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Research Papers

Synergism of abiotic stresses and Hop Stunt Viroid infections causing citrus decline in Jahrom Orchards, Iran

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Summary. Citrus is an economically important crop in subtropical regions including Jahrom, Iran. Productivity of these fruit crops can severely decline due to biotic and abiotic stresses. Interactions were investigated between Hop Stunt Viroid (HSVd) infections and abiotic stress factors (drought and soil salinity) for effects on orchard decline. HSVd infection, a key biotic factor causing citrus cachexia, along with abiotic stresses of drought, high temperatures, and poor orchard management, compromise tree health by disrupting water balance and causing stunting and bark damage. Combined effects of HSVd infection and environmental stresses on citrus decline were assessed. Experimental treatments of healthy (experimental control), HSVd infected, abiotic decline-affected, and HSVd + abiotic decline was assessed in three citrus orchards. Compared with controls, trees from the other three treatments had reduced yields, plant heights, crown sizes, and fruit diameters. Plant heights varied among orchards, while fruit diameters did not. Healthy (control) plants grew and yielded best, while yields from plants infected with HSVd and subjected to climate change yielded least. Trees under climate-induced decline had increased fruit diameters, likely due to reduced fruit set under drought stress and redirected assimilates to reduced numbers of developing fruits. Similar responses have been reported in citrus under water stress conditions. Molecular diagnostics (RT-PCR and dot-blot hybridization) confirmed HSVd presence exclusively in symptomatic trees (samples of 10 trees per orchard), while assays for citrus Tristeza Virus (CTV) were negative. These results are consistent with HSVd being the primary biotic agent associated with the observed citrus decline, although causal confirmation requires controlled inoculation studies. Stresses negatively impacted citrus productivity in 85 orchards in Jahrom. Given the observed associations between HSVd presence, abiotic decline indicators, and reduced productivity, integrated citrus crop management is recommended. This should include reductions of drought (through optimized irrigation scheduling), and soil salinity (mitigation of sodium adsorption ratio), and appropriate phytosanitary measures. Phytosanitation should include use of certified viroid-free planting material, and targeted molecular surveillance for viroid infections. Implementation of these strategies should be guided by follow-up studies that verify absence/presence of other graft-transmissible pathogens, quantify rootstock effects, and test mitigation strategies in controlled and replicated field trials.

Keywords. Abiotic stresses, HSVd infections, citrus decline, Lisbon lemon.

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INTRODUCTION

Citrus includes a diverse group of fruit plant species and cultivars, that produce oranges, limes, grapefruit, and sour oranges (Wu *et al.*, 2018). These fruits are internationally valued for their distinctive flavours, vibrant colours, and nutritional content (Lv *et al.*, 2015). These fruits are native to subtropical and tropical regions, typically found between latitudes 40°N and 40°S (Raju *et al.*, 2024). Citrus is one of the most economically important fruit crops cultivated in these regions, including the Jahrom area in Fars Province, Iran.

The Jahrom region (28°30′N, 53°33′E), in the semiarid Fars Province of Iran, exemplifies the environmental challenges facing citrus production in the era of climate change. This region has average annual rainfall of less than 170 mm, occurring almost entirely outside the winter-spring growing season, necessitating heavy reliance on groundwater irrigation for fruit production. These aquifers are increasingly stressed and often salinized, with reported sodium adsorption ratios (SAR) exceeding 13 in some agricultural wells (Bagherian *et al.*, 2021). Summertime temperatures regularly surpass 40°C, accompanied by low relative humidity (often <25%) and high evapotranspiration rates (>8 mm d^{-1}). These common conditions of water scarcity, endemic soil salinity, and high temperatures challenge citrus cultivation; and create persistent abiotic stress that pre-disposes trees to secondary pathogens. Consequently, Jahrom region offers a representative case study for investigating how orchard decline may be affected a synergy between abiotic factors (drought, salinity, high temperature) and the biotic agent *Citrus hop stunt viroid* (HSVd).

HSVd interferes with host plant regulatory and metabolic networks (Marquez-Molins *et al.*, 2021; Di Serio *et al.*, 2024). Although not typically lethal, HSVd causes chronic damage to citrus plants, that includes stunted growth, reduced fruit size and quality, stem pitting, bark gummoses, and overall decline. While HSVd infections may remain asymptomatic or produce mild symptoms under normal conditions, their effects are intensified under environmental stress (Hadidi *et al.*, 2024).

Citrus orchards internationally, including those in Jahrom, Iran, have experienced significant declines in productivity and vitality, which have been due to a complex interplay of biotic and abiotic factors. A key biotic constraint are infections by HSVd, the causal agent of citrus cachexia (Vamenani *et al.*, 2019). Concurrently, several abiotic stressors, including extreme temperature fluctuations, prolonged drought, flooding, and elevated soil and water salinity, have been exacerbated by climate change, posing severe threats for citrus cultivation (Dahro *et al.*, 2023). Notably, the HLB-associated bacterium 'Candidatus Liberibacter asiaticus' has been detected in citrus trees in Fars Province, Iran, including the Jahrom region (Rahimpour *et al.*, 2025; Faghihi *et al.*, 2025), adding another potential biotic stressor to the complex decline syndrome affecting local orchards.

