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Extreme events induced by climate change alter nectar offer to pollinators in cross pollination-dependent crops

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Both severe reductions and increases in rainfall can stress plants and modify floral traits involved in plant-pollinator interactions, such as nectar production. Animal pollination is responsible for most plant species' reproduction including several crops that rely especially on bees for fruit and seed production. Thus, extreme climate events can cause disruptions in pollination mutualism and lead to a decrease in the production of many crops worldwide. This study investigated the effects of changes in rainfall on nectar availability to pollinators at flower-, plant- and agricultural-scale, using an outcrossing bee-pollinated model crop. We experimentally simulated four scenarios: Control, Heavy rainfall, Rainfall reduction and Drought. All treatments but Rainfall reduction affected nectar traits at flower-scale. At plant- and agricultural-scale, Heavy rainfall increased nectar caloric offer (+ 79% and + 74%, respectively), while Rainfall reduction and Drought decreased it (– 37% and – 34%; – 98% and – 95%, respectively). Thus, drought treatments resulted in less resources available to pollinators The predicted rainfall shifts mediated by climate change may negatively affect cross-pollinated crops worldwide, as changes in nectar traits are prone to affect pollinator foraging behaviour and energy intake rate, decreasing pollination and fruit production. In summary, food security for humans may be closely linked to food security for pollinators.

Keywords Bee-pollination, Climate change, Drought, Floral nectar, Heavy rainfall, Zucchini

The most recent IPCC report[1], AR6, presents evidence that extreme events, such as intense rainfall and droughts, are becoming increasingly frequent and severe in different regions worldwide. On one hand, the direct effects of an excessive increase in rainfall on crop production include soil erosion and damages to crop, jeopardizing food production; on the other hand, extended periods of drought can lead to land degradation, resulting in a decrease of crop productivity[1]. Most crops depend to some degree on pollinators for their production[2], with this interaction being mediated by floral traits that may undergo important changes under different water availability scenarios[3,4].

Changes in water availability can stress plants and, consequently, lead to changes in the pattern of resource partitioning for reproduction and growth[5]. In a context of water scarcity, there is a tendency towards the distribution of fewer resources for reproduction[6], which may negatively affect floral traits[7–9]. Thus, the decrease in water availability[10] or its excessive increase[11] can affect the reproductive potential of plant species.

In particular, changes in the water regime can also lead to a decrease in nectar secretion rate[12]. A moderate increase in average temperature due to global warming can have a positive effect on nectar production but plants experiencing drought stress may decrease nectar production[13,14]. Reductions in nectar volume and/or concentration can result in a decrease in the total amount of sugar available to pollinators, which may influence the attraction and behaviour of these floral visitors[10,15]. Also, the decrease in nectar volume associated with the increase in its concentration can change nectar viscosity and affect its collection by long-tongued pollinators[15]. On the other hand, the increase in rainfall can dilute floral nectar[16] and discourage pollinator visitation[17]. All

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these changes in nectar traits that mediate flower-pollinator interactions have greater effects in plants with obligated biotic pollination leading to a decrease in the production of important crops and putting food security at increasing risks.

Changes in the regime of rainfalls can affect nectar production with direct impacts on the population maintenance of pollinators, with negative effects in the long-term for plant-pollinator interactions[18]. Additionally, due to the potential loss of pollinators, climate change has negative effects on these animals' ecosystem services for humans, hindering the production of several crops in Latin America[19]. In Brazil, the most pronounced decline in pollinators is expected to occur in the southern region, but all other regions will likely suffer major losses by 2050[20]. Thus, considering that bees are main pollinators of crops used for human consumption[21], the further decline of bee populations could be catastrophic for food production[20].

In this study, we selected a model plant species belonging to Cucurbitaceae, *Cucurbita pepo* L., for the experiments because it is totally dependent on bee-pollination for fruit production by having separate male and female flowers (diclinous-monoecious plants)[22]. American native bees have been described as important and specialized pollinators of many species of *Cucurbita*[23–25]. Pistillate flowers in different species of *Cucurbita* produce higher amount of nectar than staminate flowers[26,27] and flowers reabsorb nectar if pollinators do not collect the secretion[28]. Bumblebees show preferences for female *C. pepo* flowers[27]. Additionally, the total production of nectar can be affected by changes in meteorological conditions[29]. The chosen model plant species, *C. pepo*, has been cultivated in the Americas for more than 10,000 years[30], being currently cultivated worldwide[31]. In Brazil, it is one of the ten crops with the highest production and economic value[32]. Its cultivation is mainly carried out by small farmers[33], which are more prone to suffer with the negative effects of the climate change on crop production.

Nectar secretion under strong warming scenarios and drought stress as predicted by climate change models is expected to be reduced and can thus decrease available resources for pollinators[13,14]. Thus, in this study, we experimentally investigated whether simulated scenarios of changes in rainfall, as predicted by the IPCC (AR-6)[1], negatively affect the nectar traits of *C. pepo* at different scales (flower, plant, and per crop area), discussing the potential impacts of the results for pollinators under different agricultural scenarios for the next decades.

