Water stress and nitrogen supply affect floral traits and pollination in the white mustard, *Sinapis alba* (Brassicaceae)

Asma Akter Corresp., [1, 2], **Jan Klečka** [2]

[1] Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
[2] Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

Corresponding Author: Asma Akter
Email address: asma.akter@entu.cas.cz

Changes in environmental conditions are likely to have a complex effect on the growth of plants, their phenology, plant-pollinator interactions, and reproductive success. The current world is facing an ongoing climate change along with other human-induced environmental changes. Most research has focused on the impact of increasing temperature as a major driving force for climate change, but other factors may have important impacts on plant traits and pollination too and these effects may vary between different seasons. In addition, it is likely that the effects of multiple environmental factors, such as increasing temperature, water stress, and nitrogen enrichment are not independent. Therefore, we tested the impact of three key factors – season, water, and nitrogen supply – on plant traits, pollination, and seed production in *Sinapis alba* (Brassicaceae). Plants were grown in three seasons with two levels of water and nitrogen supply in a factorial design. We collected data on multiple vegetative and floral traits and assessed the response of pollinators in the field. Additionally, we evaluated the effect of growing conditions on seed set in plants exposed to pollinators and in hand-pollinated plants. Our results show that water stress impaired vegetative growth, decreased flower production, reduced visitation by pollinators and seed set, while nitrogen availability played an important role in nectar production. Seasonality modulated the effect of water and nitrogen availability on vegetative and floral traits and strongly affected flowering phenology and flower production. We demonstrated that changes in water and nitrogen availability alter plant vegetative and floral traits, which impacts flower visitation and consequently plant reproduction. We conclude that ongoing environmental changes such as altered precipitation regimes and nitrogen enrichment may thus affect plant-pollinator interactions with negative consequences for the reproduction of wild plants and insect-pollinated crops.

Water stress and nitrogen supply affect floral traits and pollination in the white mustard, *Sinapis alba* (Brassicaceae)

Asma Akter[1,2] & Jan Klečka[1]

[1] Institute of Entomology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic
[2] Department of Zoology, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Corresponding Author:
Asma Akter
Email address: asma.akter84@gmail.com

Abstract

Changes in environmental conditions are likely to have a complex effect on the growth of plants, their phenology, plant-pollinator interactions, and reproductive success. The current world is facing an ongoing climate change along with other human-induced environmental changes. Most research has focused on the impact of increasing temperature as a major driving force for climate change, but other factors may have important impacts on plant traits and pollination too and these effects may vary between different seasons. In addition, it is likely that the effects of multiple environmental factors, such as increasing temperature, water stress, and nitrogen enrichment are not independent. Therefore, we tested the impact of three key factors – season, water, and nitrogen supply – on plant traits, pollination, and seed production in *Sinapis alba* (Brassicaceae). Plants were grown in three seasons with two levels of water and nitrogen supply in a factorial design. We collected data on multiple vegetative and floral traits and assessed the response of pollinators in the field. Additionally, we evaluated the effect of growing conditions on seed set in plants exposed to pollinators and in hand-pollinated plants. Our results show that water stress impaired vegetative growth, decreased flower production, reduced visitation by pollinators and seed set, while nitrogen availability played an important role in nectar production. Seasonality modulated the effect of water and nitrogen availability on vegetative and floral traits and strongly affected flowering phenology and flower production. We demonstrated that changes in water and nitrogen availability alter plant vegetative and floral traits, which impacts flower visitation and consequently plant reproduction. We conclude that ongoing environmental changes such as altered precipitation regimes and nitrogen enrichment may thus affect plant-pollinator interactions with negative consequences for the reproduction of wild plants and insect-pollinated crops.

Introduction

Ecosystems worldwide are facing accelerating global change characterised by increasing temperature and changing levels of precipitations, coupled with increasing supply of nitrogen and other nutrients, biological invasions, and habitat loss (Hoover *et al.,* 2012). Testing the effects of these environmental changes on plant growth and reproductive fitness are necessary to understand potential impacts of climate change on the productivity and functioning of natural and agricultural ecosystems (Rustad, 2008). For pollinator-dependent plants, changes of these factors may impact their relationship with pollinators and consequently the success of pollination and plant reproduction (Scaven and Rafferty, 2013; Gérard *et al.,* 2020). Reproductive success of animal-pollinated plants generally depends on floral traits, which act as an advertisement of rewards to their pollinators (Hegland and Totland, 2005; Basnett *et al*., 2019). Despite strong selection from pollinators, plant populations naturally show significant variation in their morphological, phenological, and floral traits. A part of this variation results from heritable genetic differences among individuals, while the rest (phenotypic plasticity) is caused by local environmental factors (Holtsford and Ellstrand, 1992; Gray and Brady, 2016). Changing environmental factors may thus alter plant-pollinator interactions as a consequence of changing plant traits (Carroll *et al.,* 2001; Scaven and Rafferty, 2013).

Increasing temperature and water stress can have a major effect on the physiological and phenological development of plants (Schweiger *et al*., 2010). The global average annual temperature is rising gradually, with higher increases in the average and minimum temperatures reported during the winter than the summer months (NOAA, 2021). Consequently, phenological shifts are visible in many early-flowering plants (Kehrberger and Holzschuh, 2019). Increased average temperatures may allow them to initiate growth and flowering earlier because of earlier snowmelt and higher spring temperatures (Fitter and Fitter, 2002; Güsewell *et al.,* 2017).

Water availability is changing in a complex way as many regions of the world are facing water scarcity and other regions are facing increased precipitation (Christensen *et al.,* 2007). Although water availability is an important determinant of plant growth, its effect on floral traits is less clear. Water availability can directly influence the flowering time and duration (Bernal *et al.,* 2011; Lasky *et al.,* 2016) and plants with adequate water supply may achieve greater height and floral abundance (Galen, 2000; Carromero and Hamrick, 2005), increased nectar production (Zimmermann and Pyke, 1988, Carroll *et al*., 2001), and higher nectar sucrose content (Wyatt *et al.,* 1992). Water stress may lead to reduced floral resources (Rering *et al*., 2020) including impaired pollen and seed development (Barnabás *et al.,* 2008; Hedhly, 2011; Snider and Oosterhuis, 2011). Changes in water availability can also affect various stages of phenological growth differently in the same species, for example, severe water deficiency in maize delays silking but not anthesis (Blum, 1996). The consequences of water stress for flower visitation by insect pollinators are still poorly known, but there is evidence that alteration of floral rewards by water stress may lead to decreased flower visitation (Descamps *et al.,* 2018).

Variation in nitrogen supply is another key driver of local plant diversity (Bobbink *et al.,* 2010). At the global scale, anthropogenic nitrogen deposition increased more than 10 times over the last century (Galloway *et al.*, 2004; Fowler *et al.,* 2013) and is now around 200 Tg N per year with a wide range of negative environmental consequences (Battye *et al.*, 2017). In a plant community, small-scale heterogeneity of soil nitrogen content at the scale of a few meters can lead to variation in plant size and reproductive success (Scott-Wendt *et al.,* 1988), possibly including their mutualistic relationship with pollinators. Under the conditions of nitrogen limitation, increasing nitrogen supply can enhance plant growth and enable plants to produce floral rewards of higher quality (Gardener and Gillman, 2001; Burkle and Irwin, 2009; Burkle and Irwin, 2010). In particular, nitrogen enrichment can increase the amount of nectar produced per flower and alter the concentration and composition of amino acids in nectar, which may affect pollinator preferences and foraging behaviour (Pyke, 1981; Baker and Baker, 1983; Inouye and Waller 1984; Gardener and Gillman, 2002; Hoover *et al*., 2012). For example, several studies on fruit and vegetable plant species showed that pollinator preferences for their flowers strongly depended on nectar volume or sugar concentration (Jabłoński *et al.,* 1984; Schmidt *et al.,* 2015; Roldán-Serrano and Guerra-Sanz, 2005). A study on *Citrus* plants also showed that honeybee visitation rate was positively correlated with nectar volume (Albrigo *et al.,* 2012). These investigations indicated that both the quantity and quality of nectar influences flower preferences of pollinators.

Changing environmental conditions can alter plant traits and disrupt interactions of plants with pollinators, but the consequences for pollination and seed production remain poorly known. Given the pace of ongoing climate change, which alters not only the temperature but also water availability for plants, and still increasing anthropogenic nitrogen deposition, it is important to investigate how these factors act interactively. Such interactive effects have been rarely considered in experimental studies on plant-pollinator interactions (Hoover *at al.*, 2012). To fill this gap, we examined the interactive impact of water and nitrogen supply on vegetative and floral traits, pollination, and seed production in *Sinapis alba* across three different seasons. It is an economically important crop, cultivated over a wide geographic range for oil and fodder and is partly self-incompatible with seed production strongly dependent on pollination by insects. In our study we aimed to answer the following questions: 1) What are the interactive effects of water and nitrogen supply on vegetative and floral traits of *S. alba?* 2) Are these effects consistent in different seasons? 3) How does intraspecific trait variation caused by growing conditions affect flower visitation by pollinators, pollination efficacy, and seed production?

