



## Temperature-Driven Variability in *Candidatus Liberibacter asiaticus* Titer Across Citrus Hosts, Tissues, and Seasons in Saudi Arabia

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### Abstract

Huanglongbing (HLB), caused by *Candidatus Liberibacter asiaticus* (CLAs), continues to challenge citrus production in Saudi Arabia. Although HLB is widespread, there is still limited understanding of how temperature, season, host species, and the type of tissue sampled influence the reliability of CLAs detection. In this regard, 123 CLAs-positive samples sourced from major citrus-growing areas were analyzed. These samples included data from 4 major citrus species and 4 tissue types. Additionally, cycle threshold (Ct) values obtained through quantitative PCR for each sample were used to examine how climate and CLAs interact in bacterial detection. As lower Ct values indicate higher bacterial abundance, an analysis of covariance (ANCOVA) was conducted to assess the influence of season, tissue type, citrus species, and average seasonal temperature on Ct values. The results demonstrated significant differences among tissue types, with petiole and midrib tissues exhibiting consistently lower Ct values. Additionally, lower Ct values were observed in spring and autumn, and higher Ct values in warmer summers and colder winters, based on average temperatures. Among the citrus species, only *Citrus limon* illustrated a prominent increase in CLAs levels as temperatures increased. CLAs levels in *C. limon* showed only minor fluctuations with temperature. Taken together, these results indicated that temperature gradients strongly shape CLAs population levels in Saudi Arabia and influence the reliability of pathogen detection. Considering climatic context, along with tissue type and season can substantially improve sampling strategies and enhance the effectiveness of HLB monitoring programs in arid and semi-arid citrus production systems.

**Keywords:** bacterial population dynamics; citrus pathology; disease epidemiology; seasonal variation; temperature

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### INTRODUCTION

Huanglongbing (HLB), also known as citrus greening, is one of the most destructive citrus diseases worldwide (Garnier and Bové, 1983; da-Graca, 1991; Bové, 2006; Gottwald, 2010). First described in southern China in the early twentieth century, the disease has since spread to nearly all major citrus-producing regions of Asia,

Africa, and the Americas, with documented occurrences in the Caribbean and Latin America (Gottwald, 2010; Nehela and Killiny, 2010; Wang et al., 2017; Collins et al., 2025; Paredes-Tomás et al., 2025). HLB is caused by phloem-limited  $\alpha$ -proteobacteria in the genus *Candidatus Liberibacter*, of which *Ca. Liberibacter asiaticus*

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(CLas) is the most prevalent and economically damaging species (Jagoueix et al., 1994; Halbert and Manjunath, 2004; Grafton-Cardwell et al., 2013). The Asian citrus psyllid (ACP), *Diaphorina citri*, serves as the principal vector (Bové, 2006; Gottwald, 2010).

Infected citrus trees exhibit a range of symptoms, including blotched and mottled leaves, distorted fruits, aborted seeds, premature fruit drop, reduced canopy density, and decreased fruit quantity and quality (Graham et al., 2020). Detection of *Candidatus Liberibacter* has always been a challenge because of its highly irregular presence in infected tissues (Teixeira et al., 2005; Tatineni et al., 2008; Li et al., 2009) and significant seasonal variations in bacterial density (Hu et al., 2014; Sauer et al., 2015). These have made quantitative polymerase chain reaction (qPCR) the most accurate means for diagnosing infection (Li et al., 2006). However, the efficacy of qPCR detection has consistently shown strong dependence on environmental conditions, sampling time, host species, and, most importantly, the types of tissue sampled (Lopes et al., 2009, 2013; Trivedi et al., 2009).

Tissue type has a considerable influence on detection sensitivity. Midribs, petioles, young stems, and peduncles contain more bacterial counts. Thus, cycle thresholds (Ct) are lower than those for leaf lamina tissue or fruit rind tissue samples (Li et al., 2006; Johnson et al., 2014; Canale et al., 2017). Root and peduncle tissue samples often perform best for variable infections and/or infections that occur earlier in a tree (Louzada et al., 2016). However, other studies conducted in a field setting have found only minor variations among tissues for specific infections (Razi et al., 2014).

Temperature is another key ecological factor shaping CLas population dynamics. Previous studies conducted in Brazil, China, Florida, and Iran have clearly shown that CLas titers rise with moderate temperatures but drop sharply with severe heat (Irey et al., 2011; Zhang et al., 2011; Lopes et al., 2013; Bahari et al., 2021). The titers in Brazil were highest at lower temperatures (20 to 22 °C); conversely, low titers were observed during peak summer conditions, when temperatures were extremely high (Sauer et al., 2015). A similar observation was made for CLas titers in a tropical region of southeastern Iran, where maximum titers were observed in late summer to autumn (temperatures 22 to 30 °C); dramatic reductions were observed when temperatures exceeded 34 to 35 °C (Irey et al.,

2011; Bahari et al., 2021). Seasonal downturns have also been observed for CLas titers in Florida (Manjunath et al., 2008; Zhang et al., 2011). Although such a trend has clearly evolved worldwide, data regarding CLas titers in extremely arid and semi-arid climatic conditions remain limited.

Saudi Arabia offers a very favourable environment for exploring this phenomenon due to substantial climatic variations. In the summer season, temperatures range from 27 to 43 °C, while in the winter temperatures in northern and mountainous regions drop below 6 °C. In contrast, lower and more stable temperatures are observed in other zones. Variations in climatic parameters such as humidity levels, elevation ranges, and rainfall may affect CLas counts, psyllid abundance, and qPCR detection efficiency. Although CLas was identified in Saudi Arabia several decades ago, previous studies primarily focused on its identification and mapping (Bové, 2006; Ibrahim et al., 2024), with less emphasis on the climatic parameters that influence detection efficiency.

