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SIMULACIÓN DE EVENTOS CLIMÁTICOS ATÍPICOS (OLAS DE CALOR Y LLUVIA) EN ARÁNDANOS DE ARBUSTO ALTO (*Vaccinium corymbosum* L.), Y SU IMPACTO EN LA FIRMEZA DE FRUTO EN COSECHA Y POSTCOSECHA.

SIMULATION OF ATYPICAL CLIMATE EVENTS (HEAT WAVES AND RAIN) IN TALL-BUSH BLUEBERRIES (Vaccinium corymbosum L.), AND ITS IMPACT ON FRUIT FIRMNESS AT HARVEST AND POST-HARVEST.

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Contenido

CAPITULO I

1. INTRODUCCION

En casi 20 años, la superficie mundial cultivada con arándanos ha aumentado considerablemente, alcanzando para el año 2018 109.270 ha (FAO, 2020). Chile es el segundo país con mayor superficie cultivada, seguido por Estados Unidos (Brazelton & Young, 2017), el país alcanzó las 15.600 ha de *Vaccinium corymbosum* L. para el año 2019 (Moya-Elizondo *et al*., 2019), de las cuales el 71% se concentran en las regiones de Maule, Ñuble y Biobío. La cantidad de fruta exportada por Chile para la temporada 2021-22 fue de 107.142 toneladas, siendo superado por Perú, quien se posiciona como el principal exportador de la región, con 213.208 toneladas (iQonsulting, 2022).

Durante el periodo reproductivo de las plantas, la evolución del crecimiento y desarrollo de los frutos tienen un comportamiento doble sigmoidea (Ismail and Kender, 1974; Godoy et al., 2008), con tres etapas: i) I: caracterizada por un crecimiento por división celular; ii) II: desarrollo de la semilla; y iii) III: con el mayor incremento en volumen y la madurez (pigmentación, aumentos en sólidos solubles, disminución de acidez titulable, disminución de la firmeza, entre otros (Edwards *et al*., 1972;Ribera *et al*., 2006; Sun *et al*., 2018;).

Los frutos de esta especie presentan una asincronía en la maduración dentro del racimo, entre racimos de la misma planta, y entre plantas, lo que obliga a realizar múltiples colectas (Moggia et al., 2022). Producto del índice de cosecha que utiliza la industria (100% azul) y dependiendo del intervalo de cosecha, frutos con el mismo color de cubrimiento presentan importantes diferencias de madurez fisiológica; mientras mayor intervalo mayor proporción de frutos sobremaduros. Estas características se traducen en una alta variabilidad en la firmeza a cosecha, la que es muy superior a los reportados en otras especies frutales (Lobos et al., 2018).

El ablandamiento durante el almacenaje, es la principal limitante para envíos que puede llegar hasta los 60 d en barco (Giongo *et al*., 2013; Vicente *et al.*, 2007). Lo anterior, frente al evidente escenario de cambio climático, donde las temperaturas máximas se incrementan por temporada y la ocurrencia de lluvias durante el verano dejó de ser algo esporádico, el impacto sobre la perdida de firmeza durante el almacenaje puede ser crítico.

Por ende, dada la asincronía en la maduración, cualquier anomalía medioambiental impactará a frutos en diferentes estados de desarrollo. Por ello, surge la necesidad de estudiar el posible impacto que eventos climáticos adversos (lluvia y olas de calor), tendrían sobre las características fisicoquímicas de los frutos a cosecha y en postcosecha, con énfasis en la firmeza. Así, se hipotetiza que, en la simulación de eventos climáticos atípicos, el efecto combinado de lluvia y calor debiera impactar en mayor medida la deshidratación de los frutos en guarda.

Objetivo

- En dos cohortes cercanas a cosecha, determinar el efecto de la ocurrencia de eventos climáticos atípicos (lluvia, olas de calor, y su combinación) sobre los parámetros fisicoquímicos de frutos de arándano de arbusto alto (*Vaccinium corymbosum* L.) cvs. 'Duke' y 'Brigitta', en cosecha (0 d) y postcosecha (45 y 60 d).

Objetivos específicos

1. De acuerdo a la posición de la fruta en la planta, determinar el momento de mayor impacto en firmeza, la proporción de fruta blanda (< 140 g mm-1) y el peso de fruto, en cosecha como durante la postcosecha.
2. Determinar el momento de la postcosecha en el que el efecto de eventos atípicos en dos cohortes durante la temporada, será más detrimental.
3. Determinar la etapa de desarrollo de los frutos en la que el impacto de eventos atípicos en dos cohortes (C1 y C2) será más detrimental a postcosecha.

2. REVISION BIBLIOGRAFICA

1) Distribución e importancia

Los arándanos han aumentado significativamente su área cultivada en las últimas décadas en todo el mundo, alcanzando para el año 2018 109.270 ha (FAO, 2020). Chile es el segundo país con mayor superficie cultivada después de Estados Unidos (Brazelton & Young, 2017), el país alcanzó las 15.600 ha de *Vaccinium corymbosum* L. para el año 2019 (Moya-Elizondo *et al*., 2019), de las cuales el 71% se concentran en las regiones de Maule, Ñuble y Biobío. La exportación de Chile para la temporada 2021-22 fue de 107.142 toneladas, 9% inferior respecto al periodo anterior. El principal exportador de la región, Perú, en la temporada 2021/22, alcanzó 213.208 toneladas de exportación en fresco (iQonsulting, 2022).

Este aumento de superficie está dado por la creciente demanda impulsada, en gran medida, a las propiedades benéficas para la salud (Koca & Karadeniz, 2009; Yashin *et al*., 2010), repercutiendo en un boom de las plantaciones, especialmente durante las últimas décadas.

1.1 Asincronía del crecimiento y madurez del fruto

La floración del arándano dura, en promedio, cerca de un mes, por lo que tanto la cuaja como el crecimiento/madurez del fruto se desarrollan de manera asincrónica. Así, es común observar simultáneamente flores, frutos recién cuajados, y otros en los diferentes estados de desarrollo y madurez. Lo anterior implica que múltiples cosechas son requeridas por temporada.

Los frutos presentan una curva de crecimiento doble sigmoidea, con tres etapas: i) I: caracterizada por un crecimiento por división celular; ii) II: desarrollo de la semilla; y iii) III: con el mayor incremento en volumen y la madurez (pigmentación, aumentos en sólidos solubles totales (TSS), disminución de acidez titulable (TA), incremento de TSS/TA, disminución de la firmeza, entre otros) (Edwards *et al*., 1972; Ribera *et al*., 2006; Sun *et al*., 2018).

Sin embargo, la coloración, que es el principal índice de cosecha, es muy difícil de percibir debido a que los racimos son muy compactos, dificultándose su completa apreciación (Station, 1974) y por la presencia de la cera característica de los frutos, que genera visualmente un tono más oscuro del que realmente posee. También, la exposición al sol y la ubicación de los frutos en la planta pueden distorsionar su percepción del color. Considerando además que los frutos expuestos avanzarían rápidamente en madurez comparado a bayas a la sombra, su ubicación dentro de la planta repercutirá en el avance del desarrollo y maduración (Kliewer & Lider, 1968; Lobos *et al.*, 2018); de acuerdo con la orientación del huerto, en las primeras cosechas, la proporción de fruta madura a cada lado de la planta también puede ser diferente (Lobos et al., 2018).

