




Article

Mild Water Stress Makes Apple Buds More Likely to Flower and More Responsive to Artificial Forcing—Impacts of an Unusually Warm and Dry Summer in Germany

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Received: 31 January 2020; Accepted: 11 February 2020; Published: 14 February 2020



Abstract: Climate change may result in increasingly frequent extreme events, such as the unusually dry conditions that occurred in Germany during the apple growing season of 2018. To assess the effects of this phenomenon on dormancy release and flowering in apples, we compared irrigated and non-irrigated orchard blocks at Campus Klein-Altendorf. We evaluated bud development, dormancy release and flowering in the following season under orchard and controlled forcing conditions. Results showed that irrigated trees presented longer (39.2%) and thinner shoots compared to non-irrigated trees. In both treatments, apical buds developed a similar number of flower primordia per cyme (4–5), presenting comparable development and starch dynamics during dormancy. Interestingly, buds on non-irrigated shoots exposed to low chill levels responded earlier to forcing conditions than those on irrigated shoots. However, chill requirements (~50 Chill Portions) and bud phenology under field conditions did not differ between treatments. In spring, buds on non-irrigated trees presented a higher bloom probability (0.42) than buds on irrigated trees (0.30). Our findings show that mild water stress during summer influenced vegetative growth during the same season, as well as the response of buds to forcing temperatures and flowering of the following season. The differences between irrigation levels in the phenological responses of shoots under low-chill conditions point to a so-far understudied impact of water supply on chilling requirements, as well as subsequent bud behavior. Accounting for the effects of both the water status during summer and the temperature during the dormant season may be required for accurately predicting future tree phenology in a changing climate.

Keywords: chill requirements; drought; starch reserves; blooming; bud development; temperate trees

1. Introduction

Apple production, and the cultivation of other temperate fruit trees, is affected by short and long-term weather variation, which is increasingly being modulated by anthropogenic climate change. In Germany, annual mean temperature has risen by approximately 1.9 °C since 1881 (based on a linear regression until 2018 [1]). Relative to the period 1971–2000, mean winter temperature is expected to keep increasing throughout the 21st century, with a total increment between 1.2 and 3.2 °C projected for the representative concentration pathway (RCP) scenario RCP4.5 (characterized by the expectation of 4.5 W m⁻² of additional radiative forcing by 2100 compared to pre-industrial conditions) [2]. Compared

to 1986–2005 and using the scenario RCP4.5, total precipitation during April–September is predicted to change between about –20% (25th percentile of multi-model distribution) and +10% (75th percentile) in central Europe by 2046–2065 [3]. These changes may result in more frequent extreme events, similar to the extraordinary heat and dry weather observed during the apple growing season in June–September of 2018. Such seasons do not only have immediate impacts, but they may also affect orchard performance in the following growth period. In fact, tree development in a given season depends on processes that occurred during the previous year, including the accumulation of reserves and dormancy induction, as well as flower initiation and differentiation [4,5].

Apple trees are cultivated in a wide range of climates, including temperate and tropical regions [4]. Accordingly, growing techniques have been adapted to a range of temperature and water regimes. Under drought conditions, where water scarcity limits apple production, regulated deficit irrigation (RDI) allows maintenance of productive orchards [5,6], although this practice may have undesirable implications for fruit maturity, quality, and shelf life [7]. On the other hand, RDI has been proposed as a useful approach to control excessive vegetative growth [8]. In general, water scarcity during the growing season may affect fruit production by reducing fruit yield and quality [9], as well as by modifying morphological and physiological traits of trees [10]. According to Naor et al. [11], the yield of apple trees decreased by 65% in response to reducing irrigation from 7 to 1 mm day⁻¹ under a medium crop load of ~300 apples per tree. This observation may be explained by water stress directly affecting fruit growth by influencing the cell division process [12], reducing cell turgor [13] and/or by limiting energetic resources due to a decrease in photosynthetic rate [14]. Along the same lines, Bolat et al. [10], working on one-year-old M9 apple rootstocks, demonstrated that cutting irrigation by 50% between mid-July and the onset of dormancy decreased relative shoot length by 67%, probably caused by a reduction in auxin concentrations. A decrease in soil water content has been associated with physiological modifications, such as hormone signaling and the activity of reactive oxygen species (ROS) in plants [15]. Chen et al. [16] concluded that water stress promoted growth cessation during summer and affected the ontogeny of apple shoots by increasing the production of abscisic acid (ABA) in roots [17].

The end of the growing season in deciduous forest and fruit trees is characterized by leaf fall and dormancy induction. During dormancy, all meristems in buds or other plant structures are unable to perform cell division and cell expansion [18,19]. To resume growth, buds require exposure to low and subsequent warm temperatures [20]. These thermal needs, denoted by the concepts of chill and heat requirements (CR and HR, respectively, Faust et al. [21], Luedeling [22]), have been widely studied in the past [23–25] and used in orchard planning and management [22]. Most studies on CR and HR estimation assume a sequential relationship between chilling and heating phases, defining a specific need for each of them [26]. However, some evidence suggests that chill and heat may compensate for each other under particular circumstances [27,28]. The effect of temperature on dormancy completion is commonly evaluated by experiments with shoots exposed to forcing conditions [29] or through statistical approaches applied to long-term datasets [24,30]. An accurate temperature response model would allow estimation of thermal requirements, as well as the relationship between chill and heat accumulation during dormancy. This may then facilitate the adaptation of species and/or varieties to different regions and future climate conditions [22].

In deciduous fruit trees, flower initiation and flowering occur in different growing seasons [31], since dormancy interrupts flower development during the winter months. Flowers in apple trees normally differentiate during summer in apical buds on spurs (short shoots) and current-season shoots (long shoots). However, apical buds can also remain vegetative under particular circumstances (e.g., in shoots with less than 16 vegetative buds in cv. ‘Golden Delicious’) [4]. The transition from vegetative to flower buds is modulated by both environmental and physiological factors. Wilkie et al. [4] highlighted that floral initiation in apples is affected by light conditions, crop load of the previous season, and leaf area. These factors may lead to physiological changes, such as increasing concentrations of gibberellins (GAs) due to high crop load, preventing flower induction. Similarly, smaller leaf area due to high

crop load has been proposed to decrease the concentration of starch in almond spurs, with negative effects on flower initiation and flowering rate in the following season [32]. After dormancy has been established, buds only resume growth after fulfilling their temperature requirements (CR and HR) [18] during a period when trees do not have mature leaves. This implies that bud burst and flowering rely on stored reserves [33]. Starch has been proposed to play a key role in flower development [34] and dormancy progression [35]. Starch content in the pistil and anther has been suggested to sustain growth and cellular differentiation in both *Arabidopsis thaliana* [36], the annual model plant, and temperate fruit trees [37,38]. The starch detected in flowers before bloom has been associated with dormancy and flower quality [34,39], as well as with key reproductive processes, such as pollination [40,41] and fruit set [39,42,43]. Flower differentiation and the storage of reserves, which occur during the previous growing season, may be affected by unfavorable environmental conditions during summer and early fall [44].

