

RESEARCH ARTICLE

Reduced seed set under water deficit is driven mainly by reduced flower numbers and not by changes in flower visitations and pollination

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Handling Editor: Sandra Varga**Abstract**

1. Water deficit can alter floral traits with cascading effects on flower-visitor interactions and plant fitness. Water stress induction can diminish productivity, directly resulting in lower flower production and consequently seed set. Changes in floral traits, such as floral scent or reward amount, may in turn alter pollinator visitations and behaviour and consequently can reduce pollination services resulting in lower reproduction output. However, the relative contribution of this indirect in comparison to the direct effects to changes in seed set are not fully understood.
2. We manipulated water availability using rain-out shelters in a field experiment and measured effects on floral scent bouquet, morphology, phenology, flower-visitor interactions, pollination and seed set. Plant individuals of *Sinapis arvensis* (Brassicaceae) were randomly assigned to one of three treatments: mean precipitation (=control), reduced mean precipitation or drought period treatment.
3. Our results show that decreasing water availability lowers the number of flowers and seed set. This indicates a direct link between water stress and seed set, as seed mass increases with increasing flower number. The indirect link of water stress via floral traits, pollinator visits and pollination has weaker effects on seed set. However, floral traits remain relatively stable under decreased water availability, whereas plant growth and flower abundance decrease, potentially in order to allow investment in more resources in fewer flowers to maintain pollination success. Thus, plants are able to compensate for water stress and can maintain floral trait expression, such as a stable scent emission and bouquet, to retain pollinator attraction.
4. These findings indicate that the direct link from water stress to seed set has a stronger impact on plants reproductive success than the indirect link through altered floral trait expression and pollinator visits in a generalist plant species.

KEYWORDS

Brassicaceae, climate change, drought, floral traits, plant-pollinator interactions, *Sinapis arvensis*

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1 | INTRODUCTION

Reduced precipitation can influence the reproductive success of plant individuals directly and indirectly. Here, we define “directly” as the effect of stress induction on the total flower production during flowering time as lower flower production directly leads to reduced seed production (“abundance effect”; Cariveau et al., 2004; Fang et al., 2010; Lemoine et al., 2013). “Indirect” effects mean changes in floral traits such as reduced floral size or altered scent bouquet, which alter pollinator visitations and behaviour (Descamps, Jambrek, et al., 2021; Descamps, Quinet & Jacquemart, 2021; Höfer et al., 2021; Kuppler et al., 2021) and hence reduce pollination services, for example, pollen grains on stigma, resulting in lower reproductive output (“pollination effect”). General changes in seed set under water stress are well studied (Al-Ghzawi et al., 2009; Boutraa & Sanders, 2001; Gallagher & Campbell, 2017; Mantel & Sweigart, 2019; Turner, 1993). However, the contribution of direct and indirect effects to changes in seed set and, thus, the mechanisms involved in the effects of climate change on pollination and plants are not fully understood. These aspects are of particular importance, because drought events will expand in spatial extent and duration (European Environment Agency, 2017; Grillakis, 2019; IPCC, 2014), and water deficits will increase with strong effects on plant physiology (Beier et al., 2012) and phenotype (Cornwell & Ackerly, 2009; Jung et al., 2014; La Rosa et al., 2019).

Water deficit reduces resources for reproduction (Lemoine et al., 2013), resulting in lower flower production (Burkle & Runyon, 2016; Descamps et al., 2018; Descamps et al., 2020; Glenny et al., 2018) and consequently fewer ovules, thereby directly reducing the total number of seeds produced (Cariveau et al., 2004). Additionally, water-stressed plants have higher floral abortion rates of up to 50% (Fang et al., 2010; Aiguo Dai, 2013; Guo et al., 2013; Descamps et al., 2018) in order to reduce water loss and costs of flower maintenance (Galen et al., 1999; Lambrecht & Dawson, 2007). Moreover, the abortion rates of ovaries before (Alqudah et al., 2011; Smith & Zhao, 2016) and after fertilization (Sehgal et al., 2019) are increased under water stress.

In addition to direct effects on flower production, water stress can affect vegetative growth, floral morphology and scent, and floral rewards (pollen and nectar), depending on plant species, growing conditions and stress level (Descamps, Quinet, & Jacquemart, 2021; Kuppler & Kotowska, 2021). In *Sinapis arvensis* and *Sinapis alba*, water deficit reduces plant growth (Akter & Klečka, 2022), number and size of inflorescences, and floral morphology traits such as floral size, nectar tube depth, and petal width and length (Kuppler et al., 2021). These phenotypic changes decrease the water needed, as smaller corollas lose less water through evaporation and transpiration (Galen et al., 1999; Lambrecht & Dawson, 2007; Teixido & Valladares, 2014). Floral scent emission is not affected by water stress in *S. arvensis* (Höfer et al., 2021); however, in other species floral scent emission rate and the composition of compounds can change (Burkle & Runyon, 2016; Glenny et al., 2018; Rering et al., 2020). For example, water-stressed plants emit more α -pinene, (E)- β -ocimene and

(E,E)- α -farnesene and less 1,3-octadiene and benzyl alcohol, and their overall scent emission increases (Campbell et al., 2019).