2.3 Postcosecha del arándano

Los frutos son altamente perecibles y susceptibles a un rápido deterioro (Chen *et al*., 2015), lo que hace aún más compleja la postcosecha. Como regla general, una vez que la fruta se desprende de la planta, la calidad puede mantenerse, pero nunca mejorar (Retamales & Hancock, 2012). Los frutos frescos tienen una durabilidad de una a ocho semanas, este periodo es dependiente de factores como la etapa de maduración del fruto en que este se cosecha (maduro *vs*. sobre maduro), el intervalo de cosecha (3 *vs*. 9 d), la temperatura del fruto a la hora de cosecha, y condiciones de almacenaje (Duan *et al.*, 2011; Matiacevich *et al*., 2013), entre otros. Desde este punto, la máxima vida postcosecha se obtiene con un rápido enfriamiento y almacenaje a temperaturas de 0°C y humedad relativa (RH) de 90-95%, disminuyendo así la respiración al máximo posible (Falagán *et al.*, 2020).

La fruta exportada por Chile puede tomar hasta 6 semanas a los destinos más lejanos como Asia. Dentro de los atributos de calidad de la fruta, la firmeza es quizás la más determinante en el mercado de destino (Chiabrando *et al*., 2009). El ablandamiento de frutos de arándanos está asociado a la disminución de las pectinas solubles en agua, y al desmontaje de la pared celular primaria, en donde la despolimerización de hemicelulosa y la pérdida de arabinosa, son las principales modificaciones sufridas (Giongo *et al.,* 2013).

Además de la firmeza, los arándanos se caracterizan por la presencia de cera cuticular, también conocida como "bloom", que es altamente deseable por parte del consumidor. La ausencia de bloom le da al fruto un aspecto de sobre maduro y una tonalidad más cercana a un color negro que al azul claro característico (Chu *et al.*, 2017). Fisiológicamente, la cera cuticular provee de barreras al fruto, permite controlar la transpiración o pérdida de agua, ya sea cuando este se encuentra en la planta, como también en almacenaje (Jenks & Ashworth, 2010; Loypimai *et al*., 2017). En estudios en los que a la fruta se le removió la cera cuticular, se observó una mayor pérdida de peso durante almacenaje comparada a la fruta intacta; la fruta con cera removida presentó menor firmeza (21%) comparado al control (Chu *et al.,* 2017; Loypimai *et al.,* 2017; Valdés, 2020).

La calidad final de la fruta al llegar a los mercados de destino, depende de las características fisicoquímicas del fruto a cosecha, las que a su vez son el resultado de la interacción de las condiciones ambientales presentes durante la temporada de crecimiento y a los manejos agronómicos (Tyagi *et al*., 2017; Brasil & Siddiqui. 2018). Por ejemplo, Lobos *et al.* (2018) sugieren que las mayores precipitaciones y temperaturas máximas fueron responsable de una menor firmeza en cosecha y postcosecha.

2.4 Efecto medioambiental

Dado la permeabilidad del arándano a las variaciones macro- (variación entre temporadas) y micro-climáticas (variación diaria dentro de la planta), es evidente que el clima es un factor determinante en el proceso de maduración y senescencia de los frutos, (Hidalgo-Gálvez *et al.*, 2018).

Diversos autores han presentado información respecto a temperaturas optimas de desarrollo en cultivares de arándanos. En general Hancock (2009), establece como condiciones óptimas para la mayoría de cultivares 25-30°C día, y 20°C noche. Zheng *et al* (2017), especifica que, para los cvs. 'Duke' y 'Brigitta', las condiciones óptimas para acumulación máxima de biomasa, son 30.4 y 31.8 °C, respectivamente; cuando la temperatura aumenta de 25 a 30°C, esta variable se incrementa 20.3 y 14.2%, respectivamente. Los mismos autores, encontraron efectos negativos cuando se enfrentaban a condiciones de temperatura más extremas, como la disminución de la acumulación de biomasa (sobre 40°C) y de la fotosíntesis neta (sobre 34°C dependiente de cultivar).

Las anomalías meteorológicas durante la temporada (lluvia y olas de calor), a causa del cambio climático, podrían repercutir negativamente en las características de la fruta en destino. Sin embargo, el impacto de dichos eventos climáticos atípicos dependerán del estado de madurez de cada fruto dentro del racimo. Por ejemplo, en vides (*Vitis vinifera*) se ha reportado que un fruto en estado inmaduro (verde) es menos susceptible que otro que esté cercano a su madurez de cosecha (Santos *et al*., 2020). Para el caso de las altas temperaturas en esta especie cv. 'Shiraz' originan bayas con menor peso (Guot *et al.,* 2019) y turgor, con mayor puntaje de ablandamiento y pedicelos fuertemente unidos (Bonada *et al.*, 2013), y una reducción de la producción de antocianinas (Ighbareyeh y Carmona, 2018). En cv. Semillon, estas condiciones desencadenan una mayor concentración de solidos solubles en bayas en cosecha, y una disminución de la fotosíntesis en plantas (Greer y Weedon, 2013). Por su parte, temperaturas superiores a 30°C en periodos de floración y maduración de bayas de cvs. 'Cardinal' y 'Carignane', originaron un menor peso de baya, menor acidez titulable y mayor pH (Kliewer y Schultz, 1973).

En arándanos, la información es bastante limitada. Tanto Ehlenfeldt & Martin (2002) como Lobos *et al.* (2018) asocian la menor firmeza a temporadas en donde se presentaron precipitaciones por sobre la media. Frente a eventos de altas temperaturas en cvs. 'Aurora' y 'Elliott', Yang *et al.* (2019) encontraron efectos negativos en firmeza y deshidratación de bayas de arándanos, siendo más susceptibles estados inmaduros.

Dada la perecibilidad del arándano, eventos climáticos adversos podrían tener especial impacto en la firmeza y en especial la tasa de ablandamiento entre cosecha y postcosecha.

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CAPITULO II

Within-plant variability in blueberry (*Vaccinium corymbosum* L.) III: modulation of postharvest softening by simulated adverse weather events near harvest.

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Abstract

There is a significant geographical area where blueberries are grown, where daily temperatures exceed those recommended for this species, as is the case of the central valley of Chile and other producing regions of the world without marine influence. In view of the evident scenario of climate change, where maximum temperatures increase seasonally and rainfall during summer is no longer sporadic, detrimental impact on loss of firmness during storage is expected. The objective of this work was, close to harvest and according to the position of the fruit within the canopy (east and west), to determine the timing of greatest postharvest genotypic sensitivity (i.e., firmness, soft fruit proportion, and fruit weight) to simulated climatic events (heat wave, rain, and their combination).

Keywords: Climate change, environmental effect, precipitations, rainfall, thermic.