Although multiple studies have shown that CLas titer and qPCR detectability vary with temperature, season, host, and tissue type, these factors are often examined independently or under climatic regimes that do not represent arid and semi-arid production systems. Consequently, quantitative evidence remains limited on how seasonal temperature profiles interact with host species and sampling tissue to shape Ct values under the extreme thermal conditions typical of Saudi Arabia. This knowledge gap constrains the development of evidence-based sampling recommendations, potentially leading to inconsistent detection outcomes and suboptimal HLB surveillance.

To address this gap, this study analyzed qPCR Ct values from 123 CLas-positive samples collected across major citrus-growing regions of Saudi Arabia, representing 4 citrus hosts and 4 tissue types, and linked these results to mean seasonal temperature. Specifically, the research aimed to: (i) quantify seasonal and temperature-associated variation in Ct values; (ii) compare Ct values among hosts and tissue types to identify sampling conditions that maximize detection sensitivity; and (iii) test whether temperature modifies host-, tissue-, or season-related patterns using an analysis of covariance (ANCOVA) framework. To our knowledge, this is the first study in Saudi Arabia to evaluate CLas detectability using an integrated framework that

simultaneously considers host, tissue, season, and temperature, thereby providing practical guidance for HLB monitoring under arid and semi-arid conditions.

## MATERIALS AND METHOD

### Sample collection and qPCR dataset

This study analyzed 123 qPCR-positive samples from a larger nationwide survey conducted in 2023 to 2024 across 8 regions of Saudi Arabia (Supplementary Table 1). Samples were collected from both symptomatic and asymptomatic trees and represented 4 commonly cultivated hosts: *Citrus limon*, *C. reticulata*, *C. sinensis*, and *C. aurantiifolia*. For each sampled tree, one of four tissue types, midrib, petiole, combined midrib + petiole, or fruit rind, was collected following the procedures outlined in the original study. Each record contained metadata on host species, region, tissue type, and collection season. Seven samples were removed to maintain consistency across regions and host types: 2 samples from a Riyadh nursery and 4 from the Qassim region, both located outside the primary citrus-production zones, and 1 grapefruit sample from Najran, which did not belong to the 4 focal citrus hosts. Because the dataset was unevenly distributed across hosts, regions, and tissue types, the statistical analysis used Type II sums of squares to account for this imbalance.

### Environmental data acquisition

To describe the temperature conditions associated with each sample, regional climate data were obtained from World Weather Online (<https://www.worldweatheronline.com>). For each sampling location and month, the corresponding daily maximum and minimum temperatures were extracted. These values were assigned to one of four climatic seasons, spring, summer, autumn, or winter, and averaged to generate a seasonal mean temperature (MeanTemp) for each region. Seasonal averages were used instead of monthly values to reduce short-term variability and to align with the sampling design, which did not include repeated monthly collections at the same site. Although this approach smooths short-term extremes, it provides a reliable representation of the broader thermal regime relevant to CLas population dynamics.

### Statistical analysis

To explore how biological and environmental variables influenced CLas detection, a linear model based on ANCOVA was fitted with Ct

values as the response variable. The categorical predictors included host species, tissue type, and season, while mean seasonal temperature (MeanTemp) was incorporated as a continuous covariate. Interaction terms between MeanTemp and each categorical factor were included to evaluate whether temperature modified their effects. Because the dataset was unbalanced across categories, Type II sums of squares were used.

Standard regression diagnostic checks were conducted to verify model assumptions. Residual normality was assessed visually using histograms and normal probability (Q–Q) plots of standardized residuals, which indicated that the residuals approximately followed a normal distribution. Homoscedasticity was evaluated using plots of standardized residuals versus fitted values. Multicollinearity among predictors was examined using variance inflation factors (VIF), and influential observations were assessed using Cook's distance and leverage statistics. These diagnostics indicated that variance heterogeneity and influential observations did not materially affect model interpretation. All results were interpreted with consideration of the inverse relationship between Ct values and bacterial titer.

To complement the full model, separate simple linear regressions of Ct against MeanTemp were performed for each host species using individual sample values. For each host, the slope estimate, confidence interval, and *p*-value were reported. Because sample sizes varied among hosts, results for hosts with fewer observations (e.g., *C. aurantiifolia*) should be interpreted cautiously. All statistical analyses were conducted in Python (v3.11) using the packages pandas, NumPy, statsmodels, SciPy, and Matplotlib, with statistical significance set at  $\alpha = 0.05$  (McKinney, 2010; Virtanen et al., 2020).

### Data visualization

Spatial, seasonal, and host-related trends were visualized using several graphical approaches, including heatmaps, boxplots, and interaction plots generated in Python (v3.11) using the Matplotlib and Seaborn libraries (Hunter, 2007; Waskom, 2021). Host temperature responses were illustrated using scatterplots with fitted regression lines, while differences among tissue types were presented using boxplots and Host  $\times$  Tissue bar charts. Regional comparisons among citrus-growing areas were conducted using clustering based on Euclidean distances calculated from mean Ct values. All graphics were produced using

consistent formatting and plotted on a common Ct scale to facilitate direct comparisons across figures. In interpreting the visualizations, the inverse relationship between Ct values and bacterial titer was considered, where lower Ct values indicate higher CLas abundance.