Flower visitors respond to intraspecific differences in floral phenotypes (Conner & Rush, 1996; Kuppler et al., 2016; Mothershead & Marquis, 2000; Thomson et al., 1982). Water-stress induced changes in floral traits therefore might lead functional and recognition mismatches between host plants and flower visitors (Descamps et al., 2020; Gérard et al., 2020; Miller-Struttmann et al., 2015). A behavioural experiment with bumblebees on drought stressed *S. arvensis* revealed that bumblebees need more time to make their first visit in cases of decreasing flower and inflorescence size, and visits are significantly shorter on water-stressed plants (Höfer et al., 2021), as flower visitors prefer well-watered plants over water-stressed plants with reduced height, flower number, and size (Al-Ghzawi et al., 2009; Burkle & Runyon, 2016; Descamps et al., 2018; Gallagher & Campbell, 2017; Kuppler et al., 2021). Though, the duration of visits can strongly affect pollination efficiency, because less pollen is transferred from the anther to the pollinator's body during shorter visits (Sahli & Conner, 2007), and pollen reception by the stigma is less likely (King et al., 2013; Ohara & Higashi, 1994), thereby diminishing female and male plant reproductive success. In addition to behaviour, pollinator assemblage can be important for pollination, as changes in the pollinator community can change pollen deposition and influence seed set (Bruckman & Campbell, 2014).

In this study, we investigated the direct impact of water stress on reproductive success through changes in flower production and the indirect links of water stress on pollen deposition and on seed set through changes in pollinator visits. We manipulated water availability using rain-out shelters and measured effects on floral and vegetative traits, floral rewards (i.e. nectar and pollen), pollinator visits, and reproductive success. We hypothesized that limited water availability in the drought treatment would (1) directly impact seed set through reduced total floral abundance and (2) change floral traits (e.g. decrease floral height, daily flower number and size) leading to reduced pollinator visits and less pollen deposition on stigmas, representing the indirect link between water stress and seed set.

2 | MATERIALS AND METHODS

2.1 | Plants and irrigation treatment

We used *Sinapis arvensis* L. (wild mustard, Brassicaceae), which is a self-incompatible plant species visited by a broad range of insect species such as bees and syrphid flies (Kunin, 1993) and which grows in meadows and agricultural landscapes. We used seeds from a common garden experiment at the Botanical Garden of the University of Salzburg, Austria. Seeds were selected from 23 different mother plants and were potted, separated by mother plants, into 7×7×7 cm pots until seedlings emerged. Seedlings were subsequently transferred individually into 5-litre pots (see details in Methods S1).

We reared 10 individuals per mother plant for 4 weeks in a greenhouse in the Botanical Garden of the Ulm University. Each individual from each mother plant was randomly assigned to one of three irrigation treatments: (1) well-watered (WW): plants were watered daily once by hand with 90 ml water; (2) reduced-watered (RW): plants were watered daily with 70 ml water, a reduction based on regional climate projection models for 2071–2100 (LUBW, 2013); (3) drought period (D): plants were watered with 90 ml as needed, when plants showed signs of wilting (after ~4–6 days without water). Irrigation treatments started 26 days after sowing when plants were 10 cm high. We grouped 20 individuals into each treatment ($N = 60$ in total). However, only 53 individuals survived after being planted outside (WW: $N = 17$, RW: $N = 19$, and D: $N = 17$).

2.2 | Experimental setup

On an open field in the Botanical Garden of Ulm University, we set up five rain shelters (1.8 × 4.5 m) covered with PET plastic glazing foil (Gutta Werke GmbH) that allowed 80% PAR transmission. Under each shelter, we established three plots (0.5 m²), each for one treatment of four plant individuals (see details in Method S1). Permission for fieldwork was not necessary.

On 19 May 2020 (4 weeks after sowing), the potted plants were transferred under the rain shelters and were watered using a drip-irrigation system (X-CORE XC-801-E, Hunter Industries), except for the drought-treated plants, which were further watered by hand when they showed severe signs of wilting. After 10 days, we had to adjust watering regime and doubled the water amount for all treatments, otherwise the plants would have withered or would have died. Overall, the experiment lasted 13 weeks from treatment initiation to seed harvest (May 15, 2020 until August 17, 2020).

Temperature and relative air humidity were recorded under each rain shelter (Climate data logger BL30, Trotec GmbH) and near to the shelters (DK655-3S rugged Visual, Driesen+Kern GmbH). Conditions were the same under all shelters, except for shelter 1, which was warmer than the others (see details in Notes S1).

2.3 | Trait measurement and reproductive success

To test for differences in plant phenotypes between treatments, we measured several vegetative and floral traits including morphological, phenological, and chemical traits, all of which are known to mediate flower-visitor interactions (e.g. Junker et al., 2015; Thomson et al., 1982; Table S1). We measured the following parameters once in three freshly open flowers per plant from a low, middle, and high position to avoid position and age effects: stamen, style, and calyx length, petal length and width, flower display size (after 3–6 weeks under treatments), and nectar volume (after 3 weeks under treatment). On two flowers per plant, we collected one anther each in order to count pollen grains by means of a microscope (after 3 weeks under treatment). For pollen and nectar collection, nearly open flowers were

covered with mesh bags the day before collection in order to prevent access by flower visitors. The inflorescence size (greatest expansion) was measured once on five inflorescences per plant (after 3–6 weeks under treatments). Floral height (height of the highest flower) was measured weekly with a folding yardstick; means were used for statistical analyses. The number of flowers and inflorescences were counted every 4 days. Weekly measurements started 2 weeks under treatments. Total sum of flowers and the daily flower number (total number of flowers divided by number of observation days) were calculated for further analysis. Furthermore, we noted the date that the first flower emerged and the last day of flowering per plant individual (see details of measurements in Table S1, Methods S2).

