Chapter 9
Effect of Extreme Climatic Events on Plant-Pollinator Interactions in Blueberry

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Abstract Pollination is a key ecosystem service that is, however, under threat due to multiple environmental pressures, such as climate change, compromising crop production. The main goal of this study was to investigate how extreme events due to climate change affect flower traits and plant-pollinator interactions, and how this impacts fruit production, using the insect-dependent blueberry crop as study system. For this, we set up a controlled pot experiment using two blueberry cultivars (Bluecrop and Duke). At the time of bud swelling, half of the plants (12 per cultivar) were placed for two weeks in a glasshouse under stress conditions (no water and increased temperature), while the other half remained outdoors and watered. At flowering, flower traits were measured, and plants were exposed to pollinators; the identity of pollinators visiting blueberry flowers was registered as well as their behavior and the number of flowers visited. Later, mature fruits were randomly collected and weighed individually. Results showed that in our study site the most frequent visitor of blueberry flowers was *Anthophora plumipes* (Fabricius, 1781). Results also showed that stress conditions did not affect flower traits and insect pollinator visitation rates, regardless of blueberry cultivar, but affected insect preferences for Bluecrop cultivar, with *A. plumipes* preferring control over stressed plants. However, for Duke cultivar, control plants produced heavier fruits than plants under stress conditions. Our study provides some insights into the effects of climate changes on plant-pollinator interactions, but further research is necessary to better understand the impacts of climate change on plant-pollinator interactions and how this may impact food production.

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Introduction

Pollination is a key ecosystem service maintaining the stability of agricultural food production, with animal pollination affecting the yield and quality of over 75% of crops worldwide (Klein et al. 2007). However, pollination services are under threat due to multiple environmental pressures. Land use changes, such as fragmentation and agriculture intensification, pesticide use, biological invasions and eutrophication have been shown to negatively impact plant-pollinator interactions (Potts et al. 2010). Climate changes may be a further threat to pollination services by altering plant and pollinator traits, phenologies and behavior, causing phenological mismatches in plant-pollinator interactions (Gérard et al. 2020; Keeler et al. 2021). Climate prediction trends for the Mediterranean region point to higher drought, increased inter-annual variability of precipitation and increases in extreme climatic events such as heatwaves and severe droughts (IPCC 2021). According to the IPCC (2021) report, Portugal is already experiencing climate change, and climate projections point to an increase in drought stress. This will bring challenges to the agricultural sector with expected increases in water demand and decreases in crop productivity. Further challenges arising from climate change with negative impacts on crop production are related to disrupting plant mutualistic relations with their pollinators (Keeler et al. 2021). For example, water stress has been shown to decrease the quantity and quality of nectar and the quality of pollen with effects on the survival and productivity of developing honeybees and bumblebees (Wilson Rankin et al. 2020). However, most of this information comes from natural systems. The effect of climate change on the pollination of crops has been poorly addressed so far, despite its relevance for food production and security. This chapter addresses the impacts of climate change on flower traits and plant-pollinator interactions, using the insect-dependent blueberry crop as a study system.

Climate change leading to water deficit and increased temperature cause an increase in plant physiological stress, affecting crop production both directly and indirectly. Direct effects include the decrease in resources available for investment in reproduction, including flower and fruit production (Eziz et al. 2017). Information from published studies, mostly performed in natural ecosystems, indicates that climate change, in particular increased drought and temperature, influences flower visual traits and olfactory cues (*e.g.*, Burkle and Runyon 2017; Descamps et al. 2020a; Gallagher and Campbell 2017). Producing and maintaining flowers requires carbon, nutrients and water, representing a considerable cost to plants and implying trade-offs with vegetative growth (Obeso 2002). Therefore, under water stress, plants tend to produce fewer and smaller flowers (Kuppler and Kotowska 2021). While for flower visual traits drought stress seems to consistently have negative effects, for nectar, pollen and olfactory clues, which are not directly linked to transpiration and water losses, the effects of drought stress are less clear and its effects are species dependent, and may reflect species adaptations to drought (Burkle and Runyon 2016; Phillips et al. 2018). For example, reported effects of drought stress on nectar quantity and sugar content range from decrease to no change (*e.g.*, Descamps et al. 2018, 2020a; Phillips et al. 2018; Rering et al. 2020), depending on the species studied.