## RESULTS AND DISCUSSION

Seasonal climatic conditions across the 8 major citrus-producing regions showed pronounced spatial and temporal variations (Table 1). Summer temperatures were particularly high across most regions, reaching approximately 42.2 °C in the Makkah highlands, whereas northern regions such as Al-Jouf, Tabuk, and Asir experienced winter minimums below 6 °C.

Spring and autumn were characterized by intermediate temperatures, representing transitional periods between thermal extremes. These climatic gradients provide essential context for interpreting the spatial and seasonal variation in CLas Ct values across the study regions.

The ANCOVA analysis revealed that season and tissue type exerted significant effects on Ct variation, with a strong interaction detected between mean temperature and season (Table 2). While temperature alone showed marginal significance, its effect became more pronounced when evaluated in combination with seasonal categories, indicating that seasonal temperature profiles are a major determinant of CLas titer dynamics. This interaction highlights the

Table 1. Seasonal mean daily maximum and minimum air temperatures (°C) in major citrus-growing regions in Saudi Arabia

Province	Season	Mean max (°C)	Mean min (°C)
Al-Bahah	Autumn	24.5	12.5
Al-Bahah	Spring	26.0	13.3
Al-Bahah	Summer	29.5	18.5
Al-Bahah	Winter	20.6	8.6
Al-Jouf	Autumn	28.0	13.0
Al-Jouf	Spring	30.0	14.3
Al-Jouf	Summer	41.0	24.5
Al-Jouf	Winter	18.3	5.6
Al-Madinah (Al-Ula)	Autumn	28.0	11.5
Al-Madinah (Al-Ula)	Spring	32.0	14.0
Al-Madinah (Al-Ula)	Summer	37.7	21.0
Al-Madinah (Al-Ula)	Winter	22.0	5.6
Asir (Abha)	Autumn	22.5	10.5
Asir (Abha)	Spring	24.0	11.3
Asir (Abha)	Summer	27.2	16.2
Asir (Abha)	Winter	18.6	6.6
Hail	Autumn	27.0	12.0
Hail	Spring	29.6	13.3
Hail	Summer	38.0	22.5
Hail	Winter	18.6	5.0
Makkah (highland)	Autumn	34.5	20.0
Makkah (highland)	Spring	36.6	22.0
Makkah (highland)	Summer	42.2	27.2
Makkah (highland)	Winter	28.0	15.0
Najran	Autumn	30.0	15.0
Najran	Spring	33.6	18.0
Najran	Summer	37.7	23.0
Najran	Winter	26.6	10.6
Tabuk	Autumn	27.0	11.0
Tabuk	Spring	29.0	13.3
Tabuk	Summer	38.7	22.5
Tabuk	Winter	18.3	5.0

Note: Values represent long-term averages for spring (March–May), summer (June–August), autumn (September–November), and winter (December–February) in 2024

Table 2. Analysis of variance for the ANCOVA linear model evaluating the effects of host species, tissue type, season, and mean seasonal temperature on CLas Ct values across citrus-growing regions in Saudi Arabia

Source of variation	Df	Sum of squares	Mean square	F-value	<i>p</i> -value
Host	3	86.89	28.96	2.34	0.078
Tissue	3	97.50	32.50	2.62	0.05
Season	3	521.67	173.89	14.04	$9.6 \times 10^{-8}$
MeanTemp	1	39.63	39.63	3.20	0.077
Season $\times$ MeanTemp	3	162.67	54.22	4.38	0.006
Host $\times$ MeanTemp	3	54.61	18.20	1.47	0.23
Tissue $\times$ MeanTemp	3	83.52	27.84	2.25	0.087
Residual	103	1,275.46			

Table 3. Host-specific linear regression results (Ct ~ MeanTemp) showing temperature–titer relationships for 4 citrus species based on sample-level qPCR data

Host species	N	Intercept	Slope ( $\beta_1$ )	<i>p</i> -value*	R <sup>2</sup>
<i>C. limon</i>	13	43.54	-0.6048	0.01	0.47
<i>C. reticulata</i>	44	33.28	-0.0706	0.59	0.012
<i>C. sinensis</i>	58	30.85	-0.0294	0.79	0.0012
<i>C. aurantiifolia</i>	8	35.72	-0.0386	0.58	0.054

importance of considering temperature within a seasonal framework rather than as an isolated variable.

Host-specific regression analyses further clarified temperature-dependent responses (Table 3; Figure 1). *Citrus limon* exhibited a strong negative relationship between Ct values and mean seasonal temperature (slope = -0.6048), indicating increased CLas titers under warmer conditions. In contrast, *C. reticulata*, *C. sinensis*, and *C. aurantiifolia* showed slopes near zero, suggesting limited temperature sensitivity. These findings explain the non-significant main host effect detected in the ANCOVA and indicate that host-level responses to temperature are species-specific.

Seasonal trends in Ct values across citrus hosts (Figure 2) were consistent with the regression outcomes. Ct values for *C. limon* increased progressively from spring to winter, reflecting its capacity to sustain higher bacterial loads during warmer periods. *Citrus reticulata* and *C. sinensis* showed elevated Ct values during autumn, whereas *C. aurantiifolia* maintained relatively high Ct values throughout the year. These patterns further support the significance of the temperature  $\times$  season interaction in shaping CLas detectability.

Temperature emerged as the dominant environmental factor influencing Ct variation, primarily through seasonal modulation. This finding aligns with extensive research from major citrus-growing regions demonstrating that temperature strongly influences phloem

physiology, CLas population density, and ACP activity (Lopes et al., 2009; Zhang et al., 2011; Bahari et al., 2021). Moderate temperatures generally favor bacterial proliferation, whereas prolonged exposure to high temperatures suppresses CLas activity. Controlled environment studies have shown that CLas replication declines above approximately 32 °C and may cease entirely at temperatures exceeding 35 °C (Hoffman et al., 2013). Recent field-scale and modeling studies further reinforce the role of temperature as a key regulator of CLas persistence under variable climatic conditions (Collins et al., 2025; Tardivo et al., 2025).

