

To what extent does pre-harvest variability affect chilling injury susceptibility of citrus postharvest?

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Abstract

Chilling injury (CI) is a physiological rind disorder that commonly occurs during the long-time cold storage of citrus. The occurrence of CI on citrus reduces fruit quality and shelf-life, leading to market rejection. The susceptibility of citrus to CI during long cold storage period depends on the variability of several pre-harvest factors. However, the impact of pre-harvest variability on the postharvest occurrence of CI for citrus remains largely unknown. To address this, the impact of the variability in production area, cultivar, orchards and fruit harvest maturity on CI of citrus was investigated over two growing seasons. Four main citrus production regions in South Africa were considered, namely, Citrusdal, Nelspruit, Letsitele and Sundays River Valley (SRV). Five cultivars representing the main citrus subgroups were selected; 'Turkey' and 'Midknight' Valencia oranges, 'Nova' and 'Nadorcott' mandarins, and 'Star Ruby' grapefruit. For the variability in harvest maturity, fruit were harvested with two week harvest intervals from 8 weeks before commercial harvest until 6 weeks after. Our result revealed that differences in production area, cultivar, orchards, and fruit maturity have a significant impact on CI susceptibility. However, among these factors, orchard variability had the lowest impact on CI susceptibility, while the regional weather variability between production areas had the most significant impact. Fruit from the SRV, a coastal region, showed the highest risk of CI for all cultivars. They were up to 70 % more susceptible than those from Citrusdal, Nelspruit, and Letsitele. The CI susceptibility of 'Turkey' and 'Midknight' Valencias is ~60 % more than those of mandarins and grapefruit. Fruit harvested early in the season (e.g., 8 weeks before commercial harvest) have a very high risk of CI, while those at the commercial maturity stage will have the highest CI tolerance. This study provides insight into which major pre-harvest factors affect the quality of citrus postharvest.

Keywords: Food quality; pre-harvest variability; growing conditions; climate differences; cold chain; thermal damage

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1 INTRODUCTION

Citrus is one of the most cultivated fruit crops in the world, and is grown in more than 80 countries. They belong to the genus *Citrus* of the family Rutaceae, and are well known for their thirst-quenching ability, refreshing fragrance, and abundant vitamin C (Ladaniya, 2008a; Vashisth and Kadyampakeni, 2019). Citrus fruit and its products are also a rich source of minerals and dietary fibre essential for overall human nutritional well-being. They contain several other phytochemicals which serve as nutraceuticals, such as flavonoids, limonoids, phenolics, antioxidants, and carotenoids (lycopene and β -carotene) (Goulas and Manganaris, 2012; Ladaniya, 2008b, 2008a). The flavonoid from citrus juice improves blood circulation in humans and has anticarcinogenic, antiallergic and antiviral properties (Kumar et al., 2022; Ladaniya, 2008a). Some common types of citrus fruit are oranges (e.g., Valencia, Malta Blood Red, and Naval), mandarin (*Citrus reticulata*, Blanco) (e.g., Nagpur, Clementine, Nova, and Nadorcott), and grapefruit (*Citrus paradisi* Macfadyen) (e.g., Star Ruby, Marsh). Others are lemon (*Citrus limon* (L.) Burm. F) (e.g., Eureka, Lisbon, and Genoa), pummelo (*Citrus grandis* or *Citrus maxima*) (e.g., Chandler, Mato, and Goliath), lime (e.g., Persian, Makrut and Desert) and citron (*Citrus medica* Lin.) (Ladaniya, 2008c; Vashisth and Kadyampakeni, 2019). These fruit contain between 0.3 -13 g kg⁻¹ of vitamin C (Bozkir, 2020; Ladaniya, 2008b; Onwude et al., 2021), and 0.01 – 0.4 g kg⁻¹ of carotenoid (Ladaniya, 2008b). These values, however, vary considerably depending on the cultivar. Oranges generally contain 4 – 13 g of vitamin C per kg, while the vitamin C content for lemons, grapefruit, and mandarines, is between 2 – 5 g kg⁻¹ (Ladaniya, 2008b; Onwude et al., 2021). The recommended daily intake (RDI) of vitamin C for human is between 4 – 9 g kg⁻¹ (Gil et al., 2012; Onwude et al., 2021; U.S. National Institutes of Health, 2019). Since fruit are mostly eaten raw, the RDI of vitamin C per person per day is already realized by consuming one orange per day. Despite their high nutritional value and enormous health benefit, citrus are susceptible to physiological disorders as a result of dysfunction of physiological processes of fruit tissue due to abiotic stress such as temperature, relative humidity, and moisture stress. This leads to significant postharvest losses, so economic losses in the cold citrus supply chain due to degradation and spoilage. Chilling injury is a common physiological disorder of citrus during long cold storage, especially at a temperature below 7 °C.

Chilling injury (CI) is a physiological rind disorder that commonly occurs during the long-term cold storage of citrus. Extended exposure of citrus to cold temperatures (< 7 °C) could result in changes in the physical properties of the cell membrane and that of structural proteins such as tubulin and enzymes (Ashebre, 2011; Ladaniya, 2008d). The changes in the physical properties of cell membrane result in the movement of lipids, ions, enzymes and metabolites in and out of the rind cell. This movement causes metabolic imbalances and the eventual disruption of the cell membrane, cellular compartment breakdown, or cell death, leading to chilling injury (Ladaniya, 2008d). This manifests as sunken, brown, necrotic areas that increase in size as the severity of the injury progresses, thereby reducing fruit quality and shelf-life, and rendering the fruit aesthetically undesirable (Yuen et al., 1995). Such fruit in the postharvest supply chain are often sorted out and thrown away; in some cases, the full shipment is completely discarded, leading to food waste and loss of revenue.

The susceptibility of citrus to CI during a long cold storage period depends on several pre-harvest factors, including fruit maturation stage, metabolic status, horticultural practices during growing, genetic and climatic factors (Siboza et al., 2014). The fruit maturity stage contributes to CI sensitivity, as some citrus cultivars are less susceptible to CI during mid-season harvest compared to early-or late-season fruit (Ferguson et al., 1999; Mohammed and Brecht, 2002; Schirra et al., 2000). Seasonal variation also affects the metabolic rind conditions of citrus (Houck, et al., 1990). Horticultural practices, such as water management, nutrition, fertilizer application, pruning and rootstock selection influences citrus rind physiology, like rind color (Iglesias et al., 2007; Morgan et al., 2009; Mudau et al., 2005). Genetic differences between citrus cultivars result in variation in rind physiology due to significant differences in internal and external fruit quality, such as rind thickness, color, and juice content. Hence, every citrus cultivar has a unique minimum safe temperature for maximum storage life. In fact, the critical point at which CI develops varies among species and even within the same varieties grown under different weather and microclimatic conditions (Ladaniya, 2008d). As such, the prevailing weather conditions during all stages of fruit development could also significantly impact CI sensitivity of citrus (Hordijk, 2013).

For several decades, only few studies have been conducted to demonstrate how several pre-harvest factors (Holland et al., 1999; Lafuente et al., 1997; Lo'ay and Dawood, 2019), seasonal changes (Gonzalez-Aguilar et al., 2000), plant hormone regulator (Serna-Escolano et al., 2021) and abscisic acid (ABA) (Lafuente et al., 1997; Manzi et al., 2022) affects chilling injury in citrus. Rarely, the impact of differences in major pre-harvest factors such as genetic variability, orchards, maturity stages and weather between production regions are accounted for. To our best knowledge, we still do not exactly know to what extent pre-harvest variability affect CI susceptibility postharvest. To

this end, the relevant question is: among the various differences in the major growing conditions, which one exerts the greatest influence on the susceptibility of citrus to chilling injury during long cold storage. It is crucial to know if the differences in the weather conditions between citrus producing regions, the differences in citrus cultivars, the variability in orchards or the variability in the fruit maturity at harvest have the most significant impact on CI susceptibility.

To address this, this study used a controlled cold storage experiment over 32 days, mimicking the actual postharvest storage period from farm to retail of citrus exported to EU from South Africa. The goal was to quantify the impact of production area, cultivar, orchards and fruit maturity at harvest on CI of citrus. We considered citrus produced in four main citrus production regions in South Africa: Citrusdal, Nelspruit, Letsitele and Sundays River Valley (SRV), for 2018/2019 and 2019/2020 production seasons. For this, five main citrus cultivars representing various subgroups, including 'Turkey' and 'Midknight' Valencia, 'Nova' and 'Nadorcott' Mandarin, and 'Star Ruby' Grapefruit, were evaluated. For the impact of fruit harvest maturity on CI, we evaluated citrus fruit harvested from 8 weeks prior to commercial harvest time until 6 weeks after harvest. Finally, the impact of the pre-harvest factor with the largest impact on the chilling injury susceptibility of citrus postharvest was assessed.

2 MATERIALS AND METHODS

2.1 Study area and fruit material preparation

The study was carried out for citrus of five different cultivars over the 2019 and 2020 harvest seasons in four distinctly different climatic areas that are representative of the main citrus production regions of South Africa (Figure 1). These areas are Letsitele in Limpopo, Nelspruit in Mpumalanga, Sunday's River Valley in Eastern Cape, and Citrusdal, in Western Cape. Additional information regarding the production details of the experimental sites is available in (North-Dewing, 2023). The five cultivars evaluated in this study are (1) 'Turkey' Valencia, (2) 'Midknight' Valencia, (3) 'Nova' Mandarin, (4) 'Nadorcott' Mandarin, and (5) 'Star Ruby' Grapefruit. Except where stated otherwise, all fruit were harvested at commercial harvesting maturity (Caxton Magazines, 2012; Hussain et al., 2022) as determined by producers in each area, thus resulting in different harvesting times for the four respective areas. To reduce sample variation, fruit of similar diameter, comparable color plate scores, and free from defects, were harvested. In all experimental orchards, standard commercial management practices recommended for producing export quality fruit were applied (North-Dewing, 2023). For each production area and cultivar, we harvested citrus fruit from five commercial orchards. Within each orchard, ten healthy, uniformly sized adjacent trees were carefully selected and tagged to ensure consistent sampling across two production seasons (Onwude et al., 2024). To minimize variability and provide a representative sample of orchard conditions, fruit were harvested from the outer canopy, up to 30 cm into the tree. A total of 15 fruits per tree were collected, resulting in 150 fruits per orchard.

