissions from four plants per treatment and repeated with a separate set of plants for each of five sampling dates (\( n = 20 \) plants per treatment). To avoid inducing unequal damage to the more fragile tissues of drought-stressed plants, as is often unintentionally inflicted when enclosing samples within a container for attached flower sampling, all available inflorescences from each plant were cut immediately prior to sampling and the flower stems (i.e., rachises) immediately submerged into autosampler vials (Agilent Technologies, Palo Alto, California, USA) containing 2 mL Optima grade water. As a result, induced damage was equal between treatments. Here, our analyses and interpretations center on the relative differences...
in volatile emission between the treatments. Flowers were harvested between 730 and 1530 and immediately transferred in the above-described autosampler vials from the greenhouse to the laboratory where they were then placed within a 16 oz Ball jar sealed with O-rings and lids prepared from Teflon sheets (0.04” PTFE; ePlastics, San Diego, California, USA). Modified lids were prepared to accommodate sampling by drilling a small hole through the Teflon; this hole was initially sealed with Teflon and Scotch tape. The flowers were sealed within the jars for 2 h to accumulate volatiles, and then, solid-phase microextraction fibers (SPME; Supelco, Bellefonte, Pennsylvania, USA; 50/30 µm, 2 cm, divinylbenzene/carboxen/polydimethylsiloxane) were pierced through the tape layers to sorb volatiles for 15 min. Because samples were neither highly concentrated, nor highly divergent in their volatile blends, SPME fibers accurately captured relative trends in compound abundance. Glassware and lids were baked for ≥10 h at ≥100°C prior to use. Blank samples, containing water-filled vials with no flowers, were analyzed as above, and compounds arising from ambient air, SPME fibers, or containers were not included in the floral volatile data set. Floral volatiles were discriminated from background volatiles by exhaustively comparing chromatographic features in floral and blank samples (walking chromatograms) for 20% of collected floral samples and all blanks.

To aid in analyte identification, technical replicates were collected on a subset of floral samples by simultaneously collecting volatiles on duplicate SPME fibers and analyzing with two gas chromatograph mass spectrometers (GC-MS) fitted with analytical columns of differing stationary phases. Volatiles were stored on the fiber for ≤1 min before thermal desorption in the injector ports (6 min) in splitless mode onto an Agilent 7890B GC coupled to a 5977B MS in electron ionization (EI) mode and equipped with a J&W Scientific (Folsom, California, USA) DB-Wax column (60 m × 320 µm × 0.25 µm), and an Agilent 7890A GC coupled to a 5975C MS in EI mode outfitted with a J&W Scientific DB-1 column (60 m × 320 µm × 0.25 µm).

Volatiles were analyzed using parameters identical to those previously described (Rering et al. 2018) except that both GC inlets were held at 230°C. Data from the GC-MS fitted with a DB-Wax column were used for the semi-quantitative comparison of compounds. Retention indices were calculated using a homologous series of n-alkanes on both the DB-1 and DB-Wax columns. Retention indices from both columns were used to assist with initial identification, and identities were further confirmed by comparison to retention times and fragmentation patterns of standards. Compound identities not verified on both instruments with a commercially standard were marked as tentatively identified. Additionally, if peaks could not be authenticated and the library matches were poor, their identities were labeled as unknown, with the 10 most abundant ions for unknowns presented in the supplemental section (Appendix S1: Table S1). For each compound, a quantitative ion was selected for integration and one or more qualitative ions were monitored to confirm peak identity using MassHunter Quantitative Analysis (Agilent Technologies, B.07.01; Appendix S1: Table S1). After integrations were completed, box plots were prepared to compare compound abundance between flower and blank volatile samples. Compounds with similar abundance in the blank were removed from the data set, while compounds with reduced abundance in blanks were retained in the data set, but sample peak areas were blank-subtracted.