Apple is the most important fruit crop in Germany, representing about 50% of the country's total fruit production [45]. To evaluate the impacts of the unusual conditions in summer of 2018 in Germany on apple trees, we compared two orchard blocks under different irrigation regimes (irrigated vs. non-irrigated). While fruit development during the season allowed maintaining adequate yield in both blocks, we focused the attention on how natural drought affected bud performance in the following season. This general aim has been achieved by the following approaches: i) morphological observations of vegetative growth and reproductive buds; ii) evaluation of dormancy response and temperature requirements under greenhouse and field conditions; iii) estimation of the level of reserves (starch) accumulated in the flower meristems prior to flowering; and iv) estimation of the probability of buds to flower in the subsequent spring.

2. Materials and Methods

2.1. Site and Plant Material

This study was conducted in an experimental orchard of the University of Bonn at Campus Klein-Altendorf (CKA) in Germany (6° 59' 32" E, 50° 37' 51" N, and 160 m.a.s.l.). CKA is located in the temperate oceanic climate zone, according to the Köppen-Geiger classification [46]. Specific climatic conditions of this zone are mild winters and mild summers without dry periods. The records of the years 1956–2014 show an annual mean temperature of 9.4 °C, total annual precipitation of 603 mm, and a growing season length between 165 and 170 days. The orchard soil is a loess loam (silty loam), with relatively high water retention capacity.

To evaluate summer drought effects on flowering during the following season, we investigated six-year-old apple trees cv. 'Elstar' grafted onto M9 rootstock, cultivated with a spacing of 1.5 m within rows, and 3.5 m between rows. The experiment was carried out from June 2018 to May 2019, with the beginning of this period (June–September 2018) constituting an extraordinarily warm (Figure 1A) and dry (Figure 1B) growth period. Two independent blocks with similar soil conditions and crop load were exposed to different irrigation regimes (treatments). One of the blocks (of 0.27 ha and approximately 505 trees) was not irrigated, only receiving water through rainfall: 49 mm in June, 29 mm in July, 19 mm in August, and 37 mm in September. The other block (of 0.39 ha and approximately 743 trees) was irrigated according to standard orchard procedures, receiving an additional 63 mm in July and 70 mm in August (Figure 1B), split over a total of nine dates. According to a weather station located in the orchard, reference evapotranspiration (ET_0) was 102 mm in June, 172 mm in July, 136 mm in August, and 95 mm in September (Figure 1B). Since recommended crop coefficients (k_c) for computing the water needs of apple during the growing season range between 0.9 and 1.2 [47], these figures closely approximate the trees' water needs, indicating that trees in this orchard experienced water shortage during all summer months. Neither of the blocks was fertilized during the growing season to preclude nutrition-related effects on tree development. Similarly, selected trees for this experiment were not pruned during winter to preserve the total number of buds formed during the previous season. In this

study, we made use of what may be considered a natural experiment, exploiting the conditions that presented themselves in the aftermath of the 2018 drought.

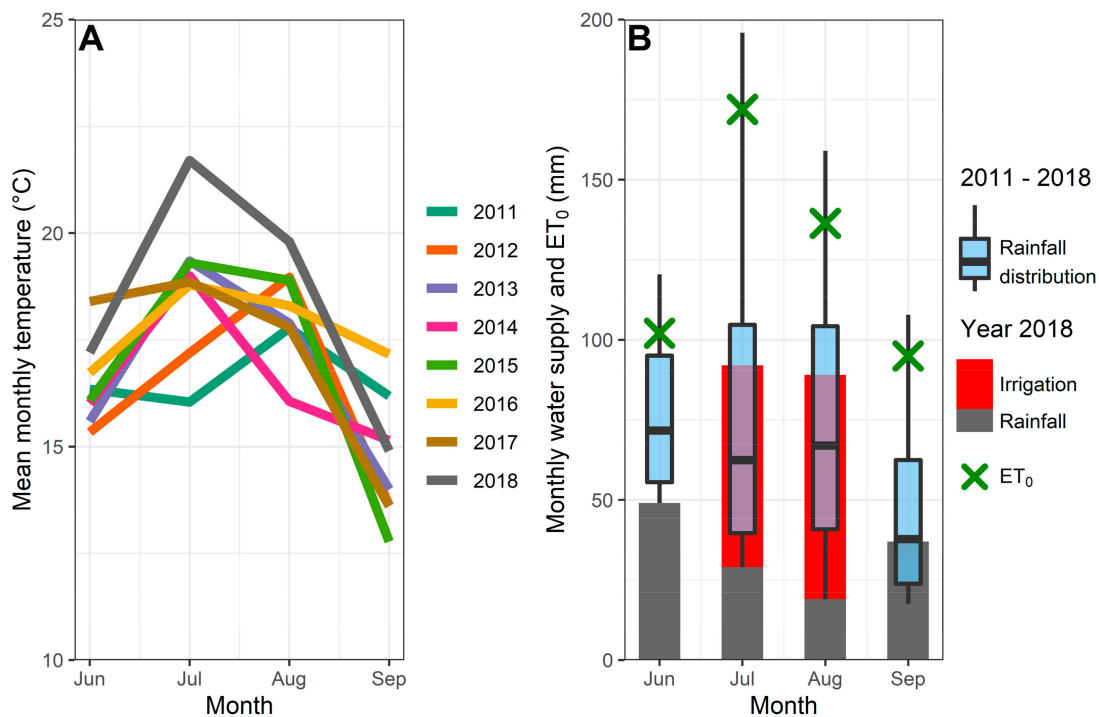


Figure 1. Weather conditions at Campus Klein-Altendorf from 2011 to 2018 for June, July, August, and September. (A): Mean monthly temperature (°C) for each year. (B): Precipitation, irrigation, and reference evapotranspiration (ET₀, in mm, indicated by green crosses). In B, boxplots correspond to the distribution of monthly precipitation for the period 2011–2018. Grey bars represent the precipitation for the year 2018, whereas red sections correspond to the additional water supplied by irrigation in July and August.

2.2. Morphological and Developmental Observations on Shoots and Apical Buds in the Field

Once dormancy had been established in December, a total of 240 one-year-old shoots were randomly selected and labelled in a set of 12 trees per treatment (120 one-year-old shoots per block). These shoots were characterized by measuring their length, their diameter, at 2 cm from the branch insertion, and the number of buds. Shoot length and number of buds were used to compute the average internode length.

Another set of seven trees in each treatment was used for weekly bud sampling from January to April, when natural bud burst occurred. In these seven trees, a total of four apical buds were randomly collected on each date from one-year-old shoots and spurs (short shoots <5 cm in length and older than one year) and subsequently dissected and observed under a stereoscopic microscope (Zeiss Discovery, V12 Stereo Microscope, Germany) to determine the developmental stage of the flower primordia. Micrographs were taken with a digital camera (The Imaging Source Camera DFK41BU02.H, Germany), linked to image processing software (IC Capture 2.4, Germany). Similarly, a total of 60 apical buds were used to determine, through microscopic observations, the number of flower primordia that composed the cyme.

2.3. Phenology Observations under Greenhouse and Field Conditions

The phenology of the vegetative and reproductive buds was recorded according to the BBCH scale [48], which has recently been adapted for apple trees [49]. The phenology of the vegetative buds was covered by the scale for “principal growth stage 0: bud development” (sub-stages from 00 to 09)

and “principal growth stage 1: leaf development” (sub-stages from 10 to 19). The phenology of the reproductive buds was described by the scale for “principal growth stage 5: inflorescence emergence” (sub-stages from 50 to 59), and bloom time was classified by the scale for “principal growth stage 6: flowering” (sub-stages from 60 to 69) [48].

