

Spectral Detection of Soybean Aphid (Hemiptera: Aphididae) and  
Confounding Insecticide Effects in Soybean

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## **Dedication**

To Alexandre Barrigossi, who has been my career counselor, mentor, and good friend since before I applied for a Ph.D. degree.

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## Dissertation Introduction

Soybean, *Glycine max* (L.) Merrill is an important legume crop for human and animal consumption worldwide (Wilson 2008). In the north central United States, where 70% of the US soybean is produced (USDA-NASS 2016), soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) is the primary insect pest since its invasion in 2000 (Ragsdale et al. 2011). Soybean aphid reproduces parthenogenically and has a relatively short developmental time that leads to a rapid occupation and buildup to economically damaging levels of soybean fields (Tilmon et al. 2011). Soybean aphid injury can cause stunted plants, leaf discoloration, plant death and negatively affect pod and seed number, seed weight, and seed oil concentration (Hill et al. 2004, Mensah et al. 2005, Ragsdale et al. 2007, Beckendorf et al. 2008). Soybean aphid can also transmit viral plant diseases (Hill et al. 2001, Wu et al. 2004) and affect the performance of other soybean pests (McCarville et al. 2012). Ultimately, soybean aphid can cause up to 40% yield loss (Ragsdale et al. 2007).

Pyrethroids (e.g.,  $\lambda$ -cyhalothrin) and organophosphates (e.g., chlorpyrifos) are the most common insecticide classes used to control soybean aphids (Johnson et al. 2009). Sampling plans have been developed to estimate soybean aphid densities, prevent economic yield losses, and facilitate pest management by using the economic threshold of 250 aphids per plant (Hodgson et al. 2004, 2007, Onstad et al. 2005, Ragsdale et al. 2007). Existing scouting methods for soybean aphid require traversing soybean fields to count aphids on plants. While sampling plans are essential for the effectiveness of integrated pest management (IPM) programs, growers' perception of time involved in

direct insect counts has contributed to a lower adoption of traditional pest scouting methods (Olson et al. 2008). Innovative scouting methods, based on remote sensing, have the potential to decrease costs and increase, or maintain, efficacy of IPM programs (Hatfield et al. 2008, Mirik, Ansley, Michels Jr., et al. 2012, Reynolds et al. 2012). Detection of soybean aphid using remote sensing could improve adoption of pest scouting methods and threshold-based insecticide applications.

Remote sensing has also shown promising results for mapping weed distributions, assessing plant nutritional requirements, and assessing soil properties (Christy 2008, Felderhof et al. 2008, Reynolds et al. 2012). Industry reports estimates that agriculture will absorb most of the 89 billion USD of investments in the remote sensing market expected for the next decade (AIS 2013, Harrison 2013). Despite the massive investments and interest on remote sensing, the effect of insect injury on plant spectral responses is not entirely characterized and needs more research.

### Remote Sensing

*Man must rise above Earth to the top of the universe and beyond, for only then will he fully understand the world in which he lives (Socrates, 500 B.C.).*

Remote sensing can be defined as a science of recording and processing electromagnetic radiation emanating from target areas at the field of view of the spectral sensor (Fischer et al. 1976, White 1977). The use of remote sensing with entomological purpose is relatively new compared to the history of this science. The first documentation of plant spectral changes used to detect insect damage on annual crops occurred in 1968

(Hart and Myers 1968). When Aristotle (384–322 BC) philosophized about the properties of light, it was hard to imagine that light could also be used hundreds of years later to describe plant characteristics from a distance and distinguish subtle spectral changes caused by insect damage. The aerial and satellite photography as we see today depended on many previous inventions and principles described by well-known scientists such as Isaac Newton (1642–1727), Carl Scheele (1742–1786), and Thomas Young (1773–1829) (Campbell and Wynne 2011a). Remote sensing instruments were initially developed for exclusive military use. Only in the last three decades has remote sensing become accessible to the general public. The declassification of the technology aroused a great diversification of uses by civilians around the world (Field et al. 1995, Sellers et al. 1995, Moran et al. 1997). A more-complete history of remote sensing can be found in Simonett et al. (1983), Riley (1989), and Morain (1998).