After harvesting, the fruit were transported along a short dirty farm road and then a long paved road at ambient temperature to the Department of Horticultural Science, Stellenbosch University, South Africa. Within 24 hours of harvesting, the samples were kept in cold storage at a controlled delivery air temperature of $-0.6\text{ °C} \pm 0.1$ for 32 days to induce chilling injury. Citrus fruit are known to develop chilling injuries when exposed to air temperatures $\leq 5\text{ °C}$ (Cronje, 2022; Golding, 2019). We selected a delivery air temperature similar to that required for the transcontinental sea export of citrus to attain a pulp temperature of -0.6 °C (PPECB, 2022, 2019). In a similar manner, the selected cold storage period represents a typical commercial cold chain duration of citrus from South Africa to Europe. Following cold storage, the samples were kept at room temperature ($20 - 25\text{ °C}$) for seven days before CI incidence evaluation. This duration and temperature were chosen to simulate typical shelf-life conditions (so after cold storage at retail), during which chilling injury (CI) symptoms are most likely to manifest. Specifically, the seven-day period reflects the average time fruits such as citrus spend in retail environments before consumption, ensuring that any potential CI symptoms are adequately observed (Collins and Tisdell, 1995; Onwude et al., 2022). Seven fifty fruit for each cultivar per production area per year were sampled, and data were collected for internal and external quality analysis (see section 2.3.2).

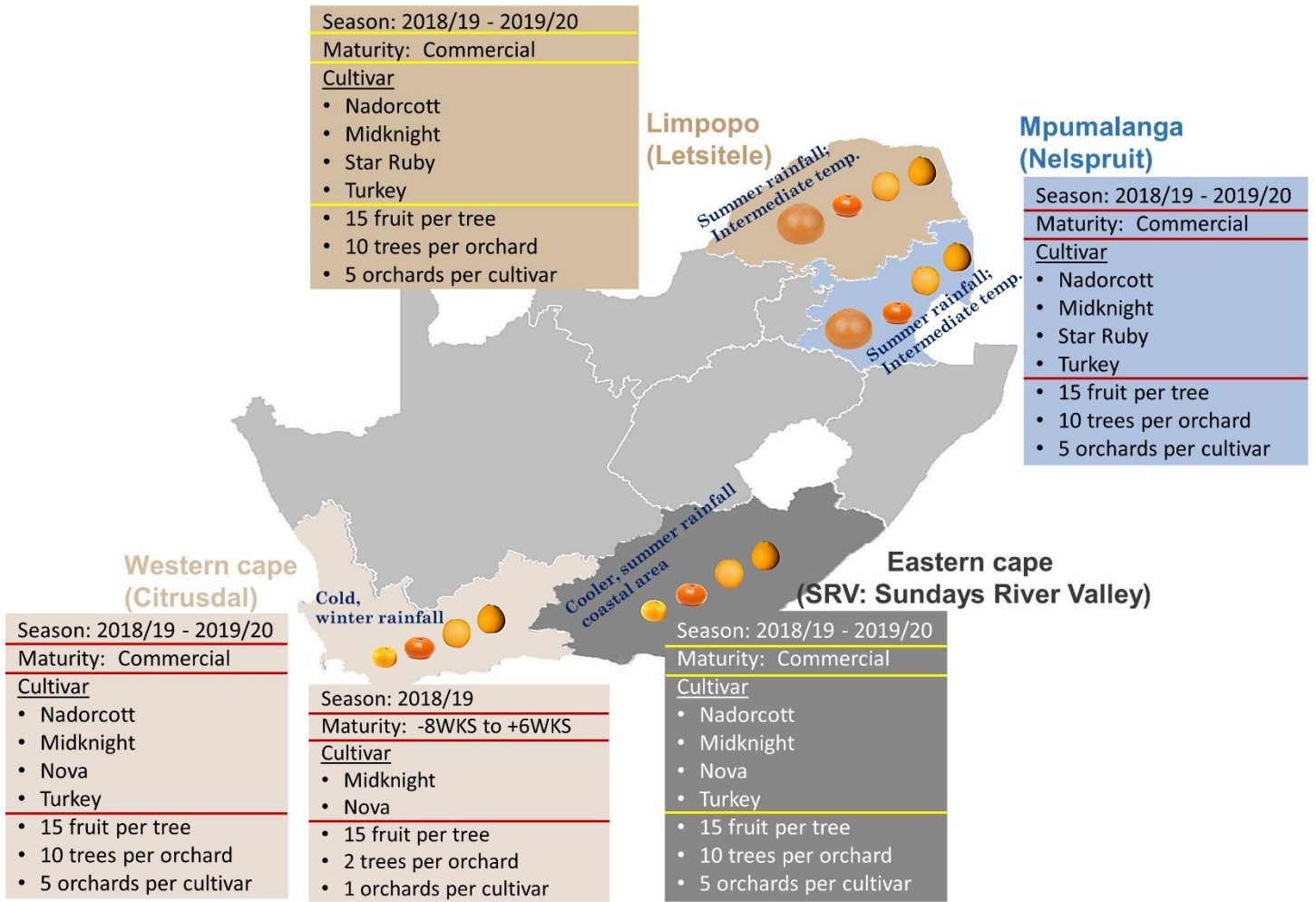


Figure 1. Map of South Africa showing the major citrus production regions and cultivars from the 2018/2019 – 2019/2020 harvest season. For the fruit harvest maturity experiment, citrus fruit were harvested at two-week intervals from 8 weeks prior to commercial harvest (-8WKS) until 6 weeks after (+6WKS) to obtain eight sampling periods. WKS = weeks.

2.2 Experimental designs and treatments

2.2.1 Production area, cultivar and orchards

We quantified the impact of the weather differences in production area, cultivar variability and variability in orchards on CI susceptibility of citrus. Five main commercial cultivars representing major citrus subgroups from five commercial orchards produced in 4 different areas over 2018/2019 – 2019/2020 production season were considered (Figure 1). Within each orchard, ten healthy adjacent trees, uniform in size and vigor, were selected and tagged and were reserved for harvesting throughout the two production seasons (North-Dewing, 2023). Fifteen fruit per tree were harvested from the outside up to 30 cm into the canopy, to obtain a total of 150 fruit per orchard. A complete randomized cold storage experimental design was explored, where 10 fruit per tree from 5 orchards served as replicates and the number of sampled trees within each orchard was considered treatment. On arrival at the laboratory, 10 fruit tree replicates per orchard for each cultivar were placed in cold storage as described in section 2.1 to induce CI, with the remaining five fruit used for maturity indexing, representing day 0, at harvest. Each fruit was then assessed separately. The experiment was completely randomized, with the production area per cultivar (n= 5 x 4) as treatments and orchards (n=5) within a production area as statistical replicates for the different harvest seasons.

To assess the impact of variability in production area on CI susceptibility of citrus, we evaluated for 'Turkey', 'Midnight', 'Nadorcott' and 'Nova' produced in Nelspruit, Letsitele, Sunday's River Valley (SRV), and Citrusdal over 2018/2019 – 2019/2020 production seasons. Concerning the impact of the variability in citrus cultivars, we evaluated for 'Turkey' Valencia, 'Midnight' Valencia, 'Nova' mandarin, 'Nadorcott' mandarin, and 'Star Ruby' grapefruit

were evaluated for CI susceptibility. This was done for 4 production areas (Citrusdal, SRV, Letsitele and Nelspruit) over 2018/2019 – 2019/2020 production seasons.

We also investigated whether CI susceptibility varies between fruit of the same cultivar, harvested from different orchards in a particular area. For this, we considered only Turkey' Valencia, and 'Nova' mandarin from five orchards produced in SRV over two production seasons (2018/2019 – 2019/2020).

2.2.2 Fruit harvest maturity

To determine the impact of fruit maturity at harvest on CI susceptibility of citrus, two cultivars, namely, 'Midknight' Valencia and 'Nova' mandarin, were considered. These citrus cultivars were harvested at two-week intervals from 8 weeks prior to commercial harvest until 6 weeks after to obtain eight sampling periods (Figure 2). The fruit were sampled from commercial orchards in Citrusdal, Western Cape, for the 2018/2019 season (see Figure 1). On each sampling date, 20 fruit were harvested per cultivar. The experimental layout was a complete randomized design where the sampling period was considered the treatment. Five of the 20 fruit sampled per tree were subjected to maturity indexing on day 0 (at harvest), while the remaining fruit were placed in cold storage to induce CI (see section 2.1).

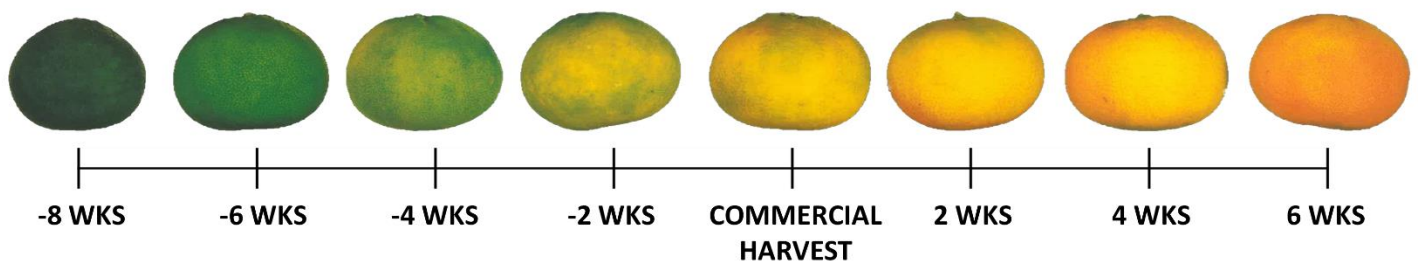


Figure 2. Citrus harvest maturity stages showing color changes with two-weeks harvest interval from 8 weeks before commercial harvest until 6 weeks after. Note that the harvest maturity of citrus produced in South Africa is also influenced by internal quality parameters beyond external color (Caxton Magazines, 2012; Hussain et al., 2022).