To estimate the chill requirement of apple buds and to study its relationship with the heat accumulation phase of dormancy, we observed bud phenology under greenhouse conditions after buds had been exposed to different chill levels under field conditions. To this end, we applied the procedure described in Fernandez et al. [35] with some modifications. In brief, 24 (12 from each block) out of the previously selected shoots (Section 2.2 of Materials and Methods) were sampled weekly from 7th December 2018 to 8th February 2019. After sampling, shoots were transferred into a heated greenhouse (environmental conditions: 13.8 ± 5.1 °C; $72.9 \pm 12.5\%$ relative humidity) and placed vertically in 1 L containers with 250 mL of a 5% (w:v) sucrose solution after a cut under water to avoid cavitation and disinfection treatment (3% CaCl₂ w:v). Shoots were maintained inside the greenhouse for 10 weeks or until an advanced developmental stage (BBCH 60) was recorded in most of the shoots. The cut was refreshed and disinfected, and the sucrose solution changed every week.

In addition, flower bud phenology was tracked directly on the trees under field conditions. Twenty individual apical buds were selected and labelled in two trees (10 buds each) per irrigation treatment. Half of the buds were located on one-year-old shoots and the other half on spurs, where fruits are usually produced. Observations were carried out weekly from early March to late April.

2.4. Proportion of the Buds that Generated a Cyme

The proportion of apical flower buds that developed a cyme was evaluated in the field on three trees per treatment, for which five main branches per tree at different heights were selected and labeled. On 19th February 2019, while trees were still dormant, we determined the total number of apical flower buds on shoots and spurs. In total, 602 buds were recorded. Out of these, about 65% were located on one-year-old shoots and about 35% on spurs. At full bloom, on 22nd April 2019, we recorded the number of buds with fully developed (BBCH 65) cymes.

2.5. Chill and Heat Quantification and Chill Requirement Estimation

Hourly temperatures were obtained from a weather station placed in the orchard and recorded with a data logger (Tinytag TGP-4500) inside the greenhouse to estimate chill and heat accumulation. We used the R package chillR [50] to compute chill in Chill Portions (CP) according to the Dynamic Model [51–53], since this model appears to be the most accurate available model of chill accumulation during tree dormancy [54,55]. Winter chill was quantified from the 1st of November, the approximate onset of the dormant season, until the day before shoot sampling (Table 1). Once shoots had been transferred into the greenhouse, heat accumulation was quantified according to the Growing Degree Hour model [56]. Following common practice [35], we used the default parameters proposed in the original publications for both the Dynamic Model and the Growing Degree Hour model.

Table 1. Chill accumulation (in Chill Portions (CP)) in the orchard according to the Dynamic Model.

Date of Sampling	Chill Received in the Field (in CP)
7th December 2018	24.9
14th December 2018	29.7
21st December 2018	34.4
28th December 2018	39.4
4th January 2019	44.9
11th January 2019	49.6
18th January 2019	55.5
25th January 2019	56.3
2nd February 2019	63.7
8th February 2019	68.6

The date of dormancy breaking, or the date the chill requirement was fulfilled, was defined as the moment (sampling date) for which most of the buds showed a steady response to heat in the greenhouse and presented at least stage BBCH 51 at the end of the experiment. This was determined by visual assessment of the heat response plots (shown in Section 3.2). We concluded that the chilling requirement was fulfilled when additional chill accumulation did not lead to further changes in the buds' responsiveness to heat (as indicated by the shape of the heat response curves).

2.6. Starch Evaluation on King Flower Primordia

A total of 48 apical buds were sampled on four dates (24th January, 1st and 13th February, and 7th March) from shoots and spurs of both the irrigated and non-irrigated treatments (six buds per treatment per date; three from spurs plus three from one-year-old shoots). These buds were fixed in Carnoy fixative (ethanol: acetic acid, 3:1 v:v) for 24 h and then moved to 75% ethanol and stored at 4 °C.

King flower primordia were separated from the fixed buds and directly stained using a whole-mount clearing and starch staining solution. Herr's 4½ clearing solution makes plant samples transparent [57], allowing detection of starch stained with lugol [58]. This solution is composed of lactic acid, chloral hydrate, phenol, clove oil, xylene, iodine, and potassium iodide (mixed at a weight-by-weight ratio of 2:2:2:2:1:0.1:0.5). The solution was applied for 30–45 min, after which the excess liquid was absorbed with a blotting paper. The stained primordia were photographed under identical light conditions and camera settings under a stereoscopic microscope (Zeiss Discovery, V12 Stereo Microscope, Germany) with a digital camera (The Imaging Source Camera DFK41BU02.H, Germany) linked to image processing software (IC Capture 2.4, Germany). Starch was qualitatively evaluated by comparing the photographs and classifying them according to a three-level color scale: low (Figure 2A), medium (Figure 2B), and high (Figure 2C) starch content.

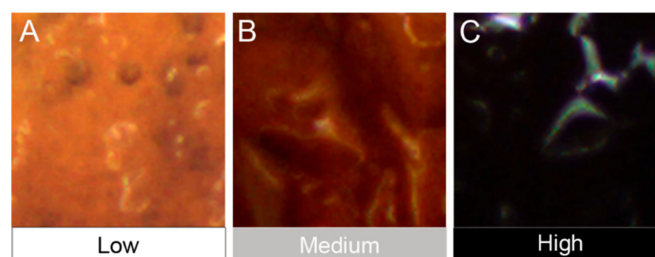


Figure 2. Qualitative starch evaluation according to a three-level color scale. (A): Low starch content (light brown). (B): Medium starch content (brown). (C): High starch content (dark grey-black). Samples were taken from flower primordia of apical buds of apple trees.

2.7. Statistical Analysis

All analyses and figures were developed in the R programming environment (version 3.5.3) [59]. Morphological variables, such as the length of internodes and the diameter, were modeled via one-way analysis of variance (ANOVA). The number of flower primordia observed within the apical buds was analyzed through generalized linear models (GLMs), assuming a Poisson distribution and using treatment, bud type, and the interaction of both factors as predictors.

Variation in starch concentrations was analyzed using the Wilcoxon–Mann–Whitney test [60]. We performed separate analyses for each sampling date to test for the effect of irrigation treatment on the starch concentration. Additionally, we treated the date as a factor to test for differences in the concentration of starch during winter, independent of the irrigation treatment. We compared starch concentrations in the flower primordia between all subsequent pairs of sampling dates. Since this implied testing for multiple null hypotheses (i.e. no differences between observations on subsequent dates), we applied Bonferroni's method [61] to adjust the criteria for identifying statistical significance (*p*-value correction).