Field observations across Saudi Arabia were consistent with these established thermal thresholds. Regions experiencing extreme summer heat, such as Al-Jouf and Tabuk, exhibited elevated Ct values during summer, indicative of reduced bacterial titers. Conversely, regions with warm but moderate thermal regimes, including the Makkah highlands, Al-Baha, and Najran, showed lower Ct values during spring and autumn, corresponding to temperature ranges optimal for CLas multiplication. These patterns indicate that prolonged high-temperature exposure typical of arid and semi-arid environments imposes strong constraints on CLas persistence and in planta population growth.

Tissue type significantly influenced detection sensitivity (Table 2; Figure 3). Midrib and petiole tissues consistently yielded the lowest Ct values across hosts and regions, confirming

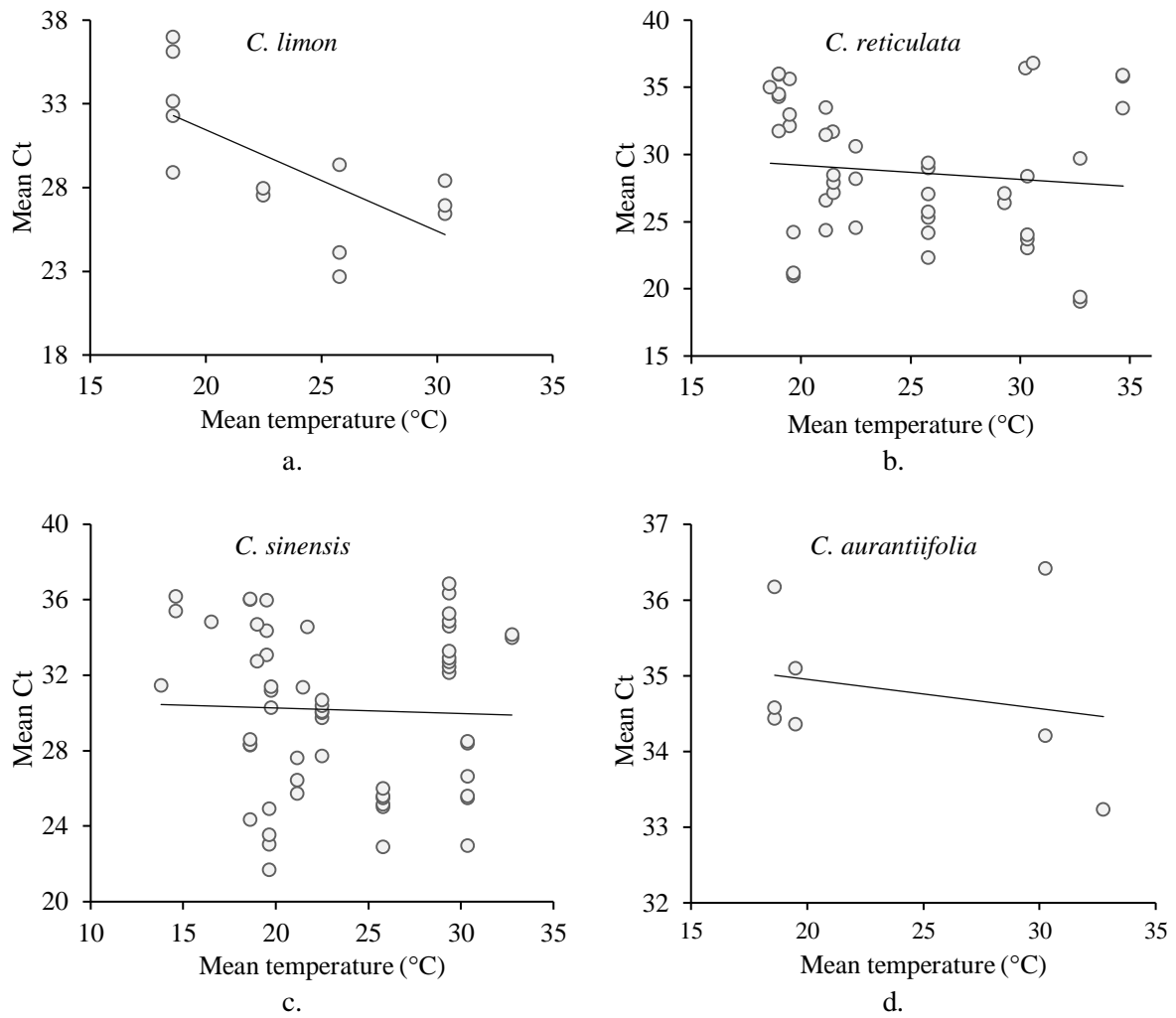


Figure 1. Relationship between seasonal mean temperature and CLas bacterial titer (Ct values) across 4 citrus hosts: *C. limon* (a), *C. reticulata* (b), *C. sinensis* (c), and *C. aurantiifolia* (d)