Results

Nectar production per flower

From the applied treatments simulating different rainfall-scenarios predicted by IPCC[1], only Drought affected nectar volume, nectar concentration and milligrams of sugar per flower (Supplementary Table S1), leading to a reduction when compared to the Control in pistillate and staminate flowers ($p < 0.0001$, Fig. 1A, B; $p < 0.001$, Fig. 1C, D; $p < 0.0001$, Fig. 1E, F, respectively; multiple comparisons are shown in Supplementary Tables S2–S4). Nectar concentration remained comparable in flowers of both types in the other treatments ($p > 0.05$, Fig. 1C, D; Supplementary Table S3). The Heavy rainfall treatment produced higher mg S per flower in both types ($p = 0.0059$; Fig. 1E, F; Supplementary Table S4). However, it is important to note that, in pistillate flowers, while nectar production was significantly lower in the Drought treatment, our sample size was very low due to a pronounced reduction in flower production in this treatment.

Number of flowers and nectar production per plant

The treatments affected the total production of pistillate flowers ($p < 0.05$; see Supplementary Table S5 for multiple comparisons), with plants from the Heavy rainfall treatment producing more pistillate flowers than plants from Control treatment ($p = 0.0460$; 6.4 ± 2.1 flowers; Fig. 2A; Supplementary Table S5). Meanwhile, plants from Rainfall reduction and Drought treatments produced a lower number of pistillate flowers than plants from Control treatment (3.3 ± 1.4, 0.9 ± 1.1 and 5.0 ± 2.0 flowers, respectively; mean ± sd; Fig. 2A).

The only treatment that affected the production of staminate flowers was Drought ($p < 0.0001$; Supplementary Table S5), which lead to a lower number of flowers per plant (7.0 ± 2.4 flowers; mean ± sd) than Control treatment (9.7 ± 2.7 flowers; Fig. 2B).

The treatments affected the total amount of sugar (mg S) per plant ($p < 0.05$; Supplementary Table S6), with plants from the Heavy rainfall treatment producing higher amounts of sugar than those from Control treatment ($p < 0.0001$; 358.8 ± 82.9 mg S; Fig. 3; See multiple comparisons in Supplementary Table S6). While plants from Rainfall reduction (127.1 ± 34.0 mg S; mean ± sd) and Drought (4.5 ± 3.2 mg S; mean ± sd) treatments (Fig. 3) produced less sugar amount than plants from Control (200.2 ± 43.7 mg S; mean ± sd).

Nectar production per crop area

When compared to the Control, the Heavy rainfall treatment led to a 74% increase in caloric value potentially offered by the crop to pollinators per hectare (from 1325.2 kg to 2304.9 kg). Meanwhile the Rainfall reduction treatment resulted in a 34% reduction in caloric value (from 1325.2 kg to 862.8 kg) and the Drought treatment caused a 95% reduction in caloric value potentially offered to pollinators per hectare of zucchini (from 1325.2 kg to 71.4 kg).

Discussion

The applied experimental treatments considering different scenarios of rainfall changes, as predicted by the IPCC, affected nectar production, leading to increases or reductions in caloric offer, according to the simulation of rainfall events (Fig. 4). Heavy rainfall led to an increase in mg S per flower in both flower types and in pistillate flower production, which led to a higher caloric offer per plant. Rainfall reduction did not affect nectar volume and concentration, and consequently mg S per flower; however, it led to a decreased in mg S per plant, due to the reduction in the number of pistillate flowers produced per plant. While Drought decreased nectar volume,