Phenological observations under both greenhouse and field conditions were analyzed using ordinal linear regression models (also known as proportional odds models). This approach assumes that the phenological stages (which are ordered from 0 to 69) and the transitions between them depend on either continuous or discrete variables, in this case on chill, heat, and irrigation treatment. A small variation of this approach is the use of nominal effects, which allows the thresholds between stages to vary in response to some predictors. In brief, we modeled the BBCH stages as a function of chill and heat accumulation (and their interaction) and used the irrigation treatment as a nominal effect factor. This enabled us to evaluate whether the irrigation treatment shifted the thresholds between BBCH stages of apple buds. With the original phenology classification (the BBCH scale), the fitting procedure of the cumulative link model (maximum likelihood method) did not converge on a well-defined set of parameters for data collected under greenhouse conditions. Following common procedures to address this computational problem [62], we reclassified the phenology data. We defined six new classes according to BBCH stages: 1 for buds at BBCH stage 0; 2 for buds at BBCH stages 51 and 52; 3 for buds at BBCH stages 53 and 54; 4 for buds at BBCH stages 55 and 56; 5 for buds at BBCH stages 57 and 59; and 6 for buds at BBCH stage 60. Data collected under field conditions did not need reclassification, since the analysis worked with the original classes. We then compared the thresholds between BBCH states in irrigated and non-irrigated treatments under greenhouse and field conditions.

Finally, the probability of success in buds to generate a cyme in the following spring was modelled using GLMs, assuming a binomial distribution of the data and including shoot type and treatment as factors. In the case of the total number of buds (independent of shoot type), we only considered treatment as a factor. To identify differences between groups (i.e., irrigated versus non-irrigated), we contrasted means using Tukey's test [63].

3. Results

3.1. Morphological Observations on Shoots and Microscopic Monitoring of Apical Buds

Shoot growth during the summer period was clearly affected by the irrigation treatment. Although shoots presented similar numbers of buds in both treatments (median of 11 buds per shoot), shoots from the irrigated treatment had longer internodes (median of 2.27 cm) than those from the non-irrigated treatment (median of 1.37 cm) ($p < 0.001$) (Figure 3A). In contrast, the shoot diameter was shorter (but no significant effect was detected using this data set; $p = 0.11$) in the irrigated treatment (median of 4.37 mm) than in the non-irrigated treatment (median of 4.76 mm) (Figure 3B).

Apical buds placed on shoots and spurs presented cymes composed of 3–6 flower primordia, surrounded by leaf primordia and numerous bud scales. No clear effect of irrigation or bud type on the number of flower primordia that composed the cymes was determined in this experiment. The majority of cymes (78–100%) presented 4–5 flower primordia (Figure 3C). It is worth noting that in irrigated trees, buds with six-flower cymes only differentiated at a low rate (6%) (Figure 3C).

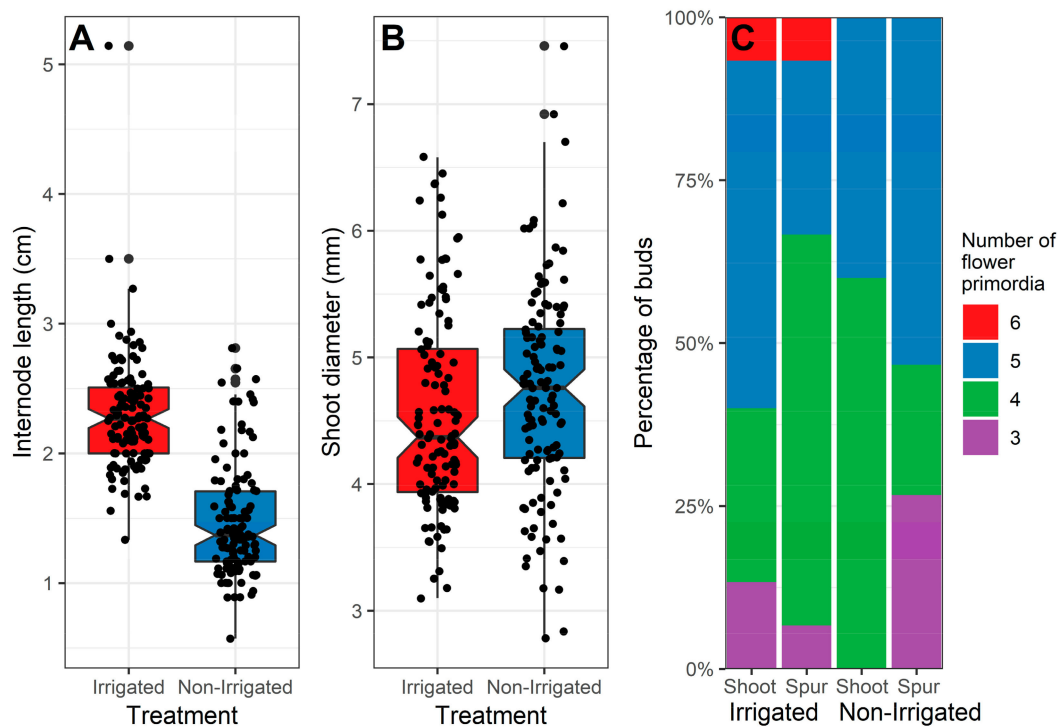


Figure 3. Morphological traits of one-year-old apple shoots cv. ‘Elstar’ sampled during the winter season of 2018/2019 (Table 1). Shoots were sampled from trees grown in Campus Klein-Altendorf, exposed to two water regimes during the growing season of 2018. (A): Internode length. (B): Shoot diameter. In (A) and (B), boxplots represent the distribution of the data, while black dots represent actual observations. In boxplots, the colored box represents 50% of the data (percentile 25%, 50%, and 75%), whereas vertical black lines mean the distance between the percentile (25% or 75%) and the smallest or largest value, respectively. Notches represent an approximation of the 95% confidence interval for comparing medians. In total, 240 shoots were sampled for internode length and shoot diameter measurements. (C): Percentage of buds with a given number of flower primordia per cyme (colors) in one-year-old shoots and spurs considering 60 apical buds in total.

Morphological observations of the king flower primordia before bud burst revealed that flower primordia developed within the dormant buds (phenological stage BBCH 00) showed no major differences between irrigated and non-irrigated trees. The general development of the flower primordia of both treatments can thus be illustrated with only one set of photographs (Figure 4). During winter, the king flower (kf) primordia were centrally placed and significantly larger than primordia of lateral flowers (lf) (Figure 4A). The sepals (se) that enclosed the flower primordia presented abundant trichomes (tr). Pistils and stamens were not differentiated and appeared as rounded domes (rd) in the king flower primordium (Figure 4B). By the end of February and early March, flower primordia had grown significantly and differentiated despite the absence of external phenological changes. Stamens presented short filaments and the anther acquired its characteristic shape with two thecae and four locules. The five styles (st) were also distinguished at this moment (Figure 4C). Once buds showed the first signs of burst, by the end of March or early April (BBCH 51), the anthers (an) acquired a yellowish appearance and the stigmas (ss), composed of numerous papillae, were distinguishable (Figure 4D).