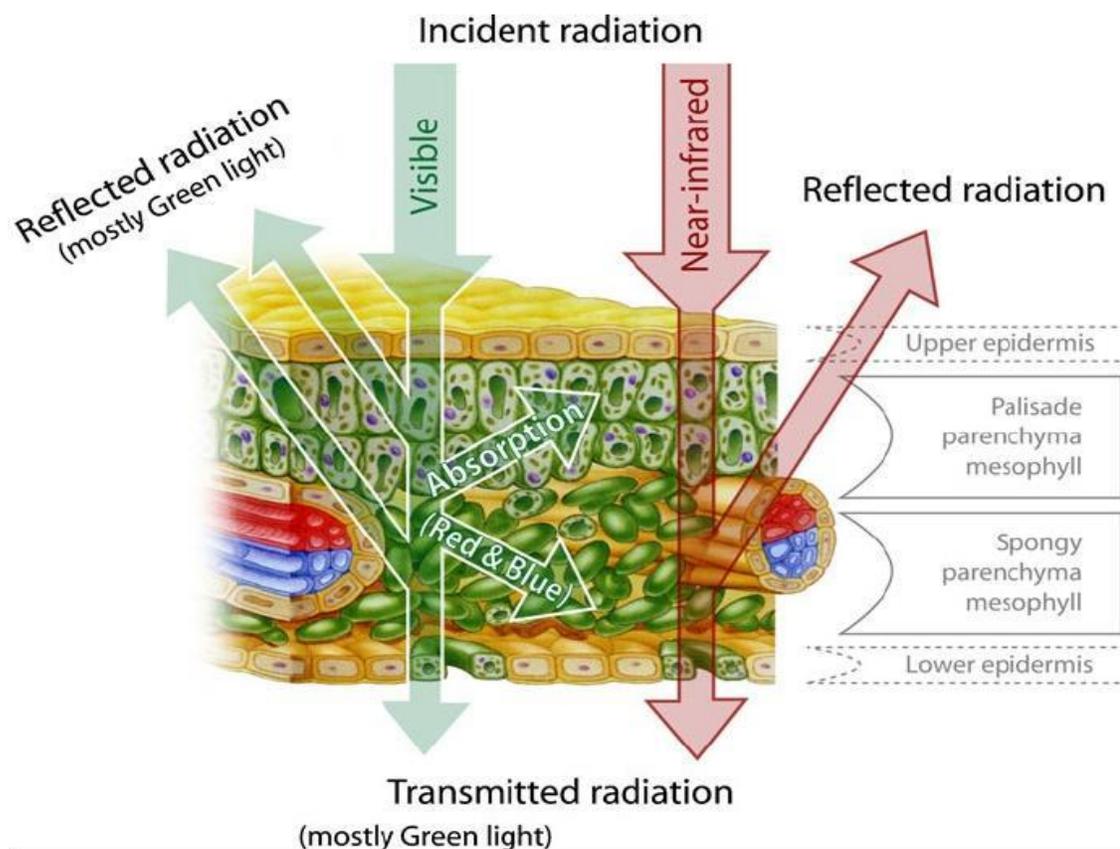
Electromagnetic energy (or light) can be generated by spectral sensors with active beam radiation, but remote sensing with entomological purpose typically relies on sunlight as the source of radiation for data collection (Iqbal 2012). Physicists commonly agree that electromagnetic radiation propagates as a series of waves through space or other media per oscillation. The series of waves are characterized by properties such as wavelength, frequency, amplitude and phase (Parrish et al. 1978, Gibson 2000, Campbell and Wynne 2011b). Wavelength is the distance between two adjacent wave crests and is measured in units of length (e.g., nm and  $\mu\text{m}$ ). Wavelength is inversely proportional to the wave frequency. Frequency is the number of crests passing a fixed point in a given period and is often measured in *hertz*. Amplitude (or spectral irradiance) is a measure of the amount

of energy that is transported by the wave and is often expressed as watts per square meter per micrometer. Phase is the alignment angle between adjacent crests.

For convenience among different disciplines, divisions of the electromagnetic spectrum are arbitrarily defined by wavelength or frequency, even though the full range of electromagnetic radiation does not have real sharp divisions. Ordered by increasing length, the major divisions of the electromagnetic spectrum are gamma-rays, X-rays, ultraviolet, visible, infrared, microwaves, TV waves, and radio waves. The boundaries of these spectral regions do not have fully accepted terminology and sometimes differ in the literature (Barrett 2013). Radiation from ultraviolet ( $\approx 300$  nm) to infrared ( $\approx 2,500$  nm) can be transmitted through the human cornea (Boettner and Wolter 1962, Kramarenko and Tan 2003), but the retina cannot distinguish tones outside the visible spectral range. Visible wavelengths (380–720 nm) comprise a small portion of the electromagnetic spectrum. The interpretation of non-visible wavelengths can only be performed with the aid of remote-sensing instruments.

Incident electromagnetic radiation can be absorbed, reflected and transmitted when interacting with plant surface and leaf ultrastructure (Fig. 1). Gamma rays ( $< 0.3$  nm) and X-rays (0.3–300 nm) are usually transmitted without reflection (Hideg and Vass 1996, Kovács and Keresztes 2002, Wi et al. 2007). Plant pigments (e.g., chlorophyll) absorb most of the incident energy at visible wavelengths. A healthy plant with intact pigments typically absorbs 85-95% of the radiant flux of visible wavelengths. The remaining 5-15% is reflected in the absence of stress. In general, plants do not use low-

energy wavelengths and may reflect around 40-70% of the electromagnetic energy of the near-infrared spectral range.



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**Fig. 1.** Schematic of the interaction between electromagnetic radiation at visible and near-infrared wavelengths and plant leaf. Diagram was reproduced from Verhoeven (2012). Healthy plants mostly absorb electromagnetic radiation of wavelengths at red and blue spectral ranges and reflect most of the near-infrared radiation. Green wavelengths are typically reflected or transmitted through the epidermis.

### Spectral Detection of Insects

The visible and near-infrared spectral ranges contain most potential wavelengths used for detection of plant stress. Wavelengths shorter than 300 nm (e.g., gamma-rays and X-rays) and longer than 3,000 nm (e.g., thermal-infrared) are not typically involved

with morpho-physiological processes affected by insect injury (Knipling 1970). Spectral sensors can be carried by ground-based, airborne, or spaceborne platforms. Ground spectroradiometers may be integrated with leaf clip assembly, calibration spheres, and fiber-optic cables to provide reflectance values of individual leaves or canopy (e.g., Reisig and Godfrey 2007). Ground-based instruments are usually used as a reference for aerial or space-borne spectral data (i.e., ground truthing).

Photograph-like images are the traditional and predominant route to deliver the extracted information from physical objects recorded by a spectral sensor. Digital images are composed of non-divisible picture elements known as *pixels* which consist of reflectance values with spatial and spectral aspects that represent the portrayed scene (Fisher 1997). However, remote sensing systems do not necessarily convert the recorded electromagnetic radiation into a pictorial image. Some instruments used in insect studies only deliver values of reflected or emitted electromagnetic radiation rather than pictures (e.g., Xing et al. 2008, Mirik, Ansley, Michels, et al. 2012). Nevertheless, there is no real difference in the information conveyed by pictures or only reflectance values in the new instruments recording digital image formats (Riley 1989). Whether the data is converted into an image or purely consisted of mean reflectance values, brightness of objects and features is typically represented by a dimensionless number (between 0 and 1) of reflectance values, commonly expressed as a percentage and analyzed by similar analytical methods (e.g., Reisig and Godfrey 2006, Sanches et al. 2014).