Dynamic headspace sampling was used to examine the effect of the different watering regimes on the quality and quantity of scent emission. All flowers per plant were enclosed within an oven bag (Toppits®, Minden, Germany). The emitted volatiles were trapped for 60 min on 1.5 mg Tenax (mesh 60–80; Supelco) and 1.5 mg Carbotrap B (mesh 20–40; Supelco) in a quartz vial (length 20 mm, inner diameter 2 mm) by using a membrane pump (G12/O1 EB; ASF Rietschle-Thomas) with a flow rate of 200 ml min⁻¹. Samples were collected between 08:30 and 12:00 h (after 3–6 weeks under treatments). Scent samples were analysed using a GC–MS. (details see in Methods S1).

To confirm water stress in a plant, we measured mid-day stem water potential (Ψ_{stem}) on one leaf per plant by using a pressure chamber. The more negative the measured values, the more the plants were water-stressed. Ψ_{stem} was measured 4 weeks after watering treatments started (see details in Methods S2).

To measure how much pollen a flower had received, we marked nearly open flowers that opened the next day and dissected them after 3 days in order to count pollen grains on the stigma by means of a microscope.

2.3.1 | Reproductive success

To investigate pollination success after the flowering period, five stalks per plant were randomly chosen, and the number of pods and aborted (=non-fertilized) flowers were counted, after which we divided the number of aborted flowers by the number of pods per stalk. Additionally, all pods per plant were counted, cut and placed in paper boxes for after-ripening. We calculated the total seed numbers by multiplying the number of pods with the mean seed number per pod (calculated from 12 pods per plant). Additionally, we weighed the total seed mass per plant. To obtain the aboveground biomass, all plant material (without leaves) of each individual was oven-dried for 72 h at 65°C and were weighed directly afterwards. The total root mass was obtained in the same way.

2.3.2 | Flower-visitor interactions

Flower-visitor interactions were recorded on non-rainy days (in total 20 days) between 08:00 and 12:00 h until the end of the flowering period

(31 July). An interaction was defined as a visit by an arthropod to one or more flowers of one plant, that is, a single flower visitor that visited several flowers of one plant consecutively was regarded as one interaction. The observations were conducted by up to two persons within 1 day, and on most days, all 53 plant individuals were observed. The daily observation time of each plant individual was adjusted depending on weather conditions and interaction frequency of floral visitors. Each person simultaneously observed between four (high floral abundance and high interaction frequency) and 12 (low floral abundance and low interaction frequency) individual plants at a given time. On most days, all plants were observed for the same amount of time in the morning. In the afternoon, it was too hot for observations as the number of flower visitors drastically decreased around midday. All interactions on flowers were recorded, and single visitors were collected to identify them immediately after the observations (Table S2). Following identification, the arthropods were released. The total observation time per plant ranged from 100 to 260 min (mean (SD) 191.89 (44.98) min), with plants being observed for mean (SD) 12.45 (3.65) days.

2.4 | Data analysis

All data were analysed and plotted using R (version 4.1.0, R Core Team, 2021). As the stem water potential showed a high variability within the treatments (mean (SD) MPa: well-watered: -0.15 (0.09), reduced-watered: -0.15 (0.06), drought treatment: -0.19 (0.09)), we used water potential as a continuous variable rather than using treatment as a categorical variable for the analyses (Gallagher & Campbell, 2017; Waser & Price, 2016). To test the association between stem water potential (Ψ_{stem}) and measured floral traits and seed mass, we fitted linear mixed-effects models with Ψ_{stem} as the fixed factor and mother plant and rain shelter as the random factors, to account for differences in the microclimate under each shelter. All models were fitted by using the *lmer*-function from the *LME4*-package 1.1-27 (Bates et al., 2015) with restricted maximum likelihood (REML). Total scent emission was square-root transformed to achieve a normal distribution. If no model convergence was reached after the default 10,000 iterations, we restarted the model from the previous fit with a maximum of 100,000 iterations. For testing the differences in mean values of the categorical treatment models, we performed the same analyses but used treatment as fixed factor. Nectar volume and total scent emission were square-root transformed to achieve a normal distribution. Significance was ascertained via Type II Wald chi-square tests implement in the *Anova()* function of the *CAR*-package 3.0-10 (Fox & Weisberg, 2018). The fit of each model was assessed using the *DHARMA*-package 0.4.1 (Hartig, 2020).

The effect of water stress on floral scent bouquet and visitor assemblage was assessed by NMDS analysis by using a permutational multivariate ANOVA (PERMANOVA, 999 permutations, Bray–Curtis similarity distance matrix) in the *VEGAN*-package 2.5-7 (Oksanen et al., 2020). One scent sample from the well-watered treatment was empty and was excluded from analysis. Selected compounds were found in at least 20 plant individuals.