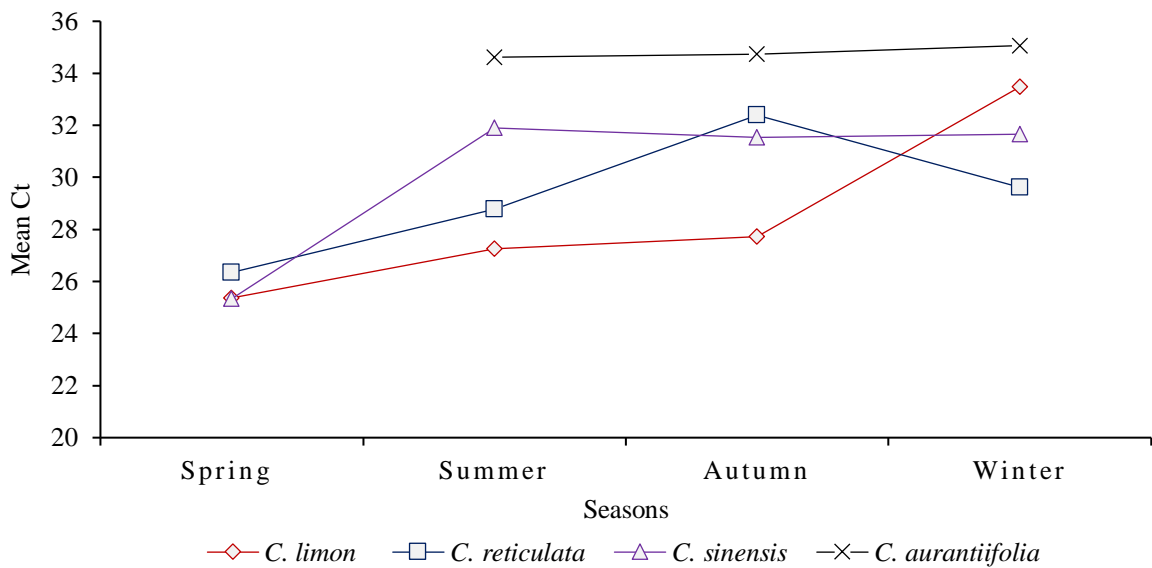


Figure 2. Interaction between season and citrus host species on mean CLas Ct values in Saudi Arabia  
 Note: Points represent mean Ct values for each Season × Host combination, averaged across tissues; vertical bars indicate the standard errors of the mean. Lower Ct values indicate higher CLas titers

their diagnostic superiority. This observation is consistent with the phloem-limited nature of CLAs and previous reports documenting higher bacterial titers in phloem-enriched tissues such as midribs, petioles, young stems, and peduncles (Li et al., 2006; Johnson et al., 2014; Canale et al., 2017). Root and peduncle tissues also performed well for detecting early or sporadic infections (Louzada et al., 2016). In contrast, fruit rind tissues exhibited consistently higher Ct values, confirming their limited diagnostic reliability. Host  $\times$  tissue patterns are illustrated in Figure 4, showing that these tissue-specific differences were largely conserved across citrus species. The lack of a significant tissue  $\times$  temperature interaction indicates that tissue-level detection differences remain stable across climatic

gradients, supporting tissue selection as a climate-robust predictor for CLAs detection.

Seasonal and regional analyses further emphasized the central role of temperature (Figure 5). Regions characterized by moderate temperatures during spring and autumn (20 to 30 °C) exhibited lower Ct values, whereas areas experiencing severe summer heat showed higher Ct values during those periods. Heatmap clustering revealed 2 major regional groups: regions with lower Ct values during moderate seasons (Makkah highlands, Al-Baha, Najran, Hail, and Tabuk) and regions with consistently higher Ct values (Asir, Al-Jouf, and Al-Ula). These spatial patterns are consistent with the temperature-driven effects identified through ANCOVA and host-specific regression analyses.

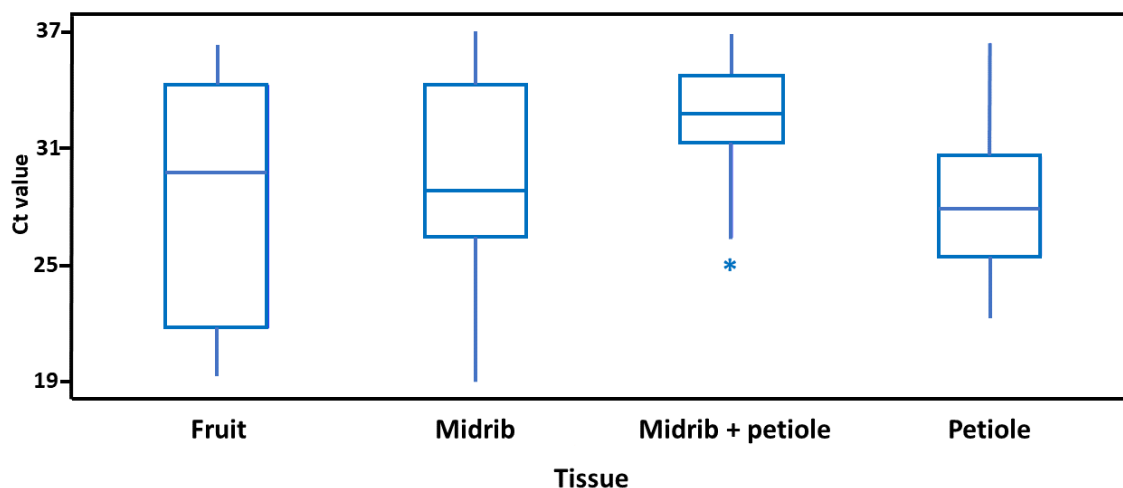


Figure 3. Boxplots of CLAs Ct values in different citrus tissues (fruit, midrib, midrib + petiole, and petiole), pooled across hosts and seasons

Note: Boxes show the interquartile range, horizontal lines indicate the medians, and whiskers represent the data range excluding outliers