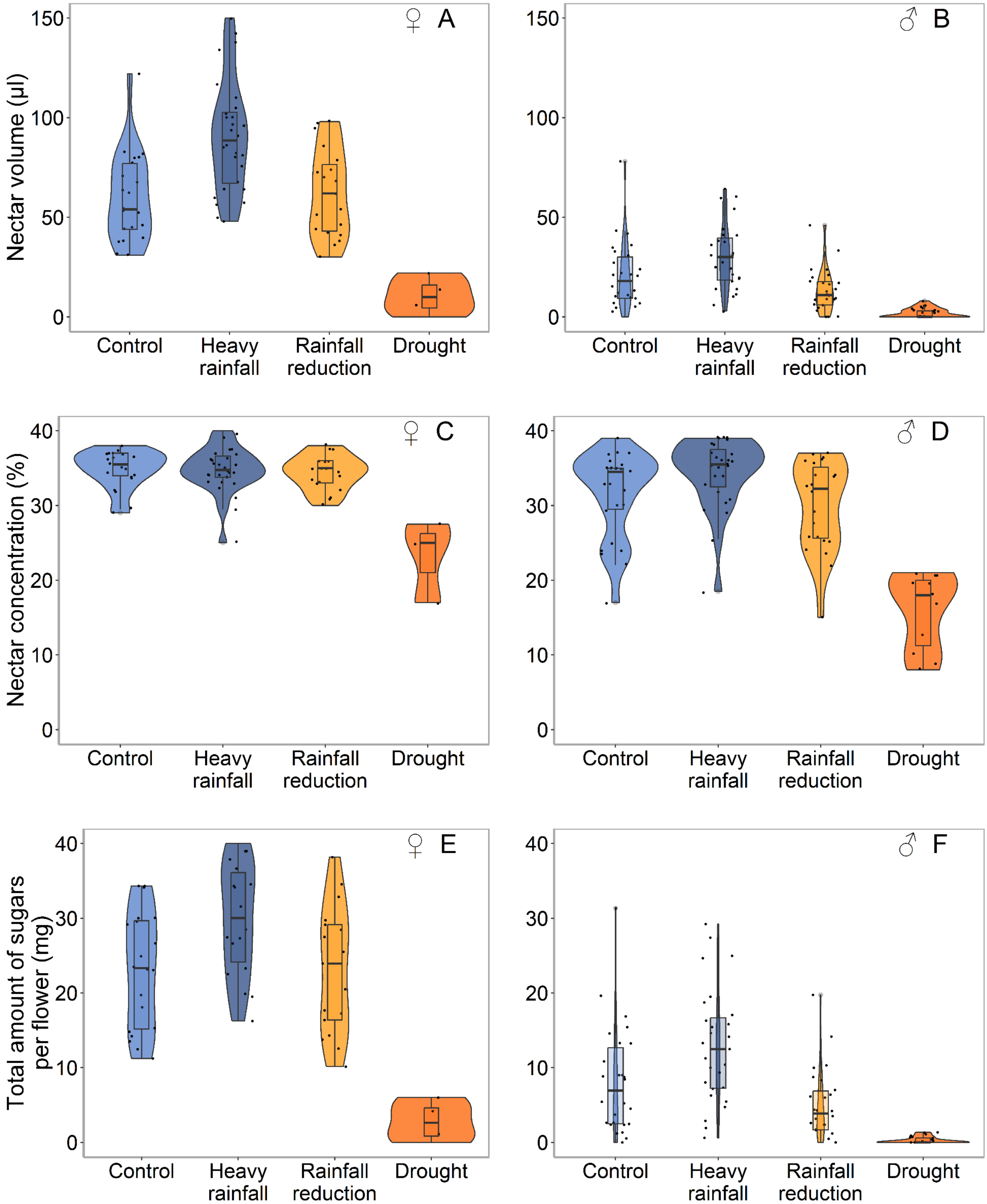

Fig. 1. *Cucurbita pepo* L. (Cucurbitaceae) floral nectar traits. (**A**) Volume (microliters), (**C**) concentration (%) and (**E**) total amount of sugars (mg S) of nectar collected from pistillate flowers. (**B**) Volume (microliters), (**D**) concentration (%) and (**F**) total amount of sugars of nectar collected from staminate flowers during the whole flowering period under the four treatments simulating different rainfall scenarios: Control, Heavy rainfall, Rainfall reduction, and Drought. The plot shows the median (horizontal line through the box), the 25th and 75th percentiles (lower and upper bounds of the boxes), the lower and upper tails, which correspond to the smallest and largest data points that are less than 1.5 times from the box of the interquartile distance, and outliers.

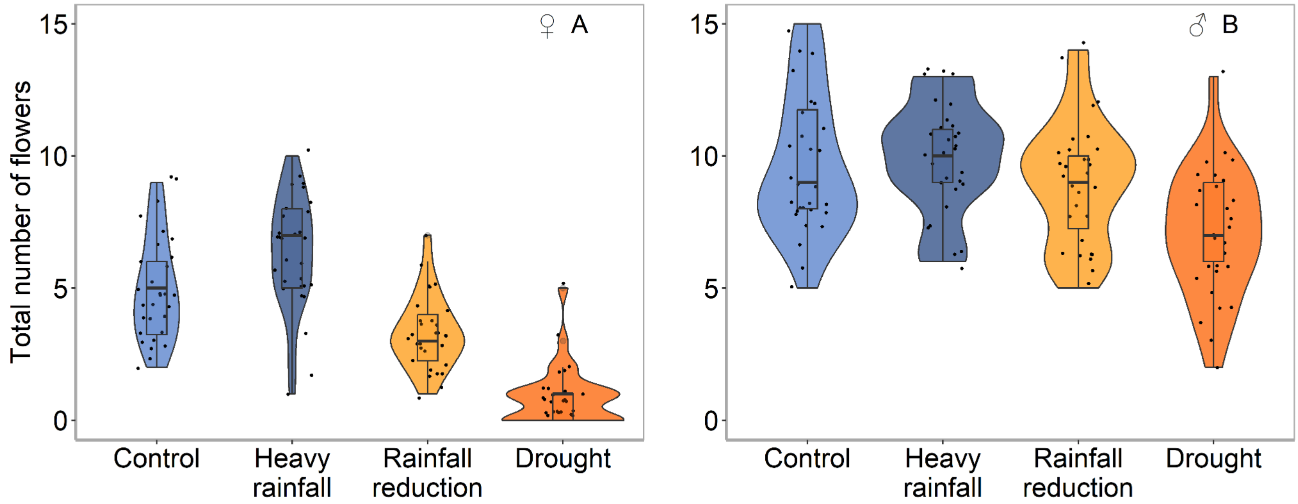

Fig. 2. Total number of (**A**) pistillate and (**B**) staminate flowers produced per plant of *Cucurbita pepo* L. (Cucurbitaceae) during the whole flowering period under the four treatments simulating different rainfall scenarios: Control, Heavy rainfall, Rainfall reduction, and Drought. The plot shows the median (horizontal line through the box), the 25th and 75th percentiles (lower and upper bounds of the boxes), the lower and upper tails, which correspond to the smallest and largest data points that are less than 1.5 times from the box of the interquartile distance, and outliers.