Finally, we conducted structural equation modelling (SEM) analysis to assess the way in which water stress affected reproductive success directly through total sum of produced flowers per plant or indirectly through the contributions of floral traits and resulting number of visits. The SEM was constructed with the gradient of Ψ_{stem} as the exogenous variable and with floral traits (floral height, daily flower number, total sum of flowers, diameter of flowers and inflorescences, nectar volume, pollen amount, time to first flower, flowering period, total scent emission), number of visits per flower per day, and seed mass (output variable) as endogenous variables. SEM analysis was repeated with pollen number on stigma as the output variable. All data were centred and scaled by function: $x - \text{mean}(x)/\text{sd}(x)$. Data were fitted using linear mixed-effects models with motherID and shelterID as random factors for all models with *lmer*-function, and SEM was constructed using *pssem*-function from the *PIECEWISE* SEM-package 2.1.2 (Lefcheck, 2016). Standardized path coefficients (β -value) extracted from the model are a measure for strength of connection between two variables/paths with $0.3 < \beta < 0.5$ defined as a weak connection, $0.5 < \beta < 0.7$ as a moderate, and $\beta > 0.7$ as a strong connection. We performed an additional SEM to check for singularity that had no influence on the output (see Notes S4). For the analysis with seed mass, we had to exclude three individuals, two from the drought treatment, as we could not measure the water potential, and one individual from the well-watered treatment, as we had no scent data. For the analysis of pollen on stigma, we used 28 individuals as we were not able to obtain flowers from all individuals after they had opened for 3 days to receive pollen.

3 | RESULTS

3.1 | Linkage between water stress, floral traits, pollinator visits and reproductive success

There is a direct link from water stress to seed set as the total number of produced flowers played a dominant role in seed production (Figure 1; Table S3 for details). The β -value = 0.790 shows a strong positive link between the sum of produced flowers and seed mass ($p < 0.001$) and flower abundance was significantly affected by water stress ($p = 0.031$), with increasing abundance when water stress decreased ($\beta = 0.334$). For the other floral traits, only floral height was significantly affected by water stress ($p = 0.033$), with a weak positive connection strength ($\beta = 0.332$). Overall, aboveground biomass of well-watered plants was twice as much as in drought stressed plants (LMM: $\chi^2 = 9.25$, $p = 0.010$; means (SD) in g: WW: 39.92 (28.12), RW: 33.47 (24.53), D: 17.89 (15.14), details in Notes S2).

For the indirect link via floral traits and pollinator visits, a weak negative connection was detected between number of visits and the daily flower numbers ($\beta = -0.389$) and nectar volume ($\beta = -0.399$), as the per-flower visit decreased with higher flower number ($p = 0.007$) and more nectar ($p = 0.034$). However, more days until flowering and longer flowering time increased the number of visits ($\beta = 0.295$, $p = 0.027$; $\beta = 0.252$, $p = 0.029$). With increasing number of visits, the seed mass