The number of flowers, as well as their size and colour, are important cues for pollinator attraction, while nectar, pollen and flower morphology are important determinants of flower handling and pollinator's efficiency (Parachnowitsch et al. 2019). Factors, such as water stress, that affect these visual and olfactory cues used by pollinators when searching for food, as well as reward availability, have the potential to change the behavior, preferences and fidelity of insect pollinators (Klatt et al. 2013) and indirectly impact fruit production. However, few studies have addressed pollinator responses to changes in flower traits and floral rewards resulting from drought stress, with results pointing to variable effects on pollinator visitation rates regardless of the negative effects on such traits (Burkle and Runyon 2016; Descamps et al. 2018; Glenny et al. 2018; Rering et al. 2020; Kuppler et al. 2021). Additionally, the response of pollinators to drought mediated effects on flower traits and rewards may also be dependent on the pollinator species (Burkle and Runyon 2016; Kuppler et al. 2021).

While it is clear that water stress will directly and negatively affect crop growth and yield, the impact on plant-pollinator interactions and pollination services to crops remains uncertain. Additionally, and despite its relevance, floral traits and pollinator attraction in the context of climate change are still poorly studied (Byers 2017) and empirical studies explicitly focusing on the effects of climate change on pollination services to crops are almost inexistent (Vaissière et al. 2011). However, evaluating the effects of drought on flower traits and plant fitness and how changes in flower traits affects plant-pollinator interactions and fruit production and quality is key to understand how crops could cope with predicted future climate changes.

Blueberries require insect pollination to produce marketable fruits (Klein et al. 2007). Although this crop presents varying levels of self-fertility (depending on the species, cultivar and genotype), they are primarily outcrossing, showing larger fruit size and earlier fruit ripening when cross-pollinated (Dogterom et al. 2000; Song and Hancock 2011; Taber and Olmstead 2016). Blueberries are nectar rewarding plants, bearing bell-shaped flowers, with poricidal anthers, and with nectar producing structures located at the basis of the corolla. Its cultivars present particular floral attributes (Rodriguez-Saona et al. 2011; Huber 2016), which can also be affected by different water availabilities. Flower size and morphology limit pollinators' access to rewards, limiting potential pollinator species, and may encourage nectar-robbing behavior (Courcelles et al. 2013).

Considering all this, in this work, our main objective was to investigate how extreme events due to climate change affect flower traits and plant-pollinator interactions, and how this impacts fruit production, using the insect-dependent blueberry crop as study system. To achieve this, we set up a water-controlled pot experiment and quantified flower traits, plant-pollinator interactions, and fruit traits. We hypothesize that stress conditions: (1) will lead to changes in flower traits relevant for insect attraction; (2) that these changes will affect interactions with pollinators, and (3) that this will have an impact on fruit production and fruit weight.

Methods

Experimental Design

Two blueberry cultivars commonly grown in Portugal with similar flowering times, Duke and Bluecrop, were selected for this study. A total of 48 plants (24 per cultivar), were purchased at a nursery shop specialized in blueberry plants. The plants were two-years old, as they is the stage at which they are available at nurseries, and were transplanted into 6L pots filled with professional blueberry suitable soil and placed in the Botanical Garden of the University of Coimbra. At the beginning of bud swelling, 12 plants of each cultivar were placed in a glasshouse until the beginning of flowering, which took two weeks. During this period, plants were not watered, and they experienced an increase by 5.1 °C in mean air temperature when compared to 12 control plants of each cultivar, which remained outdoors. At the end of these two weeks, we observed the opening of the first flowers in plants inside the greenhouse. At this stage all the plants were moved to an open patch dominated by small sized grasses, in the Botanical Garden (lat. 40.206403°, long. −8.425170°, 65 m a.s.l.) and were exposed to pollinators.

Flower Morphology and Rewards

For all the 48 plants in the experiment, one, young but fully open, flower per plant was selected for morphological measurements. Blueberry flowers are urn-shaped, and we recorded measurements that capture the variation of this shape among cultivars. Following the methodology described by Courcelles et al. (2013), we measured corolla length, diameter at widest area, and diameter of corolla opening (flower throat). Measurements were made using a digital caliper. Floral volume (corolla volume) was calculated considering the shape of a cylinder and using the length of the corolla and the diameter at the widest portion.

Nectar amount was quantified in the morning on flowers bagged the day before, using micro-capillaries and following Dafni et al. (2005). The percentage of sugars (ºBrix) was determined with a hand-held refractometer.

