Detecting Sugarcane Aphid (*Melanaphis sacchari*) infestation in Grain Sorghum (*Sorghum bicolor*) using leaf spectral response

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Grace Craigie

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Approved by:

Major Professor
Dr. Brian P. McCornack

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Abstract

Sugarcane aphids (*Melanaphis sacchari*) are major agricultural pests to sorghum and infestation can cause up to 70% yield loss without timely insecticide applications. Populations can build exponentially on susceptible plants and require frequent field monitoring to determine when densities reach injurious levels. Current monitoring practices for sugarcane aphids (SCA) are time consuming and not practical for high acreage fields. Our overarching goal in Integrated Pest Management (IPM) is to develop more efficient monitoring techniques for SCA using remote sensing technologies, but this requires a better understanding of the interactions between aphids and leaf damage. Therefore, we studied the effect of SCA density on sorghum spectral responses near the feeding site and quantified potential systemic effects (i.e., plant-induced response) to see if the aphid feeding can be detected on leaves distal to the infestation. A leaf spectrometer, 400-1000 nm range, was used to measure reflectance changes in the range of 400-1000 nm by varying levels of SCA density on lower leaves and those distant to the caged infestation. Our results show that SCA infestation can be determined by changes in reflected light, especially between the green-red range (500-650 nm) and that sorghum plants respond systemically. This research is an important first step in developing more effective pest management strategies for SCA, as it shows that leaf reflection sensors can be used to identify aphid feeding regardless of where the infestation occurs on the plant. Future research should address whether such reflectance signatures can be observed autonomously using small unmanned aircraft systems or sUAS equipped with comparable sensor technologies. The goal is to improve sampling efficiency and overall decision making for this invasive species and reduce potential yield losses for growers through timely decisions.

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(1.) Introduction: Sugarcane Aphids, a Sorghum Pest

The Sugarcane Aphid

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), is a major agricultural pest of sorghum (*Sorghum* sps.) and sugarcane (*Saccharum officinarum*) [(Singh et al. 2004)]. They have a worldwide distribution range and sorghum-feeding populations are invasive to many regions in the Western Hemisphere (Singh et al. 2004; Nibouche et al. 2014; Bowling et al. 2016). Sugarcane aphids are particularly destructive to sorghum in North America, including the United States, after a reintroduction into the region (Michaud et al. 2017). Since 2013 sugarcane aphid populations build exponentially in sorghum, particularly under hot and dry environments (Brewer et al. 2016; Elliot et al. 2017), feed on the majority of sorghum growth stages (Rensburg 1973; Bayoumy et al. 2016), and can cause up to 75-100% yield loss when insecticide treatments are not used or ineffective (Catchot et al. 2015).

Origin of Sorghum-Feeding Sugarcane Aphids in United States

Sugarcane aphids inhabit a wide span of geographic regions including Asia, Africa, South and Central America, the United States, and Australia (Singh et al. 2004; Nibouche et al. 2014). Regardless of this expansive range, sugarcane aphid populations have low genetic diversity (Nibouche et al. 2014) and a limited host range of gramineous plants (Singh et al. 2004). Sugarcane aphids were found to have different lineages based on preference and specialization

for sugarcane or sorghum hosts (Nibouche et al. 2015). These diversified lineages are of particular importance to populations in the United States, whereas sugarcane aphids have historically only been minor pests of sugarcane (Nibouche et al. 2018). Early reports documented sugarcane aphids in Hawaii in 1896 and in the continental states in 1977 but only feeding on sugarcane (Mead 1978; White et al. 2001; Singh et al. 2004). This changed in 2013, when sugarcane aphids were documented in Texas feeding heavily on sorghum (Armstrong et al. 2015; Nibouche et al. 2018). In subsequent years, sorghum-feeding sugarcane aphid populations spread throughout the United States and dispersed eastward from Texas to Florida (Bayoumy et al. 2016) and northward to Kansas and Kentucky by 2015 (Bowling et al. 2016).

There is some debate over the origin of sorghum-infesting sugarcane aphids in the United States. It was unknown whether native populations naturally expanded their feeding range or whether sorghum-feeding populations were introduced from another geographic region. Nibouche et al. (2014) conducted genetic analysis of sugarcane aphid feeding on sorghum and found that lineages stem from West Africa, Australia, and China (Nibouche et al. 2014; Nibouche et al. 2018). This has led to the hypothesis that sorghum-feeding sugarcane aphids were re-introduced into the United States and are genetically different from native sugarcane-feeding populations (Nibouche et al. 2014). These invasive sugarcane aphids have caused significant yield reduction in sorghum production fields since their re-introduction in 2013 and provide novel pest management challenges in a high-acreage, low-value crop.

(2.) Sugarcane Aphid Biology and Life History

Biology and characteristics

Like most aphids, sugarcane aphids have both apterous (wingless) and alate (winged) morphologies (Dixon 1998; Bowling et al. 2016) and are around 1-2 mm in length (Akbar et al.

2010). Apterous sugarcane aphids vary in color from yellow to tan and have black cornicles, antenna tips, and tarsi (Bowling et al. 2016; Brewer et al. 2016). Alates have similar coloration except for dark bands on their abdomen and wings (Brewer et al. 2016). The majority of sugarcane aphids are purely anholocyclic (lacking males) and parthenogenetic with females producing genetically identical offspring without a mate (Dixon 1998; Bowling et al. 2016). They are viviparous (produce live nymphs) and live an average of 30-38 days while reproductive females typically deposit 8-9 offspring per day (Chang et al. 1982). Sugarcane aphid also have short maturation times as newborn nymphs can mature into reproductive adults in as little as 4 days- depending on environmental conditions such as temperature, humidity, and sorghum growth stage (Chang et al. 1982; Singh et al. 2004). This means that one female can produce an average of 34-96 nymphs during its lifetime, depending on environmental conditions, with each nymph being able to develop and reproduce in less than a week (Chang et al. 1982; Bowling et al. 2016). One of the reasons sugarcane aphids can mature rapidly and produce a staggering number of nymphs is due to telescoping of generations. Telescoping of generations occurs when a pregnant female aphid has two generations of embryos, both daughters and granddaughters, developing inside simultaneously (Dixon 1998). Essentially, aphid nymphs start forming inside immature mothers before the mother is born. This provides a great competitive advantage to aphids as they are able to mature, reproduce, and build up populations faster than other insect species of the same size (Dixon 1998). These characteristics are some of the primary contributing factors leading to rapid sugarcane aphid population growth in fields that can result in intense damage to sorghum plants.

Sugarcane Aphid Feeding Damage

High densities of sugarcane aphid can cause a wide range of injury to sorghum including severe damage or death to plants. To effectively control sugarcane aphid populations and reduce plant damage, insecticide should be applied once population levels exceed economic threshold level of 50-125 aphids per leaf in 20-30% of plants (McCornack et al. 2017)Sugarcane aphids can feed on the majority of sorghum growth stages (Rensburg 1973; Bayoumy et al. 2016) and can infest almost any part of the shoot, including the grain head (Brewer et al. 2016). Both nymphs and adults actively feed on sorghum and tend to feed on leaf undersides, favoring lower canopy leaves (Armstrong et al. 2015). As sugarcane aphids feed, dense populations directly damage host plants by removing phloem contents, called phloem sap, which is used for transportation of various compounds throughout the plant (Ruiz-Medrano et al. 2001; Singh et al. 2004; Kehr 2006; Merchant et al. 2010). The exact content of phloem sap can vary, especially between growing or senescing plants versus mature ones, but it typically contains large levels of sugar with various amino acids and low nitrogen content (Dixon 1998; Giordanengo et al. 2010). Reduction of grain quality and quantity (Berg et al. 2003; Singh et al. 2004), delayed plant development (Brewer et al. 2016), and leaf chlorosis and death (Colares et al. 2015) have been reported due to high sugarcane aphid infestation. Sugarcane aphids also cause indirect damage to host plants as they excrete a clear, sticky excrement called honeydew (Dixon 1998; Douglas 2003; Brewer et al. 2016) which coats the leaf epidermis underneath active feeding sites. High levels of honeydew on leaves promotes black sooty mold growth, thus blocking photosynthesis, (Narayana 1975; Singh et al. 2004; Brewer et al. 2016) and can cause mechanical issues during harvest when populations are left untreated (Bayoumy et al. 2016). The exact level of plant damage varies based on infestation levels, plant developmental stage, and combination of other stresses, such as drought, on sorghum plants (Singh et al. 2004).

Conditioning Hosts Plants

Another contributing factor that allows sugarcane aphids to heavily infest and damage sorghum is its near continual feeding habits while on an acceptable host plant. Once an aphid has found a suitable host they can uptake phloem contents for long time periods without having to cease for digestion or reproduction (Tjallingii 1995; Tjallingii 2006). However, feeding almost incessantly comes at a cost. Aphids need mechanisms to overcome the natural occlusion or wound response exhibited by most host plants (Goggin 2007; Will and van Bel 2008). During feeding, the aphid stylet pierces into sieve tube elements located in phloem tissue; these structures are responsible for transporting sugar and photoassimilates (Will et al. 2007). Sieve tubes elements allow for mass solute transportation and are connected to each other through pores that allow phloem sap to travel from one sieve element to another (Will and van Bel 2006; Taiz and Zeiger 2010). When a sieve element is pierced, the plant responds with a calcium triggered occlusion response that blocks interconnected pores and cuts off the damaged cell from adjacent ones (Will et al. 2007). This prevents the flow of phloem sap to the damaged cell, resulting in a loss of food for actively feeding aphids (Will et al. 2009). Penetrating the phloem is a time consuming activity, between 30 min to a few hours depending on aphid species (Pollard 1973; Morris and Foster 2008), so blocking plant occlusion to avoid plugged plant cells, prevents aphids from needing to find new feeding sites.

Aphids in general have been seen to condition host plants for continuous feeding through the release of compounds found in aphid saliva (Will and van Bel 2008). Generally, when an aphid feeds, it uptakes phloem sap through a food canal while the adjacent salivary canal emits watery saliva and gelling saliva (Dixon 1998; Tjallingii 2006). Proteins in watery saliva inhibit

the influx of calcium and prevent plants from blocking off damaged cells at active feeding sites (Will and van Bel 2008). This provides aphids with extended access to phloem elements and therefore a prolonged food source. Salivary compounds in watery saliva play an even larger role in aphid biology by lubricating the stylet during feeding and facilitating digestion and excretion processes (Tjallingii 2006; Moreno et al. 2011). Gelling saliva also enhances feeding by protecting the stylet from physical damage by covering the mouthpart with a protective sheath during intercellular movement through the plant (Tjallingii 2006; Will et al. 2012). The ability of aphids to manipulate host defense responses provides a large competitive advantage to sugarcane aphids by providing long durations for active feeding. Conditioning sorghum, combined with other biological traits including short maturation times, telescoping of generations, and high fecundity rates are some of the main factors that allow sugarcane aphid populations to build rapidly in sorghum fields.

Field Infestation

As populations can build rapidly in sorghum, sugarcane aphid outbreaks are difficult to manage in a short window of time before infestations reach economically damaging levels (Elliot et al. 2017). Within a week, sugarcane aphid numbers can increase from 50 to 600 aphids on a single leaf (Brewer et al. 2016; Brewer et al. 2017) with population densities reaching upwards of 10,000 aphids per plant (Brewer et al. 2016). Frequent monitoring of fields is needed to assess sugarcane aphid population levels since they amass in high numbers with exponential population growth, especially under low levels of biological control and on susceptible hybrids (Bowling et al. 2016). When field wide infestation levels have reached 50-125 sugarcane aphids in 20- 30% of plants, depending on the developmental stage of the sorghum, a foliar insecticide

is needed to control sugarcane aphid and prevent injury to crops (McCornack et al. 2017; Bowling et al. 2016). Despite viable insecticidal treatment options against sugarcane aphid, sorghum growers still experience yield loss, especially when needing to manage high-acreage fields, as monitoring entire production fields on foot is time consuming and impractical. In order to effectively decrease yield loss due to sugarcane aphids and identify treatable areas within a field, more efficient and effective methods for monitoring, and therefore controlling, sugarcane aphids are required.

(3.) Sugarcane Aphid Migration and Host Selection

Annual Sugarcane Aphid Migration in the US

Geographic location plays a major role when managing aphids due to the migratory patterns of this species. Sugarcane aphids have developed an annual cycle of migration in the US with populations overwintering in southern states and northern Mexico on Johnson grass (*Sorghum halepense*) or volunteer sorghum (Bowling et al. 2016; Michaud et al. 2017). As the sorghum growing season progresses across states, sugarcane aphids migrate from wintering locations to northern sorghum crops (Bowling et al. 2016). Migration occurs sporadically into northern Plain states, such as Kansas, making it difficult to predict when sugarcane aphids will infest fields (Michaud et al. 2017). The reason for this annual migration pattern is due to the unique sugarcane aphid life cycle and prolonged freezing periods in northern areas of the country. Most aphid species go through both asexual and sexual phases in which eggs are produced in the fall and survive harsh winter conditions until spring when asexual females emerge (Sullivan 2008). Sugarcane aphids reproduce asexually, without disposition of eggs, within the United States for overwintering (Bowling et al. 2016). Additionally, aphids in general

are soft-bodied insects, which often lowers overwinter survival of aphids at below-freezing temperatures. This is the case for sugarcane aphids, as no aphids have been documented overwintering at northern latitudes (Strathdee et al. 1995; Bale 2013). Consequently, sugarcane aphids produce a "winter phenotype" when temperatures decrease below 4.4 ˚ C (Michaud et al. 2017). This phenotype has dark-grey body coloration and, unlike the summer morph, has the ability to survive brief freezing conditions (Michaud et al. 2017). This trait provides the necessary means for sugarcane aphids to survive in a purely asexual life cycle in habitats experiencing sub-optimal temperatures. While regions in the northern US are often too cold to support year-round survival, sugarcane aphids survive in southern areas and disperse to northern latitudes during the growing season (Brewer et al. 2016).

Flight of the Alate

The dispersal of sugarcane aphids from overwintering sites occurs annually with populations moving northward during summer months (Michaud et al. 2018). Although the exact mechanisms surrounding sugarcane aphid migration are not fully understood, observational data shows similar patterns to other seasonal migrating aphid species (Bowling et al. 2016). Long-distance flight could be difficult for sugarcane aphids, as aphids in general are not known to be strong fliers (Powell and Hardie 2001; Powell et al. 2006). Alates in general have limited flight capacity due to low flight speed and interference from other environmental factors, such as low temperature (Kring 1972; Parry 2013). A few aphid species have been shown to remain in flight for a few hours before exhaustion. Cockbain (1961) found that in laboratory settings, black bean aphids (*Aphis fabae*) were able to sustain flight for 3-9 hours before exhaustion while other species only retain flight between 1-3 hours in laboratory and field settings (Cockbain 1961;

Kring 1972). Thus, to accomplish long-distance migration and sustained flight, alates rely on wind currents to carry them to new locations (Parry 2013; Bowling et al. 2016). Studies in the United States have found that several aphid species use low-level jet streams for northward migration with the duration and seasonality of the jet stream playing a large role in the time of population movement (Wallin and Loonan 1971; Zhu et al. 2006; Parry 2013). Other favorable weather conditions, such as low precipitation and warmer temperatures, also heavily contribute to seasonal migration (Irwin et al. 1988; Carlson et al. 1992; Zhu et al. 2006), with low-level jet streams carrying alates over a 1 km in some cases (Parry 2013). Other weather patterns have been found to carry alates up to 1600 km, between New Zealand and Australia, and over trans-oceanic distances (Loxdale et al. 1993; Parry 2013).

In addition to prevailing wind patterns, existing field conditions can affect when alates migrate to new locations. While hot and dry temperatures in infested sorghum fields are particularly inductive to sugarcane aphid exponential population growth (Singh et al. 2004), overcrowding and decline of a host plant are triggers for sugarcane aphids to produce alate offspring, which can in turn move to new fields (van Rensburg 1973; Singh et al. 2004; Michaud et al. 2017). Additional factors, including developmental stage of the host plant, wind speed, and abundance of natural enemies, can also effect alate production and take-off ability (Parry 2013). When existing field locations becomes inhospitable, apterous aphids produce alate morphs, which then search for new host plants (Singh et al. 2004; Sullivan 2008). The conditions in the infested fields along with wind patterns are thought to contribute to sugarcane aphid migration and, as these conditions vary from year to year, make the exact time of sugarcane aphid movements fluctuate.