3. Introduction

The area of blueberries (*Vaccinium corymbosum* L.) has increased considerably in recent decades; in almost 20 years it has doubled from 52,000 to 110,000 ha (FAO, 2017). During this period, new actors have joined the international market scenario, either because fruit is produced closer to the destination or because it is possible to reach farther ports with firmer fruit. In fact, during season 2021, Peru became the largest counter-season blueberry producer, placing Chile in a second position.

Handling during the whole blueberry supply chain, from the field to consumption is key to keep fruit quality (Forney, 2009; Ktenioudaki et al., 2021). Although it is also generally understood that a fruit that grows and develops under adverse environmental conditions will be at a disadvantage for fruit quality at harvest (Mainland 1989; Connor et al., 2002; Ehlenfeldt and Martin, 2002; Yañez et al., 2009; Lobos et al., 2009, 2012 and 2013; Konarska, 2015; Lobos et al, 2018; Yang et al., 2019), there are not many studies that delve into the direct impact of climate on the postharvest life of Vaccinium (Chen et al., 2012; Estrada et al., 2015).

Unfortunately, blueberry show asynchrony in fruit set and maturity dynamics (Gorchov, 1985; Vander Kloet and Cabillo, 2010; Moggia et al., 2022), and several pickings are required, further complexifying the determination of the causes and effects of adverse events. In *Vitis vinifera*, a species that develops in clusters, whose berries show a double sigmoid growth and ripening pattern like in blueberries (Gough, 1994; Routray and Orsat; 2011; Forney et al., 2012; Coombe and McCarthy, 2000), the information point to fruit veraison as the phenological stage in which climatic events are relevant (Kliewer y Schultz, 1973; Greer y Weedon, 2013; Sadras et al., 2013; Bonada et al., 2013). For example, high temperatures in *V. vinifera* 'Shiraz' have been reported to generate berries with lesser weight (Guot et al., 2019) and turgor, and greater softening scores (Bonada et al., 2013).

Because of the harvest index used by the industry (100% blue), fruits with the same cover color show important differences in physiological maturity (i.e., ripe *vs*. overripe) (Lobos et al., 2018). During storage, softening of the berries is the main obstacle for long term shipments, which can take up to 60 d by ship (Vicente et al., 2007; Giongo et al., 2013).

In view of the evident scenario of climate change, where maximum temperatures increase seasonally and rainfall during summer is no longer sporadic, detrimental impact on loss of firmness during storage is expected.

Thus, given the genotypic plasticity to micro- and macroclimatic conditions (Moggia et al., 2017; Lobos et al., 2018), determining the consequences on postharvest life is crucial. Even more so if, due to the asynchrony of blueberry in ripening, any environmental anomaly will have a different effect depending on the proportion of fruit at each developmental stage. Consequently, the objective of this work was, according to the position of the fruit within the canopy (east and west), to determine the timing of greatest postharvest genotypic sensitivity (i.e., firmness, soft fruit proportion, and fruit weight) to simulated climatic events (heat wave, rain, and their combination) in two cohorts close to harvest.

4. Material and Methods

- *Plant material and trial establishment*

Trial was conducted on mature highbush blueberry plants (*V. corymbosum*), cvs. 'Duke' and 'Brigitta' (11- and 10-years-old, respectively), established on a commercial field located in Río Claro, Maule Region, Chile (35°15′33.80″ S; 71°14′17.70″ W; 339 m.a.s.l.; N – S orientation: 331.75°), during season 2019/20; at budbreak, bushes of similar appearances (i.e., number and age of canes, plant height and volume) were selected for each cultivar.

Four environmental conditions were studied: (Fig. 1A): i) control (***C***) (Fig. 1A): field conditions; ii) rain (***R***): only during the third day, 15 – 18 mm of precipitation per cohort between 12:00 and 15:00 h (details in Fig. 1B); iii) heat (***H***): 5 d heat wave (i.e., ~5°C above ambient temperature) using an open-top chambers OTC (Hannah, 2015; Tasnim et al., 2020) for seven plants, attached to the ground and covering the first three quarters of the plant height (detailed in caption Fig. 1A); and iv) rain and heat (***R+H***): OTC to keep a 5 d heat wave, and precipitation (15 – 18 mm) in the third day.

To identify the timing of greatest genotypic sensitivity to the stimulus, two cohorts were considered: 7 d prior (**C1**) and 7 d after (**C2**) the first commercial harvest. Since a significant portion of the annual production comes from the first two commercial pickings, each simulated conditions cohort was sampled twice: the day after treatment window (**S1**) and 5 d after that (**S2**).

To unify the maturity stages of the first cohort under study (i.e., S1), when the orchard reached ~2 – 4% of ripe fruit within the plant (i.e., one day before the establishment of the first cohort of treatments), a cluster cleaning was performed, removing all fruit with blue color coverage above 90% (Moggia et al., 2022). Since all field was harvested at S1, it was not necessary to perform a cluster cleaning at the beginning of C2 (Fig 1E).

On each of the five replicas of seven plants (Supplementary Fig. 1), fruit was sampled from the five central ones; as detailed in Lobos et al. (2018), plants were divided into east (E), top, and west (W) sectors, but fruit were studied only from the E and W sides.

At the field, automatic sensors (HOBO S-THB, Onset Computer, Bourne, MA, USA) were installed to record (e.a. 15 min @ 2 m heigth) ambient temperature (°C) and relative humidity (RH; %); same measurements were established within each simulated environmental condition (HOBO U23 Pro v2 Temperature/Relative Humidity, Onset Computer Corporation, MA, USA). To record the amount of water dropped to ground level in *R* and *H+R*, millimeter vessels were placed under each of the three central plants.

2.2 Fruit sampling and evaluations

At each sampling date (Table 1), fruit was taken to the laboratory (Plant Breeding and Phenomics Center, Universidad de Talca, Talca, Chile), and three lots of fruit were generated to assess fruit quality at harvest (i.e., P1 and P2), and after 45 and 60 d of postharvest (0°C and 90% of RH). The following characters were recorded: (1) fruit weight (g) with an electronic balance (LSV-6200g, Veto y Cía. Ltda., Santiago, Chile), 25 fruit per replicate; (2) firmness (N) by a compression device (FirmTech 2, BioWorks Inc., KS, USA), using the maximum slope of the curve as compressive force (15 to 20 g force; loading rate of 16 mm s^{-1}), gram force was converted to N (× 0.0098), 25 fruit per replicate; and (3) the soft fruit (< 1.4 N) proportion of each lot was calculated.

2.3 Statistical analyses

The analysis was performed using Generalized Linear Model (GLM) in R 3.0.0 (R Development Core Team, 2008). When significant differences were found, Tukey's multiple comparison test ($p \leq 0.05$) was applied.

To determine the environmental effect relative to the control treatment, the information from each canopy side was combined, and blueberry firmness (N), soft fruit (%), and fruit weight (g) of cvs. 'Brigitta' and 'Duke' for each studied cohort (C1 and C2), picking sample (P1 and P2), and evaluation time (0, 45 and 60 d) were contrasted.

3. Results

3.1 Environmental characteristics of simulated weather events

Ambient field temperature during establishment of the treatments ranged 25.6 – 27.1 °C for 'Duke' and 27.4 – 29.2 °C for 'Brigitta'; similarly, maximum temperature ranged 30.4 – 31.4 °C and 29.3 – 33.5 °C, respectively (Supplementary Table 1).

