Light manipulation with next-generation indoor farming affects the onset and severity of viral disease symptoms in citrus indicators

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Introduction

The majority of the source trees used in citrus propagation in California are introduced, grown, and/or diagnosed by the Citrus Clonal Protection Program (CCPP). The CCPP uses the Variety Introduction index (VI index) to detect graft-transmissible pathogens for new citrus varieties which must undergo a quarantine period of disease testing and therapy procedures that require the use of biological indexing (BI) assays (Cal. Admin. Code tit. 3, § 3250 Citrus pests exterior quarantine). Alternative agriculture practices using next-generation indoor vertical farming techniques can potentially influence viral disease expression in BI procedures by reducing the amount of time and materials needed for the CCPP's VI index.

Plant defense responses and symptom development are controlled and modulated by abiotic signals such as light, circadian rhythm, and temperature (3). For example, disease symptoms of tomato mosaic virus on pepper plants developed faster and more severely when grown under 99% red wavelength LED light, compared to plants grown with broad spectrum light sources that contained blue and UV-A wavelengths (8). While plant symptom development and light signaling are connected, the responses to different quality of light seems to be pathogen and host-dependent (1, 2, 8). The connections to light and defense responses support the hypothesis that quality of light can induce changes in symptom expression of viral diseases.

Currently, no research has been conducted on how light quality affects viral disease symptom expression and host physiological responses in citrus. Next-generation indoor vertical farming techniques utilizing hydroponics and different ratios of red and blue wavelengths from artificial LED lighting influenced viral disease expression onset, severity, and persistence in sensitive citrus indicators used in BI quarantine protocols. These alternative and sustainable agriculture practices can be used to reduce the time and materials needed for the CCPP's VI index, better meeting the demand for new pathogen-free citrus varieties in California.

Goals

- Optimize viral disease expression in sensitive citrus indicators used in BI assays
- Reduce the time and materials needed for the CCPP's VI index
- Determine if environmental manipulation affects graft success and/or truck growth of indicators

Materials & Methods

Citrus pathogen sources and indexing hosts. Six economically important endemic viruses of California were chosen from the CCPP's disease bank located at the University of California, Riverside. The citrus indicators used for pathogen inoculation were selected based on sensitivity for symptom expression (7) **(Table 1)**.

Table 1: Graft-transmissible citrus pathogens and sensitive citrus indexing hosts for BI assays

Viral disease agent	Genus	Pathogen isolates[a]	Citrus indicator
Citrus tristeza virus (CTV)	*Closterovirus*	CTV516 (T30) CTV519 (VT)	Mexican lime
Citrus psorosis virus (CPsV)	*Ophiovirus*	CPsV205	Pineapple sweet orange
Citrus virus A (CiVA)	–[b]	CiVA301	Pineapple sweet orange
Citrus tatter leaf virus (CTLV)	*Capillovirus*	CTLV100	Rusk on rough lemon
Citrus vein enation virus (CVEV)	*Enamovirus*	CVEV704	Mexican lime
Citrus yellow-vein associated virus (CYVaV)	–[c]	CYVaV920	Mexican lime

[a] Isolate numbers from the Citrus Clonal Protection Program archives
[b] Probable *Coguvirus* (5)
[c] Probable *Umbravirus*-like unclassified RNA (4)

Next-generation indoor vertical farming. Adjustable spectrum LED lights were used to achieve four different ratios of red: blue light (R, 660nm; B, 450nm) treatments, one full-spectrum (FS) light treatment, and then compared to standard greenhouse conditions (SGC) at the CCPP **(Figure 1a-f)**. The citrus indicators were placed in vertical farming systems with recirculating hydroponics that uses the ebb and flow irrigation with standard vegetative growth nutrients, and monitored weekly for pH and electrical conductivity. All treatments were in temperature controlled environments averaging 24-27°C and included non-inoculated control trees.

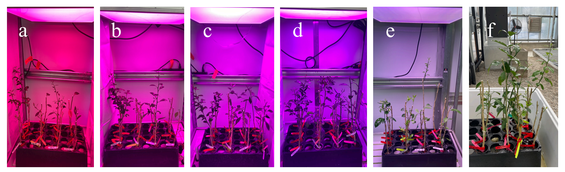

Figure 1: Experiment environmental conditions **(a)** 95R:5B **(b)** 85R:15B **(c)** 75R:25B **(d)** 65R:35B **(e)** FS and **(f)** SGC.