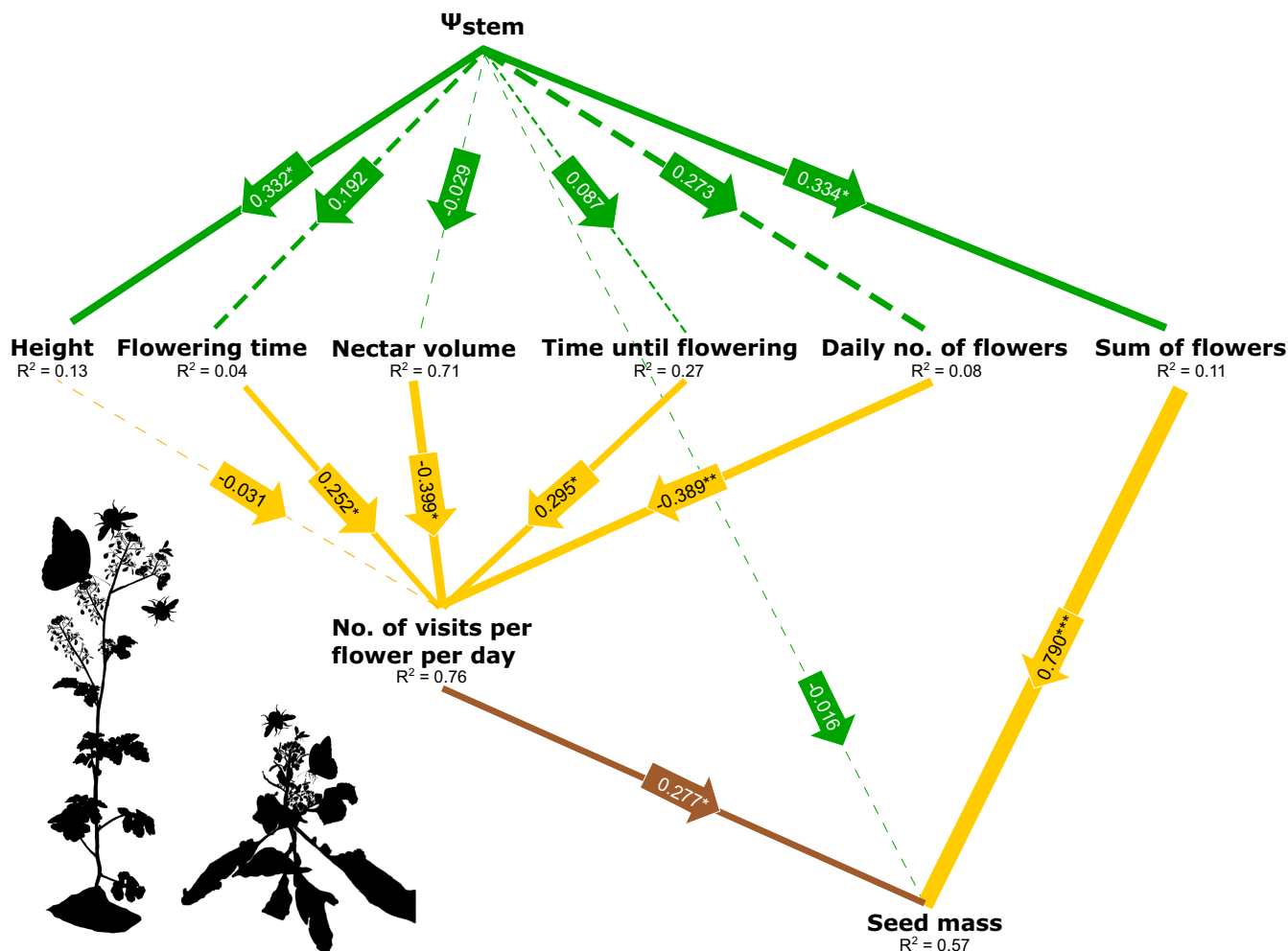


FIGURE 1 Structural Equation model showing the paths through which water stress affects seed mass as an indicator of reproductive success ($N = 50$ plant individuals, well-watered: $N = 16$, reduced-watered: $N = 19$, drought-treated: $N = 15$). Standardized path coefficients are shown in the arrows. To provide a better overview, we have shown only significant paths of floral traits. Line width is proportional to path strength; solid and dashed lines represent significant ($p < 0.05$) and non-significant ($p > 0.05$), respectively. Asterisks on path coefficients represent * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. R^2 (conditional) denotes the proportion of variance explained by the model. Global goodness-of-fit for the model: Fisher's $C = 152.92$, $df = 94$, $p = 0$.

increased ($P = 0.041$), but the connection was weaker ($\beta = 0.277$) than between the sum of flowers and seed mass ($\beta = 0.720$). Overall, 57% of variance in seed mass could be explained by the variables included in the SEM. Along the Ψ_{stem} gradient (-0.376 to -0.041 MPa), the predicted seed mass increased by 34%; however, it was not significant (LM: $F_{1,49} = 0.89$, $p = 0.350$; Figure S1B; Table S5). The number of pods and seeds per plant also increased with decreasing water stress ($p = 0.030$, $p = 0.018$, respectively; Table S5). However, the number of seeds within a single pod was unaffected by water stress ($p = 0.930$). Differences between the categorical treatments are shown in Figure S1A and Table S6. Pollination success (ratio of aborted flowers divided by pods per stalk) was not affected by water stress; however, the ratio decreased by 11% along the Ψ_{stem} gradient (LM: $F_{1,48} = 0.178$, $p = 0.675$), as the number of pods increased with less water deficit.

In the SEM analysis with pollen deposition, we found no significant links between Ψ_{stem} and floral traits (Figure 2; Table S4); although, $\beta = 0.700$ revealed a strong connection for time until

flowering and number of visits per flower/day ($p = 0.010$). However, number of visits did not affect the pollen amount on stigmas ($p = 0.507$). On the other hand, stamen length was strongly connected with pollen on stigma ($\beta = -0.869$), as with increasing stamen length the pollen deposition reduced ($p = 0.036$).

We further tested if the flower visitor assemblage was affected by watering treatment; however, it did not differ significantly between treatments ($F_{2,50} = 0.542$, $p = 0.857$, see Notes S3). The correlation coefficient of visitor taxa and floral traits ($RV = 0.153$) did not differ significantly from chance (permutation test, 999 permutations: $p = 0.226$, Notes S3).

3.2 | Water stress and floral traits

Along the Ψ_{stem} gradient (Figure 3; Table S7 for statistical details), predicted floral height increased by $\sim 47\%$ ($p = 0.007$), daily flower