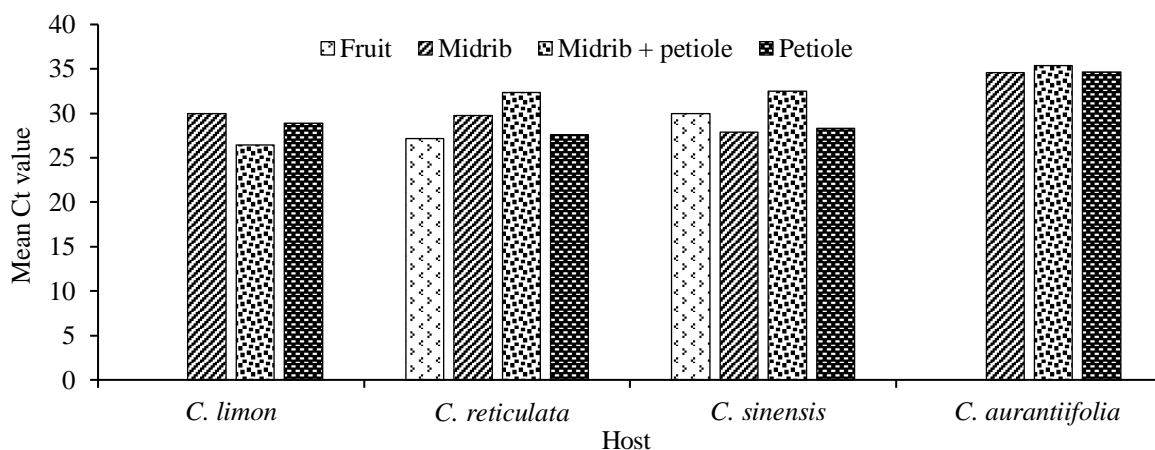


Figure 4. Interaction between citrus host and tissue type on mean CLAs Ct values

Note: Bars represent mean Ct values for each Host  $\times$  Tissue combination; error bars represent the standard errors of the mean

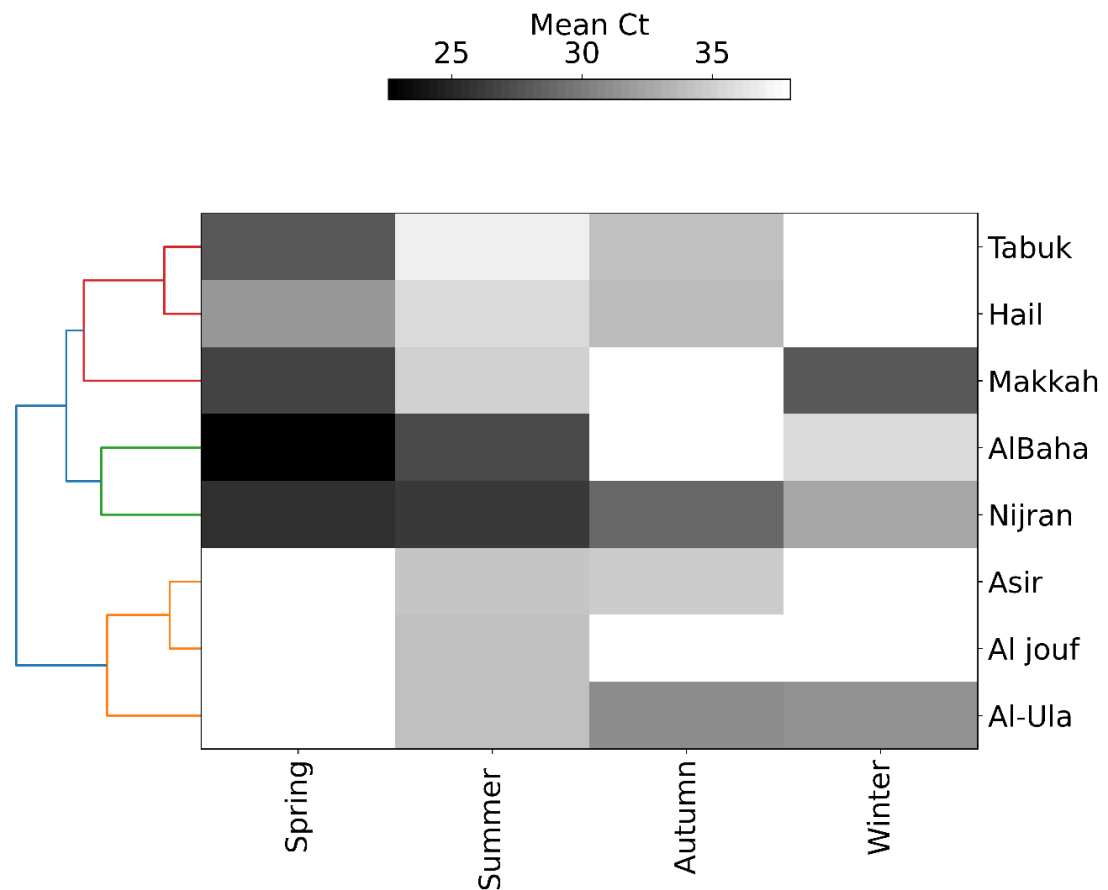


Figure 5. Heatmap of mean CLas Ct values across major citrus-producing regions of Saudi Arabia in relation to season

Collectively, these results demonstrate that temperature is the primary ecological driver influencing CLas population dynamics and qPCR detectability in Saudi citrus orchards. Although host species, tissue type, and season contribute to Ct variability, their effects are largely mediated by environmental temperature. Integrating climatic information with optimized tissue selection offers a practical strategy to improve HLB surveillance and management. Specifically, sampling midrib or petiole tissues during spring or autumn under moderate temperature conditions is likely to maximize diagnostic sensitivity in arid and semi-arid citrus production systems.

