

Advanced 2D and 3D Image-Based Plant Phenotyping of Citrus Morphological Responses to *Candidatus Liberibacter asiaticus* Infection

Juan C. Cifuentes-Arenas¹, Annika Janßen², Chiara Lunewski², Fabian Keil², Mônica N. Alves¹, Nelson A. Wulff¹

¹ Fund for citrus protection – Fundecitrus. Araraquara, Brazil

² Fraunhofer Institute for Integrated Circuits IIS, Erlangen, Germany

Abstract

Huanglongbing (HLB), associated with *Candidatus Liberibacter asiaticus* (CLas), is considered the most destructive citrus disease worldwide and threatens the long-term sustainability of citrus production because all commercially cultivated varieties remain susceptible. Identification of tolerant or resistant genotypes has therefore become a major priority for breeding programs. Conventional phenotyping based on visual scoring and manual measurements is labor intensive, subjective, and often unable to detect subtle morphological changes associated with disease progression. This study evaluated complementary two-dimensional (2D) and three-dimensional (3D) image-based phenotyping approaches for detecting structural responses of citrus plants to CLas infection. Healthy and CLas-inoculated ‘Valencia’ and ‘Pera’ sweet orange plants grafted onto ‘Rangpur’ lime were analyzed using automated RGB image processing and 3D reconstruction approaches. Image-derived traits captured reductions in plant architecture and leaf morphological alterations associated with infection, supporting digital phenotyping as a scalable framework for genotype evaluation and early disease characterization.

Keywords: 3D plant architecture, high-throughput plant phenotyping (HTPP), disease response, computational phenomics, trait quantification

Introduction

Huanglongbing (HLB), associated with *Candidatus Liberibacter asiaticus* (CLas), is the most devastating citrus disease worldwide. Since its introduction into major citrus-producing regions, HLB has caused severe economic losses due to reductions in yield, fruit quality, tree vigor, and orchard lifespan (da Graça et al. 2016). In Brazil, management strategies rely on integrated practices including removal of symptomatic trees, vector control targeting *Diaphorina citri*, and the use of disease-free planting material (Bassanezi et al. 2020). However, despite intensive management efforts, no commercially cultivated citrus variety exhibits complete resistance. Consequently, the identification of tolerant or resistant genotypes has become a major objective of citrus breeding programs (Alves et al. 2021; Ramadugu et al. 2016).

Conventional breeding approaches require evaluation of large populations derived from hybrids, rootstocks, and germplasm collections. However, phenotypic characterization remains a major bottleneck because it largely depends on visual assessments and manual measurements (Furbank and Tester 2011; Reynolds et al. 2019). Such approaches are labor intensive, susceptible to observer variability, and often unable to detect subtle structural changes during early disease progression.

Over the last decade, high-throughput plant phenotyping (HTPP) has transformed plant characterization by enabling rapid, objective, and reproducible measurements through imaging technologies and computational tools (Fahlgren et al. 2015; Yang et al. 2013). Image-based phenotyping has increasingly been used to quantify plant growth,

architecture, biomass, and stress responses across different crops. Among available technologies, RGB imaging remains particularly attractive because of its low cost and ease of implementation (Li et al. 2014). Combined with image processing and machine learning approaches, RGB images can generate quantitative descriptors of plant morphology and canopy structure.

Several computational platforms have been developed for automated trait extraction, including PlantCV, a widely used open-source framework for plant image analysis. Applications of digital phenotyping have expanded across crops such as wheat, maize, rice, and soybean (Schuhl et al. 2026), while in citrus, image-based methods have been explored for disease detection and crop monitoring in the field (Garcia-Ruiz et al. 2013).

Despite these advances, conventional 2D approaches still present important limitations because plant architecture is inherently three-dimensional (Paulus 2019). Projected images may lose information due to leaf overlap, occlusion effects, and viewpoint dependency (Lou et al. 2014; Pound et al. 2017), leading to erroneous parameters and potentially masking subtle disease-related changes.

Recent computer vision approaches such as Structure-from-Motion (SfM), multi-view stereo, and Neural Radiance Fields (NeRF) enable reconstruction of detailed 3D plant representations even with the inherent determination of semantic traits (Meyer et al. 2024). These approaches allow extraction of architectural traits including leaf orientation, curvature, insertion angles, and canopy organization (Hu et al. 2024; Mildenhall et al. 2020; Paulus 2019), potentially improving sensitivity for detecting stress responses.