**Pollinator attraction**

To examine the effects of drought stress on pollinator attraction, a single research-sized bumble bee colony of approximately 70 workers (Koppert Biological Systems, Howell, Michigan, USA) was placed in a separate greenhouse three days prior to the first data collection period in each location. For the first 24 h after arrival, bees were fed from a sugar water bag. After 24 h, we removed the sugar water and allowed bees to forage in the greenhouse on well-watered, non-experimental buckwheat plants. In the evening before the data collection period, the colony door was set to “entrance only” to collect all bees, and on the following morning, all background plants were removed from the greenhouse. We then brought in experimental plants arranged in single-treatment trays, with six cone-tainers per tray and three trays per treatment for a total of 18 cone-tainers per treatment. Trays were set in two rows on a greenhouse bench approximately 5 m
in front of the bumble bee colony, alternating trays of each treatment within rows. Trays were placed close together within and between rows such that spacing between containers within a tray was approximately equal to spacing between containers in adjacent trays. Observations began 5 min after the bumble bee colony door was opened.

We collected data for a 30-min period in mid-morning (900–1000) during which an observer continuously walked around the plant array recording each bumble bee visitor observed on a flower as they passed each plant. This observation method takes into account both number of visits to individual plants and visit length since bees spending more time on a plant may be recorded repeatedly and is thus a good indication of a plant’s overall attractiveness to bees. Data were collected across five sampling dates in North Dakota, while in Florida, due to premature colony decline, data were collected across three sampling dates. After observations, containers were returned to the original greenhouse, arranged in the same manner as other experimental plants, and treated with the appropriate watering treatment. The same set of plants was utilized across the sampling dates, but container placement within trays, and placement of trays on the greenhouse bench, varied across dates to prevent any bee forager learning. In between observations, bumble bees foraged on non-experimental buckwheat plants and were additionally provided with a sugar water bag.

Additionally, to measure attraction to other pollinators, we brought a separate set of plants outside of the greenhouse and conducted similar observations in the field. In late morning (~11:00–11:30), 18 plants per treatment were arranged as described above at a designated field site near the greenhouse. After a 5-min acclimation period, we conducted observations as described above, recording visitors to the following morphogroups: honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.), other bees, flies, and wasps. We then left plants in the field, returned approximately 1 h later, and conducted a second set of 30-min observations in early afternoon (~12:30–13:00), summing pollinator counts per plant across both observation sets. We repeated this across five sampling dates per location with a separate set of plants per date (as plants brought outside could not be returned to the greenhouse). We only received enough visits by honey bees (in Florida only) and flies (in North Dakota only) to analyze; visits by other groups were absent or infrequent. For both visitation observations in the greenhouse and in the field, the number of open flowers per plant was recorded in order to account for the effects of flower density on pollinator attraction.

**Seed set**

After the last data collection period, plants utilized for the above-described bumble bee observations remained under their respective watering treatments in the greenhouse until seed formation. Additionally, a set of plants not visited by bees was also kept under watering treatments in the same greenhouse until seed formation. We counted fully mature seeds on each plant once per week for a period of five weeks from late June 2018 to late July 2018 after which we harvested all seeds and recorded the number and mass of mature seeds per plant. We additionally calculated average mass per seed (total seed mass/total seed count) for each plant.

**Statistical analyses**

To confirm our treatments were successful, we analyzed pre-dawn and mid-day water potential (absolute values, sqrt-transformed) using linear models including treatment, time (days since start of experiment), interaction between treatment and time, and greenhouse (Florida or North Dakota) as explanatory variables. We additionally compared plant height relative growth using a linear model with factors of treatment and greenhouse. To investigate how drought treatments affected floral rewards, we used linear models including treatment, time, and their interaction, and greenhouse, on the following response variables: nectar volume per 10 flowers per plant (µL, sqrt-transformed), total nectar sugar concentration (mol/L), proportion sucrose in nectar (sucrose/total sugars, mol/mol, sqrt-transformed), and average number of pollen grains per 1 µL sample per plant (sqrt-transformed).