2.3 Data collection

2.3.1 Chilling injury evaluation

This study represented chilling injury severity (CIS) as the index of chilling injury (CI). CI index was assessed after 32 days of cold storage at $-0.6\text{ }^{\circ}\text{C}$ plus a seven-day shelf life period at ambient temperature using a four-stage rating scale (Figure 3). They are (i): 0 = no damage, without any chilling injury symptoms (no pitting), (ii): 1= slight pitting of the rind, (iii): 2= moderate pitting of the rind, and (iv): 3= severe chilling injury symptoms, covering more than 30 % of the flavedo rind, rendering the fruit completely unmarketable (Siboza et al., 2017). This was done for each replicate according to the following formula (Equation 1):

$$\text{CI index} = \frac{\text{Chilling injury score (0-3)} \times \text{number of fruit in each score class}}{\text{Total number of fruits evaluated in replicate}} \quad (1)$$

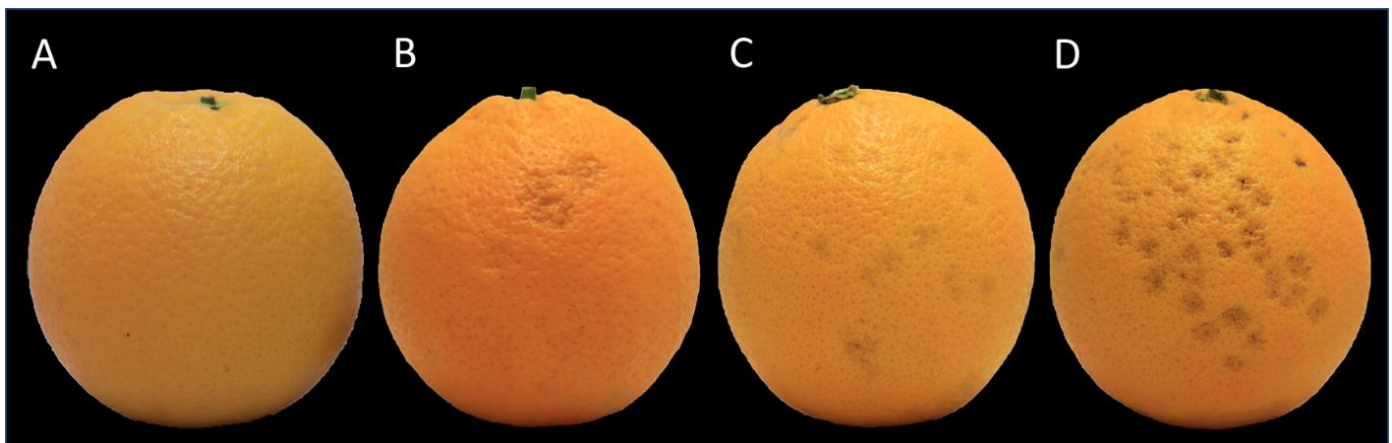


Figure 3. A photographic representation of the scoring system used to calculate the CIS index (North et al., 2024). The scoring scale ranged from 0 = no damage (A), 1 = slight pitting of the rind (B), 2 = moderate pitting of the rind (C), 3 = severe chilling injury symptoms (D).

2.3.2 Weather data

As weather differences were considered one of the main factors differentiating the four respective areas and temperature, rainfall and humidity data were collected from the point of anthesis to harvest. This was done from August 2018 to December 2020 for Nelspruit, Letsitele, Sunday's River Valley (SRV) and Citrusdal areas. Daily minimum and maximum temperature (°C), minimum and maximum relative humidity (%RH) and rainfall (mm) data were obtained using automated weather stations (data supplied by the Agricultural Research Council), for the different production areas.

Vapour pressure deficit (VPD) was derived from these various parameters using the following calculation (Equation 2) (Koverda, 2018):

$$VPD = 610.78e^{\left(\frac{T}{T+23.3}\right)^{17.27}} \times \left(1 - \frac{RH}{100}\right) \quad (2)$$

where T is the average temperature $\left[\frac{(\text{Maximum} + \text{Minimum})}{2}\right]$ (°C)

We also calculated the Heat Units (HU) for each day in the respective areas using the following formula (Equation 3) (Hardy and Khurshid, 2021):

$$\text{Daily HU} = \frac{(\text{Minimum temperature} + \text{Maximum temperature})}{2} \quad (3)$$

Heat units can be used in citrus to assess the suitability of a region for growing citrus (Hardy and Khurshid, 2021). It shows the effect of temperature on the growth and development of a crop. In this study, we used the effective heat unit (EHU) for each area for 2018 - 2020 period.

2.4 Statistical data analysis

Data from the two consecutive growing seasons (2019 and 2020) were used in this analysis. The impact of production area, cultivar, orchard, and fruit harvest maturity stage variability on CI susceptibility of citrus were evaluated. CI susceptibility data were analyzed using 3-way ANOVA for cultivar and orchard variability. Data concerning the differences in CI susceptibility in fruit from various production areas, and fruit maturity were analyzed using a one-way ANOVA. Results for production area and maturity stages were presented as median (centre line), 75th upper and 25th lower quartiles (box limits), and 1.5 × the interquartile range (IQR, whiskers) with a 0.95 confidence level. Fisher LSD test at $p \leq 0.05$ significant level and 95 % confidence interval was used to assess the significant differences in CI susceptibility within groups. This was done for differences in cultivar, orchards, production area, and maturity stages. A fitted probability distribution function and rug plot were applied to visually determine the statistical differences in the impact of production area and maturity stage variability on CI susceptibility. Additionally, a two-sample t-test, assuming equal variances at $p \leq 0.05$ significant level, was used to compare the mean significant difference in CI susceptibility. All analyses were conducted using ORIGINPRO 2022 (64-bit) SR1 (Government) (OriginLab, Northampton, Massachusetts, USA).

3 RESULTS AND DISCUSSION

3.1 How do cultivar, and orchard variations within a production area affect the chilling injury susceptibility of citrus?

We evaluate if differences in cultivars and orchards within a production area have a significant effect on the chilling injury (CI) susceptibility of citrus. During the 2018/2019 – 2019/2020 production seasons (Figure 4), the CI susceptibility was significantly different between cultivars in most production areas, especially SRV (Figure 4C) and Letsitele (Figure 4B). Significant higher CI was reported for 'Turkey' Valencia compared to 'Midnight', 'Nadorcott', 'Nova', and 'Star Ruby' cultivars for Nelspruit, SRV and Citrusdal. This was closely followed by 'Midnight' Valencia. Statistical analysis (Table 1) shows that cultivar has the strongest influence on CI susceptibility, with significant differences across cultivars ($P = 8.33 \times 10^{-92}$). Additionally, interaction effects indicate that responses vary by production area and season (Table 1). For example, 'Turkey' Valencia produced in SRV during the 2019/2020 season recorded the highest CI susceptibility (Mean = 1.74179), significantly higher than all other cultivar and area combinations. In contrast, 'Star Ruby' grapefruit and 'Nadorcott' Mandarin consistently had low CI susceptibility for Nelspruit, Letsitele, and SRV (Figure 4A–C). Importantly, the CI susceptibility for Star Ruby, Midnight and

Nadorcott produced in Nelspruit was similar for both the 2018/2019 and 2019/2020 seasons, reflecting the observed lack of seasonal influence on CI for these cultivars in this area.

These findings demonstrate that CI susceptibility varies significantly among cultivars, and these differences are further modulated by production area and cultivar-area interactions. Seasonal effects, while generally minor, were significant for some cultivar-area combinations (e.g., Citrusdal and 'Turkey' Valencia). This means that the CI susceptibility of fruit varies from cultivar to cultivar within a single production area and season. Similar results have been reported by (North-Dewing, 2023).

Interestingly, the susceptibility of 'Star Ruby' grapefruit to CI was lower than that of Valencia oranges and mandarins, despite grapefruit generally being more sensitive to CI (Rey et al., 2020; Toivonen, 2016). A recent study has demonstrated that rootstocks play a significant role in influencing chilling injury (CI) susceptibility in citrus (Cronje, 2021). In particular, rootstocks were found to enhance the CI resistance of 'Star Ruby' grapefruit, contributing to its lower CI severity compared to Valencia oranges. This was evidenced by a reduction of more than 40% in CI severity for 'Star Ruby' across two growing seasons compared to Valencia oranges. This difference may also reflect the varying harvest timings of cultivars within the region, such as the early harvest of 'Star Ruby' versus the later harvest of 'Midknight' Valencia oranges. These harvest practices align with differences in fruit development rates, which proceed through four stages from flowering to maturity.

We further buttress this interesting result using a correlation heat map (Figure 5). The Pearson correlation coefficient shows how weather affects chilling injury (CI) differently in 'Star Ruby' grapefruit and 'Turkey' Valencia orange, emphasizing cultivar-specific responses. For 'Star Ruby,' minimum relative humidity (RHn) showed a moderate positive correlation with CI, suggesting that higher RHn increases the likelihood of chilling injury. In contrast, total radiation (Rs) was moderately negatively correlated with CI, indicating that higher radiation levels help reduce CI. For 'Turkey' Valencia orange, the correlations were more pronounced: minimum temperature (Tn), minimum relative humidity (RHn), and heat units (HU) were all strongly positively correlated with CI, meaning that increases in these parameters consistently increased the CI susceptibility.

This finding also helps explain why the 'Star Ruby' grapefruit exhibited greater tolerance to chilling injury compared to the Valencia orange. The weaker correlations between weather parameters and CI in 'Star Ruby' suggest that weather variability has a limited effect on its chilling injury, likely due to a combination of genetic factors and environmental influences. While genetic composition largely governs traits like flowering time, fruit development, harvest timing, and inherent CI susceptibility, rootstock selection and pre-harvest weather conditions significantly shape the fruit's physiological response to chilling stress. In regions like Letsitele and Nelspruit, these environmental conditions, coupled with appropriate rootstock selection, likely enhanced the chilling tolerance of 'Star Ruby' grapefruit, making it more resilient compared to Valencia oranges and mandarins. On the other hand, the Valencia orange's strong sensitivity to specific weather parameters, such as minimum temperature (Tn), minimum relative humidity (RHn), and heat units (HU), makes it more vulnerable to CI. These strong positive correlations indicate that environmental variability plays a greater role in intensifying CI susceptibility in Valencia oranges. Together, this highlights the importance of integrating genetic, environmental, and management factors into cultivar-specific strategies to mitigate chilling injury.

We also observed that the CI index was below 2 for all treatment combinations (Figure 4). The relatively modest levels of CI observed may be due to the controlled nature of the chilling treatments, which were designed to simulate commercial storage and shipping conditions rather than extreme scenarios. This approach ensures that the findings are directly applicable to real-world postharvest handling practices. Besides, our results highlight that even relatively low levels of CI can pose significant commercial challenges, particularly for markets that demand high fruit quality and long storage durations.

