Unmanned aerial vehicles (UAVs) or drones are being studied for many agricultural applications. One application is plant phenotyping to reduce the time and effort required in collecting field data. This study aims to explore the use of a UAV, 4K-color camera and a commercial image analysis service to measure citrus plant parameters that are important to a crop scientist or grower with limited technical background and resources. Citrus spp. are important crops in Puerto Rico and the United States. Currently, the citrus industry is struggling to contain the devastating effects of citrus greening or Huanglongbing disease. The disease is associated with a phloem-limited bacteria, Candidatus Liberibacter asiaticus (CLAs), vectored by the Asian citrus psyllid (ACP), Diaphorina citri Kuwayama. The use of insecticides for vector control is the primary strategy used in nurseries and orchards. However, once the citrus plant is infected, there is no effective control available for the disease. In Puerto Rico this disease has reduced Citrus spp. yields by more than 50%; studies are underway to find effective control measures such as supplemental nutrients, vector management practices, planting disease-free vegetative material and protective screen structures. An experiment at the Fortuna Agricultural Experiment Substation, in
Juana Díaz, Puerto Rico, was conducted to address the challenges posed by citrus greening. The experiment was established in a four-year-old grove of Tahiti lime (Citrus latifolia Tan.) on Cleopatra mandarin (Citrus reshni hort. ex Tanaka), naturally infected with Candidatus Liberibacter asiaticus. The experiment was arranged in a randomized complete block design with four replicates and three treatments: supplemental nutrients, supplemental nutrients + salicylic acid, and granular fertilization. Tree growth parameters were measured, and laboratory analyses were carried out to determine nutrient levels and disease severity levels from the leaf samples. The color camera, on board the UAV, was employed to acquire images of the experimental plot. Drone Deploy application was used for planning the UAV flights and image analysis. Field-measured plant height and canopy diameter compared well with the parameters determined from the color images. The average errors in measuring canopy diameter (14.5%) and plant height (22.4%) could be considered within an acceptable range, especially for comparing different treatments or crop varieties. However, the average errors in measuring canopy volume (47.5%) were high and can be considered unacceptable. It appears that the assumed conical shape of the trees could be one of the main reasons, besides the algorithms used in calculating plant volume, and built-in inaccuracies of the single frequency GPS (global positioning system) used in estimating altitude. Further studies could help in reducing errors and exploring other applications. The method used can be of importance in evaluating fruit trees.

Key words: UAV, drone, citrus, plant phenotype, automation, image processing

RESUMEN

Imágenes a color tomadas por drones para determinar parámetros fenológicos en cítricos

El uso de vehículos aéreos no tripulados (UAV, por sus siglas en inglés) o drones en aplicaciones agrícolas se está estudiando. Una de las aplicaciones es determinar parámetros fenológicos de las plantas para reducir el tiempo y los esfuerzos requeridos en la recolección de datos de campo. Este estudio tiene como objetivo explorar el uso de un UAV, una cámara de color 4K y un servicio de análisis de imágenes disponible comercialmente para medir los parámetros de las plantas de cítricos que son importantes para un científico agrícola o para el agricultor con recursos técnicos y recursos limitados. Los cultivos de Citrus spp. son importantes en Puerto Rico y Estados Unidos de América. Actualmente, la industria de los cítricos está luchando por contener el efecto devastador del enverdecimiento de los cítricos o la enfermedad de Huanglongbing. Esta enfermedad se asocia a la bacteria, Candidatus Liberibacter asiaticus (CLA), que se aloja en el tejido del floema y es acarreada por el psílido asiático de los cítricos (ACP), Diaphorina citri Kuwayama. El uso de insecticidas para el control de vectores es la estrategia principal utilizada en viveros y huertos. Sin embargo, una vez la planta de cítricos está infectada, no hay control efectivo disponible para la enfermedad. En Puerto Rico, esta enfermedad ha reducido el cultivo de Citrus spp. en más del 50%, y se están realizando estudios para encontrar medidas de control efectivas, como la aplicación de nutrientes suplementarios, prácticas de manejo de vectores, el uso de material vegetativo libre de enfermedades y estructuras protegidas. Este trabajo presenta los resultados parciales de un experimento que se llevó a cabo en la Subestación Experimental Agrícola de Fortuna en Juana Díaz, Puerto Rico, para abordar los desafíos
planeados por el enverdecimiento de los cítricos. El experimento se estableció en un huerto de lima Tahití de cuatro años (Citrus latifolia Tan.) injertado en mandarina Cleopatra (Citrus reshni Hort. Ex Tanaka), infectada naturalmente con Candidatus Liberibacter asiaticus. El experimento se organizó en un diseño de bloques completos al azar con cuatro repeticiones y tres tratamientos: aplicación de nutrientes suplementarios, nutrientes suplementarios + ácido salicílico y fertilización granular. Se midieron los parámetros de crecimiento de los árboles y se llevaron a cabo análisis de laboratorio para determinar los niveles de nutrientes y los niveles de gravedad de la enfermedad a partir de las muestras de hojas. La cámara a color, a bordo del UAV, se empleó para adquirir imágenes del experimento. La aplicación ‘Drone Deploy’ se utilizó para planificar los vuelos del UAV y el análisis de imágenes. En el campo se tomaron medidas de la altura de la planta y el diámetro del dosel y se compararon con los parámetros determinados a partir de las imágenes en color. Los errores promedio en la medición del diámetro del dosel (14.5%) y la altura de la planta (22.4%) podrían considerarse dentro del rango aceptable, especialmente para comparar diferentes tratamientos o variedades de cultivos. Sin embargo, los errores promedio en la medición del volumen del dosel (47.5%) fueron altos y pueden considerarse inaceptables. Al parecer, la forma cónica asumida para el cálculo del volumen de los árboles podría ser una de las principales razones para el margen de error, además de los algoritmos utilizados para calcular el volumen de la planta. Estudios adicionales podrían ayudar a reducir los errores, como también explorar otros algoritmos y aplicaciones. El método utilizado puede ser importante para realizar evaluaciones de árboles frutales.