Pollinator's Observation

Pollinator observations followed standard methodologies (Dafni et al. 2005). Pollinators were observed at the peak of flowering, between 22nd of March and 12th of April 2020, on sunny days with temperatures above 13 °C, i.e., weather conditions favorable for pollinators activity. Visits were recorded for 10 min periods, at different times of the day from 9 A.M. to 5 P.M. (CET + 1 h), totaling 13 h 18 min of net observation. We recorded the number of individual insects that visited the flowers, the insect species, and the treatment, cultivar and number of flowers visited. Additionally, the total number of open flowers per treatment and cultivar were also recorded.

Indexes of floral preference and constancy were calculated for the main pollinator species [*Anthophora plumipes* (Fabricius, 1781)], excluding visits with less than three visited flowers (Dafni et al. 2005; Castro et al. 2020). Floral preference was calculated for each cultivar, as the ratio between the number of visits to plants under control conditions and the total number of visits. Values of 0.5 indicate no preference by the pollinator, values of 0 indicate preference for plants under stress conditions, and values of 1 indicate preference for plants under control conditions. Floral constancy was calculated for each cultivar as the ratio between the number of movements within the same treatment and the total number of movements during the visit. A value of 0 indicates alternating foraging behavior, a value of 0.5 indicates random foraging behavior and a value of 1 indicates constancy in foraging behavior within treatment.

Fruit Sampling and Processing

The percentage of flowers that set fruit (fruit set) was calculated as the ratio between the mean number of fruits produced per inflorescence and the mean number of flowers per inflorescence, by counting the number of flowers and fruits in five inflorescences per plant.

All fruits produced by the plants were collected when ripe, counted and weighed in an analytical scale (accuracy 0.1 mg). To determine mean fruit weight a subset of 15 fruits per plant was taken and each fruit was weighed individually.

Data Analysis

A *t*-test was used to explore differences between control and stress treatments within each blueberry cultivar in flower morphology, floral rewards and fruit parameters (fruit set, number of fruits per plant and fruit weight). The *t*-test was also used to explore differences in flower traits between blueberry varieties. For this analysis we pooled control and stressed plants of each cultivar, as no significant differences were found between treatments (see ‘Results’).

Floral preference and constancy indices were used as a measure of pollinator behavior and were analyzed as the deviation from 0.5 which represents the randomly expected value (Dafni et al. 2005) using a one-sample *t*-test.

All analyses were done using *R* version 3.3.2 (Core Development Team 2016) and differences were considered statistically significant at $P < 0.05$.

Results

Flower morphology differed between blueberry cultivars, but it was not affected by stress conditions (Figs. 9.1 and 9.2), with exception of the corolla volume in Duke cultivar, where stress led to marginally significantly larger volume than control conditions (Table 9.1). Duke flowers had significantly larger corolla throat and wider corollas than Bluecrop flowers, which is reflected in a larger corolla volume in Duke flowers when compared to Bluecrop (Fig. 9.2; Table 9.1).

The mean number of flowers per inflorescence was not significantly affected by stress conditions, although Bluecrop plants under stress conditions produced a higher number of flowers than control plants (Table 9.2). The number of inflorescences per plant was not significantly affected by stress conditions (Table 9.2), although, for both cultivars, there was a tendency for a higher number of inflorescences in stressed plants. We did not find significant differences in the mean number of flowers per inflorescence ($t = 1.089$, $P = 0.285$) or total number of inflorescences ($t = -0.405$, $P = 0.689$) between cultivars.

Stress conditions triggered earlier flowering in Bluecrop plants, but not in Duke plants (Fig. 9.3).

Nectar volume and sugar content were not affected by stress conditions, as shown, for both cultivars, by the lack of significant differences between control and stressed plants (Fig. 9.4, Table 9.1). Despite not significant, stressed plants presented a slight decrease in nectar volume and a slight increase in sugar content when compared to control plants.

Overall, there were no significant differences in nectar volume and sugar content (Table 9.1) between blueberry cultivars, but there was a trend for higher nectar volume in Bluecrop (5.04 ± 0.95 μl) than in Duke (4.09 ± 0.53 μl), and for higher sugar content in Duke ($19.79 \pm 0.95\%$) than in Bluecrop ($16.23 \pm 0.53\%$).