In Jahrom, additional challenges including use of substandard planting materials, degraded soil quality, inconsistent rainfall patterns, and suboptimal orchard management practices, further contribute to citrus decline. Among abiotic stressors, drought remains the most pressing issue, driven by high temperatures, inadequate rainfall, poor irrigation management, and soil water deficits. Physical constraints, including soil compaction, poor drainage, and excessive salinity, also limit root access to water and nutrients, intensifying the effects of drought stress.

High temperatures, particularly when accompanied by elevated evaporation losses, severely affect citrus physiology by disrupting the balance between water absorption and transpiration (Moore *et al.*, 2021; Chen *et al.*, 2025). In the Jahrom region, this imbalance is a key contributor to physiological decline observed in citrus trees. The optimal temperature range for citrus plant growth is between 22 and 34°C; temperatures exceeding this range cause fruit drop, reduced fruit size, and impaired overall tree productivity. Although some citrus varieties can tolerate temperatures above 40°C without showing immediate visible symptoms, the long-term physiological stress incurred leads to reduced growth and low fruit yields.

Infections by viroids, which are circular, single-stranded RNA molecules ranging from 246 to 401 nucleotides, are another important factor in citrus decline. They are classified into two families, the *Pospiviroidae* and *Avsunviroidae* (Di Serio *et al.*, 2014). Citrus trees are natural hosts to several viroid species, all belonging to *Pospiviroidae*. Among these, pathogenic variants of HSVd are responsible for citrus cachexia, a disease that prevails in most citrus-producing regions, including Jahrom (Belabess *et al.*, 2021). HSVd is mechanically transmissible *via* sap and contaminated tools. Infected trees typically exhibit symptoms of stunting, leaf chloroses, bark gummoses, stem pitting, and general decline, although leaf and fruit symptoms may be absent in HSVd-infected plants (Marquez-Molins *et al.*, 2021).

The present study aimed to evaluate the combined effects of abiotic stresses and HSVd infection on citrus trees in the Jahrom region. Disease severity was assessed in the context of local climatic conditions, and the interactive roles were assessed of environmental and pathogen factors in citrus orchard decline. This knowledge could support development of integrated management strategies to enhance citrus resilience and sustainability under changing environmental conditions. The study controlled for potential confounding factors, by focusing on one citrus rootstock across the sampled orchards, and also made assessments for other major pathogens.

MATERIALS AND METHODS

This study was carried out during 2022 and 2023, across multiple commercial citrus orchards in the Jahrom region, Fars Province, Iran. Hereafter, 'growth condition' refers to pre-existing orchard health categories (healthy, HSVd-affected, abiotic decline, or HSVD/abiotic decline combined) used for comparative analyses, but that were not experimentally imposed treatments. Specifically, the treatments corresponded to orchard health/status categories, which were: (A) Healthy control orchards (no visual decline symptoms, no molecular evidence of HSVd); (B) HSVd-affected orchards (trees exhibiting stem-pitting/shaqqaq symptoms, and HSVd detection confirmed by molecular assays); (C) abiotic decline orchards (trees meeting predefined abiotic decline criteria described below); or (D) orchards showing both HSVd-associated symptoms and abiotic decline indicators. Each "treatment" thus represented a natural orchard category used for observational comparisons.

The following abiotic stress factors were quantified in this study:

- Soil salinity: Measured as sodium adsorption ratio (SAR), using ICP-OES, with SAR > 13 classified as severe salinity stress.
- Drought stress: Assessed by actual irrigation volume applied (L per tree per day) compared to recommended rates (80–100 L per tree per day in summer; 40–50 L per tree per day in winter).
- Heat stress: Recorded as number of days per growing season with maximum temperatures exceeding 40°C.
- Soil structural degradation: Evaluated using assessments of soil organic matter content and texture (hydrometer method), with low organic matter (< 1%) considered a contributing factor.
- Canopy light limitation: Measured as percentage light interception using a ceptometer (AccuPAR LP-80), with < 60% interception indicating poor canopy development.
- Evaporative water loss: Assessed indirectly by presence/absence of organic mulch (target: 10–15 cm depth under canopy).

Trees were classified as experiencing "abiotic decline" (treatment category 3) if they exhibited SAR > 13, light interception < 60%, and at least two visual decline symptoms (leaf chlorosis score ≥ 3, canopy thinning > 30%, or terminal branch dieback).

All sampled trees had the same scion cultivar, rootstock, age, and phenological stage, to minimize variability unrelated to the factors under study. Specifically, the study focused on 'Lisbon' lemon (*Citrus limon* L. 'Lisbon') grafted onto sour orange (*Citrus aurantium* L.) rootstock. All selected trees were 12 to 15 years old, at full commercial maturity, and had been consistently fruit-bearing for at least 6 years prior to the study. Trees exhibiting obvious signs of juvenility or senescence were excluded from samplings. This uniformity ensured that observed differences in decline severity could be attributed primarily to treatment effects (HSVd infection, abiotic stress, or their combination) rather than to genetic or developmental variation.