## CONCLUSIONS

This study confirms temperature, acting through seasonality, as the primary driver of CLas detectability in Saudi citrus orchards. CLas titers were highest during spring and autumn under moderate temperatures and were suppressed during extreme summer and winter conditions. Host effects were generally minor, except for

*C. limon*, which maintained higher titers at warmer temperatures. Midrib and petiole tissues consistently provided the most sensitive detection regardless of climate. Collectively, these findings support seasonally and tissue-targeted sampling as an effective strategy to improve HLB surveillance and management under arid conditions. Future research should incorporate multi-year monitoring datasets and continuous environmental measurements to better quantify the long-term effects of climatic variability on CLas population dynamics. Additionally, integrating vector population data, host physiological responses, and high-resolution climatic variables may further improve predictive models of HLB risk and optimize detection strategies across diverse citrus production systems in arid and semi-arid regions.

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Supplementary Table 1. Results of real-time quantitative polymerase chain reaction detection (Cq value) of “*Candidatus Liberibacter asiaticus*” in citrus tissues collected from trees in 8 study regions in Saudi Arabia during 2023-2024

Sample number	Province	Citrus species	Plant part sampled	RNR Cq value	Season
1	Al Bahah	<i>C. reticulata</i>	Midrib	19.04	Autumn
2	Al Bahah	<i>C. reticulata</i>	Fruit	19.39	Autumn
3	Al Bahah	<i>C. reticulata</i>	Fruit	20.94	Spring
4	Al Bahah	<i>C. reticulata</i>	Fruit	21.10	Spring
5	Al Bahah	<i>C. reticulata</i>	Fruit	21.15	Spring
6	Al Bahah	<i>C. sinensis</i>	Fruit	21.71	Spring
7	Al Bahah	<i>C. sinensis</i>	Midrib	23.07	Spring
8	Al Bahah	<i>C. sinensis</i>	Midrib	23.57	Spring
9	Al Bahah	<i>C. reticulata</i>	Midrib	24.21	Spring
10	Al Bahah	<i>C. sinensis</i>	Fruit	24.95	Spring
11	Al Bahah	<i>C. reticulata</i>	Fruit	29.68	Summer
12	Al Bahah	Mexican lime	Midrib	33.24	Summer
13	Al Bahah	<i>C. sinensis</i>	Midrib	34.17	Summer
14	Al Bahah	<i>C. sinensis</i>	Midrib	34.56	Summer
15	Al Bahah	<i>C. sinensis</i>	Fruit	34.85	Autumn
16	Asir	<i>C. sinensis</i>	Midrib + petiole	35.41	Winter
17	Asir	<i>C. sinensis</i>	Midrib + petiole	36.20	Winter
18	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	26.38	Spring
19	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	27.10	Spring
20	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	27.14	Winter
21	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	27.90	Winter
22	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	28.45	Winter
23	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	30.30	Autumn
24	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	31.21	Autumn
25	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	31.41	Autumn
26	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	31.47	Winter

Supplementary Table 1. *Continued*

Sample number	Province	Citrus species	Plant part sampled	RNR Cq value	Season
27	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	32.16	Summer
28	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	32.45	Summer
29	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	32.74	Summer
30	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	32.94	Summer
31	Al Ula-Madinah	<i>C. sinensis</i>	Midrib + petiole	33.30	Summer
32	Makkah	<i>C. reticulata</i>	Midrib	33.45	Summer
33	Makkah	<i>C. reticulata</i>	Midrib	34.62	Summer
34	Makkah	<i>C. reticulata</i>	Midrib	34.89	Summer
35	Makkah	<i>C. reticulata</i>	Midrib	35.28	Summer
36	Makkah	<i>C. reticulata</i>	Midrib	35.79	Summer
37	Makkah	<i>C. reticulata</i>	Midrib	35.91	Summer
38	Makkah	<i>C. reticulata</i>	Midrib	36.37	Summer
39	Makkah	<i>C. reticulata</i>	Midrib	36.88	Summer
40	Nijran	<i>C. reticulata</i>	Petiole	22.29	Spring
41	Nijran	<i>C. limon</i>	Midrib	22.67	Spring
42	Nijran	<i>C. sinensis</i>	Midrib	22.91	Spring
43	Nijran	<i>C. sinensis</i>	Midrib	23.00	Summer
44	Nijran	<i>C. reticulata</i>	Petiole	23.01	Summer
45	Nijran	<i>C. reticulata</i>	Petiole	23.70	Summer
46	Nijran	<i>C. reticulata</i>	Midrib	24.01	Summer
47	Nijran	<i>C. limon</i>	Petiole	24.10	Spring
48	Nijran	<i>C. reticulata</i>	Petiole	24.17	Spring
49	Nijran	<i>C. sinensis</i>	Petiole	24.37	Winter
50	Nijran	<i>C. reticulata</i>	Petiole	24.52	Autumn
51	Nijran	<i>C. sinensis</i>	Midrib + petiole	25.05	Spring
52	Nijran	<i>C. sinensis</i>	Petiole	25.18	Spring
53	Nijran	<i>C. reticulata</i>	Petiole	25.30	Spring
54	Nijran	<i>C. sinensis</i>	Petiole	25.52	Spring
55	Nijran	<i>C. sinensis</i>	Petiole	25.53	Summer
56	Nijran	<i>C. sinensis</i>	Petiole	25.60	Summer
57	Nijran	<i>C. sinensis</i>	Midrib	25.61	Spring
58	Nijran	<i>C. reticulata</i>	Petiole	25.71	Spring
59	Nijran	<i>C. sinensis</i>	Petiole	26.02	Spring
60	Nijran	<i>C. limon</i>	Midrib + petiole	26.43	Summer
61	Nijran	<i>C. sinensis</i>	Midrib + petiole	26.66	Summer
62	Nijran	<i>C. limon</i>	Petiole	26.93	Summer
63	Nijran	<i>C. reticulata</i>	Petiole	27.04	Spring
64	Nijran	<i>C. limon</i>	Midrib	27.53	Autumn
65	Nijran	<i>C. sinensis</i>	Petiole	27.73	Autumn
66	Nijran	<i>C. limon</i>	Petiole	27.94	Autumn
67	Nijran	<i>C. reticulata</i>	Petiole	28.18	Autumn
68	Nijran	<i>C. sinensis</i>	Petiole	28.32	Winter
69	Nijran	<i>C. sinensis</i>	Petiole	28.34	Winter
70	Nijran	<i>C. reticulata</i>	Midrib	28.36	Autumn
71	Nijran	<i>C. limon</i>	Midrib	28.39	Autumn
72	Nijran	<i>C. sinensis</i>	Petiole	28.42	Autumn
73	Nijran	<i>C. sinensis</i>	Midrib	28.50	Autumn
74	Nijran	<i>C. sinensis</i>	Petiole	28.61	Winter
75	Nijran	<i>C. limon</i>	Midrib	28.88	Winter
76	Nijran	<i>C. reticulata</i>	Midrib	29.00	Spring
77	Nijran	<i>C. limon</i>	Petiole	29.34	Spring
78	Nijran	<i>C. reticulata</i>	Midrib	29.35	Spring