Palabras clave: UAV, drones, cítricos, fenotipos de plantas, automatización, procesamiento de imágenes

INTRODUCTION

The UAVs (Unmanned Aerial Vehicles), or UAS (Unmanned Aerial Systems), or drones promoted for agricultural applications are inexpensive and comparable to hobbyist grade UAVs (Freeman and Freeland, 2015). Yet, they have potential because of lower initial cost, easy availability of software and global positioning system (GPS) based navigation control. Further, they provide higher resolution images with less processing related delays compared to satellite and manned aircraft based imaging systems. It appears that by employing UAVs, even specialty crop growers will be able to practice precision agriculture at an affordable cost (Ehsani, 2011). A typical UAV-based system of remote sensing is shown in Figure 1 (Rokhmana, 2015). Similar to conventional manned aerial mapping, a UAV system uses an aerial vehicle with a remote controller for directing digital cameras to acquire aerial images at predetermined positions, or time intervals as per the flight plan configurations and satellite based navigation. The aerial images are processed by digital photogrammetric techniques to produce an ortho-mosaic image and a point cloud of digital elevation model.
There are numerous studies deploying UAVs for agricultural application and some relevant ones are briefly described here. “Monitoring water stress and fruit quality in an orange orchard under regulated deficit irrigation using narrow-band structural and physiological remote sensing indices” was the topic of research by Stagakis et al. (2012). In grapefruit, Romero-Trigueros et al. (2017) reported significant correlations between: red (R) wavelength with chlorophyll and potential turgor; near infra-red (NIR) wavelength with gas exchange; and normalized difference vegetation index (NDVI) with gas exchange. Similarly, significant correlations were found in mandarin between NIR with stem water potential and gas exchange, and NDVI with stem water potential (Romero-Trigueros et al., 2017). Weed classification accuracy from the UAV images was 94.5% and the coefficient of determination was 0.89 between the detected weeds and their ground truth densities (Gao et al., 2018). Thermal images obtained from a UAV were used to correlate soil moisture and water stress in sugar beet with limited success (Quebrajo et al., 2018). A multi-temporal study showed that caution must be taken when results from one sensor are compared to results from a different sensor or image processing scheme (Aasen and Bolten, 2018).

Another application of UAVs studied is plant phenotyping (i.e., measurement of plant observations such as plant height, canopy diameter, canopy volume and others). Comparable accuracies in detecting citrus greening symptoms were found for manned and unmanned aerial im-

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**Figure 1.** Typical architecture of UAV- (unmanned aerial vehicle) based remote sensing system (Adapted from Rokhmana, 2015).
aging systems using the same sensor (Garcia-Ruiz et al., 2013). Sankaran et al. (2015) published a comprehensive review of the technological aspects of integrating unmanned aerial vehicles with imaging systems to enhance field phenotyping capabilities, the state-of-the-art of unmanned aerial vehicle technology and many agricultural applications. Furthermore, the review discussed the potential of using aerial imaging to evaluate resistance/susceptibility to biotic and abiotic stress for crop breeding and precision agriculture (Sankaran et al., 2015). A study investigated black sigatoka disease detection from plantain images (Mathanker and Pérez-Alegría, 2016). A field phenotyping system was developed to assess potato late blight resistance with a coefficient of determination 0.73 (Sugiura et al., 2016). Detection of citrus greening and determination of plant parameters were investigated using a color camera (Mathanker et al., 2017a, 2017b). The vegetation fraction and plant height determined from UAV imagery and actual measurement showed a correlation of 0.7 for white radish and napa cabbage vegetables (Kim et al., 2017).