Materials & Methods

The experimental plant, *Sinapis alba*

Sinapis alba (white mustard) is a rapidly growing annual plant from the Brassicaceae family with a short vegetation period. This crop is widely cultivated for seeds, oil, fodder, or as a catch crop. Flowers are yellow, produced in an elongated raceme, have four petals, four sepals, and 6 stamens, of which four are long and two are short. Fruit is a pod with usually four seeds but can have up to eight seeds (Jauzein, 2011). A wide range of pollinating insects visit this plant but the European honey bee (*Apis mellifera*), bumble bees and solitary bees are the main pollinators in Europe (Flacher *et al.,* 2020).

Growing *S. alba* under variable conditions in the greenhouse

This experiment was conducted in the greenhouse, where *S. alba* seedlings were grown in the winter (30 plants per treatment), spring (60 plants per treatment), and summer (45 plants per treatment) in 2017-2018. Growing conditions varied among the three seasons in temperature and sunlight. The temperature in the greenhouse fluctuated in a near-natural way and was on average 21°C in the winter 2017-2018, 25°C in the spring 2017, and 29°C in the summer 2018. A minimum 10 hours of daylight (natural daylight + artificial light) was maintained for all seasons. Plants grown in the winter received 10-12 hours of daylight, plants grown in the spring received 12-16 hours, and plants grown in the summer received 16 hours of daylight. The plants were grown in a combination of garden soil: compost soil: sand = 2:2:1 by volume. Seeds were germinated in germination trays and seedlings were transferred to individual pots (11 x 11 x 11 cm) four days after germination.

We divided the seedlings from each season into four treatment groups which received a different combination of two watering regimes and two levels of nitrogen supply. Based on the average ca. 700 mm annual precipitation in the Czech Republic (source: Czech Hydrometeorological Institute, https://www.chmi.cz/), we determined the lower level of water for one pot as 20 ml and the higher level of water as 40 ml per day. As the minimum N application recommended for the *S. alba* is 12 kg N ha^{-1} with a ≤ 330 mm annual precipitation (Brown *et al.,* 2005) and the N deposition is 100 kg N ha^{-1} for Europe for two years, we applied NPK fertiliser, corresponding to 0.242 g N pot^{-1} (~ 100kg N ha^{-1}) as higher level of application and 0.121 g N pot-1 (~50 kg N ~~pot~~$^{-1}$) as lower level, once a week for 8 weeks according to Vitousek *et al.,* 1997. Before conducting the main experiment, we performed a preliminary trial to determine the described water level, N~~$_2$~~ application, and soil mixture to optimize the growth conditions for *S. alba*. The position of plants in the greenhouse was altered regularly to avoid any possible impact of environmental gradients, e.g. the light level, within the greenhouse. Plants infected with diseases or attacked by aphids were discarded from the experiment.

Assessment of plant morphology, phenology and nectar production

We measured several vegetative and flora traits of individual plants in each treatment group. Plant height and leaf number were measured several times throughout the growing period and final height was taken after the end of flowering. The number of leaves was counted for the main shoot only. Stem diameter of each plant was measured 20 cm above ground. The number of days until the onset of flowering was counted from the day of the seedling transfer to the opening of first flower and the total number of flowers bloomed were counted until the end of flowering. Nectar was collected from 4 flowers per plant in each treatment group after one day of flowering by using calibrated 0.5 µl capillary tubes (Drummond Microcaps®), which allowed us to measure the volume of nectar. Additional data were collected on the plant fresh weight and dry weight of plants grown in the spring to determine the effect of different combinations of water and nitrogen availability on plant biomass. Overall, we collected data on plant height, the number of leaves, and stem diameter from 11-20 plants, sampled nectar from 15-25 plants, measured the onset of flowering in 15-30 plants, and counted the total number of flowers in 12-15 plants per treatment and season. All data collected during the experiments are deposited in Figshare (https://doi.org/10.6084/m9.figshare.13317686).

Pollination efficacy treatment and field pollination observations

To determine the impact of water and nitrogen supply on the pollination efficacy in *S. alba*, we carried out self- and cross-pollination in 6 plants per treatment grown in the winter. We marked flowers selected for the experiment, performed cross-pollination manually using pollen from a different plant, and recorded fruit and seed production after the completion of seed development. Plants grown in the spring and the summer were brought outside and placed in a sunny location nearby the Institute to assess the pollinator response and natural pollination efficacy under field conditions. First part of the pollinator observations was carried out in the spring 2017 (May 17 – May 31) and a second part in the summer 2018 (July 26 – July 31). We always exposed four plants at the same time (one plant from each of the four water x nitrogen combinations), placed 1 meter apart in a square configuration. We observed their visitation by naturally occurring pollinators for 30 minutes, from 9:00 to 14:00 each day. Altogether, we carried out observations of forty-four groups of four plants, which resulted into a total 22 hours of observation. Pollinators were observed, collected, and identified in the field. After the end of each observation, the plants were brought back to the greenhouse, their open flowers were marked, and seed production through the natural pollination was measured after fruiting (data: https://doi.org/10.6084/m9.figshare.13317686).

Statistical analyses

We used generalised linear models (GLM) to assess the individual and interactive impact of water, N_2, and season on the plant vegetative and floral traits. The availability of water, nitrogen, and the season were used as factors in the analyses. Depending on the type of the response variable, we specified the GLM with either Gaussian error distribution, overdispersed Poisson (“quasipossion”, the number of flowers), or Gamma distribution with a log link function (onset of flowering and nectar volume). We analysed data from the outdoor flower visitation experiment using GLM with water, nitrogen level, and season as factors, using the overdispersed Poisson (“quasipossion”) error distribution. We analogously analysed also data on seed set of plants depending on growing conditions. We always examined the distribution of residuals to verify that the models fitted the data well. We conducted all analyses in R Version 3.6.3 (R Core Team 2020). Most plots were created using GraphPad Prism (Version 6.01, for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com).

Results

Vegetative traits

We observed a complex response of the selected vegetative traits of *S. alba* to differences in the growing conditions (Table 1, Fig.1). Plant height was affected by the three-way interaction of water availability, nitrogen supply, and season, i.e. the effect of each variable was dependent on the values of the other two variables (F = 4.94, P =0.008, Fig. 1A). While higher water availability made the plants taller, increasing nitrogen availability made the plants shorter. The magnitude of these effects varied across the three seasons (Fig. 1A). In addition, plants grown in the summer were almost 50% shorter than those grown in the winter. Stem diameter was larger in plants grown under high water availability (F = 40.84, P < 0.001, Fig. 1B) and varied also depending on nitrogen supply in interaction with the season (F = 9.51, P < 0.001) – higher nitrogen supplies increased stem diameter in plants grown in the winter and in the summer, but not in the spring (Fig. 1B). On the other hand, the number of leaves was affected only to a limited degree by water availability (F = 16.20, P < 0.001, Fig. 1C) and the season (F = 7.48, P <

0.001), while nitrogen supply had no measurable effect (*F* = 0.09, *P* = 0.768). Finally, dry weight of the plants grown under high water availability was 2.6 times higher compared to those grown under low water availability, with a positive effect of nitrogen supply only at high water availability (water x nitrogen interaction: *F* = 5.52, *P* = 0.023, Fig. 1D).

Phenology and flower resource variation

The onset of flowering was delayed by ca. 21 days in the winter compared to the spring and summer (*F* = 69.45, *P* < 0.001) and slightly delayed also by high nitrogen supply (*F* = 6.21, *P* = 0.013, Table 2, Fig. 2A). The number of flowers produced over the plants' flowering period was affected positively by water (*F* = 24.24, *P* < 0.001) and slightly negatively by nitrogen supply (*F* = 6.83, *P* = 0.010), with a week evidence for their interactive effect (*F* = 3.60, *P* = 0.060), and varied among the three seasons (Table 2, Fig. 2B). Finally, nectar volume showed a complex dependence on the interaction of water, nitrogen supply, and season (*F* = 3.56, *P* = 0.030). Higher water availability increased nectar volume in the winter, but not in the spring and summer, while higher nitrogen availability increased nectar volume under low water availability in both spring and summer (Fig. 2C).