Fig. 9.1 Blueberry flowers from Bluecrop (a) and Duke (b) cultivars

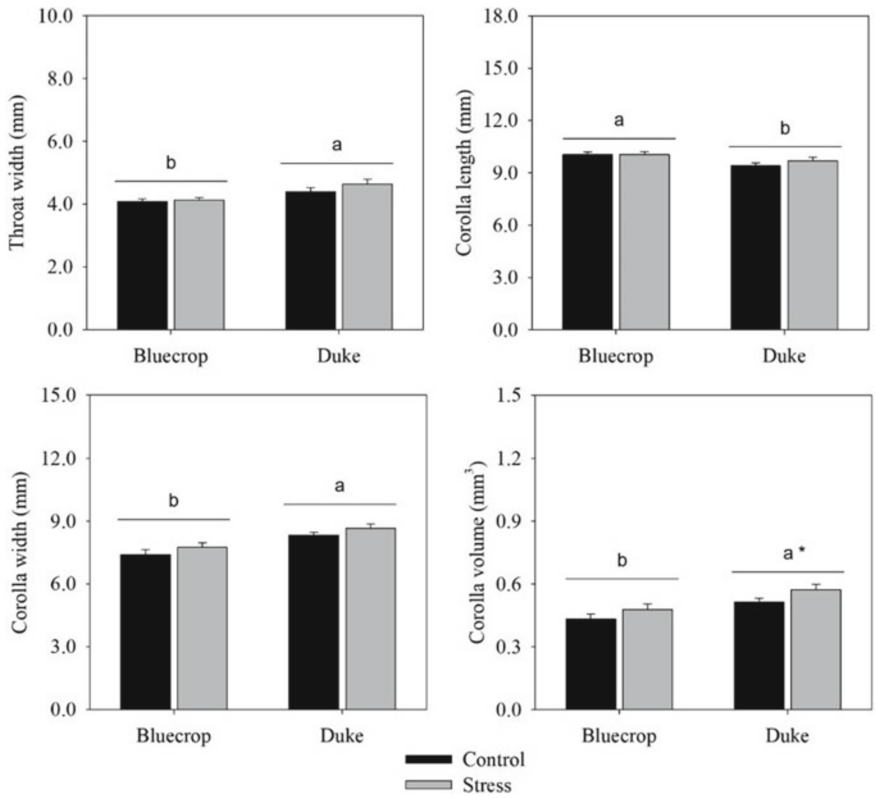

Fig. 9.2 Mean (±SE) throat width (upper-left), corolla length (upper-right), corolla width (down-left) and corolla volume (down-right) of blueberry cultivars, Bluecrop and Duke, under control and stress conditions. Different letters indicate significant differences between blueberry varieties ($P < 0.05$) after a *t*-test, while * indicates marginal significant differences between control and stress plants within cultivar Duke ($P = 0.09$)

A total of seven pollinator species were observed visiting blueberry flowers, but the main pollinator visiting blueberry flowers was *Anthophora plumipes*, accounting for 93.7% of all visits (Table 9.3).

The percentage of visited flowers was not affected by the imposed conditions, although for Bluecrop, pollinators tended to visit a higher percentage of flowers from plants under control conditions (Fig. 9.5).

Pollinator behavior as indicated by floral preference and constancy indices showed that *A. plumipes* for Bluecrop preferred control plants while, for Duke, it showed no preference (Table 9.4). Data also shows that *A. plumipes* preferred Bluecrop plants rather than those from Duke cultivar (preference index = 0.40, *P*-value = 0.009). Regarding constancy, *A. plumipes* significantly visited flowers within the same treatment rather than among treatments (Table 9.3).

Table 9.1 Statistical results (t-test and P-value) from t-tests used to test differences in flower traits, nectar volume and sugar, and fruit weight between stressed and control Bluecrop and Duke plants and between cultivars. Significant differences ($P < 0.05$) and marginally significant differences ($P < 0.05$) are highlighted in bold

	Treatment effect		Cultivar effect
	Bluecrop	Duke	
Flower traits			
Throat width	$t = -0.322$, $P = 0.751$	$t = -1.214$, $P = 0.238$	**$t = -3.509$, $P = 0.001$**
Corolla length	$t = 0.031$, $P = 0.976$	$t = -1.115$, $P = 0.278$	**$t = 2.974$, $P = 0.005$**
Corolla width	$t = -1.116$, $P = 0.277$	$t = -1.354$, $P = 0.192$	**$t = -4.509$, $P < 0.001$**
Corolla volume	$t = -1.238$, $P = 0.230$	**$t = -1.792$, $P = 0.088$**	**$t = 3.555$, $P = 0.001$**
Nectar			
Nectar volume	$t = -0.810$, $P = 0.441$	$t = 0.412$, $P = 0.688$	$t = 0.952$, $P = 0.352$
Nectar sugar	$t = -1.513$, $P = 0.156$	$t = -0.625$, $P = 0.545$	$t = -1.863$, $P = 0.076$
Fruit			
Fruit set	$t = 0.2936$, $P = 0.772$	$t = -1.580$, $P = 0.129$	$t = -0.963$, $P = 0.341$
Fruit weight	$t = -1.585$, $P = 0.128$	**$t = 2.736$, $P = 0.012$**	$t = 0.642$, $P = 0.524$