Apart from the second cohort of 'Brigitta' in the *R+H* treatment, the CTOs used for each cohort and cultivar were able to maintain 5°C above the control treatment (Supplementary Table 1). Rain simulation were also around 18 mm.

3.2 Treatment impact in harvest and postharvest

In general, simulated environmental conditions did not affect fruit firmness to a significant extent (Fig. 2). In addition, differences associated with fruit position within the plant (i.e., E *vs*. W) were also minor, although they increased as postharvest lengthened (i.e., 0, 45, and 60 d). In genotypic terms, 'Brigitta' proved to be more tolerant than 'Duke' to environmental stimuli (Fig. 2); in particular, for 'Duke', C1 generated a greater effect, at P1 for 45 d shipments and at P2 for 60 d shipments.

In contrast, when studying the proportion of soft fruit (< 1.4 N), more significant differences were observed among treatments and between canopy sides (Fig. 3). Regarding genotypic differences at harvest (i.e., 0 d), 'Brigitta' had a greater proportion of soft fruit (~20 – 40%) while in 'Duke' did not exceed 10%. In postharvest, however, this pattern was inverted; compared to 'Brigitta' (except in P1 from C2), 'Duke' shows a greater tendency to increase the proportion of soft fruit.

Regarding fruit weight (Fig. 4), there were no major differences between the time of evaluation, treatment, cultivar, or position of the fruit within the plant.

3.3 Impact relative to the control treatment

In general, when comparing the responses under simulated conditions with respect to the control (Fig. 5), both cultivars showed different behavior throughout storage.

Among the variables analyzed, the proportion of soft fruit (<1.4 N) showed the greatest variation. At harvest (0 d), 'Brigitta' was the only cv. that displayed a marked difference in both cohorts (C1P2 and C2P1: 2- and 1-times, respectively). During postharvest, the greatest effect was generated in the second cohort with the combined environment at 45 d for both 'Brigitta' (C2 P1) and 'Duke' (C2 P2), with no significant differences at 60 d of storage.

As for firmness, except for C1P1 and C2P2 values, there seems to be no significant impact on this variable. During storage, treated fruit in C2 of both cultivars showed similar responses, however in C1 (P1 and P2) the greatest differences were observed in 'Duke' at 60 d.

Fruit weight was also affected, varying mostly in C2, with a greater influence on P1 than on P2.

4. Discussion

The physicochemical characteristics of fruit at harvest are highly influenced the environmental conditions during annual vegetative and reproductive growth. Thus, along with genotype, these seasonal interactions are determinants of post-harvest softening characteristics; especially in blueberries, a species that lacks root hairs (Gough, 1994), limiting its ability to cool through transpirational flow (Bryla and Strik, 2007; Valenzuela-Estrada et al., 2008 and 2009). For example, Moon et al. (1987a) reported a 50% reduction in stomatal conductance when vapor pressure deficit (VPD) was increased from 1 to 3 kPa.

Unfortunately, most of commercial growing areas are characterized by ambient temperatures and solar radiation that are significantly greater than those of the species' natural habitats (Hancock and Siefker, 1982; Vander Kloet, 1988; Luby et al., 1991;

Hancock, 2006; Lobos and Hancock, 2015). Thus, the environment is always challenging in orchards located far from the marine influence (Darnell, 2000 and 2006; Chen et al., 2012; Lobos and Hancock, 2015), where maximum temperatures easily exceed 25 °C during the veraison window (Lobos et al., 2018). In fact, in Globes (MI, USA), an area predominantly planted with highbush blueberries, Lobos et al. (2012) reported leaf temperatures ~36 °C on 'Elliott'.

Since asynchronous ripening of blueberries involves multiple harvests, the impact of a given weather event will depend on the stage of development of each of the growing and ripening fruits, making it difficult to study and describe. Asynchronous ripening is thought to improves the reproductive success throughout the evolution (Gorochov, 1985) by enhancing the number and diversity of possible frugivores species dispersing the seed along the season (Gleditsch at al., 2017). However, it would also prevent isolated adverse climatic events, such as those described in this work, from compromising at once the necessary dispersal of the seeds (Stapanian, 1982; McDonnell et al., 1984).

Although not widely documented, it has been suggested that extremely warm seasons (i.e., faster fruit development and ripening) as well as adverse events (e.g., heat waves, extemporaneous rain), can result in significant rejections at long-distance destination (Forney, 2009; Lobos et al., 2018). For example, according to the literature, rainfall during the harvest period can negatively affect blueberry fruit quality by increasing fungal diseases, softening berries, wetting pedicel scars, and producing berry splitting (Pritts and Hancock, 1992). In consequence, a heat wave combined with a precipitation event could be expected to represent an even more unfavorable scenario for long-term shipments. Contrary to expectations, in the present study these R did not show large differences with H+R. It is possible that evaporation after the rain event (15 – 18 mm between 12:00 –15:00 h) decreased fruit temperature at the hottest time of the day. However, it would not be surprising that, given the origin of the species (Vander Kloet, 1998; white xxxxxxx), where rainfall during the year is common, over the centuries the plants developed a greater tolerance to rainy periods within the fruit growth/ripening period. In fact, the visible heavy epicuticular wax layer know as bloom acts as a hydrophobic layer covering fruit (Shepherd and Wynne, 2006; Qi et al., 2019); quantity and composition of the epicuticular waxes constitute a possible strategy with which each genotype would deal with the rain, varying according to the genotype and the environmental conditions of each season (Moggia et al., 2017).

Environmental temperature is a key variable for blueberry performance under field conditions. In general, northern highbush blueberry cultivars, such as those used in this study, have lower heat tolerance than those from the south (Estrada et al., 2015). As an example, Hancock et al. (1992) proved that an increase in ambient temperature from 20 to 30 ºC reduced the CO_2 assimilation rate by 23.7% in 'Elliott'. Similar results were found by Moon et al. (1987a) working with *V. corymbosum* L. 'Jersey' and 'Bluecrop', finding the optimum being 18 – 26 ºC and 14 – 22 ºC, respectively. More recently, in blueberry fruit 'Aurora' and 'Elliott', Yang et al. (2019) demonstrate how extreme heat (>35 °C) causes necrosis and shriveling compared to normal conditions. Although in grapevine it is suggested that the most sensitive period to environmental factors is after fruit veraison (Martinez et al.,2015 and Savé et al., 2020), in blueberries fruit at more juvenile stages (green) showed a lower damage threshold (>32 °C) than blue fruit (>35 °C) (Yang et al., 2019). In fact, Yang et al. (2019) suggests the benefits of elevated irrigation as a technique to reduce temperature damage to the fruit, as may have occurred in *R* and *H+R*.

5. Conclusions

There is a significant geographical area where blueberries are grown, where daily temperatures exceed those recommended for this species, as is the case of the central valley of Chile and other producing regions of the world without marine influence. Increasing temperatures and the evident weather variability resulting from climate change indicate that blueberry growers will face even greater firmness variability, especially at destination. Therefore, it will be critical to establish the major environmental factors contributing to and predisposing to fruit softening.