The dependence of flower visitation on growing conditions

We observed flower visitation by eight major types of flower-visiting insects in the spring and summer which we distinguished as: the honeybee (*Apis mellifera*), solitary bees, wasps, bumblebees, rapeseed beetles (*Brassicogethes* (=*Meligethes*) sp.), other beetles, hoverflies, and other flies. Rapeseed beetles were the most abundant flower visitors in the spring, followed by honeybees, while solitary bees were dominant in the summer, followed by hoverflies (data: https://doi.org/10.6084/m9.figshare.13317686).

Plants grown with high amount of water were visited more frequently than the plants grown with low amount of water (*F* = 23.57, *P* < 0.001) and the total number of flower visitors was higher in the spring than in the summer (*F* = 14.19, *P* < 0.001) (Fig. 3A, 3B). Nitrogen supply under which the plants were grown did not consistently affect their flower visitation (*F* = 0.26, *P* = 0.612). Flower visitation was also affected by the number of open flowers (*F* = 18.92, *P* < 0.001, Fig. 3C) and by plant height (*F* = 8.89, *P* = 0.003, Fig. 3D), but the effect of water availability and season remained significant even after accounting for the variation in flower number and height (GLM, $F = 5.46$, $P = 0.021$ for the effect of water and $F = 6.58$, $P = 0.011$ for the effect of the season), i.e. the differences in flower visitation between plants grown under different conditions could not be explained simply by differences in plant height and flower number. In addition to differences in total flower visitation, we detected changes in the composition of the flower visitors observed on plants grown under different water availability according to a redundancy analysis (RDA) performed separately for observations from the spring ($F = 4.0$, $P = 0.004$) and summer ($F = 3.1$, $P = 0.028$), while nitrogen supply did not affect the composition of flower visitors ($F = 0.37$, $P = 0.869$ for the spring data and $F = 0.57$, $P = 0.669$ for the summer data). Some flower visitors visited plants grown under high water availability more frequently, particularly solitary bees and rapeseed beetles, while other flower visitors did not show a clear preference (Figs. 4 and 5).

Pollination efficacy and seed production

Our hand pollination experiment confirmed that *S. alba* is partially self-incompatible. Plants cross-pollinated by hand using a brush produced ca. 3.9 times higher number of seeds per flower than the self-pollinated ones; on average 2.9 compared to 0.7 seeds per flower (Fig. 6A). However, the seed set depended not only on the mode of pollination (self-pollinated compared to cross-pollinated) but on its interaction with nitrogen availability ($F = 10.64$, $P = 0.002$). Specifically, higher nitrogen availability increased seed set in self-pollinated plants, but decreased seed set in cross-pollinated plants. In addition, higher water availability increased seed set in both self-pollinated and cross-pollinated plants irrespective of the nitrogen level ($F = 5.24$, $P = 0.028$) (Fig. 6B).

Plants exposed to natural pollination in the spring and summer produced a variable number of seeds per flower depending on the interaction of water availability and season ($F = 8.93$, $P = 0.004$) and on the nitrogen availability ($F = 5.08$, $P = 0.028$). We observed a slightly higher number of seeds per flower in plants grown under high water availability in the spring 2017, but lower in the summer 2018. In addition, plants grown under higher nitrogen availability produced a lower number of seeds per flower (Fig. 6C). As we showed above, plants grown under different combinations of water and nitrogen availability varied in their total production of flowers. Combined with the variation in the number of seeds produced per flower, this led to differences in the total seed set per plant (Fig. 6D). Specifically, total seed set was higher in plants grown under high water availability, but the effect was stronger in the summer than in the spring (the interaction between water availability and season: $F = 5.03$, $P = 0.029$).

Discussion

The effect of environmental changes on plant traits

Our results highlight that water stress is a key factor for both vegetative and floral traits (Descamps *et al.,* 2018), while changes of nitrogen supply had a more limited impact in our case. The effects of these factors were mostly interactive and dependent on the season. Higher amount of water positively affected plant growth, especially in the spring and summer. Nitrogen enrichment played a more complex role in the vegetative growth of *S. alba* and its effect was modulated by the season and water availability. Interestingly, previous research has shown that while water deficiency may lead to reduced biomass production and diminished nitrogen uptake in plants (Cossani *et al*., 2012), sufficient nitrogen supply may enhance their drought tolerance and increase water use efficiency (Quemada and Gabriel, 2016).

Flowering phenology, the number of flowers, and nectar production of *S. alba* were also affected by growing conditions. In particular, nectar production was affected by a complex interaction of all three variables, where nectar production increased with higher nitrogen supply to a variable degree depending on water availability and season. This is a complex relationship, not reported by previous studies on other plant species. Contrary to our results, several studies showed that nectar production may decline in response to water reduction and increased temperature (Keasar *et al*., 2008; Scaven and Rafferty, 2013; Takkis *et al*., 2015). In our case, nectar production did not decrease under these conditions and also showed an opposite result compared to Hoover *et al.* (2012), where nectar production of *Cucurbita maxima* decreased with higher nitrogen supply and increased with increasing temperature. However, our results are in line with a previous study by of Villarreal and Freeman (1990), who showed that nectar production of *Ipomopsis longiflora* was positively affected by water supplementation. It seems that the effects of varying environmental conditions on nectar production are highly species-specific (Lu *et al*., 2015).

Both vegetative and floral traits displayed significant differences among plants grown in different seasons under the same water and nitrogen supply levels. In particular, plants grown in the winter were generally smaller and their onset of flowering was significantly delayed compared to spring and summer. Generally, the growth rate and reproductive success of plants is the highest within a certain range of optimal temperatures and decreases rapidly beyond this optimal range (Vasseur *et al*., 2014, Hatfield and Prueger, 2015). Phenological shifts in many plants are also closely related to temperature (Jagadish *et al*., 2016; Kehrberger and Holzschuh, 2019). Previous studies on *Borago officinalis* also showed that increasing temperature may diminish flower production or lead to flower bud abortion, which may reduce the total number of flowers produced during the plant's flowering period (Saavedra *et al*., 2003; Descamps *et al.,* 2018). Similarly, the total number of flowers of *S. alba* significantly dropped in our experiment in the summer when the temperature in the greenhouse averaged ca. 29°C. However, the fact that plants produced more flowers with high amount of water, even during the summer, shows that the impact of thermal stress on flower production can be reduced by water supplementation (see also Mahan *et al*., 1995; Li *et al*., 2020). An optimal temperature is also required for the maximum nectar secretion (Pacini and Nepi, 2007, Lu *et al*., 2015). In our case, *S. alba* produced comparatively higher amount of nectar in the spring when the temperature reached intermediate values (average ca. 25°C). Apart from the temperature, seasonal differences in vegetative and floral traits in our plants could have been driven also by differences in day length and light intensity.

Impact on pollination and seed production

Our experiments showed that differences in traits among plants grown under different environmental conditions had a cascading effect on the number and identity of flower visitors of *S. alba* and on its reproduction. Flower visitation rate of insect-pollinated plants depends on visual cues indicating high floral reward such as the number of open flowers (Conner and Rush, 1996; Akter *et al.,* 2017) and the size of floral display (Grindeland *et al*., 2005; Parachnowitsch *et al*., 2010; Biella *et al*., 2019), and on the amount and quality of nectar and pollen (Cresswell, 1999; Grindeland *et al*., 2005). Other morphological features can also influence plant detection by potential pollinators, such as plant height (Junker *et al*., 2013; Klecka *et al.,* 2018a; Hernández-Villa *et al*., 2020), local plant clustering (Elliot and Irwin, 2009; Akter *et al.,* 2017), and flower colour (Reverté *et al.,* 2016). Measurements of flower visitation with plants grown in the spring 2017 and plants grown in the summer 2018 revealed that and in both cases plants grown with higher amount of water had a significantly higher number of flower visitors compared to plants grown under low amount of water irrespective of nitrogen supply. This is likely a consequence of differences in vegetative and floral traits induced by differences in water availability. As discussed above, plants grown with high amount of water were taller and produced more flowers and these characteristics had a positive effect on the visitation of individual plants as reported in other plant species (e.g. Mitchell *et al.* 2004; Akter *et al.,* 2017; Klecka *et al.,* 2018a). However, other modifications of plant traits induced by water stress also apparently decreased the visitation of plants grown with low amount of water, because the effect of water availability on the number of flower visitors per plant persisted even after accounting for differences in the number of open flowers and plant height in our analysis. We believe that the remaining unexplained variation could be related to nectar chemistry (Petanidou *et al.,* 2006; Hoover *et al.,* 2012) or flower scent (Farré-Armengol *et al.*, 2020).