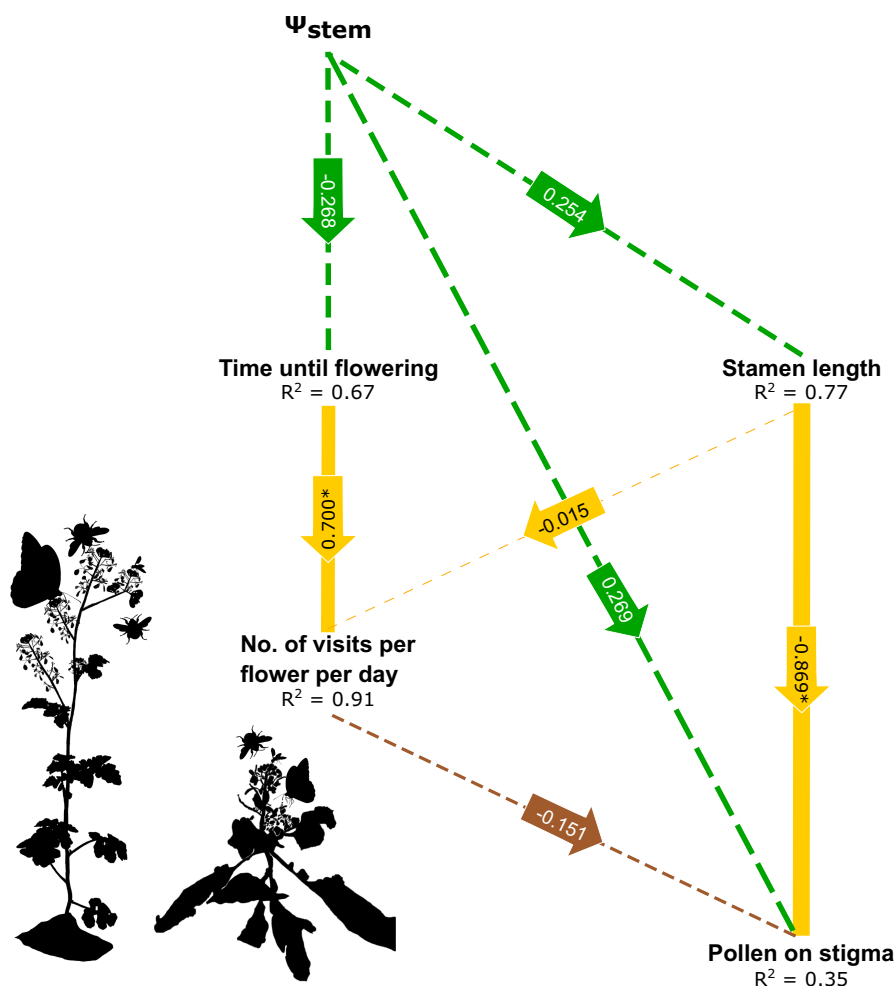


FIGURE 2 Structural equation model showing the paths through which water stress affects pollen deposition on stigmas as an indicator of reproductive success ($N = 28$ plant individuals, well-watered: $N = 7$, reduced-watered: $N = 10$, drought-treated: $N = 11$). Standardized path coefficients are shown in the arrows. To provide a better overview, we have shown only significant paths of floral traits. Line width is proportional to path strength; solid and dashed lines represent significant ($p < 0.05$) and non-significant ($p > 0.05$), respectively. Asterisks on path coefficients represent $*p < 0.05$. R^2 (conditional) denotes the proportion of variance explained by the model. Global goodness-of-fit for the model: Fisher's $C = 297.89$, $df = 134$, $p = 0$.

number by $\sim 79\%$ ($p = 0.027$), number of inflorescences by 70% ($p = 0.027$), and stamen length by $\sim 16\%$ ($p = 0.018$). Furthermore, time until first flower increased by 9% , flowering time by 20% , inflorescence diameter by 14% , petal length by 7% , petal width by 6% , style length by 10% , calyx length by 9% , and total scent emission by 45% ; flower diameter decreased by 4% , and nectar volume and pollen load per flower decreased by 50% and by 10% , respectively. However, these effects were not significant. Differences in the mean value of the categorical treatment are shown in Figure S2; Table S8.

Scent bouquets of all three treatments did not differ significantly (PERMANOVA, Bray–Curtis similarity measures, $F_{2,49} = 1.01$, $p = 0.413$; Figure S3; Table S9 for detailed list of scent compounds).

4 | DISCUSSION

Water stress lowers productivity and resources for flower production (Cariveau et al., 2004; Lemoine et al., 2013) or alters floral traits leading to changed pollinator visitations (Descamps, Jambrek, et al., 2021; Descamps, Quinet, & Jacquemart, 2021; Höfer et al., 2021; Kuppler et al., 2021). These effects can reduce pollination services and ultimately decrease the reproductive success of plants. Knowledge of the proportion of direct and indirect effects

on seed set is nevertheless important in order to improve our understanding of the impacts of increasing drought events. As shown here, the direct impact of water stress through reduced investment in floral abundance has the strongest effect on seed set. The indirect link of water stress through changes in floral traits and therefore altered pollinator visits plays a subordinate role for the reproductive success of *Sinapis arvensis* plants. Water-stressed plants receive fewer visits compared with well-watered plants, although pollen deposition is not significantly affected along the Ψ_{stem} gradient, indicating that pollination success under water deficit might not be fully dependent on the number of visits per flower.

4.1 | Linkage between water stress, floral traits, pollinator visits and reproductive success

As expected, the seed set was reduced with increasing water stress, and the direct link of total number of flowers to seed mass had the strongest effect; more flowers probably leading to more ovules for seed production, in accordance with observations from other studies (Al-Ghazawi et al., 2009; Boutraa & Sanders, 2001; Gallagher & Campbell, 2017; Guillioni et al., 2003). The indirect link of water stress via flower-visitors and pollination had weaker effects on