Therefore, this study evaluated complementary 2D and 3D phenotyping approaches to characterize morphological responses of healthy and CLas-inoculated citrus plants and assess their potential for scalable genotype evaluation in citrus breeding programs.

Material and Methods

Plant material and inoculation procedure

For the 2D analysis, ‘Valencia’ and ‘Pera’ sweet orange plants grown in 300 mL conical tubes containing coconut fiber substrate were evaluated at 12 months after inoculation (MAI). For the exploratory 3D analysis, ‘Valencia’ sweet orange plants were grown in 1.5 L plastic bags containing the same substrate and evaluated at 2 MAI to investigate early responses associated with CLas infection.

Plants were inoculated according to Lopes and Cifuentes-Arenas (2021). Briefly, plants were pruned to induce flushing, and five CLas-infective *Diaphorina citri* adults were confined on a single young shoot per plant for a 7-day inoculation access period (IAP) using a sleeve mesh cage. After inoculation, insects were removed and plants were sprayed with insecticide/acaricide.

Psyllid rearing, inoculation procedures, and the first four weeks after inoculation were conducted in a controlled-environment growth room under a 14:10 h photoperiod, light intensity of approximately 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 26 ± 2 °C, and relative humidity of approximately 55%. After this period, plants were transferred to a greenhouse. CLas infection was confirmed at 4 MAI by qPCR analysis of leaf samples.

2D image acquisition and trait extraction

Before image acquisition, plant height was manually measured and used as the ground-truth reference for validation. Plants were individually photographed under

standardized conditions using an RGB camera positioned at a fixed distance and height relative to the canopy. Up to eight lateral images were acquired per plant while manually rotating plants around their vertical axis to obtain distinct canopy profiles associated with plant asymmetry and leaf distribution.

Images were processed using an automated Python-based workflow integrating PlantCV and custom scripts for plant segmentation and trait extraction. Plant height was estimated from segmented canopies after pixel-to-length calibration. Based on the validation analysis described below, at least four images per plant were used for subsequent phenotyping analyses on ‘Pera’ sweet orange. Additional morphological traits including projected 2D canopy area, convex hull area, canopy solidity, and canopy width were extracted from four canopy profiles per plant, and final trait values were calculated as the mean across images. These analyses were performed for both CLas-challenged and mock-inoculated plants.

3D reconstruction and leaf morphological analysis

An exploratory 3D analysis was performed using one CLas-challenged and one mock-inoculated control plant at 2 months after inoculation (MAI). Multiview RGB image sets were acquired from multiple lateral perspectives around each plant under standardized conditions.

Dense 3D point clouds were reconstructed from RGB images using Neural Radiance Fields (NeRF). Reconstructed point clouds were subsequently processed using the Fraunhofer Plant2Trait pipeline, in which individual leaves were segmented and fitted to a parametric leaf model. This procedure enabled extraction of leaf morphological and architectural traits including leaf length, width, area, insertion angle, and curvature. To evaluate reconstruction accuracy, image-derived leaf area estimates were compared with manual measurements obtained from representative leaves sampled from both plants.

Data analysis

To determine the minimum number of images required for reliable height estimation, only plants containing at least eight valid images were included in the analysis. All possible image combinations were generated for subsets ranging from one to seven images, whereas for eight images a single estimate corresponding to the mean value across all profiles was calculated. For each subset, estimated height corresponded to the mean value obtained from the selected image profiles.

Image-derived height estimates were compared against manually measured values using mean absolute error (MAE), root mean square error (RMSE), and coefficient of determination (R^2). Linear regression models were fitted between measured and image-derived height for each image subset size. Variability and confidence intervals for R^2 were assessed using bootstrap resampling with 500 iterations.

$$MAE = \frac{1}{n} \sum_{i=1}^n |H_i - \hat{H}_i|$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (H_i - \hat{H}_i)^2}$$

where H_i and $\hat{H}_i$ represent measured and image-derived plant height, respectively.