Besides having lower flower visitation, plants grown under low amount of water had different composition of flower visitors compared to plants grown with higher amount of water. In the spring 2017, plants grown with high amount of water received more frequent visits from rapeseed beetles, solitary bees, and hoverflies than plants grown under low amount of water, while the other flower visitors, including honeybees and bumblebees, did not discriminate among the plants. The results were similar in the summer 2018, although rapeseed beetles were almost absent. The observed differences in the flower visitation in the spring 2017 and the summer 2018 may be influenced by the differences in overall insect abundance or weather, but not necessarily by the growing conditions of the plants. For instance, increasing temperature may affect flower visitation by a number of mechanisms, from differences in plant traits caused by high temperature stress (Descamps *et al.,* 2018), through phenological shifts of plant flowering and pollinator emergence (Hegland *et al.,* 2009; Bartomeus *et al.,* 2011), to changes in pollinator foraging activity caused by their responses to temperature (Corbet *et al.,* 1993; Slamova *et al.* 2011), and direct and indirect effects of temperature on the fitness and mortality of pollinating insects (Scaven and Rafferty, 2013).

Finally, seed production of *S. alba* was also affected by water and nitrogen availability, apparently both directly through physiological mechanisms and indirectly through changes in insect pollination. Our hand pollination assessment confirmed that *S. alba* is a partially self-incompatible plant (Fan *et al*., 2007). Low water availability reduced seed production per flower in both self-pollinated and cross-pollinated plants, which is consistent with previous studies suggesting that water stress may lead to seed or pod abortion (e.g. New *et al.,* 1994; Behboudian *et al.,* 2001). However, we also observed an intriguing effect of nitrogen availability on seed set: increased nitrogen availability increased seed set in self-pollinated plants, but decreased seed set in cross-pollinated plants. We are not aware of any studies which would show that high nitrogen supply can cause seed abortion.

Seed count per flower from the naturally pollinated plants in the spring 2017 also showed a similar trend as in plants cross-pollinated by hand, where the number of seeds per flower increased in plants grown with high water availability, but decreased with high nitrogen availability. In contrast, in experiments done in the summer 2018, the number of seeds per flower was not affected by nitrogen availability and decreased in plants grown with high amount of water. Total seed set per plant was unaffected by nitrogen availability and increased in plants grown under high water availability – moderately in the spring 2017 but much more in the summer 2018. This may stem from differences in the composition of the flower visitor community between plants grown under low and high water availability and from the higher total visitation rate in the spring 2017 compared to summer 2018. The level of pollen limitation (Knight *et al.*, 2005) was thus higher in the summer 2018, which likely explains why the number of seeds per flower was lower and was more strongly reduced in plants grown with low amount of water.

Conclusions

We have shown that multiple drivers of environmental change have a complex and interactive impact on plant traits, visitation by pollinators, and seed production. Our model species, *S. alba*, is an important crop and a close relative to many other economically important crops and vegetables from the Brassicaceae family, hence our experiment shows how different climatic ~~drivers~~ may affect both vegetative growth and crop yield in plants form this family in the future extreme climatic events. We conclude that not only increasing temperature, but also reduced precipitation and nitrogen enrichment, may impact plant-pollinator interactions with negative consequences for the reproduction of wild plants as well as insect-pollinated crops.

Acknowledgements

We are grateful to Pavla Kaloušková for her valuable help during the field observations and Jana Jersáková for her help with the laboratory analyses.

References

Akter A, Biella P, Klecka J. 2017. Effects of small-scale clustering of flowers on pollinator foraging behaviour and flower visitation rate. *PLoS ONE* 12(11): e0187976. doi: 01.1371/journal.pone.0187976

Albrigo LG, Russ R, Rouseff R, Bazemore RA. 2012. Some citrus flower characteristics and honeybee preference. *Proceedings of the Florida State Horticultural Society* 125: 112–118.

Baker HG, Baker I. 1983. Floral nectar sugar constituents in relation to pollinator type. In: Jones C. E., Little R. J. (eds.) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, 117–141.

Barnabás B, Jäger K, Fehér A. 2008. The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell and Environment* 31(1):11-38.

Bartomeus I, Ascher JS, Wagner D, Danforth BN, Colla S, Kornbluth S, Winfree R. 2011. Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences* 108 (51): 20645-20649.

Basnett S, Ganesan R, Devy SM. 2019. Floral traits determine pollinator visitation in Rhododendron species across an elevation gradient in the Sikkim Himalaya. *Alp Botany* 129: 81–94.

Battye, W., Aneja, V. P., & Schlesinger, W. H. (2017). Is nitrogen the next carbon? Earth's Future, 5(9), 894-904.

Behboudian MH, Ma Q, Turner NC, Palta JA. 2001. Reactions of chickpea to water stress: yield and seed composition. *Journal of the Science of Food and Agriculture* 81(13): 1288-1291.

Bernal M, Estiarte M, Peñuelas J. 2011. Drought advances spring growth phenology of the Mediterranean shrub *Erica multiflora*. *Plant Biology* 13: 252-257.

Biella P, Akter A, Ollerton J, Tarrant S, Janeček Š, Jersáková J, Klecka J. 2019. Experimental loss of generalist plants reveals alterations in plant-pollinator interactions and a constrained flexibility of foraging. *Scientific Reports* 9: 7376.

Brown, J., J.B. Davis, and A. Esser. 2005. Pacific Northwest condiment yellow mustard (Sinapis alba L.) grower guide. Subcontract report - National Renewable Energy Laboratory. University of Idaho. [Online]. Available at http://www.nrel.gov/docs/fy05osti/36307.pdf.

Blum A. 1996. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regul* 20: 135–148.

Bobbink R, Hicks K, Galloway J. et al. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. *Ecological Applications* 20(1): 30-59.

Burkle L, Irwin RE. 2009. The importance of interannual variation and bottom–up nitrogen enrichment for plant–pollinator networks. *Oikos* 118: 1816-1829.

Burkle LA, Irwin RE. 2010. Beyond biomass: measuring the effects of community-level nitrogen enrichment on floral traits, pollinator visitation and plant reproduction. *Journal of Ecology* 98: 705– 717.

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

Carromero W, Hamrick JL. 2005. The mating system of *Verbascum thapsus* (Scrophulariaceae): the effect of plant height. *International Journal of Plant Sciences* 166: 979– 983.

Christensen JH, Carter TR, Rummukainen M, Amanatidis G. 2007. Evaluating the performance and utility of regional climate models: the PRUDENCE project. *Climatic Change* 81: 1–6.

Conner JK, Rush S. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia, 105(4), 509-516.*

Corbet SA, Fussell M, Ake R, Fraser A, Gunson C, Savage A, Smith K. 1993. Temperature and the pollinating activity of social bees. *Ecological Entomology* 18: 17-30.

Cossani CM, Slafer GA, Savin R. 2012. Nitrogen and water use efficiencies of wheat and barley under a Mediterranean environment in Catalonia. *Field Crops Research* 128: 109-118.

Cresswell JE. 1999. The influence of nectar and pollen availability on pollen transfer by individual flowers of oil seed rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidarius*). *Journal of Ecology* 87(4): 670–677.

Descamps C, Quinet M, Baijot A, Jacquemart AL. 2018. Temperature and water stress affect plant-pollinator interactions in *Borago officinalis* (Boraginaceae). *Ecology and Evolution* 8: 3443-3456.

Elliott SE, Irwin RE. 2009. Effects of flowering plant density on pollinator visitation, pollen receipt, and seed production in *Delphinium barbeyi* (Ranunculaceae). *American Journal of Botany* 96: 912-919.

Farré-Armengol G, Fernández-Martínez M, Filella I, Junker RR, Peñuelas J. 2020. Deciphering the biotic and climatic factors that influence floral scents: A systematic review of floral volatile emissions. *Frontiers in Plant Science* 11: 1154.

Fitter AH, Fitter RSR. 2002. Rapid changes in flowering time in British plants. *Science* 296: 1689–1691.

Flacher F, Raynaud X, Hansart A, Geslin B, Motard B, Verstraet S, Bataille M, Dajoz I. 2020. Below-ground competition alters attractiveness of an insect-pollinated plant to pollinators. *AoB PLANTS* 12(4): plaa022. doi:10.1093/aobpla/plaa022

Fowler, D. *et al.* 2013The global nitrogen cycle in the twenty-first century. *Philos. Trans. R. Soc. B* 368, 20130164.

Galen C. 2000. High and dry: drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *American Naturalist* 156: 72– 83.

Galloway JN *et al.* 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry* 70: 153–226.

Gardener MC, Gillman MP. 2001. The effects of soil fertilizer on amino acids in the floral nectar of corncockle, *Agrostemma githago* (Caryophyllaceae). *Oikos* 92:101–106.