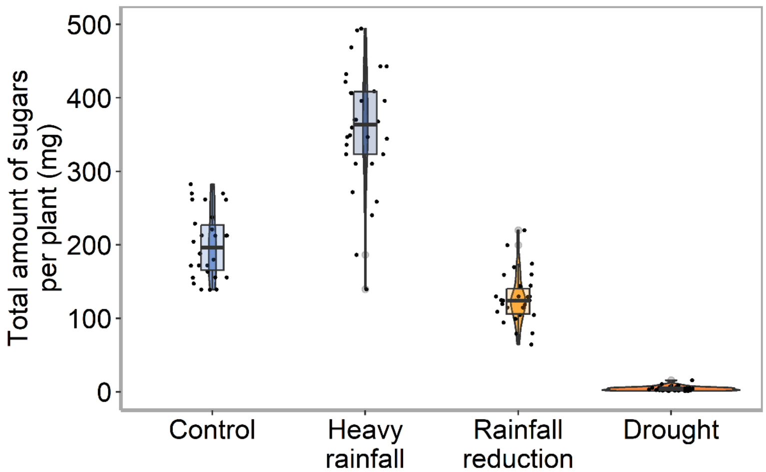

Fig. 3. Total amount of sugars produced per plant of *Cucurbita pepo* L. (Cucurbitaceae) under the four treatments simulating different rainfall scenarios: Control, Heavy rainfall, Rainfall reduction, and Drought. The plot shows the median (horizontal line through the box), the 25th and 75th percentiles (lower and upper bounds of the boxes), the lower and upper tails, which correspond to the smallest and largest data points that are less than 1.5 times from the box of the interquartile distance, and outliers.

concentration, and mg S per flower in both flower types and reduced the number of pistillate and staminate flowers per plant, leading to less mg S per plant. In summary, in this study we verified that all treatments, Heavy rainfall, Rainfall reduction and Drought, can alter nectar availability for pollinators (Fig. 4).

Effects of rainfall changes on nectar production at flower-scale

Although there were variations in nectar traits among treatments, it is interesting to note that plants display physiological mechanisms that ameliorate variations triggered by the extreme rainfall events. For example, this species can maintain nectar volume and concentration in both pistillate and staminate flowers when plants are exposed to a rainfall increase (+57%) or to a moderate rainfall decrease (−30%), suggesting a powerful plasticity for these nectar traits that could be associated with the maintenance of pollinators' visits. On the other hand, nectar volume and concentration suffered a substantial decrease under extreme drought conditions. In severe water scarcity scenarios, stomatal closure mechanisms, that reduce evapotranspiration, can also reduce CO_2 absorption and consequently photosynthetic rates, decreasing nectar production. Although plants stressed before the flowering period tend to adapt more readily to new environmental conditions[34], reductions

	Nectar production per flower						Nectar production per plant			Caloric value per crop area
	Nectar volume		Nectar concentration		mg S per flower		Number of flowers per plant		mg S per plant	mg S per hectare
Flower type	♀	♂	♀	♂	♀	♂	♀	♂	♀ ♂	♀ ♂
Heavy rainfall	–	–	–	–	↑	↑	↑	–	↑	↑ 74%
Rainfall reduction	–	–	–	–	–	–	↓	–	↓	↓ 34%
Drought	↓	↓	↓	↓	↓	↓	↓	↓	↓	↓ 95%

Fig. 4. Summary of the effects of the Heavy rainfall, Rainfall reduction and Drought treatments compared to Control on nectar traits at flower-, plant- and agricultural scale in *Cucurbita pepo* (Cucurbitaceae). Symbols: (♀) pistillate; (♂) staminate; (–) no effect; (↓) decrease; (↑) increase.

in photoassimilates can further limit plant resources, resulting in lower amounts of glucose produced per individual[35]. Indeed, this was expected for nectar volume since it is a known plastic trait[36] that can vary according to abiotic factors, such as temperature[13], ambient humidity[37], soil[38], CO_2 concentration[39], and water availability[3,8]. Moreover, the significant variability in the amount of nectar produced per flower, along with the common fluctuations in the available nectar per flower for pollinators, indicate that nectar volume per flower may not be strongly selected by pollinators. Indeed, pollinators encounter variations in the nectar available in each flower (e.g[40]). due to (a) disparities in nectar production within and between plants[41], (b) alterations from environmental factors[27,42], and (c) the consumption patterns of floral visitors[40,43–45]. Contrary to nectar volume, we expected nectar concentration to be the most stable among nectar traits in flowers with hidden nectar, as the effect of abiotic factors on nectar volume usually is not followed by changes in nectar concentration[3,8,39] and as it directly affects nectar energy supply per visit and even pollinators' ability to collect it[46].

Although nectar volume and concentration were not incremented in heavy rainfall scenarios, we observed an increasing trend in its production, which, when combined, resulted in higher amounts of sugar produced per flower. On the other hand, we registered a reduction in mg S per flower in drought scenario, following the reduction in nectar volume and its concentration, which represents a decrease in nectar energetic value per flower[47]. Calculating amounts of sugar per flower can be particularly useful for comparing floral resources and can provide us with direct clues about what pollinators may prefer when looking for an ideal energy gain or to select a more stable floral resource[48].