Harvesting from various orchards within a single production area significantly affected the susceptibility of citrus fruit to CI during the 2019 and 2020 seasons (Figure 6). This was particularly evident for Nadorcott, Turkey, and Midknight cultivars, where CI susceptibility varied significantly across orchards. ANOVA results (Table 2) confirmed that orchard ($P = 2.22 \times 10^{-5}$) and its interaction with growing season ($P = 7.59 \times 10^{-8}$) and cultivar ($P = 9.21 \times 10^{-46}$) significantly influenced CI susceptibility. Furthermore, CI susceptibility differences among orchards were less pronounced in 2020 than in 2019 (Table 2). For the 2020 season, only 2 out of 5 orchards demonstrated significantly different CI susceptibility levels, compared to 3 out of 5 orchards in 2019. This variation reflects the importance of pre-harvest conditions and orchard-specific management practices in determining CI susceptibility. Additionally, the Fisher test highlighted that CI susceptibility was strongly influenced by cultivar ($P = 1.13 \times 10^{-97}$), with Turkey showing the greatest variability and Nadorcott consistently exhibiting the least susceptibility (Table 2). These results

demonstrate the critical role of orchard and cultivar interactions in reducing CI susceptibility, further emphasizing the need for tailored management strategies for each orchard within a production area.

In summary, when growers in a certain region are growing citrus, they should expect statistically significant differences in chilling injury to occur: (1) between different orchards; (2) between different citrus cultivars, especially for 'Turkey' and 'Midnight' Valencia; and (3) between different harvest seasons. Based on our findings, data for the two harvest seasons, four production areas, five different cultivars and orchards were used to investigate the impact of weather differences in production areas on CI susceptibility.

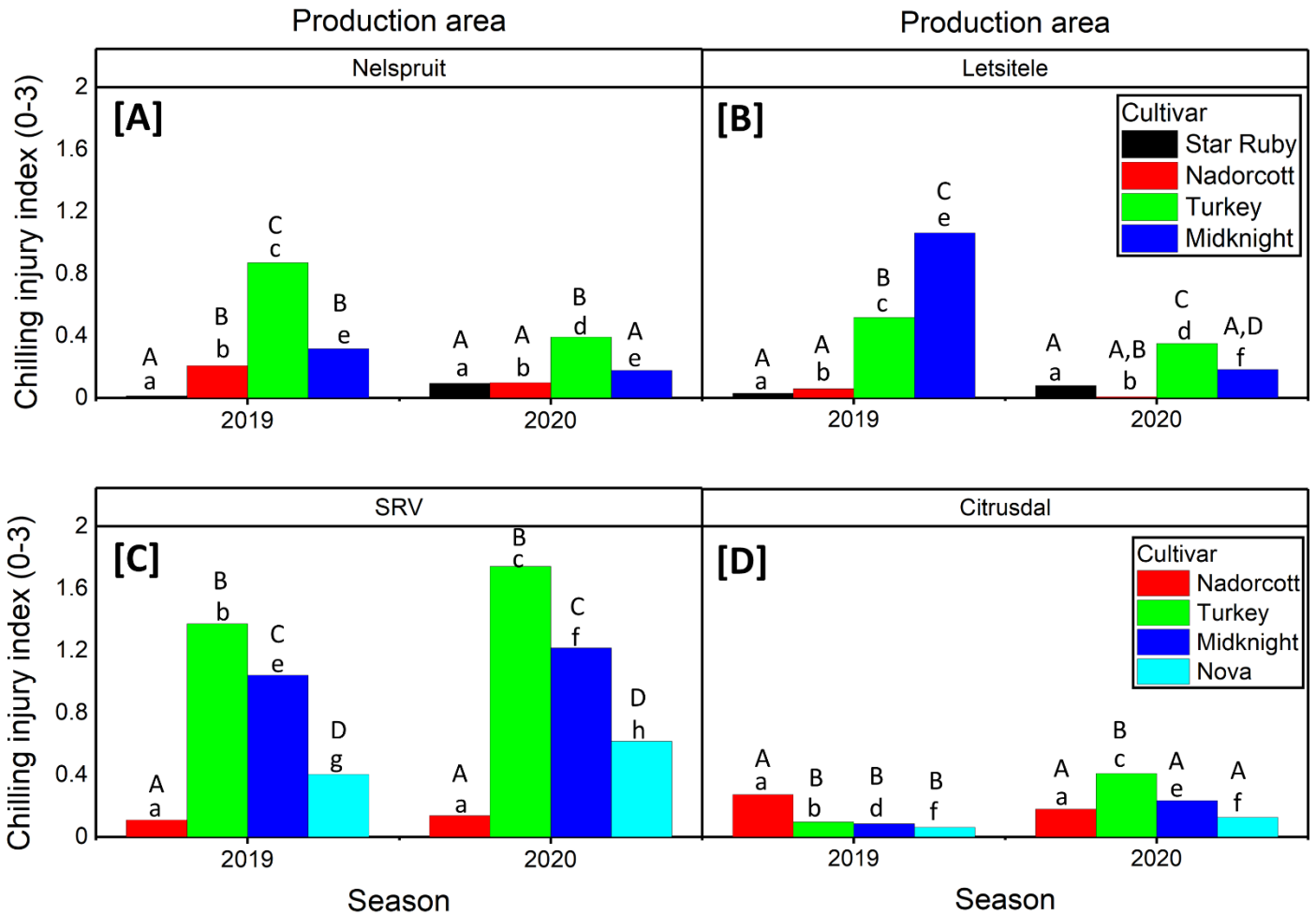


Figure 4. The interaction between harvest seasons and citrus cultivars produced in different regions on chilling injury susceptibility. Bars for each cultivar with different lowercase letters (e.g., 'a', 'b', 'c') indicate significant differences between different seasons at $p < 0.05$. Bars with different uppercase letters (e.g., 'A', 'B', 'C') indicate significant differences between cultivars within the same season at $p < 0.05$. The letters are assigned independently within each production area and are not indicative of absolute rankings across seasons, areas or cultivars. Statistical comparisons were conducted using ANOVA followed by the Fisher LSD test at $p \leq 0.05$.

Table 1. Overall ANOVA showing the variability of production area, cultivar, and production season into main effects, two-way interactions, and three-way interactions.

	DF	Sum of Squares	Mean Square	F Value	P Value
Cultivar	4	71.69	17.92	123.78	8.33×10^{-92}
Season	1	3.41×10^{-13}	3.41×10^{-13}	2.36×10^{-12}	1
Area * Cultivar	12	71.38	5.95	41.09	1.96×10^{-84}
Area * Season	3	6.82×10^{-13}	2.27×10^{-13}	1.57×10^{-12}	1
Cultivar * Season	4	1.73	0.43	2.98	0.018
Area * Cultivar * Season	12	14.14	1.18	8.14	5.29×10^{-15}
Model	39	306.13	7.85	54.22	4.95×10^{-258}
Error	1557	225.43	0.145		
Corrected Total	1596	531.55			

P value at 0.005 level; DF = degree of freedom

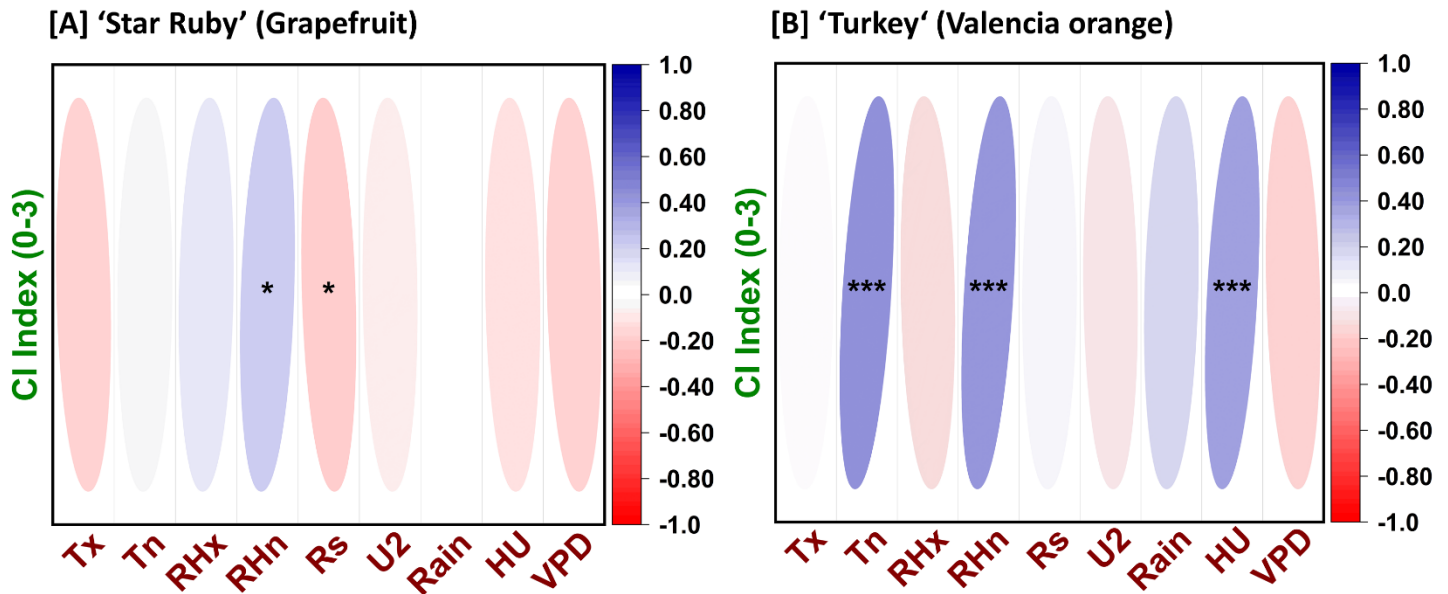


Figure 5. Pearson's correlation coefficients between different climatic parameters (x-axis in red) and chilling injury (CI) index of citrus (y-axis in green), for [A] 'Star Ruby' grapefruit, [B] 'Turkey' Valencia orange produced in Nelspruit and Letsitele. The positive (red) and negative (blue) correlation are indicated, and the color gradient depicts each correlation's strength. Black asterisks represent p-value as *** for $p \leq 0.001$, ** for $p \leq 0.01$, and * for $p \leq 0.05$. The orientation of the ellipse indicates the direction of the correlation, while its shape illustrates the strength of the correlation. The size of the ellipse suggests the confidence level of the correlation, with a smaller ellipse representing higher confidence, indicating that the correlation is more likely to be reliable. Tx: Daily Maximum Temperature ($^{\circ}\text{C}$); Tn: Daily Minimum Temperature ($^{\circ}\text{C}$); Rain: Total Rainfall [Calculated From Hourly Data] (mm); Rs: Total Radiation [Calculated From Hourly Data] (MJ/m^2); U2: Average Wind Speed [Calculated From Hourly Data] (m/s); RHx: Daily Maximum Relative Humidity (%); RHn: Daily Minimum Relative Humidity (%); HU: Total Heat Units [Calculated From Hourly Data] (Unitless); VPD: Vapour Pressure Deficit [Calculated From Hourly Data / 06:00 - 18:00]. Note that CI index represents chilling injury susceptibility.

