ADVANCED TECHNIQUES FOR ASSESSING PHYTOPHTHORA ROOT ROT (PRR) SEVERITY IN AVOCADO ORCHARDS: PROXIMAL AND REMOTE SENSING APPROACHES

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NOTE REGARDING PUBLICATION
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- Duncan, S.J., McLeod, A. and Poblete-Echeverría, C. (2024). Application of UAV and satellite technologies for assessing Phytophthora Root Rot (PRR) severity in avocado orchards. Frontiers (*Submitted - under revision*).

INTRODUCTION

While avocados have become a beloved staple in diets worldwide, few realise that a silent killer, *Phytophthora* root rot (PRR), threatens this lucrative industry. The good news? Cutting-edge remote sensing (RS) technology is offering a lifeline.

PRR, caused by the soil-borne pathogen *Phytophthora cinnamomi*, is a major threat to avocado orchards worldwide, leading to significant economic losses and reduced fruit production (Ploetz, 2003; Ploetz *et al.*, 2007). The disease attacks the roots, hindering their ability to absorb water and nutrients, which compromises the health and productivity of the trees (Erwin and Ribeiro, 1996). Traditionally, assessing PRR severity has required labour-intensive field surveys and visual inspections, which can be time-consuming and subjective (Coffey *et al.*, 2017).

In recent years, advancements in RS technologies have offered promising avenues for monitoring and managing PRR in avocado orchards. RS techniques, such as multispectral imaging (MSI) and hyperspectral imaging (HSI), provide a non-invasive means to detect subtle changes in vegetation health and canopy structure associated with PRR infection (Mahlein *et al.*, 2012; Mahlein, 2016). These technologies enable the collection of high-resolution data over large areas, supporting early detection and the spatial mapping of PRR-infected areas within orchards (Gómez *et al.*, 2020).

Additionally, proximal sensing (PS), using handheld sensors or low altitude flying unmanned aerial vehicles (UAVs) equipped with various sensors, have emerged as powerful tools for assessing PRR severity on a more detailed scale by gathering information about plant physiological parameters (Mahlein *et al.*, 2013; Calderón *et al.*, 2013). By measuring specific indicators such as canopy temperature, PS provides insights into the physiological responses of avocado trees to PRR infection, offering valuable data for disease management strategies (Caffier *et al.*, 2021).

This article summarises research findings from field experiments that evaluated the suitability of different RS and PS techniques for mapping the severity of PRR in avocado orchards, with focus on laboratory HSI and remote MSI data collection techniques.

MATERIALS AND METHODS

1. Experimental sites

Two mature, commercial avocado blocks with the same cultivar and rootstock but with different canopy management practices were selected. Block 1 underwent intense pruning, while block 2 received mild pruning.

To establish suitable research blocks, a RS-based screening analysis was implemented to evaluate the intra-block changes in vigour expressed as Normalized Vegetation Index (NDVI) from ZZ2 and Westfalia Fruit, relying on visual assessments using the 'Ciba-Geigy' rating scale. For the MSI analysis, a total of 69 trees were selected: 34 trees in block 1 and 35 trees in block 2. Data were collected in May, September and December 2021 and February 2022.

In September 2021, a subsample of target trees with rankings of 1, 4 and 8/9 were identified in each study block for the HSI analysis. The locations of the target trees were captured using a Global Navigation Satellite System (GNSS) rover, and their coordinates were saved in the WGS-84 reference system. The positions of the trees were measured in the middle of the rows, perpendicular to the trees.

For the HSI analysis in the laboratory, samples were collected in November 2021. Branchlets were sampled to allow for better preservation of the leaves during transport.

3. Severity ranking

Visual assessments of the target trees in each block were conducted using the 'Ciba-Geigy' rating scale for each assessment period (Darvas *et al.*, 1984). To maintain consistency, the visual assessments were performed by the same two personnel throughout the experiment. Figure 1 shows the locations of target trees and the severity rankings measured in the field in during May 2021.

4. Hyperspectral imaging (HSI) acquisition

The HSI acquisition was conducted at the Central Analytical Facility (CAF), Vibrational Spectroscopy Unit at Stellenbosch University. At the CAF, the leaves were carefully removed from the branchlets at the base of the leaf and positioned flat on a sheet of black paper. The adaxial (upper) side of the leaves was scanned using a HySpex dual VNIR-1800/SWIR-384 camera system (Neo, Oslo, Norway) as shown in Figure 3 in Poblete-Echeverría *et al.* (2023).