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Figures and Tables

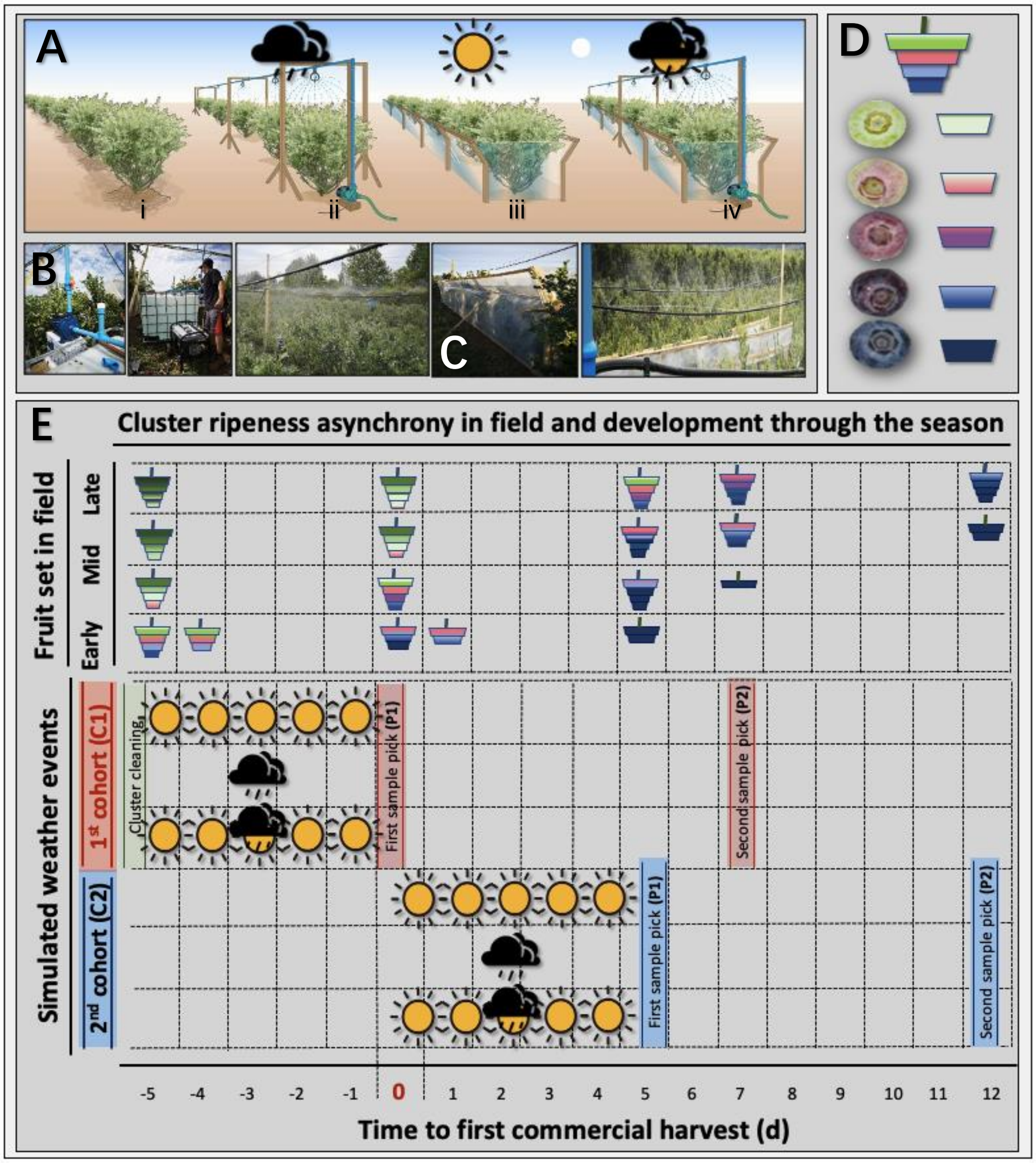


Figure 1. (A) Representation of field simulated adverse weather events: (**i**) control: field conditions; (**ii**) rain: only during the third day, 15 – 18 mm of precipitation, between 12:00 and 15:00 h; **iii**) heat: open-top chambers OTC fixed to the ground to keep 5 °C above control during 5 d; and **iv**) rain and heat: OTC to keep 5 °C above control during 5 d, and precipitation (15 – 18 mm) in the third day. **(B)** rain was simulated by pumping well-water through

microjet emitters (@ 80 cm, 60 L h^{-1}, 360 °) inserted in a polyethylene pipe (12.7 mm diameter) located at 70 cm above the canopy top-edge. Through a PVC pipeline (32 mm diameter), a 1 HP centrifugal pump (BCM158, Bestflow, ciudad, China) fed with a 2.8 HP portable generator (EU20i, Honda, Tokio, Japan), prompted water from a container (1000 L). **(C)** Open-top chambers OTC (Hannah, 2015; Tasnim et al., 2020) were built with a rectangular base (8.5 x 1.9 m) and height according to the cv. (i.e., 'Duke': 1.6 m and 'Brigitta': 1.8 b), and fixed to the ground to keep 5 °C above *C* during 5 d, in the lower two thirds of the plant. According to the representation of the asynchrony within the cluster **(D)**, the simulated events were established in two cohorts, which were sampled 5 days after the application of the event and 7 days later **(E)**.