To analyze differences in floral volatile emissions, we ran a linear model on total volatile emissions (sum of all volatile organic compound peak areas divided by flower count) with fixed effects of treatment, time, and an interaction between...
treatment and time. We next analyzed differences in flower count-normalized volatile composition (individual compound peak areas divided by flower count) with a permutational analysis of variance (PERMANOVA) on a Bray–Curtis distance matrix, including factors of treatment, time, and their interaction, and visualized differences between treatments with a non-metric multi-dimensional scaling plot. Dispersion in the dissimilarity matrix based on the volatile emission data was compared between treatments and among collection days. To evaluate which volatiles differed in relative abundance between the treatments, we first evaluated homogeneity of treatment variance using median distance-based tests and then conducted a differential analysis based on the negative binomial distribution.

To compare honey bee (Florida only) and fly (North Dakota only) counts on each plant, we used generalized linear mixed-effects models with a zero-inflated Poisson distribution. Models included fixed effects of treatment, time, and their interaction, number of flowers per plant as a covariate, and a random effect of tray (1–6) nested in sampling round (1–5) to account for correlated errors between plants situated within the same tray. To compare bumble bees counts per plant, we used a similar model but with a random intercept of plant ID nested within greenhouse and random slopes of time for each plant to account for repeated measures of the same plants across time in each greenhouse. For all plants included in observations, we also compared flower number between drought and well-watered plants; for plants used in the honey bee and fly observations, we used generalized linear models with a negative binomial distribution including the number of open flowers per plant as the response variable and treatment, time, and their interaction as fixed effects. For plants used in the bumble bee observations, we included a random intercept of plant ID nested within greenhouse and random slopes of time to account for repeated measures on the same plants across time.

Finally, seed count, total seed mass, and average mass per seed were examined as both a function of treatment (drought n = 60; well-watered n = 59) and visitation by pollinators, with plants recorded as either having been visited by a bumble bee (n = 73) or never visited by a bee during the experiment (n = 31). Total seed mass per plant (sqrt-transformed), total number of mature seeds per plant, and average mass per seed per plant were compared across treatments using linear models with explanatory variables of treatment, bee visitation (yes/no), and their interaction, and greenhouse. Statistical significance for model parameters was determined using the function ANOVA (car package, R v. 3.4.2; R Core Team 2018). All models and analyses were performed in R v. 3.4.2 using the packages and functions: lm (linear models), glm/glm.nb (generalized linear models), glmmTMB (generalized linear mixed-effects models), DESeq2 (differential analysis; Love et al. 2014), and vegan (PERMANOVA, betadisper; Oksanen et al. 2019).

RESULTS

Drought stress and vegetative traits

Plants in the drought treatment were significantly more drought-stressed than plants in the control treatment for both pre-dawn ($F_1 = 7.44$, $P = 0.008$) and mid-day ($F_1 = 5.01$, $P = 0.03$) drought stress measurements. Furthermore, there was no significant treatment by time interaction for pre-dawn and mid-day drought stress measurements, indicating consistent differences across time ($F_1 = 0.55$, $P = 0.46$; $F_1 = 0.52$, $P = 0.47$, respectively). Additionally, drought-stressed plants had significantly lower relative growth rates over the duration of the experiment as compared to control plants ($F_1 = 124.78$, $P < 0.001$; Fig. 1a).

Floral rewards: nectar and pollen production

Flowers on drought-stressed plants produced significantly less nectar, 44% less, than flowers on control plants (drought-stressed 0.41 ± 0.04; control 0.73 ± 0.07 μL per 10 flowers per plant; Fig. 1b; Table 1). Furthermore, nectar from drought-stressed plants had a significantly lower proportion of sucrose (drought 0.197 ± 0.017; control 0.233 ± 0.013 mol/mol; Fig. 1c), though there was no difference in total sugar concentration (drought 3.4 ± 0.1; control 3.5 ± 0.2 mol/L; Table 1). These differences were consistent across the experiment as indicated by insignificant treatment by time interactions (Table 1). However, while treatment affected nectar traits, it did not affect the number of pollen grains produced.
by buckwheat flowers (drought 14.8 ± 0.98; control 16.7 ± 1.7 grains per 1 µL sample per plant), a result that was consistent over time (Table 1).