Differences between mock-inoculated and CLas-challenged plants for image-derived morphological traits, including plant height, canopy width, projected 2D canopy area, convex hull area, and canopy solidity, were evaluated using Student's t-tests. For each plant, final trait values corresponded to the mean obtained from four canopy profiles. Statistical analyses and graphical visualizations were performed in R software (R Core Team, Vienna, Austria).

Results and Discussion

Validation of image-based height estimation

The image acquisition and processing workflow provided accurate estimates of plant height for both 'Pera' and 'Valencia' sweet orange plants. Strong linear relationships were observed between manually measured (ground-truth) and image-derived height estimates, with coefficients of determination exceeding 0.95 for both genotypes (Figure 1). The high agreement between methods confirms that canopy segmentation and trait extraction procedures were able to reliably capture plant dimensions despite natural variation in canopy architecture and image perspective.

c

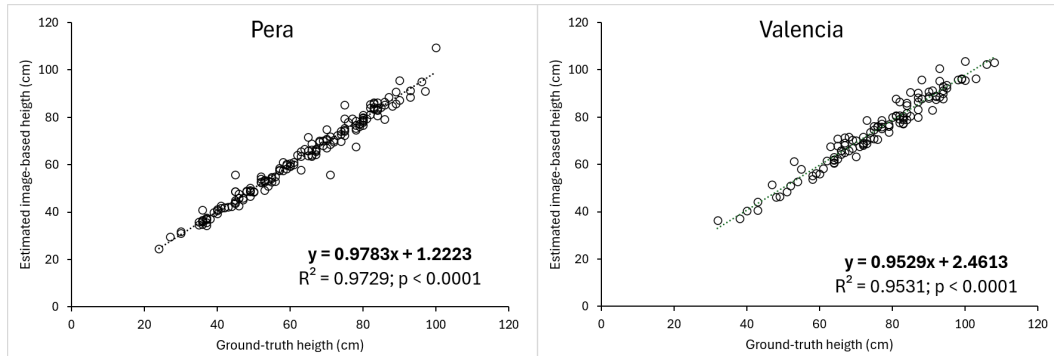


Figure 1. Relationship between ground-truth plant height measurements and image-derived height estimates for potted 'Pera' and 'Valencia' sweet orange plants. Each point represents an individual observation, and dashed lines indicate fitted linear regression models.

Validation of image-based height estimation

The image acquisition and processing workflow provided accurate estimates of plant height for both 'Pera' and 'Valencia' sweet orange plants. Strong linear relationships were observed between manually measured (ground-truth) and image-derived height estimates, with coefficients of determination exceeding 0.95 for both genotypes (Figure 1). The high agreement between methods confirms that canopy segmentation and trait

extraction procedures were able to reliably capture plant dimensions despite natural variation in canopy architecture and image perspective.

CLas-induced alterations in canopy architecture revealed by 2D phenotyping

The automated RGB-based phenotyping workflow successfully detected significant differences between mock-inoculated and CLas-challenged ‘Pera’ sweet orange plants at 12 months after inoculation (Figure 2). All evaluated traits indicated a substantial reduction in plant growth and canopy development associated with CLas infection.