Citrus is an important agricultural crop in both Puerto Rico and the USA, valued at roughly $3.3 billion in 2015-2016, out of which fresh market oranges alone were valued at $861 million (USDA, 2016). However, citrus yield and fruit quality are adversely affected by citrus greening, also known as Huanglongbing (HLB) or yellow dragon disease. Citrus greening has emerged as one of the most serious citrus plant diseases and has devastated millions of acres of citrus crops throughout the United States and abroad (USDA, 2018). There appears to be no cure, but some control measures have been developed such as spraying insecticides (Chen et al., 2017) and horticultural mineral oil (Tansey et al., 2015) for vector control, use of tolerant rootstocks (Bowman et al., 2016), better nutrition (Estévez de Jensen et al., 2010), removal of infected trees (Bassanezi et al., 2013) and improved irrigation (Kadyampakeni and Morgan, 2017). Furthermore, there are other research studies underway to develop measures for controlling citrus greening. One such study was conducted at the Fortuna Agricultural Experiment Substation in Puerto Rico to evaluate the effect of different nutrient treatments in improving Citrus sp. nutrition and ameliorating the effect of the disease. Besides laboratory analysis, the study involved measuring citrus plant phenotypes such as plant height, canopy diameter, canopy volume and others. This process is quite cumbersome and time consuming. To reduce scouting and field data collection costs, the objective of this preliminary study was to explore the possibility of determining plant parameters by analyzing color images of the citrus experiment taken from a UAV.
MATERIALS AND METHODS

An unmanned aerial vehicle, DJI Phantom 3 Professional, with a 4K-color camera (DJI Corporation, China)6 (Figure 2), was used to acquire images of an experimental orchard at the Fortuna Substation. The experiment was established in a four-year-old grove of Tahiti lime (Citrus latifolia Tan.) on a Cleopatra mandarin (Citrus reshni hort. ex Tanaka), naturally infected with Candidatus Liberibacter asiaticus. The experiment was arranged in a randomized complete block design with four replicates and three treatments: 1) a standard essential nutrients supplement applied to the foliage; 2) the standard essential nutrients

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6Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.
supplement + salicylic acid; and 3) a control consisting of a standard granular fertilizer applied to the soil at the tree base. The plants were spaced at about 4.6 x 6.8 m. Disease severity was assessed using a modified scale of 1 to 6 where: 1 = healthy, no symptoms and 6 = dead tree (Rouse et al., 2010). The trees were tested for CLAs using an HLB detection kit (Enviroloxic) at the beginning of the experiment. Plant height and canopy diameter were measured for 48 plants that were part of the experiment. The canopy volume was assumed to be conical and was calculated using the cone volume formula (canopy volume = \( \frac{22}{7} \times \text{canopy diameter}^2 \times \text{plant height} \)). The plant parameters were recorded on the same day of the UAV flights employing the Drone Deploy application.

The Drone Deploy is an application for UAV image analysis that includes an automated flight module and image data processing on a cloud server (https://www.dronedeploy.com). The acquired UAV images tagged with GPS (Global Positioning System) coordinates are uploaded to the cloud server for image stitching and calculating three dimensional point clouds. After image analysis, the application provides an ortho-mosaic, terrain elevation map and three-dimensional map. The application supports a variety of interactive tools for analysis and visualization. One of the tools lets a user measure distance, area and volume of a desired area on the map.

The UAV was flown around noon to avoid shadow effects, at three flight altitudes: 20, 27.4 and 36.6 m (66, 90 and 120 ft) with an 80% image overlap using the Drone Deploy application. The images acquired were uploaded to the Drone Deploy cloud server to create image mosaics and elevation maps. An interactive tool was used to select an individual plant’s canopy on the image mosaic or the elevation map. The selected individual plant canopy was used to record canopy diameter calculated, plant height calculated and canopy volume calculated.

RESULTS AND DISCUSSION

The data analysis consisted of comparing the field measured and cloud server calculated plant parameters from the images that were acquired employing the UAV and color camera. Some of the individual images acquired from the 20 m altitude are shown in Figure 3. The image mosaic generated by the Drone Deploy application using the images similar to those shown in Figure 3, taken at 20 m altitude is shown in Figure 4A, and the elevation map generated is shown in Figure 4B. In the elevation map, the red color intensity shows higher elevation and blue color intensity shows lower elevation. The image mosaics and elevation maps at 27.4 and 36.6 m flight altitudes were of poor quality and were not used in further analysis.
The measured canopy diameter compared well with the calculated canopy diameter (Figure 5A), and the average error was 14.5% with a standard deviation of 9.9% (Table 1). The measured average canopy diameter was 2.32 m and calculated average canopy diameter was 2.01 m. The errors may be due to human bias, analysis methodology and other factors, and the errors could be further improved by better image acquisition and data analysis.