Table 9.2 Number of flowers per inflorescence and number of inflorescences per plant for both blueberry cultivars under control and stress conditions. Results are given as mean ± standard error of the mean (SE). The P value after a t-test comparison is also provided

Cultivar		No. of flowers/inflorescence		No. of inflorescences/plant	
		Mean ± SE	t-test, P value	Mean ± SE	t-test, P value
Bluecrop	Control	7.00 ± 0.50	$t = -1.800$, $P = 0.085$	7.83 ± 1.40	$t = -1.344$, $P = 0.196$
	Stress	8.23 ± 0.47		11.42 ± 2.27	
Duke	Control	7.04 ± 0.18	$t = -0.060$, $P = 0.952$	9.17 ± 0.69	$t = -1.988$, $P = 0.063$
	Stress	7.06 ± 0.21		11.92 ± 1.20	

Also, for Duke cultivar the mean number of fruits harvested in stressed plants (60.42 ± 7.15) was significantly higher ($t = -2.369$, $P = 0.030$) than that of control plants (40.83 ± 4.15). For Bluecrop cultivar the mean number of fruits harvested from stressed (50.50 ± 8.44) and control (40.08 ± 6.98) plants was not significantly different ($t = -0.952$, $P = 0.352$).

Fruit set was not significantly affected by the imposed conditions for either blueberry cultivar (Fig. 9.6, Table 9.1). Regarding fruit weight, control plants of Duke cultivar, yielded significantly heavier fruits than those exposed to stress (Fig. 9.7, Table 9.1).

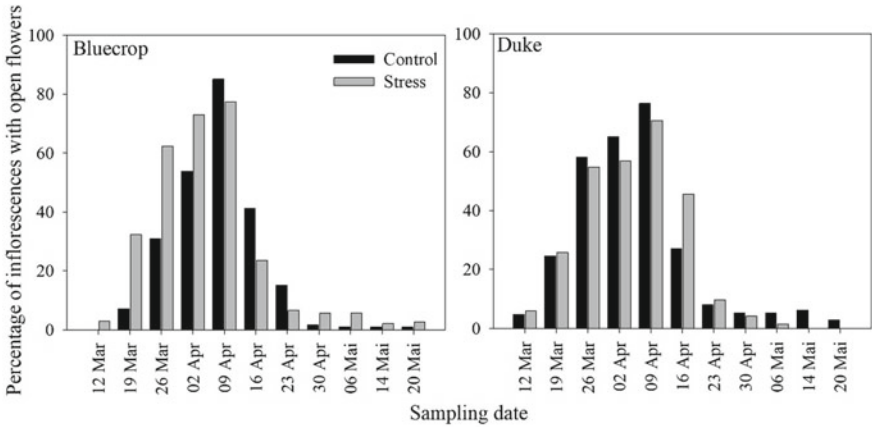

Fig. 9.3 Flowering phenology of blueberry cultivars, Bluecrop and Duke, under control and stress conditions

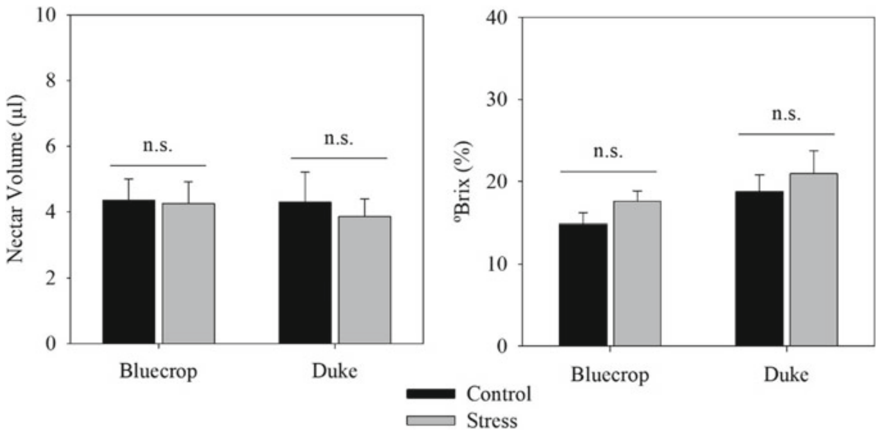

Fig. 9.4 Mean (±SE) of nectar volume (left) and sugar content (ºBrix; right) of the flowers of blueberry cultivars, Bluecrop and Duke, under control and stress conditions