Indeed, optimizing caloric intake from flowers can be crucial for bees, since almost half of the energy obtained from nectar is spent in its collection[49]. Therefore, one can expect that bees would prefer to forage on flowers that yield the highest caloric intake with the least investment of time and energy, favouring the collection of more concentrated nectar if bees are physically able to collect it. Under this assumption, the establishment of Drought scenarios in the future, could make foraging on *C. pepo* flowers not worth to bees in terms of caloric intake per flower, which could lead to the abandonment of this plant species. Bees use both previous memory and associative learning of floral signals to visit more suitable resources[50], and they are quite constant in their visit pattern, especially stingless bees such as *Melipona*[51], which is an efficient pollinator of *C. pepo*[52]. The probability of bees visiting flowers of the same species sequentially is significantly higher than seeking out flowers of a new species[53]. However, the systematic encounter with smaller amounts of floral resources can lead to flower abandonment[17]. Although some bees tend to carry the same amount of nectar whether it is concentrated or not, they prefer a more concentrated nectar to a more diluted one[51]. It is noteworthy that the Drought treatment made the average concentration of nectar produced by staminate flowers to be lower than the range commonly collected by bees, from 20–70%[51].

The fact that pistillate and staminate flowers changed in the same way means that the original differences[54] in nectar traits among flower types are maintained in new rainfall scenarios. Such differences may constitute an important evolutionary mechanism underlying the diversification of floral traits in dimorphic flowers[55], since they are responsible for maintaining the sequence of pollinator visitation, ensuring that visits occur first to the staminate flowers and then to the pistillate ones.

Our findings suggest that there is a threshold of drought tolerance and a gradual negative effect of water regime reduction. This gradual effect of drought could be expected since the effects of stress conditions on plants can vary according to stress type and its intensity[34]. Moreover, the effects of drought on floral traits can be highly variable when plants are subjected to milder (e.g. Rainfall reduction) or more extreme drought conditions[56]. Thus, a forthcoming challenge is to determine the threshold of plant tolerance to rainfall changes in which floral traits and pollination are not compromised.

Effects of rainfall changes on nectar production at plant-scale

The total amount of nectar sugar per plant changed in all treatments at least partially due to the simultaneous changes in the number of flowers produced per plant. The differences in water availability led to distinct responses regarding the number of pistillate and staminate flowers produced per plant during the whole flowering. Pistillate flower production was more affected by fluctuations in water regime than staminate flowers, which may be associated with a higher water demand for ovary and ovule production[57]. This resulted in a higher production of pistillate flowers when rainfall increased and fewer flowers produced when it decreased, even as slightly as in Rainfall reduction scenario. On the other hand, only drought conditions affected staminate flower production. Thus, the decrease in flower production added to fewer amounts of sugar produced per flower, led to a decrease in caloric offer to pollinators at plant scale. This means that pollinators will face climatic scenarios with a reduction in the availability of flowers per plant, especially pistillate flowers that present higher nectar sugar content than male flowers. This might negatively impact *C. pepo* pollination as higher nectar production has been correlated with more pollinator visits[58]. Another possibility is that pollinators will need to move further and visit more plants to obtain the same calories in drought scenarios, which will negatively impact their rate of energy gain due to the energetic expense of moving between flowers of different plants[49].

Effects of rainfall changes on nectar production at agricultural-scale

The total amount of sugar per plant was higher in Heavy rainfall treatment and lower in Rainfall reduction and Drought treatments, compared to natural rainfalls Control. When extrapolating these findings to an agricultural crop scale, one can expect a substantial increment (+ 74%) in caloric value available for pollinators in scenarios with increased rainfall. However, when extrapolating our findings for drought scenarios, one can expect a gradual decrease of these resources, which can reach an alarming low (− 95%) resource offer for pollinators in crops under extreme drought conditions.

A reduction in nectar's total amount of sugars, along with less flowers produced during flowering, resulted in substantial changes in the caloric offer available to bees that pollinate *C. pepo*, such as *Melipona*, *Bombus*, *Apis*, *Peponapis* and *Xenoglossa* species. A decrease in nectar's total milligrams of sugars is closely linked to a reduction in its total calories, and this reduction may not cover pollinators' energy needs[10]. This might be especially relevant in crop scenarios, in which a single plant species might be the only nectar source in the pollinator foraging area and, on the other side, crops with cross-pollination dependence will reduce their yields. These effects can propagate through food webs[59], threatening the maintenance of animal populations that depend directly on floral resources and, consequently, on their predators. Currently, we are facing sharp declines in bee populations, caused by changes in the resources they collect, in their habitats and diet, among other factors[60]. In reduced water regime scenarios, the decrease in caloric availability in cultivation environments foraged by bees can cause changes in their feeding pattern and in the maintenance of their populations[61], putting at risk food production.

Although in heavy rainfall scenarios we showed an increase in caloric offer availability to pollinators at an agricultural scale, it is necessary to consider the consequences of increased intense precipitation events in a broader ecological approach. Indeed, the high frequency and intensity of precipitation can have devastating consequences for plant species, floral visitors, and the maintenance of plant-pollinator interactions[62–64]. For example, most pollinators decrease their activities in rainy periods[65], as heavy rainfall events affect the mechanics behind their flight and even their thermoregulation[66], increasing the energy required to perform foraging[67]. Additionally, frequent events of heavy rainfall could produce negative effects on crop production because increased soil erosion and nutrient washout. In this case, it would be necessary to perform crop fertilization more regularly to maintain productivity, which may also have important economic impacts on smallholder agriculture. Besides causing an increase in production cost, a larger use of fertilizers could lead to the soil and water bodies pollution[68], negatively impacting the local biota[69].