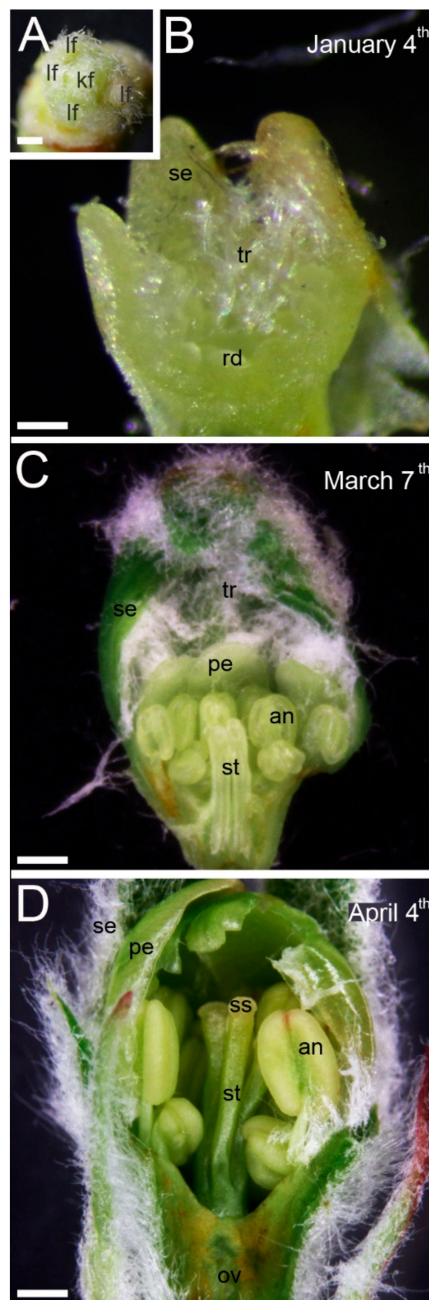


Figure 4. Flower development and growth during dormancy until bud burst. (A): Apple cyme during winter (4th of January 2019); (B): King flower primordia presenting sepals with numerous trichomes and undifferentiated flower verticiles during winter (4th of January 2019); (C): Distinguishable stamens and pistils at the end of winter (7th of March 2019); and (D): Anthers with yellowish appearance and stigmas at the end of the styles (4th of April 2019). Abbreviations: lateral flower (lf), king flower (kf), sepals (se), trichomes (tr), rounded dome (rd), petals (pe), anthers (an), style (st), stigma (ss), and ovary (ov). Scale bars: A, C, and D = 500 μm ; B = 100 μm .

Starch concentrations in the king flower primordia changed over the course of the observation period (Figure 5). These dynamics were comparable across irrigation treatments, with similar starch concentrations in irrigated and non-irrigated trees on all sampling dates (Figure 5). We observed that flower primordia tissue stained lightly for starch at the end of January. Just a week later in early February, strong differences were observed among the flower primordia ($p < 0.05$). In mid-February, an intense dark reaction revealed that the flower primordia had significantly ($p < 0.05$) more starch

compared to the previous sampling date (Figure 5). Subsequently, flower primordia presented first signs of growth in March and the starch content was significantly reduced ($p < 0.001$) in comparison with the concentration observed on 13th February.

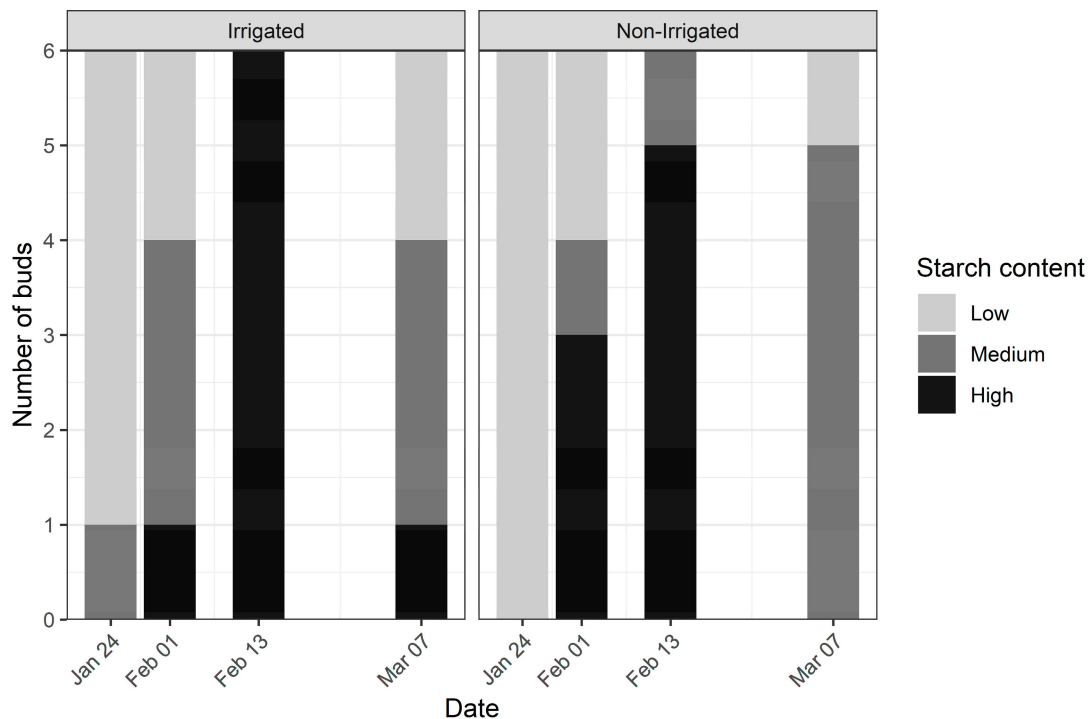


Figure 5. Frequency of buds in a given starch content class (low, medium, or high) according to qualitative observations on flower primordia tissue (see Section 2.6. in Materials and Methods). Samples were taken from shoots and spurs of apple trees cv. ‘Elstar’ grown in Campus Klein-Altendorf exposed to two irrigation regimes (‘irrigated’ and ‘non-irrigated’ panels) during the summer of 2018. For the figure, we used all analyzed buds (48) collected on four dates during the winter of 2019.

3.2. Bud Development on One-Year-Old Shoots under Greenhouse Conditions

Only a small number of lateral buds on sampled shoots were able to reach bud burst or another more advanced phenological stage under greenhouse conditions.

Apical bud development during this experiment was affected by both the irrigation treatment and the chill received in the field (Figure 6). Using the first sampling date (after 24.9 CP had accumulated), the percentage of buds that had reached at least BBCH stage 51 at the end of the experiment was about 25% in shoots from irrigated trees, while about 50% in shoots from non-irrigated trees reached such stages. From 39.4 CP accumulated under field conditions, most of the buds reached at least BBCH stage 51 by the end of the experiment in both irrigated and non-irrigated treatments.

The amount of heat necessary to force bud burst decreased with higher levels of chill accumulated in the field. For samples taken at 24.9 CP, buds required 11,000–15,000 Growing Degree Hours (GDHs) to reach BBCH stages 51 and 52. After accumulating 29.7 CP by the subsequent sampling date, heat requirements for reaching the same BBCH stages had decreased from 6000 to 9400 GDH (Figure 6). For samples taken in Week 9 (63.7 CP), the first bud burst (BBCH stages 51 and 52) was recorded after about 3000 GDH for both irrigated and non-irrigated shoots. Along the same lines, the transition between BBCH states was faster when more chill had been accumulated by the buds under field conditions. For samples taken at 24.9 CP, about 12.5% of the buds remained at BBCH stages 51 and 52 at the end of the experiment (up to 16,000 GDH), while for samples taken at 55.5 CP all the buds had overcome this stage after about 7500 GDH.

