Introduction

As the climate changes, the viticulture industry will need to adapt. Climate change is predicted to raise temperatures 1-3°C in winegrowing regions across the world, which could drive major changes in the viticulture industry. Research suggests the industry will shift growing areas towards the poles and to higher elevations to maintain ideal growing temperatures for winegrapes {Schultz, 2010 #33}{Hannah, 2013 #10}. In the Southern Hemisphere, where there is less landmass closer to the poles, climate change could lead to a loss in total viticultural land. There is also concern that vineyards could move to land that is currently conserved {Hannah, 2013 #10}.

Alternatively, vineyards could take advantage of the great genetic variety that already exists by planting varieties better suited to the new climate {Wolkovich, 2017 #32}. *Vitis vinifera* subsp*. vinifera* (winegrape) has at least 6000 genetically distinct varieties grown for many purposes, but only 1100 are grown for the viticulture industry, and an even smaller number dominate the global market {Lacombe, 2012 #45}. However, for this adaptation to be effective, the differences in phenology among these varieties must be better understood, so that the varieties could be matched with compatible climates.

Studying the phenology of different varieties of winegrapes would help viticulturists better adapt to climate change, because winegrape phenology is extremely sensitive to temperature {Jones, 2013 #36}{Chuine, 2017 #41}. Timing for leafout and flowering of diverse plant species have advanced six to 20 days in the last 30-40 years of warming {Root, 2003 #44}{Menzel, 2006 #42}, equivalent to four to six days per degree Celsius. A similar advance is seen for winegrape harvest dates, which can change about 6 days per degree Celsius {Benjamin, 2016 #31}. In winegrapes, phenological timing varies across varieties, and it is this variation that could be used to better adapt to future climates. Generally, timing of phenology can vary from three to six weeks across varieties {Wolkovich, 2017 #32}{Boursiuot, 1995 #62}. However, most varieties have very little phenological data from which to infer where they could best be grown. Harvest dates are the only data available for over 90% of varieties, so for phenological data to be used for adaptation, more varieties need to be studied {Parker, 2013 #46;Parker, 2011 #47}. Expanding the amount of data on flowering can also help improve models, which seek to understand how winegrapes will be affected by climate change and how the industry could change in order to endure.

Because successful flowers become berries, understanding how climate change will affect winegrape flowering is an important aspect of the overall effect on phenology and directly relates to harvest yields. Petrie and Clingeleffer (2004) found that Chardonnay buds exposed to elevated temperatures just before or just after budburst produced 24.2-32.6 less flowers per °C warming. Other research has found that Semillon winegrapes exposed to four days of elevated temperatures (40 °C during the day and 25 °C at night) during flowering aborted all flowers {Greer, 2010 #34}. Studies of vegetative growth and photosynthesis in other perennial crops exposed to a range of temperatures exhibited that extreme temperatures tend to slow or inhibit certain processes in the plants {Zaka, 2017 #61;Zaka, 2016 #60}. It is possible that flowering will follow a similar bell-shaped response curve.

Here we studied the phenology of 50 varieties in the field and lab, and examined the flowering response of mixed varieties across a wide range of temperatures in growth chambers. We were particularly interested in the effect of heat stress on phenological timing. This information could be used to broaden the understanding of phenology for little-studied varieties of winegrapes.

Methods

Dormant winegrape cuttings were taken from the UC Davis Robert Mondavi Institute vineyard in December 2015, where phenology was monitored in the 2015 growing season. Observations using the modified Eichorn-Lorenz (EL) scale {Coombe, 1995 #37}began 6 March 2015 and continued generally every 3-4 days until 2 April 2015, when almost all plants had reached EL stage 11 or higher.

Following collection, cuttings were chilled for 21 days (4° C, 21 d) at the Arnold Arboretum, then forced in greenhouses in 26 cm diameter pots in January 2016. After several months of growth, on 27 May they were placed in growth chambers with day/night temperatures of 6/4 °C and an 8-hour photoperiod to induce dormancy, though the plants did not appear visibly dormant until 20 June.