**Floral traits: floral volatile emissions**

Total volatile emissions were not significantly different between treatments ($F_1 = 3.08, P = 0.09$) and with no significant treatment by time interaction ($F_1 = 0.006, P = 0.94$). Additionally, the same 54 constituent chemicals of the buckwheat floral aroma were detected in both control and drought plants (Appendix S1: Table S1). The relative abundance of volatiles, however, was significantly different between treatments ($F_1 = 2.37, P = 0.018$; Fig. 2) and with no significant interaction between treatment and time ($F_1 = 0.10, P = 0.455$). Emissions of 2-methylbutanal, 3-methylbutanal, (Z)-3-hexenol, and isobutyaldehyde contributed to the significant treatment differences and were 3.36, 3.38, 2.47, and 1.99 log2-fold greater in drought-stressed plants than in control plants, respectively ($P < 0.001$ for all four compounds; Fig. 3a–d). Dispersion based on the dissimilarity matrix was similar between drought and control plants and among collection days ($P > 0.5$).

**Pollinator attraction**

There were significantly fewer counts of bumble bees, honey bees, and flies on drought-stressed plants (3.7 ± 0.31; 3.4 ± 0.55; 1.1 ± 0.17 counts/plant/period, respectively) as compared to control plants (6.4 ± 0.50; 6.1 ± 0.94; 2.4 ± 0.39 counts/plant/period, respectively; Fig. 4a–c, Table 2). Counts of all three types of pollinators increased with the number of flowers per plant (Table 2; Appendix S1: Fig. S1), and control plants had more flowers than drought plants for those plants visited by bumble bees (58.8 ± 4.9; 44.7 ± 3.3 flowers per plant; $\chi^2 (1) = 13.24, P < 0.001$), honey bees (78.8 ± 5.0; 57.4 ± 3.8 flowers per plant; $\chi^2 (1) = 16.40, P < 0.001$), and flies (44.2 ± 3.6; 18.6 ± 1.2 flowers per plant; $\chi^2 (1) = 117.26, P < 0.001$). However, even after accounting for the overall effect of flower number on pollinator counts, there was still a significant effect of drought treatment on pollinator counts (Table 2).

**Seed set**

Drought-stressed plants produced significantly fewer seeds, 45% less, as compared to control plants (drought 17.4 ± 2.2; control 31.6 ± 2.7 seeds per plant) and had significantly lower total seed mass, 48% lower (drought 0.65 ± 0.08; control 1.24 ± 0.11 g per plant; Fig. 5a, b, Table 3). Additionally, plants that had not been visited by bumble bees had significantly fewer seeds, 57% fewer, as compared to plants visited by bumble bees (unvisited 12.7 ± 3.2;...
Table 1. Linear model parameter estimates ± SE for treatment (control vs. drought), time, and their interaction, and greenhouse site (Florida, North Dakota) on the following buckwheat response variables: nectar volume per 10 flowers per plant, proportion sucrose relative to total sugars in nectar, nectar sugar content (all sugars), and number of pollen grains per sample per plant.

| Response variable by source | Estimate ± SE | \(F_{1,116}\) | \(P\) |
|----------------------------|---------------|--------------|-----|
| Treatment                  |               |              |     |
| Nectar volume (μL)         | 0.20 ± 0.13†  | 21.63        | <0.001** |
| Proportion sucrose/total sugars (mol/mol) | 0.05 ± 0.05† | 8.64         | 0.003** |
| Nectar sugar (mol/L)       | −1.63 ± 1.07† | 0.46         | 0.50 |
| Pollen quantity            | 0.22 ± 0.64†  | 0.31         | 0.58 |
| Time                       |               |              |     |
| Nectar volume (μL)         | −0.007 ± 0.006| 3.13         | 0.08 |
| Proportion sucrose/total sugars (mol/mol) | −0.001 ± 0.002 | 0.59 | 0.44 |
| Nectar sugar (mol/L)       | −0.024 ± 0.05 | 0.27         | 0.60 |
| Pollen quantity            | −0.04 ± 0.03  | 4.01         | 0.045* |
| Treatment × time           |               |              |     |
| Nectar volume (μL)         | −0.0002 ± 0.007| 0.0006   | 0.98 |
| Proportion sucrose/total sugars (mol/mol) | 0.0001 ± 0.003 | 0.001 | 0.97 |
| Nectar sugar (mol/L)       | 0.085 ± 0.06  | 1.90         | 0.17 |
| Pollen quantity            | 0.066 ± 0.04  | 0.03         | 0.86 |
| Greenhouse site            |               |              |     |
| Nectar volume (μL)         | −0.037 ± 0.046‡ | 0.64       | 0.43 |
| Proportion sucrose/total sugars (mol/mol) | −0.16 ± 0.02‡ | 73.68 | <0.001*** |
| Nectar sugar (mol/L)       | 1.22 ± 0.39‡  | 9.77         | 0.002** |
| Pollen quantity            | 0.77 ± 0.23‡  | 11.07        | 0.001*** |