5. Multispectral imagery (MSI)

Multispectral images were acquired over four phenological periods using UAV and commercial satellite platforms (for more details, refer to Duncan *et al.*, (2024)).

5.1 Unmanned aerial vehicle (UAV)

A DJI Phantom 4 Pro multicopter (DJI, Shenzhen, China) equipped with a 16-bit RGB camera (model FC6310) and a 12-bit RedEdge™ 3 multispectral camera (MicaSense Inc., Seattle, U.S.A.) was utilised for acquiring red, green, and blue (RGB) and multispectral images respectively. Flights were scheduled as close to solar noon as possible to minimise shadows, and the UAV followed a predefined flight aligned with the tree rows (Fig. 2).

5.2 Satellite imagery

High-resolution satellite imagery (0.50 - 0.75 m) was obtained from third-party suppliers, including Maxar Technologies and GEO Data Design (Pty) Ltd. This imagery came pre-georeferenced and underwent radiometric corrections. Due to their short revisit times, the satellite imagery acquisition dates were

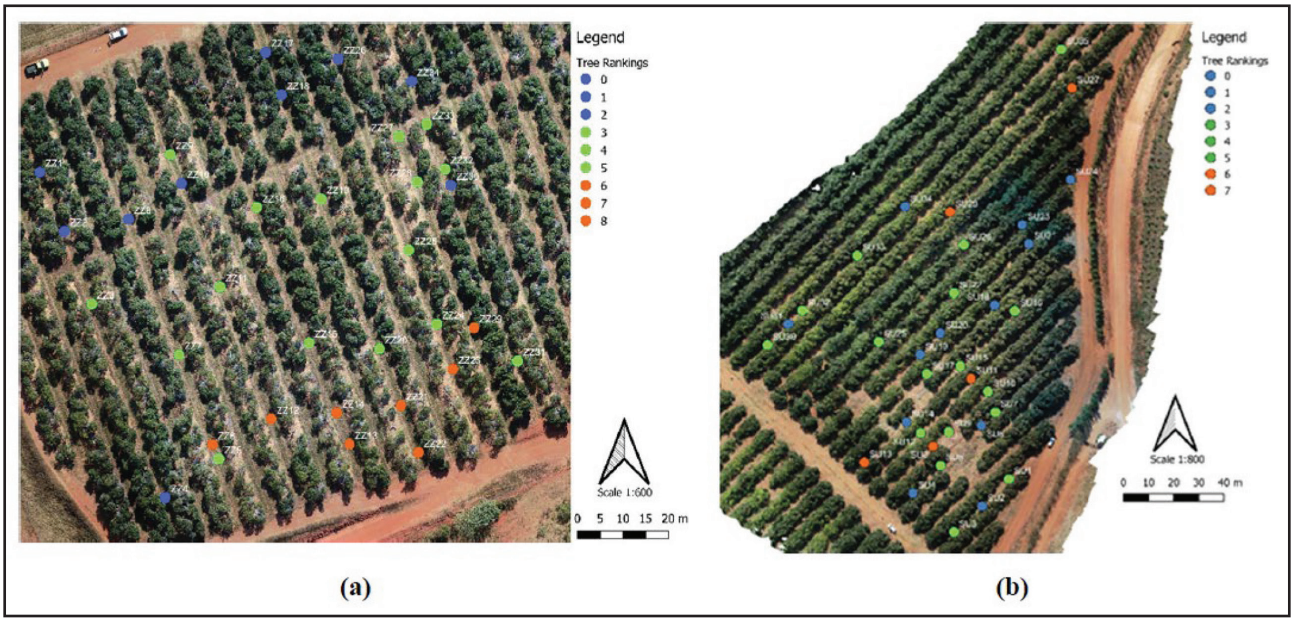


Figure 1: Locations of target trees and severity rankings (a) Block 1 and (b) Block 2. Images acquired from UAV data (flown in May 2021).

closely lined up with the UAV data collection dates.

6. Data analysis

6.1 HSI analysis

The HSI data was initially processed by performing spectral calibration to convert radiance data to reflectance data using Breeze software by Prediktera (Umeå, Sweden). Subsequently, the reflectance hyperspectral images were analysed using a customised MATLAB script (MathWorks, U.S.A.).

Partial Least Squares-Discriminant Analysis (PLS-DA) was employed as a supervised classification method to categorise the severity of PRR into three classes. The spectral values extracted from the images were used for this analysis and PLS-DA was conducted using the PLS-Toolbox for MATLAB (version 8.6.1, Eigenvector Research Inc., U.S.A.) as detailed in Poblete-Echeverría *et al.* (2023).