Pathogen inoculation and symptom monitoring. Each pathogen was graft-inoculated from single-infection disease bank trees with mild pathogen isolates **(Table 1)**. Successful grafts (1 out of 2 surviving grafts) were considered positively inoculated after three weeks. Symptoms severity was monitored weekly and rated for severity on a 0-5 scale. Symptom persistence was determined by how many weeks visible symptoms were apparent in young citrus flush.

Results

Clear and distinct viral symptom expression in sensitive citrus indicators from mild isolates (Figure 2a-f).

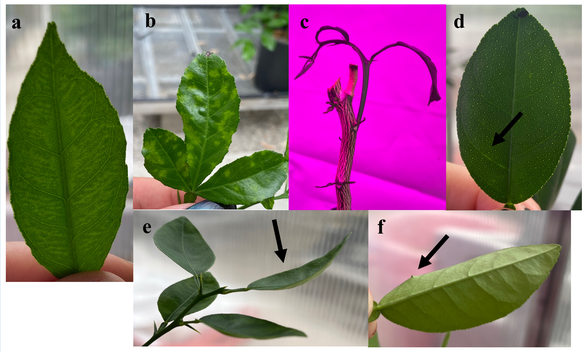

Figure 2: Viral symptom expression **(a)** CiVA301 young leaf pattern and oak leaf pattern **(b)** CTLV100 chlorotic lesions and leaf deformities **(c)** CPsV205 shock **(d)** CTV519 delicate vein clearing **(e)** CTV516 leaf cupping **(f)** CVEV704 vein enation

Highest ratios of red light produced more distinct symptoms of CiVA for longer persistence periods in young flush (Figure 3a-c).

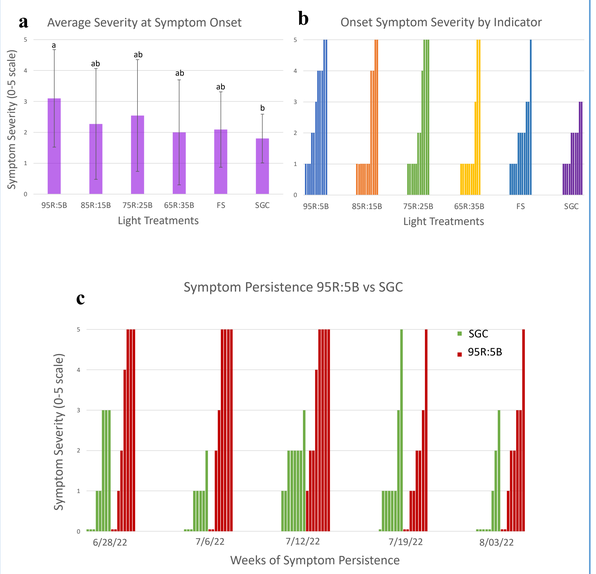

Figure 3: CiVA symptom onset and persistence in sweet orange indicators **(a)** average symptom severity per light treatment, n=11 **(b)** symptom onset severity per sweet orange indicator, n=11 and **(c)** symptom persistence comparing 95R:5B and SGC, n=8.

Results

Highest ratios of blue light produced more distinct symptoms of CPsV in similar persistence periods (Figure 4a-b).

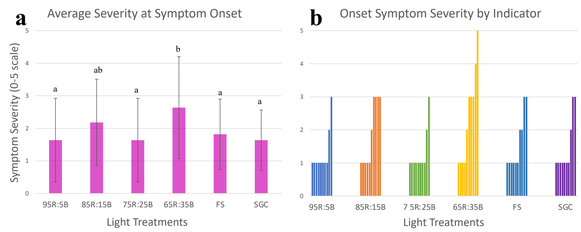

Figure 4: CPsV symptom onset severity in sweet orange indicators **(a)** average symptom onset severity per light treatment, n=11 and **(b)** symptom onset severity per sweet orange indicator, n=11

Conclusions

- **The mildest pathogen isolates show clear and distinct symptoms in all light treatments**
- **Light spectra manipulation can influence viral symptom expression in CiVA and CPsV, increasing symptom severity and persistence**
- **Further replicates are necessary to understand host physiological responses**

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