Healthy orchards (control group) were selected based on optimal tree growth, high fruit quality, and the absence of significant pests and diseases. HSVd infections were diagnosed by symptomatology and confirmed molecularly. Leaf and symptomatic bark samples were tested by RT-PCR with the HSVd-specific primers described by Bagherian and Izadpanah (2010). Representative RT-PCR amplicons (n = 6) were gel-purified and Sanger-sequenced; BLASTn comparison of these sequences against NCBI nt confirmed HSVd identities with ≥ 99% nucleotide similarity to reference HSVd sequences (Bagherian and Izadpanah, 2010). Amplification controls included a positive control (RNA from HSVd-infected sweet lime), and a negative control (RNA from viroid-free seedlings). Dot-blot hybridization using DIG-labelled HSVd probes corroborated RT-PCR results. Young, fully expanded leaves and symptomatic bark tissues were collected from ten randomly selected trees per orchard, including symptomatic and asymptomatic trees. The samples were promptly placed in sterile polyethylene bags, stored on ice, and transported to the laboratory within 6 h, before being assessed using RT-PCR. Total nucleic acids were extracted using the optimized high titer viroid extraction method described by Semancik *et al.* (1975). For RNA extraction and viroid enrichment, citrus tissues were homogenized in extraction buffer containing 0.1 M Tris–HCl, 0.1 M NaCl, 0.01 M EDTA, and 1% SDS, pH 8.0. After centrifugation, nucleic acids were fractionated by adding 2 M LiCl, selectively precipitating high-molecular-weight RNA and DNA. The viroid-enriched supernatant was then precipitated using ethanol. The resulting RNA pellets were resuspended in TKM buffer (10 mM Tris–HCl, 10 mM KCl, 0.1 mM $MgCl_2$, pH 7.4), and were then stored at −80°C until used. Reverse transcription-polymerase chain reaction (RT-PCR) detection of HSVd, was carried out using HSVd-specific primers targeting the full-length viroid genome (Bernad and Durán-Vila 2006):

Forward primer: 5′-GGGGCAACTCTTCTCA-GAATCC-3′
Reverse primer: 5′-GGGGCTCCTTTCTCAGGTAA-GTC-3′

Complementary DNA (cDNA) syntheses were carried out using RevertAid M-MuLV reverse transcriptase (Thermo Scientific), followed by PCR amplification with Taq DNA polymerase (Invitrogen). Thermal cycling conditions were as follows: initial denaturation at 94°C for 3 min; 35 cycles, each of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 45 sec; then a final extension at 72°C for 7 min. PCR products (~300 bp) were separated on 1% agarose gels, were stained with SYBR Safe DNA gel stain, and were visualized under UV illumination.

To rule out co-infections with other prevalent pathogens, all sampled trees were also tested for citrus tristeza virus (CTV) using CTV-specific primers (Ayllón *et al.*, 2001). Each CTV-specific RT-PCR run included a positive control (RNA from CTV-infected citrus tissue) and a negative control (RNA from viroid- and virus-free citrus seedlings). All sampled trees tested negative for CTV. To confirm RT-PCR results, dot-blot hybridization assays were carried out, following the method of Li *et al.* (1995). Full-length HSVd cDNA probes were labelled with digoxigenin (DIG) using the PCR DIG Probe Synthesis Kit (Roche). Nucleic acid extracts were applied to positively charged nylon membranes, and were hybridized with DIG-labelled probes at 50°C. Detection was carried out using anti-DIG alkaline phosphatase-conjugated antibodies, followed by chromogenic development with NBT/BCIP substrate.

Abiotic citrus decline was diagnosed based on a combination of field symptoms and environmental stress indicators, following the protocol of Bagherian *et al.* (2021). Citrus trees were classified as experiencing only abiotic decline if they met all the following criteria:

- Soil sodium adsorption ratio (SAR) > 13 (determined by ICP-OES)
- Canopy light interception < 60% (measured by ceptometer at midday)
- At least two of the following visual decline symptoms: persistent leaf chlorosis (score ≥ 3); canopy thinning > 30%; annual terminal branch dieback.

Soil analyses

Composite soil samples (0–30 cm depth) were collected and analyzed for texture using the hydrometer method (Mwendwa, 2022), and sodium adsorption ratios (SAR) were calculated from Na^+, Ca^{2+}, and Mg^{2+} concentrations (Pansu and Gautheyrou, 2006) determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

Canopy shading

Light interception was measured using a ceptometer (AccuPAR LP-80, Decagon Devices), at midday on clear days. Ten readings were taken under each assessed canopy, and ten outside (open sky), in each tree quadrant, and percentage light interception was calculated.

Tree vigour assessments

Tree trunk circumferences, shoot growth rates, and incidence of branch dieback were assessed as indicators of tree vigour. Trees were classified as experiencing abiotic decline only if they met all of the following criteria:

(a) Soil SAR > 2 (determined by ICP-OES),
(b) Canopy light interception < 60% (based on ceptometer readings), and
(c) At least two visual symptoms of decline, including: Persistent leaf chlorosis (chlorosis score ≥ 3), Canopy thinning > 30% or Annual terminal branch dieback.