Optical instruments used to assess plant spectral response generally works in similar manner. A greatly simplified example of a typical optical device is a

spectroradiometer held above plant canopy to record electromagnetic radiation emanating from the plant surface. The photosensitive material of the spectral sensing system is excited by photons coming from the target scene. The excitation of the electrons on the surface of the sensitive material triggers electric chains that measure radiation from selected regions of the electromagnetic spectrum (Silva 1978). Detailed information about the instruments used for the acquisition of spectral information can be found in Silva(1978) and Campbell and Wynne(2011b).

Improvements on radiometric, spectral, spatial, and temporal resolutions provide an excellent opportunity for using remote sensing to detect insect-induced stress. Spectral resolution refers to the number of bands that a sensor can differentiate. Multispectral sensors usually record less than twenty bands. Hyperspectral sensors record spectral information using narrower spectral bands and provide data at hundreds or thousands of wavelengths. The radiometric resolution can be defined as the number of brightness levels that can be distinguished by a sensor. Finer radiometric resolution has more brightness levels and may provide more information about plant stress. Spatial and temporal resolutions previously limited satellite use in precision agriculture, but newer satellites, such as QuickBird and WorldView, provide finer resolutions for sub-meter characterization of plant stress on an almost daily basis from any point on the Earth's surface. Unmanned Aerial Systems (UAS) platforms can provide even better spatial and temporal resolutions. An UAS flying at 100 m from the Earth's surface may describe single-plant changes in reflectance virtually at any day that weather conditions are adequate.

## Plant Spectral Response to Insect Injury

Insect-induced effects on plant morpho-physiology underlie spectral detection of insect injury because most of the remote sensing instruments are unable to record spectral information of the insects themselves. Biochemical components, leaf surface properties, and internal structures govern how plants reflect, scatter, absorb and emit electromagnetic radiation (Peñuelas et al. 1998). Infested plants may have spectral signatures different than that of insect-free plants. The common insect-induced effects that alter plant spectral responses include changes in cellular content of plant pigments, internal leaf structures, and biomass (Riedell and Blackmer 1999, Stone et al. 2001, Franzen et al. 2007). Plant spectral responses induced by insect herbivory are variable and depend on the type of damage (Meyer 2000). Insect orders can have species in one or more of the seven feeding guilds used to categorize the effects of agricultural insect pests on plant spectral responses (adapted from Denno(1995)):

- i. Inducers of Morpho-Physiological Changes.** This guild comprises of sap feeders, such as aphids, leafhoppers, stinkbugs, and thrips. Phloem and xylem feeders may affect plant pigments (e.g., chlorophylls), morphology (e.g., parenchyma structures), and physiology (e.g., photosynthesis). Spectral responses due to morpho-physiological effects of insect injury typically correspond to increased plant reflectance at visible wavelengths and decreased plant reflectance at near-infrared wavelengths (e.g., Mirik, Ansley, Michels, et al. 2012, Yuan et al. 2014).

- ii. **Honeydew producers.** Examples of honeydew producers include aphids, soft scales, white flies, mealybugs, psyllids, planthoppers, and leafhoppers. These insects can indirectly affect plant reflectance by producing sticky substances that cover external plant tissues. In high densities, many phloem-feeding insects can secrete enough quantities of honeydew to cover plant surface entirely. Because honeydew mostly consists of water, sugars and amino acids (Douglas 1993, Völkl et al. 1999), only plant reflectance at the far-infrared spectral range ( $>2,500$  nm) is expected to be affected (Gausman and Hart 1974). Presence of honeydew on plant surfaces may also contribute to the development of a black fungus, known as sooty mold that may affect photosynthesis rates and plant reflectance at visible and near-infrared wavelengths. Because sooty mold is typically associated with pest densities above economic thresholds, insect detection using plant reflectance should occur preferentially before sooty mold appearance. Thus, honeydew producers are not expected to confound the use of spectral reflectance for detection of insect pests before economic yield loss occurs.
- iii. **Defoliators.** Beetles, caterpillars, grasshoppers, and ants are the most common defoliators attacking plants in agriculture. Leaf area removal caused by these mandibulate foliage feeders can be detected over large areas with few spectral bands and coarse spatial resolution (e.g., Townsend et al. 2012). A simplistic approach used for spectral detection of defoliators consists of quantifying pixels that represent green plant mass (Rullan-Silva et al. 2013). Because plants have very distinct spectral signatures differing from soil background, the detection of leaf area removal

using remote sensing is relatively easier than the detection of stress of other guilds.

Nonetheless, defoliators affecting the physiology of intact tissues may require more complex classification systems to detect changes in photosynthesis, transpiration, and water content (Oleksyn et al. 1998, Aldea et al. 2005, Nabity et al. 2008).