Notes: All model parameters excluding nectar sugar content were square-root transformed. The intercepts for response variables are as follows: nectar volume, 0.75; proportion sucrose/total sugars, 0.52; nectar sugar content, 3.49; pollen quantity, 3.98. Statistical significance for model parameters was determined using ANOVA function, car package, R v. 3.4.2. SE, standard error.

† Parameter estimate for Control.
‡ Parameter estimate for North Dakota.

visited 29.7 ± 2.0 seeds per plant) and had significantly lower total seed mass, 53% lower (unvisited 0.53 ± 0.13; visited 1.13 ± 0.08 g per plant; Fig. 5a, b, Table 3). The average mass per seed did not differ significantly between watering treatments but was significantly lower on plants that had not been visited by bumble bees (Table 3). For all seed set measurements, there was no significant interaction between treatment (drought/control) and bumble bee visitation (Table 3).

**Discussion**

Environmental conditions such as temperature and precipitation can affect plant reproductive success, potentially through altered plant–pollinator interactions resulting from changes in floral traits and rewards (Scaven and Rafferty 2013). These effects are increasingly important to understand in changing climates and landscapes under human disturbance. In this study, we found that drought stress to buckwheat during its flowering period affected both vegetative and floral traits. Plants under drought stress had reduced floral rewards, including fewer flowers per plant, reduced nectar volume per flower, and lower proportion of sucrose in nectar, though pollen quantity per flower was not affected. Furthermore, both visual and olfactory signals were affected by water availability, with drought-stressed plants having a smaller floral display (i.e., lower floral density) and altered floral volatile emissions. Drought-stressed plants subsequently had lower pollinator attraction and activity. Thus, drought stress had direct and potential indirect effects on buckwheat reproductive success; drought stress directly resulted in lower seed set and, additionally, may have had indirect effects through reducing pollinator visitation, which was shown to increase seed set. Our results suggest that under drought stress, the value of buckwheat for pollinators and as a
honey plant will decrease. Additionally, our results suggest drought stress can affect crop yields not only through direct effects on plant physiology, but also through reducing pollination services.

Plants produce two main rewards for pollinators, nectar and pollen, and the effects of drought stress on the quantity and quality of these rewards could have consequences for pollinator fitness. In our study, while per-flower nectar volume was reduced under drought stress, per-flower pollen quantity was unaffected. While the timing of our drought treatment, initiated at the first sign of flower bud formation, may have reduced the potential for effects on pollen production in our first data collection period, there was no treatment effect even for flowers sampled one month later, and no interaction between treatment and time. Buckwheat flowers are produced over months, though individual flowers are typically only open for one day (Jacquemart et al. 2007). We would thus expect to see a treatment effect over the course of the experiment. Furthermore, our results mirror those of previous findings. While nectar production rates are widely variable and sensitive to environmental factors including drought (Boose 1997, Carroll et al. 2001, Leiss and Klinkhamer 2005, Halpern et al. 2010), the effects of drought stress on pollen production are less well documented and include both no and asymptotic (hump-shaped) effects (Waser and Price 2016, Descamps et al. 2018). Nectar functions primarily to reward pollinators, and plasticity in nectar production may promote not only adaptation to environmental contexts but also pollinator visitation patterns that increase cross-pollination (Boose 1997). Alternatively, pollen is not only a floral reward, but a gametophyte and therefore a direct determinant of plant male fitness. Pollen production may therefore be less plastic, if such plasticity is not adaptive, or it may be under greater genetic control. However, since drought stress also affected the number of flowers per plant, total pollen quantity per plant was lower even if per-flower pollen production was unaffected. Furthermore, pollen quality (i.e., protein content) could change under drought stress, but was not examined herein. While previous studies have found reduced pollen viability under drought stress (Descamps et al. 2018), we are unaware of any research examining how drought stress changes the value of pollen as a food resource to pollinators. Based on the findings of this study, we conclude that drought stress during buckwheat’s flowering period is likely to reduce both total nectar and pollen quantity, but with greater effects on nectar production at both the whole-plant and per-flower level.