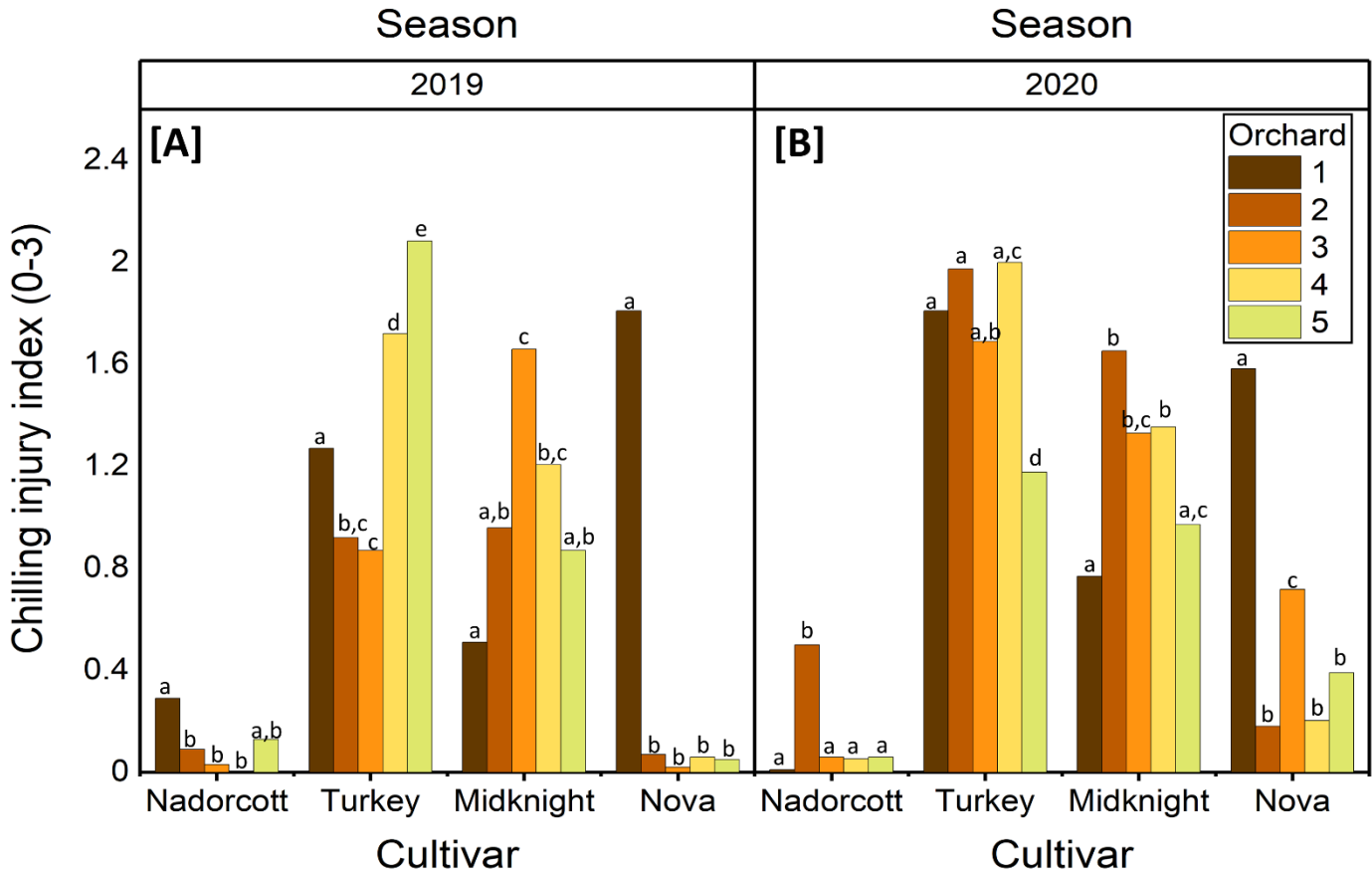


Figure 6. The interaction between the orchards for different citrus cultivars produced in SRV on chilling injury susceptibility for [a] 2018/2019 season and [b] 2019/2020 season. Letters above bars represent statistical groupings based on the Fisher test. Bars with the same letter between orchards of a particular cultivar are not significantly different ($P > 0.05$) within the same season. The letters are assigned independently within each season or cultivar and are not indicative of absolute rankings across seasons or cultivars.

Table 2. Overall ANOVA showing the main effects, two-way interactions, and three-way interactions of the variability in orchards, cultivars, and production season for SRV.

	DF	Sum of Squares	Mean Square	F Value	P Value
Season	1	3.73	3.73	27.87	2.25×10^{-7}
Orchard	4	3.70	0.93	6.93	2.22×10^{-5}
Cultivar	3	120.82	40.27	301.29	1.13×10^{-97}
Season * Orchard	4	5.46	1.37	10.22	7.58×10^{-8}
Season * Cultivar	3	1.37	0.456	3.41	0.018
Orchard * Cultivar	12	46.40	3.87	28.93	9.21×10^{-46}
Season * Orchard * Cultivar	12	12.18	1.01	7.59	1.50×10^{-12}
Model	39	194.45	4.99	37.30	4.43×10^{-103}
Error	359	47.99	0.13		
Corrected Total	398	242.44			

P value at 0.005 level; DF = degree of freedom

3.2 What is the impact of differences in production area on chilling injury susceptibility of citrus?

We evaluate the impact of production area variability on CI susceptibility. We do this only for 'Turkey' and 'Midnight' Valencia, and 'Nadorcott' and 'Nova' Mandarin cultivars. The impact of the variability in production area on Star Ruby was not considered as it has the lowest CI compared with other cultivars (North-Dewing, 2023; North et al.,

2024). Chilling injury susceptibility differed significantly between production areas during both 2018/2019 and 2019/2020 growing seasons (Figure 7). In 2018/2019 season, the Valencia oranges from SRV sustained a higher CI compared to the other production areas (Figure 7A - B). This result is similar to that of the 2019/2020 season. On the other hand, the Citrusdal production area had the lowest CI for Turkey, Midnight and Nova during the 2019 season. The CI sustained by Valencia oranges from Citrusdal did not differ significantly from that sustained by the fruit from Letsitele and Nelspruit during the second season. 'Nadorcott' mandarin produced in SRV had similar CI with those of Letsitele in the first year, but differed significantly in the second season. The CI susceptibility of mandarin produced in Citrusdal is significantly different from those produced in SRV for both seasons, but is similar to those from Nelspruit. The coastal SRV production area, which is known for its high summer temperatures, displayed the highest CI susceptibility. The reason for the high CI susceptibility of citrus in SRV is related to the local weather conditions and the fruit's internal and external quality attributes (North-Dewing, 2023).

SRV had the highest average minimum temperature (11 °C) compared to Citrusdal (9 °C), with the lowest score for the two harvest seasons (Figure 8). The average temperature of the two seasons for SRV is ~8 % higher than that of Citrusdal. However, the minimum and maximum temperature difference for Citrusdal is 6 % higher than that of SRV. This suggests that fruit tissues may better withstand low postharvest temperatures when exposed to low pre-harvest temperatures that remain above the CI threshold for citrus in South Africa. A CI threshold of 4 °C was identified for several citrus varieties, including soft citrus, grapefruit, and Valencia under tested conditions (Cronje, 2022). Studies have shown that high pre-harvest temperature increases the risk of CI (Botina A et al., 2019; Bugaud et al., 2016; Lange and Cameron, 1997). Also, Citrusdal has higher average relative humidity, with more constant maximum relative humidity than SRV for the two seasons. The average rainfall, for Citrusdal, a winter rainfall region, was 50% higher compared to SRV for both harvest seasons. In fact, the rainfall distribution over the 65 days before harvest was more pronounced for Citrusdal compared to SRV (Figure 8C). This climatic condition tends to produce citrus fruit with thicker skin and higher cell turgor (Albrigo, 1975; Reuther, 1988) compared to those from a more humid climate. So differences in CI between production areas could be linked to local weather conditions. If we compare production areas with respect to chilling injury incidence, we are actually comparing different weather conditions.

We examined the correlation between different climatic parameters and CI index to identify the factors influencing the CI susceptibility of citrus (Figure 9). The correlation analysis confirms that regional weather variations significantly influence CI susceptibility in citrus fruit, with notable differences across the four production areas.

Letsitele showed the strongest correlations between weather parameters and CI susceptibility, with significant negative relationships observed for minimum temperature (T_n), relative humidity (RHx and RHn), solar radiation (Rs), heat units (HU), and vapor pressure deficit (VPD) (Figure 9B). This highlights the importance of pre-harvest climatic conditions in this region, where high relative humidity and low VPD may predispose fruit to moisture retention and subsequent CI during cold storage. In contrast, Nelspruit demonstrated moderate CI susceptibility, with negative correlations pointing to the influence of temperatures (T_n) and wind speed (U2) on fruit vulnerability.