Discussion

Flower number and density are considered important visual cues for pollinator attraction, and it has been shown in the literature that a decrease in such traits may bear consequences for pollinator visitation rates and the species of pollinators visiting the flowers (Kuppler and Kotowska 2021). Flower production requires plant resources and is a source of water loss under water stress conditions. Therefore, plants under water deficit are expected to produce fewer and smaller flowers, as often reported

Table 9.3 List of insect species visiting blueberry flowers, overall percentage of visits carried out by each species and abundance per unit of observation time (10 min)

Insect species	Visits (%)	Abundance per 10 min
Anthophora plumipes (Fabricius, 1781)	93.71	1.74
Andrena nigroaenea (Kirby, 1802)	0.75	0.03
Bombus terrestris (Linnaeus, 1758)	0.90	0.01
Lasioglossum malachurum (Kirby, 1802)	3.29	0.19
Lasioglossum sp.1 Curtis, 1833	0.30	0.03
Lasioglossum sp.2 Curtis, 1833	0.75	0.04
Vespula sp. Thomson, 1869	0.30	0.01

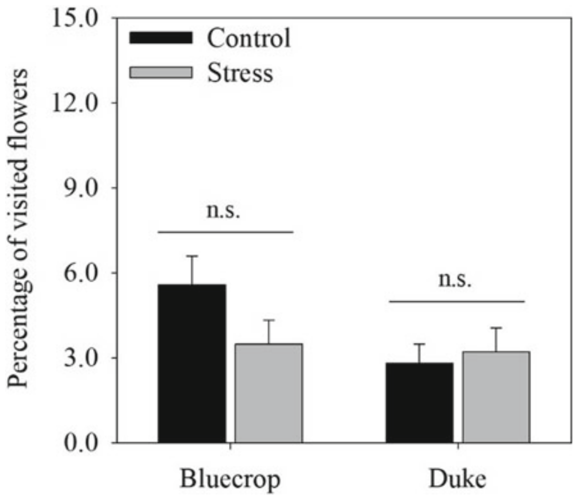

Fig. 9.5 Percentage of flowers visited for blueberry cultivars Bluecrop and Duke under control and stress conditions

in studies evaluating the effect of water stress on floral traits (*e.g.*, Descamps et al. 2020b; Gallagher and Campbell 2017; Kuppler et al. 2021). In our study, water deficit did not affect flower size significantly, contrary to our hypothesis. A few factors may have contributed for this results: (1) the water stress treatment was imposed before flowering; a recent meta-analysis by Kuppler and Kotowska (2021) on the effects of water stress on flower traits showed that a reduction in water availability before flowering has a lower impact than if applied at the beginning of flowering; (2) Blueberry flower production follows a dormancy period and is mainly dependent on stored resources, as vegetative growth starts simultaneously or even after flower bud

Table 9.4 Pollinator behavior as indicated by floral preference and constancy indices of the main pollinator of blueberry cultivars, Bluecrop and Duke, under control and stress conditions. Results are given as mean ± standard error of the mean (SE). Floral preference: values of 0.5 indicate no preference by the pollinator, values of 0 indicate preference for plants under stress conditions, and values of 1 indicate preference for plants under control conditions. Floral constancy: values of 0 indicate alternating foraging behavior, values of 0.5 indicate random foraging behavior and values of 1 indicate constancy in foraging behavior within treatment. The P value after a t-test comparison is also provided. Significant differences at $P < 0.05$ are highlighted in bold

Species	Preference index				Constancy index			
	Bluecrop		Duke		Bluecrop		Duke	
	Mean (±SE)	t-test, P-value	Mean (±SE)	t-test, P-value	Mean (±SE)	t-test, P-value	Mean (±SE)	t-test, P-value
A. plumipes	**0.61 ± 0.05**	**t = 2.099, P = 0.042**	0.49 ± 0.05	t = −0–104, P = 0.918	**0.84 ± 0.04**	**t = 8.801, P < 0.001**	**0.88 ± 0.03**	**t = 11.432, P < 0.001**