On the other hand, in scenarios of reduced rainfalls and global warming, maintaining favourable conditions for large-scale production will require more frequent and abundant irrigation of the crops, as plant water-requirement depends on its evapotranspiration rate, which is directly influenced by weather conditions[70]. These drought scenarios, in addition to increasing the overall cost of zucchini production, could lead to more serious water scarcity problems than the already occurring in several countries[1]. The increase in water demand for crop irrigation can be particularly harmful to family farms, which occupy ~ 75% of the world's agricultural land[71], and to poor rural communities worldwide, considering that 70% of them rely on agriculture as their primary source of income[60].

What to expect in IPCC climate change prediction scenarios

According to the expectations of future scenarios of climate change, we fitted a linear model to discuss the trends in percentual changes in mg of nectar sugars produced per hectare of zucchini in function of the percentual changes in rainfall ($r^2 = 0.9993$; Fig. 5). This model highlights the linear nature of the caloric availability to pollinators at crop stand scale, allowing to predict the resource availability for pollinators as a function of the different future scenarios of rainfall. According to the model, we can expect that nectar offered to pollinators will decrease or increase depending on the scenario until the physiological limits of plants are reached in the extremes.

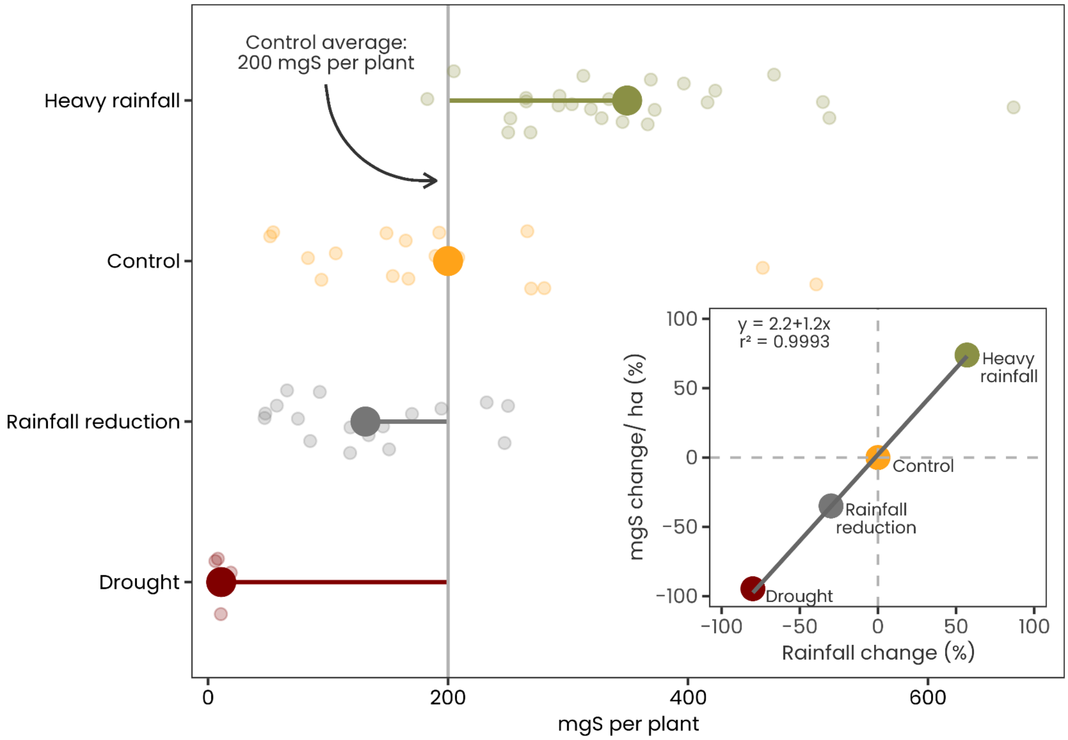

Fig. 5. Amount of nectar offered to pollinators (mg S) per plant of *Cucurbita pepo* L. (Cucurbitaceae) under the different models of climatic change (treatments in rainfall: Heavy rainfall, Rainfall reduction and Drought) simulating different rainfall scenarios, highlighting the differences between each treatment compared to the control (current rainfall regime). Inserted linear relationship between the mean percentual change in rainfall and the mean percentage change in caloric offer to pollinators per hectare. Small dots correspond to data for each plant and the large dots to the mean for each treatment.

Based on this linear model and on the least severe near-term IPCC projections, in which CO_2 emissions will be cut to net zero around 2075 (SSP1-2.6), we can expect that rainfalls will decrease (~16%) in some regions and increase (~42%) in others[1]. In this more optimistic model, we can predict a decrease in ~17% in the caloric offer to pollinators at the zucchini stand scale and an increase in ~53%, respectively. Whereas when considering this linear model and the most severe near-term IPCC projections, in which CO_2 emissions will triple by 2075[1], we can expect that pollinators will face potential reductions of ~23% in nectar production in regions affected by drought and potential increases of ~79% in regions affected by heavy rainfalls. Thus, these trends for nectar production in zucchini under different scenarios of climate change might be useful to simulate what may happen to the most important and universal caloric value mediating plant-pollinator interactions worldwide.