Detecting Host Plants: Visual Cues

Predicting when sugarcane aphids will move into an area and how they locate hosts is one of the challenges with controlling infestation in sorghum in northern US growing regions. To successfully infest northern production fields, sugarcane aphid alates must be able to differentiate between host and non-host plants. Sugarcane aphids have a narrow host range that is limited to a few Gramineae species- whereas other, poorer, hosts like corn, wheat, and millet do not sustain high aphid populations (Singh et al. 2004; Armstrong et al. 2015). To increase the chance of locating a suitable host, sugarcane aphids need to have some degree of flight control and the means to distinguish a host field of sorghum even when traveling in air currents. Wind-mediated flight is not completely passive as alates have limited control mechanisms to land if a potential host is spotted (Thomas et al. 1977; Reynolds and Reynolds 2009; Parry 2013). Visual cues are used initially to locate vegetation (Hardie 1989; Powell et al. 2006; Parry 2013). Plants reflect different wavelengths of light, such as green and near infrared (NIR) regions, and aphids use difference in signal to distinguish hosts from non-hosts or other landscape features such as soil or water (Jensen 2010; Parry 2013). Several aphid species respond to specific wavelengths including green and UV light (Hardie 1989; Nottingham et al. 1991). Kirchner et al. (2005) determined specific photoreceptors in pea aphids, *Myzus persicae,* which respond to light bands in green, blue-green, and near UV regions of the spectrum (Kirchner et al. 2005). Other aphid species are positively attracted to yellow and the blending of specific colors, such as yellow with orange or green (Kring 1967). While detecting leaf reflectance can help aphids locate vegetation from non-vegetation, it does not provide enough sensory information to distinguish between host and non-host plants (Powell et al. 2006). Aphids rely on the combination of visual cues and plant

volatiles, olfactory cues, to distinguish a suitable host from a non-host plant in flight (Pickett et al. 1992; Nottingham and Hardie 1993; Dixon 1998).

Detecting Host Plants: Chemical Cues

Alates have a number of specialized receptors on each antenna called primary and secondary rhinaria that are used to detect airborne odors (Pickett et al. 1992; Park and Hardie 2002). Once a plant odor has been detected, an alate lands on the plant surface and then uses antennal receptors to further explore chemicals emitted from vegetation by tapping antenna on the leaf surface (Powell and Hardie 2001; Fereres and Moreno 2009). In addition, aphids feel for appropriate feeding sites on the leaf surface by using tactile receptors on the proboscis apex (Dixon 1998). If probing the surface results in a favorable stimulus for feeding, the aphid will penetrate its stylet, mandible and maxillary mouthparts, into plant tissues and uptake cellular components (Pickett et al. 1992; Dixon 1998). Several initial probes into the upper layer of plant tissue, epidermis, may occur before the aphid rejects the plant, and flies away, or accepts it and penetrates the stylet past the epidermis (Powell et al. 2006). To access food, the stylet moves along an intercellular pathway through the upper plant tissue layers until the phloem is located (Dixon 1998; Tjallingii 2006). Once the stylet has penetrated into the phloem and into sieve tube elements, which are used for plant sugar and nutrient transport (Tjallingii and Esch 1993; Taiz and Zeiger 2010), it uptakes phloem sap and continues until the sap is no longer an acceptable food source by the aphid (Powell et al. 2016). This process is critical for migrating alates as they need to accurately identify a suitable host, which support individual aphid reproduction and subsequent colony growth.

New Monitoring Methods Needed for Sugarcane Aphids

Given the high level of temporal variability in alate migration, scouting and subsequent management of sugarcane aphids can also vary based on geographic location. In southern states, sugarcane aphid scouting is recommended when sorghum shoots first emerge while scouting in northern areas is more sporadic and dependent on when sugarcane aphids move into an area after long-distance migration (Brewer et al. 2016; Elliott et al. 2017). In addition, wind direction is important for northern growers to estimate where alates will initially land and infest fields (Bowling et al. 2016). Sugarcane aphids have a tendency to aggregate in fields (Elliott et al. 2015; Elliott et al. 2017), which can create patches of infestation based on which plants alates successfully located. These pockets of infestation make estimation of overall aphid density difficult to determine as well as when populations have surpassed economic threshold and need insecticidal treatments. With sugarcane aphid exponential population growth rates, there can be serious yield loss to growers if insecticidal treatments are not applied timely to plants (Elliott et al. 2017). Therefore, vigilant field scouting with large quantity of sample plants is required in northern regions to locate sugarcane aphid infestation pockets and accurately estimate populations. However, as growers physically scout sorghum fields this monitoring process is not time efficient and is impractical for large fields. A more efficient means of monitoring fields would be to implement remote sensing technology to detect plant stress without having to physically scout fields.

(4.) Remote Sensing: Leaf Spectral Response

Remote Sensing to Detect Crop Stress

Remote sensing is a process that uses external and non-intrusive techniques for assessing crop health (Richardson et al. 2002; Pinter et al. 2003). It encompasses a wide range of systems, from satellites to hand-held devices, equipped with spectral sensors that detect plant responses to environmental stress (Pinter et al. 2003; Zhang and Kovacs 2012). Remote sensing in agricultural systems is especially useful as measurements are nondestructive and can be taken repeatedly, thus allowing temporal analysis of crop stress (Prabhakar et al. 2012; Sims and Gamon 2002). More recently, unmanned aircraft systems (UAS) are being used to capture data from agricultural fields and such systems can provide more timely and on-demand data about field-wide plant health as compared to satellites or manned aircrafts (Zhang and Kovacs 2012; Stanton et al. 2017). Implementing UAS with sensors capable of determining changes in plant vigor or stress may provide an alternative to current sugarcane aphid monitoring methods by detecting the presence of aphids more efficiently and accurately than walking through fields. It is not known whether such technologies are capable of detecting aphid-infested sorghum.

Leaf Spectral Reflectance

Modern remote sensing equipment measures wavelengths of light reflected off leaves, which is then used to derive information about the overall health of the plant (Warren and Metternicht 2005; Reisig and Godfrey 2006). When sunlight reaches plant tissues, light between 400-2600 nm wavelength range in the electromagnetic spectrum is either absorbed or reflected by plant cells based on a variety of factors (Jensen 2000). For example, green and near-infrared light (NIR) is reflected while other wavelengths in the red and blue region of the spectrum are essential to energy acquisition and used in sugar production (Carter 1993; Jensen 2010; Campbell et al. 2008). More specifically, photosynthesis by leaf pigments, predominately

chlorophyll *a* and *b*, determine the specific wavelengths in the visible spectrum that are absorbed or reflected (Knipling 1970; Jensen 2000). Light in the visible range (between 350-700 nm) is used as an energy source for photosynthesis by absorbing blue and red light (Taiz and Zeiger 2010; Prabhakar et al. 2012). Green light is reflected off leaves, as these wavelengths are not useful for photosynthesis, thus giving "healthy" or unstressed plants green coloration (Campbell et al. 2008).

Light in the NIR region or between 700-1200 nm reaching leaf tissues in the palisade layer is largely reflected (Knipling 1970; Jensen 2000). Reflecting NIR light protects internal cell structures by scattering NIR light at the cell wall membrane and air interfaces and prevents excessive heat buildup (Jensen 2000; Prabhakar et al. 2012; Ustin and Jacquemoud 2020). Finally, wavelengths between 1300-2600 nm or middle-infrared (MIR) provides unique reflection and absorption patterns due to water levels in leaf tissue (Allen and Richardson 1968; Knipling 1970; Jensen 2000). Overall, the wavelength ranges in the visible, NIR, and MIR each exhibit different patterns of absorption or reflection or "signatures", based on leaf pigment content, leaf structures, and water content respectively within a plant (Pinter et al. 2003). These signatures also differ between plant species and even within a species, given differences in host phenotypes (Knipling 1970; Jensen 2000; Klančnik and Gaberščik 2016). Measuring differences in reflection patterns, also referred to as the vegetative spectral reflectance curve (Fig. 1), is the basis of remote sensing as such changes in values indicate the internal state of a plant (Prabhakar et al. 2012).

Leaf Spectral Response to Stress

When plants experience environmental pressures, such as herbivory, these stressors can cause a detectable physiological response by plants, which basically alters the spectral reflectance curve (hereafter referred to as the leaf spectral response) observed in the absence of stress (Carter and Knapp 2001; Prabhakar et al. 2012). Physiological responses vary based on type of stress and plant species but overall comprise of altered leaf pigment content, cell and tissue structure, or water content (Knipling 1970; Pinter et al. 2003). As these three physiological responses are correlated with reflection changes in the visible, NIR, or MIR regions of the electromagnetic spectrum, measuring which wavelength range is altered can provide a diagnosis into the physical and chemical state of the stressed plant (Carter et al. 1996; Jensen 2000). This technique is the foundation for diagnosing crop stress as these measurements can be taken multiple times without damaging plant tissue (Prabhakar et al. 2012).

Although remote sensing of plant stress has been analyzed throughout 400-2600 nm range, studies have found that most spectral responses to stress can be estimated within visible and NIR wavelengths (Hatfield and Pinter 1993; Prabhakar et al. 2012). One general spectral response to environmental stress, reflectance increases in the visible range and the green light reflectance peak widens (Adams et al. 1999; Pinter et al. 2003). Another trend is NIR light decreasing and causing a shift towards shorter wavelengths in the "red-edge" between 650-700 nm (Jensen 2000; Pinter et al. 2003; Prabhakar et al. 2012). Despite these common plant responses to stress, reflectance is highly correlated to leaf structure and pigment content (Croft et al. 2014). The exact wavelength changes due to stress can differ as internal and external leaf structures, along with conditions during development, can vary significantly (Jensen 2000; Klančnik and Gaberščik 2016; Hallik et al. 2017). As the precise physiological response, and thereby leaf spectral response, can differ based on plant species and type of stress, a sorghum-

sugarcane aphid specific study is needed to determine if there is a signature spectral to sugarcane aphid feeding.

(5.) Leaf Spectral Response to Aphid Feeding

Stress-Induced Changes to Leaf Pigments

In order to use remote sensing to detect sugarcane aphid feeding, the physiological stress response of sorghum needs to cause changes in leaf pigments, and internal cell structures, so there is a detectable leaf reflection change (Prabhakar et al. 2012). Several aphid species, including sugarcane aphids, have been documented decreasing chlorophyll content in their respective host plants (Riedell and Blackmer 1999; Diaz-montano et al. 2007; Golawska et al. 2010; Armstrong et al. 2018). In general, "healthy" or unstressed leaves contains active chlorophyll pigments, which dominates the reflective effects of accessory leaf pigments, as evident by reflected green coloration on the leaf's surface (Jensen 2000; Taiz and Zeiger 2010). Reduction of chlorophyll leaf pigments, specifically chlorophyll *a* and *b*, causes cessation of photosynthesis and overall health of the leaf (Green and Durnford 1996; Jensen 2000; Taiz and Zeiger 2010). With enough stress and chlorophyll reduction, especially during leaf senescence, carotenoids and anthocyanin accessory leaf pigments dominate (Merzlyak et al. 1999; Jensen 2000; Sims and Gamon 2002).

Carotenoids and anthocyanins are present in "healthy" leaves but in lower quantities than chlorophyll (Jensen 2000; Sims and Gamon 2002). Carotenoids provide additional energy for photosynthesis by augmenting absorption in the blue-green region (Demmig-Adams and Adams III 1996; Green and Durnford 1996; Barker et al. 1997; Sims and Gamon 2002; Havaux 2013) while both accessory pigments function in photoprotection or protecting leaf tissues from excess

light energy and heat (Holton and Cornish 1995; Demmig-Adams and Adams III 1996; Green and Durnford 1996; Sims and Gamon 2002). As chlorophyll is more susceptible to degradation, compared to carotenoids and anthocyanins, the higher proportion of accessory pigments in stressed leaves causes leaves to turn yellow or red (Merzlyak et al. 1999; Jensen 2000; Carter and Knapp 2001; Sims and Gamon 2002; Gitelson et al. 2003). This is due to unique absorption ranges in carotenoids between 450-500 nm, causing yellow light reflection, and anthocyanins having a peak absorption around 550 nm causing reflection of pink, purple, or red light (Sims and Gamon 2002; Gitelson et al. 2003; Albert et al. 2009). These internal leaf pigment changes, and subsequent changes in visible light reflection, are a well-studied means of detecting plant stress through remote sensing.

Problems Detecting Sugarcane Aphids

When accessory pigments are present in higher proportions to chlorophyll, external leaf coloration will change which can provide visual evidence of plant stress (Jensen 2000). Changes in leaf color is a useful indication of plant stress for monitoring strategies as altered colors can even vary between similar herbivores on the same plant. In the case of sorghum, greenbug aphid (*Schizaphis graminum*) feeding completely breaks down chlorophyll at the feeding site causing external leaf colors to change from green to red or rust (J.P. Michaud et al. 2017) while yellow sugarcane aphids will cause general leaf chlorosis that starts from the leaf tip (Kindler and Dalrymple 1999). Sugarcane aphids can also cause leaf chlorosis, yellowing, and discoloration in sorghum (Singh et al. 2004; Michaud et al. 2017), but chlorosis and other external sorghum coloration changes does not manifest until sugarcane aphids have reached high population levels (GC personal observation; Brewer et al. 2016). This allows infested leaves to remain green for

longer periods of time despite sugarcane aphid infestation (Brewer et al. 2016) making it difficult to visually detect sugarcane aphids while walking through a field before they reach high density levels. This phenomenon complicates monitoring strategies, as sugarcane aphid infestation needs to be detectable before populations exceed economic threshold, 50-125 aphids per leaf in 20-30% of plants, and reach damaging levels to crops (McCornack et al. 2017). Therefore, the nature of response in sorghum to sugarcane aphid feeding further exemplifies the need for remote sensing to detect populations before they reach damaging quantities. To efficiently monitor sugarcane aphids, chlorophyll degradation due to feeding needs to be detected before external chlorosis; when populations are still at low infestation levels, below economic threshold of 50 aphids (McCornack et al. 2017; Hernández et al. 2021).

As altered visible reflectance correlates to degraded chlorophyll pigments in stressed leaves, detecting the proportion of chlorophyll to accessory pigments, before high sugarcane aphid density, is critical. However, detecting chlorophyll degradation can be challenging as the absorption ranges between chlorophyll, carotenoids, and anthocyanin overlap (Sims and Gamon 2002). Furthermore, only a relatively small amount of chlorophyll is needed for absorption making it difficult to determine its content level (Sims and Gamon 2002). Remote sensing at the edge of chlorophyll's wavelength range, around 700 nm, is more indicative of degradation due to plant stress (Sims and Gamon 2002). In this red-NIR transition region or "red-edge," there is little overlap between chlorophyll and other pigments and a high level of chlorophyll is needed for absorption (Curran 1983; Horler et al. 1983; Cibula and Carter 1992; Datt 1999; Carter and Knapp 2001; Sims and Gamon 2002).

Species-Specific Response to Aphid Feeding

Several remote sensing studies in crop systems have used leaf spectral response in the red-edge, or indices such as NDVI that compare changes in the red and NIR ranges, to detect aphid infestation in their respective host plants (Yang et al. 2005; Elliott et al. 2007; Yang et al. 2009; Prabhakar et al. 2012). However, this response in not universal to all host-aphid interactions due to varied leaf structures in different plant species (Sims and Gamon 2002). For example, Reisig and Godfrey (2006, 2007) found that instead of the common 700 nm range, cotton aphids caused detectable reflection changes in the NIR range in cotton plants (Reisig and Godfrey 2006; Reisig and Godfrey 2007). Similar reflection changes in NIR were also seen when wheat was infested with Russian wheat aphids, albeit there were slight variations in wavelength ranges due to irregularity in planting conditions, species variety, and developmental stage of the wheat (Riedell and Blackmer 1999; Mirik et al. 2007). As the specific spectral response of sorghum to sugarcane aphids is not fully understood, it is important to include both visible and NIR wavelength ranges in a sugarcane aphid-sorghum study.

Detecting Low Aphid Densities

In addition to studying sorghum's signature wavelength range in response to sugarcane aphid feeding, other factors need to be studied before an effective monitoring strategy can be developed against these pests. It is currently unknown how many sugarcane aphids are needed to cause enough physiological changes to sorghum to alter leaf reflection in a way that can be detected using remote sensing technology. Detection of low aphid densities is critical to effectively monitor and control sugarcane aphids as populations can build exponentially in sorghum (Elliott et al. 2017). If changes in light reflection due to aphid feeding are only measurable once populations have reached high infestation levels than sorghum will have

already incurred enough damage to cause economic losses to growers. Sorghum's stress response to sugarcane aphids should be detectable before populations exceed economic threshold levels, 50-125 aphids per leaf in 20-30% of plants, so insecticides can be effectively applied to control populations and reduce plant damage (McCornack et al. 2017). Different densities of sugarcane aphids should be tested to determine if there is a threshold number of aphid feeding needed to elicit a measurable leaf spectral response in sorghum.

