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Proximal hyperspectral sensing of stomatal conductance to monitor the efficacy of exogenous abscisic acid applications in apple trees

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ABSTRACT

Stomatal conductance is a critical regulating factor in plant water relations and responses to abiotic stress. Abscisic acid (ABA) is one of the plant hormones that regulates stomatal conductance and leaf transpiration. The presence of endogenous or exogenous ABA induces stomatal closure, which reduces leaf transpiration rates and increases tolerance to abiotic stress. In this study, visible near-infrared (Vis-NIR) spectroscopy, as well as proximal multispectral and thermal imaging were used to evaluate changes in stomatal conductance through exogenous ABA applications to apple trees. ABA was applied twice at 500 mg kg^{-1} in 2016, with five control and five ABA-treated trees in a three-year-old apple orchard. Proximal Vis-NIR spectral reflectance (350–2500 nm) data, and multispectral and thermal infrared images were acquired from control and treated trees after 1–3 days of exogenous ABA application to the trees. Ground reference stomatal conductance was also measured to compare the data with proximal sensing data. Partial least square regression (PLSR), linear support vector machines (SVM), and quadratic SVM algorithms were applied to classify the control and ABA-treated leaves, before and after feature selection using rank features technique and stepwise regression analysis. The average classification accuracy ranged between 80 and 85% at 3 days after treatment with the entire Vis-NIR spectra, while the accuracies ranged between 74 and 80% with five selected spectral bands. The ABA treatment effects could not be observed with crop water stress index extracted from thermal images, although the leaf temperature in ABA-treated trees were higher than the untreated control trees. Green normalized difference vegetation index extracted from multispectral images also did not show any differences between control and ABA-treated trees. Overall, results suggest that the hyperspectral Vis-NIR sensing was able to acquire spectral changes pertinent to the dynamic processes such as stomatal conductance, independent from non-responsive traditional vegetation indices that lacked responsive spectral bands.

1. Introduction

The measurement of plant water status is important to improve upon the ability to optimize irrigation decisions. Improving water-use efficiency can have ecological as well as horticultural benefits. Changes in plant water status can affect tree growth, physiology, productivity and crop quality (Espinoza et al., 2017). The dynamic management of plant water in horticultural crops can be a valuable tool to improve growth, productivity, and quality (Shackel et al., 1997). The success of above strategy depends on the ability to rapidly and accurately measure changes in plant water status to make informed irrigation decisions. In many crops, stomatal conductance has been shown to be closely linked to plant water status. Traditional measures of stomatal conductance have been focused on measuring water loss from a defined leaf area surface. However, emerging strategies are focused on rapid, proximal sensing to detect changes in plant water status (Espinoza et al., 2017). Proximal sensing of leaf stomatal conductance has implications that extend beyond irrigation management and is important for maintaining optimum plant health.

Abscisic acid (ABA) is a growth regulator that is produced in both the roots and shoots and is translocated to other parts of the plant through xylem during the transpiration process (Coggins and Lovatt, 2014). ABA manages plant responses to water stress by coordinating stomatal conductance with the available water supply (Aasamaa and Sõber, 2011). ABA regulates stomatal closure (Correia et al., 1995), leaf transpiration, and plant water potential (Freitas et al., 2011). Under water stress conditions, ABA induces stomatal closure to conserve water (García-Mata and Lamattina, 2003) and to reduce the risk of damage to

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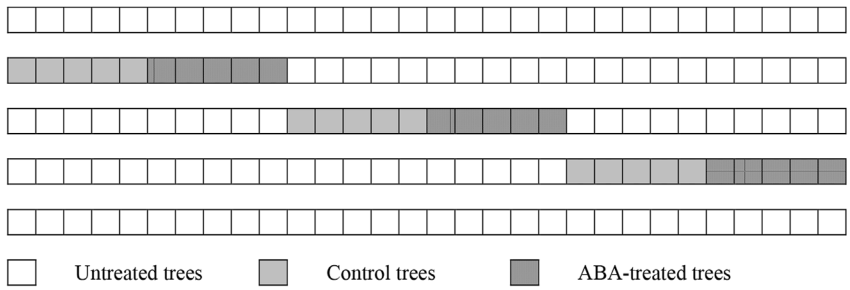

Fig. 1. Plot design describing the treatments in the Sunrise orchard.

the plant. ABA accumulates in shoots that reduces leaf transpiration without significantly affecting the fruit transpiration (Freitas et al., 2011).

Plant water use efficiency is influenced by water uptake and transport wherein leaf transpiration plays a major role. Exogenous or synthetic ABA can also be used to induce drought stress responses in the absence of stress conditions (Shinozaki and Yamaguchi-Shinozaki, 2007). McArtney et al. (2014) reported that exogenous synthetic ABA applications to apple canopy induces stomatal closure and decreases stomatal conductance. There is growing interest in being able to monitor the effect of exogenous ABA applications on stomatal conductance and plant water status. ABA can limit transplant shock (Berkowitz and Rabin, 1988), induce dormancy (Hilhorst and Karssen, 1992), increase fruit quality (Kobashi et al., 1999), and has been reported to induce greater fruit calcium accumulations in tomato (Freitas et al., 2011).