Nelspruit (Figure 9A) and SRV (Figure 9C) showed fewer weather parameters significantly influencing CI susceptibility compared to Letsitele and Citrusdal. In both regions, minimum temperature (T_n), total radiation (Rs), and heat units (HU) emerged as critical factors, consistently showing significant correlations with CI susceptibility. However, in the SRV region, parameters such as maximum temperature (T_x), minimum temperature (T_n), Rs, wind speed (U2), HU, and vapor pressure deficit (VPD) were all strongly and positively correlated with CI, unlike the patterns observed in Nelspruit and Letsitele. These findings further support the earlier observation that high pre-harvest temperatures significantly increase the risk of CI. Additionally, wind speed (U2) showed a region-specific impact on CI susceptibility. In SRV, U2 was positively correlated with CI, indicating that higher wind speeds exacerbate factors that make citrus fruit more prone to CI during the postharvest cold chain. In contrast, U2 was negatively correlated with CI in Nelspruit and had no significant effect in Letsitele and Citrusdal. The positive correlation between U2 and CI in SRV suggests that higher wind speeds in this region may contribute to increased fruit stress, potentially amplifying the susceptibility to chilling injury during postharvest storage and transport. This could be attributed to several potential mechanisms including transpiration and moisture stress, microclimatic condition alteration, and mechanical stress (Bock et al., 2010; Cataldo et al., 2013; Duvenage, 2023). Nonetheless, the relatively limited number of significant correlations between weather parameters and CI in these regions already suggests that other factors, such as cultivar-specific traits, rootstock, and harvest maturity also have a significant influence.

Across all regions, minimum temperature (T_n) consistently emerged as the most influential weather parameter, with lower nighttime temperatures associated with increased CI susceptibility. Relative humidity (RHx and RHn), heat

units (HU), and VPD were also significant contributors, varying in their impact across regions. These findings emphasize the need for region-specific management strategies, particularly in SRV, where CI risk is highest, and Letsitele, where weather conditions strongly influence susceptibility. By tailoring pre-harvest and postharvest practices to each region's climatic profile, the risk of CI can be effectively mitigated during the export cold chain.

By comparing the impact of orchards, cultivar, and production area variability on CI susceptibility of citrus (Figure 10), we see that the differences in the production area have the most impact on CI. The local weather conditions, therefore strongly determine the incidence of chilling injury.

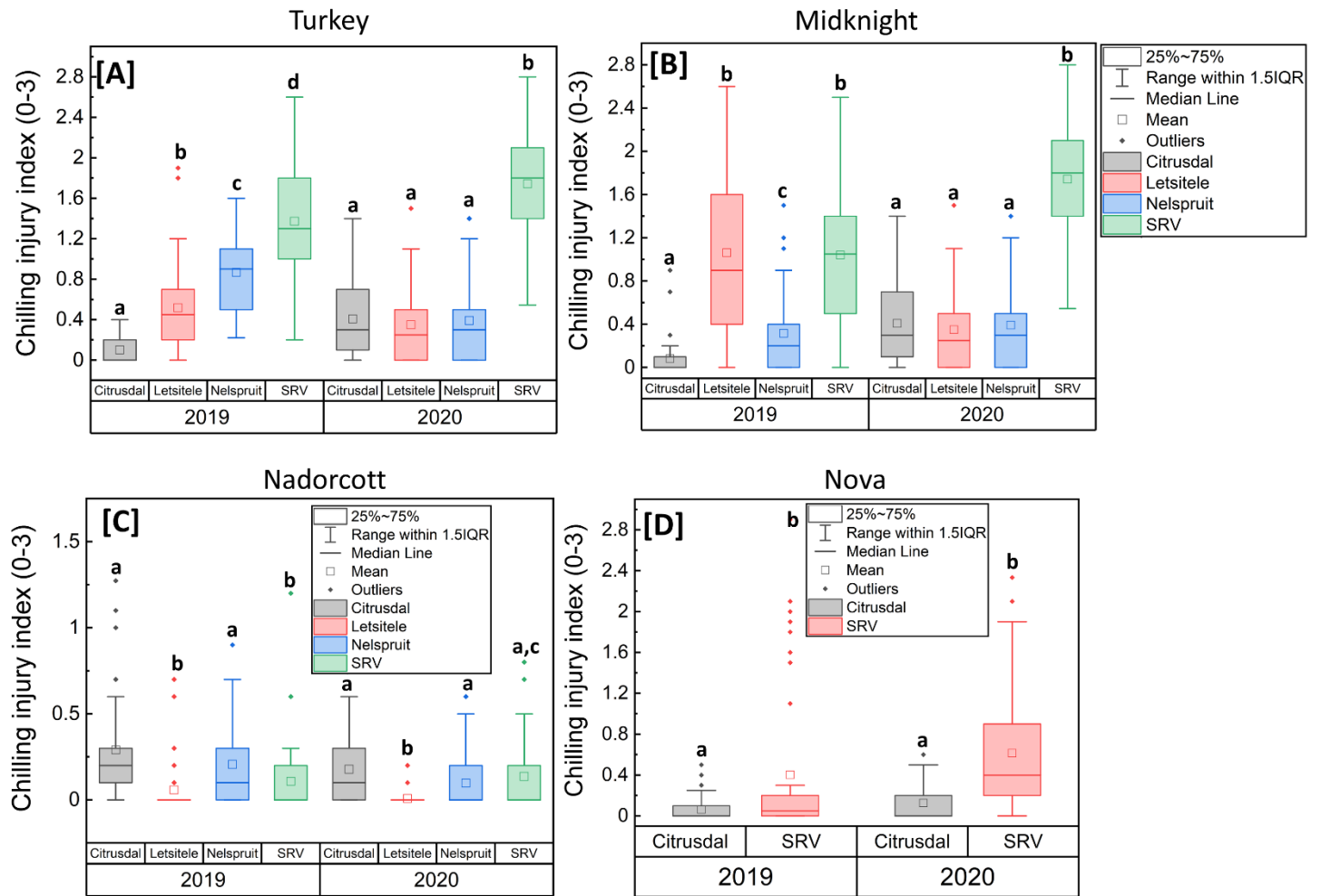


Figure 7. Impact of production area on chilling injury susceptibility of [A] 'Turkey' Valencia, [B] 'Midnight' Valencia, [C] 'Nadorcott' Mandarin, and [D] 'Nova' Mandarin for 2018/2019 and 2019/2020 seasons. The boxplots represent the median (centre line), 75th upper and 25th lower quartiles (box limits), and 1.5× the interquartile range (IQR, whiskers). Different lowercase letters between production areas per season, namely, Citrusdal, Letsitele, Nelspruit, and SRV, signify statistically significantly different groups at $p \leq 0.05$.

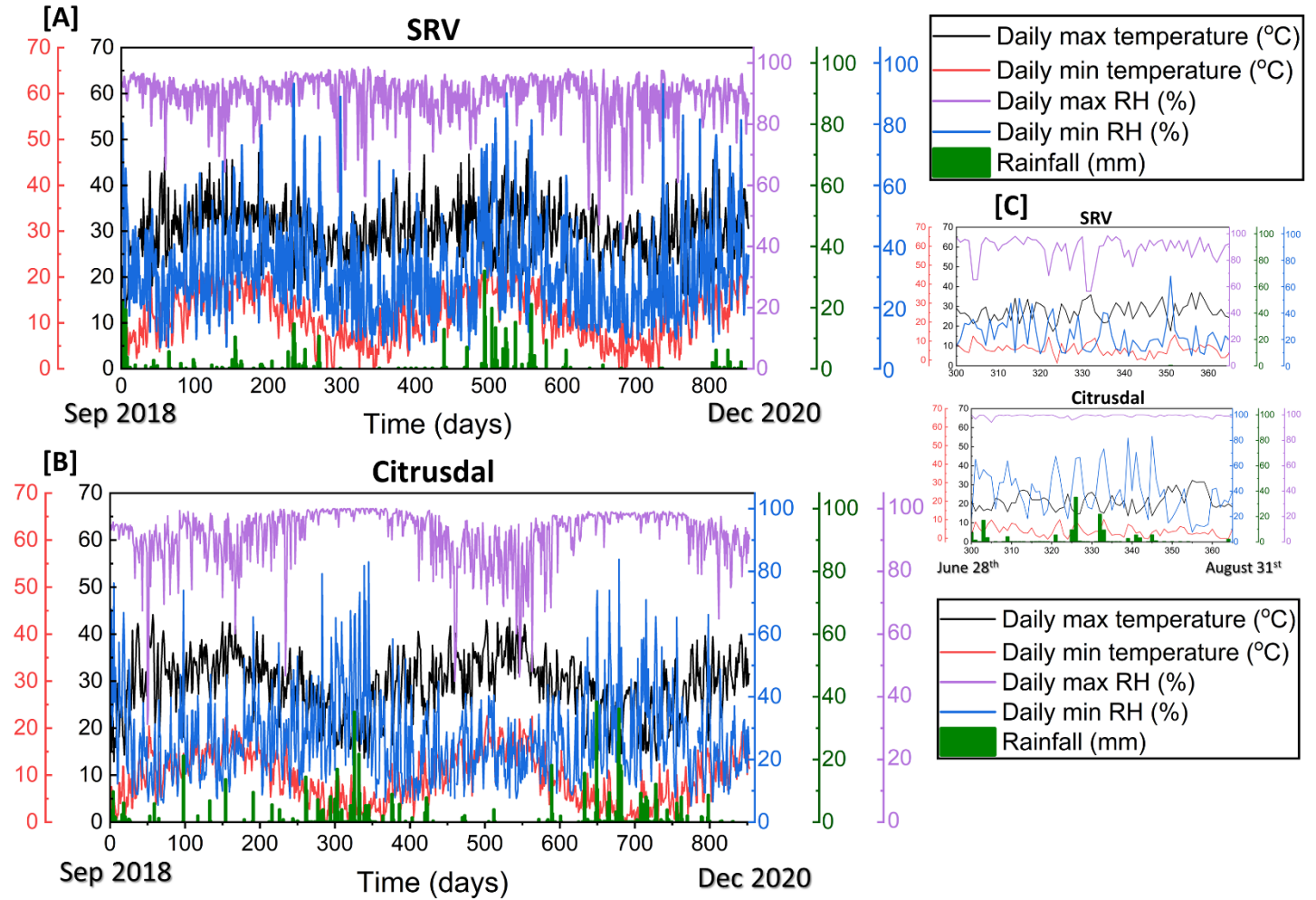
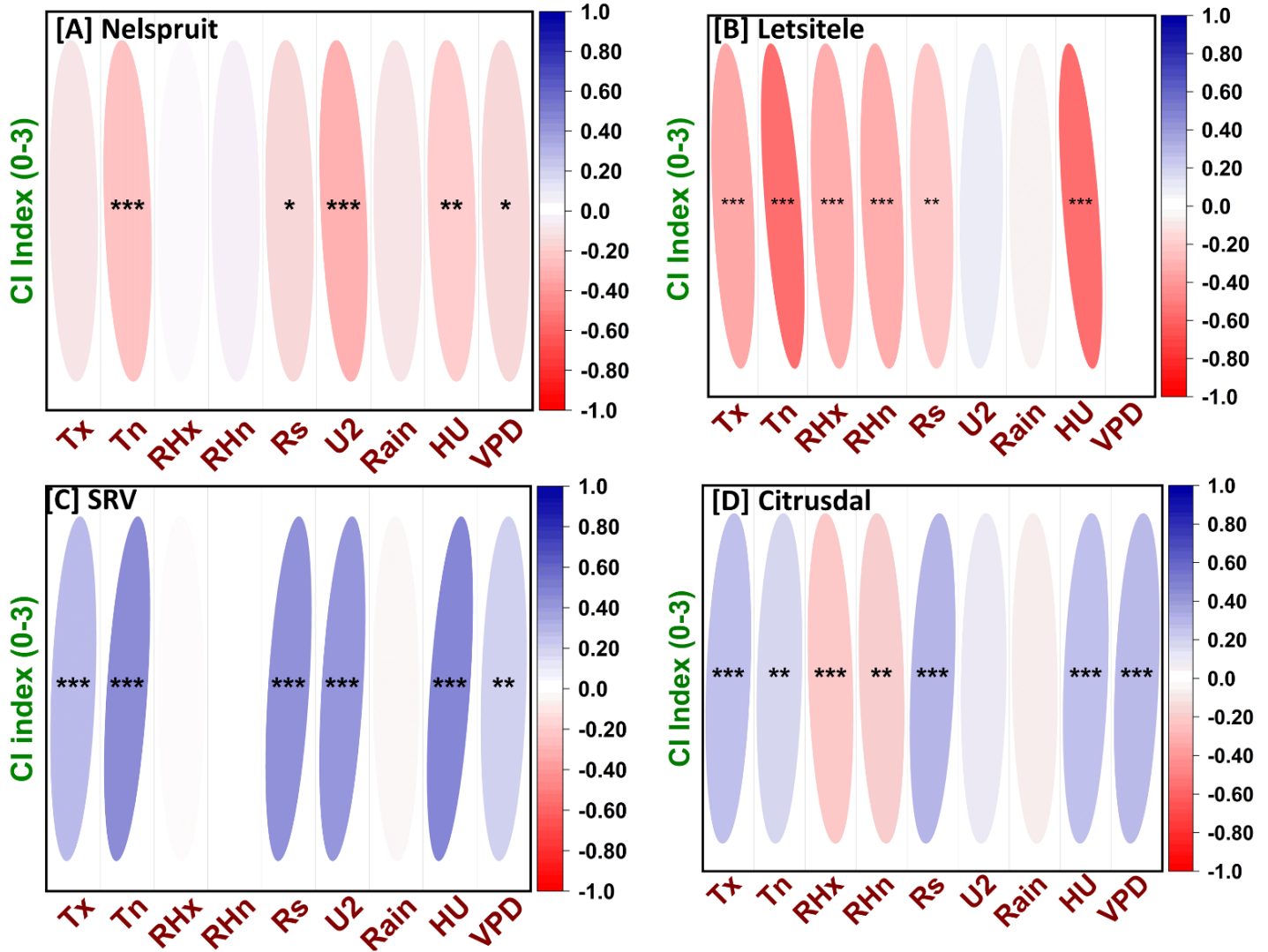