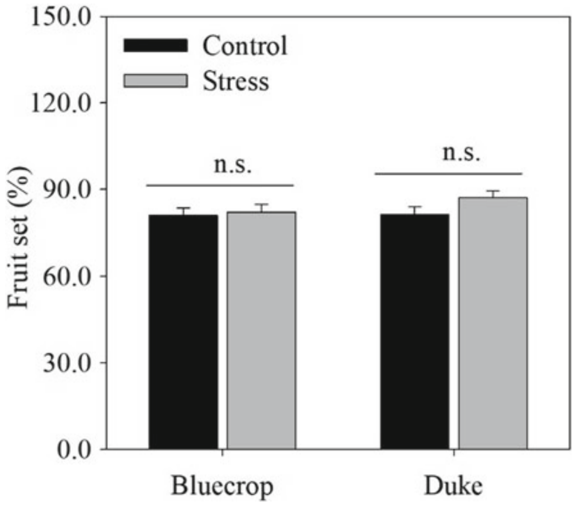

Fig. 9.6 Mean (±SE) fruit set for blueberry cultivars, Bluecrop and Duke, under control and stress conditions

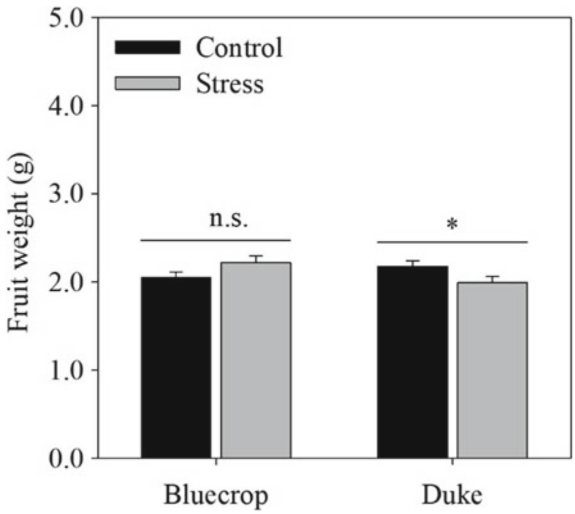

Fig. 9.7 Mean (±SE) of fruit weight for blueberry varieties, Bluecrop and Duke, under control and stress conditions. *Indicates significant differences ($P < 0.05$) between Duke plants under control and under stress conditions after a *t*-test

growth; (3) Being late winter, the humidity in the air was high and soil water evaporation low. The combination of points 2 and 3 may have resulted in a very mild water stress (difference between control and stress was around 8%; data not shown), which combined with the application of water stress before flowering have likely resulted in no or low effect of water stress on the floral traits of blueberry cultivars Bluecrop and Duke.

Floral morphology differed significantly between both cultivars. Duke flowers presented a significantly larger corolla throat and wider corollas than Bluecrop flowers. This was expected from intrinsic traits of the cultivars and similar results were obtained by other authors (*e.g.*, Courcelles et al. 2013; Huber 2016) for the same cultivars. The differences in corolla size between blueberry cultivars, however, did not affect pollinator visitation rate. This result is in contrast with those of the study by Courcelles et al. (2013), which showed higher visitation rates by honeybees and bumblebees to Duke than to Bluecrop, and with the authors pointing to a relationship with the larger corolla of Duke. However, the response to flower traits may be related with differences in the pollinator community present during the flowering period in the surrounding landscape and interacting with the blueberry flowers, which in our case was scarcely represented by bumblebees and lacked honeybees. Indeed, at our study site, the main pollinator was *A. plumipes*, which is an insect with long proboscis and relatively large body, being mostly active early in the flowering season (Ornai and Keasar 2020), when blueberries flower in this geographic region. Species of the same genus have been reported as pollinators of blueberries under field conditions and were even used as managed pollinators in lowbush blueberry [e.g., *A. pilipes villosula* Smith, 1854 (Bushmann and Drummond 2020)]. The second most common pollinators in our study belong to the genus *Lasioglossum* Curtis, 1833, here represented by small bodied species that can fit inside blueberry flowers, regardless of the cultivar (author's observation). Thus, the behavior of the small *Lasioglossum* spp. observed here were not impacted by throat width of blueberry flowers.