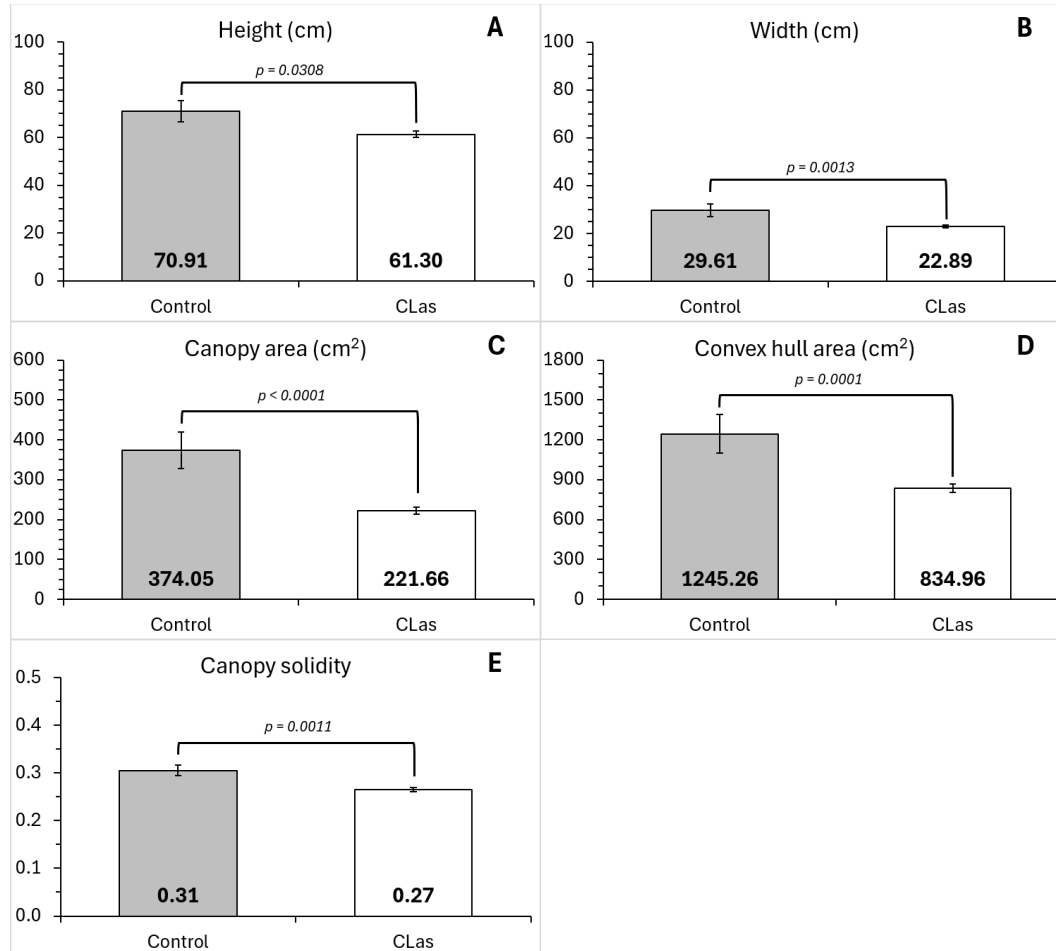


Figure 2. Image-derived morphological traits of mock-inoculated (Control) and CLas-challenged ‘Pera’ sweet orange plants at 12 months after inoculation. (A) Height, (B) canopy width, (C) projected canopy area, (D) convex hull area, and (E) canopy solidity. Bars represent mean $\pm$ standard error. P-values were obtained using Student’s *t*-tests.

In CLas-infected plants, there was a significant reduction in height (by 13.6%; $p = 0.0308$) and width (by 22.7%; $p = 0.0013$). These reductions indicate that CLas infection affects not only vertical growth but also lateral canopy expansion, resulting in smaller and less vigorous plants, a response commonly associated with CLas infection and disruption of phloem transport and carbohydrate allocation (Achor et al. 2020; da Graça et al. 2016).

The strongest effects were observed for canopy area and convex hull area. Projected canopy area decreased from 374.05 cm² in healthy plants to 221.66 cm² in

infected plants (-40.7%; $p < 0.0001$). Likewise, convex hull area decreased from 1245.26 cm² to 834.96 cm² (-33.0%; $p = 0.0001$). Also, canopy solidity also differed significantly between treatments, decreasing from 0.31 in control plants to 0.27 in CLas-challenged plants ($p = 0.0011$). These results indicate a pronounced loss of canopy volume and occupation, reflecting the cumulative effects of reduced shoot growth, leaf production, and canopy expansion commonly associated with HLB progression in the field (Livingston et al. 2023).

These results show that image-derived traits are sensitive to structural changes induced by CLas infection and can quantitatively capture multiple dimensions of canopy decline. In addition, the automated workflow processed approximately 91 images per minute, highlighting its potential to substantially increase phenotyping throughput while reducing observer-related variability.

3D morphological leaf traits and early infection responses

Figure 3 presents the NeRF-based 3D reconstructed point clouds and the modelled leaves of both healthy and infected plants, two months after inoculation. The automatically calculated 3D leaf traits derived from these models were validated against manually measured values for leaf length, width, and area. The comparison (Figure 4) demonstrates a very high correlation between model-derived and manual measurements, with R^2 values ranging from 0.83 to 0.97.