Figure 8. Daily maximum and minimum temperature ($^{\circ}\text{C}$), daily maximum and minimum relative humidity (RH) (%), and average rainfall (mm) in [A] SRV and [B] Citrusdal production areas, over 2018/2019 to 2019/2020 seasons. Figure [A] and [B] were scaled to show only 65 days prior to harvest (June 26th to August 31st 2019) and represented in [C]. Temperature, and relative humidity are depicted by line graphs and rainfall is indicated by bar graphs.



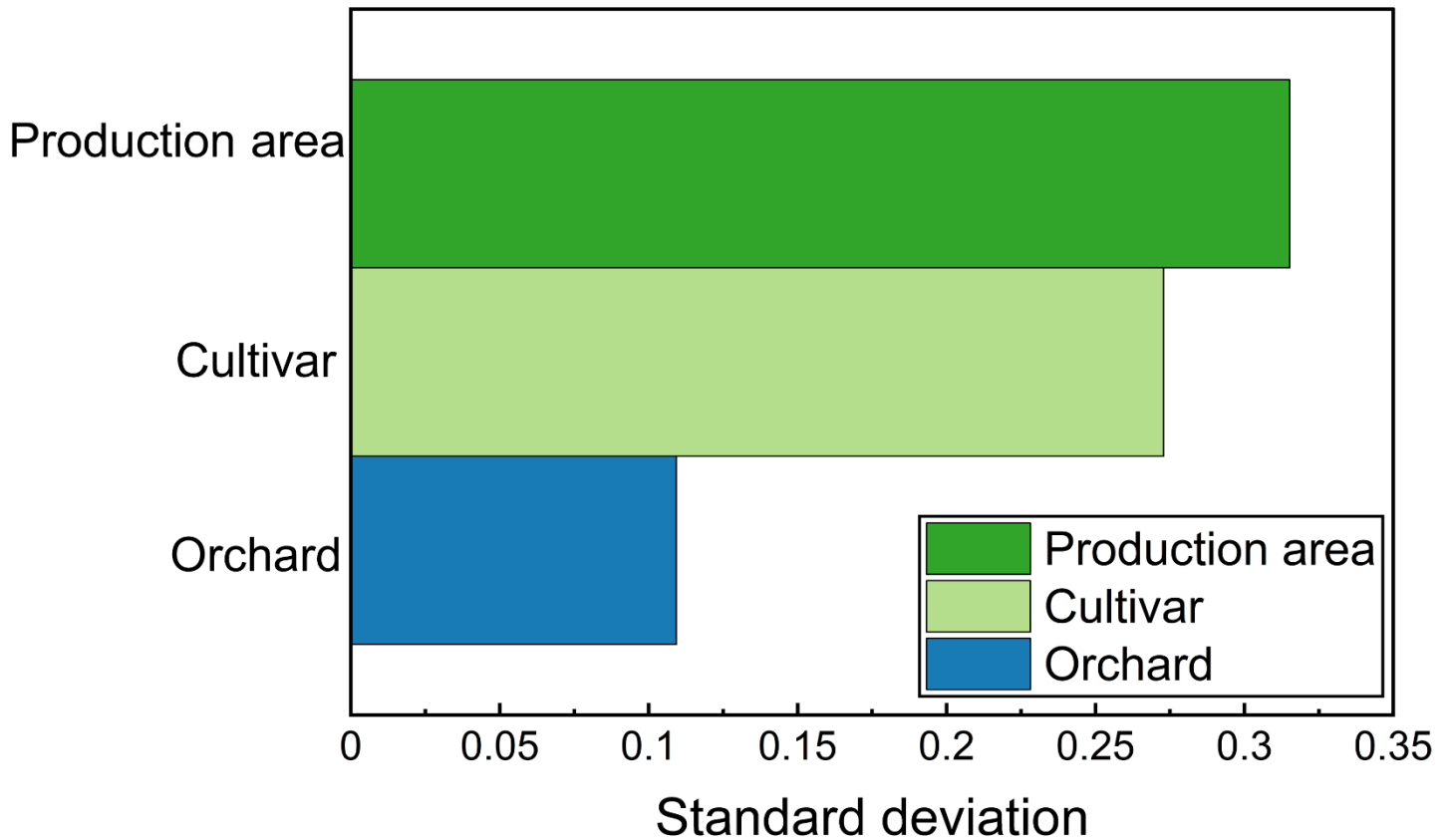


Figure 10. Ranking the impact of various pre-harvest factors on postharvest chilling injury susceptibility of citrus

3.3 What is the impact of the variability in fruit harvest maturity on the chilling injury susceptibility of citrus?

We evaluate the impact of the variability in fruit maturity over a 14-weeks harvesting period on citrus's chilling injury (CI) susceptibility. We do this for 'Nova' Mandarin and 'Midnight' Valencia cultivars (Figure 11). The susceptibility of citrus to chilling injury greatly depends on the cultivar and the day of harvest (Figure 11). The maturity stages at harvest significantly impact CI susceptibility for 'Nova' mandarin (Figure 11A), but not so much for 'Midnight' Valencia (Figure 11B). This means that 'Nova' Mandarin was more sensitive while 'Midnight' Valencia was resistant. For Midnight cultivar, the early-season fruit (-8 weeks before commercial harvest) were most susceptible to CI and were significantly different from those harvested afterwards (Figure 11B). For this cultivar, fruit harvested later in the season had very low susceptibility to CI. However, this was not the case for Nova cultivar, where the highest CI susceptibility was observed for fruit harvested 2 weeks after the commercial harvest (Figure 11A). The CI index for fruit at this harvest maturity stage was 37 %, 87 %, 62 %, 93 %, 100 %, 95 %, and 75 % higher than those harvested at maturity stages -8, -6, -4, 0, 4, and 6, respectively.

Nonetheless, the early season fruit (-8 weeks before commercial harvest) also had a significantly higher CI susceptibility than those harvested at different times. For both cultivars, fruit harvested at the commercial maturity stage were least susceptible to CI. A pattern emerged with very low CI susceptibility for fruit harvested 2 weeks before and 4 weeks after the commercial maturity stage of harvest for both cultivars. If CI is expected for a certain production area, producers could be advised always to harvest a certain cultivar pre or post mature. These findings are slightly different from those in the literature. Early-season harvested 'Fortune' mandarins were more resistant to CI than mid-season fruit (Lafuente et al., 1997). However, our findings for Midnight were similar to those of grapefruit, where early-season harvest fruit exhibited the highest susceptibility compared to those at later stages (Schirra et al., 1998). As already observed for other pre-harvest factors, the impact of variability in the maturity stage at harvest highly depends on the variability in cultivars, production areas, and orchards.