The experimental groups represented distinct phytosanitary and edaphic conditions found in the region. To minimize variability unrelated to the factors under study, all sampled trees were of the same cultivar and age, grafted on a common rootstock, and managed under similar horticultural practices prior to the onset of decline symptoms. For consistency, all sampled trees belonged to one citrus cultivar, were of similar age, and exhibited uniform pre-treatment health conditions. To reduce variability, the trees were at 6 m spacings and were managed under identical cultural practices, apart from the treatment-specific interventions.

Three orchards were selected based on similar cultivar, age, and general management practices. Although irrigation systems and soil types varied slightly, these were accounted for as random effects in the statistical model to minimize confounding variability between orchards. At the fruit ripening stage, data were collected using systematic random sampling. Each selected tree was divided into four quadrants (north, south, east, or west), and samples were taken from three canopy levels (upper, middle, or lower) to account for intra-canopy variations.

The experimental design was a Repeated Measures Completely Randomized Design (RM-CRD). Treatments were randomly assigned to experimental units. The following morphological and yield-related traits were measured for each tree:

- Fruit yield (kg per tree): All harvested fruits were weighed using a high-precision digital scale.
- Tree height (m): Measured from the tree base to the canopy apex using a telescopic measuring pole.
- Canopy diameter (m): Calculated by averaging two measuring tape measurements at right angle to each other.
- Fruit diameter (cm): Measured from a random sample of 100 fruits per tree, collected from various canopy positions. A digital caliper with ±0.1 mm accuracy was used, and all measurements were carried out in triplicate.

Data obtained were assessed for normality prior to analyses. Statistical analyses were carried out using Minitab software. A one-way analysis of variance (ANOVA) was conducted to examine effects of treatments on tree growth and fruit production. Where statistically significant effects were detected, Tukey's *post hoc* test was used to identify pairwise differences among treatment means. Results were expressed as means ± standard errors (SE), with statistical significance set at $P < 0.05$.

Although citrus greening (HLB) caused by 'Candidatus Liberibacter asiaticus' has been reported in Fars Province (Faghihi *et al.*, 2025), the sampled trees in the present study did not exhibit characteristic HLB symptoms, such as asymmetrical chloroses, blotchy mottle, or lopsided fruit with aborted seeds. Therefore, HLB was not considered an important biotic factor in the present study, although its potential presence in the region underscores the complexity of citrus decline etiology.

RESULTS

RT-PCR assays successfully amplified approx. 300 bp fragments specific to HSVd in all leaf and bark samples collected from trees showing stem pitting symptoms (Groups B and D). In contrast, samples from asymptomatic trees (Groups A and C) did not show any amplifications. Positive (infected sweet lime) and negative (virus-free seedlings) controls confirmed specificity of the assay. Dot-blot hybridization further confirmed these results. All RT-PCR-positive samples exhibited strong hybridization signals with DIG-labeled HSVd probes, while RT-PCR-negative samples did not give detectable signals. These molecular results indicated direct association between HSVd infections and the stem pitting symptoms observed in the field. Nevertheless, given the limited sample size (ten trees per orchard) and the observational design, these data demonstrate HSVd association rather than causation.

The ANOVA results showed significant differences in fruit yields across the treatments ($P = 0.001$). This indicates that the treatments affected yield, and at least one treatment produced a significantly different result compared to the others. This indicates that the applied treatment type influenced yield in comparison to the control (Table 1). Combined HSVd + abiotic-decline trees experienced the greatest yield losses (≈85% relative to controls). To evaluate whether this reduction represented synergy rather than independent effects, the observed combined loss (85%) was compared with the expected combined loss under an independent (multiplicative) model: expected combined loss = $1 - (1 - 0.38) \times (1 - 0.60) = 75.2\%$. Because the observed loss (85%) exceeded the expected independent effect (75.2%) and the interaction term in the mixed-effects model was statistically significant ($\chi^2 = 12.7$, $P < 0.001$), these results are consistent with a synergistic interaction between HSVd infection and abiotic decline.

The *post hoc* Tukey's test analysis grouped the treatments into four distinct four groups. Group A: the control (greatest yield), Group B: Hop Stunt Viroid-infected trees, Group C: climate change threatened decline, and Group D: combined effect of HSVd and decline (least yield). Healthy plants yielded more than plants in the other three treatments. The combined HSVd and decline treatment yielded the least (Figure 1). The ANOVA for plant height data showed statistically significant differences between the treatments ($P = 0.001$), and between the orchards ($P = 0.035$), indicating that treatments and orchards affected plant height (Table 2).

Plant height was the most affected of the growth parameters assessed. and the orchards also affected

Table 1. Analysis of Variance (ANOVA) for fruit yield data obtained in this study.

Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Treatment	3	3924427	1308142	634.15	< 0.001
Orchard	2	5008	2504	1.21	0.297
Error	2258	4657836	2063		
Lack-of-Fit	6	18300	3050	1.48	0.181
Pure Error	2252	4639536	2060		
Total	2263	8587484			

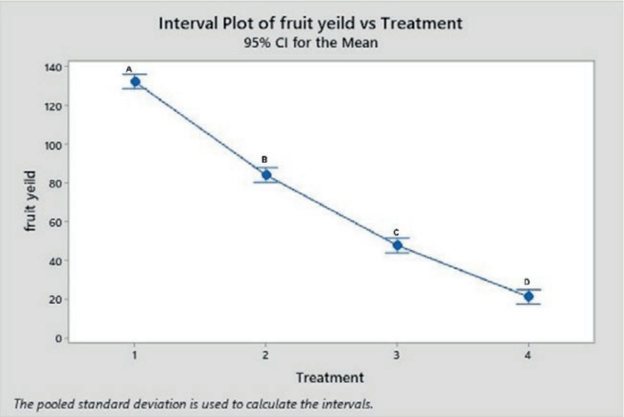

Figure 1. Mean Citrus plant yields (kg) from "healthy" plants (Treatment 1) or plants with Hop Stunt Viroid (HSVd) infections (Treatment 2), climate change-induced decline (Treatment 3), or combined HSVd plus climate-induced stress (Treatment 4). Means accompanied by different letters are ($P < 0.05$; Tukey's HSD tests).

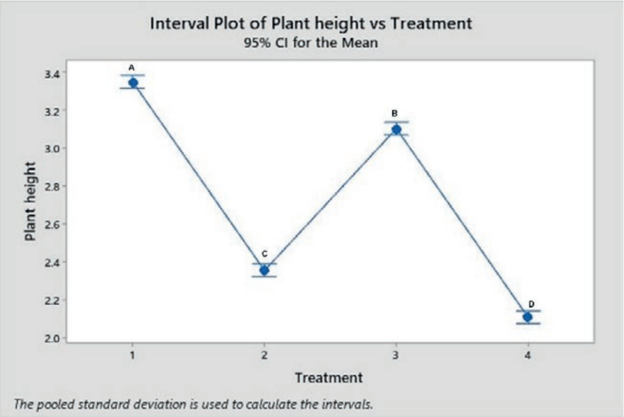

Figure 2. Effects of Hop Stunt Viroid Infection, climate change-induced decline, and their interaction on citrus tree height (m). (1) healthy control, (2) HSVd-infected trees, (3) trees exhibiting decline due to climate-related stressors, and (4) trees exposed to both HSVd infection and climate-induced decline. Means with different letters are significantly different at $P < 0.05$ (Tukey's HSD).

Table 2. Analysis of Variance (ANOVA) for plant height data obtained in this study.

Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Treatment	3	598.215	199.405	1199.67	0.001
Orchard	2	1.120	0.560	3.37	0.035
Error	2258	375.317	0.166		
Lack-of-Fit	6	0.493	0.082	0.49	0.814
Pure Error	2252	374.824	0.166		
Total	2263	974.571			

Table 3. The Analysis of Variance (ANOVA) for plant crown diameter data obtained in this study.

Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Treatment	3	207.31	69.1049	88.67	0.001
Orchard	2	0.47	0.2358	0.30	0.739
Error	2258	1759.77	0.7793		
Lack-of-Fit	6	4.60	0.7663	0.98	0.435
Pure Error	2252	1755.17	0.7794		
Total	2263	1967.54			

this parameter. However, the primary source of plant height differences was due to the treatments. *Post-hoc* Tukey's test results showed the following growth condition grouping: Group A: the control (tallest plants), Group B: climate changes threatened decline, Group C: HSVd infected trees, and Group D: HSVd infected and decline (shortest plants). The healthy plants were the tallest plants, which were taller than the climate changes threatened decline, the Hop Stunt Viroid-infected trees, and for the trees in the HSVd plus decline treatment. Although HSVd-infected trees were shorter than the climate plus decline trees, the overall growth suppression (including canopy densities and yields) was more severe in the climate plus decline trees, indicating the broader physiological limitation of these two factors. The combined effect of HSVd plus decline resulted in the shortest plants, indicating that the treatment interaction caused greatest growth reduction (Figure 2). The ANOVA for tree crown diameter indicated significant differences between the treatments ($P = 0.001$). Growth condition was also associated with crown diameter (Table 3).

Post hoc, the Tukey tests showed that the control plants had the largest crown diameters. The mean crown diameters in HSVd-infected trees, climate change threatened trees, and the combined effects of HSVd and decline were all less than for the controls. This indicated that the combined effect HSVd plus decline had the greatest impact on crown diameter, which could impede overall plant growth and development (Figure 3). For fruit diameter, there were significant differences between the treatments ($P = 0.001$), differences between the three orchards were not significant ($P = 0.125$) (Table 4).

Post hoc analyses grouped the treatments as follows: Group A: climate changes threatened decline (greatest fruit diameter), Group B: the control, Group C: HSVd infected trees, and Group D: combined HSVd and decline (least fruit diameter). Although fruit diameter was greater in climate stressed trees,

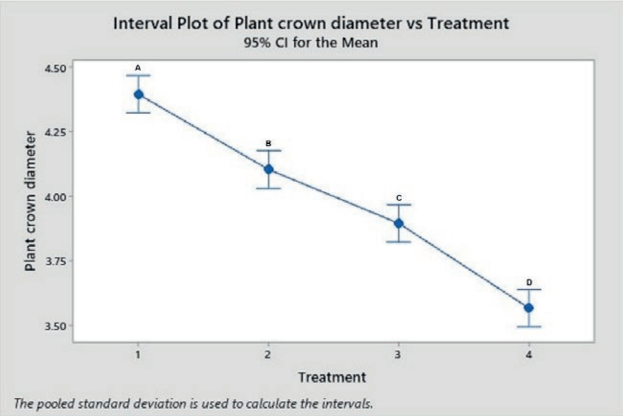

Figure 3. Effects of Hop Stunt Viroid Infection, climate change-induced decline, and their combination on crown diameter of citrus trees (m). (1) healthy control, (2) HSVd-infected trees, (3) trees exhibiting decline due to climate-related stressors, and (4) trees exposed to both HSVd infection and climate-induced decline. Means with different letters are significantly different at $P < 0.05$ (Tukey's HSD).