- iv. Gall inducers.** Deformities on leaf mesophyll are caused by gall-inducing species of wasps, scales, mites, midges, aphids, and psyllids. Gall inducers can locally affect photosynthesis efficiency, plant pigment content and, consequently, plant spectral responses (Nutter et al. 2002, Dorchin et al. 2006, Huang et al. 2011). Gall inducers may also affect undamaged tissues that surround deformities (Fay et al. 1993, Larson 1998). Because gall-inducing insects are highly host-specific (Hardy and Cook 2010), plant spectral responses to gall inducers may be much more consistent than that of other guilds. UV, visible, and near-infrared are the main spectral ranges used to detect gall inducers.
- v. Plant miners.** Moths, sawflies, flies, beetles, and wasps are the most important plant miners in agriculture. Feeding of immature stages on succulent tissues of fruits and leaves creates brownish or greenish winding spaces in irregular, serpentine or zigzag patterns (Xu et al. 2007). Feeding damage can greatly increase the transmittance of visible wavelengths on the feeding windows, but typically do not affect intact tissues (Pincebourde and Casas 2006). Less absorption of UV radiation is also observed on the feeding windows (Connor and Taverner 1997).
- vi. Plant borers.** Beetles, sawflies, and moths are the most important plant borers in agriculture. The larva is typically the stage that excavates tunnels into stems, kernels,

barks, buds, and trunks. Changes in protein content of infested kernels can be detected using near-infrared wavelengths between 1,000–1,350 and 1,500–1,680 nm (Dowell et al. 1998). Plant reflectance at red and near-infrared wavelengths is associated with effects of trunk or stalk borers on chlorophyll, leaf area, canopy water content, leaf gas exchange, and whole-plant transpiration (Campbell et al. 2004, Pontius et al. 2008, Marino et al. 2014).

- vii. **Root feeders.** Aphids, thrips, larvae of beetles, moths, and flies are the most important root feeders in agriculture. The use of remote sensing to detect stress of root feeders seems to be the least explored among the seven insect feeding guilds. X-ray computed tomography holds promise to understand the role of root feeders in managed and natural systems (Asseng et al. 2000, Johnson et al. 2007, Taina et al. 2008), but a three-way trade-off between sample size, scanning time, and permissible energy dosage has limited the use of X-rays for detection of root feeders (Mankin et al. 2008). X-rays can be used to determine changes in root architecture, root length and root diameter (Kaestner et al. 2006, Hargreaves et al. 2009, Tracy et al. 2010). Near-infrared spectroscopy of root samples can also distinguish between living and dead roots (Picon-Cochard et al. 2009). Leaf-level plant reflectance can characterize under-ground insect infestations when root feeders affect leaf pigments or plant stand (Everitt et al. 2003, Blanchfield et al. 2006, Powell et al. 2006).

This research explored the use of remote sensing of soybean reflectance for detection of soybean aphids and determined the effects of foliar insecticides on

subsequent use of soybean spectral reflectance. Chapter 1 aimed to determine the effects of soybean aphid on soybean spectral reflectance at leaf- and canopy-level at two narrowband wavelengths and a commonly used vegetation index. Chapter 2 aimed to use hyperspectral data and simulated wide-band sensors for detection of soybean aphid. Chapter 3 aimed to determine the effects of foliar insecticides on spectral response of soybean plants under greenhouse and field conditions. The information provided in this research may help to design optimized sensors for soybean aphid detection and contribute to the understanding of insect- and insecticide-induced effects on plants. Results may also improve the current field-wide management tactics by making decisions for pest control when plant spectral reflectance indicates that soybean aphid abundance reached its economic threshold.

## **1. Chapter I. Soybean Aphid (Hemiptera: Aphididae) Affects Soybean Spectral Reflectance**

### **1.1. Summary**

Our objective was to determine whether plant spectral reflectance is affected by soybean aphid feeding. Field trials were conducted in 2013 and 2014 using caged plots. Early-, late- and non-infested treatments were established to create a gradient of soybean aphid pressure. Whole-plant soybean aphid densities were recorded weekly. Measurements of plant spectral reflectance occurred on two sample dates per year. Simple linear regression models were used to test the effect of cumulative aphid-days (CAD) on plant spectral reflectance at 680 nm (RED) and 800 nm (NIR), normalized difference vegetation index (NDVI), and relative chlorophyll content. Data indicated that CAD had no effect on canopy-level RED reflectance, but CAD decreased canopy-level NIR reflectance and NDVI. Canopy- and leaf-level measurements typically indicated similar plant spectral response to increasing CAD. CAD generally had no effect on relative chlorophyll content. The present study provides the first documentation that remote sensing holds potential for detecting changes in plant spectral reflectance induced by soybean aphid. The use of plant spectral reflectance in soybean aphid management may assist future IPM programs to reduce sampling costs and prevent prophylactic insecticide sprays.

### **1.2. Introduction**

Soybean, *Glycine max* (L.) Merrill, is one of the most important legume crops for the global demand for protein and vegetable oil (Hartman et al. 2011). Approximately

30% of the annual worldwide soybean production is harvested in the north central United States (USDA 2012). The invasive soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Sternorrhyncha: Aphididae), is the most economically important insect pest of soybean throughout the north central United States (Ragsdale et al. 2011). Soybean aphid reproduces parthenogenically and has a relatively short developmental time that leads to rapid development of infestations in soybean fields (Beckendorf et al., 2008; Rhainds et al., 2008; Ragsdale et al., 2011; Hodgson et al., 2012). This insect can cause economic damage to soybean through most of the vegetative and reproductive growth stages (Myers et al. 2005, Ragsdale et al. 2007). In particular, soybean aphid can cause stunted plants, leaf discoloration, and plant death (Hill et al. 2004, Mensah et al. 2005, Ragsdale et al. 2007). Prolonged sap removal negatively affects plant biomass and yield components, such as pod number, seed number, seed weight, and seed oil concentration (Beckendorf et al. 2008). Soybean aphid can also transmit viral plant diseases (Hill et al. 2001, Wu et al. 2004) and affect the performance of other soybean pests (McCarville et al. 2012). Ultimately, soybean aphid can cause up to 40% yield loss (Ragsdale et al. 2007).