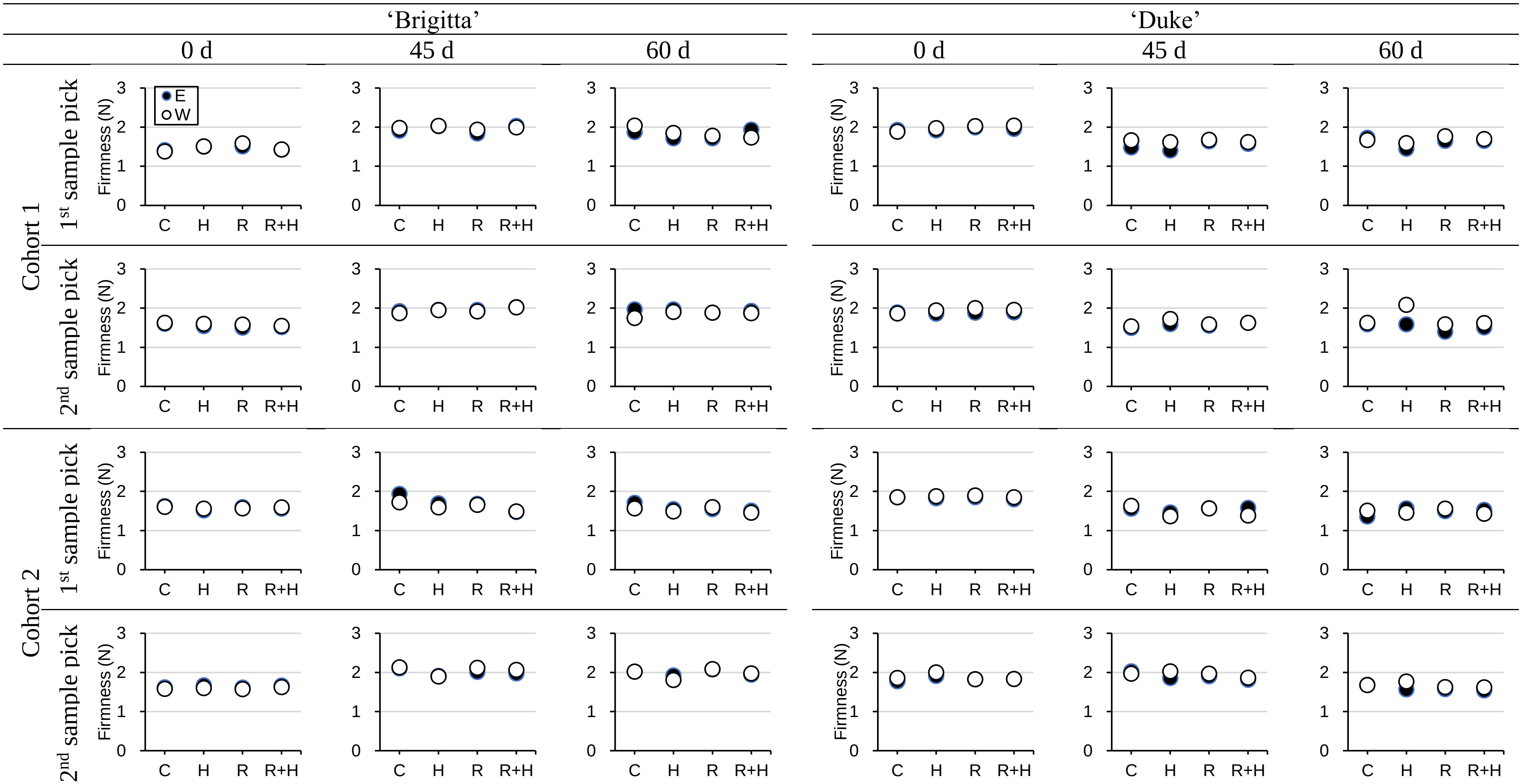


Figure 2. Blueberry Firmness (N) of cvs. 'Brigitta' and 'Duke' for each studied cohort (C1 and C2), picking sample (P1 and P2), evaluation time (0, 45 and 60 d), and orientation (E and W), for each treatment: control (C), heat (H), rain (R), and heat + rain (H+R).

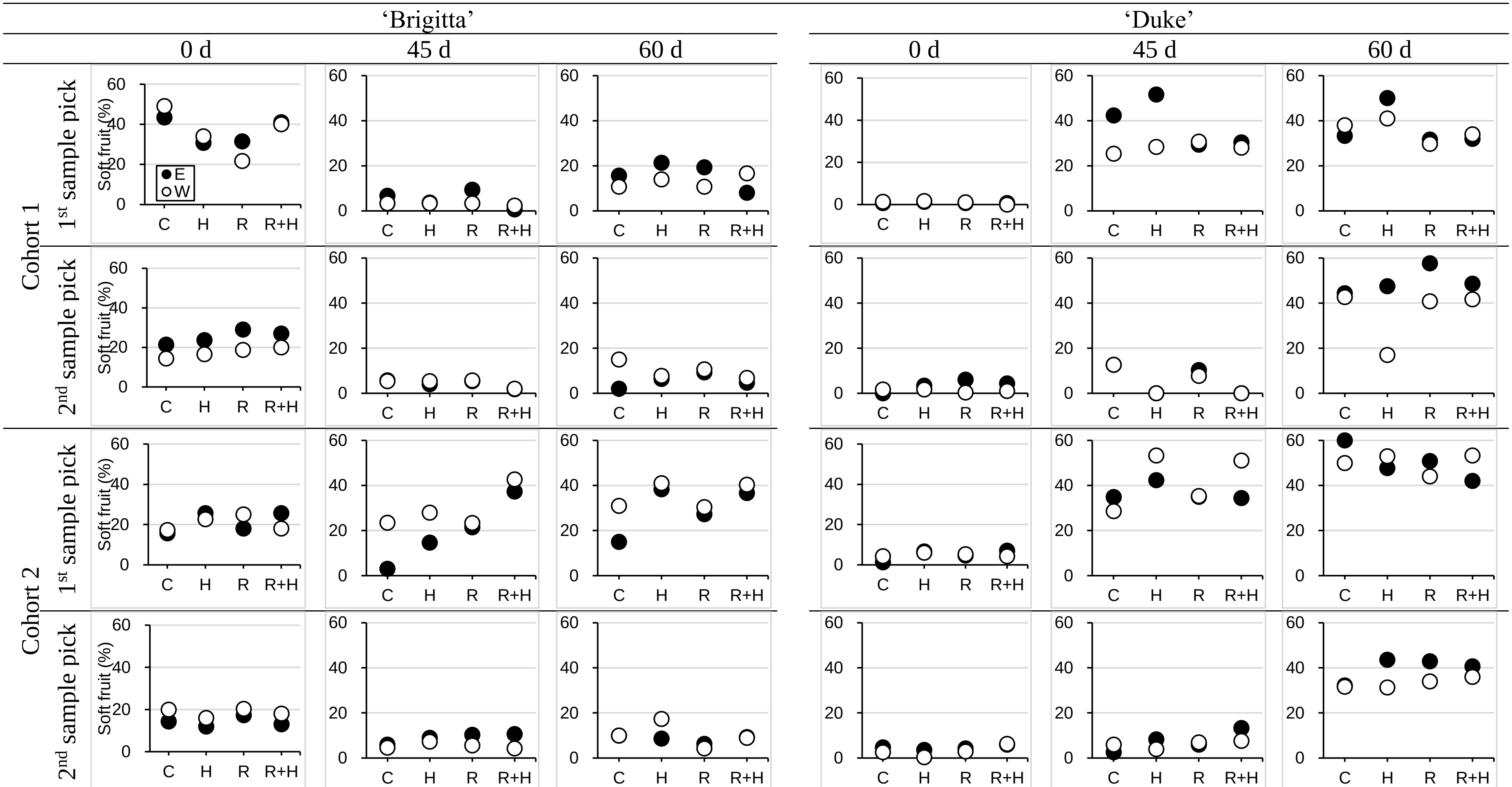
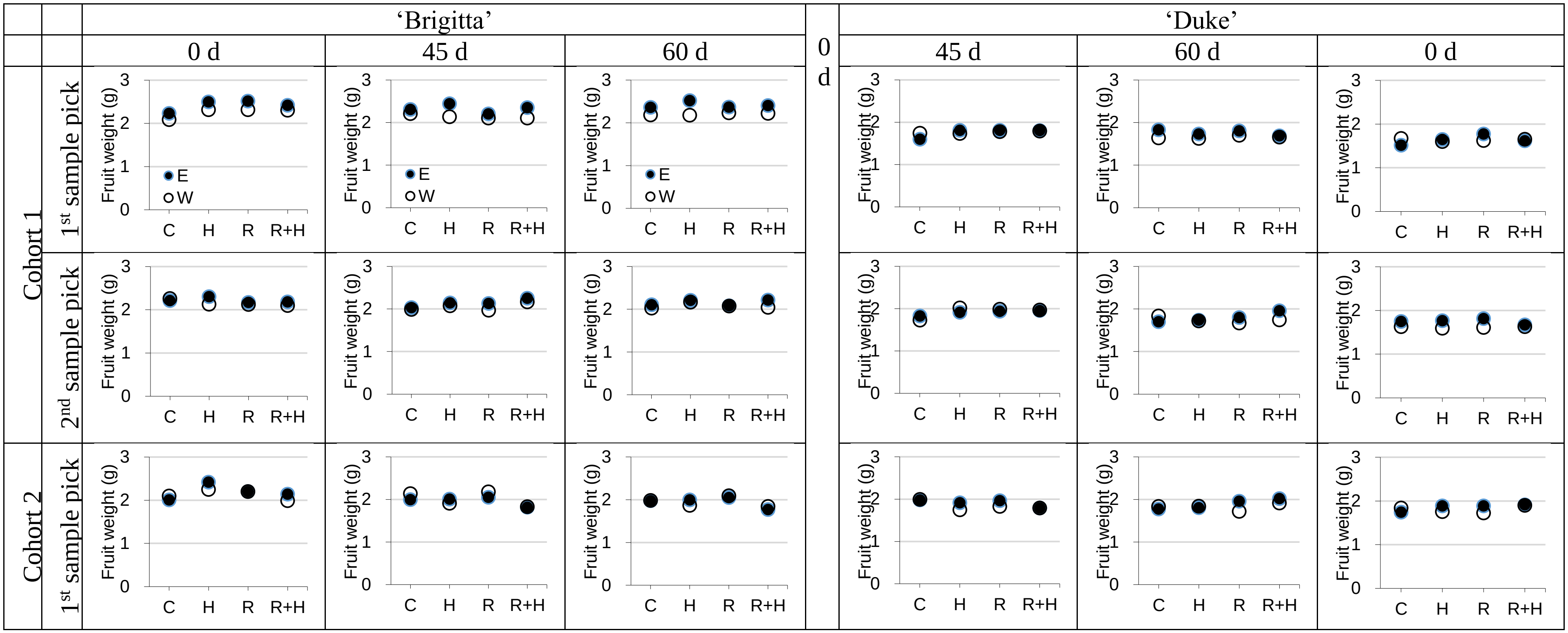