Nectar chemistry is thought to remain generally consistent among plants of a given species, in part as a function of co-evolution with pollinators which have explicit nutrition preferences, and as a result of conserved nectary structures and excretion methods (Heil 2011, Roy et al. 2017). However, we found that drought stress resulted in a small but significant change in nectar sugar composition. Such changes in nectar chemistry can influence pollinator attraction; for example, bees preferred sunflower and zucchini cultivars with higher ratios of sucrose to hexose sugars (Pham-Delegue et al. 1990, Roldan-Serrano and Guerra-Sanz 2005). One possible explanation for these findings is that nectar sucrose is converted into its hexose monomers in greater rates in drought-stressed plants due to a greater accumulation of reactive oxygen species (ROS) under drought.
stress. The increased oxidative stress and subsequent enhanced formation of ROS in plants under drought stress are well known (Cruz de Carvalho 2008), and non-enzymatic reactions of ROS with plant carbohydrates have been proposed to protect plant tissues (Matros et al. 2015). The hydroxyl radical, a ubiquitous environmental ROS (Vione et al. 2014) which has been documented to occur in floral nectar (Carter and Thornburg 2000), preferentially splits disaccharides like sucrose into its hexose monomers including glucose and fructose (Peshev et al. 2013). This mechanism potentially explains the reduced sucrose content of nectar relative to fructose and glucose sugars under drought stress.

Few studies have examined the influence of soil moisture on floral volatiles; in those that have, total floral volatile emissions tended to be higher and volatile community compositions different under drought stress, though results varied across species (Burkle and Runyon 2016, Campbell et al. 2018, Glenny et al. 2018). Though we did not observe a significant increase in total buckwheat floral volatile emissions under drought stress, we observed increases in particular components of the floral aroma blend, specifically increased emissions of (Z)-3-hexenol and three aldehydes (2-methylbutanal, 3-methylbutanal, isobutyraldehyde). (Z)-3-hexenol is a member of a group of compounds called “green leaf volatiles” that are released by plants upon damage and stress including abiotic stressors (Cofer et al. 2018). Previous studies have found that drought-stressed Potentilla recta flowers produced greater amounts of (Z)-3-hexenol (Burkle and Runyon 2016, Glenny et al. 2018), suggesting that this response may be more general across plant species. The three other volatiles emitted in greater quantities

Fig. 3. Box plots showing differences in volatile emissions (volatile peak area) between buckwheat plants grown under drought stress and control treatments. The four floral volatile compounds that significantly differentiated drought stress and control samples according to a differential analysis based on the negative binomial distribution are shown, including (a) isobutyraldehyde, (b) 3-methylbutanal, (c) (Z)-3-hexenol, and (d) 2-methylbutanal peak areas.
by drought-stressed plants are aldehydes that may be formed from branched chain amino acid degradation with ROS, a reaction termed the Strecker degradation (Schonberg and Moubacher 1952). Branched chain amino acids accumulate in leaf and flower tissue in response to osmotic stress (Joshi et al. 2010), a strategy thought to provide an alternative substrate for respiration during periods of drought stress or light deprivation (Araújo et al. 2011, Engqvist et al. 2011, Hildebrandt et al. 2015). One of these aldehydes, 2-methylbutanal, was emitted in greater quantities by drought-stressed grapevine leaves (Griesser et al. 2015). Thus, increased emission of aldehydes due to increased branched chain amino acid accumulation may also be a relatively widespread response to drought stress.