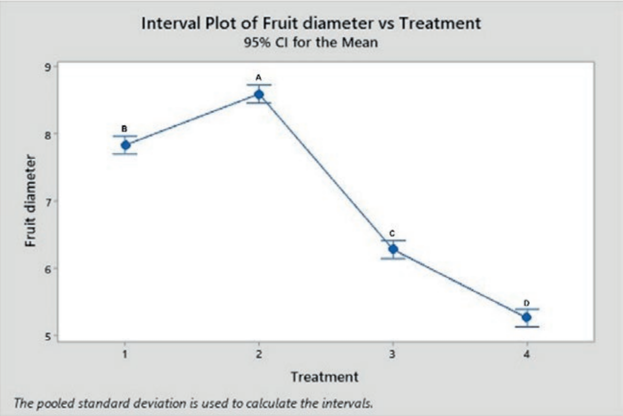

Figure 4. Effects of Hop Stunt Viroid Infection, climate change-induced decline, and Their Interaction on Fruit Diameter in Citrus Trees (cm). (1) healthy control, (2) HSVd-infected trees, (3) trees exhibiting decline due to climate-related stressors, and (4) trees exposed to both HSVd infection and climate-induced decline. Means with different letters are significantly different at $P < 0.05$ (Tukey's HSD).

Table 4. Analysis of Variance (ANOVA) of fruit diameter data obtained in this study.

Source	DF	Adj SS	Adj MS	F-Value	*P*-Value
Treatment	3	3831.25	1277.08	482.97	0.001
Orchard	2	10.99	5.49	2.08	0.125
Error	2258	5970.62	2.64		
Lack-of-Fit	6	18.99	3.17	1.20	0.304
Pure Error	2252	5951.63	2.64		
Total	2263	9813.23			

this should be interpreted with caution due to the concurrent yield reductions, which would undermine the practical value of increased fruit size. However, the combined effect of HSVd plus decline produced the smallest fruits (Figure 4). A strong negative correlation was observed between leaf chlorosis index and fruit yields (r = -0.79, $P < 0.001$), confirming that leaf chlorosis is an indicator of reduced citrus productivity. There was also a negative correlation between SAR and fruit yields (r = -0.72, $P < 0.01$).

Overall, healthy plants gave the greatest fruit yields, were the tallest, and had the greatest crown diameters compared with the other three treatments applied in this study. Climate change threatened decline was the best for enhancing fruit diameter, suggesting it might be more suitable for improving fruit size. The combined effect of HSVd plus decline consistently resulted in the greatest negative effects on plant growth and fruit productivity.

DISCUSSION

This study has provided field-based evidence for synergistic interaction between HSVd infections and abiotic stress as causes of decline of Lisbon lemon trees grafted on sour orange rootstock in Jahrom. The study has also demonstrated a direct link between the observed biotic stress to HSVd, by confirming HSVd infections molecularly and excluding CTV co-infections. Citrus decline is an increasing concern for international citrus production, and is particularly severe in Jahrom, a region recognized for its vibrant and productive citrus industry. Once a hallmark of agricultural success in southern Iran, Jahrom now faces a serious threats to citrus sustainability. While numerous factors contribute to this decline, the two interrelated stressors of climate change and HSVd infections have emerged as the most detrimental and complex challenges facing citrus growers in this region (Kovalskaya and Hammond, 2014; Belabess *et al.*, 2021). Furthermore, while HLB has been detected elsewhere in Fars Province (Faghihi *et al.*, 2025), the absence of characteristic HLB symptoms in the trees sampled in the present study indicate that decline in Jahrom is primarily caused by HSVd and abiotic stresses rather than by Liberibacter infections.

The present study has provided field-based evidence of individual and combined effects of HSVd infection, manifesting as stem pitting ("shaqqaq"; Bagerian and Izadpanah, 2010), and abiotic stress-induced reductions on growth, productivity, and survival of Lisbon lemon (*Citrus limon* L. 'Lisbon') trees in a semi-arid environment. These results provide valuable insights into the pathophysiological mechanisms affecting citrus tree degeneration under complex, multifactorial stress conditions.