On 15 August 2016, the 351 potted cuttings were moved out of the chambers and into a greenhouse where the initial day temperature was 18.5 ± 1.5 °C and night temperature was 16.75 ± 1.25 °C. After the first week, the temperatures were slowly raised to 25.5 ± 2.5 °C during the day and lowered to 10 °C at night. The cuttings were pruned the day they were removed from dormancy so that each cutting had two spurs and each spur had two nodes. Then, the diameter of each spur and node and the distance between the two nodes on each spur were measured with calipers.

Twice a week, beginning 22 August, each plant’s development was recorded using the modified Eichorn-Lorenz scale {Coombe, 1995 #64} and soil moisture was measured with a probe in three locations in each pot. Each spur was kept at two shoots, but only the dominant shoot on each spur had observations recorded. Each shoot was trained up a stake for support. When an inflorescence had developed (EL stage 12), the plant was randomly assigned to one of five growth chambers if it was a part of the heat tolerance experiment. Otherwise, observations on it continued in the greenhouse. Varieties were chosen for inclusion in the experiment to include a diversity of phenology from those varieties for which there were five or more replicates growing.

The five chambers all had a 12-hour photoperiod with 800 m-2s-1 of fluorescent light, but varied in their temperature: Chamber 1 was set at 17/23 °C, Chamber 2 was set at 23/29 °C, Chamber 3 was set at 27/33 °C, Chamber 4 was set at 31/37 °C, and Chamber 5 was set at 34/40 °C. Initially, CO2 levels were set at 400 ppm during the day and 600 ppm at night, because plants respire at night, increasing CO2 levels. Each inflorescence was contained in a paper bag to collect the flower caps as they fell.

Observations on the percent of buds flowering, leaf number, stem length, and number of fallen flower caps were made three times a week, along with soil moisture. On 19 September, it was noted that some inflorescence bags also contained aborted buds that had yet to flower, and thereafter observations of aborted buds were also recorded. Once a plant had reached 100% flowering, or, in the case of plants where the entire inflorescence had died and fallen off, the plant had spent a minimum 14 days in the chamber, it was returned to the greenhouse.

All analyses performed in R version 3.3.3 (R Core Team).

Results

The plants underwent budbreak (EL 4) between 17 August and 6 September (mean = 29 August) and leafout (EL 7) between 22 August and 22 September (mean = 4 September). All plants had at least one bud that burst, but two plants never leafed out. The first inflorescence formed on 5 September, and 51 plants eventually reached this stage (EL 12), though only 26 were of the varieties pre-selected for the experiment.

Budbreak and leafout timing among the varieties were similar in the lab and field (Figure #, budburst: F(1,47)=14.55, p=0.0004; leafout: F(1,47)=18.51, p<0.0001). Few plants developed inflorescences (see Table #). Plants that had thicker spurs were more likely to develop inflorescence (Figure in supplement, Z(##)=XX, p=XX), and more likely to reach 50% flowering (Figure in supplement, Z(##)=XX, p=XX).

Soil moisture in the chambers varied by chamber temperature (F(1,24)=8.05, p=0.009), ranging from 69% to 76% over time. There was no directional relationship between the moisture levels and the chamber temperature (i.e., the warmest chambers were not the driest) and means were similar across treatments, ranging from 71% to 74%.

There was also no directional relationship between chamber temperature and either change in stem length or change in leaf number (stem length: F(1,24)=0.5347, p=0.4717; leaf number: F(1,24)=0.0455, p= 0.8329). Plants in Chamber 3 had the greatest change in stem length during their time in the chamber (FIGURE #). Similarly, plants in Chamber 2 had the greatest change in leaf number during the experiment (FIGURE #).