The comparison of model-based leaf parameters between healthy and infected plants reveals that healthy plants tend to have leaves with a larger area, which is primarily due to greater leaf width (Figure 5a). Additionally, the curvature of infected leaves is slightly but measurably increased, especially in the lower part of the canopy (Figure 5b). No differences were observed for the leaf inclination angle (Figure 5c). These findings highlight the potential of high-resolution 3D leaf morphology analysis for plant phenotyping. Leaf curling is a known symptom of HLB infection, but it typically becomes apparent only in later stages of the disease, several months after infection. 3D leaf modelling enables precise quantification of changes in leaf geometry and is therefore capable of detecting deviations from normal growth at very early stages.



Figure 3. NeRF-based 3D point clouds and modelled leaves using the Fraunhofer Plant2Trait-Pipelining for healthy (left) and HLB infected (right) plant.

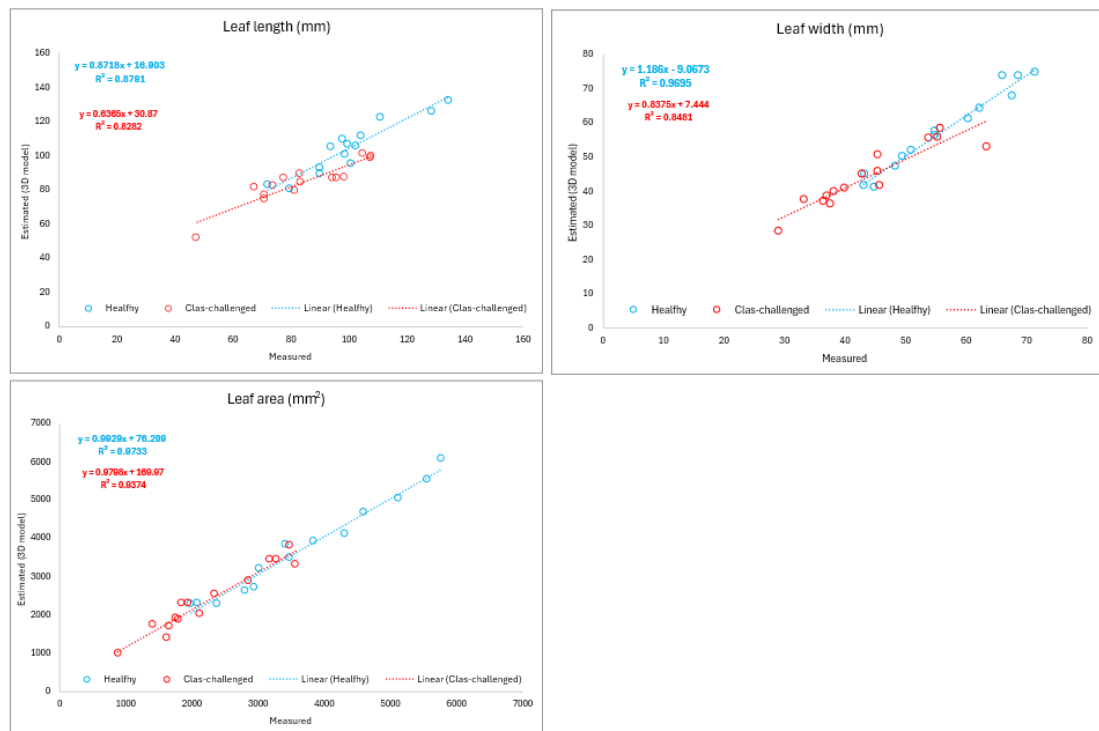


Figure 4. Validation of automatically derived leaf traits with manual reference measurements.

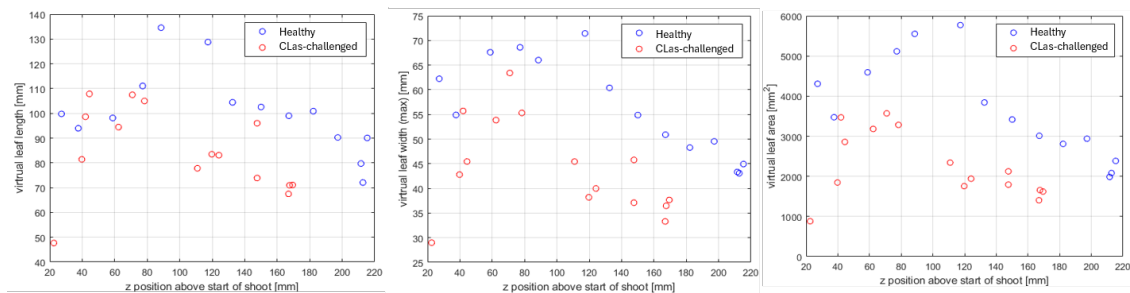


Figure 5a. Leaf length, width and height derived from 3D leaf model



Figure 5b. Comparison of leaf curvature from healthy and infected plant as derived from 3D leaf model