Soybean aphid infestations can be effectively managed with threshold-based (i.e., 250 aphids per plant) sprays of foliar insecticides to prevent populations from reaching the economic injury level (EIL) (i.e., 5,563 cumulative aphid-days) (Ragsdale et al. 2007, Johnson et al. 2009). Sampling plans have been developed to estimate soybean aphid densities and facilitate pest management decision-making (Hodgson et al. 2004, 2007, Onstad et al. 2005). Song and Swinton (2009) predicted that the use of integrated pest

management (IPM) for soybean aphid can generate a net economic benefit of \$1.3 billion over 15 years. However, existing methods for scouting soybean aphid require traversing soybean fields to count aphids on plants. The extensive size of soybean fields, large numbers of aphids to count, and difficulties walking through fields after canopy closure are the main factors contributing to reluctance of growers to implement scouting programs (Hodgson et al. 2004, Olson et al. 2008, Bueno et al. 2011). Some soybean pest managers resort to the prophylactic use of insecticides to control soybean aphid (Olson et al. 2008). Innovative scouting methods that decrease costs while increasing or maintaining efficacy can lead to wider adoption of pest management based on estimates of pest population size (Ragsdale et al. 2007, Bueno et al. 2013). Among such alternatives for scouting, remote sensing has been considered a promising method to characterize plant health condition (Hatfield et al. 2008, Mirik, Ansley, Michels Jr., et al. 2012, Reynolds et al. 2012).

The increasing availability of optical instruments and unmanned aerial systems (UAS) has driven the recent development and lower cost of remote sensing techniques, providing greater spatial and temporal resolution (Lee et al. 2010, Zhang and Kovacs 2012). Remote sensing can accurately provide estimates of insect stress before economic yield losses (Reisig and Godfrey 2007) for a broad variety of plant species (Sims and Gamon 2002). Nevertheless, distinguishable spectral regions affected by insect injury remain to be documented for most economically important insect pests. Plant spectral response has also been used to map weed distributions (e.g., López-Granados 2011, Rasmussen et al. 2013), assess plant nutritional requirements (e.g., Felderhof et al. 2008,

Felderhof and Gillieson 2012) and make other significant contributions to the development and implementation of precision agriculture (e.g., Yue et al. 2012, Zhang and Kovacs 2012). The use of remotely sensed plant spectral reflectance could facilitate precision management of pests such as soybean aphid by exploiting within-field variability of pest infestation, rather than using the traditional whole-field approach (Brisco et al. 1998, Seelan et al. 2003).

The potential of remote sensing for detecting insect populations is typically associated with insect-induced changes in plant morpho-physiological characteristics (Franzen et al. 2007, Yang et al. 2009, Prabhakar et al. 2011). Decreasing photosynthesis rates and changes in leaf mesophyll are usually associated with decreasing reflectance of wavelengths within the near-infrared (NIR) spectral range (Carter and Knapp 2001). Decreased chlorophyll content caused by insect pests is usually associated with increasing reflectance of wavelengths of the visible spectrum, including those within the red spectral range (Riedell and Blackmer 1999). Chlorophyll meters can be used to indicate the relative status of chlorophyll in plant tissues (Vos and Bom 1993, Markwell et al. 1995). Vegetation indices, such as the Normalized Difference Vegetation Index (NDVI), are commonly used to contrast the stronger chlorophyll absorption of red wavelengths with the higher reflectance of NIR wavelengths (Rouse Jr et al. 1973, Carlson and Ripley 1997, Hmimina et al. 2013). NDVI is used as a measure of plant 'greenness' directly related to the absorbed photosynthetically active radiation and canopy photosynthetic capacity (Sellers 1985). In agriculture, the simultaneous use of red ( $\approx 680$  nm) and NIR ( $\approx 800$  nm) wavelengths in the NDVI equation is usually a better

predictor of plant morpho-physiological changes than individual narrowband wavelengths (Gamon et al. 1995, Gamon and Surfus 1999, Richardson et al. 2002).

In greenhouse conditions, the chlorophyll content of susceptible soybean cultivars is significantly reduced by soybean aphid injury (Diaz-Montano et al. 2007). Soybean photosynthesis rates are also affected by accumulated soybean aphid injury (Macedo et al. 2003). The purpose of this study was to investigate whether the season-long exposure to soybean aphids affects RED reflectance, NIR reflectance, NDVI, and chlorophyll content of soybean under field conditions. The use of plant spectral reflectance to detect stress induced by soybean aphid has a potential to increase the adoption of scouting-based IPM programs.

### **1.3. Materials and Methods**

#### **1.3.1. Research Plots**

Two research trials were conducted at UMore Park, University of Minnesota, Rosemount, MN, USA, in 2013 and 2014. Each trial was seeded with approximately 495,000 seeds per hectare with 17 cm row spacing into sandy loam soil. In 2013, the soybean cultivar Pioneer 91Y92 was planted on 8 June. In 2014, the soybean cultivar Pioneer P19T01R was planted on 27 May. The cultivars used in the two years had similar harvest standability, field emergence, herbicide resistance, relative maturity (both were 1.9); and both were considered to be broadly adapted for southern Minnesota (DuPont-Pioneer 2014). Fertilizers were not applied. Weeds were managed with a pre-emergent herbicide and application of glyphosate according to standard production practices (Egel et al.

2012). Approximately 40 d after planting, 3-m alleys were tilled to establish the experimental units (1×1-meter plots). When plants had three fully-expanded trifoliolate leaves [V3 growth stage, Fehr and Caviness (1977)] plots (twenty-four plots in 2013 and twenty-one plots in 2014) were individually enclosed by 1×1×1-meter cages built with PVC tubing and white fine-mesh (0.02 cm mesh size, 100% polyester, Quest Outfitters Inc., Sarasota, USA).