Fruit calcium absorption is important in preventing some physiological disorders in fleshy horticultural crops (Ho and White, 2005). Calcium is absorbed from the soil through root water uptake. Xylem water movement is required for calcium uptake and delivery to sink organs such as leaves and fruit. The difference in transpiration rates between leaves and fruit has been hypothesized as a mechanism that leads to calcium imbalances between these two competing organs. When ABA production is induced in the plant by water stress or when exogenous ABA is applied, stomata closure leads to declined transpiration rate. This may influence the transpirational balance between fruit and leaves and produce an increase in calcium uptake in developing fruit. Freitas et al. (2011) reported that calcium uptake and distribution into the developing tomato fruit was greater when exogenous ABA was applied because of reductions in leaf transpiration. Exogenous ABA increased calcium uptake into the fruit and reduced blossom end rot in tomato (Freitas et al., 2011). Similar experiments in apricot (Montanaro et al., 2010) and kiwi (Montanaro et al., 2012) have demonstrated a close association between transpiration and calcium accumulation. In apple, ABA has been reported to affect calcium-related genes (Falchi et al., 2017) and to reduce transpiration that can have a strong effect on calcium levels in the fruit. Although the effect of ABA on plant physiology has been well documented, practical limits on the real-time monitoring of plant responses to ABA are difficult and time consuming to measure. As such, there needs to be better monitoring method developed for the use of exogenous ABA treatments.

The effect of ABA on leaf and plant physiology can be estimated through the direct measure of evapotranspiration, photosynthetic rate, and stomatal conductance (Astacio and Iersel, 2011). Advanced high-throughput plant sensing tools to accurately monitor the effect of ABA on plants/trees are essential in optimizing exogenous ABA applications. Proximal and remote sensing techniques such as visible near-infrared (Vis-NIR) spectroscopy and imaging have been used to evaluate biotic stress status (Calderón et al., 2013; Gomez-Candon et al., 2014; Naidu et al., 2009; Sankaran et al., 2011). High-throughput vegetation temperature measurement can also serve as an indicator of transpiration rate and stomatal conductance changes (Berni et al., 2009; Calderón et al., 2014; Zarco-Tejada et al., 2012). Stomata closure results in lower transpiration rate that increases the leaf temperature (Osakabe et al., 2014). Thus, thermal imaging may be useful in estimating the plant water use efficiency, and consequently abiotic and biotic stress in plants (Araus and Cairns, 2014; Chaerle et al., 2005). For example, the spatial variability of water stress in a vineyard has been assessed with extracted crop water stress index (CWSI) using temperature data (Bellvert et al., 2014). The CWSI was found to be highly correlated with leaf water potential (coefficient of determination, $R^2 = 0.83$).

Currently, stomatal conductance is monitored using handheld or stationary instrumentation that monitors shifts in relative humidity gradients, which can be used to estimate plant transpiration, a measure of stomatal conductance. In this study, we hypothesize that decreases in stomatal conductance induced by exogenous ABA applications to apple trees can be monitored using proximal visible-near infrared and thermal sensing systems. The objectives were to evaluate the response of the apple trees to exogenous ABA application using proximal visible near-infrared spectroscopy, and agricultural utility vehicle (AUV)-based thermal infrared and multispectral imaging with controlled experimentation and provide comparisons of such data with direct physiological measurements.

2. Materials and methods

2.1. Field site, experimental design, and ABA treatments

The experiment was conducted at Washington State University's Sunrise Research Orchard located in Rock Island, WA (47°18′35.27″N, 120° 4′0.16″W). The ‘Honeycrisp’ apple orchard on M9-T337 rootstock was planted in 2015 and did not contain fruit at the time of measurements. Three replicates of 10 trees were selected for uniformity and health from the four rows of 60 trees in a split-plot experimental design. Each replicate was split into two groups of five trees; one to act as an untreated control and the other for ABA to be applied (Fig. 1). ABA was applied at a rate of 500 mg kg^{-1} to the point of drip formation on the leaves using a backpack sprayer on two dates; June 20 and August 16, 2016. ABA has been shown to be biologically active for approximately 21 days in young apple trees (McArtney et al., 2014) and the time between treatments was chosen to exceed that period.

2.2. Ground reference measurements

Stomatal conductance was measured immediately prior to sensor-based measurements in the same location. Measurements were made using a Decagon SC-15 handheld porometer (Meter Group Inc., WA, USA) between 09:00 and 11:00 a.m. on sunny days when the photosynthetically active radiation was between 1200 and 1500 μmol m^{-2} s^{-1} on two sun-exposed leaves that were approximately 1.5 m from the ground. The porometer used in this study considers the air relative humidity and temperature in the calibration process. For leaf measurement, the sensor obtains leaf humidity and estimates the stomatal conductance according to the difference between relative humidity in two conductance elements inside the sensor. Air temperature during measurements was between 18 and 20 °C (AgWeatherNet at