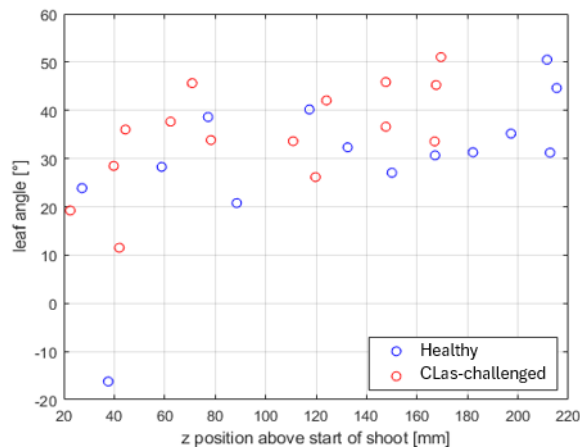


Figure 5c: Leaf inclination angles for healthy and HLB infected plants derived from 3D leaf model

References

- Achor, D., Welker, S., Ben-Mahmoud, S., Wang, C., Folimonova, S. Y., Dutt, M., Gowda, S., and Levy, A. 2020. Dynamics of *Candidatus Liberibacter asiaticus* Movement and Sieve-Pore Plugging in Citrus Sink Cells. *Plant Physiol.* 182:882–891. <https://doi.org/10.1104/pp.19.01391>.
- Alves, M. N., Lopes, S. A., Raiol-Junior, L. L., Wulff, N. A., Girardi, E. A., Ollitrault, P., and Peña, L. 2021. Resistance to ‘*Candidatus Liberibacter asiaticus*’ the Huanglongbing Associated Bacterium, in Sexually and/or Graft-Compatible Citrus Relatives. *Front. Plant Sci.* 11:1–16. <https://doi.org/10.3389/fpls.2020.617664>.
- Bassanezi, R. B., Lopes, S. A., de Miranda, M. P., Wulff, N. A., Volpe, H. X. L., and Ayres, A. J. 2020. Overview of citrus huanglongbing spread and management strategies in Brazil. *Trop. Plant Pathol.* 45:251–264. <https://doi.org/10.1007/s40858-020-00343-y>.
- Fahlgren, N., Gehan, M. A., and Baxter, I. 2015. Lights, camera, action: high-throughput plant phenotyping is ready for a close-up. *Curr. Opin. Plant Biol.* 24:93–99. <https://doi.org/10.1016/j.pbi.2015.02.006>.
- Furbank, R. T., and Tester, M. 2011. Phenomics--technologies to relieve the phenotyping bottleneck. *Trends Plant Sci.* 16:635–44. <https://doi.org/10.1016/j.tplants.2011.09.005>.
- Garcia-Ruiz, F., Sankaran, S., Maja, J. M., Lee, W. S., Rasmussen, J., and Ehsani, R. 2013. Comparison of two aerial imaging platforms for identification of Huanglongbing-infected citrus trees. *Comput. Electron. Agric.* 91:106–115. <https://doi.org/10.1016/j.compag.2012.12.002>.
- da Graça, J. V., Douhan, G. W., Halbert, S. E., Keremane, M. L., Lee, R. F., Vidalakis, G., and Zhao, H. 2016. Huanglongbing: An overview of a complex pathosystem ravaging the world's citrus. *J. Integr. Plant Biol.* 58:373–387. <https://doi.org/10.1111/jipb.12437>.
- Hu, K., Ying, W., Pan, Y., Kang, H., and Chen, C. 2024. High-fidelity 3D reconstruction of plants using Neural Radiance Fields. *Comput. Electron. Agric.* 220:108848. <https://doi.org/10.1016/j.compag.2024.108848>.
- Li, L., Zhang, Q., and Huang, D. 2014. A Review of Imaging Techniques for Plant Phenotyping. *Sensors* 14:20078–20111. <https://doi.org/10.3390/s141120078>.
- Livingston, T., Levy, A., and Vashisth, T. 2023. A Simple Low-cost Method for