6.2 MSI analysis

For the images captured by the UAV, the following steps were undertaken using Agisoft Metashape version 1.7.6 (Agisoft LLC., St. Petersburg, Russia): radiometric calibration, orthomosaic generation, and digital elevation model (DEM) creation. Once the orthomosaics and DEMs were generated, the individual target trees were extracted from the orthomosaics using QGIS (Quantum GIS, London, United Kingdom). The size of each clipping region was based on row and tree spacing, canopy size, and canopy overlap.

Multispectral vegetation indices (VIs) were computed using the MSI canopy data. MATLAB was employed to calculate the mean reflectance values for each band for each canopy. Subsequently, these mean reflectance values were utilised to compute various VIs for each individual canopy.

To correlate with the 'Ciba-Geigy' visual disease rankings, simple linear regression was applied to the computed VIs. Duncan *et al.* (2024) provide a comprehensive list of all calculated VIs. The VIs demonstrating the most significant correlations with the visual rankings were identified, and descriptive statistics were performed on them.

RESULTS AND DISCUSSION

Hyperspectral imaging (HSI)

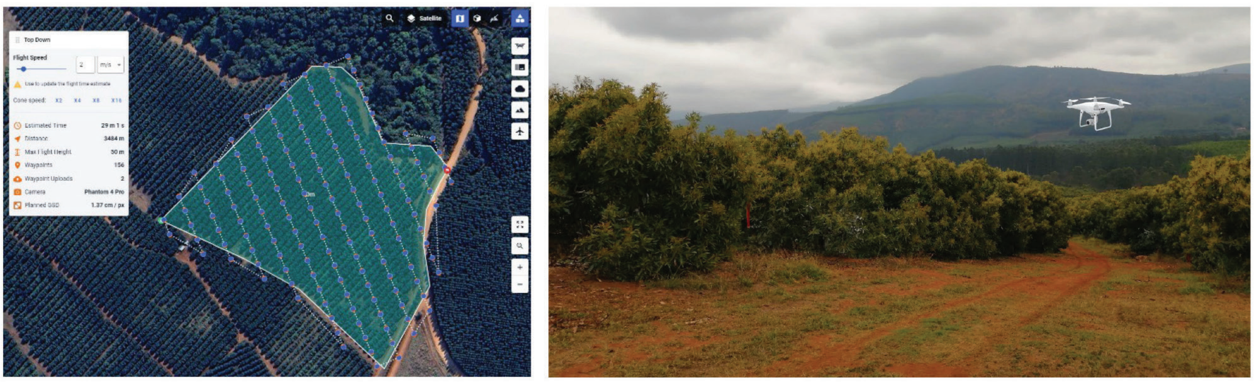
Figure 4 in Poblete-Echeverría *et al.* (2023) illustrates the spectral signatures for different severity rankings, highlighting significant variability among samples within the same ranking. Consistent patterns were observed across all samples, with notable magnitude changes, particularly in the near-infrared (NIR) region.

Table 2 in Poblete-Echeverría *et al.* (2023) presents the results of the PLS-DA method which was used to classify the three levels of PRR severity (Class 1, Class 4, and Class 8/9) in both study blocks. The PLS-DA model was able to classify the rankings with a low error in both blocks. In block 1, the PLS-DA model achieved effective classification with a low error rate. However, Class 9 exhibited the highest error rate, reaching a Cross-Validation Error Rate (CER) of 25%, with some samples misclassified as Class 4. Better results were obtained in block 2, with Class 1 showing the highest error rate at 13%. However, some samples misclassified as Class 4 and Class 8. This was unexpected given the clear differences in tree conditions.

Reflectance signals of leaves contain essential information used for non-destructive evaluation of plant status, and each part of the electromagnetic spectrum has been associated with certain properties. Based on the wavelength selection analysis, the wavelengths centred near 726, 710, 713, and 2517 nm were identified as crucial for estimating PRR rankings in block 1. A similar trend was observed in block 2, with several NIR and short-wave infrared (SWIR) bands also identified among the top 20 wavelengths.