(6.) Local and Systemic Effects of Aphid Feeding

Local Effects of Aphid Feeding

Another unknown factor in developing a remote-based sugarcane aphid-sorghum monitoring technique is where on the sorghum plant can a response can be detected. If an aphid is feeding on one part of a leaf, does it only elicit a local response or is it a more wide-spread, systemic plant responses? In general, aphids have been shown to cause local physiological changes in sieve tube elements where aphid feeding release salivary compounds to block occlusion and allow a continual flow of phloem sap (Will et al. 2007). However, conditioning effects on sieve tube elements are not isolated to the cell where the aphid is directly feeding. Sieve tube elements are connected through pores in sieve plates, which allows contents from ejected watery saliva to be transported from the feeding site to nearby cells; pre-conditioning nearby sieve tubes elements for enhanced feeding (Will and van Bel 2006; Dugravot et al. 2007; Will and van Bel 2008; Taiz and Zeiger 2010). Pre-conditioned cells have lowered occlusion, and down-regulated local defense responses, which allows easier access and improved feeding for nearby aphid feeding (Will and van Bel 2008). This could explain why sugarcane aphids tend to aggregate in groups on sorghum leaves as one aphid would allow easier feeding for adjacent

nymphs or incoming aphids. As nearby sieve tube elements are affected by aphid feeding this presumably would also alter light reflection in neighboring cells and allow detection of aphid feeding in neighboring uninfested cells. However, an aphid's ability to pre-conditioning other sieve tube elements only occurs on a local scale as salivary contents become diluted farther away from active feeding sites (Will and van Bel 2008).

Measuring light reflectance locally, near feeding sites, can provide a method of detecting and studying sorghum response to sugarcane aphids feeding. The exact leaf spectral response or which specific wavelength ranges are altered due to sugarcane aphids can provide insight into what physiological changes are occurring within infested leaves. If a specific altered wavelength range can be determined it would allow sugarcane aphid feeding to be distinguishable from other herbivore stress responses. In addition, temporal studies through remote sensing can be conducted of sorghum's stress response changes over time, such as how rapidly chlorophyll degrades with building infestation levels. Overall, detecting sugarcane aphid infestation locally, near active feeding sites, can provide information about the nature of sorghum's stress response to sugarcane aphid feeding and the differential effects this species has on altering leaf pigments content as compared to analogous aphid species. The ability to study these effects over time, using non-destructive remote sensing technology, can also provide insight into the density level of sugarcane aphids needed to elicit a measurable leaf spectral response if low or high infestation levels are needed to cause noticeable change in light reflection.

Systemic Effects of Aphid Feeding: Jasmonic Acid Defense Response

Although aphids can condition host plants at the site of feeding (i.e. local effects), they can also stimulate a more systemic plant defense response that can negatively impact feeding

(Will and van Bel 2008). Like aphids, insects in general deposit saliva into plant tissue while feeding which acts as a trigger or elicitor for stimulating plant defense response (Taiz and Zeiger 2010). When an aphid penetrates sieve tube elements, it releases byproducts of gelling saliva that trigger plant defenses (Moreno et al. 2011; Will et al. 2007). One induced response is the jasmonic acid pathway (JA), -which uses jasmonic acid, or jasmonate, as a mediator in signal cascades against wounding and herbivory stress (Turner et al. 2002; Morkunas et al. 2011). As the jasmonic acid pathway can be induced against abiotic and biotic stress, using saliva as an elicitor allows the plant to distinguish between mechanical and herbivory damage (Turner et al. 2002; Hilker and Meiners 2010). Saliva composition in insect and subsequent damage type, such as chewing versus phloem damage, are detectable by the plant, allowing for a species-specific exact defense response (Zhu-Salzman et al. 2004; Taiz and Zeiger 2010).

Systemic Effects of Aphid Feeding: Salicylic Acid Defense Response

In addition to the jasmonic acid defense response, plants have a secondary response to aphid feeding that is not typically expressed against other types of herbivory (Zhu-Salzman et al. 2004). When plants are exposed to aphids they upregulate the salicylic acid (SA) defense response, which is typically stimulated against pathogens and similar microorganisms (Moran et al. 2002; Martinez de Ilarduya et al. 2003; Zhu-Salzman et al. 2004). In general, this pathway generates hydrogen peroxide, a type of reactive oxygen species, that triggers production of the salicylic acid hormone and pathogenesis-related (PR) proteins to generate defenses against identified pathogens (Wu et al. 1997; Zhu-Salzman et al. 2004). The salicylic acid pathway provides an additional and unique response by plants to phloem-feeders and is thought to aide in distally upregulating defense responses (Morkunas et al. 2011). The connectivity of the phloem

provides a means for systemic communication by transporting signaling proteins associated with stress and defense (Ruiz-Medrano et al. 2001; Kehr 2006). This allows plants to "activate" defense genes in regions that have yet to be exposed to the stressor (Ruiz-Medrano et al. 2001). The systemic nature of this response would therefore allow distal, uninfested parts of the plant to elevate defenses before aphids begin to feed and potentially provide a detectable change in spectral response due to sugarcane feeding.

Unknown Sorghum Response to Sugarcane Aphids

Despite a large range of studies showing localized plant conditioning as well as induction of both the jasmonic acid and salicylic acid pathways due to aphid feeding (Moran and Thompson 2001; Will and van Bel 2008) the exact response of sorghum to sugarcane aphid feeding has yet to be determined. As plants can detect salivary elicitors during insect feeding, it allows upregulation of species-specific defense responses (Felton and Tumlinson 2008). For example, Yang et al. (2009) conducted a remote sensing study that discriminated between Russian Wheat aphid (*Diuraphis noxia*) and greenbug aphid (*Schizaphis graminum*) feeding in wheat (Yang et al. 2009) that demonstrates a species-specific response through distinguishable leaf spectral changes between the two aphid pests. In the case of sorghum, both jasmonic acid and salicylic acid defense pathways have been induced in response to greenbug aphids (*Schizaphis graminum*) (Zhu-Salzman et al. 2004). However, as plants can induce species-specific response, it cannot be assumed that sorghum will respond in exactly the same way to sugarcane aphid feeding. In addition, external leaf coloration in sorghum differs between sugarcane aphids and greenbug feeding (Zhu-Salzman et al. 2004; Singh et al. 2004) indicating that each species is likely affecting the plant differently. As responses to similar insect pests can

differ within the same plant, a specific sorghum-sugarcane aphid study is further justified. To determine if sorghum induces jasmonic acid and salicylic acid pathways in a detectable way, both locally and systemically, the internal changes within sorghum need to alter pigment content and/or leaf structure in a way that changes patterns of leaf reflectance (Prabhakar et al. 2012). Therefore, before remote sensing monitoring of sugarcane aphids in sorghum is implemented a more exact understanding of sorghum-sugarcane aphid interactions and responses needs to be studied.

(7.) Project Goals and Objectives

There is limited information on how sorghum plants respond to sugarcane aphids in terms of defense pathways and alteration of leaf pigments. A species-specific study is needed between sorghum and sugarcane aphids (SCA) to first determine if aphid feeding causes a detectable leaf spectral response within the visible and near-infrared regions of the electromagnetic spectrum. Measuring which wavelength ranges alter in response to SCA feeding will provide insight into what physiological changes, such as altered leaf pigment content and internal leaf tissues, sorghum undergoes in response to SCA stress. In addition, to practically implement SCA monitoring system using remote sensing, populations need to be detected before they reach high densities as they populations can exponentially grow to damaging levels within fields. It is additionally important to detect SCA at low densities as there is not an immediate, visible change in leaf composition until sugarcane aphids have feed extensively on a leaf. Varying densities of sugarcane aphids will be tested to see how many aphids are needed to induce a detectable response in sorghum.

In addition to the exact leaf spectral response and the density levels of SCA needed to elicit leaf reflection changes in sorghum, whether this response is local and systemic spectral is also critical to test. To implement a practical monitoring system, that is more time efficient than traditional monitoring of fields on food, remote sensing unmanned aircraft systems (UAS) are used as these drones take field-wide spectral readings in relatively short periods of time (Zhang and Kovacs 2012; Stanton et al. 2017; Barbedo 2019). However, unmanned aircraft systems take spectral measurements from above the field of upper canopy leaves as they move above a field. If the leaf spectral response is only local, taking readings from above the field would lead to an inaccurate infestation estimations as sugarcane aphids tend to feed and aggregate on lower canopy leaves (Armstrong et al. 2018). In order to have more precise measurements using UAS technology, aphid infestations need to induce a systemic leaf spectral response so that spectral measurements can be taken accurately, regardless of feeding locations. Based on previous studies that have successfully detected aphid feeding using remote sensing in agricultural crops (Yang et al. 2005; Elliott et al. 2007; Yang et al. 2009; Prabhakar et al. 2012), I hypothesize that SCA, at low density levels, will cause enough physiological changes to sorghum to be detectable on infested leaves, local response, and at distal leaves from the active feeding site, using this technology. In addition, I hypothesize our results are likely to show both a local and systemic response to SCA feeding as similar aphid species, such as greenbugs (Zhu-Salzman et al. 2004), have been demonstrated to induce both JA and SA defense pathways in sorghum implying that sorghum responds to aphid infestation systemically.

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(9.) Figures

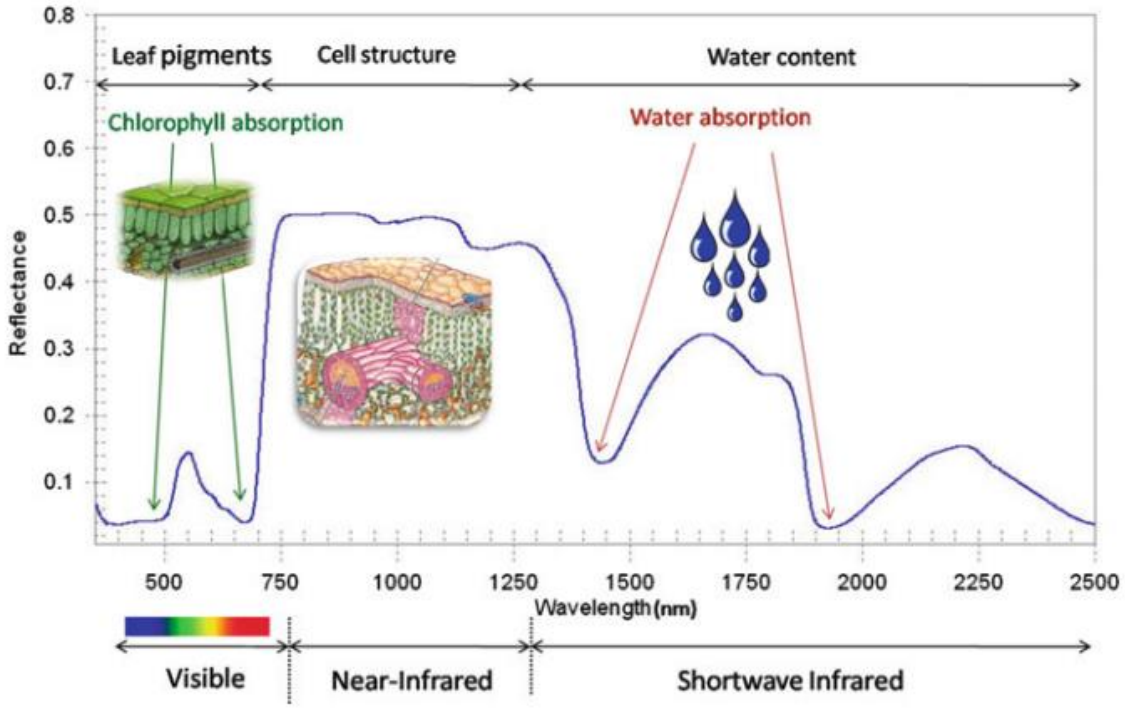

Figure 1. Vegetative spectral reflectance curve of healthy vegetation in the visible, near-infrared (NIR) and shortwave infrared (also called middle-infrared) range- reproduced from (Prabhakar et al. 2012)

Chapter 2- Detecting Sugarcane Aphid (*Melanaphis sacchari)* Infestation in Grain Sorghum (*Sorghum bicolor)* Using Leaf Spectral Response

(1.) Introduction

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), is a major agricultural pest of sorghum (*Sorghum* sps.) and sugarcane (*Saccharum officinarum*) (Singh et al. 2004). In the United States, sugarcane aphid populations have historically only feed on sugarcane until 2013 when they were first documented infesting sorghum fields in Texas (Armstrong et al. 2015; Nibouche et al. 2018). In the subsequent years, sugarcane aphids spread into other regions of the US and in 2015 were first observed in Kansas sorghum fields (Bayoumy et al. 2016; Bowling et al. 2016). Nationwide, sorghum-feeding sugarcane aphids have caused significant losses to growers as populations can grow exponentially, particularly under hot and dry environments (Brewer et al. 2016; Elliot et al. 2017). Infestations can cause up to 75-100% yield loss to growers without timely application of an insecticidal treatment (Catchot et al. 2015).

Sugarcane aphid feeding directly damages sorghum as it can cause reduction of grain quality and quantity (Berg et al. 2003; Singh et al. 2004), delayed plant development (Brewer et al. 2016), and leaf chlorosis and death (Singh et al. 2004; Colares et al. 2015) depending on the developmental stage and conditions in which sorghum is infested. Sugarcane aphids also cause indirect damage to sorghum host plants as they excrete a clear, sticky excrement called honeydew (Brewer et al. 2016), which coats the leaf epidermis underneath active feeding sites (Singh et al. 2004). High levels of honeydew on leaves promotes black sooty mold growth, which blocks photosynthesis, (Narayana 1975; Singh et al. 2004; Brewer et al. 2016) and causes mechanical issues during harvest (Bayoumy et al. 2016).

Due to high levels of plant damage and the potential for rapid population growth, frequent field scouting is needed to control sugarcane aphid outbreaks in sorghum fields (Bowling et al. 2016). Growers and scouts must locate sugarcane aphid infestations manually, estimate populations, and determine when plants need to be treated with insecticide. However, physically walking through fields to visually locate populations is not time efficient and often impractical for detecting crop pests (Prabhakar et al. 2012), especially for farmers with high-acreage sorghum fields (e.g., >1000 acres).The overarching goal behind my research is to overcome these monitoring limitations by developing a more efficient monitoring technique by using remote sensing technologies. Remote sensing equipment measures wavelengths of light reflected off leaves that relays information about the overall "health" of the plant (Warren and Metternicht 2005; Reisig and Godfrey 2006). Plants undergoing environmental stress will undergo different physiological changes, based on the type of stressor, which alters light reflection patterns from that of an unstressed plant (Jensen 2000; Carter and Knapp 2001; Prabhakar et al. 2012). This leaf spectral response is due to internal physiological responses by the plant which include altered leaf pigment content and tissue structure (Knipling 1970; Pinter et al. 2003). These physiological responses are correlated with reflection changes in the visible or near-infrared (NIR) regions of the electromagnetic spectrum, respectively, that can provide a diagnosis into the physical and chemical state of the stressed plant based on which wavelength range has altered (Carter et al. 1996; Jensen 2000). This technique is the foundation for diagnosing crop stress as these measurements can be taken multiple times without damaging plant tissue (Prabhakar et al. 2012).

Airborne remote sensing technology, such as unmanned aircraft systems (UAS), has been used to capture leaf spectral data from agricultural fields and such systems can provide timely

and cost-effective data about field-wide plant health (Zhang and Kovacs 2012; Stanton et al. 2017). However, the exact leaf reflectance response can vary between crop systems based on the type of environmental stressor, variations in internal leaf structures, and growing conditions during development (Jensen 2000; Sims and Gamon 2002; Klančnik and Gaberščik 2016; Hallik et al. 2017). As leaf spectral responses can vary widely, several key variables such as spectral signature, aphid density needed to elicit a response, and local versus a systemic response, need to be addressed before implementing a sorghum-sugarcane aphid monitoring system to assure populations are being detected accurately and efficiently.

One of the first steps to developing a UAS-based monitoring system is to determine the signature spectral response or specific changes in wavelength reflection due only to sugarcane aphid stress in sorghum. These precise changes in wavelength ranges are not currently known, making it difficult to distinguish sugarcane aphid feeding from similar insect pests and determine what physiological effects sugarcane aphids have on sorghum, such as altered leaf pigment content or cell structures. In addition, the density level of sugarcane aphids needed to elicit a detectable leaf spectral response also needs to be studied as aphid populations can grow exponentially and therefore needs to be detectable at low densities. In this study, we wanted to assess any reflectance changes to sugarcane aphid feeding on sorghum in greenhouse conditions.

To ascertain a signature response to sugarcane aphids, a hand-held CI-710 miniature leaf spectrometer, which measures spectral reflectance in the visible and NIR wavelength range between 400-1000 nm, was used to measure leaf reflectance of infested sorghum leaves. This reflectance range coincides with remote sensing UAS but allows measurements to be taken on a single leaf, in a controlled greenhouse setting, so sorghum's precise leaf spectral response to sugarcane aphid feeding can be studied (Fig. 2). Leaves were also infested with low or high

starting densities of sugarcane aphids and the aphids could reproduce over time to determine the aphid density needed to detect infestation.