Gardener MC, Gillman MP. 2002. The taste of nectar-a neglected area of pollination ecology. *Oikos* 98: 552-557.

Gérard M, Vanderplanck M, Wood T, Michez D. 2020. Global warming and plant–pollinator mismatches. *Emerging Topics in Life Sciences* 4 (1): 77–86.

Gray SB, Brady SM. 2016. Plant developmental responses to climate change. *Arid Soil Research and Rehabilitation* 2: 49-58.

Grindeland JM, Sletvold N, Ims, RA. 2005. Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Functional Ecology* 19: 383-390.

Güsewell S, Furrer R, Gehrig R, Pietragalla B. 2017. Changes in temperature sensitivity of spring phenology with recent climate warming in Switzerland are related to shifts of the preseason. *Global Change Biol*ogy 23: 5189– 5202.

Hatfield JL, Prueger JH. 2015. Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes* 10 (A): 4-10.

Hedhly A. 2011. Sensitivity of flowering plant gametophytes to temperature fluctuations. *Environmental and Experimental Botany* 74: 9– 16.

Hegland SJ, Totland Ø. 2005. Relationships between species' floral traits and pollinator visitation in a temperate grassland. *Oecologia* 145: 586–594.

Hegland SJ, Nielsen A, Lázaro A, Bjerknes AL, Totland Ø. 2009. How does climate warming affect plant-pollinator interactions? *Ecology Letters* 12(2): 184– 195.

Hernández-Villa V, Vibrans H, Uscanga-Mortera E, Aguirre-Jaimes A. 2020. Floral visitors and pollinator dependence are related to floral display size and plant height in native weeds of central Mexico. *Flora* 262: 151505.

Hoover SER, Ladley JJ, Shchepetkina AA, Tisch M, Gieseg SP, Tylianakis JM. 2012. Warming, CO_2, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecology Letters* 15: 227-234.

Holtsford TP, Ellstrand NC. 1992. Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). *Evolution* 46: 216–225.

Fan HL, Sun WC, Yan N, Zhu HX, Wu JY, Zhang YH, Zeng J, Ye J, Liu YL. 2007. Analysis of self-compatibility in *Sinapis alba* (L.) Boiss. In Proceedings of the 12th International Rapeseed Congress, Wuhan, China, 26–30 March 2007, 368–369.

Inouye DW, Waller GD. 1984. Responses of honeybees (*Apis mellifera*) to amino acid solutions mimicking nectars. *Ecology* 65: 618– 625.

Jabłoński B, Król S, Pliszka K, Żurowska Z. 1984. Nectar secretion and pollination of the blueberry (*Vaccinium corymbosum* L.). *Acta Horticulture* 165: 133–144.

Jagadish SK, Bahuguna RN, Djanaguiraman M, Gamuyao R, Prasad PV, Craufurd PQ. 2016. Implications of high temperature and elevated CO_2 on flowering time in plants. *Frontiers in Plant Science* 7: 913.

Jauzein P. 2011. *Flore des champs cultivés.* 2nd edn. Versailles, France: Editions Quae.

Junker RR, Blüthgen N, Brehm T, Binkenstein J, Paulus J, Schaefer MH, Stang M. 2013. Specialization on traits as basis for the niche-breadth of flower visitors and as structuring mechanism of ecological networks. *Functional Ecology* 27(2): 329-341.

Keasar T, Sadeh A, Shmida A. 2008. Variability in nectar production and standing crop, and their relation to pollinator visits in a Mediterranean shrub. *Arthropod-Plant Interactions* 2: 117–123.

Kehrberger S, Holzschuh A. 2019. Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. *PLoS ONE* 14(6): e0218824.

Klecka J, Hadrava J, Koloušková P. 2018a. Vertical stratification of plant–pollinator interactions in a temperate grassland. *PeerJ* 6: e4998. doi:10.7717peerj.4998

Knight, T. M., Steets, J. A., Vamosi, J. C., Mazer, S. J., Burd, M., Campbell, D. R., ... & Ashman, T. L. (2005). Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution and Systematics, 36, 467-497.*

Lasky JR, Uriarte M, Muscarella R. 2016. Synchrony, compensatory dynamics, and the functional trait basis of phenological diversity in a tropical dry forest tree community: effects of rainfall seasonality. *Environmental Research Letters* 11: 115003.

Li Y, Guan K, Peng B, Franz TE, Wardlow B, Pan M. 2020. Quantifying irrigation cooling benefits to maize yield in the US Midwest. *Glob Change Biology* 26: 3065– 3078.

Lu NN, Li XH, Li L, Zhao ZG. 2015. Variation of nectar production in relation to plant characteristics in protandrous *Aconitum gymnandrum. Journal of Plant Ecology* 8(2): 122–129.

Mahan JR, McMicheal BL, Wanjura DF. 1995. Methods for reducing the adverse effects of temperature stress on plants: A review. *Environmental and Experimental Botany* 35: 251–258.

Mitchell RJ, Karron JD, Holmquist KG, Bell JM. 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Functional Ecology* 18: 116-124.

New B, Duthion C, Turc O. 1994. Phenological response of pea to water stress during reproductive development. *Crop science* 34(1): 141-146.

NOAA National Centres for Environmental Information, State of the Climate: Global Climate report for the Annual 2020, published online January 2021, retrieved on September 13, 2021 from https://www.ncdc.noaa.gov/sotc/global/202013

Pacini E, Nepi M. 2007. Nectar production and presentation. In: Nicolson S.W., Nepi M., Pacini E. (eds) Nectaries and Nectar. Springer, Dordrecht.

Parachnowitsch AL, Kessler A. 2010. Pollinators exert natural selection on flower size and floral display in *Penstemon digitalis*. *New Phytologist* 188(2): 393-402.

Petanidou T, Van Laere A, Ellis WN, Smets E. 2006. What shapes amino acid and sugar composition in Mediterranean floral nectars? *Oikos* 115(1): 155–169.

Pyke GH. 1984. Optimal Foraging Theory: A Critical Review. *Annual Review of Ecology and Systematics* 15(1): 523-575.

Quemada M, Gabriel JL. 2016. Approaches for increasing nitrogen and water use efficiency simultaneously. *Global Food Security* 9: 29-35.

R Core Team 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Rering, CC, Franco, JG, Yeater, KM, Mallinger, RE. 2020. Drought stress alters floral volatiles and reduces floral rewards, pollinator activity, and seed set in a global plant. *Ecosphere* 11(9): e03254.

Reverté S, Retana J, Gómez JM, Bosch J. 2016. Pollinators show flower colour preferences but flowers with similar colours do not attract similar pollinators. *Annals of Botany* 118(2): 249-257.

Roldán-Serrano AS, Guerra-Sanz JM. 2005. Reward attractions of zucchini flowers (*Cucurbita pepo* L.) to bumblebees (*Bombus terrestris* L.). *European Journal of Horticultural Science* 70: 23–28.

Rustad LE. 2008. The response of terrestrial ecosystems to global climate change: towards an integrated approach. *Science of the Total Environment* 404: 222–235.

Saavedra F, Inouye DW, Price MV, Harte J. 2003. Changes in flowering and abundance of *Delphinium nuttallianum* (Ranunculaceae) in response to a subalpine climate warming experiment. *Global Change Biology* 9: 885-894.

Scaven VL, Rafferty NE. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. *Current Zoology* 59(3): 418-426.

Schmidt K, Filep R, Orosz-Kovács Z, Farkas A. 2015. Patterns of nectar and pollen presentation influence the attractiveness of four raspberry and blackberry cultivars to pollinators. *The Journal of Horticultural Science and Biotechnology* 90: 47–56.

Schweiger O, Biesmeijer JC, Bommarco R, Hickler T, Hulme PE. 2010. Multiple stressors on biotic interactions: How climate change and alien species interact to affect pollination. *Biological Reviews* 85: 777–795.

Scott-Wendt J, Chase RG, Hossner LR. 1988. Soil chemical variability in sandy Ustalfs in semi-arid Niger, West Africa. *Soil Science* (June): 414.

Slamova I, Klecka J, Konvicka M. 2011. Diurnal behavior and habitat preferences of *Erebia aethiops*, an aberrant lowland species of a mountain butterfly clade. *Journal of Insect Behavior*, *24*(3), 230-246.

Snider JL, Oosterhuis DM. 2011. How does timing, duration, and severity of heat stress influence pollen-pistil interactions in angiosperms? *Plant Signaling & Behavior* 6(7): 930–933.

Takkis K, Tscheulin T, Tsalkatis P, Petanidou T. 2015. Climate change reduces nectar secretion in two common Mediterranean plants. *AoB Plants* 7: 111.

Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, Savage V, Tunney TD, O'Connor MI. 2014. Increased temperature variation poses a greater risk to species than climate warming. *Proceedings of the Royal Society B: Biological Sciences* 281(1779): 2013-2612.

Villarreal AG, Freeman CE. 1990. Effects of temperature and water stress on some floral nectar characteristics in *Ipomopsis longiflora* (Polemoniaceae) under controlled conditions. *Botanical Gazette*151: 5-9.

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, W. *et al.* (1997). Human alteration of the global nitrogen cycle- sources and consequences. *Ecol. Appl.*, **7**, 737– 750.

Wyatt R, Broyles SD, Derda GS. 1992. Environmental influences on nectar production in milkweeds (Asclepias syriaca and A. exaltata). *American Journal of Botany* 79: 636-642.

Zimmermann M, Pyke GH. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. *American Naturalist* 131: 723-738.

Figure 1

The impact of water and nitrogen supply on vegetative traits of *S. alba* across three seasons.

Four vegetative traits were measured: A) plant height, B) plant diameter, C) the number of leaves, and D) dry weight. The boxplots show the median and interquartile range. The results of statistical tests are summarised in Table 1.

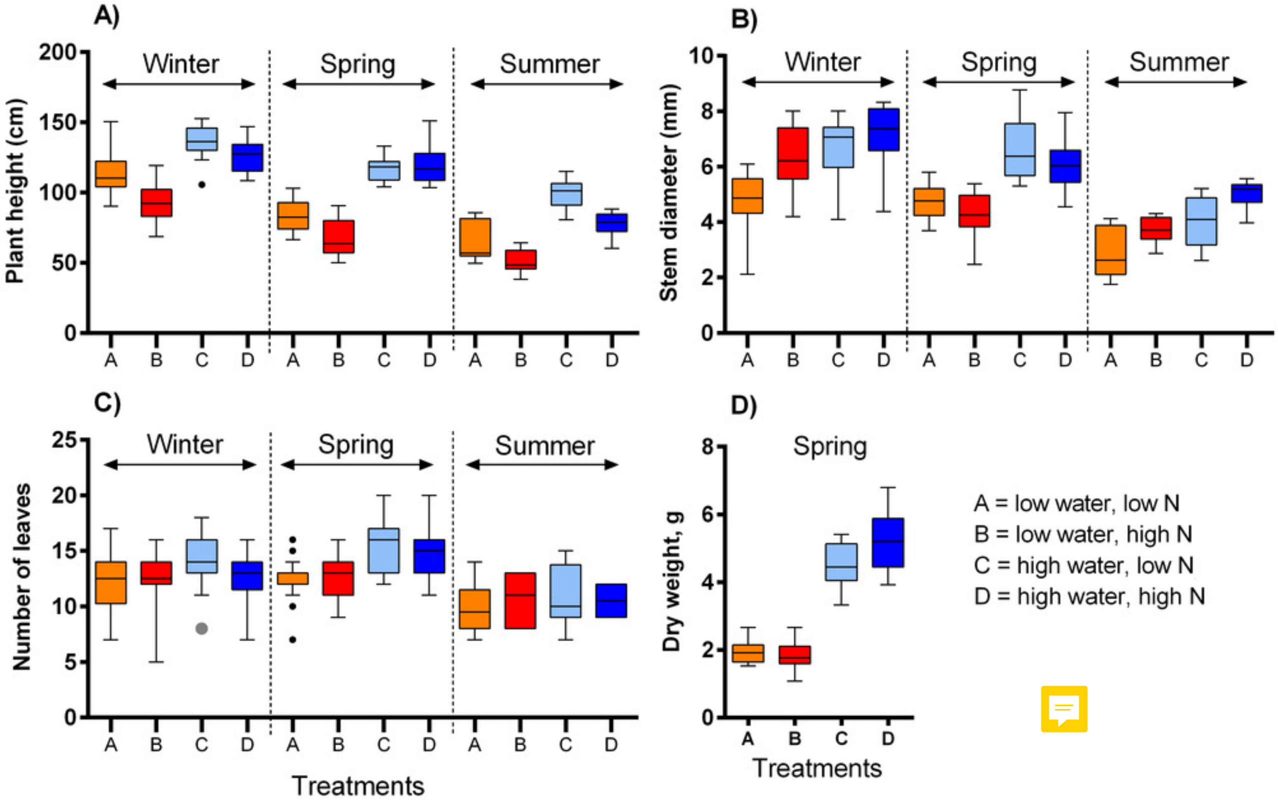

Figure 2

The impact of water and nitrogen supply on floral traits of *S. alba* across three seasons.

A) the onset of flowering (the day of the opening of the first flower), B) the number of flowers produced per plant, and C) nectar production (median and interquartile range is shown). The results of statistical tests are summarised in Table 2.

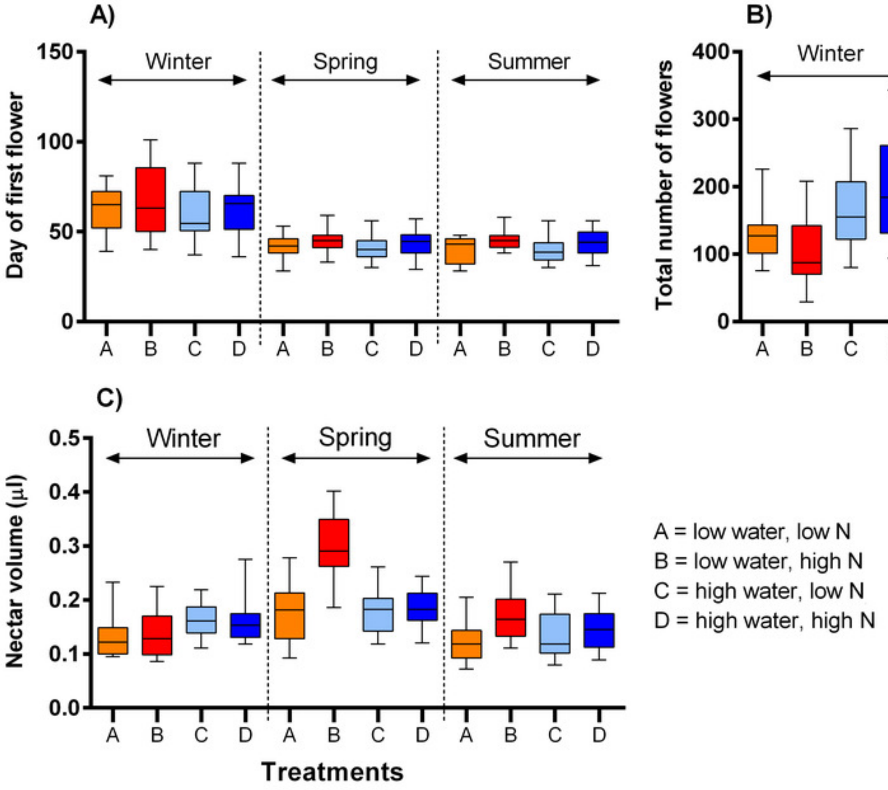

Figure 3

Flower visitation of plants grown under varying water and nitrogen availability.

The number of flower visitors per plant per 30 minutes during two observation periods are shown: A) spring 2017 and B) summer 2018 (median and interquartile range is shown). Flower visitation also varied depending on the number of open flowers (C) and plant height (D).