Contrary to expectations, chamber temperature did not affect the days it took for the plants to reach 10% and 50% flowering and there was no trend in the duration of flowering (10%: F(1,20)=0.4324, p=0.5183; 50%: F(1,15)=0.4987, p=0.4909). Within treatments, the number of days it took plants to reach 10% flowering ranged from 34 to 51 days (mean = 42.6 ± 0.9).

The number of buds aborted per plant was significantly affected by the chamber temperature (Figure #, F(1,24)=7.4285, p=0.01179). The two warmest chambers saw the greatest number of buds lost during the time in the chamber, with the greatest average number of buds aborted seen in Chamber 5 (mean number of buds aborted Chamber 1: 4.5, Chamber 2: 2.8, Chamber 3: 5.8, Chamber 4: 27.6, Chamber 5: 57.3). .

Discussion

Overall, we studied the effects of temperatures between 20 and 37 °C on flowering for 26 winegrape plants. We found no directional relationship between temperature and soil moisture, stem length, leaf number, or the number of days it took to reach 10% or 50% flowering. Contrary to expectations of most phenological models {C. Cuccia, 2014 #65}{Garcia de Cortazar-Atauri, 2010 #48} we found that phenology was not delayed in either the coldest or warmest chambers. However, plants in the hotter treatments aborted a higher number of flower buds than those in the cooler treatments.

While phenological timing was not affected, the plants in the two warmest chambers showed signs of stress, because plants in those chambers aborted a significantly higher number of flower buds. Thus, it appeared that the plants sacrificed their reproduction for the growing season to ensure they were able to survive the elevated temperatures. Semillon grapes subjected to day/night temperatures of 40/25 °C for four days at flowering saw similar effects. Inflorescences grew much less—gaining only 22 mm in length compared to the 85 – 90 mm of growth seen in plants treated with heat after flowering—and subsequently aborted all flowers {Greer, 2010 #34}.

The majority of literature on winegrape heat tolerance focuses on the effects of heat on berry ripening (TABLE #). In their aforementioned 2010 study of Semillon winegrapes, Greer and Weston noted that plants treated with elevated temperatures at fruit set and veraison were much less vulnerable and suffered few ill-effects when compared with those treated at flowering and mid-ripening {Greer, 2010 #34}. This could mean that winegrapes are more vulnerable to high temperatures during flowering than they are later in development. If winegrapes are especially susceptible to heat during flowering, viticulturists could take extra precautions during this period to ensure the survival of the flowers through to fruit set.

Because the majority of the plants’ development was stalled before the flowering stage (EL stage 11), the sample sizes in the chambers were small (each chamber had four to six plants). This meant there were not enough plants of each variety in each chamber to test for a difference in varietal response to the heat treatments. In fact, most varieties were only represented in a single treatment. Still, it is important to note that we studied nine different varieties in the chambers, which greatly increased the genetic diversity of the experiment. It has been shown that controlled ecological experiments in labs that include greater genetic diversity are more easily replicated {Milcu, 2018 #63}. The increased diversity helps to prevent unaccounted for environmental factors unique to each lab from preventing the replication of results, allowing conclusions to be tested and verified.

The rate of development seen in the plants grown in the greenhouse was significantly correlated with that seen in the winegrapes grown in the Robert Mondavi Institute Vineyard, from which the cuttings in this experiment were taken (FIGURE #). This suggests that the overall progression and timing of phenological development was not negatively affected or altered by the lab setting, and it can be used in models along with field data to better predict winegrape response to climate change.

While heat treatments during flowering did not affect the phenology of the grapes we studied, we still saw a significant impact from the elevated temperatures that could become a harsh reality for viticulturists around the world. Fewer flowers means reduced yields for wine grape producers. These findings also underscore the importance of modeling more than the plants’ phenology to fully understand the impact climate change will have on the viticulture industry. Future research should strive to include a greater diversity of *Vitis vinifera* varieties so that the results can be used by a larger number of researchers and wine growers to plan for future climates around the world.