### **1.3.2. Establishment of Aphid Populations**

Soybean aphid populations were manipulated using three caged treatments arranged in a randomized complete block design with eight replications per treatment in 2013 and seven replications per treatment in 2014. In 2013, natural colonization of the soybean field by soybean aphid did not occur before the establishment of the plots. The three treatments used in 2013 to create different soybean aphid populations were: Treatment 1) aphid-free caged plots maintained with an insecticide regime that consisted of spraying the labeled rate of  $\lambda$ -cyhalothrin (11.35 g a.i. per ha, Warrior II<sup>®</sup>, Syngenta Crop Protection Inc., Greensboro, NC, USA) at two-week intervals or more frequently if ten or more aphids were found in a plot; Treatment 2) caged plots which received 140 mixed-age (i.e., nymphs + adults) wingless soybean aphids per plot at the V3 growth stage (on 22 July 2013) (i.e., early infestation); and Treatment 3) artificially infested caged plots infested with 250 mixed-age wingless aphids at the V6 growth stage (on 2 August 2013) [i.e., late infestation when plants had six fully expanded trifoliolate leaves, Fehr and Caviness (1977)].

Prior to the establishment of the cages in 2014, 75% of the soybean plants in the experimental area were naturally colonized by ten or fewer aphids per plant. The treatments were adjusted to utilize the natural infestation occurring in 2014. The three treatments in the 2014 trial were: Treatment 1) aphid-free caged plots maintained with the standardized insecticide regime used in 2013; Treatment 2) caged plots artificially infested with 150 mixed-age wingless soybean aphids per plot at the V3 growth stage (on 10 July 2014) in addition to those that colonized the plants naturally; and Treatment 3) caged plots in which aphids that naturally colonized the plants were allowed to develop.

Aphid-free treatments provided the baseline of plant morpho-physiological characteristics in the absence of aphid feeding. Aphid-infested treatments produced a range of aphid densities from low-level infestation to infestation levels that could significantly affect plant morpho-physiology over time. Aphids used in the artificial infestations (i.e., treatments 2 and 3 in 2013 and treatment 2 in 2014) were obtained from a laboratory colony of soybean aphids maintained at the University of Minnesota. For infestation, mixed-age wingless soybean aphids were carefully transferred using a fine-tipped brush from infested plants in the colony to pieces of filter paper (5.5 cm diameter). Pieces of filter paper containing the aphids were placed on the uppermost expanded trifoliate leaf of five to six plants per plot and secured to the plants with paper clips.

### **1.3.3. Aphid Assessments**

Aphid densities were estimated by non-destructive, visual, whole-plant inspection. The fine-mesh cages were temporarily opened during aphid assessment.

Soybean aphid densities per plant were recorded weekly. Nymphs and adults were summed together during evaluations. Sampling occurred from 30 July to 28 August 2013 and from 10 July to 5 August 2014. The number of plants inspected per plot was the same for all treatments on a given sample date. Moreover, this number was adjusted based on the mean percentage of plants infested with at least one aphid in the aphid-infested treatments during the preceding week (Seagraves and Lundgren 2012). Twenty randomly-selected plants per plot were initially sampled because aphids were present on less than 80% of the plants in the aphid-infested treatments. When 80-99% of the plants were infested, a total of ten plants per plot were sampled in the following week. Five plants per plot were sampled when all plants were infested with at least one aphid. As the season progressed, the number of plants per plot was not increased if the mean percentage of plants infested decreased. On each sample date, aphid-free plots were usually sampled before aphid-infested plots to prevent an unintentional movement of aphids to the uninfested plots. When an aphid-free plot was evaluated after an infested plot, hands and clothing were inspected to avoid an unintentional infestation of the uninfested plots. The fine-mesh cages were reclosed after sampling and kept closed between evaluations.

#### **1.3.4. Spectral Reflectance Measurements**

Plant spectral reflectance was measured using a handheld spectroradiometer immediately after aphid counts (FieldSpec<sup>®</sup> 4 Hi-Res spectroradiometer, ASD Inc., Boulder, USA). The spectroradiometer was capable of determining the percentage of

relative reflectance within the ultraviolet, visible and infrared spectral ranges. Relative reflectance values were obtained by the ratio between plant reflectance and reflectance of the white reference panel accompanying the spectroradiometer (ASD Inc. 2002). Plant reflectance was measured on 21 August and 28 August in 2013 (i.e., 74 and 81 days after planting, respectively), and on 30 July and 5 August in 2014 (i.e., 64 and 70 days after planting, respectively). The spectroradiometer pistol grip assembly (A145653, ASD Inc., Boulder, USA) was held at a 45-degree angle from approximately 0.6 m above the canopy to record canopy-level measurements. The self-illuminated plant probe assembly (AK101800, ASD Inc., Boulder, USA) of the spectroradiometer was used to record leaf-level reflectance of five randomly-selected plants per plot. The leaf-level reflectance measurements were obtained from the adaxial leaf surface of uppermost trifoliates and middle trifoliates (i.e., newly-developed trifoliates and trifoliates located at halfway between upper and lowermost trifoliates, respectively). Spectral measurements occurred between 10:00 am and 2:00 pm. Although leaf-level measurements using the self-illuminated plant probe assembly would not be affected by cloud cover, the spectral reflectance was recorded on dates that had more than 70% cloudless skies to minimize the atmospheric effect on canopy-level measurements. Relative chlorophyll content was assessed using a chlorophyll meter (Spad-502 DL Plus, Konica Minolta Sensing Inc., Osaka, Japan) on the same five plants previously sampled with the spectroradiometer plant probe. The chlorophyll meter was consistently positioned on central leaflets of uppermost fully expanded trifoliates in 2013 and 2014. Chlorophyll was also measured on central leaflets of middle trifoliates in 2014. The chlorophyll meter provides unitless

values of indexed chlorophyll content between 0 and 99.9. Lower indexed chlorophyll content values are considered to represent less chlorophyll content (Markwell et al. 1995, Richardson et al. 2002). All remote sensing measurements were taken before visible signs of sooty mold on plant tissues.