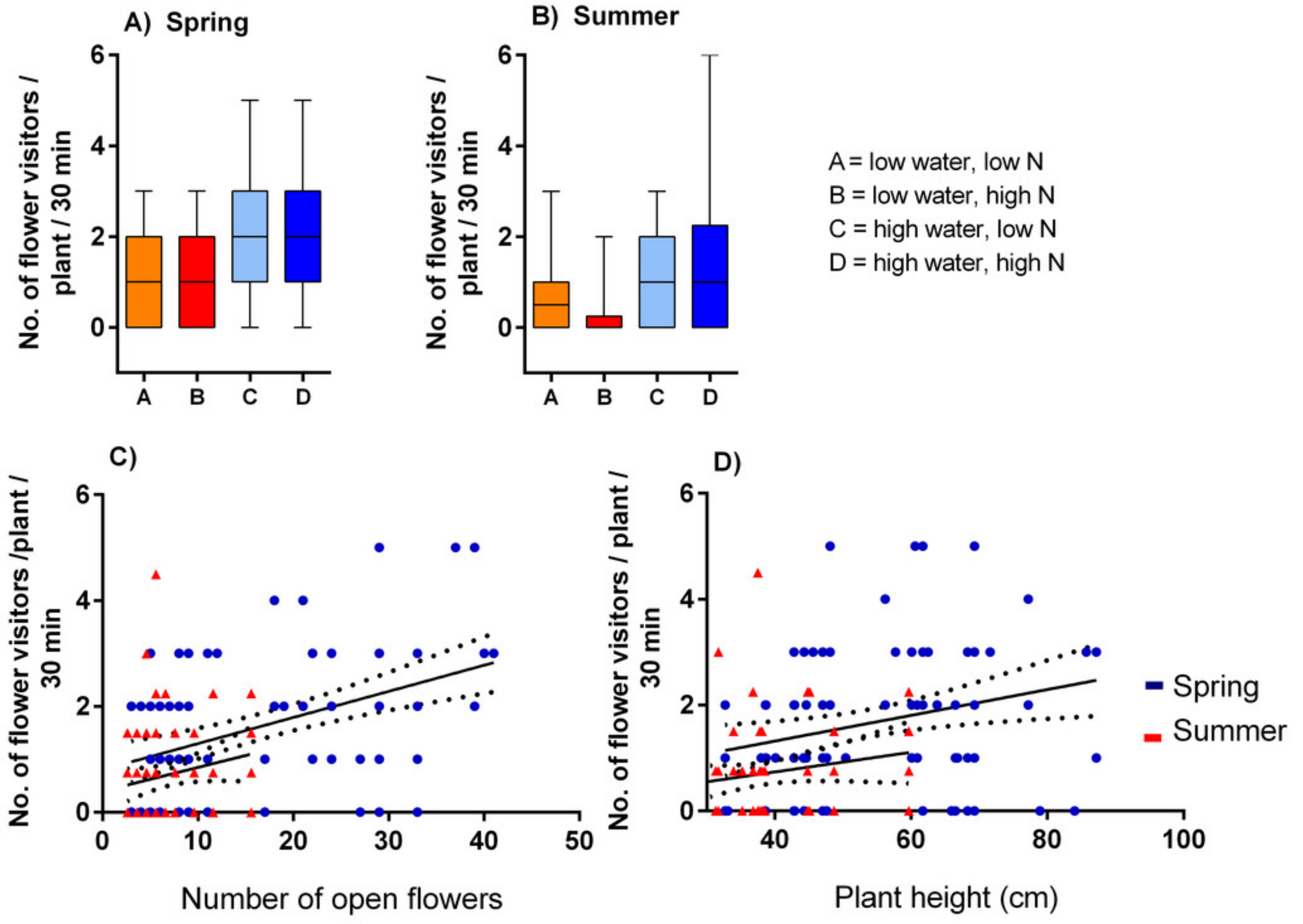

Figure 4

The composition of the flower visitor community varied depending on water availability under which the plants were grown.

The results of RDA show the effect of water availability and the lack of an effect of nitrogen supply during the plant growth period on the composition of the flower visitor community in the spring 2017 (A) and the summer 2018 (B).

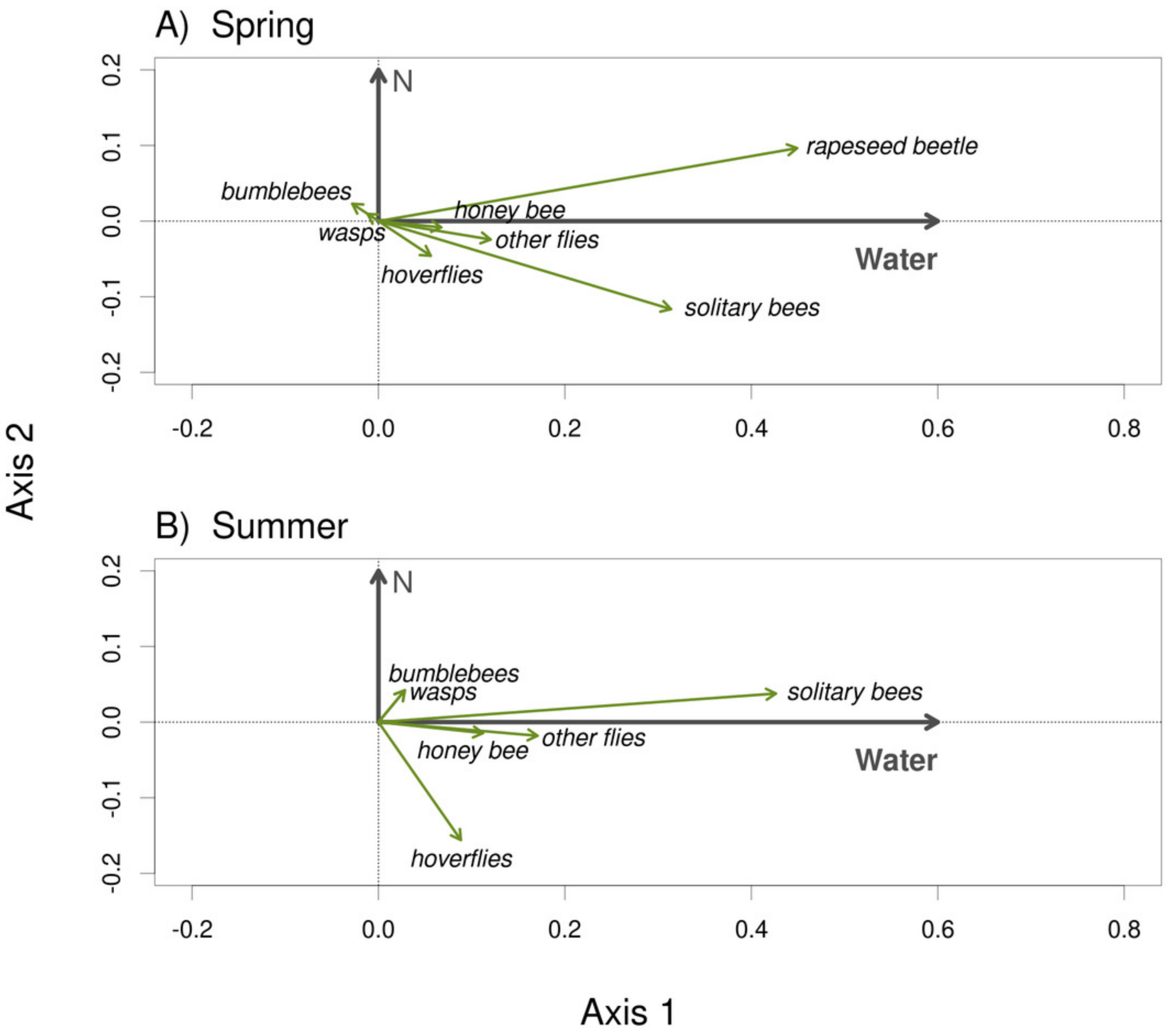
A) Spring
N
rapeseed beetle
bumblebees
honey bee
wasps
other flies
Water
hoverflies
solitary bees
B) Summer
N
bumblebees
wasps
solitary bees
other flies
honey bee
Water
hoverflies
Axis 2
Axis 1
0.2
0.1
0.0
-0.1
-0.2
-0.2
0.0
0.2
0.4
0.6
0.8

Figure 5

Flower visitation by major flower visitor groups.

A) plants grown in the spring 2017, and B) in summer 2018. The number of visitors per 30 min. is shown (mean ± SE).