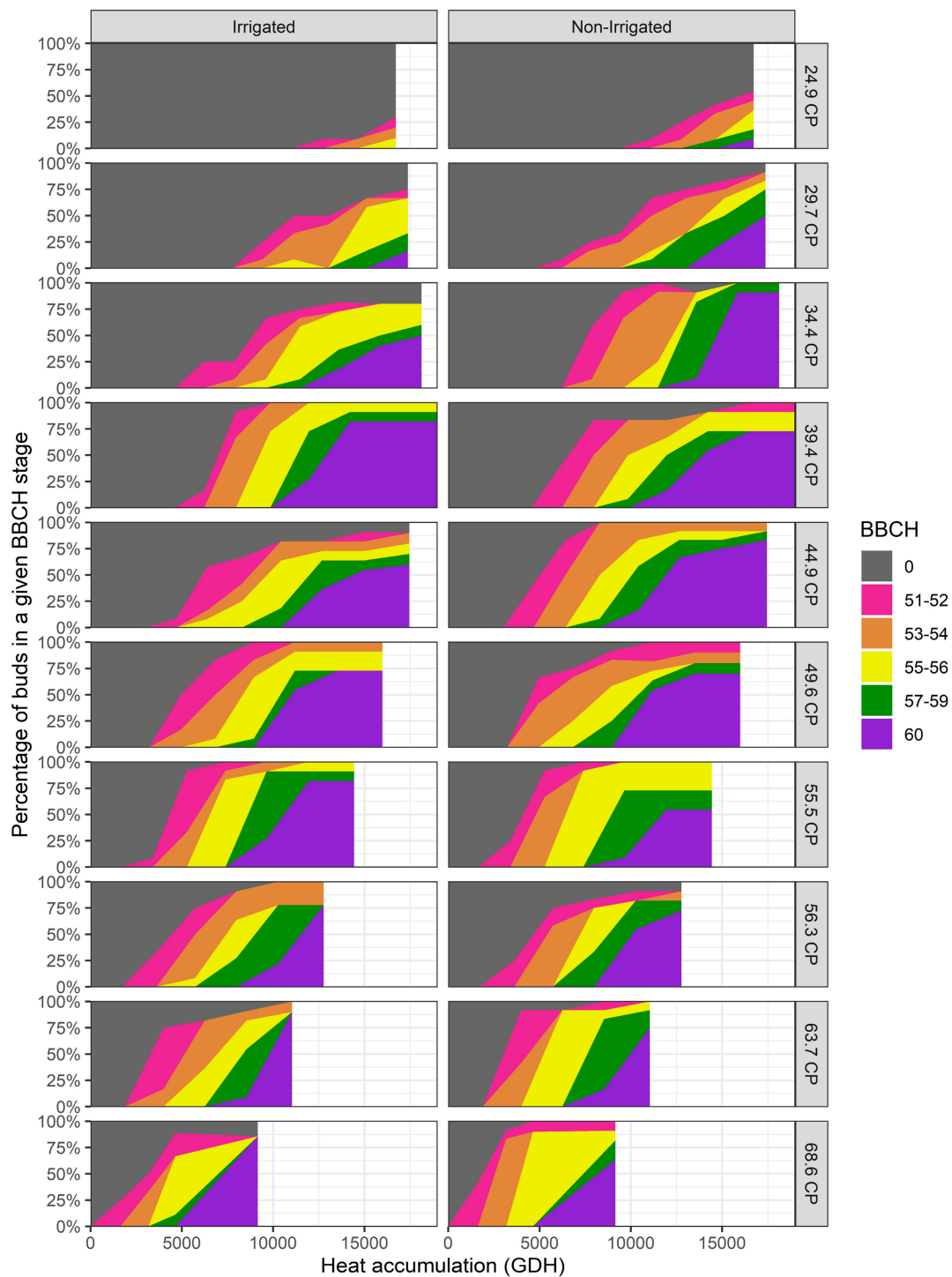


Figure 6. Apical bud development in one-year-old apple shoots (cv. ‘Elstar’) sampled during the winter season of 2018/2019 (Table 1). Shoots sampled from trees grown in Campus Klein-Altendorf were exposed to two water regimes (‘irrigated’ and ‘non-irrigated’ panels) during the growing season of 2018. Each row of panels corresponds to a particular sampling date represented by the chill accumulated in the field (in Chill Portions (CP)). The x-axis represents heat accumulation (in Growing Degree Hours (GDHs)) under greenhouse conditions after the shoots had been sampled. The colors represent the sub-stages for the principal growth stages 5 (inflorescence emergence) and 6 (flowering) of reproductive buds, according to the BBCH scale. We monitored a total of 213 apical reproductive buds (27 apical vegetative buds were excluded).

The cumulative link model indicated both chill and heat, as well as the interaction between them, as highly significant predictors of the BBCH stages of buds under both irrigated and non-irrigated conditions ($p < 0.001$). Regarding the effects of the irrigation treatment on the transition points between BBCH stages, we determined that the estimates for the parameters significantly differed for the two first thresholds. From BBCH 0 to BBCH 51 and 52, the estimate was 0.69 in irrigated buds, whereas in the non-irrigated buds this value decreased to 0.07 ($p < 0.001$). Similarly, the difference between the estimates of the thresholds from BBCH 51 and 52 to BBCH 53 and 54 in irrigated versus non-irrigated buds was 0.59 ($p < 0.001$) (Table 2). From the third transition point (e.g., BBCH 53 and 54) onwards, the estimates tended to show similar values among treatments, with only the fourth transition point indicated as significantly different between treatments.

Table 2. Differences between estimated thresholds for transition points between non-irrigated and irrigated buds through cumulative link modelling. Data in the confidence interval column reflects results on significance from Wald's test (z-value). '***' indicates $p < 0.05$, while 'n.s.' means that treatments were not significantly different at this confidence level.

Transition Point (from Stage x to Stage y)	Difference in Threshold Estimates (Non-Irrigated – Irrigated)	Confidence Interval for the Difference (95%)
BBCH 0 to BBCH 51-52	−0.62	−0.90 to −0.34 ***
BBCH 51-52 to BBCH 53-54	−0.59	−0.88 to −0.30 ***
BBCH 53-54 to BBCH 55-56	−0.27	−0.57 to −0.05 n.s.
BBCH 55-56 to BBCH 57-59	−0.52	−0.91 to −0.13 ***
BBCH 57-59 to BBCH 60	−0.09	−0.61 to −0.42 n.s.

3.3. Phenology Progression and Flowering Rates under Field Conditions

Based on the previous results, we concluded that endo-dormancy was overcome around the 11th of January in both treatments, after 50 CP had accumulated (Figure 6). Under field conditions, buds from both the irrigated and non-irrigated treatments showed similar phenological patterns at bloom time (Figure 7). The first changes in flower bud phenology were observed on the 7th of March, after 1876 GDHs had accumulated in both treatments. Only minor differences were observed on subsequent dates. On the 11th of April, after 4500 GDHs had accumulated, about 85% of the buds in the non-irrigated and 70% in the irrigated treatment presented some open flowers (BBCH 60 or higher). Full bloom (BBCH 65) occurred in a small proportion of the buds simultaneously in both orchards on the 18th of April, after at least 5000 GDHs had accumulated.