All pollinators, including honey bees, flies, and bumble bees, showed reduced attraction to drought-stressed plants, a result that may be explained by both floral display size (i.e., flower density) and additional differences between drought-stressed and control plants. Flower density and/or display size can positively affect pollinator visitation rates per plant, though this relationship is not necessarily linear (Ohashi and Yahara 1998, Thompson 2001, Makino and Sakai 2007, Ishii et al. 2008, Mallinger et al. 2019).

Fig. 4. Box plots showing differences in activity for (a) bumble bees (b) honey bees, and (c) flies to plants grown under drought stress and control conditions. Statistical significance for the difference between drought and control plants comes from zero-inflated generalized linear mixed-effects models including fixed effects of treatment, time, and their interaction, and number of flowers per plant as a covariate. For full model results, see Table 2.

| Response variable by source | Estimate ± SE | $\chi^2$ | P   |
|-----------------------------|--------------|----------|-----|
| Treatment                   |              |          |     |
| Bumble bees                 | 0.15 ± 0.34† | 5.5      | 0.02*|
| Honey bees                  | 1.31 ± 0.24† | 35.33    | <0.001***|
| Flies                       | 1.40 ± 0.72† | 4.69     | 0.03*|
| Time                        |              |          |     |
| Bumble bees                 | 0.02 ± 0.09  | 0.03     | 0.87 |
| Honey bees                  | 0.26 ± 0.10  | 7.45     | 0.006**|
| Flies                       | −0.05 ± 0.03 | 13.83    | <0.001***|
| Treatment × time            |              |          |     |
| Bumble bees                 | 0.01 ± 0.03‡ | 0.19     | 0.66 |
| Honey bees                  | −0.06 ± 0.02 | 6.95     | 0.008**|
| Flies                       | −0.05 ± 0.04‡| 1.73     | 0.19 |
| No. flowers                 |              |          |     |
| Bumble bees                 | 0.006 ± 0.01 | 19.83    | <0.001***|
| Honey bees                  | 0.009 ± 0.01 | 54.33    | <0.001***|
| Flies                       | 0.01 ± 0.004 | 12.55    | <0.001***|

Notes: Intercepts for response variables are as follows: bumble bees, 1.18; honey bees, −4.49; flies, 0.90. Statistical significance for model parameters was determined using Wald’s chi-squared test (ANOVA function, car package, R v. 3.4.2). SE, standard error.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
† Parameter estimate for Control.
‡ Parameter estimate for Control: time.
Indeed, we found that pollinator activity increased with flower number per plant, but that there was still a significant, negative effect of drought stress on pollinators after accounting for the effect of flower number. Drought-stressed plants, in addition to having fewer flowers per plant, also had reduced nectar volume per flower and a reduced proportion of sucrose in nectar, both of which have been shown to affect bee preferences (Pham-Delegue et al. 1990, Abrol 1992, Silva and Dean 2000, Roldan-Serrano and Guerra-Sanz 2005, Makino and Sakai 2007, Mallinger and Prasifka 2017). Previous research has also shown that while floral display size is important in initial decision-making, later visits are explained best by reward status irrespective of floral display size (Ohashi and Yahara 1998).

Pollinators can learn the location of rewarding flowers and can also learn to associate visual and olfactory signals with more rewarding plants, thus using these cues to facilitate decision-making (Cartar 2004, Knauer and Schiestl 2015). In our study, both floral display size and floral volatile emissions differed between treatments and may have been used as signals of reward status. However, as it is difficult to disentangle correlated traits that affect pollinator behavior, we cannot conclude the mechanism driving pollinator preferences.