In addition to the exact leaf spectral response and aphid density needed to elicit a response, another key variable was tested using a leaf spectrometer to verify use of a UAS monitoring system. It is currently unknown if sorghum initiates a localized (i.e. near the feeding site), or systemic response (i.e. plant-wide) to sugarcane aphid infestation in a way that can be detected using leaf spectral response. This knowledge gap poses a problem in implementing UAS remote sensing for sugarcane aphids as they tend to feed on the underside of lower canopy leaves (Armstrong et al. 2015), whereas drones take spectral readings from upper canopy leaves (Hassler and Baysal-Gurel 2019). If sugarcane aphid feeding response is only localized, then populations feeding on lower leaf levels may not be detectable using a UAS system. A measurable systemic response to sugarcane aphids is needed so that the location of aphid infestation does not disrupt the efficiency of UAS sensor.

Based on previous studies that have successfully detected aphid feeding using remote sensing in agricultural crops (Yang et al. 2005; Yang et al. 2009; Elliott et al. 2007; Prabhakar et al. 2012), I hypothesize that sugarcane aphids, at low density levels, will cause enough physiological changes to sorghum to be detectable using remote sensing technology. In addition, our results are likely to show both a local and systemic response to sugarcane aphid feeding as a similar aphid species, greenbugs (*Schizaphis graminum*), has been demonstrated to induce defense pathways in sorghum (Zhu-Salzman et al. 2004). If the presence and feeding of sugarcane aphid on a lower leaf, for example, induces a systemic defense pathway than enough physiological alteration throughout the plant should occur to allow aphid infestation to be detectable regardless of infestation sites. Overall, determining a specific spectral signature,

aphid density levels, and localized versus systemic response in sorghum will provide critical information to ensure that a UAS remote sensing is a time effective and accurate monitoring strategy against sugarcane aphid infestations.

Objectives

The first objective of the study was to analyze changes in sorghum response to sugarcane aphid feeding by comparing leaf reflectance between infested and uninfested plants. This will hypothetically allow us to discern a signature leaf spectral response of sorghum to sugarcane aphid feeding by measuring spectral reflectance near active feeding sites. The second objective was to correlate the level of aphid density, feeding on the same leaf, needed to elicit a detectable response. The final objective was to determine if changes in sorghum response to sugarcane aphid feeding is only be detectable locally or systemically using remote sensing technology. To accomplish these objectives, three runs of the experiment were conducted under greenhouse conditions during June and July 2017 (R1), February 2018 (R2), and March 2018 (R3) respectively.

(2.) Materials and Methods

Aphid and Plant Material

Sugarcane aphids used for the study were wild caught from naturally infested sorghum fields during the summer of 2016 and 2017. Apterous aphids were collected from fields within the Ashland Bottoms Research Farm and the Department of Agronomies North Farm (Kansas State University) in Manhattan, KS. Fine-tipped paintbrushes were used to transfer sugarcane aphids from infested plants to Eppendorf tubes. Aphids were then transported to Kansas State University greenhouses in Manhattan, KS where they were transferred to potted sorghum plants

(DKS29-28, Dekalb). Newly infested plants were placed into Bugdorms (cage-2400, BugDorm; Taiwan) rearing tents (Fig. 2) and all sugarcane aphid colony Bugdorms were placed in the same greenhouse space.

Prior to infestation, DKS29-28 sorghum plants were grown in a separate greenhouse room from aphid colonies to prevent unwanted infestations. DKS29-28 (Pioneer, Corteva Agriscience, Iowa, U.S.A.) sorghum is an early-maturity hybrid that is considered susceptible to sugarcane aphids. All potted plants were grown using Sungro Professional Growing Mix (SunGro; Agawam, MA). Seeds were first planted in cone-shaped pots (dimensions: 3.81 cm diameter x 20.955 cm depth x 238.91 ml volume), referred to as "Cone-tainers" (model SC10, Ray Leach Cone-tainers, Tangent, OR) with 18 cm of soil per pot. All sorghum was grown in a greenhouse under artificial lights with 16:8 hr. (light: dark) photoperiod, maintained at 22 ± 3°C, fertilized with "Peter's Professional 20-20-20 General Purpose Fertilizer," and were regularly inspected to ensure that no aphid or other insect pests were present. Sorghum plants were allowed to grow until they reached 3-leaf stage or Vegetative Stage 1 (Fig. 3). Plants were then transplanted into 15.24 cm diameter plastic pots, to provide adequate room for growth, and placed in clean Bugdorms (Fig. 2) with no prior contact with aphids. All plants were grown to the 5-7 leaf stage or Stage 2 (Fig. 3) before being used for greenhouse experiments. Sorghum plants in the 5-7 leaf stage were selected to accommodate the width of the clip cage, meaning they needed plenty of height for growth.

Sugarcane aphid colonies were kept in Bugdorms (Fig. 2) to contain colonies, and prevent unwanted infestation to other greenhouse spaces, and were regularly supplied with sorghum plants. For rearing SCA colonies for use in further experiments, high numbers of sorghum plants were needed to maintain aphid colonies between 2016, when sugarcane aphids

were first collected, up through 2018 when the experiments took place. Both plant-rearing and aphid colony rooms were maintained under similar greenhouse conditions under artificial lights at 16:8 hr. (light: dark) photoperiod and maintained at 22 ± 3°C temperatures throughout the year. Sugarcane aphid voucher specimens (*Melanaphis sacchari* apterous and alate adults) were deposited in the KSU Museum of Entomological and Prairie Arthropod Research Department (voucher # = 263).

Experimental Set-Up

Each run of the greenhouse study was set up in a randomized complete block design and were carried out 13 days post initial aphid infestation. Sorghum pots were placed in 32 mesh SL2.0 exclusion tents (dimensions: 21.59 W x 21.59 L x 96.52 H cm) created from cut pvc pipe pieces and pvc pipe joints, wrapped in sewed mesh sleeves, and had the top sealed with a binder clip (Fig. 4). Each tent had 1 sorghum pot per tent and were randomly assigned different treatments of 0, 2, or 10 apterous, adult sugarcane aphids. As sugarcane aphid feeding needed to be measured near active feeding sites, both for determining a signature spectral response and to analyze local versus systemic sorghum responses, aphid movement was restricted by housed aphids in clip cages. Clip cages (dimensions: 2W x 2L x 2.5H cm) were created with a cut piece of pvc pipe surrounded on the bottom by a ring of foam for padding and with the top covered in mesh for ventilation (Fig. 5). Each cage was secured to the leaf using a metal utility clip with a plastic backing placed between the metal clip and the leaf to decrease chance of tissue damage (Fig. 6). In addition, rods with metal loops were attached to each clip cage to support the weight of the cage. All clip cages were positioned on the abaxial side of the leaf as sugarcane aphids tend to feed on the underside of leaves. In addition, all clip cages for the experiments were

placed in the middle section of the leaf and at the third leaf position from the bottom and position was kept consistent for all replications. The third leaf position, from the bottom, was used as leaves in lower, older leaves senesced during pre-tests within the duration of the experiment.

Using clip cages to house aphids also played into the size of the sorghum plants used in each run of experiment. Clip cages have a 2 cm width which means that sorghum leaves needed to have a width > 2 cm to prevent aphid escape and ensure that all aphids were given equal access to leaf tissue. In pre-tests, sorghum plants that were in the 5-7 leaf stage consistently had leaves wide enough to encompass the clip cages and were less likely to exhibit structural damage when the clip cage was secured to the leaf. Smaller leaves had higher chances of damage due to the mechanical pressures of the clip cage and metal clip used to secure the cage. Therefore, all plants used in each experiment were in the 5-7 leaf stage as leaves were wide enough for the clip cage.

Although using clip cages are important to restricting aphid movement, cage effects on leaves was expected to elicit a physiological response from sorghum for several reasons; added shading, temperature and humidity differences, and the chance that the metal clip used to secure the clip cage could cause minor, mechanical damage to leaf tissues. Therefore, two control groups (i.e. non-infested treatments), were used in the study to account for cage effect on sorghum leaves. The first control (C1) had no clip cage, and no aphids present on the plant to provide a baseline comparison between infested and uninfested plants. The second control (C2) had an empty clip cage on the plant with no sugarcane aphids. C2 was added to measure any stress, and potential leaf spectral response due to the clip cage, which could be distinguished from stress due to sugarcane aphid feeding.

In addition to the two control groups, two aphid infestation treatment groups were tested. To determine the density of sugarcane aphids needed to elicit a leaf spectral response, two different starting densities of aphids, low and high, were used by infesting sorghum leaves. For the low aphid density treatment (T1), only 2 adult, apterous aphids were initially placed in each low-density clip cages. Conversely, high aphid-density cages (T2) started with 10 adult, apterous aphids. Both aphid groups in T1 and T2 aphid populations were allowed to reproduce within respective clip cages throughout the duration of each experiment. Aphid densities were recorded on the same days as spectral readings, which was every other day over 13 days (except for the R2 experiment were several days were missing due to scheduling conflicts), to measure population growth over time. To do so, the clip cage was temporarily removed from the leaf, a picture was taken of the sugarcane aphid population, and then the clip cage was put back in the original position (Fig. 9).

Measuring Leaf Spectral response

Once the experiment was set up in the greenhouse, light reflectance was measured 24 hrs. after initial aphid infestation (Day 1) and continued once a day for 13 days post initial infestation (Day 13). Leaf spectral response on individual sorghum leaves was measured using a hand-held CI-710 Miniature Leaf Spectrometer (SpectraVue Leaf Spectrometer,CID Bio-Science) (Fig. 7 and 8). All spectral readings were taken in the morning starting around 9:00 am for a 2-hour period, and always starting with control tents (C1 and C2) and then the aphid treatment groups (T1 and T2) to prevent accidental infestation of sugarcane aphids to control tents. The spectrometer uses a broadband light source and measures reflectance in the visible and near-

infrared (NIR) wavelength ranges, between 400-1000nm (Fig. 10). This wavelength range is analogous to hyperspectral sensors currently available for remote deployment using small UAS.

For the first run of the experiment (R1), the objective was to detect sugarcane aphids near the feeding site, next to the clip cage (11.1), and determine what population density of aphids are needed for spectral detection. The leaf spectral response of sorghum over time, as aphid population increased, was also analyzed. The second (R2) and third runs (R3) also measured spectral response near the active feeding site and how spectral response may have been affected over time. However, an additional variable was added to these two runs as spectral measurements were also taken at a distal location, on a different uninfested leaf, from aphid infested cages to see if the presence of sugarcane aphids could be detected systemically (Fig. 11.2). Local spectral measurements, labeled A position, were taken near clip cages for the C2, T1, and T2 treatments. Measurements were consistently taken directly next to the clip cages on the side of the leaf near the petiole. A second reading, labeled D position, was taken distal to the feeding site. This was done by taking a reading that was two leaf positions from the clip cage towards the newest leaf. Since cages were placed on the third leaf position, distal readings were taken on the fifth leaf. All treatments, with or without clip cages/aphids, measurements were taken in the same location of the middle and abaxial side of the sorghum leaf for consistency's sake. In addition, control tents (C1 and C2) were always measured before treatment (T1 and T2) tents to reduce the chance of unwanted sugarcane aphids dispersing to control tents. It should be noted that spectral readings were not consistently taken on the same days between the 3 experiments (R1, R2, and R3) due to scheduling conflicts.

Statistical Analysis of Experimental Data

We jointly analyzed all three replicates (i.e., R1, R2, and R3) of the experiment to determine the influence of sugarcane aphid infestation on sorghum leaf reflectance using a machine learning approach known as boosted regression trees (Elith et al. 2008; Ch. 10 in Hastie et al. 2009). This machine learning approach enables prediction of the expected leaf reflectance in the visible wavelength range of 500-799 nm, green to red light (Fig. 10), for sorghum plants at both the feeding site and distal location between 1- and 14-days post infestation under the two treatments (T1 and T2; low and high aphid density) in addition to the two controls (C1 and C2; no aphids without cage and no aphids with cage). All graphical representations of the boosted regression tree results can be found at: https://trevorhefley.shinyapps.io/bm_aphid_1/. A range of only 500-799 nm was used for this study because data output from CI-710 Miniature Leaf Spectrometer has a lot of "noise" that interfered with getting accurate leaf reflectance readings at lower wavelengths, which has been reported in previous studies. There was no way to correct for this to our knowledge.

The output for this analysis includes heatmaps that show predicted expected leaf reflectance. Like any map (e.g., geographic map), there is an x-coordinate and a y-coordinate that determine the "location" of a point. For our analysis, the x-coordinate is the day since aphid infestation and the y-coordinate is wavelength. That is, for a chosen "location" (i.e., a specific day and wavelength) a heatmaps provides a prediction of the expected leaf reflectance. Furthermore, since there are two aphid densities (T1 and T2), two controls (C1 and C2) and two points of measurements (adjacent and distal to feeding site), a total of eight heatmaps were generated.

To infer the treatment effects, we compared the predicted expected reflectance among the eight heatmaps. The difference between the predicted expected reflectance for a chosen

comparison was used as an estimate of the main effect. For example, we compared the predicted expected reflectance for T2 to the predicted expected reflectance of T1 to estimate the effect that aphid density had on leaf reflectance. As another example, we compared the predicted expected reflectance for C2 to C1 to determine the impact of cages on leaf reflectance. Similar to our heatmaps, a one-dimensional visualization of all predictions are reported. The one-dimensional visualization shows the predicted leaf reflectance for any given sample date for a pre-selected wavelength.

Our "predictive approach" to infer the effects sugar aphid infestation on sorghum leaf reflectance departs from traditional approaches used for designed experiments (e.g., hypothesis testing using analysis of variance). There are two important reasons why a predictive approach was selected in addition to at least one important benefit. The first reason why a predictive approach was used is that treatment affects are likely to have complex dependence on the interaction between wavelength and day since day since aphid infestation. While traditional approaches such as analysis of variance do enable the quantification and testing of interaction effects, the major drawback is the need to conduct for every wavelength and day combination for each of the eight combination of treatment, control and location of measurement; this is clearly infeasible because the number of comparisons is too large for a human to interpret. Boosted regression trees are ideally suited for detecting and quantifying high-order interactions like those mentioned above (Elith et al. 2008). The second reason a predictive approach was used is that the data set is rather large (i.e., >450K responses) and with near certainty all treatment, control, and location effects would be "statistically significant" using traditional hypothesis testing, using p-values for example (Lin et al. 2013). As a results, the magnitude of the effect (e.g., what is the impact on leaf reflectance of having a high vs. low aphid density on day 12 at a wavelength of

603) would still need to be quantified. Thus, this innovative approach circumvents the need to find “statistical significance”, which almost surely can be found in a large data set, by using modern, predictive approaches that are well-equipped and designed to quantify such effects. Finally, there is at least one benefit to a predictive approach. In the next paragraph, we explain how we validate our predictions. Because of this validation step, we were able to produce heatmaps (and other predictions) that quantified the reflectance for a given day and wavelength that have an assessed level of accuracy. Like other types of maps (e.g., geographic), it is important to quantify and communicate their accuracy. Finally, and regardless if the method of analysis is traditional (e.g., analysis of variance) or modern, it is important to validate the analysis, which typically requires either a second data set or a large data set that can be split into two with one portion reserved for validation.

As mentioned above, boosted regression trees were used to analyze data from all three replicates (i.e., R1, R2, and R3) of our experiment. Boosted regression trees are a machine learning approach that are known to have high predictive accuracy and are capable of detecting and quantifying high-order interactions. We chose to use boosted regression trees because we desired that our predicted heatmaps be as accurate as possible and because we anticipated complex interactions among the predictor variables day, wavelength, treatment, control and location. We split our data into a training and validation set by randomly allocating 50% of the observations to each set. This resulted in 276,151 observations in the training data set and 276,151 observations in the test data set. We then fit multiple boosted regression tree to the training data set using different combinations of the predictor variables (Table 1). We fit our boosted regression trees using the gbm package in program R using a Laplace distribution (i.e.,

absolute loss function), a total of 100 trees, an interaction depth of 30, a bag fraction of 1.0 and a shrinkage rate of 0.1.

To quantify the predictive accuracy of each boosted regression tree, we predicted the expected leaf reflectance for all observations, all control and treatment groups, in the validation data set. We then calculated the absolute difference between the recorded leaf reflectance and the predicted reflectance. We then took the average of this difference (i.e., the average predictive error) across all 276,151 observations in the validation data set. When evaluating predictive performance, it is important to have "dummy" metrics for comparison (i.e., a method of prediction that is simple to obtain that has some predictive accuracy). As such, we used the average leaf reflectance obtained from the training data set, which was 0.1769. Using this value of 0.1769 as a predictor for all 276,151 leaf reflectance measurements in the validation data set results in a predictive error of 0.1362. That is, the average absolute difference between the recorded value of leaf reflectance and the predicted (using 0.1769 for all predictions) was 0.1362.