### 1.3.5. Data Analysis

The number of aphids per plant was averaged per plot and then converted to cumulative aphid-days (CAD) over sample dates. CAD was calculated using the formula:  $\sum_{i=1}^n = [(x_i + x_{i-1})/2] \times (t_i - t_{i-1})$ , where  $n$  is the number of sample dates,  $x_i$  is the mean number of aphids per plant on sample date  $i$ , and  $(t_i - t_{i-1})$  is the number of days between two consecutive sample dates (Ruppel 1983, Hanafi et al. 1989). Simple linear regressions [ $\alpha = 0.05$ , R Development Core Team (2015)] were used to predict the effect of CAD on plant spectral reflectance in two narrowband wavelengths (i.e., RED = 680 nm and NIR = 800 nm) and NDVI (Normalized Difference Vegetation Index). NDVI was computed as a ratio of the difference between the NIR and RED narrowband wavelengths, over the sum of both narrowband wavelengths [ $NDVI = (NIR - RED) / (NIR + RED)$ ](Rouse Jr et al. 1973). Reflectance data from canopy, middle trifoliates and uppermost trifoliates were analyzed separately for each sample date. Simple linear regression models were also used to test the effect of CAD on relative chlorophyll content of middle and uppermost trifoliates. Transformation was not necessary to stabilize the variance and achieve statistical assumptions. Residual plots using original values did not show evidence to reject simple linear regression models for the effect of

CAD on NIR reflectance, RED reflectance, NDVI or relative chlorophyll content. Formal tests also did not show evidence of violations of parametric assumptions [gvlma package, Peña and Slate (2006), R Development Core Team (2015)].

## **1.4. Results**

### **1.4.1. Aphid Population Growth**

On 21 August 2013, the first date of spectral reflectance measurements, mean CAD ranged from 0 to 1,635 among the treatments. By 28 August 2013, the second date of reflectance measurements, mean CAD ranged from 0 to 14,611 among the treatments. On 30 July 2014, the first date of spectral reflectance measurements, mean CAD ranged from 0 to 6,844 among treatments. By 5 August 2014, the second date of spectral reflectance measurements, mean CAD ranged from 0 to 21,004 (Fig. 1.1).

### **1.4.2. Spectral Reflectance of Canopy**

CAD did not affect canopy reflectance of the RED narrowband wavelength on either sample date in either year ( $P > 0.05$ , Table 1.1). However, CAD decreased the canopy reflectance of the NIR narrowband wavelength on 28 August 2013 ( $P = 0.005$ ) and 5 August 2014 ( $P = 0.006$ ), but did not affect NIR canopy reflectance on the earlier sample dates of either year (Table 1.1). CAD decreased canopy NDVI on 28 August 2013 ( $P = 0.001$ ) and 5 August 2014 ( $P < 0.001$ ), but did not affect canopy NDVI in the earlier sample dates of each year (Table 1.1).

### 1.4.3. Spectral Reflectance of Individual Leaves

On 21 and 28 August 2013 and 30 July 2014, CAD did not affect the RED reflectance of middle trifoliates (Table 1.2) or uppermost trifoliates (Table 1.3). However, on 5 August 2014, CAD decreased RED reflectance of middle (Table 1.2) and uppermost trifoliates (Table 1.3).

CAD decreased NIR reflectance of middle trifoliates on 21 August 2013 ( $P = 0.044$ ), 28 August 2013 ( $P < 0.001$ ) and 5 August 2014 ( $P = 0.001$ ), but did not affect NIR reflectance of middle trifoliates on 30 July 2014 (Table 1.2). CAD decreased NIR reflectance of uppermost trifoliates on 28 August 2013 ( $P < 0.001$ ) but did not affect NIR reflectance of uppermost trifoliates on the other three sample dates (Table 1.3).

CAD decreased NDVI of middle trifoliates on 28 August 2013 ( $P < 0.001$ ), but did not affect NDVI of middle trifoliates on 21 August 2013, 30 July, and 5 August 2014 (Table 1.2). CAD decreased NDVI of uppermost trifoliates on 28 August 2013 ( $P < 0.001$ ) and increased NDVI of uppermost trifoliates on 5 August 2014 ( $P < 0.001$ ). CAD had no effect on NDVI of uppermost trifoliates on 21 August 2013 or 30 July 2014 (Table 1.3).

CAD did not affect indexed chlorophyll content of middle trifoliates on 30 July 2014 (Table 1.4). CAD also had no effect on the indexed chlorophyll content of uppermost trifoliates on 21 and 28 August 2013 and 30 July 2014 (Table 1.4). On 5 August 2014, CAD decreased indexed chlorophyll content of middle trifoliates ( $P = 0.013$ ) and increased indexed chlorophyll content of uppermost trifoliates ( $P = 0.017$ ) (Table 1.4).