According to the ordinal linear regression analysis, only heat and the interaction between chill and heat significantly ($p < 0.05$) affected the phenology of both irrigated and non-irrigated buds. After chill requirements had been met (which appeared to be the case for chill accumulation greater than 50 CP), additional chill (without heat) was not found to produce significant differences ($p = 0.25$). Regarding the effect of irrigation on the transition points, we detected that under field conditions, most of the transition thresholds did not significantly differ between treatments. The difference between only 2 out of 12 transition points were found statistically significant under field conditions at a p level of 0.05. These were from BBCH 52 to BBCH 53 ($p = 0.014$) and from BBCH 53 to BBCH 54 ($p = 0.039$), for which non-irrigated buds showed lower thresholds for the transition points compared to irrigated buds.

The probability of a bud generating a cyme was higher on buds that developed on shoots compared to buds from spurs in both irrigated ($p < 0.001$) and non-irrigated ($p = 0.07$) treatments. Furthermore, buds from non-irrigated trees presented a higher probability of bloom than those from irrigated trees (Figure 8). This impression was maintained when the type of bud was also considered, with buds from non-irrigated shoots presenting a higher chance of flowering than buds from irrigated shoots ($p = 0.02$). The latter was also observed on buds located on spurs ($p < 0.001$).

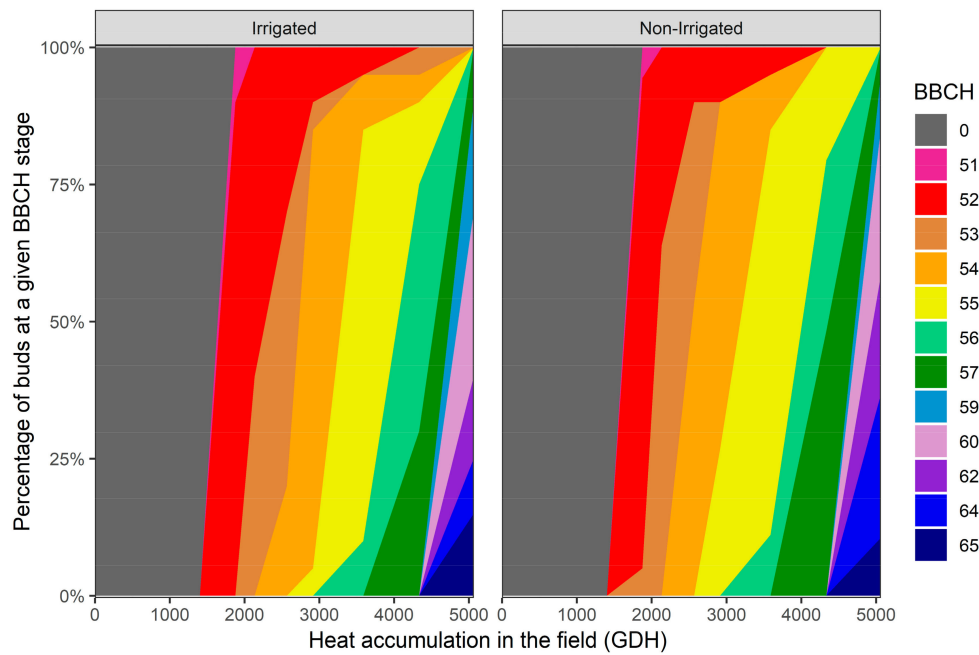


Figure 7. Phenology progression of the apical buds in apple trees cv. ‘Elstar’ grown in Campus Klein-Altendorf exposed to two irrigation regimes (‘irrigated’ and ‘non-irrigated’ panels) during the growing season of 2018. The x-axis represents the accumulation of heat (in Growing Degree Hours (GDHs)) under field conditions after a minimum chill requirement of 50 CP had been fulfilled. The colors represent the sub-stages for the principal growth stages 5 (inflorescence emergence) and 6 (flowering) of reproductive buds, according to the BBCH scale. We monitored 208 apical buds in total.

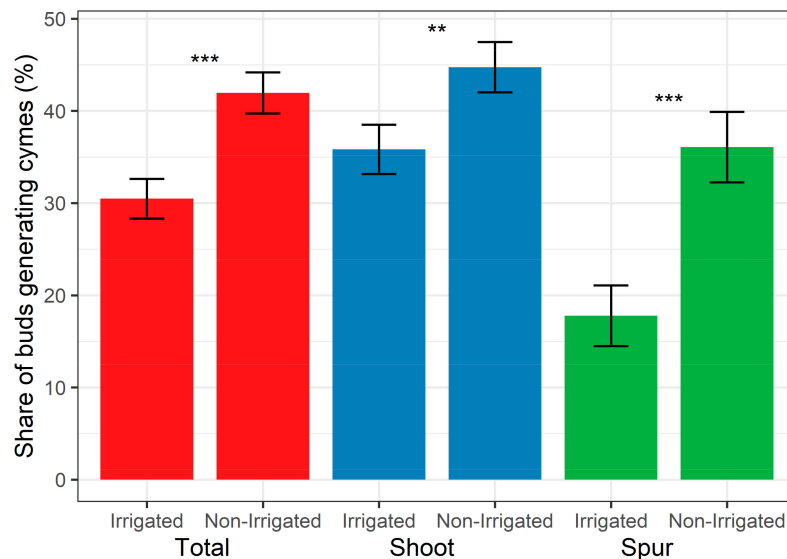


Figure 8. Share of apical flower buds generating cymes for apple trees cv. ‘Elstar’ exposed to two irrigation regimes. Measurements were taken on the 22nd of April 2019 in Campus Klein-Altendorf. Bar colors indicate the structure on which the bud developed (one-year-old shoots versus spurs), with red bars illustrating results for all sampled buds. Error bars in each category show the standard error of the mean. In total, 602 buds were counted, out of which 212 (35%) were from spurs and 390 (65%) were from one-year-old shoots. *** represents $p < 0.001$ and ** means $p < 0.05$.

4. Discussion

We evaluated the impacts of two irrigation regimes on apple trees grown under the unusual moisture conditions in the summer of 2018 at the experimental station at Campus Klein-Altendorf. Although we made use of what may be considered a natural experiment, we were able to document that the additional water supplied during summer increased vegetative growth (i.e. shoot length) during the growing season. Shoots on irrigated trees were significantly longer (by 39%) and thinner compared with trees grown without irrigation. However, this did not affect the apical buds at the top of the shoots, which developed a similar number (4–5) of flower primordia per cyme. Observations during the winter season showed that the flower primordia and the starch dynamics in bud tissue followed a similar pattern in both treatments. Our CR estimations resulted in about 50 CP for both non-irrigated and irrigated trees. The use of one-year-old shoots exposed to forcing conditions revealed that, after low exposure to chill (i.e., 24.9–34.4 CP), buds from non-irrigated trees developed significantly faster and at higher rates compared to buds from irrigated trees. However, these differences became less pronounced after longer exposure to chilling conditions. Under field conditions, differences in phenological development between treatments disappeared, but buds on non-irrigated trees presented a significantly higher probability of flowering (0.42) than buds on irrigated trees (0.30) during the following spring.