'Brigitta'
'Duke'
0 d
45 d
60 d
Cohort 1
Cohort 2
1st sample pick
2nd sample pick
Soft fruit (%)
E
W
C
H
R
R+H

Figure 3. Blueberry soft fruit proportion (< 1.4 N; %) of cvs. ‘Brigitta’ and ‘Duke’ for each studied cohort (C1 and C2), picking sample (P1 and P2), evaluation time (0, 45 and 60 d), and orientation (E and W), for each treatment: control (C), heat (H), rain (R), and heat + rain (H+R).



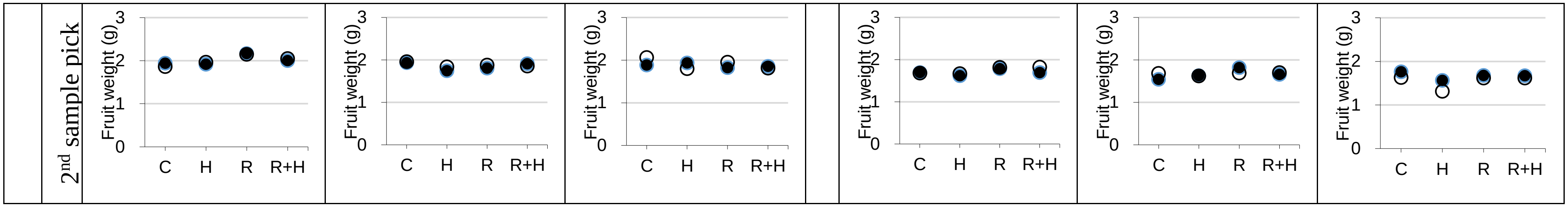


Figure 4. Blueberry fruit weight of cvs. ‘Brigitta’ and ‘Duke’ for each studied cohort (C1 and C2), picking sample (P1 and P2), evaluation time (0, 45 and 60 d), and orientation (E and W), for each treatment: control (C), heat (H), rain (R), and heat + rain (H+R).