The correlation between stem pitting symptoms and molecular detection of HSVd (and absence of CTV) strengthens evidence that HSVd was the biotic component in tree decline, which is consistent with the report of Bagherian *et al.* (2021). HSVd-infected trees were also smaller than the healthy trees (~30% compared to healthy trees) and had moderate canopy contractions, results that are similar to those of Bagherian and Izadpanah (2010). Viroid-induced disruption of phloem function, characterized by callose deposition and collapse of vascular tissues, has been previously reported, and may play a central role in symptom development, particularly growth stunting and impaired assimilate transport (Di Serio *et al.*, 2023). Phloem disruption It likely causes the stunted growth phenotype by impairing assimilate transport and meristem activity. Shaqqaq-only trees had yield losses of approx. 38%, consistent with previous results for HSVd-infected citrus (Navarro *et al.*, 2012), highlighting potential economic, though sublethal, effects of these infections. The mild to moderate chloroses indicate impaired nutrient transport or water relations rather than complete systemic failure. The genetic diversity of HSVd populations in Mediterranean regions (Alaxin *et al.*, 2023) could explain the variability in symptom expression.

Over the past decade, climate patterns in Jahrom have shifted, with increasing average temperatures, prolonged drought periods, erratic rainfall, and increased evapotranspiration. These abiotic stressors impose adverse physiological effects on citrus trees, disrupting water and nutrient uptake, impairing photosynthesis, and causing hormonal imbalances (Kumari *et al.*, 2022; de Souza Junior *et al.*, 2025). Direct consequences include reduced size, yield and quality of fruit, stunted vegetative growth, and general plant vigour decline.

Abiotic decline independently exerted adverse effects on tree performance, including yield reductions exceeding 60%, reductions in crown diameters (~11%), and moderate foliar chlorosis. These results are consistent with multi-year studies conducted in comparable semi-arid citrus-growing regions, where factors such as soil compaction, elevated sodium adsorption ratio (SAR), and excessive shading have been identified as key contributors to citrus decline (Navarro *et al.*, 2012; Di Serio *et al.*, 2023). Salt stress disrupts plant osmotic balance, induces ion toxicity, and promotes oxidative damage, while poor soil aeration impairs root respiration and nutrient uptake (Tian *et al.*, 2022; Ahmed *et al.*, 2023). Trees with abiotic decline showed minimal height reductions, indicating that root-zone stressors primarily limited canopy development and fruit production, rather than plant height. In the decline-only orchards, the moderate plant death (~15%) reflected the progressive nature of abiotic decline, where tree vigour gradually weakens over several growing seasons before tree death. In addition, a significant negative correlation was detected between leaf chlorosis and yield, emphasizing the importance of monitoring chlorosis as a reliable indicator of citrus decline. Negative correlation was also detected between SAR and fruit yields, indicating that salinity stress adversely affected tree productivity.

Drought stress, in particular, exacerbated by suboptimal irrigation practices and water scarcity, further weakens tree health and compromises defense systems (Seleiman *et al.*, 2021). Reduced fruit set under drought stress may lead to the accumulation of photosynthates in the remaining fruits, resulting in increased fruit diameters (Liu *et al.*, 2023). Under these conditions, citrus trees become more vulnerable to opportunistic pathogens such as HSVd. This abiotic-biotic interaction creates a compounding effect, where climate stress amplifies the host susceptibility and the impacts of viroid infections. This effect was particularly evident in Jahrom, where stressed trees exhibited severe viroid-induced symptoms and failed to recover or maintain productivity.

The symptoms caused by HSVd, including leaf chlorosis, reduced vigour, and fruit deformation, can be mistaken for, or masked by, drought-related damage. This overlap complicates early diagnoses, delays disease management interventions, and facilitates the spread of HSVd, often through contaminated tools and inadequate sanitation protocols.

The results of the present study reinforce the impacts of the HSVd–climate stress interaction. Growth condition type was found to influence all the measured plant performance indicators, including fruit yield, tree height, canopy diameter, and fruit size. Healthy control trees consistently outperformed all the other growth condition groups, highlighting the importance of maintaining plant health for optimal growth and productivity.

The observed 85% yield loss under combined stress conditions exceeded the theoretical additive effect of HSVd infection and abiotic decline (38% + 60% = 98%; $P < 0.001$), indicating a synergistic rather than additive effect. The increasing incidence of HSVd in Mediterranean citrus orchards further emphasizes the importance of stress interactions and their impacts on orchard sustainability. In the present study, trees with infected by HSVd and affected by abiotic stress had yield reductions (> 85%), severe chlorosis (mean index = 4.2), mortality rates (> 40% within 2 years), and reductions in tree height and crown diameter. These results provide strong evidence for the synergistic interaction between biotic and abiotic stressors. Several mechanisms may underlie this interaction. Compromised vascular function may result from HSVd-induced stem pitting which disrupts phloem integrity, compounding the effects of water stress associated with salinity and impaired root function. Enhanced oxidative stress can result from simultaneous presence of biotic and abiotic stressors to synergistically increase reactive oxygen species (ROS) accumulation, overwhelming antioxidant systems and triggering programmed cell death. Suppression of host defense mechanisms occurs when viroid infections modulate host gene expression, downregulating pathways involved in abiotic stress resistance and increasing the host plant vulnerability to environmental challenges. Synergistic interactions between viral pathogens and drought or salinity stress have been documented in other perennial crops (Prasad *et al*., 2022). However, quantitative characterization of these interactions involving viroids in citrus under commercial field conditions remains scarce, highlighting the novelty and importance of the present study results. Abiotic stresses probably exacerbate viroid replication by disrupting the host RNA silencing mechanisms.