Concluding remarks

This study provides a multi-scale analysis of nectar availability to pollinators under predicted climate change scenarios, showcasing increases or reductions in caloric offer according to rainfall events. These results call for further investigations on the effect of rainfall changes on nectar production at field conditions, to better explore how phenomena observed in a greenhouse relates to field results. Moreover, they call for investigations on how pollinators would react to such changes in nectar availability at agricultural scale. Expanding our knowledge about rainfall changes and nectar production per hectare will allow us to evaluate if this strong linear relationship is widespread and valid for other plant species. If so, we could expect an alarming global scenario in terms of food insecurity for both pollinators and people under extreme drought conditions. Additionally, there is a growing interest in making food production more sustainable for small- and mid-scale farmers, and in addressing economic and social problems alongside the climate and biodiversity crises[72]. Furthermore, our study highlights the importance of guaranteeing pollinator food security to ensure the maintenance of people's food production, especially in developing nations where more than 2 billion people rely on smallholder agriculture[73].

Materials and methods

Experimental design

The experiment was conducted in May and June 2021, at the São Paulo State University (Unesp), city of Botucatu, São Paulo, Brazil. Seeds from Horticeres®'s lot 033712 referring to the 1028/2018 harvest with 90% germination and 99% purity of *Cucurbita pepo* L. cultivar Caserta were sowed in 6 L pots containing 1.67 kg of Carolina Soil® each, fertilized with 4 g of Single Superphosphate (SSP), 4 g of Thermophosphate, 1 g of potassium chloride (KCl) and 1.5 g of ammonium nitrate (NH_4NO_3) to prevent the results from being influenced by nutrient deficiency. In consequence, the initial growing conditions were standardized and the individuals ($n = 120$ plants; one per pot) were cultivated in a greenhouse with temperature ranging between 26 and 30 °C, and irrigated daily with 278 mL of water, to maintain the plants at field capacity until the first leaves had expanded. Then, plants were randomly assigned to treatments simulating different rainfall scenarios expected by the IPCC (AR-6) for the next decades ($n = 30$ plants/treatment): Control each pot was irrigated daily with 177 mL of water as a control condition, corresponding to the average natural rainfall in September for the region, calculated based on data for the last 40 years obtained from the Meteorological Station from the School of Agriculture, Unesp, month in which this crop is cultivated in the southern Brazil. The daily irrigation (177 mL) in control treatment correspond to a total of 108.14 mm of simulated rainfall per month; Heavy rainfall each pot was irrigated daily with 278 mL of water simulating heavy rainfall events, totalizing 170.20 mm of rainfall per month (57% of rainfall increase); Rainfall reduction each pot was irrigated daily with 124 mL of water simulating a 30% reduction in the natural rainfall average in September, totalizing 75.92 mm per month; and Drought each pot was left without irrigation until up to 70% of the plants showed signs of wilting, followed by irrigation with 278 mL, in order to simulate extreme events of prolonged drought followed by heavy rainfall, totalizing 22.69 mm of rainfall per month (80% of rainfall decrease).

Nectar production

We investigated the effects of changes in rainfall on nectar production at three different scales, per flower, per plant and per crop area (Fig. 6). During the whole experiment, the plants were maintained in a greenhouse completely isolated from pollinator visits. Thus, nectar data correspond to each unmanipulated flower directly measured in each plant (i.e., neither removed nor visited by pollinators).

Nectar production per flower

We sampled floral nectar in newly opened flowers, at 8 am, which corresponds to the peak time of its secretion of the 1-day lived flowers[28]. We sampled nectar from one pistillate and one staminate flower per plant ($n = 30$ plants per treatment; see Supplementary Table S7 for sample sizes). We measured nectar volume with graduated syringes and its concentration with a manual refractometer (0–32% brix). Then we used nectar volume and concentration data to calculate the total amount of sugars (mg S) per flower using the exponential regression proposed by Galetto and Bernardello[42].

Estimate of nectar production per plant

We counted the number of flowers opened each day during the whole experiment. We then use this information to calculate the average number of pistillate and staminate flowers produced per plant throughout the entire flowering period in each treatment ($n = 30$). To calculate the total amount of sugars produced per plant in each treatment, we used the mean amount of mg S per pistillate and staminate flowers, separately. Then we multiplied these values by the number of pistillate and staminate flowers produced per plant during the entire flowering period for each treatment. Lastly, we summed the mg S from all pistillate and staminate flowers together to quantify the total mg S available to pollinators per plant.

Estimate of resource abundance per crop area

We used our data on total mg S per plant to estimate the caloric offer to pollinators per hectare. We adopted the spacing guidelines by Emater-MG[74] and calculated that there are approximately 6600 plants of zucchini per hectare. We then simulated a scenario with 6600 plants in each treatment (i.e., we did not assume differential plant mortality among treatments), using bootstrap with replacement of data on total mg S per plant in each treatment. This procedure allowed us to obtain an estimate of how future scenarios of rainfall variation due to climate change can affect the availability of caloric offer for pollinators of this crop in a large-scale agricultural setup. As we did not assume differential plant mortality among treatments, resource availability may be overestimated, especially in the most extreme climate scenarios.

Statistical analyses

We analysed nectar volume using a generalized linear model (GLM) with treatment, flower type, and their interaction (treatment*flower type) as predictor variables. For nectar concentration, we used GLM with treatment and flower type as predictor variables. For mg S per flower and mg S per plant we used generalized linear mixed models (GLMMs) with treatment and flower type as predictor variables and plants and blocks as random effects. We performed GLMs and GLMMs with gamma error distribution, except for nectar concentration, for which we used Gaussian error distribution. Additionally, we tested the effects of the treatments on flower production using a linear model (LM) with treatment, flower type, and their interaction (treatment*flower type) as predictor variables. We carried out model selection to obtain the most parsimonious model for each variable by comparing the models through likelihood ratio tests[75]. First, we obtained the optimal random structure for each model, removing random effect variables if there was no variability between plants and/or blocks across all treatments, as indicated by the variance in random effects. Then, we obtained the optimal fixed structure for each model, starting with full models encompassing treatment, flower type, and the interaction between them as predictor variables, but removing the interaction term when it was not explanatory. We performed post hoc pairwise comparisons by calculating contrast per treatment and per flower type, considering the interaction between variables when appropriate.

