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Latent detection of anthracnose on strawberry crop using multi-spectral imaging

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Abstract: The purpose of this research was to develop a novel approach that involves the integration of small unmanned aerial systems (UAS) equipped with multispectral imaging (MSI) sensors to accurately identify the most appropriate vegetation indices (VI) for early detection of AFR on strawberries. At the Virginia Tech Tidewater Agriculture Research and Extension Center (AREC) located in Suffolk, VA, a field trial was conducted in a randomized complete block with four replicates in the 2022–2023 season. The treatments in a block consisted of three groups: i) plants that were infected with *Colletotrichum nymphaea*, ii) plants that were infected with *Colletotrichum fioriniae*, and iii) control plants that were treated with Switch 62.5WG before being transplanted into the field. A study investigated 22 VIs as markers for detecting anthracnose fruit rot on strawberries. Results showed that all VIs were significantly lower for infected leaves with *Colletotrichum acutatum* subspecies than healthy leaves, except for GLI and IPVI. This suggests stress on infected leaves may decrease chlorophyll content. The highest contrast and significant differences were observed in VIs of infected and healthy strawberry plants throughout the season. Further, the study has shown that the five spectral vegetation indices (VIs) Chlorophyll Index (CI), Difference Vegetation Index (DVI), Enhanced Vegetation Index (EVI), Green Optimized Soil Adjusted Vegetation Index (GOSAVI), and Non-Linear Index (NLI) have proven to be highly effective in detecting and identifying AFR on strawberries with a high degree of accuracy. This method can be of great assistance to growers as it enables them to monitor the severity of disease infestations using field maps and drones to apply appropriate management techniques.

Introduction: Anthracnose fruit rot (AFR), caused by *Colletotrichum acutatum* J. H. Simmonds, can cause severe yield losses of up to 80% under favorable conditions (Mertely et al., 2019). Both pre-harvest and post-harvest activities are susceptible to its impact, leading to a reduction in overall yield. The management of AFR on strawberry production system heavily relies on the early detection of the disease which plays a fundamental role in preventing this disease from spreading (Ciofini et al., 2022). The diagnosis of AFR typically involves identifying the host, observing signs and symptoms, and examining orange acervuli and conidia under a microscope which require extensive experience. The disease may be identified with high specificity and sensitivity using laboratory test methods on plant tissue samples, such as loop-mediated isothermal amplification (LAMP), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR)(Garrido et al., 2009; Theerthagiri et al., 2016; Wu et al., 2019). However, these detection methods are expensive, time-consuming, labor-intensive, requiring professional expertise, and have limited applicability due to the constraint of testing only a limited number of samples that can be tested each time (Sanzani et al., 2016). Therefore, the absence of an effective protocol to identify and discard infected plants or implement appropriate measures further complicates the management of AFR, making it a complex task to control the disease under favorable weather conditions (Aljawasim et al., 2023; Ciofini et al., 2022). Further, AFR exhibits a latent period during which the strawberry plugs are infected without exhibiting visible symptoms, making it a suitable candidate for assessing the scalability of disease detection using spectral imaging techniques. Early detection plays a pivotal role in not only selecting the most suitable disease management techniques but also curbing the transmission of infections among healthy plants (Barbedo, 2018). Therefore, there is a pressing need to develop an early, precise, and automated method for detecting strawberry diseases. We have proposed a novel approach that involves the integration of small unmanned aerial systems (UAS) equipped with multispectral imaging (MSI) sensors to accurately identify the most appropriate vegetation indices for early detection of AFR on strawberries.

Materials and Methods: Remote sensing (RS)- a combination of small unmanned aerial systems (UAS) equipped with multispectral imaging (MSI) sensors, was used to evaluate latent infections of the anthracnose fruit rot (AFR) in strawberry plants. The study was conducted at the Virginia Tech, Tidewater Agriculture Research and Extension Center (AREC), Suffolk, VA, with total of 240 strawberry plants in four blocks. Each block contains three bed rows that is 7.3 m long each, 2.1 wide on the bed tops, and 0.15 m high and each row contained 20 plants. Treatments within a block were comprised of i) plants inoculated with *Colletotrichum nymphaea*, ii) plants inoculated with *Colletotrichum fioriniae*), and iii) control plants preplant dipped with Switch 62.5WG before transplanting in the field (Lorente et al., 2015). The preparation of the *Colletotrichum* spp. inoculum was executed as per the recommended procedure (Turechek et al., 2006). In order to demonstrate the practical applicability of this study in real-world scenarios, the flight altitude was established at 15 meters above ground level (AGL). By utilizing multispectral images, the system was able to produce ground sampling resolutions of 1.2 cm/pixel, providing a high level of detail and accuracy. Multispectral imagery data was gathered 15 days after the transplanting process on November 31, 2022. Subsequently, data collection was carried out on a monthly basis throughout the season until the end of harvest on June 15th, 2023.