The difference we observed in total shoot length was explained by the length of the internodes rather than a different number of lateral buds. On average, additional irrigation of 133 mm during July and August of 2018 effected an increase of 39.6% in the internode length of one-year-old shoots. Similar results on shoot length have previously been observed on one-year-old M9 apple rootstocks exposed to a water deficit of 50% of tree water needs [10]. Chen et al. [16] reported that water stress during the summer promoted the cessation of vegetative growth, probably due to an increase in the production of ABA in the roots [17]. Moreover, the drought stress responses of other hormones regulating growth, development, and senescence (i.e. auxins and ethylene) have also been studied (reviewed in [64]). Changes in the concentration of these hormones during the growing season may explain why shoots on irrigated trees were longer than shoots on non-irrigated trees.

Meristems inside the apical buds differentiated into cymes composed of a similar number of flower primordia (4–5), with the king flower presenting a similar state of development during winter in both treatments. This suggests that these traits were not greatly affected by this level of water stress. In agreement with previous studies on apple flower differentiation [65], the king flower did not present differentiated flower verticilles, but remained in an earlier development stage (i.e. round domes). This early flower developmental stage remained during endo- and beginning of early eco-dormancy. This observation indicates a less mature stage of development during dormancy in apples compared to *Prunus* spp., where flower verticilles are already distinguishable (sepals, petals, anthers, and pistil) during winter [66,67]. The king flower started to accumulate starch at this early developmental stage in late January, showing peak starch reserves in mid-February. Later, the starch content was reduced and the king flower presented the first signs of growth. Starch dynamics have been reported in the flower primordia of sweet cherry during winter as a source of energy for subsequent flower development [68,69]. In apple primordia, maximum starch content occurred when about 63.7 CP–68.6 CP had accumulated, which was after fulfillment of the estimated chill requirement (50 CP), but corresponded with a rapid growth response under forcing conditions. In sweet cherry, maximum starch accumulation has been shown to occur earlier in winter and to be closely associated with fulfillment of the chill requirement [68].

Compared to irrigated trees, apical buds from non-irrigated shoots showed a tendency towards lower thresholds for transition points between BBCH stages, indicating that they developed faster than buds from irrigated trees. This tendency was clearly observed under forcing conditions for low-chill sampling dates (i.e. 24.9–34.4 CP). Nonetheless, this difference was not observed for shoots that had been exposed to greater chill levels in the field, with shoots from both treatments showing the same bud burst pattern and similar thresholds between BBCH stages. This may suggest that water stress during

summer may affect the CR, especially in trees grown in warm-winter locations. Under field conditions, buds from the non-irrigated treatment showed a significantly higher probability of flowering (0.42) in spring than buds from the irrigated block (0.30). An increase in flowering rates during the following season, after experiencing water deficit, has also been observed in other temperate fruit trees, such as pear and peach [6]. In young pomelo trees (*Citrus grandis*), the number of inflorescences, flower buds, and flowers that opened increased as the duration of water stress was extended [70]. Our initial hypothesis to explain these differences hinged on disparities in the starch content of the flower primordia. In fact, one could expect less starch to accumulate in the irrigated treatment, as more energy might have been spent on shoot growth rather than storage during the previous growing season. Alternatively, less starch may have accumulated in the non-irrigated treatment because of a reduction in the photosynthetic rate due to water stress [14]. However, in light of our results, this expectation was not confirmed, with both treatments showing a similar pattern regarding starch levels in the flower primordia during winter. Thus, differences in phenological development, as well as in flowering probability, may rely on changes in hormonal regulation [71], different levels of reactive oxygen species (ROS) produced during the growing season [72], or other biochemical changes inside the buds during bud initiation in summer or bud dormancy in winter. Further experimentation is still needed to clarify these findings.

Based on the results obtained from shoots exposed to forcing conditions (Figure 6), we estimated that this variety overcame dormancy after 50 CP had accumulated in the field. Similar estimates have been reported for apples cv. 'Golden Delicious' (50 CP [73]) and cv. 'Starkrimson Delicious' (about 65 CP [74], using conversion factors for Chilling Hours to CP in North Carolina according to Luedeling and Brown [75]). However, the heat units accumulated until the first signs of bud burst differed between sampled shoots and buds on trees grown under field conditions. Buds from cuttings required about 5000 GDHs, whereas buds from trees in the field required 2000 GDHs. This difference can be explained by trees in the field accumulating more chill than 50 CP, which may have compensated for the lower heat accumulation outside the greenhouse. This dynamic relationship between chilling and heating phases during dormancy has been previously proposed by Harrington et al. [27]. Indeed, shoots sampled at 63.7 CP only required about 3500 GDHs to reach bud burst. This may also suggest that our CR estimation corresponds to the minimum amount of chill needed to reach a homogeneous bud burst pattern after a given amount of heat and might not be valid for comparison with observations under field conditions, where trees can receive additional chill. Along the same lines, while winter chill is expected to decrease in many growing regions of the world due to rising winter temperatures [76], heat accumulation during late winter and early spring is expected to increase. In this regard, an overlapping relationship between chill and heat phases may suggest that different chill and heat accumulation patterns may result in similar bloom dates. However, further experimentation on this relationship across a wide range of climates, as well as on the impact of water stress on CR at low chill levels, could greatly improve our knowledge on the dormancy of temperate fruit trees.

5. Conclusions

Mild water stress during the growing season impacts on the morphology of shoots in the same growth period and may possibly affect the phenology and bloom probability of apical buds in the following spring. Irrigated trees presented more vegetative growth compared to those that did not receive additional water. The implications of this phenomenon for orchard management are currently unclear. Based on morphological observations, we suggest that this level of water stress did not affect bud initiation, bud differentiation, or storage processes. Regarding phenological development, we observed that, after low chill exposure, apical buds from non-irrigated shoots developed faster and at higher rates than those from irrigated shoots. This suggests that summer drought may affect the chill requirement of apple buds. However, further validation and experimentation on the mechanisms behind this finding are needed. Similarly, further experimentation is required to validate our findings on flowering probability, as well as to test the effect of summer drought on subsequent phases, such as

fruit set and fruit development. Climate change is widely expected to affect the cultivation of temperate deciduous fruit trees by increasing winter temperatures as well as modifying total precipitation and rainfall distribution. We hope that our findings will contribute to understanding the medium-term (i.e., subsequent seasons) effects of summer drought on apple cultivation, as well as provide useful guidance for future research on this topic.

Author Contributions: E.F. (Erica Fadón) and E.F. (Eduardo Fernandez) designed the experiment with the assistance of E.L. and A.K., D.B. and S.V.d.V. established the experiment and recorded the data with the guidance of A.K., E.F. (Erica Fadón), and E.F. (Eduardo Fernandez). All the authors contributed to analyzing and discussing the results. E.F. (Eduardo Fernandez), E.F. (Erica Fadón), and E.L. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the project “Phenological and Social Impacts of Temperature Increase—climatic consequences for fruit production in Tunisia, Chile, and Germany” (PASIT; grant number 031B0467B of the German Federal Ministry of Education and Research).

Acknowledgments: The authors would like to thank Campus Klein-Altendorf for providing assistance during the experiment and Katja Schiffers for her support in the statistical analysis.

Conflicts of Interest: This research has been conducted in the absence of any relationship that can construct a possible conflict of interest.

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