Supplementary Table 1. *Continued*

Sample number	Province	Citrus species	Plant part sampled	RNR Cq value	Season
79	Nijran	<i>C. sinensis</i>	Midrib	29.76	Autumn
80	Nijran	<i>C. sinensis</i>	Midrib + petiole	30.04	Autumn
81	Nijran	<i>C. sinensis</i>	Petiole	30.11	Autumn
82	Nijran	<i>C. sinensis</i>	Midrib + petiole	30.40	Autumn
83	Nijran	<i>C. reticulata</i>	Petiole	30.59	Autumn
84	Nijran	<i>C. sinensis</i>	Petiole	30.69	Autumn
85	Nijran	<i>C. paradisi</i>	Petiole	31.18	Autumn
86	Nijran	<i>C. limon</i>	Midrib	32.28	Winter
87	Nijran	<i>C. limon</i>	Midrib	33.16	Winter
88	Nijran	Mexican lime	Midrib	34.44	Winter
89	Nijran	Mexican lime	Midrib	34.58	Winter
90	Nijran	<i>C. reticulata</i>	Midrib	35.00	Winter
91	Nijran	<i>C. sinensis</i>	Petiole	36.03	Winter
92	Nijran	<i>C. sinensis</i>	Midrib + petiole	36.06	Winter
93	Nijran	<i>C. limon</i>	Petiole	36.09	Winter
94	Nijran	Mexican lime	Midrib	36.18	Winter
95	Nijran	<i>C. limon</i>	Midrib	36.99	Winter
96	Tabuk	<i>C. reticulata</i>	Midrib	24.34	Spring
97	Tabuk	<i>C. sinensis</i>	Fruit	25.76	Spring
98	Tabuk	<i>C. sinensis</i>	Midrib	26.45	Spring
99	Tabuk	<i>C. reticulata</i>	Midrib	26.56	Spring
100	Tabuk	<i>C. sinensis</i>	Fruit	27.62	Spring
101	Tabuk	<i>C. reticulata</i>	Fruit	31.39	Spring
102	Tabuk	<i>C. sinensis</i>	Fruit	31.42	Spring
103	Tabuk	<i>C. sinensis</i>	Fruit	31.67	Spring
104	Tabuk	<i>C. reticulata</i>	Fruit	31.72	Autumn
105	Tabuk	<i>C. reticulata</i>	Fruit	32.09	Autumn
106	Tabuk	<i>C. reticulata</i>	Fruit	32.75	Autumn
107	Tabuk	<i>C. sinensis</i>	Fruit	32.97	Autumn
108	Tabuk	<i>C. reticulata</i>	Midrib	33.09	Autumn
109	Tabuk	<i>C. reticulata</i>	Midrib	33.49	Spring
110	Tabuk	<i>C. reticulata</i>	Midrib	34.01	Summer
111	Al Jouf	<i>C. sinensis</i>	Midrib	34.21	Summer
112	Hail	<i>C. sinensis</i>	Midrib + petiole	34.30	Autumn
113	Hail	<i>C. reticulata</i>	Midrib + petiole	34.36	Autumn
114	Hail	<i>C. reticulata</i>	Petiole	34.38	Autumn
115	Hail	<i>C. reticulata</i>	Midrib + petiole	34.45	Autumn
116	Hail	<i>C. sinensis</i>	Midrib + petiole	34.71	Autumn
117	Hail	Mexican lime	Petiole	35.10	Autumn
118	Hail	Mexican lime	Petiole	35.60	Autumn
119	Hail	<i>C. sinensis</i>	Fruit	35.94	Autumn
120	Hail	Mexican lime	Petiole	35.97	Autumn
121	Hail	<i>C. reticulata</i>	Fruit	35.99	Summer
122	Hail	<i>C. sinensis</i>	Midrib + petiole	36.42	Summer
123	Hail	Mexican lime	Petiole	36.42	Summer