The observation of plant-pollinator interactions shows two main behavioral approaches by floral visitors to blueberry flowers. First, big body insects with long proboscis (such as *A. plumipes*) would be able to reach the nectar legitimately and contact with the pollen inside the flower. Second, relatively small body insects with small proboscis (such as *Lasioglossum* spp.) have no restriction by the corolla and easily access nectar and pollen rewards, being potentially involved in blueberry pollination. The different behaviors of the flower visitors have an impact in pollination efficiency and reproductive fitness (*e.g.*, Castro et al. 2008, 2013).

Other important note was the high prevalence of male individuals of *A. plumipes* (author's observation), the main pollinator at the study site. These individuals usually start flying earlier in the season than females (Michener 2007) in an active search for matting partners (and collecting mainly nectar), contrarily to females that actively collect pollen (Michener 2007). Even though this data was not actively collected, male and female *A. plumipes* may thus have different pollination efficiencies impacting differently blueberry fruit production.

The main pollinator in our study, *A. plumipes*, preferred control plants for Bluecrop, while it showed no preference in Duke. *Anthophora plumipes*, also preferred Bluecrop plants rather than Duke. Although non-significant, the slight differences in nectar volume and sugar content found between cultivars may have contributed for the differences in *A. plumipes* behavior. Additionally, water deficits have been shown to affect floral volatile emissions and composition in natural communities, as well as other aspects of nectar composition such as secondary metabolites (Glenny et al. 2018; Descamps et al. 2021), and although we cannot confirm that these occurred in our study, it may be factor influencing *A. plumipes* behavior in our study. Variations in both nectar and floral volatile amount and composition have been linked to changes in pollinator behavior and preferences (Burkle and Runyon 2017; Parachnowitsch et al. 2019), and should be considered in future studies.

Nevertheless, the differences found in pollinator visitation were not reflected in differences in fruit production, as no significant differences in fruit set and in fruit weight were found between control and stressed plants of Bluecrop cultivar. Similarly, we did not find significant differences in fruit set between stressed and control Duke plants. This suggests that visitation by the pollinator's community to blueberry plants was not affected to the point of causing pollination deficits in stressed plants. However, we found that fruit weight was affected in Duke cultivar, with control plants yielding heavier fruits. Also, despite non-significant, fruit set and the number of inflorescences were higher in stressed than in control plants, which resulted in a significantly higher number of fruits. Considering that the plant has limited resources, producing more fruits implies investing less resources in each of them, which resulted in lighter fruits. However, considering our data, and the fact that such differences in fruit weight and number were observed in Duke cultivar where there was no preference of pollinators for a particular set of plants, it is not possible to associate fitness differences to pollinator's behavior.

Increased temperatures or heat pulses out of season resulting from climate changes may lead to early flowering of plants (Gérard et al. 2020), including crop species, with potentially negative consequences for crop production. Early flowering may result in plants missing part of the activity window of their main pollinators, which may lead to consequences for fruit production (Gérard et al. 2020). Such plant behavior may also lead to two potentially co-occurring consequences with strong impacts on crop production: (1) flower loss due to late frosts; (2) heterogeneity in flowering that may affect pollination success (Inouye 2008; Gérard et al. 2020), negatively affecting fruit production and quality, and causing heterogeneity in fruit ripening, and subsequently, increasing harvesting costs. In our study, increased temperature triggered earlier flowering in stressed Bluecrop plants, but not in Duke. Duke is considered an early flowering cultivar, even in relation to Bluecrop (Huber 2016) even though both cultivars overlap in their flowering period. The earlier flowering trait of Duke cultivar may have buffered the effect the stress imposed in this study. The response of flowering phenology to temperature increases has been shown to affect flowering phenology (Gérard et al. 2020), but it also seems to vary with plant species even within the same genus. For instance, flowering phenology was mostly unaffected in *Echium vulgare* L., while the opposite was observed in *E. plantagineum* L. (Descamps et al. 2020b).

Conclusions

We provide some insights into the effects of climate changes on plant-pollinator interactions. However, one should bear in mind that the blueberry individuals we used in our study, were small two-year-old plants entering the first productive year and therefore, a conservative approach was used when applying stress to these plants to reduce the risk of losing them due to the imposed stress. As referred above, further studies should involve drought periods during flowering, which are thought to have stronger effects on floral traits (Kuppler and Kotowska 2021). Also, there are inherent limitations related with the experimental design and its effects on pollination, as it is difficult to induce a similar stress on wild pollinators, which are usually studied in natural populations. Nevertheless, our study draws attention to key issues in pollination services to crops under climate change.

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