Data processing: Collected snapshot images for all multispectral bands were stitched and radiometrically calibrated through a sequence of image stitching procedures executed within a photogrammetry and mapping software platform called Pix4D Mapper, developed by Pix4D, Inc. based in Lausanne, Switzerland. This provided seamless imagery maps of the trial site. Next, these images from different imaging days were registered. Regions of interest were drawn around all the plants and mean feature values were extracted for each vegetation index. Under field conditions, a total of 22 VIs was investigated as important markers to detect anthracnose fruit rot (AFR) on strawberries (data not shown). These processes were conducted in QGIS software (QGIS.org (2023). QGIS Geographic Information System. Open-Source Geospatial Foundation Project. http://qgis.org). Furthermore, the effects of infection by different subspecies of *C. acutatum* on extracted mean index values were assessed using one-way analysis of variance (ANOVA) tests, and all outcomes were determined to be statistically significant at a 5% level of significance using by JMP v.14 (SAS Institute Inc., Cary, NC, USA).

Results: The photosynthesis process in healthy plants involves the reflection of green light and the absorption of blue and red light to create chlorophyll. If a plant contains more chlorophyll, it will reflect near-infrared (NIR) energy. By analyzing the reflectance of the NIR wavelength and the absorption of blue and red wavelengths, one can determine the plant's overall health (Meena et al., 2020). Many studies have been carried out to examine the VIs associated with crop traits, such as plant stress due to plant diseases. Under field conditions, a total of 22 VIs were investigated as important markers to detect anthracnose fruit rot (AFR) on strawberries (data not shown). All VIs derived from multispectral images were consistently and significantly ($p < 0.001$) lower for infected leaves with both subspecies of *Colletotrichum acutatum* (*C. nymphaea* and *C. fioriniae*) with a mean between 0.01 and 2.1. Healthy leaves had a mean between 0.21 and 2.67 except for GLI and IPVI. These observations suggest the imposition of stress on leaves that are infected, potentially resulting in a decrease in chlorophyll content. The CLI, DVI, EVI, GOSAVI, and NLI showed the highest contrast and significant differences thought out the season in the VIs of infected (Mean: 2.1, 0.16, 0.30, 0.01, and 0.36) and healthy (Mean: 2.67,0.21,0.39, 0.12 and 0.42) strawberry plants (Fig 1-2).