Statistical analyses were carried out using R software 4.2.2[76] with standard and additional R packages. For all the analyses, we used emmeans[77] and MASS[78]. For GLMM and GLM analyses, we also used packages actuar[79], fitdistrplus[80], glmmADMB[81], lme4[82], and R2admb[83]. For graphical representations, we used packages ggplot2[84], ggsci[85], ggthemes[86], patchwork[87], showtext[88], stringr[89], and tidyverse[90].

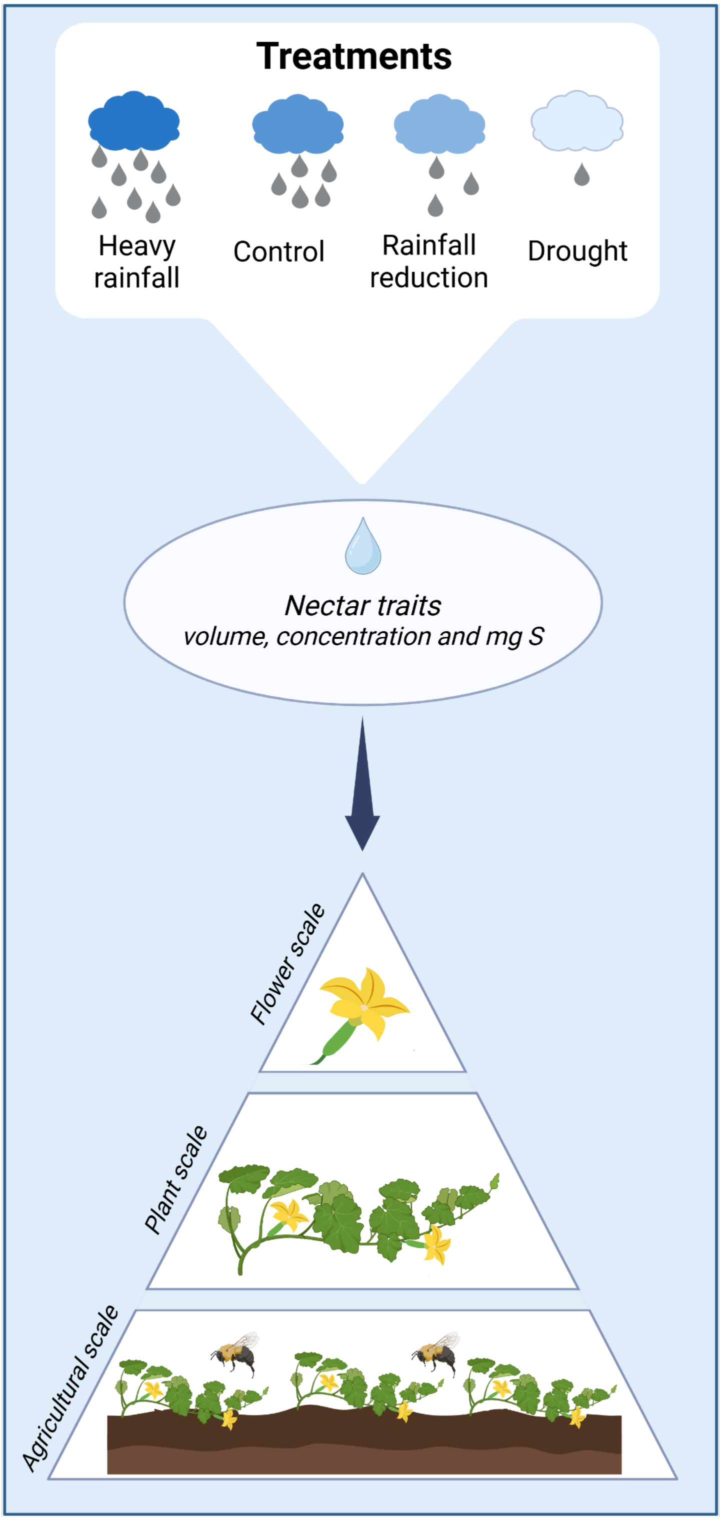

Fig. 6. Illustration portraying the experimental design used in the study to analyse the responses in nectar traits under different rainfall-scenarios (treatments) predicted by climate change models (IPCC 2021) at three different scales. Abbreviations: mg S = milligrams of sugars. Created in BioRender. Tunes, P. (2025) https://BioRender.com/o38e487.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding authors on reasonable request.

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Author contributions
P.T. and E.G. conceived and designed the study, and acquired funding; M.L.P.F. and P.T. collected samples and performed the data curation; M.L.P.F., P.T. and E.G. analysed the data, elaborated the figures, and wrote the original draft; C.S.F.B. contributed to the methods of cultivation in the experimental design and to the original draft; L.G. contributed critically to the data analysis and interpretation, and to the original draft. All the authors contributed to writing, reviewing, and editing the final version.

Declarations

Competing interests
The authors declare no competing interests.

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