(3.) Results

Gradient Boosted Regression Trees

The gradient boosted regression tree curves depict the predicted changes in leaf reflectance across a 500-799 nm wavelength range (Fig. 12). Eight maps, or panels, depict 8 different experimental groups (A-H) that were generated for each wavelength within the tested wavelength range. The top panels of each map (A-D) show the close aphid treatments of T2-C and T1-C (high and low aphid densities, -respectively) and control groups C1-C and C2-C (no cage-no aphid and cage-no aphid, respectively). The bottom panels (E-H) show the distal aphid

treatments of T2-D and T1-D (high and low aphid densities respectively) and control groups C1-D and C2-D (no cage-no aphid and cage-no aphid, respectively).

Since the analysis generated a regression tree for every tested wavelength, three example wavelengths of 550, 650, and 750 nm (Fig. 12.1, 12.2, and 12.3 respectively) were chosen to represent a cross-section of the data. The changes in leaf reflectance between each day and wavelength are visibly complex as seen in the uneven and convoluted curves within each panel. This shows the intricate interactions between day and wavelength and indicates that the predicted reflection is very accurate. When comparing the 550, 650, and 750 nm regression trees, several notable differences can be detected. The curve from Days 2-10 shows a dip around Day 4 within every experimental group except the C1 (no cage-no aphid) controls. The C1 curve, in both close and far treatments, show a relatively consistent reflectance across all 3 representative wavelengths. In addition, the level of reflectance in the 550 and 650 nm (Fig. 12.1 and 12.2 respectively) are comparatively lower than the 750 nm (Fig. 12.3), which is consistent with the spectral reflectance curve for plants (Fig. 10). All three wavelengths also had an unexpected curve pattern that was seen across all the panels. At Day 10 there is a relatively large increase in reflectance followed by an equally large decrease in reflectance at Day 12. Interestingly, this Day 10-12 pattern is the most pronounced at 550 nm, followed by 650nm, and then 750 nm where the pattern is less noticeable.

The gradient boosted regression trees (Fig.12.1, 12.2, and 12.3) show not only the complexity of the predicted reflectance in relation to day and wavelength but also similar sorghum response patterns between proximity treatments (i.e., close or distal to feeding site). All wavelengths were plotted, but only select ranges were included to show differences in response through time; all graphical representations of the results can be found at:

https://trevorhefley.shinyapps.io/bm_aphid_1/. In each example wavelength of 550, 650, and 750 nm (Fig. 12.1, 12.2, and 12,3 respectively), the close (-C) and distal (-D) locations from aphid infestation sites displayed analogous reflection curves. For example, in the 550 nm wavelength example (Fig. 12.1) the curve pattern during Days 2-10 were seen in both close (A-D) and distal panels (E-H). Both proximity treatments showed a unique pattern of having a flatter curve for C1 panels compared to C2, T1, and T2. In addition, the dramatic dip in reflectance around Day 10-12 was also witnessed in both close and distal within each example wavelength (Fig. 12.1, 12.2, and 12.3). Considering the complexity of the reflection curves, it is extraordinary that these patterns are consistently apparent between both close and distal groups within each regression tree example.

Predicted Reflectance Heatmaps

Another way of depicting the gradient boosted regression trees is to create a heatmap that shows day, wavelength, and reflectance (Fig. 13). This map shows predicted reflectance given a specific day and wavelength. That means that knowing how long sorghum has been infested with sugarcane aphids allows one to estimate the leaf reflectance at a given wavelength. Fairly minor effects can be detected since this map was created using a very large data set. The heatmaps (Fig. 13) also show a consistency between close (-C) and distal (-D) patterns, with C1 showing a lower predicted reflection around Day 2-10 as compared to C2, T1, and T2 panels, but the intricacy of the heatmaps makes it difficult to visually discern additional similarities between proximity treatments.

Predicted Scores

Both the gradient boosted regression tree and heatmap show the complex relationship between day and wavelength in predicting reflectance. However, these are predictive maps, which introduces the question of how accurate they are in predicting leaf reflectance. As described in the Materials and Methods section, predictive scores were calculated to determine accuracy (Table 1). Essentially, this table shows how accurate we can get in estimating leaf reflectance based on how many variables are known. If we take the average reflectance from all three experiments (R1, R2, and R3), you get a mean reflectance of 0.1769. From that value, we calculated a predictive error of 0.1362. In other words, if no predictive variables, such as day, wavelengths, treatment, or proximity of the leaf spectrometer to aphid feeding, are known then our prediction of reflection will be off by 0.1362.

Using the validation data set to assess predictive accuracy, we found that a boosted regression tree that uses only day and wavelength as predictor variables can predict leaf reflectance with an average error of 0.0222. By comparison if no predictor variables are used to obtain predictions (i.e., we use the value 0.1769 to predict leaf reflection regardless of the day or wavelength), we get an average error of 0.1362. This indicates that knowing the day and wavelength enables a massive improvement in our ability to predict leaf reflection; of course, this result was expected, by comparison, when we add the treatment (T1 and T2) and control (C1 and C2) as predictor variables in addition to day and wavelength, we obtain an average error of 0.0207. While the predictor variables treatment and control did not result in a major improvement in the average predictive error, we do note that this is roughly a 7% improvement in predictive accuracy compared to when the treatment and control predictor variables are not included (i.e., (0.0222 - 0.0207)/0.0207 = 0.0725). The addition of location (i.e., feeding site or distal) as a predictor variable resulted in similar but slightly smaller increases in predictive

accuracy (Table 1). Finally, the most accurate approach was the boosted regression tree that contained all predictor variables (i.e., day, wavelength, treatment, control, and location), which had a predictive error of 0.0202. These predictive scores show both the value of knowing additional variables in increasing estimation accuracy and that the system is highly predictable. As our reflection range is between 0.0 – 0.5, having an average error of only 0.0202 is very low by comparison.

Predicted Difference in Reflectance

Since the regression trees were shown to have high predictability, with only a predictive error of 0.0202 when all variables are known, we can now show that they are very accurate in determining leaf reflection. However, this high accuracy includes having a clip cage in 3 out of 4 experiment groups (C2, T1, and T2). One of the questions surrounding using clip cages to house aphids is the effect the cage might have on leaf reflectance. To tackle the potential imposition of a cage effect, we removed the experimental group C2 (cage-no aphid) from all other groups (C1, T1, and T2) (Fig. 14). The results showed a stark difference between the C1 control (no cage-no aphid) and the two aphid densities treatment groups (T1 and T2). Surprisingly, we found that in the presence of sugarcane aphids (T1 and T2), the change in reflection was visibly lower than in non-infested plants (C1). In other words, in the absence of a clip cage, uninfested sorghum had an increase change in reflectance while the infested plants displayed a comparatively decreased reflectance change. These reflection trends were seen across both the close and distal treatment groups.

Another interesting discovery was the difference between low and high aphid densities (T1 and T2). There is also a clear distinction as the lower aphid group (T1) had less reflection

change than the higher aphid group (T2). There was a 0.2 predicted change in reflectance between low and high maps as seen in the darker blue colors in the high aphid versus low aphid. In addition, the largest difference between high and low aphid maps was between 500-650 nm range which is within the visible spectrum, specifically between green to red light wavelengths (Fig. 14 and 10). This means that the sugarcane spectral signature is between 550-650 nm. Overall, the maps in Figure 4 show a clear relationship between infested and uninfested plants and between low and high aphid densities.

(4.) Discussion

This study provided additional insight about sugarcane aphid (SCA) and sorghum interactions and critical foundational information towards sensor-based UAS monitoring systems. Results in this study demonstrate a discernable spectral response in the visible range, 500-650 nm, between infested and uninfested sorghum with further distinction between low and high sugarcane aphid densities. Additionally, these effects were statistically different for both proximity treatments, close and distal to the sugarcane aphid active feeding site, showing a detectable local and systemic sorghum response. Our model system showed high predictability when identifying plants infested with aphids, with an average error of only 0.02 (Table 1); this level of confidence allows for highly accurate predictions of reflectance response to sugarcane aphids is accurate (Fig 12, 13, 14). When more predictor variables are known, including period of sugarcane aphid infestation (i.e., days post initial infestation), wavelength, aphid density, and proximity of sensor reading to active aphid feeding sites, then our accuracy in predicting spectral response for any plant in the system increases. Prediction of sugarcane infestations over the 14-

day experimental period allows for accurate predictions for every individual wavelength analyzed, which was 500-799 nm range, on any given sample date.

Our predictive analysis approach provides accurate predictions of sorghum infested with aphids near the active feeding site. There was a clear distinction between sorghum infested or uninfested with sugarcane aphid (Fig. 14). The most notable changes in reflectance values were observed in the visible green-red region of the electromagnetic spectrum or between 500-650 nm. This distinct wavelength range is where the most change in light reflectance occured on sorghum leaves where sugarcane aphids were present. However, it is not known whether such a response in sorghum is specific to the aphid species feeding on it. Future experiments need to explore specific-specific responses.

Specific wavelength range changes in plants can provide insight about how stress, like aphid feeding, effects internal, physiological processes, which can lead to altered leaf structures and changes to how light is absorbed or reflected (Knipling 1970; Carter and Knapp 2001; Pinter et al. 2003; Prabhakar et al. 2012). Spectral changes in the visible light range, 350-700 nm, is determined by changes in chlorophyll as blue and red light, about 400-500 nm and 600-700 nm respectively, is absorbed and green light, about 500-600 nm, is reflected (Gates et al. 1965; Knipling 1970; Jensen 2000; Campbell et al. 2008; Taiz and Zeiger 2010; Prabhakar et al. 2012). Therefore, most changes in reflectance in the visible range is likely due to changes in leaf chlorophyll content (Knipling1970; Jensen 2000). This correlation between changes in visible light and chlorophyll content corresponds with other studies, which show sugarcane aphids causing decreased levels of chlorophyll, thus negatively impacting photosynthesis on susceptible sorghum varieties (Paudyal et al. 2020). Continued chlorophyll degradation due to aphid feeding typically results in external leaf color changes such as chlorosis (Merzlyak et al 1999.; Jensen

2000). This means that sugarcane aphid feeding causing changes in visible reflection will have a negative impact on chlorophyll and photosynthesis, thus causing leaves to yellow or reflect less green light.

Interestingly, sugarcane aphid infestations can cause visible leaf discoloration but only at high population levels (GC personal observation, Brewer et al. 2016). This unique phenomenon allows sugarcane aphid infested leaves at low aphid densities to remain greener longer (Brewer et al. 2016) than other sorghum feeding aphids, which cause relatively rapid leaf discoloration (Michaud et al. 2017). Paudyal et al. (2020) tested sugarcane aphid density on susceptible sorghum and found that internal photosynthetic rates were impaired after only 72 hours post infestation when 100 or more sugarcane aphids were present but there were little to no external changes to the infested leaves (Paudyal et al. 2020). It is possible that extended feeding times and higher population levels are required to cause visible leaf damage. We were able to distinguish between high and low sugarcane aphid densities (Fig. 14) but not the exact aphid number needed to elicit a plant response. If higher sugarcane aphid populations are needed to cause external changes to sorghum leaves, this provides additional limitations to current sugarcane aphid monitoring practices as it makes detection of low population densities more difficult to find. In order to reduce economic damage to sorghum, insecticide needs to be applied when sugarcane aphid populations reach 50-125 aphids on 20-30% of plants (McCornack et al. 2017). This further justifies the need for sensors that can detect responses in sorghum to sugarcane aphids before high population build up and cause visible leaf damage (Zhang and Kovacs 2012; Hernández et al. 2021). These findings introduce several questions including: 1) what sugarcane aphid population density is needed to cause visible leaf chlorosis?; 2) what internal changes are sugarcane aphids causing to the leaf that allow it to stay green longer?; and 3) what does a

decrease in visible light due to feeding mean in terms of physiological changes in sorghum leaves?

Although changes in visible light correlate with a known decrease in chlorophyll content, and external leaf chlorosis at high population densities, our results show a decrease in reflectance in the green-red wavelength range due to sugarcane aphid feeding. This is unexpected as a general plant response to stressors causes an increase in visible light and a decrease in NIR (Mirik et al. 2007; Prabhakar et al. 2012). More specifically, plant stress causes an increase in red reflectance, due to decreased chlorophyll content and decreased absorption, and causes the reflectance peak of green light to widen (Adams et al. 1999; Pinter et al. 2003; Yang et al. 2009; Prabhakar et al. 2012). Limited research has been conducted to assess spectral properties in sorghum to date (Singh et al. 2017), but a few have shown similar trends in sorghum due to nitrogen (N) deficiency. Zhao et al. (2005) showed that nitrogen deficiency in sorghum caused an increase in green and red light, specifically around 555 nm and 715 nm, and a red-edge shift (Zhao et al. 2005). Singh et al. (2017) had similar findings in sweet sorghum as "nitrogen-sensitive wavebands" in the green and red region, specifically centered at 595 nm and 701 nm respectively, also increased due to changes in nitrogen (Singh et al. 2017). However, exact plant spectral responses can vary between different aphid-crop systems (Riedell and Blackmer 1999; Yang et al. 2005; Mirik et al. 2007; Elliott et al. 2009). Our findings showed a general decrease in reflectance between green and red wavelengths, 500-650 nm, but we could not discern specific wavelength changes within that range. Additional research is needed to understand why we saw decreased reflectance in the visible range, in both close and distal proximity treatments (Fig 14.), and how that could relate to internal sugarcane aphid effects on sorghum.

Another component of our study looked at the sorghum spectral response at close and distal proximities from the aphid feeding site. Remarkably, similar sorghum responses were seen between close and distal locations (Fig. 12, 13, 14), indicating that sorghum has a local and systemic response to sugarcane aphid feeding. For our close proximity treatment, measurements were taken within a few centimeters of the site of infestation next to the clip cage (Fig. 11), so we did not measure directly over the active feeding site. This indicates that sugarcane aphids can elicit a discernable spectral response in nearby plant tissues. One possible explanation is that aphids release saliva components that condition their host plant on a local level, such as impacting plant cell occlusion, and the impact of feeding can spread several centimeters from stylet penetration (Will and van Bel 2008). This provides a remarkable advantage to detecting leaf spectral response using hand-held spectrometers as aphid populations do not need to be removed from the leaf to take a spectral reading so they can continue to feed undisturbed.

Our model also shows significant differences in spectral responses at sites distant from where aphids fed, which causes a systemic response by the plant that was observable using our light sensor. One possible explanation is that sorghum in this study responded through the induction of various defense pathways. For example, aphid feeding in general elicits the jasmonic and salicylic acid defense pathways that can be upregulated systemically throughout the plant (Moran and Thompson 2001; Will and van Bel 2008). However, an underlying question is whether upregulation of these defense responses cause enough internal changes to sorghum, due to sugarcane aphid infestation, that causes the plant to absorb or reflect light differently than plants without aphids. Yang et al. (2009) observed that Russian wheat aphids and greenbugs feeding on wheat each elicited a distinct spectral response (Yang et al. 2009) and that greenbugs on sorghum upregulated the jasmonic acid and salicylic acid defenses (Zhu-Salzman et al. 2004).

Based on these studies, it is plausible a similar mechanism explains the systemic response observed in this study; however, further investigation is needed to quantify such mechanisms using tissue extractions to quantity various plant constituents. The objective of the current study was to understand whether aphid feeding can elicit a response that is detectable using light reflectance and whether such a response could be observed on uninfested sorghum leaves.

Broader Implications

The detection of both local and systemic sorghum response to sugarcane aphid feeding is a critical find towards our overarching goal of using small UAS remote sensing technologies for more efficient field monitoring of this invasive species. For small UAS equipped with sophisticated sensors to accurately detect sugarcane aphids, data from these machines need to be able to discern the presence of infestation regardless of feeding sites. Since a UAS captures images above the field canopy, measuring spectral changes is likely feasible since aphid feeding appears to be detectable in different parts of the plant. In other words, remotely sensed data from UAS do not capture reflectance of leaves deep in the canopy, which is where most sugarcane aphids are found early in the colonization process (Paudyal et al. 2019). Canopy distribution of sugarcane populations are not uniform as sugarcane aphids tend to feed on the bottom leaves resulting in a higher population density at the bottom of the canopy (Armstrong et al. 2015). Uneven canopy distribution combined with a tendency to feed on the underside of leaves (Singh et al. 2004; Bayoumy et al. 2016; Paudyal et al. 2019), makes sugarcane aphid outbreaks harder to spot. Further complications in monitoring for sugarcane aphids are that high sugarcane aphid populations are needed to cause visible changes to sorghum leaves, meaning that sugarcane

aphid populations have already exceeded economic threshold by the time visible signs of damage appear in sorghum (Bowling et al. 2016; Hernández et al. 2021).