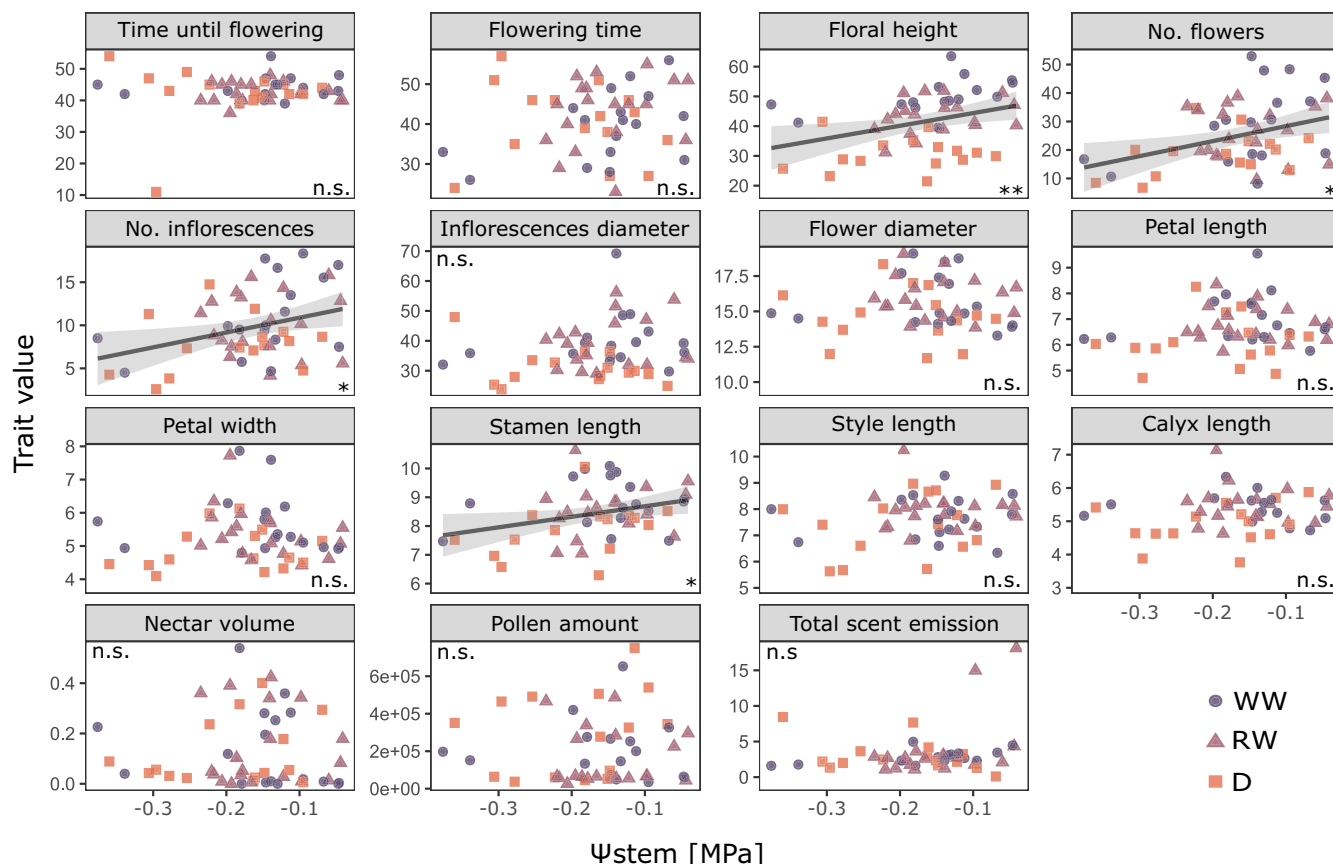


FIGURE 3 Association between floral traits and stem water potential [MPa]. Well-watered (circles): $N = 17$, reduced-watered (triangles): $N = 19$, and drought-treated (rectangles): $N = 17$. Each colored point represents one plant. Black lines are regression lines derived from a linear mixed model; gray shading represents the standard error of the regression line. * $p < 0.05$, ** $p < 0.01$, n.s. = not significant.

seed set. In general, more visits per flower are beneficial for sufficient pollen deposition on the stigma for maximization of seed set per flower (Dudash & Ritland, 1991; Karron et al., 2006). Therefore, the consistent numbers of seeds per pod in our experiment indicate that the pollen deposition per stigma was sufficient for flowers in all treatments, the amount of deposited pollen grains being decisive for the number of seeds produced (Cariveau et al., 2004; Falque et al., 1995). This suggests that indirect effects are less strong if pollen deposition does not differ or is sufficient, and thus, the reduction of flower number under water deficit affects seed set more strongly than the number of visits by pollinators.

Pollen deposition on stigmas was not significantly affected by flower visits. In general, plants with a higher number of flowers received more visits in total, but the per-flower visits to our plants tended to decrease with increasing flower number, in accordance with (Brody & Mitchell, 1997), and possibly leads to lower pollen deposition per flower of well-watered plants. On the other hand, flower-visits might have been shorter on water-stressed plants (Höfer et al., 2021) making pollen transfer to the stigma less likely (King et al., 2013; Ohara & Higashi, 1994). These two aspects might compensate each other leading to a similar pollen deposition on flowers of water-stressed and well-watered plants. A further aspect to take into consideration might be the

type of pollinators visiting the flowers. The generalist honey bee *Apis mellifera*, made by far the most visits during our observations. They visited nearly every flower per plant, and, as the plants of the different treatments were in close proximity to each other, they switched regularly between plants. This might have led to similar pollen deposition in stressed and watered plants. Honey bees show a higher flower constancy, that is, they visit the same type of flowers again, compared with other bees (Free, 1963, 1970; Grant, 1950; Slaa et al., 2003), thereby reducing handling time costs and errors, as honey bees store information about correct flower handling in their easily accessible short-term memory (Chittka et al., 1999). Thus, the honey bees in our experiments might have learned how to handle *S. arvensis* flowers and might have visited all nearby plants because of flower constancy, although the water-stressed plants with lower floral display should have been less attractive than well-watered plants. In addition, if honey bees mainly collected pollen, water-stressed plants might still have been attractive to the bees, as the pollen amount did not differ between treatments, resulting in similar amounts of visits.