Similarly, the measured plant height compared well with the calculated height (Figure 5B). The average error was 22.4% with a standard deviation of 8.3% (Table 1). The measured average plant height was 1.87 m and calculated average plant height was 1.40 m. The errors may be due to human bias, land topography and analysis methodology, and the errors could be further reduced by better image acquisition and data analysis.

Conversely, the measured canopy volume did not consistently compare well with the calculated canopy volume (Figure 5C). The average error was 47.5% with a standard deviation of 73.8% (Table 1). It appears that for some plants the error was very high, such as for plant no. 10, 34, 40, and others. The measured average canopy vol-
volume was 0.059 m$^3$ and calculated average canopy volume was 0.075 m$^3$. The errors may be due to the conical shape assumed by the plant, analysis methodology and inaccuracies in the single frequency GPS used. Single frequency GPS systems are not very accurate in estimating altitudes, and using GPS corrections from a nearby GPS base station or using a double frequency GPS can improve the accuracy of altitude estimation and thereby canopy volume estimates.

Figure 4. A) Generated ortho-mosaic image, and B) Generated elevation map, using Drone Deploy Application, from the images acquired during the 20-m altitude flight over the citrus greening experiment.
An analysis of variance showed no correlation between plant parameters and disease severity levels and nutrient levels (Table 2). However, the significant difference (< 5%) was found in disease severity levels between both the supplemental nutritional programs and the granular fertilization. After two years, no differences were found in plant height and canopy diameter with the application of the supplemental nutrients.
with and without salicylic acid or the standard granular fertilizer. This is in accordance with research conducted on Valencia sweet orange over a two-year period where no significant differences in bacterial titer dynamics, fruit yield [number of fruit per tree, fruit weight (kg) per tree, proportion of fruit dropped], or juice quality between treated trees and non-treated control trees were found (Gottwald et al., 2012). Differences found in disease severity levels between both the supplemental nutritional programs and the standard granular fertilization soil application showed an effect in the symptoms observed. There was no significant impact on macronutrient accumulation on the leaves in the different treatments. Also, no significant variations were found in the micronutrients with the exception of a slight increase of Zn and Mn in treatments 1 and 2 with the supplemental nutrients plus salicylic acid.

**CONCLUSION**

This study demonstrated use of an automated plant phenotyping method for measuring citrus canopy diameter, plant height and canopy volume for individual trees employing a UAV, color camera

| Plant parameter       | Average error (%) | Standard Deviation (%) |
|-----------------------|-------------------|------------------------|
| Canopy diameter       | 14.5              | 9.9                    |
| Plant height          | 22.4              | 8.3                    |
| Canopy volume         | 47.5              | 73.8                   |

**Table 1.**—Average errors and standard deviation of the errors between the field-measured plant parameters and calculated plant parameters using the Drone Deploy application at 20-m altitude.

**Table 2.**—Significance levels for nutrient treatments as affected by plant parameters and citrus greening severity levels.

| Variable                | Treatment         | Mean   | Significance level |
|-------------------------|-------------------|--------|--------------------|
| Plant height            | Foliar + SA       | 74.79 a| LSD 11.6           |
|                         | Granular fertilizer| 73.31 a| CV. 9.8%           |
|                         | Foliar            | 72.90 a|                     |
| Canopy diameter         | Foliar + SA       | 113.96 a| LSD 32.9           |
|                         | Granular fertilizer| 97.33 a| CV. 20.4%           |
|                         | Foliar            | 90.63 a|                     |
| Disease severity        | Foliar + SA       | 4.38 a | LSD 1.3            |
|                         | Granular fertilizer| 2.95 b | CV. 22.1%           |
|                         | Foliar            | 4.50 a |                     |
and commercially available image analysis service. The average errors in measuring canopy diameter (14.5%) and plant height (22.4%) could be considered within acceptable range, especially for comparing different treatments or crop varieties, and for the technology used in this study. However, the average errors in measuring canopy volume (47.5%) were high and are unacceptable. Using a double frequency GPS on board the UAV and a local GPS base station are recommended for improving canopy volume estimates. Further studies could help in reducing errors and also exploring other applications. The demonstrated method can be used to assess phenotypic data for investigations and to reduce scouting costs for citrus greening control measures.

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