Our research tackles the practical application of using a UAS sensor to detect sugarcane aphids in an uneven canopy distribution. Sorghum was shown to responding locally and systemically to sugarcane aphid feeding (Fig 12, 13, 14) meaning that sugarcane aphid infestations on lower leaves can be detected from UAS readings on upper-canopy leaves.

Traditionally, many remote sensing studies detecting sugarcane aphid populations in sorghum using normalized differenced vegetation index (NDVI) but Lillesand and Kiefer (2000) found that use of NDVI is not always a reliable indicator as it cannot always differentiate between different plant stressors (Lillesand and Kiefer 2000; Backoulou et al. 2018). This further justifies our novel approach to analyzing sorghum's spectral response as gradient boosted regression trees, as seen in the results (Table 1), is a very accurate method of predicting expected leaf reflectance in response to sugarcane aphids. Regression trees also allow us to analyze sorghum's response, change in reflectance, in relation to several variables including different starting densities of sugarcane aphids, length of infestation, and proximity of spectral reading from sugarcane aphid feeding site. Our analysis produced regression trees for every tested wavelength (i.e., 300 trees) within 500-799 nm range. To our knowledge, no current studies have used this machine learning approach in relation to an aphid-crop system or gained this level of detail between the multiple interactions associated with leaf spectral response and aphid infestation.

Limitations of Study

This study provides valuable information about sugarcane aphid feeding on sorghum, which is foundational study to future work involving remotely sensed data captured from autonomous vehicles. Future research should explore the mechanisms by which the plant is responding to infestation. We controlled for water, nutrients, and other potential factors that could have affected plant growth and photosynthesis. Therefore, the models in the current study are only applicable to systems where such factors are controlled, and it is not appropriate to extrapolate these findings to field conditions. We also only tested one variety of sorghum, DKS 29-28, which is considered susceptible to sugarcane aphids. However, there is a wide range of susceptible and resistant sorghum hybrids, with varied levels of antibiosis, antixenosis, and tolerance to sugarcane aphids, that result in different responses to sugarcane aphid feeding (Paudyal et al. 2019). For instance, susceptible sorghum hybrids have been shown to have higher chlorophyll loss and faster rates of photosynthetic capacities decline compared to resistant hybrids under the same conditions (Paudyal et al. 2020).

In addition to testing environment and plant variety, this study analyzed a limited wavelength range (500-799 nm), but the CI-710 Miniature Leaf Spectrometer has a range of 400-1000 nm. Since our data set was very large, we limited the wavelength range to regions of the spectrum where other studies have seen the highest sensitivity to stressors. Plant spectral responses to stress can vary but many plants show high sensitivity to stress between 535-640 nm and 685-700 nm (Jensen 2000). For discernment of chlorophyll content, in relation to light reflection patterns, other studies found 530-630 nm (green-red light) and at the red-edge, around 700-750 nm (Curran 1983; Horler et al. 1983; Cibula and Carter 1992; Datt 1999; Carter and Knapp 2001; Sims and Gamon 2002; Blackburn 2007). Lower wavelength ranges such as blue light, 400-500 nm, have overlapping absorption ranges between chlorophyll and carotenoid and

are not recommended (Alsina et al. 2016). The blue range was also excluded due to technical issues with the CI-710 Miniature Leaf Spectrometer where we saw a lot of "noise" in the spectrometer output. Based on these studies, we limited our wavelength range to 500-799 nm to provide more confidence in the accuracy of the reflection readings and inclusion of the more spectrally sensitive wavelengths for detecting stress.

Future Studies

In this study we saw that sugarcane aphids can be detected in sorghum by measuring leaf reflectance, both locally and systemically, and that changes in spectral responses are mostly observed between 500-650 nm. This allows us to focus our attention to the visible spectrum when using other remote sensing equipment, such as a UAS, to detect sugarcane aphids in sorghum fields. We were able to distinguish spectral differences between uninfested, low-density, and high-density aphid groups (Fig. 14) but these values are based on aphid densities between all three experiments (R1, R2, and R3). The exact population number needed to elicit a detectable spectral response, and whether that number is below the 50-aphid threshold, remains unknown.

Although this is an important foundational study that adds additional insight into the SCA-sorghum system, future research is needed to test the applicability of this study under different circumstances. This experiment was conducted under controlled conditions, with only sugarcane aphid feeding, and testing only one susceptible variety of sorghum. In the field, other environmental stressors, such as drought, nutrient deficiency, or other insect infestations, would be present and detection of sugarcane aphids when sorghum is undergoing multiple stressors is critical. For example, can sugarcane aphid feeding be distinguished from other aphid pests such

as greenbugs or yellow sugarcane aphids? In addition, different sorghum hybrids, such as other susceptible and resistant varieties, are needed to see if the spectral response of infested sorghum in this study is a generalized sorghum response and not hybrid specific. Looking into other factors, such as early or mature sorghum growth stages, are also needed to ensure that our results translate throughout sorghum development.

On a physiological scale, the questions about what internal changes sugarcane aphids cause sorghum, the unique decrease in visible reflectance under stress, would greatly increase our knowledge about how aphids impact plant responses to light. Overall, this is the first study to my knowledge that uses a "predictive approach" using boosted regression trees, to analyze a big data set measuring leaf spectral responses with high accuracy. In addition, this project showed additional information about sugarcane aphid and sorghum relationships and provided new data concerning detecting these insect pests that brings us closer to developing a more efficient monitoring system.

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(6.) Figures and Tables

Figure 2. BugDorm-2400 Insect Rearing Tent (Dimensions: W75 x D75 x H115 cm). Bugdorms have clear panels on the front and back with knitted mesh panels on the right and left sides to allow ventilation. Bugdorm rearing tents were chosen to effectively contain sugarcane aphids and allow adequate height for sorghum plant growth. (Image reproduced from the BugDorm Store: https://shop.bugdorm.com/bugdorm-2400-insect-rearing-tent-p-5.html)

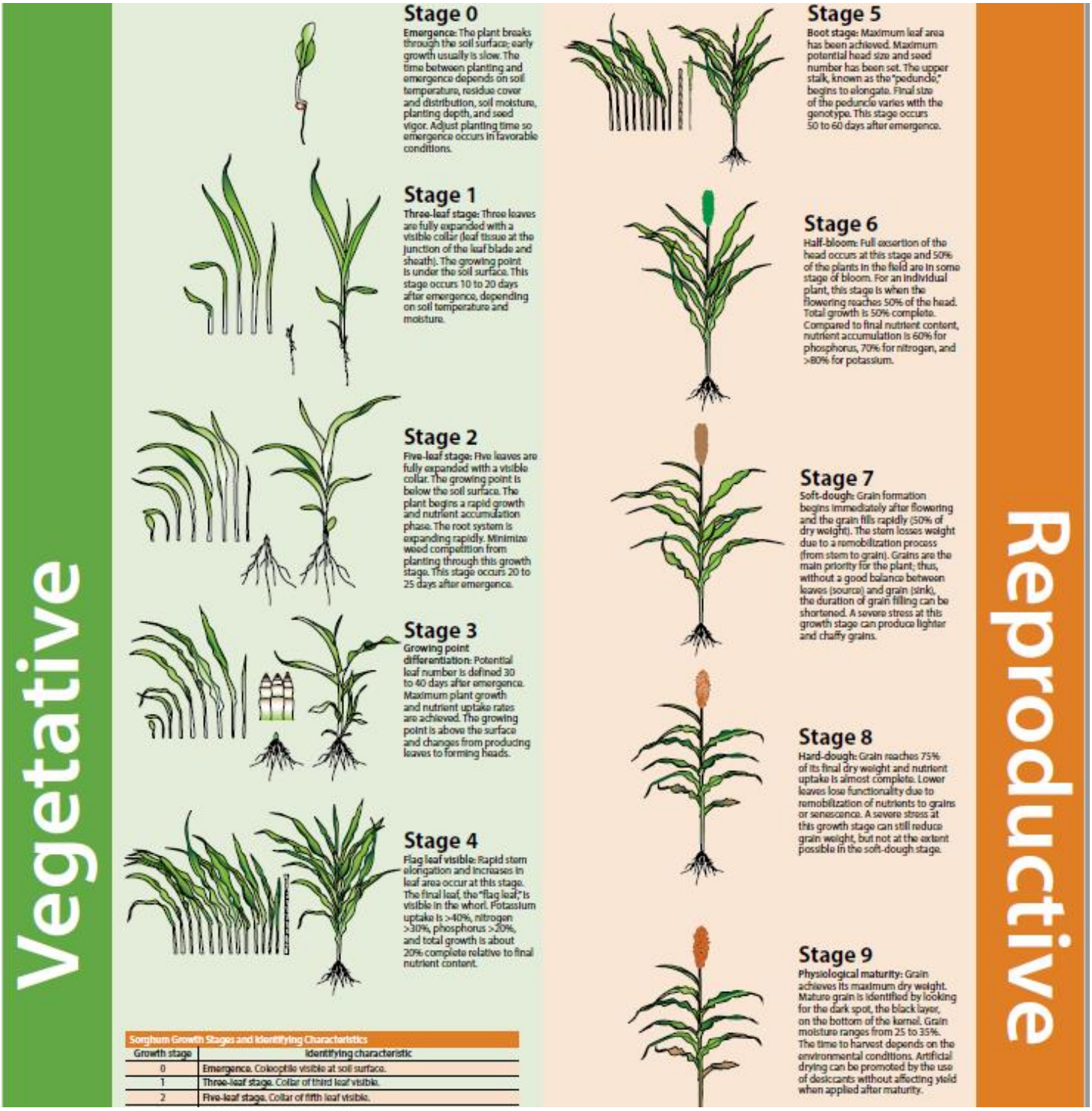


Figure 3. Sorghum Growth and Development (reproduced from Kansas State University, Ignacio Ciampitti)

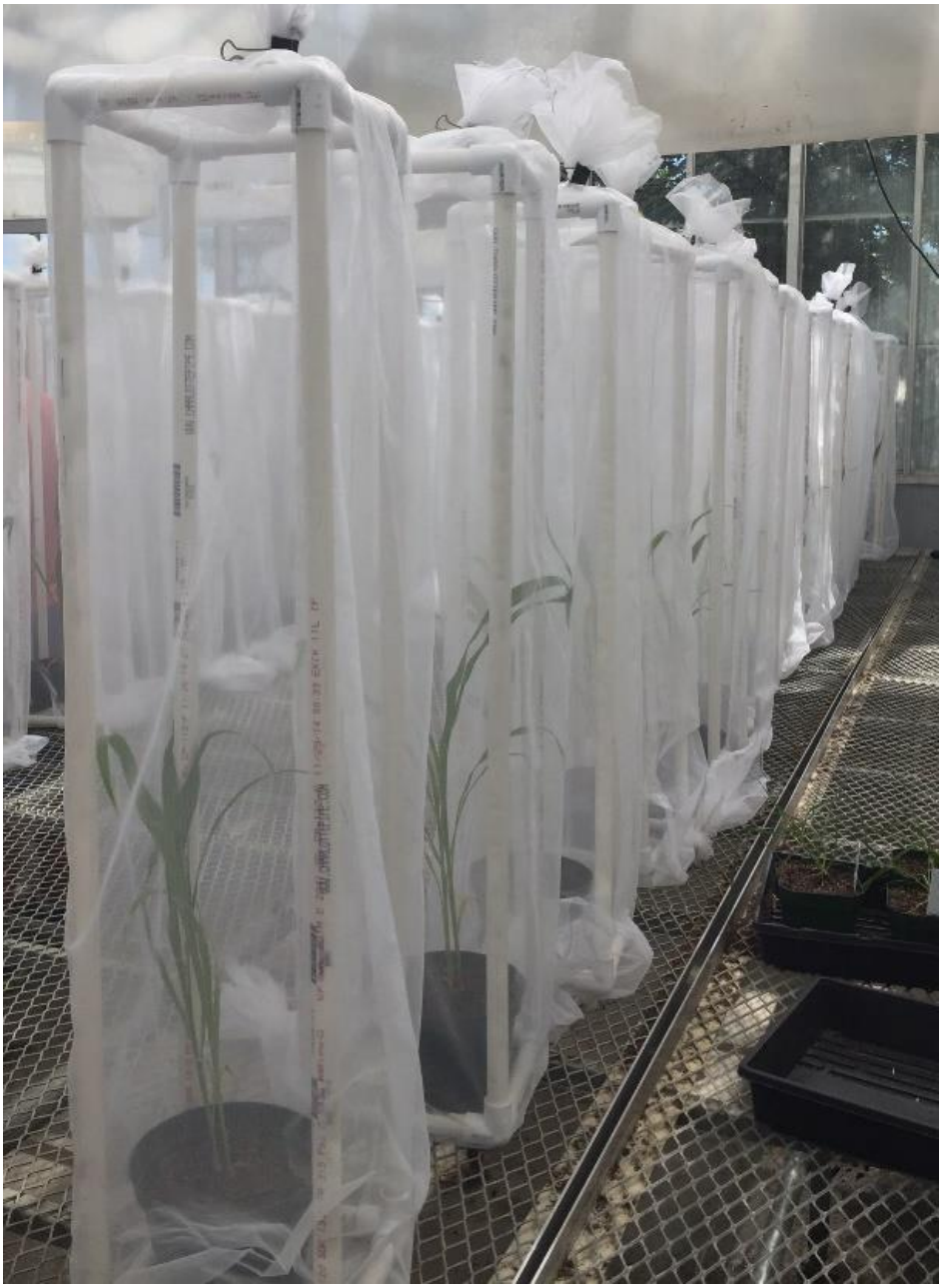


Figure 4. SL2.0 Exclusion Tent. (Dimensions: 21.59 W x 21.59 L x 96.52 H cm). Exclusion tents were created by the McCornack lab at Kansas State University using pvc pipes and pvc pipe joints. They were wrapped in mesh to prevent aphids, or other insects, from entering the exclusion tents. When the plants were not in use, no leaf reflectance readings or watering, the top of the exclusion tent's mesh was sealed with a binder clip.

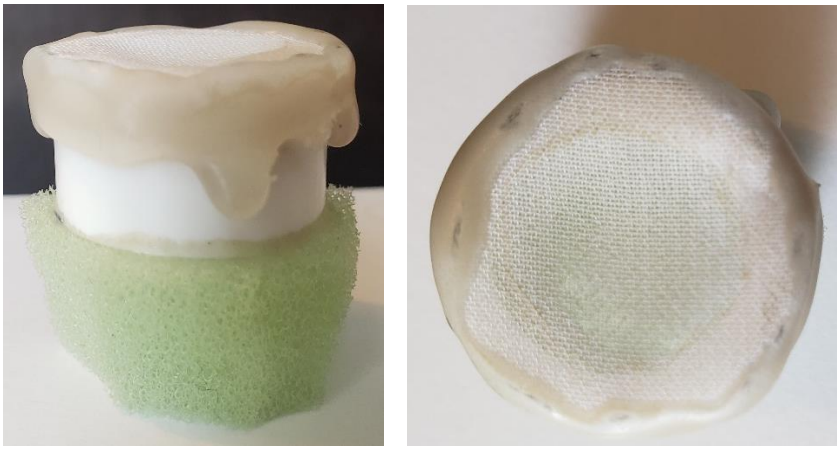


Figure 5. Clip cage used to house sugarcane aphids during experiments (Dimensions: W2 x L2 x H2.5 cm). Clip cages were created by the McCornack lab at Kansas State University. They have a foam bottom for padding that minimizes leaf damage and creates a barrier to contain the sugarcane aphids. The middle is hollow and created with a pvc pipe piece and the top is covered in mesh for ventilation. **5.1** (left) side view of clip cage. **5.2** (right) top view of clip cage.

Figure 6. Clip cage attached to a sorghum leaf. Clip cages were used to house aphids and were secured to the plant using a type of metal hairdressing double prong curl clip. **6.1** (left) front view. The metal clip prongs were bent to conform to the size of the clip cage. **6.2** (right) back view. A piece of plastic was placed under the metal clip prongs so that the clip wouldn't damage the leaf.

Figure 7. CI-710 Miniature Leaf Spectrometer. (image reproduced from CID Bio-Science, Inc.)

Figure 8. Measuring leaf reflection on a sorghum leaf using a CI-710 Miniature Leaf Spectrometer. The leaf spectrometer connects to a tablet so that reflectance data can be stored and transferred.

Figure 9. High sugarcane aphid numbers that were housed in a clip cage. To assess aphid population, the clip cage was temporarily removed from the sorghum leaf and a picture was taken so the number of aphids could be counted later.