The SEM revealed a strong negative relation between average stamen length and pollen deposition, with fewer pollen grains being found on stigmas under conditions of increasing stamen length. With an increased distance between anthers and stigma,

herkogamy increases. Herkogamy, that is, the spatial separation of anthers and stigma, prevents self-fertilization in self-compatible plants (Endress, 2004; Webb & Lloyd, 1986). With increasing water stress, the distance between the anthers and stigma shrinks. The style length of our plants was unaffected by water stress, and thus more pollen from the shortened anthers of the water-stressed plants was likely to reach the stigma. Self-incompatible *Brassica rapa*, a species from the same family as *S. arvensis*, has evolved degrees of self-compatibility and autonomous selfing under herbivory (Ramos & Schiestl, 2019). In breeding experiments with *S. arvensis*, a few examples of pseudocompatibility have been observed (Ford & Kay, 1985) suggesting that *S. arvensis* under water stress can also show self-compatibility that is attributable to phenotypic plasticity and that leads to self-fertilization.

4.2 | Water stress and floral traits

In the single analyses of floral traits, our plants exhibited reduced floral height, flower and inflorescence abundance, and above-ground biomass with increasing water stress, as a phenotypic response to lower water loss through evaporation and transpiration (Feild et al., 2009; Galen et al., 1999; Lambrecht et al., 2017; Lambrecht & Dawson, 2007; Teixido & Valladares, 2014). In contrast to other studies, our plants did not reduce their flower size (Burkle & Runyon, 2016; Carroll et al., 2001; Caruso, 2006; Gallagher & Campbell, 2017; Suni et al., 2020), indicating that *S. arvensis* plants maintain flower size to attract pollinators by reducing investment in shoot growth and flower abundance. Nevertheless, Kuppler et al., 2021 found a reduction in flower size under water stress for *S. arvensis*; however, they used a pulsed-drought treatment, which started shortly before flowering and it was shown that the timing of stress imposition is important, as water stress application shortly before flowering has a greater impact than an earlier initiation of such treatment (Kuppler & Kotowska, 2021). Thus, our plants seem to have adjusted their physiology to the new conditions by reducing plant growth in order to compensate for maintaining floral characteristics. Larger flowers are more attractive to pollinators who increase their visitation rate (Burkle & Runyon, 2016; Gallagher & Campbell, 2017; Kuppler et al., 2021) and reduce the time that visitors need for their first visit (Höfer et al., 2021). Therefore, plants need to maintain floral size expression for pollinator attraction.

Further, in our experiments, nectar volume seems to decrease under less water stress. However, water-stressed flowers have previously been reported to produce reduced amounts of nectar (Brunet & van Etten, 2019; Gallagher & Campbell, 2017; Waser & Price, 2016) and pollen (Waser & Price, 2016) compared with well-watered plants. Our plants might compensate for water stress by reducing flower number, while maintaining nectar production for higher resource investment and increased reward production in single flowers. In the SEM, we found no significant effect of water stress on daily flower number, but a trend was detected indicating that water deficit reduced the number of flowers but increased

nectar volume. This might still make the plant attractive to pollinators, as increased reward availability increases the number of pollinator visits or (Brunet et al., 2015) or increased visit length (Waser & Price, 2016). Furthermore, reduced water availability had no effect on floral scent. Along the Ψ_{stem} gradient, plants emitted the same amount of scent per flower as it was also shown previously for *S. arvensis* (Höfer et al., 2021). Further, scent bouquet did not differ between treatments. Nevertheless, the scent bouquet of water-stressed plants seems to be more variable between individuals. This implies that *S. arvensis* is able to emit a stable scent bouquet to “pretend” that a floral reward is present potentially to maintain pollinator attraction. However, in other plant species the scent emission increases and composition of scent compounds change under water stress (Burkle & Runyon, 2016; Campbell et al., 2019; Glenny et al., 2018; Rering et al., 2020), leading to possible recognition mismatches between plant and pollinators, especially in highly specialized pollinator attractive systems (Vereecken et al., 2010). If pollinators are no longer able to find their host plants, this would lower visitation rate and pollination success and reduces the reproductive success of the plant. Therefore, plants might sustain their characteristic scent in a range in which possible pollinators are still able to recognize it as their host plant.

5 | CONCLUSION

Reduced water availability causes changes in floral traits, for example, reduced growth and flower abundance, presumably in order for the plant to invest more resources in fewer flowers and thereby to maintain pollination success. Plants are able to compensate for water stress to retain floral trait expression, such as stable scent emission and bouquet, for the maintenance of pollinator attraction. However, flower-visits might be reduced because of the lower floral display in water-stressed plants, although they are still sufficient to ensure pollination success leading to full seed set per flower. We have also shown that a reduction of overall flower abundance has the strongest effect on seed set and consequently on reproductive success. These findings indicate that a direct link from water stress to seed set has a stronger impact on the reproductive success of plants than an indirect link through altered floral trait expression and reduced pollinator visits in a generalist plant species.

AUTHOR CONTRIBUTIONS

Jonas Kuppler conceived the study. Rebecca J. Höfer, Manfred Ayasse and Jonas Kuppler designed the study. Rebecca J. Höfer and Tina Lindner collected the data. Rebecca J. Höfer, Tina Lindner, and Jonas Kuppler analysed the data. Rebecca J. Höfer drafted the first version of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data and R-Code available from the Dryad Digital Repository <https://doi.org/10.5061/dryad.66t1g1k5f> (Höfer et al., 2022).

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