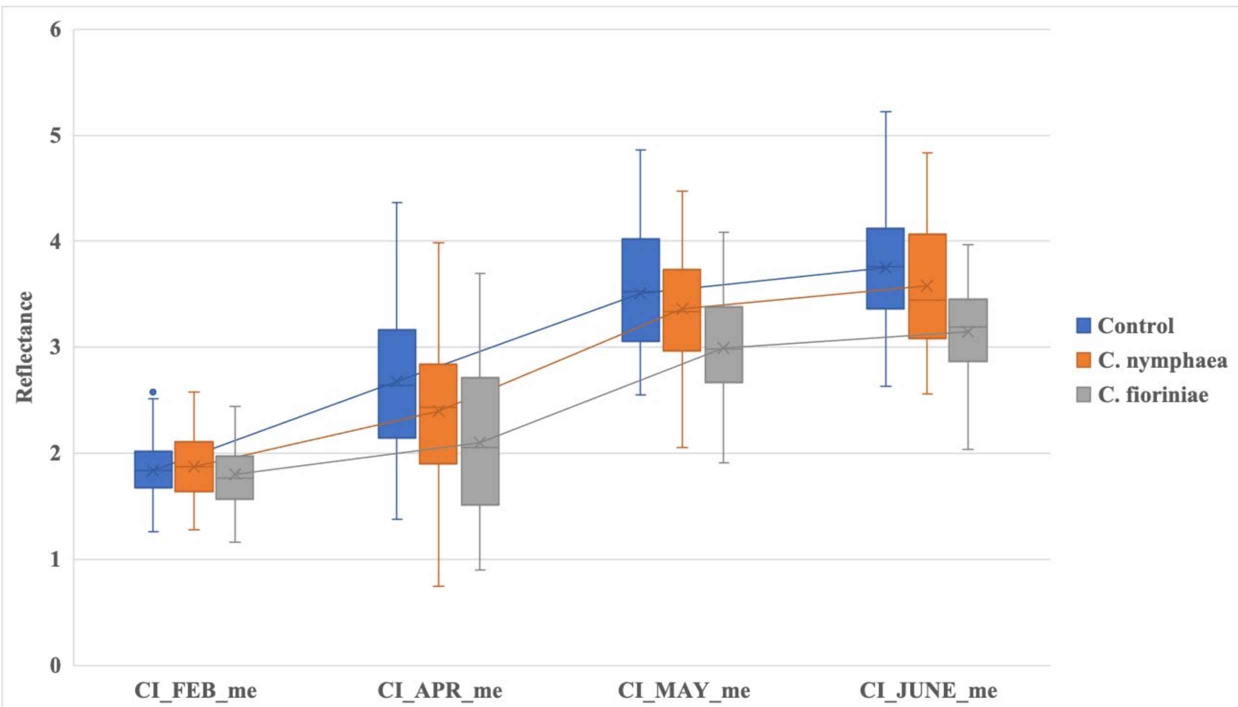

Fig. 1: Chlorophyll indices (CI) of plants inoculated with i) *C. nymphaea* ii) *C. fioriniae* and iii) untreated control. These imaging data were collected during February, April, May, and June 2023.

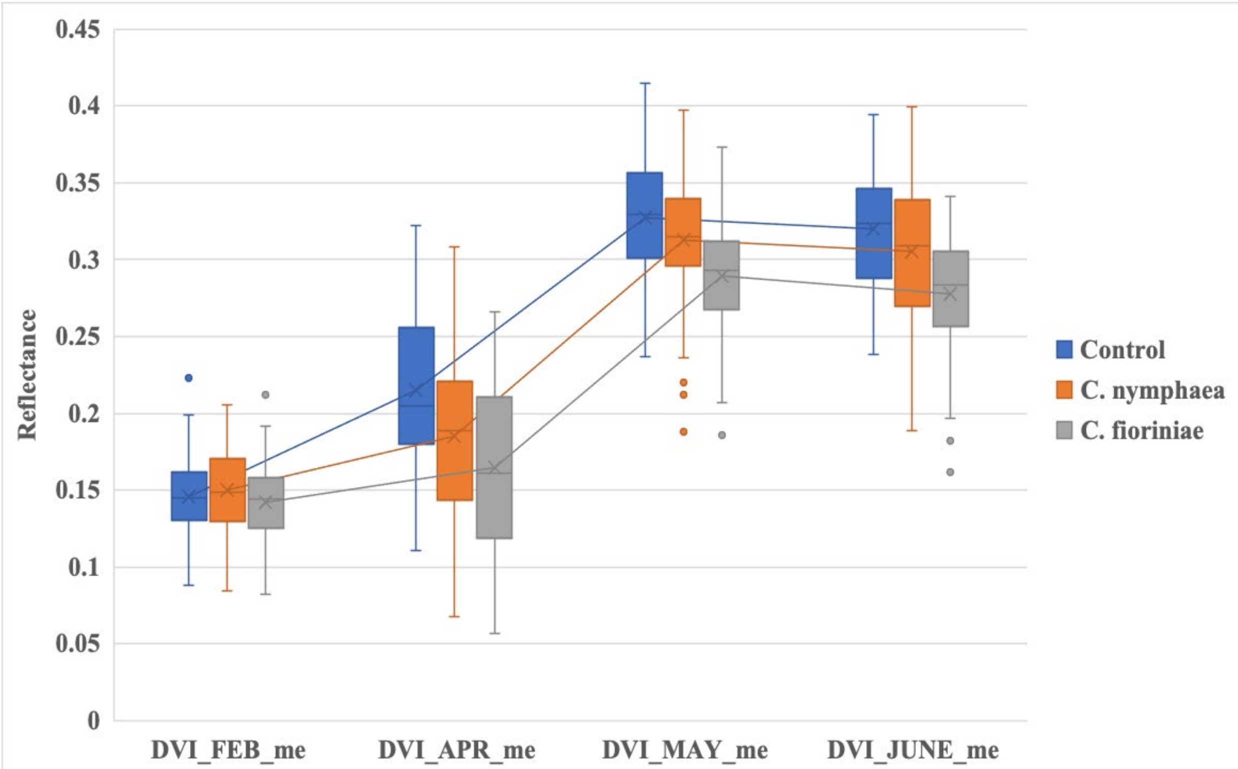

Fig. 2: Anthracnose fruit rot caused *by Colletotrichum acutatum* (*C. nymphaea* and *C. fioriniae*) on strawberry plants with the Difference Vegetation Index (**DVI**). These imaging data were collected during February, April, May, and June 2023.

Conclusion: The five spectral vegetation indices (VIs) including CLI, DVI, EVI, GOSAVI, and NLI demonstrated remarkable abilities to accurately detect and identify AFR on strawberries with high levels of specificity and sensitivity. In general, the method covered in this study may help growers directly by using field maps to monitor the severity of disease infestation and apply the suitable management technique.

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