Our study highlights both direct and potential indirect pathways by which abiotic stress can affect plant reproductive success. Drought stress had a direct effect on seed set, independent of insect pollination services, likely due to reduced flower production per plant and possibly to

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Table 3. Linear model parameter estimates ± SE for drought stress treatment (control vs. drought), bee pollinator visitation (yes/no), and their interaction, and greenhouse site (Florida, North Dakota) on the following response variables: total seed count per buckwheat plant, total seed mass per buckwheat plant, and average mass per seed per plant.

| Response variable by source | Estimate ± SE | F1,99 | P         |
|-----------------------------|--------------|-------|-----------|
| Treatment                   | Seed count   | 9.9 ± 4.2† | 24.08   | <0.001*** |
|                            | Seed mass (g)| 0.28 ± 0.12† | 24.68   | <0.001*** |
|                            | Mass per seed (g) | 0.004 ± 0.005† | 1.40   | 0.24     |
| Bee visitation              | Seed count   | 13.6 ± 4.7 | 27.08   | <0.001*** |
|                            | Seed mass (g)| 0.42 ± 0.11 | 39.66   | <0.001*** |
|                            | Mass per seed (g) | 0.007 ± 0.004 | 5.90   | 0.02*    |
| Treatment × bee visitation  | Seed count   | 7.0 ± 6.6 | 1.13    | 0.29     |
|                            | Seed mass (g)| 0.08 ± 0.15 | 0.28    | 0.60     |
|                            | Mass per seed (g) | −0.002 ± 0.005 | 0.08   | 0.77     |
| Greenhouse site             | Seed count   | −9.3 ± 3.0‡ | 9.5     | 0.03**   |
|                            | Seed mass (g)| −0.33 ± 0.07‡ | 24.20   | <0.001*** |
|                            | Mass per seed (g) | −0.01 ± 0.003‡ | 20.13  | <0.001*** |

Notes: Intercepts for response variables are as follows: seed count, 12.42; seed mass, 0.57; mass per seed, 0.03. Seed mass was square-root transformed. Statistical significance for model parameters was determined using ANOVA function, car package, R v. 3.4.2. SE, standard error.

* P < 0.05; ** P < 0.01; *** P < 0.001.
† Parameter estimate for Control.
‡ Parameter estimate for North Dakota.
reduced pollen viability or increased flower/seed abortion (Akhalkatsi and Lisch 2005, Alqudah et al. 2011, Descamps et al. 2018). Additionally, our study confirms that buckwheat benefits from insect pollination, even though wind can also be an effective pollinating agent across short distances (Adhikari and Campbell 1998). Therefore, through reducing insect pollinator activity, drought stress can have indirect effects on seed set. However, while drought stress reduced pollinator activity on flowers in a controlled context, these results may not translate to landscape-scale contexts in which pollinators are not offered choices or in which all plants are drought-stressed. On the one hand, if all plants in a landscape are drought-stressed, relative activity on a given plant species may not be affected. On the other hand, buckwheat may be more prone to drought stress as compared to other plants (Myers and Meinke 1994), therefore showing more significant responses. Furthermore, cascading effects of drought stress on pollinator populations due to reduced floral resources could result in lower pollinator abundance over time, thereby resulting in reduced visitation. We therefore expect that both direct and indirect (pollinator-mediated) effects of drought stress on buckwheat seed set would be present under field contexts.

In summary, we show that drought stress during buckwheat’s flowering period affects both floral rewards and floral signals, reducing pollinator attraction, and with cascading effects on plant reproductive success. Buckwheat is a commonly used honey plant, and under dry conditions, its reduced nectar production and proportion sucrose could limit its utility. Finally, our study illustrates that under dry conditions, the value of buckwheat in conservation plantings for pollinators and other wildlife may be diminished relative to other plants with differing responses to drought (Burkle and Runyon 2016). The response of plants to varying environmental conditions should be considered when selecting plants for conservation purposes.

ACKNOWLEDGMENTS

The authors would like to thank Jon Elmquist, Ebony Taylor, Alexandor McMillan, Alexia Rose, Bevin Ferguson and Taylor Johnson for their help conducting the experiment and processing samples.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3254/full