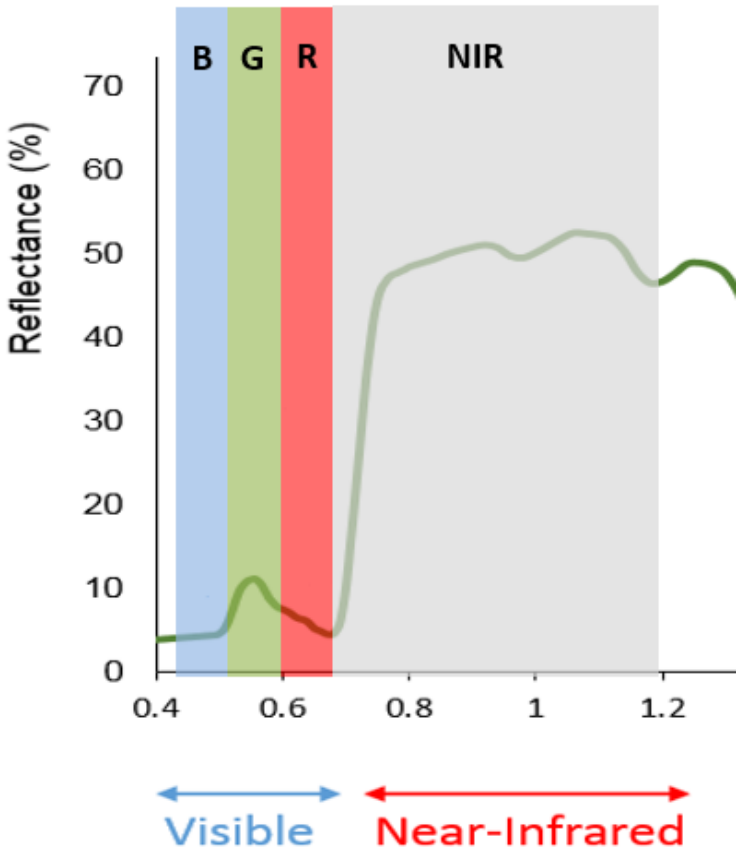


Figure 10. Spectral Reflectance curve for a healthy (unstressed) plant. Shows the electromagnetic spectrum with wavelength ranges from 400- 1200 nm. The Visible range within 400-700 nm (encompasses blue, green, and red light) and near-infrared (NIR) within 700-1,200 nm range. (image reproduced from Humboldt State University)

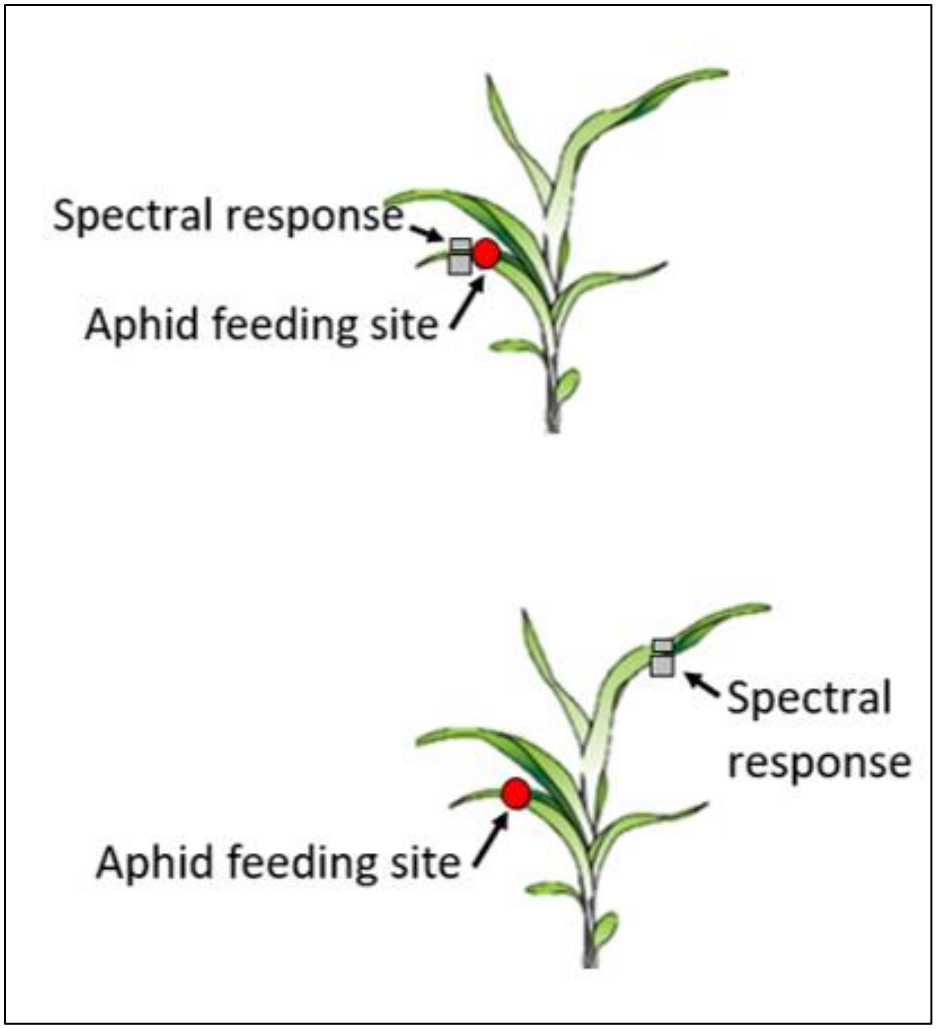


Figure 11. Leaf spectral reflectance measurements are taken in relation to location of active aphid feed (in clip cage). **11.1** (top) Spectral measurements near active feeding site. **11.2** (bottom) Spectral measurements on distal leaf from active feeding site.

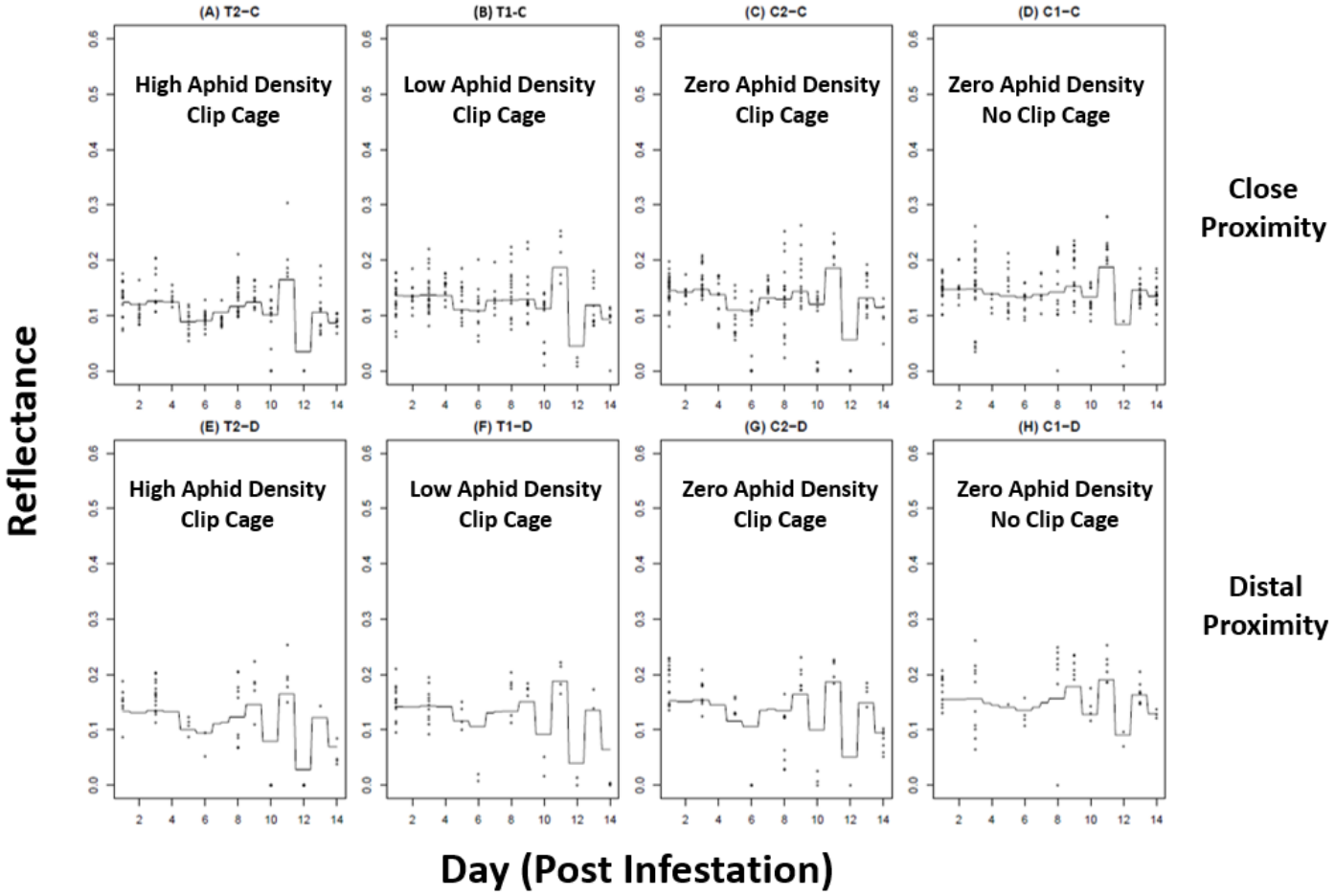


Figure 12.1. Gradient Boosted Regression Tree- 550 nm example. Graphical representation of the change in sorghum leaf reflectance, in the green light range, in response to sugarcane aphid infestation. The top panels of each map (A-D) show the close aphid treatments (close proximity) of T2-C and T1-C (high and low aphid densities, -respectively) and control groups C1-C and C2-C (no cage-no aphid and cage-no aphid, respectively). The bottom panels (E-H) show the distal aphid treatments (distal proximity) of T2-D and T1-D (high and low aphid densities respectively) and control groups C1-D and C2-D (no cage-no aphid and cage-no aphid, respectively). The curves show the complex interactions of the predicted reflectance in relation to day and wavelength. Similarities between the close proximity curves, panels A-D, and distal proximity curves, panels E-H, indicate the sorghum is responding locally and systemically.

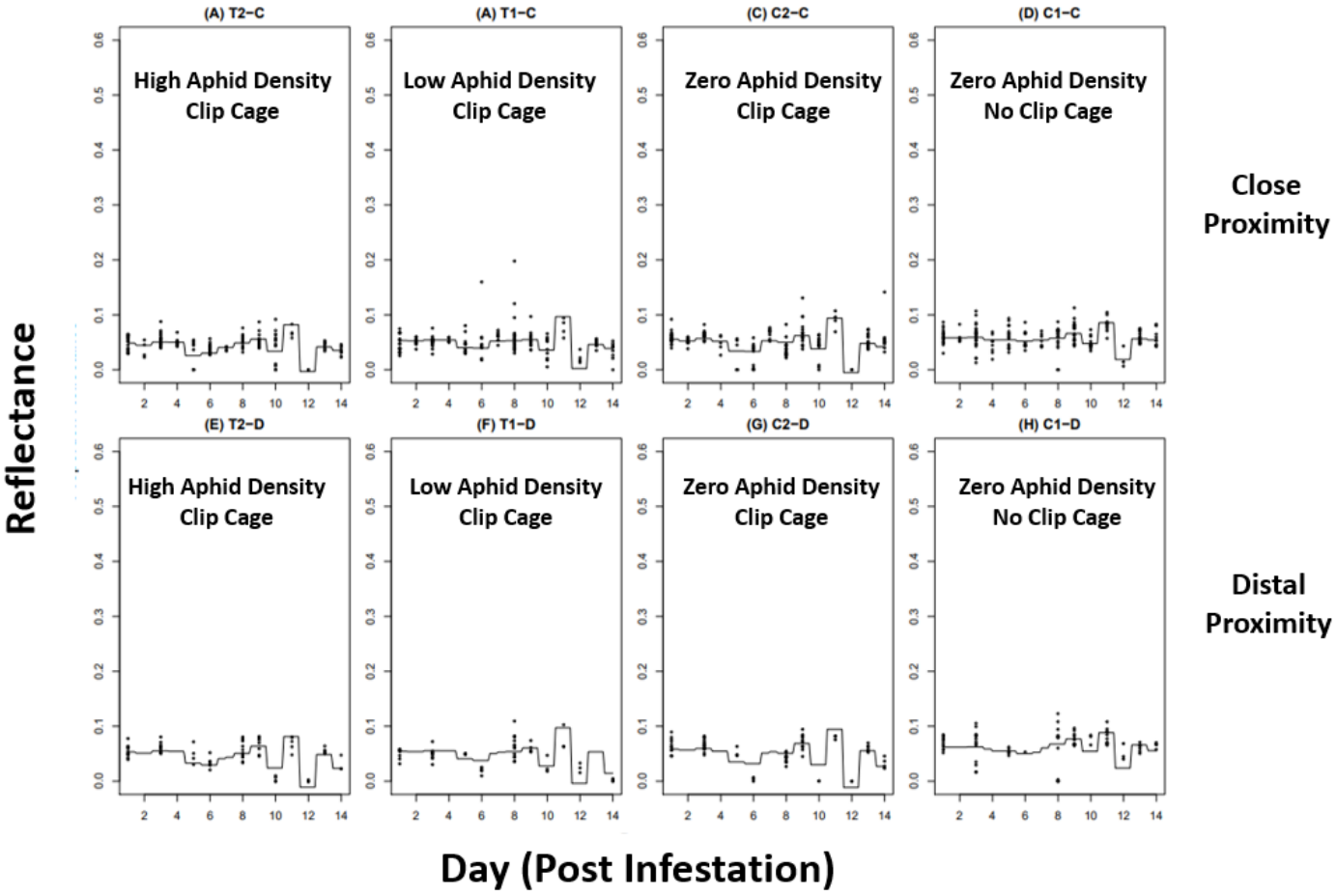


Figure 12.2. Gradient Boosted Regression Tree- 650 nm example. Graphical representation of the change in sorghum leaf reflectance, in the red light range, in response to sugarcane aphid infestation. The top panels of each map (A-D) show the close aphid treatments (close proximity) of T2-C and T1-C (high and low aphid densities, -respectively) and control groups C1-C and C2-C (no cage-no aphid and cage-no aphid, respectively). The bottom panels (E-H) show the distal aphid treatments (distal proximity) of T2-D and T1-D (high and low aphid densities respectively) and control groups C1-D and C2-D (no cage-no aphid and cage-no aphid, respectively). The curves show the complex interactions of the predicted reflectance in relation to day and wavelength. Similarities between the close proximity curves, panels A-D, and distal proximity curves, panels E-H, indicate the sorghum is responding locally and systemically.

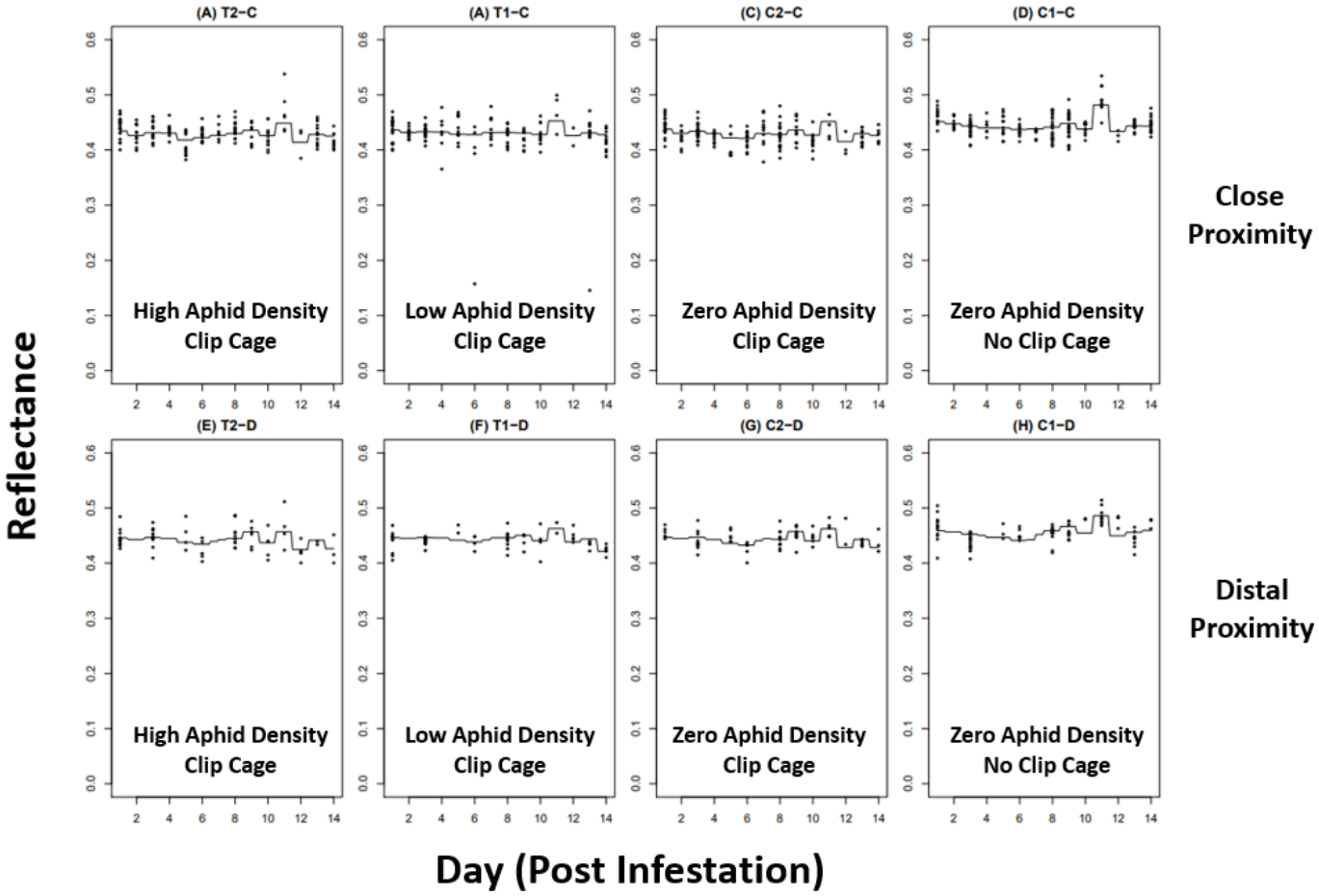


Figure 12.3 Gradient Boosted Regression Tree- 750 nm example. Graphical representation of the change in sorghum leaf reflectance, in the red-edge range, in response to sugarcane aphid infestation. The top panels of each map (A-D) show the close aphid treatments (close proximity) of T2-C and T1-C (high and low aphid densities, -respectively) and control groups C1-C and C2-C (no cage-no aphid and cage-no aphid, respectively). The bottom panels (E-H) show the distal aphid treatments (distal proximity) of T2-D and T1-D (high and low aphid densities respectively) and control groups C1-D and C2-D (no cage-no aphid and cage-no aphid, respectively). The curves show the complex interactions of the predicted reflectance in relation to day and wavelength. Similarities between the close proximity curves, panels A-D, and distal proximity curves, panels E-H, indicate the sorghum is responding locally and systemically.