Multispectral imaging (MSI)

Spectral reflectance in visible light is influenced by photosynthetic pigment concentrations, while NIR reflectance is sensitive to leaf and canopy structure (Rahman *et al.*, 2022). Additionally, orchard location factors such as topography, slope, sun angle, along the growing season (i.e. phenological phase), impact tree spectral reflectance (Robson *et al.*, 2017). Therefore, VIs can provide valuable insights for assessing PRR severity in avocado orchards. From our analysis, the best results were achieved in block 1,



(a) (b)

Figure 2: (a) Example of the flight plan and (b) Operation of the UAV in the field experiment.

demonstrating a relevant effect of the pruning intensity in the assessment of the PRR symptoms remotely.

In the case of VIs obtained from commercial satellite imagery, the best results were obtained during the bud development phase (June) with a spatial resolution of 0.50 m. The VIs Green Normalized Difference Vegetation Index (GNDVI - Louhaichi *et al.*, 2001) and Modified Soil-Adjusted Vegetation Index (MSAVI - Qi *et al.*, 1994) achieved a determination coefficient (r^2) of 0.76. However, the accuracy of the assessment increased considerably where UAV-based MSI outperformed satellite imagery across all phenological periods, achieving r^2 of 0.83 with the Modified Chlorophyll Absorption in Reflectance Index (MCARI - Daughtry *et al.*, 2000) during the flowering phenological phase.

The super high-resolution UAV imagery consistently delivered superior results obtained in both blocks, despite block 2 showing lower efficacy due to unclear symptom expression in large canopies. UAV-based MSI proves effective for assessing PRR severity but faces limitations in coverage, cost, and image processing time.

While high-resolution satellite imagery shows promise for infection assessment at the block and orchard level, challenges such as pixel mixing, cloud cover, and sun angle affect its reliability. Although satellite imagery offers flexibility in image acquisition and sensor selection comparable to UAVs, it falls short in spatial resolution and subtle plant trait detection with resolutions from 0.31 m (Zhang *et al.*, 2020).

CONCLUSIONS

In this study, we investigated the use of HSI and MSI technologies to assess PRR severity in avocado orchards. As a proximal method, HSI technology was employed at a laboratory scale to identify the spectral bands that are specific to PRR-affected trees. Despite the high variability among samples with the same ranking, the PLS-DA method successfully classified the three PRR severity levels analysed. The variable selection analysis indicated a preference for spectral bands in the NIR region, highlighting their importance in the PLS-DA model construction.

For RS over large areas, MSI with varying spatial resolutions (UAV versus high-resolution satellite images) was tested. VIs derived from this MSI were evaluated as potential indicators of PRR severity. The results showed that UAV-based MSI provided the highest accuracy across all phenological periods studied, demonstrating its efficacy in PRR severity assessment. However, UAV-MSI still faces challenges with large canopies where symptoms are occluded from aerial view.

The findings of this study underscore the potential of VIs obtained from UAV and high-resolution satellite imagery in determining and mapping of PRR severity in avocado orchards. MSI could serve as a rapid sensing technology to aid in the scouting process, reducing scouting costs and improving efficiency in identifying and managing avocados from this invisible threat.

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DIAGNOSING AVOCADO POLLEN ABNORMALITIES BY USING A NEW NOVEL COUNTING METHODOLOGY

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ABSTRACT

A new method for pollen counting in avocados needed to be developed as it is impossible to consistently cut anther samples at identical depths for light microscopy investigation. This new pollen-counting methodology therefore addresses inconsistent cutting depths in semi-thin sectioning preparations for light microscopy. This method effectively distinguishes between 'healthy', 'deformed', and 'empty' pollen in avocado (*Persea americana* Mill.) anthers, offering insights into pollen development under cold stress as preliminary findings suggest that proper pollen maturation in avocados is temperature-dependent, particularly during late flower development.

INTRODUCTION

Cold stress adversely affects avocado flowering, causing unsynchronized blooming and low yields. Avocados rely heavily on insect pollinators, and their flower structure act as a barrier to self-pollination (Radar *et al.,* 2020). Given the low pollination rates and the impact of cold temperatures on pollen viability, this study employed a novel pollen-counting method to assess pollen health in two *P. americana* cultivars, using light microscopy.

MATERIALS AND METHODS

The materials and methods of this novel counting methodology are outlined and described in Stanton and Du Toit (2022), but are summarised as follows:

Flower Collection and Anther Fixation:

Samples were taken from 12-year-old avocado trees ('Hass' and 'Fuerte') at the University of Pretoria. Flowers were collected on warmer and cooler days, and anthers were fixed in a glutaraldehyde solution.

Microscope Preparations:

Anthers were dehydrated in ethanol series and embedded in epoxy resin. Semi-thin sections were stained with Toluidine blue for analysis.