- Accurate Canopy Density Evaluation of Citrus. *HortScience* 58:747–749.
<https://doi.org/10.21273/HORTSCI17112-23>.
- Lou, L., Liu, Y., Han, J., and Doonan, J. H. 2014. Accurate Multi-View Stereo 3D Reconstruction for Cost-Effective Plant Phenotyping. In *11th International Conference Image Analysis and Recognition*, Lecture Notes in Computer Science, eds. Aurélio Campilho and Mohamed Kamel. Cham: Springer International Publishing, pp. 349–356. <https://doi.org/10.1007/978-3-319-11755-3>.
- Meyer, L., Gilson, A., Schmid, U., and Stamminger, M. 2024. FruitNeRF: A Unified Neural Radiance Field based Fruit Counting Framework. In *2024 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS 2024)*, Abu Dhabi, United Arab Emirates.
- Mildenhall, B., Srinivasan, P. P., Tancik, M., Barron, J. T., Ramamoorthi, R., and Ng, R. 2020. *NeRF: Representing Scenes as Neural Radiance Fields for View Synthesis*. https://link.springer.com/10.1007/978-3-030-58452-8_24.
- Paulus, S. 2019. Measuring crops in 3D: using geometry for plant phenotyping. *Plant Methods* 15:103. <https://doi.org/10.1186/s13007-019-0490-0>.
- Pound, M. P., Atkinson, J. A., Townsend, A. J., Wilson, M. H., Griffiths, M., Jackson, A. S., Bulat, A., Tzimiropoulos, G., Wells, D. M., Murchie, E. H., Pridmore, T. P., and French, A. P. 2017. Deep machine learning provides state-of-the-art performance in image-based plant phenotyping. *Gigascience* 6:1–10.
<https://doi.org/10.1093/gigascience/gix083>.
- Ramadugu, C., Keremane, M. L., Halbert, S. E., Duan, Y. P., Roose, M. L., Stover, E., and Lee, R. F. 2016. Long-term field evaluation reveals Huanglongbing resistance in *Citrus* relatives. *Plant Dis.* 100:1858–1869. <https://doi.org/10.1094/PDIS-03-16-0271-RE>.
- Reynolds, D., Baret, F., Welcker, C., Bostrom, A., Ball, J., Cellini, F., Lorence, A., Chawade, A., Khafif, M., Noshita, K., Mueller-Linow, M., Zhou, J., and Tardieu, F. 2019. What is cost-efficient phenotyping? Optimizing costs for different scenarios. *Plant Sci.* 282:14–22. <https://doi.org/10.1016/j.plantsci.2018.06.015>.
- Schuhl, H., Gutierrez, J., Ballenger, J. G., Chavez, L., Dimech, A. M., Hendrikse, C., Brown, K. E., Schneider, D., Sheng, H., Casto, A. L., Bhatt, P. K., Acosta-gamboa, L., Barbero, F., Braley, J., Brown, A. M., Cunningham, S., Dilhara, M., Duenwald, J. G., Hernandez, G. L., Fischer, A., Hodge, J. G., Gordon, J. M., Huber, M., Hurr, B. M., Jarolmasjed, S., Jimenez, K. M., Kenney, S., Konkell, G., Luebbert, C., Ly, N., Mudrageda, P., Pierik, R., Schultz, T. J., Sumner, J., Daalen, T. Van, Ying, K. R., Fahlgren, N., Lohbihler, M., Murphy, K. M., Polydore, S., Seigel, E., Robey, C., Rogers, T., Srivastava, D., Thompson, A. E., Tovar, J. C., Wheeler, J. J., Wilson, M. C., Gehan, M. A., Teng, C., and Watson, M. 2026. PlantCV v4 : Image analysis software for high-throughput plant phenotyping. 1–20.
<https://doi.org/10.1002/ppj2.70065>.
- Yang, W., Duan, L., Chen, G., Xiong, L., and Liu, Q. 2013. Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. *Curr. Opin. Plant Biol.* 16:180–187.
<https://doi.org/10.1016/j.pbi.2013.03.005>.