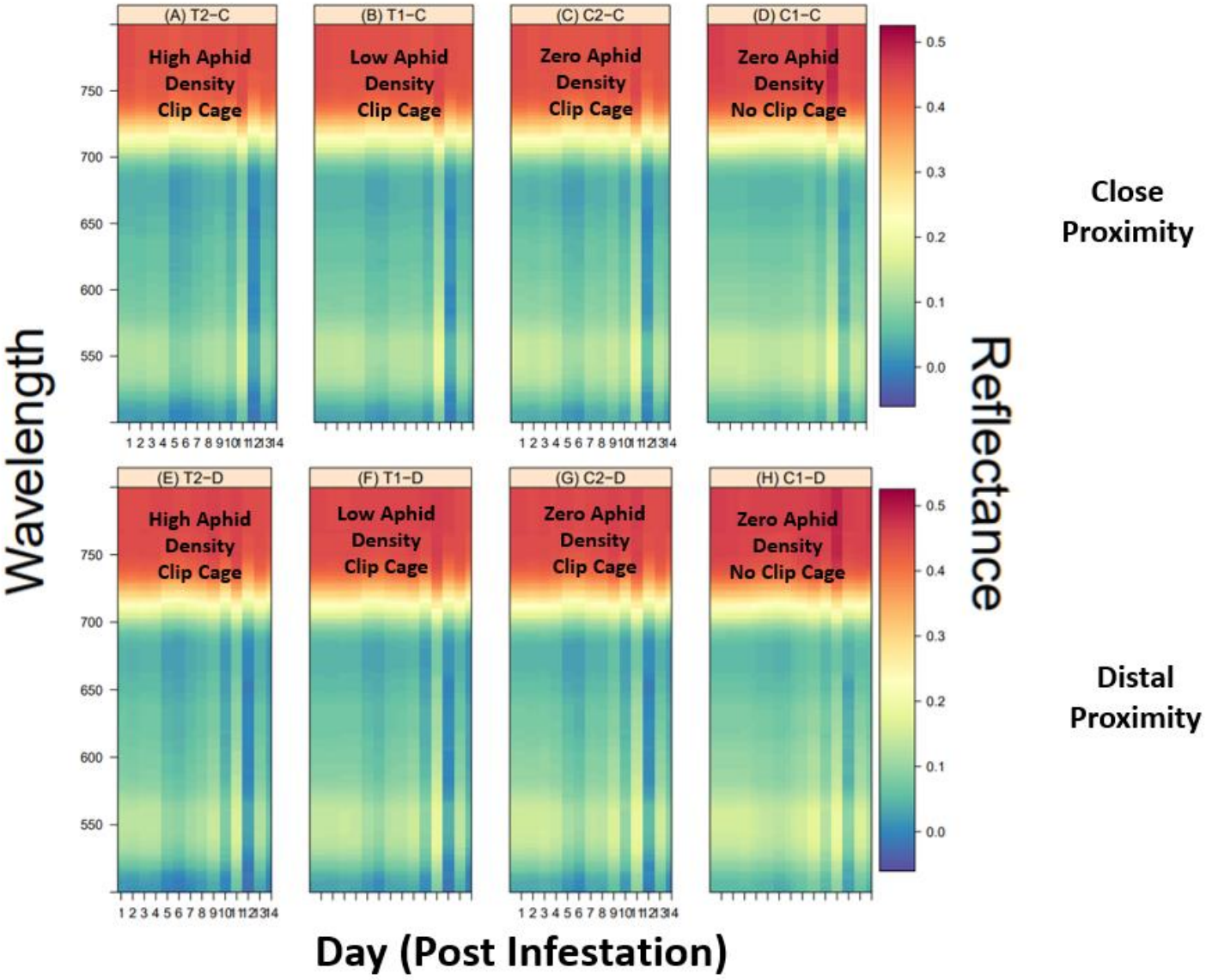


Figure 13. Predicted Reflectance Heatmap. A different graphical representation, from the boosted regression trees, that depicts the predicted reflectance given a specific day and wavelength. The top panels of each map (A-D) show the close aphid treatments (close proximity) of T2-C and T1-C (high and low aphid densities, -respectively) and control groups C1-C and C2-C (no cage-no aphid and cage-no aphid, respectively). The bottom panels (E-H) show the distal aphid treatments (distal proximity) of T2-D and T1-D (high and low aphid densities respectively) and control groups C1-D and C2-D (no cage-no aphid and cage-no aphid, respectively). The curves show the complex interactions of the predicted reflectance in relation to day and wavelength. Similarities between the close proximity curves, panels A-D, and distal proximity curves, panels E-H, indicate the sorghum is responding locally and systemically.

Predictor.variables	Predictive.accuracy
none	0.136238
Day + wl	0.022152
Day + wl + TRT	0.020739
Day + wl + Prox	0.021395
Day + wl + TRT + Prox	0.020168
Day + wl + TRT + Prox + pid	0.014966

Table 1. Predicted Scores. This table shows the additive effect of knowing the predictive variables day post infestation (Day), wavelength (wl), treatment group (TRT), proximity to the aphid feeding site (Prox), and plant ID (pid). The more predictor variables that are known, the more accurate our predicted leaf reflectance. If no predictor variables are known then the accuracy of the predicted leaf reflectance will be off by 0.1362 (predictive error) within our reflection range of 0.0-0.5. When the variables Day and wl are known our predictive accuracy will only be off by 0.022. When Day, wl, and TRT are known our predictive accuracy increases about 10% compared to only known Day and wl. If Day, wl, TRT, and Prox are known our predictive accuracy increase by 3-4%. If Day, wl, TRT, Prox and plant ID, which specific sorghum plant is being analyzed, our predictive accuracy increases by 25% compared to only knowing Day, wl, TRT, and Prox variables. Knowing pid in addition to the other forementioned variables shows how accurate out predicted leaf reflectance can get with our estimation of being off by 0.015. This high level of accuracy shows the high predictability of the sugarcane aphid-sorghum system.

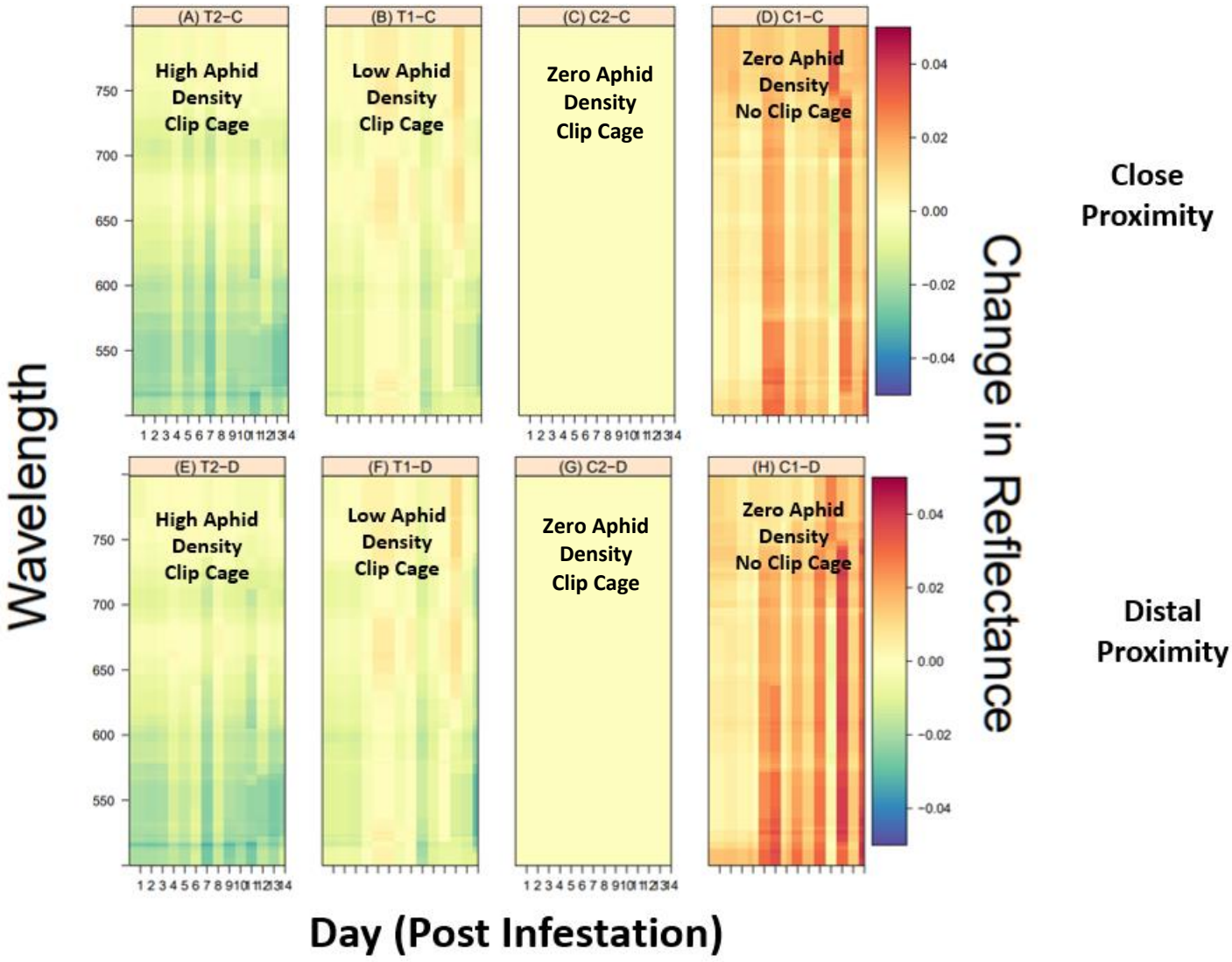
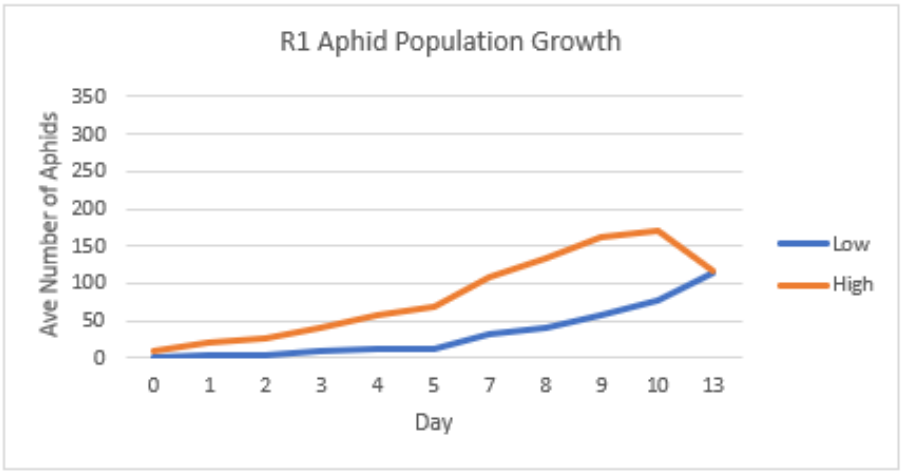
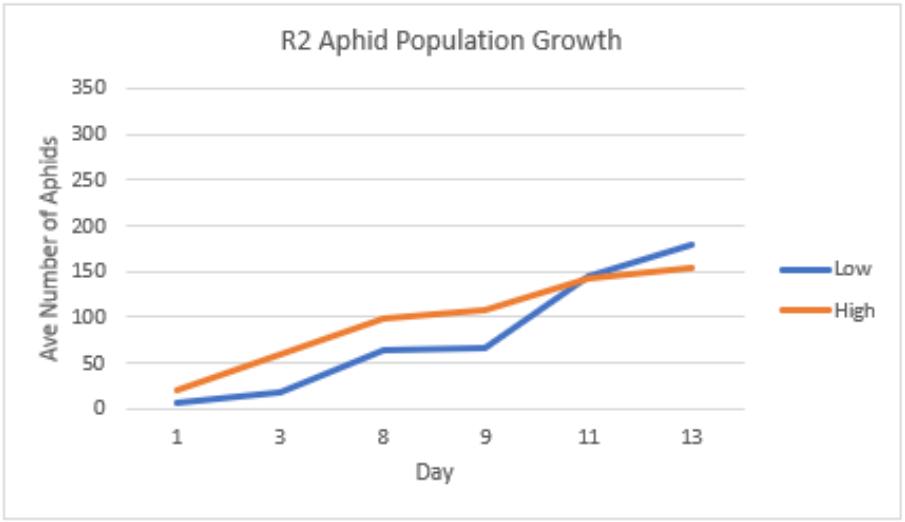


Figure 14. Predicted Difference in Reflectance. Shows the change in leaf reflectance in response to different sugarcane aphid when the clip cage is removed. This was done by subtracting the zero aphid density-clip cage (C2) group from all the other treatment and control groups (C1, T1, and T2) for both the close (panels A-D) and distal (panels E-H) proximity. The resulting figure shows an increase in reflection for the control group (zero aphid density-no clip cage) and a decrease in reflectance for the aphid treatment groups (high and low aphid density) in both close and distal proximity groups. This means that in the presence of sugarcane aphids, there is a decrease in reflection for sorghum, especially between 500-650 nm. The high aphid treatment groups had a distinctly lower reflection, more blue coloration, than the low aphid density groups.





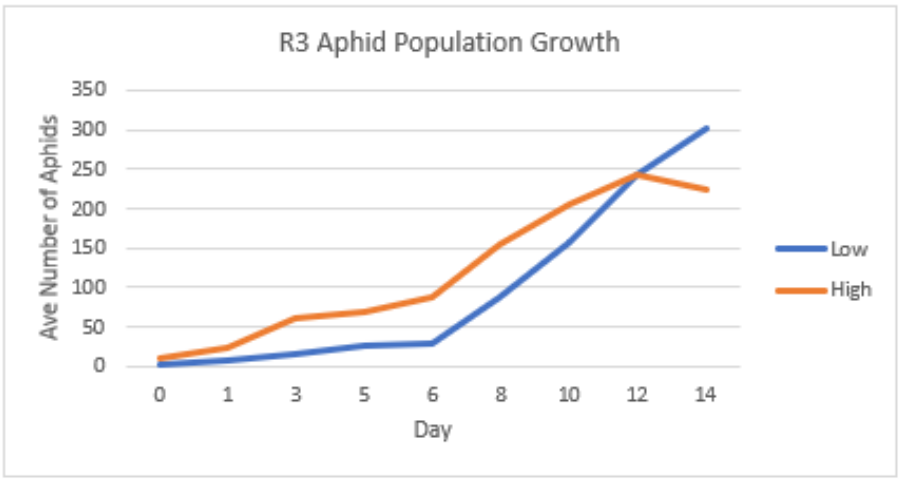


Figure 15. Aphid Population Growth over Time