The combined HSVd + climate-induced decline resulted in the most pronounced reduction in all the key measured plant performance traits. These results emphasize the urgent need to revise and improve orchard management strategies, especially in regions facing viroid infections and climate-related pressures. This interaction has socio-economic implications. Citrus production is a key source of income and employment in Jahrom, and the observed decline threatens economic stability of local farming communities. Sustaining citrus cultivation requires an integrated management approach that addresses viroid control and climate adaptation.

The present study had several limitations. Firstly, while CTV was confirmed as absent, potential interactions with other non-assessed or soil-borne pathogens cannot be ruled out. Secondly, the rootstock used (sour orange) is known to be sensitive to abiotic stresses; so the observed synergy might also be rootstock-influenced, and studies on different rootstocks are needed. Thirdly, the term "treatment" in our experimental design refers to pre-existing field conditions rather than actively applied treatments, which is a constraint of observational field studies. Fouthly, the mechanistic basis of the observed synergy requires molecular physiological investigations to confirm hypotheses involving vascular dysfunction or oxidative stress.

The present study results have implications for the development of effective orchard management strategies. These include:

- Enhanced viroid surveillance. Systematic molecular monitoring (e.g., RT-PCR, dot-blot hybridization) for HSVd should be prioritized, particularly in regions where shaqqaq symptoms are prevalent. Early detection of HSVd is important for minimizing viroid-related losses in productivity and citrus tree lifespans.
- Systematic molecular monitoring for HSVd is essential. When detected, infected trees should be immediately removed, as this is the only effective management strategy, as no known cure exists. For infection rates less than 5%, only positive trees should be removed; for rates of 5 to 20%, positive trees plus a two tree buffer should be removed; for infection rates greater than 20%, whole-orchard removal should be undertaken, followed by a fallow period before replanting with certified viroid-free stock.
- Improved soil and water management, to maintain citrus plant vigour and productivity. Drip irrigation systems should be used, to apply 80 to 100 L per tree per day in summer, or 40 to 50 L per tree per day in winter. Irrigate every 2 to 3 d during heat waves (>40°C). Soil salinity management should be used where SAR values exceed 13, and should include application of gypsum (2 to 5 tons ha^{-1} annually). For the 5 to 20% soil leaching fraction, 10 to 20 tons ha^{-1} of manure should be added each year. Organic mulch (10 to 15 cm depth) should be maintained under crop canopies to reduce evaporation and salt accumulation. Every 6 months, soil tests should be carried out to monitor SAR, EC, and pH.
- Integrated stress management. An holistic approach is essential, that simultaneously addresses biotic and abiotic stressors. Specific integrated practices include: (i) combine HSVd testing with soil SAR/EC monitoring in each citrus orchard; (ii) coordinated tree removal (rogueing) with irrigation upgrades and gypsum applications; (iii) planting of viroid-free scions on salt- and viroid-tolerant rootstocks (e.g., 'Rangpur' lime) instead of sour orange; and (iv) training for growers emphasizing that solving abiotic stress alone does not eliminate HSVd decline.
- Sanitation and use of clean planting material. This should rely on RT-PCR certified viroid-free nursery stock. Five percent of each shipment should be tested, and all plants should be rejected if viroid infections are detected. Disinfect pruning tools with 10% bleach or 2% NaOH for 30 seconds between trees. Rinse after bleach treatment, but not required for

NaOH. Immediately remove HSVd-positive trees. For 5 to 20% infections, remove a 2-tree buffer around each infected tree. After whole-orchard removals, leave fallow for 6–12 months before replanting. Carry out annual workshops on citrus crop sanitation, emphasizing that non-certified budwood is the most common route for HSVd introductions. To prevent the spread of HSVd, prioritize removing severely affected trees and using only certified viroid-free propagation material in orchards.

In conclusion, the interplay between climate change and pathogens such as HSVd is a major cause of citrus decline in the Jahrom region. Field observations and molecular assays have shown that orchard health category (growth condition type), particularly concurrence of HSVd-associated symptoms and diagnostic evidence plus abiotic decline indicators, is associated with reduced tree performance (height, crown diameter, yield). However, because this study was observational, and because potential confounders (rootstock variation, undetected co-infections) were not ruled out across all the surveyed citrus orchards, these results are evidence of association and synergistic interactions between HSVd and abiotic factors, rather than definitive causal proof. Controlled inoculation experiments and complete pathogen assessments are required to conclusively attribute causality. Without timely interventions, these dual threats pose a serious risk to the long-term viability of citrus orchards. To mitigate their impacts, a comprehensive management strategy is essential. This should include improved irrigation systems, the use of drought-tolerant and disease-resistant rootstocks, optimized nutrient and pruning practices, and robust viroid surveillance and control measures. Long-term solutions may also involve the development of HSVd-resistant citrus cultivars and adoption of climate-integrated agricultural technologies. Survival of productive citrus farming in Jahrom requires proactive, science-based, and locally tailored interventions that address environmental and pathological dimensions.

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DATA AVAILABILITY

The data supporting the findings of this study are not available elsewhere and will be made available in accordance with the data availability policy for *Phytopathologia Mediterranea*.

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