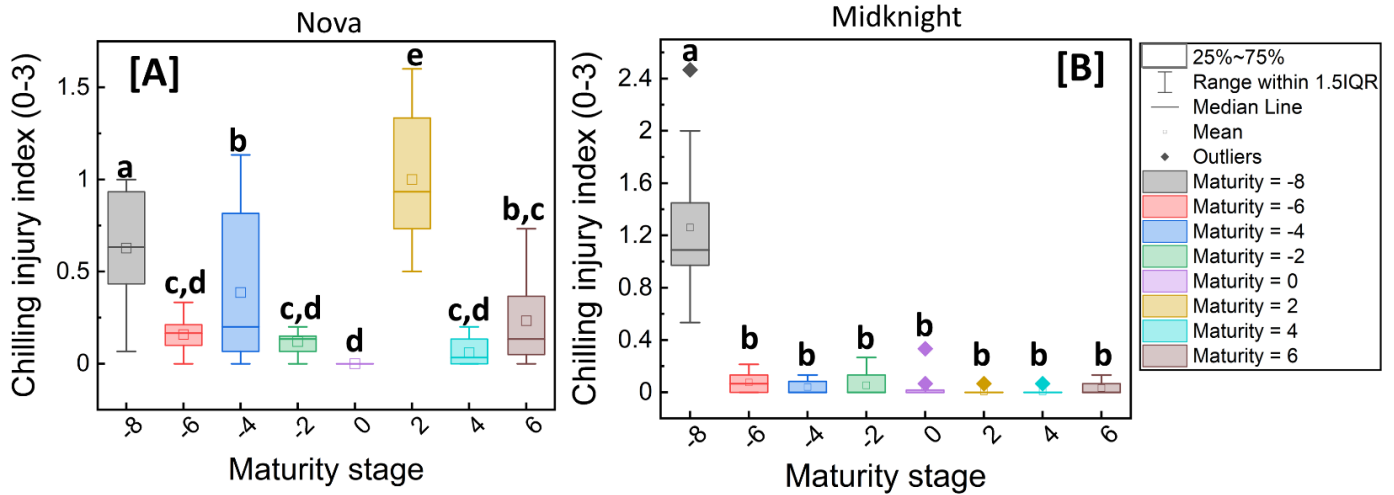


Figure 11. Impact of maturity stages at harvest on chilling injury susceptibility of [A] 'Nova' mandarin, [B] 'Midnight' valencia produced in Citrusdal for the 2019 season. The boxplots represent the median (centre line), 75th upper and 25th lower quartiles (box limits) and 1.5× the interquartile range (IQR, whiskers). Different lowercase letters between maturity stages, from 8 weeks prior to commercial harvest until 6 weeks after, signifies statistically significantly different groups at $p \leq 0.05$.

3.4 Which pre-harvest factor has more impact on chilling injury severity?

The chilling injury (CI) susceptibility of citrus due to (1) production area variability and (2) variability in fruit maturity stage at harvest was compared. A probability distribution function and rug plot were used, as shown in Figure 12. Our findings on preharvest factors affecting citrus reveal valuable insights for citrus growers and stakeholders in the supply chain. For 'Nova' mandarin, the susceptibility to chilling injury (CI) is influenced similarly by the inherent variability in the fruit maturity stage at harvest and the variability in production areas (Figure 12A). On the other hand, the regional weather variability between production areas has a larger impact on CI susceptibility of 'Midnight' Valencia compared to the variability in the maturity stages of fruit at harvest (Figure 12B). The impact is more than 70 % larger than that of the fruit maturity stage. These findings have important practical implications. Growers in their respective production areas can make informed decisions regarding the maturity index to minimize CI risk. However, this decision comes with its own set of challenges. If pre or post-mature fruit is found to have compromised other quality indicators such as taste, growers may face a dilemma. They must balance reducing CI susceptibility and ensuring fruit quality and taste meet consumer expectations.

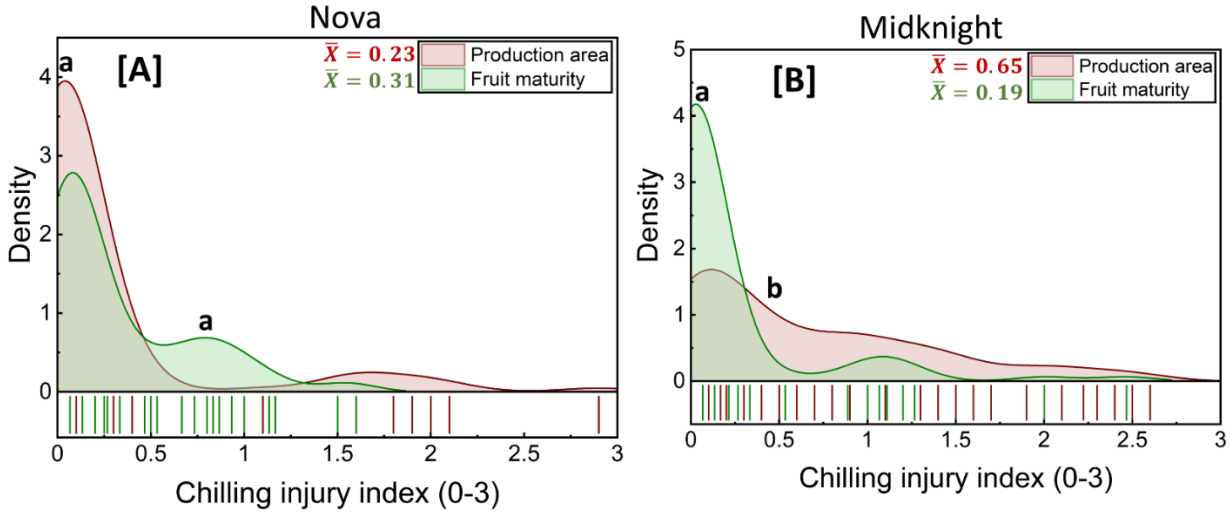


Figure 12. Rug plot with kernel density estimates for Scott bandwidth smoothers comparing the impact of production area and fruit maturity stages on chilling injury severity with Nova [A] and Midnight [B] for the 2019 season. The rug plot shows the actual data set used for the \bar{X} = standard deviation between groups. Two sample t-test with equal variance assumed at $p \leq 0.05$ significant level was used to compare the mean significant difference of chilling injury susceptibility due to production area and fruit maturity stage variability. They are indicated with letters a and b for statistically significantly different groups.

4 CONCLUSIONS

This study quantified the impact of variations in production area, cultivar, orchard, and fruit maturity stage on citrus's chilling injury (CI) susceptibility. We did this for 'Turkey' Valencia, 'Midnight' Valencia, 'Nova' Mandarin, 'Nadorcott' Mandarin, and 'Star Ruby' grapefruit produced in Nelspruit, Letsitele, Sunday's River Valley (SRV), and Citrusdal over 2019 – 2020 seasons. For each cultivar evaluated, 10 fruit per tree from 5 orchards in the different production areas were used. For the impact of the variability in fruit maturity stage at harvest on CI susceptibility, 'Midnight' Valencia, and 'Nova' mandarin harvested in 8 different periods for 14 weeks, were considered. The key conclusions derived from this study are as follows:

- Variations in cultivar, orchards, production area, and fruit maturity stage at harvest significantly impact the CI susceptibility of citrus. Compared to cultivar, and orchard variability, the regional weather differences in production area impact CI susceptibility the most.
- Fruit produced in the coastal region of SRV are most susceptible to CI during the postharvest export cold chain compared to those produced in Nelspruit (70 % less), Letsitele (67 % less), and Citrusdal (80 % less). Fruit produced from the cold-winter region of Citrusdal have the lowest risk of CI. Since fruit are not packed or shipped based on areas where they are produced, it means that fruit that get to the retailer have different levels of CI susceptibility and other thermal-driven spoilage.
- The 'Valencia' oranges (especially Turkey) have the highest risk of CI during postharvest export cold chain compared to mandarin (>60 % less) and grapefruit (> 80% less), with grapefruit (StarRuby) having the least susceptibility to CI. This is because 'Valencia' oranges are highly sensitive to temperature, relative humidity, and heat units during pre-harvest fruit growth and development, whereas grapefruit exhibits a weaker relationship to these weather factors. Our finding aligns with previous research findings. Although some studies have shown that 'Valencia' oranges are indeed more prone to CI compared to other citrus fruits like mandarins and grapefruits (Ehlers, 2016; North et al., 2024; Onwude et al., 2022), it is widely reported that grapefruit is more sensitive to chilling injury compared to oranges and mandarin (Lesar, 2006; Rey et al., 2020; Salvo et al., 2021). This contradiction presents an opportunity for further studies on CI specifically focused on grapefruit production in South Africa.
- Irrespective of the cultivar, fruit harvested very early in the season (e.g., 8 weeks before commercial harvest) have a very high risk of CI, while those harvested at the commercial maturity stage are the least susceptible to CI. This means that citrus growers should be cautious when considering early-season harvesting, as it can result in a higher likelihood of CI development. Instead, aiming for harvesting at the commercial stage can significantly reduce CI risk.

- CI susceptibility for fruit harvested 2 weeks before the commercial maturity stage of harvest is also low. This suggests that growers should aim for harvest within this optimal window to minimize the risk of CI. Our findings align with previous research on the effect of harvest maturity on the chilling injury of citrus (Cronje, 2022; Lo'ay and Dawood, 2019).
- Both production area and fruit maturity stage variations result in significant differences in the chilling injury susceptibility of citrus during cold storage. However, compared to the fruit maturity stage at harvest, the variability in production area has a more than 70 % larger impact for 'Midknight' Valencia. This means that growers in different production areas must carefully consider the maturity index to minimize CI risk, but this choice presents challenges. If pre or post-mature fruit compromises taste and other quality indicators, growers should balance CI susceptibility with meeting consumer taste and quality expectations.

This study has answered the question of which major pre-harvest factor has the highest impact on CI susceptibility of citrus during postharvest long cold storage periods. With such valuable insight, trade-offs between pre-harvest practices and supply chain logistics could also be established. Here, new supply chain strategies based on production area, cultivars, and maturity stage at harvest could be developed where shipments are sent at higher delivery air temperatures to save energy and cost. As our study is the first report on the effect of fruit maturity on the susceptibility to CI in 'Nova' mandarin and 'Midknight' Valencia, it is important for future studies also to be conducted in a broader range of cultivars, production conditions and areas, to validate our initial findings.

AUTHOR CONTRIBUTIONS

DO: Conceptualization, Methodology, Investigation, Writing Original draft, Review & Editing. **JN:** Data Collection, Methodology, Investigation, Review & Editing. **TD:** Conceptualization, Methodology, Review & Editing. **PC:** Conceptualization, Methodology, Review & Editing.

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