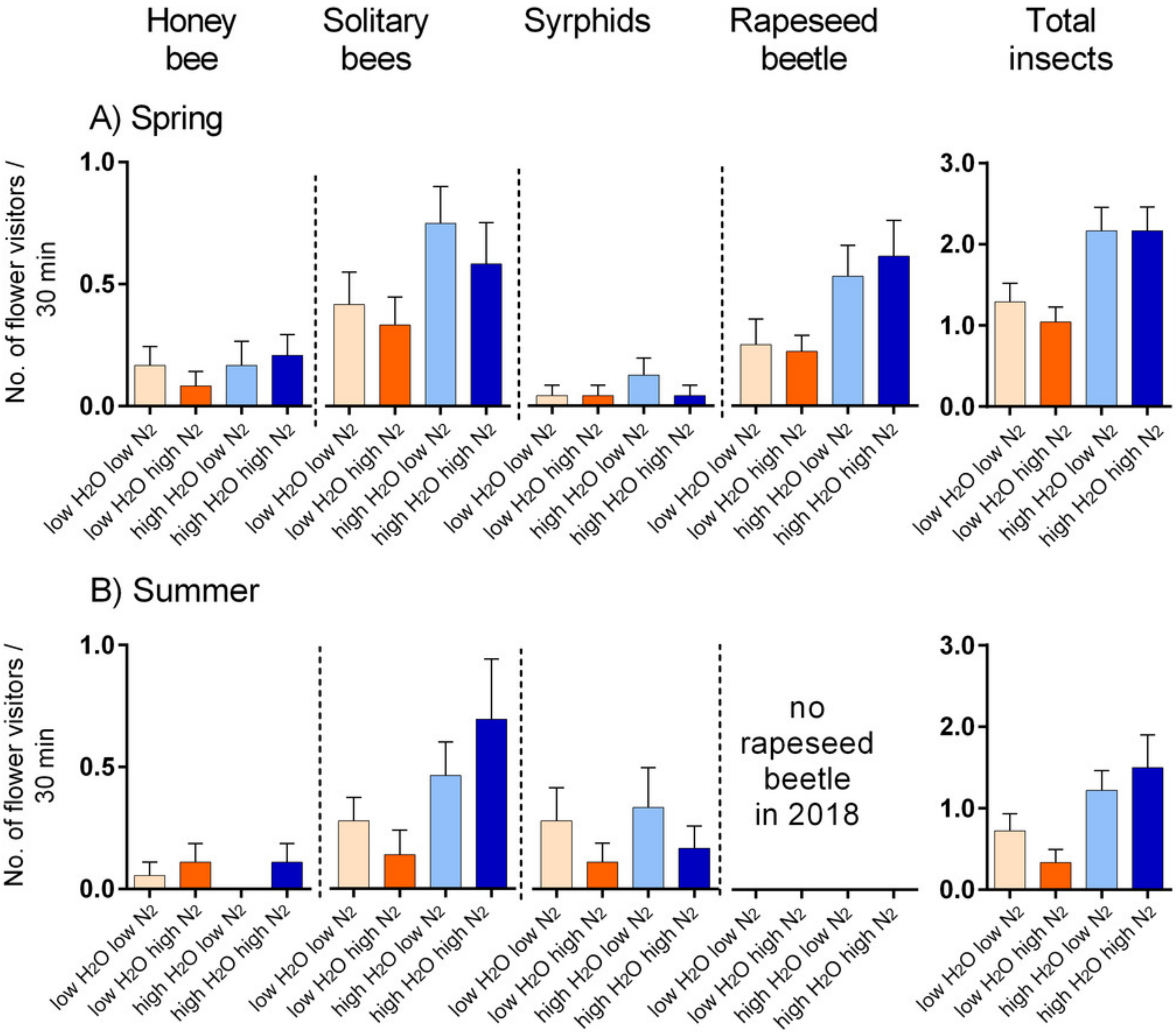

Figure 6

Seed production of *S. alba* grown under different growing conditions.

Seed production in plants subjected to self-pollination and cross-pollination by hand: A) seed production per flower and B) seed production per pod (median and interquartile range is shown). Seed production of plants subjected to natural pollination in the spring 2017 and the summer 2018: C) the number of seeds per flower, and D) the total seed set per plant (median and interquartile range is shown).

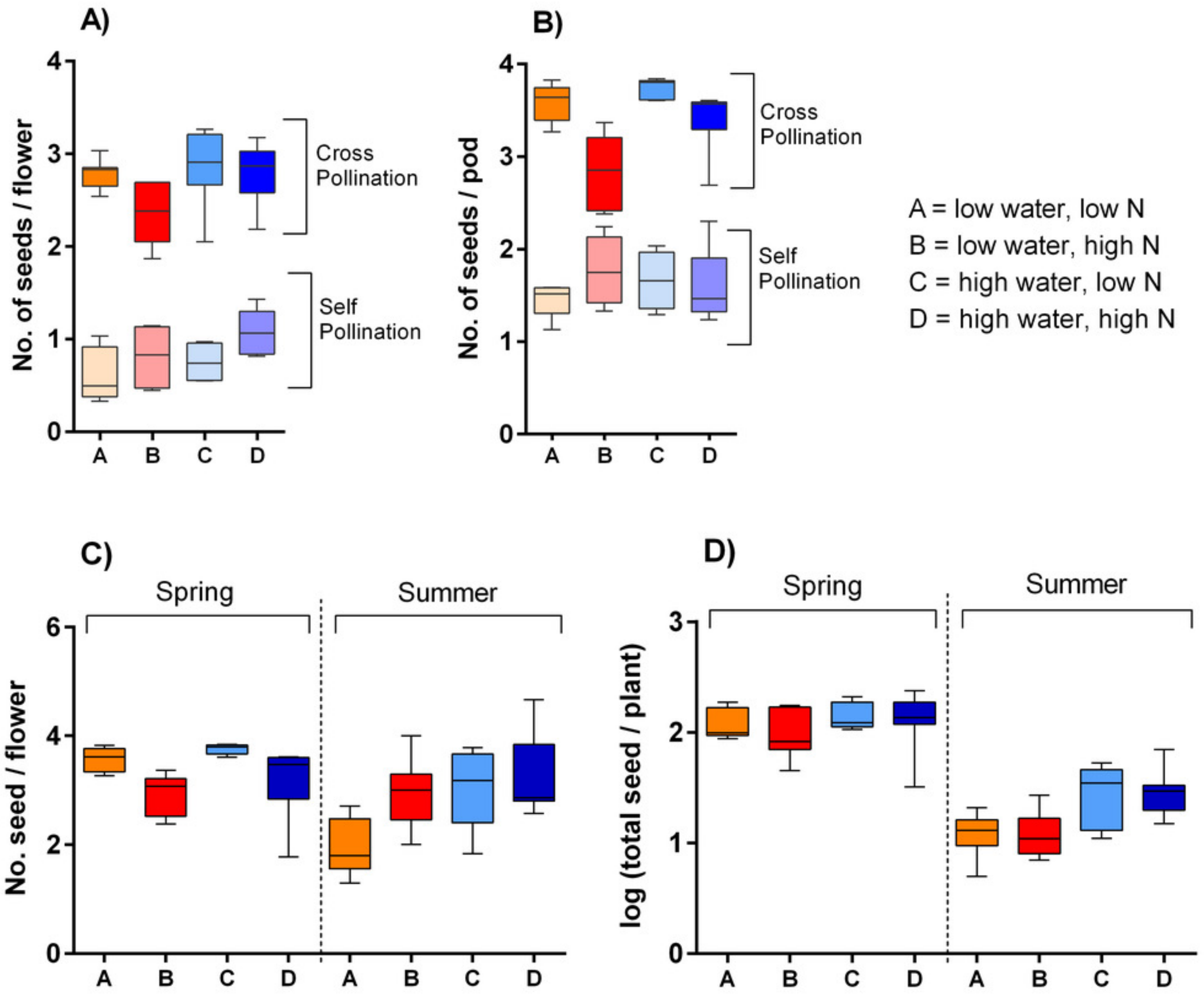

Table 1(on next page)

The effects of water availability, nitrogen supply, and season on selected vegetative traits of *S. alba*. *F* and *P* values for individual variables and their interactions estimated by generalised linear models (see Methods) are shown.

Variable	**Plant height**		**Stem diameter**		**Number of leaves**		**Dry weight**	
	F	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Water	**23.2**	**<0.001**	**32.9**	**<0.001**	**8.8**	**0.004**	**105.72**	**<0.001**
Nitrogen	**22.3**	**<0.001**	**22.3**	**<0.001**	0.1	0.78768	0.22	0.643
Season	**60.7**	**<0.001**	**41.9**	**<0.001**	**9.0**	**<0.001**	-	-
Water x Nitrogen	2.1	0.147	2.1	0.154	1.9	0.167	**5.52**	**0.023**
Water x Season	2.2	0.113	**3.1**	**0.047**	**3.3**	**0.039**	-	-
Nitrogen x Season	0.457	0.636	**14.4**	**<0.001**	0.2	0.817	-	-
Water x Nitrogen x Season	**4.94**	**0.008**	1.2	0.306	0.1	0.951	-	-

1

Table 2(on next page)

The effects of water availability, nitrogen supply, and season on floral traits of *S. alba*. *F* and *P* values for individual variables and their interactions estimated by generalised linear models (see Methods) are shown.

Variable	**Day of first flower**		**Number of flowers**		**Nectar volume**	
	F	***P***	***F***	***P***	***F***	***P***
Water	**11.6**	**<0.001**	**11.6**	**<0.001**	**4.9**	**0.028**
Nitrogen	**8.4**	**0.004**	**8.4**	**0.004**	0.2	0.622
Season	**25.1**	**<0.001**	**25.1**	**<0.001**	**10.74**	**<0.001**
Water x Nitrogen	**6.1**	**0.015**	**6.1**	**0.015**	**21.95**	**<0.001**
Water x Season	**3.5**	**0.033**	**3.5**	**0.033**	1.2	0.318
Nitrogen x Season	1.3	0.265	1.3	0.265	**8.8**	**<0.001**
Water x Nitrogen x Season	0.01	0.950	1.3	0.288	**3.6**	**0.030**

1