Early Detection of Pistachio Botryosphaeria Panicle Blight Disease Using High-Throughput Plant Phenotyping

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INTRODUCTION

Botryosphaeria panicle blight (caused by *Botryosphaeria dothidea*), a major disease of pistachios in California, first became a serious problem in the Sacramento Valley, in the late 1980s, but a couple of decades later, the disease caused a significant yield reduction in the San Joaquim Valley as well. Botryosphaeria is known as the major threat to the California pistachios. Unfortunately, Botryosphaeria disease has a long latent period, and the earliest symptoms can appear in late-April to early-May, if the temperature is warm enough. Dead or partially infected buds can show symptoms such as dead areas as early as midsummer. However, a significant portion of potentially infected (or infested) buds remain nonsymptomatic. According to a previous study, more than half of nonsymptomatic (healthy looking) buds collected from an orchard, in Butte County (December 1986), contained spores of *Botryosphaeria dothidea*. Some of these contaminated buds most likely develop infected shoots in spring. Conventional early-detection practice suggests collecting up to 100 buds from random locations throughout the orchard and processing them using the BUDMON (bud monitoring) technique. The BUDMON technique (developed by Dr. Michailides) is highly sensitive and capable of diagnosing the samples with little or no visual symptoms. However, it requires sample collection/preparation, laboratory effort/cost, and it takes at least one week to have the diagnosis results. Although the BUDMON technique is highly accurate and a reliable diagnosis method, it is not free, and it requires lab labor. Therefore, the number of samples to be collected for BUDMON assay could be limited by the available budget for disease monitoring; while, an optimum Botryosphaeria management requires maximized monitoring in which high special and temporal resolution data must be available.

For the current project, pistachio buds were collected from Botryosphaeria-infected pistachio trees as well as from healthy trees, in an experimental pistachio orchard at UC Kearney REC. After spectral measurements were conducted, the buds were tested by using the BUDMON method to determine the incidence of Botryosphaeria infection. Spectral analyses were conducted to identify the wavelengths that are relevant to the identification of Botryosphaeria positive buds, while they were in nonsymptomatic conditions.

An in-field spectral measurement tool (KOBIN Proximity) was designed and developed for in-field bud spectral data collection, in the range of ultraviolet (UV), visible, and near-infrared (NIR) (300-1650 nm). KOBIN Proximity will be used in step 2 of this project to conduct a nondestructive time-series spectral measurement of pistachio buds.

RESULTS

A total number of 508 healthy and infected pistachio buds (in nonsymptomatic condition) were collected in two datasets (223 buds in dataset 1 and 285 buds in dataset 2). Spectral reflectance of all the buds was measured in the range of 186 nm to 1031 nm with the resolution of ~0.3 nm in the Precision Agriculture Lab at UC Kearney REC. Then, the buds were given to Dr. Michailides' lab, at UC Kearney REC, to be diagnosed for *Botryosphaeria* and *Phomopsis*

species using the BUDMON assay. Based on the BUDMON results, 316 out of 508 bud samples were heathy, and they were categorized as the control class. Buds diagnosed with *Botryosphaeria numbered 186,* 14 buds were diagnosed with *Phomopsis* and only 8 buds had both *Botryosphaeria* and *Phomopsis*. Since the number of bud samples in the *Phomopsis* class was limited, compared to the other classes, Phomopsis class was not considered for the data analysis. A preliminarily spectral analysis was conducted in MATLAB software (version R2017a, MathWorks, Natick, MA) to determine the relevant wavebands for Botryosphaeria identification in the non-symptomatic stage. The rankfeatures function was used with a t-test evaluation criterion, in which reflectance in all wavelengths was employed as input features. The output of this process was a vector of cross-correlation coefficients (corresponding to all wavelengths) that indicates the relevance of wavelengths to Botryosphaeria identification. The results showed that the wavelengths in the visible range (400-700 nm) had minimum relevance to Botryosphaeria identification compared to the wavelengths in UV (below 400 nm) and NIR (above 700 nm). These results confirmed that the Botryosphaeria infected buds did not have any obvious symptom in the visible range. In other words, they were in a nonsymptomatic condition during the time of the experiment. However, a growing trend in cross-correlation coefficients was observed in the NIR region as the wavelength increased between 800-1000 nm. In the next step of this project, the spectral reflectance of pistachio buds will be analyzed in an expanded NIR band up to 1650 nm using the KOBIN Proximity device.

KOBIN Proximity is a handheld field data collection device that includes a light source, batteries (14.8V for the light source, and 7.4V for other components), two spectrometers, a geographical positioning system, a microcontroller, a LCD screen, and a customized fiber optic probe head that was developed for bud spectral measurement. KOBIN Proximity is able to conduct an in-field spectral measurement in a nondestructive manner, log the location where the measurement is done, illustrate the spectral data on a LCD screen, and store the spectral data on a flash memory. This device will be used for collecting time-series spectral data from the same pistachio buds during the winter and spring of 2018. After the last round of spectral data measurement is conducted, the bud samples will be collected and processed with the BUDMON assay to validate the disease status of the samples. A time-series spectral analysis will be conducted to determine the accuracy of Botryosphaeria identification at different stages of the growth season.

CONCLUSION AND APPLICATIONS

Pistachio buds with Botryosphaeria infection remain nonsymptomatic during the winter and early spring. The earliest symptoms appear in late-April to early-May, if the temperature is warm enough. Dead or partially infected buds can show symptoms such as dead areas as early as midsummer. Still, a significant portion of potentially infected buds remain nonsymptomatic. The bud samples used for this project were collected between March 20 to 27, 2017. The preliminarily results showed that pistachio buds, with Botryosphaeria infection, illustrate spectral characteristics similar to healthy buds in the visible band; however, the nonvisible bands of UV and NIR demonstrated the potential for Botryosphaeria identification using spectral analysis.

By the end of this project, in spring 2019, we expect to develop a practical in-field diagnosis methodology for the detection of Botryosphaeria-infected buds before the appearance of symptoms. The methodology will involve the KOBIN Proximity sensor as well as a Botryosphaeria diagnosis software that will run on the KOBIN Proximity device.