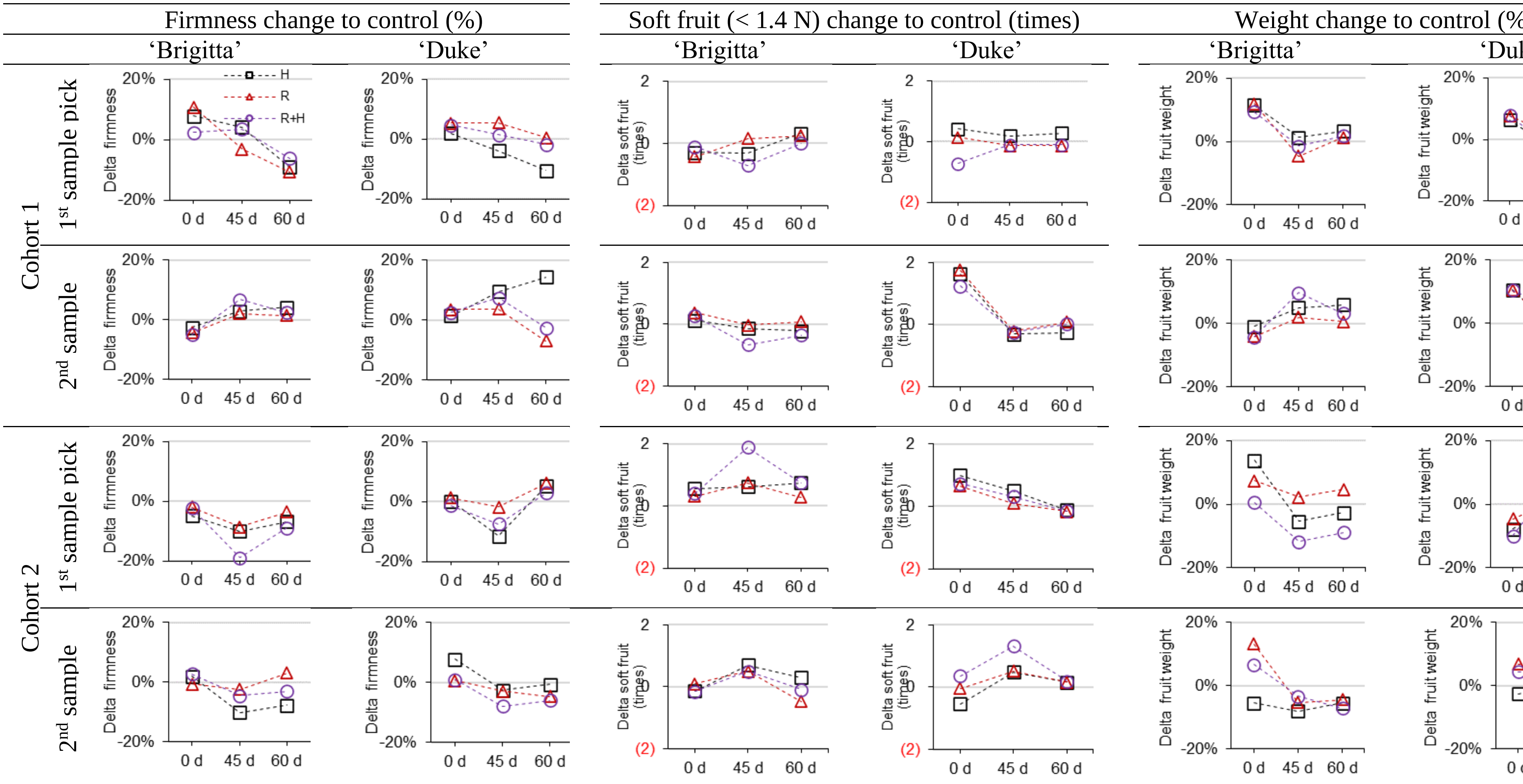
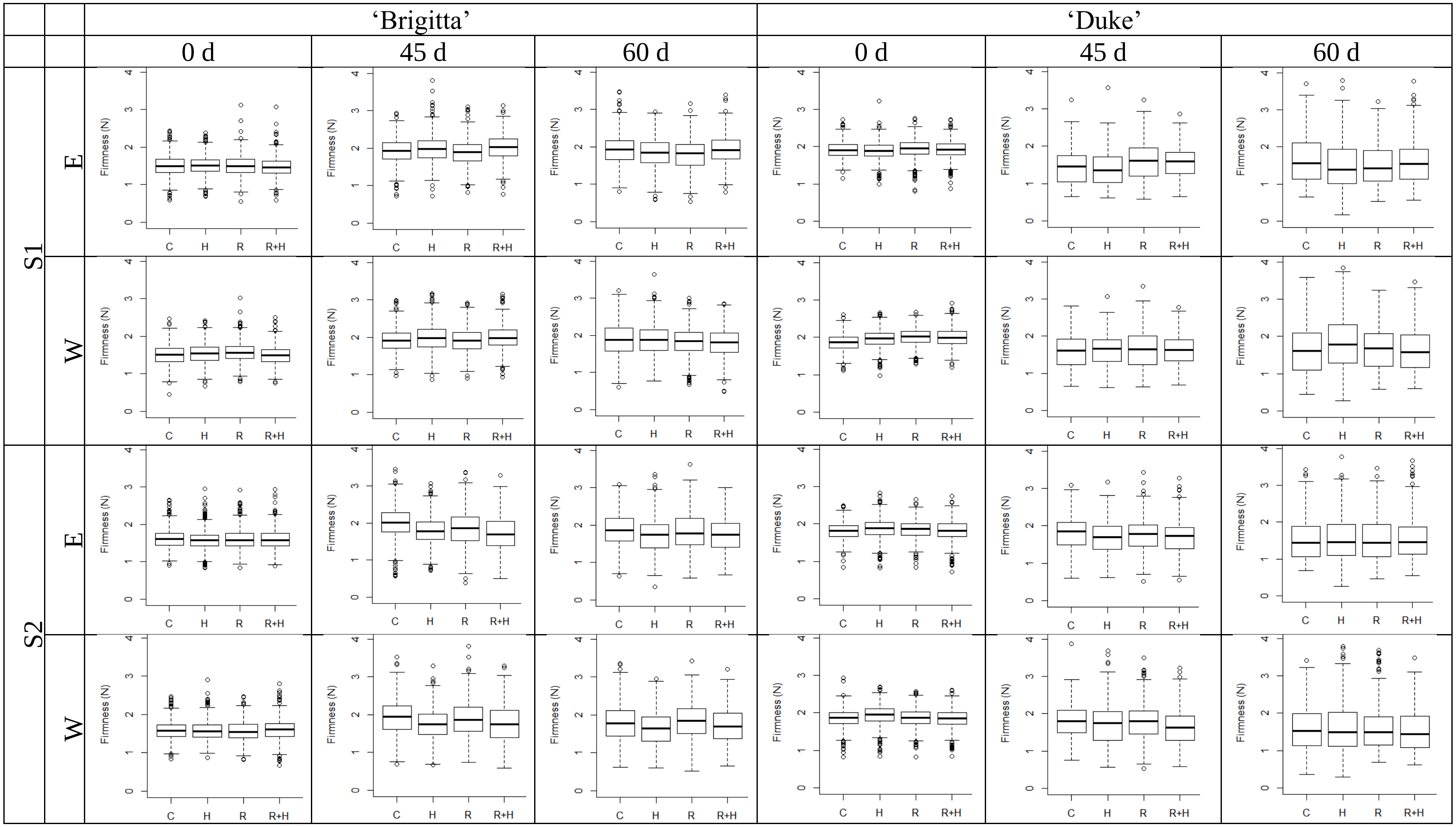

Firmness change to control (%)
Soft fruit (< 1.4 N) change to control (times)
Weight change to control (%
'Brigitta'
'Duke'
Cohort 1
Cohort 2
1st sample pick
2nd sample
H
R
R+H
Delta firmness
Delta soft fruit (times)
Delta fruit weight
20%
0%
-20%
2
0
(2)
0 d
45 d
60 d

Figure 5. Changes in firmness (%), soft fruit (times) and weight (%) with respect to the control treatment in blueberry cvs. 'Brigitta' and 'Duke' for studied cohort (C1 and C2), picking sample (P1 and P2), and evaluation time (0, 45 and 60 d) for each treatment: control (C), heat (H), rain (R), and heat + rain (H+R).


Supplementary Figure 1. OTC aerial image



Supplementary Fig 2. Box-plot for firmness of cvs. ‘Brigitta’ and ‘Duke’ for each studied cohort (C1 and C2), picking sample (P1 and P2), evaluation time (0, 45 and 60 d), and orientation (E and W), for each treatment: control (C), heat (H), rain (R), and heat + rain (H+R).

Supplementary Table 1. Ambient temperature and rainwater measured for each studied cohort (C1 and C2) and treatment: control (C), heat (H), rain (R), and heat + rain (H+R).

Cultivar	Cohort	Stimulus window	Field ambient temperature (ºC)	OTC temperatures above ambient temperature (ºC)		Rainwater (mm)	
				Heat	Rain + Heat	Rain	Rain + Heat
'Duke'	C1	20 - 25 Nov., 2019	Avg: 25.6 Max: 30.4	4.7±1.4	4.4 ±1.3	18.1 ± 0.25	17.8 ± 0.15
	C2	27 Nov. - 02 Dec., 2019	Avg: 27.1 Max: 31.4	6.7±2.1	4.4 ±1.5	17.8 ± 0.21	17.9 ± 0.36
'Brigitta'	C1	14 - 19 Dec., 2019	Avg: 27.4 Max: 29.3	6.6±3.5	4.8±2.97	18.0 ± 0.21	18.0 ± 0.38
	C2	23 - 28 Dec., 2019	Avg: 29.2 Max: 33.5	4.6±2.0	13.9±4.8	17.9 ± 0.4	18.1 ± 0.32