## 1.5. Discussion

Remote sensing has the potential to increase the adoption of scouting-based pest management decisions through reduction of costs for scouting and flexibility to record data (Swinton 2005, Pedersen et al. 2006, Yuan et al. 2014). To our knowledge, this study provides the first documentation of soybean aphid affecting the canopy spectral reflectance of soybean plants. The two-year canopy data revealed that soybean aphid injury can be detected using NDVI and NIR reflectance, but not with RED reflectance (Table 1.1). Soybean aphid populations in this study (Fig. 1.1) developed similarly to other studies with analogous experimental conditions (McCornack et al. 2004, Costamagna and Landis 2006, Gardiner and Landis 2007).

The use of canopy spectral reflectance has shown promising results for detection of stress caused by other aphids such as the English grain aphid [*Sitobion avenae* (Fabricius)] (Yuan et al. 2014) and the cotton aphid (*Aphis gossypii* Glover) (Reisig and Godfrey 2006). For soybean, canopy spectral reflectance has been used to detect the fungus *Sclerotinia sclerotiorum* (Vigier et al. 2004), to predict injury from herbicide (Huang et al. 2012), and for replanting recommendations (Gaspar and Conley 2015). In this study, canopy-level measurements did not always detect changes in soybean spectral reflectance caused by locally induced stress caused by soybean aphid. On 21 August 2013, leaf-level NIR reflectance of middle trifoliates was reduced by CAD (Table 1.3), but canopy-level measurements did not detect this effect on NIR reflectance (Table 1.1). In another instance, the RED reflectance of both middle and uppermost trifoliates was reduced by CAD (Tables 1.2 and 1.3), but canopy-level measurements again did not

detect this locally-induced effect (Table 1.1). However, similar conclusions were obtained by canopy-level and leaf-level measurements of middle and uppermost trifoliates when CAD decreased NIR reflectance and NDVI on 28 August 2013. Canopy-level and leaf-level measurements also provided similar conclusions when CAD had no effect on reflectance of middle or uppermost trifoliates [e.g., RED reflectance on 21, 28 August 2013 and 30 July 2014 (Tables 1-3)].

At both canopy- and leaf-levels, soybean aphid stress was typically better detected by NIR reflectance than by RED reflectance. Despite CAD having no effect on canopy-level RED reflectance on any evaluation date (Table 1.1), two (of four) regression models showed that increasing CAD decreased canopy-level NIR reflectance (Table 1.1). Decreased NIR reflectance has also been recorded in response to increasing insect injury in other commercial crops (Peñuelas et al. 1998, Reisig and Godfrey 2007). The better performance of NIR regression models may be related to the mechanisms most affected by soybean aphid. Stress caused by insect feeding, including aphids, may not always reduce chlorophyll content, but does typically compromise internal leaf structures that can result in lower NIR reflectance (Morgham et al. 1994, Burd 2002). Furthermore, canopy-level NDVI models were more precise than canopy-level NIR models. On 28 August 2013 and 5 August 2014, CAD explained 11% and 34%, respectively, more variability in canopy spectral reflectance using NDVI in the regression models than using NIR alone (Table 1.1). Better results with NDVI for detection of plant stress were also observed by Peñuelas et al. (1998) and Yang et al. (2009). The main advantage of NDVI for soybean aphid is that it compresses the data into a value representing both the

absorbed energy required for photosynthetic processes and the reflectance of low energy wavelengths in the NIR spectral range (Rouse Jr et al. 1973, Carlson and Ripley 1997).

Neither indexed chlorophyll content (Table 1.4) nor RED reflectance (Tables 1-3) showed any evidence of chlorophyll reduction associated with CAD at leaf-level or canopy-level on 21, 28 August 2013, and 30 July 2014. Other wavelengths within the red spectral range or combinations of wavelengths are likely to have indicated a similar non-significant effect on chlorophyll content on these evaluation dates (Wu et al. 2008, Sanches et al. 2014). Reduction in chlorophyll content, intercellular CO<sub>2</sub>, and photosynthetic rates are commonly associated with aphid feeding on susceptible cultivars (Rafi et al. 1996, Wang et al. 2004, Macedo et al. 2009). Resistant cultivars, however, may tolerate aphid feeding with no reduction in chlorophyll content (Franzen et al. 2007). In particular, soybean plants can tolerate soybean aphid injury without losing chlorophyll by up-regulation of detoxification mechanisms, such as peroxidases and faster regeneration of organic substances involved in photosynthesis (Pierson et al. 2011). CAD only significantly affected relative chlorophyll content on 5 August 2014, when some plants had above 21,000 CAD. On this sample date, the data from the chlorophyll meter indicated that CAD decreased relative chlorophyll content of middle trifoliates (Table 1.4). Contradicting the results from the chlorophyll meter, leaf-level measurements indicated that CAD decreased the RED reflectance of middle trifoliates (Table 1.2), which typically is associated with increasing chlorophyll content (Riedell and Blackmer 1999). The conflicting decrease in RED reflectance may be interpreted as evidence of decreasing anthocyanin content, rather than increasing chlorophyll content of middle

trifoliates (Monje and Bugbee 1992, Karageorgou and Manetas 2006, Manetas 2006, Wu et al. 2007).

Soybean plants surprisingly increased the relative chlorophyll content of uppermost trifoliates on 5 August 2014 (Table 1.3). It was unexpected that soybean plants would produce more chlorophyll pigments associated with accumulated soybean aphid injury. On 5 August, plants were at the V12R3 growth stage. On this reproductive growth stage, soybean aphids tend to move to lower canopy (McCornack et al. 2008). The movement of aphids within the plant would potentially decrease the effect of aphid feeding on plant tissues located in the upper canopy. Therefore, the absence of aphids in the uppermost trifoliates on 5 August might have provided an opportunity for investment in the production of chlorophyll pigments (Kennedy et al. 1950, Burd and Burton 1992). The increase in chlorophyll production might be a strategy to overcome soybean aphid injury incurred prior the development of uppermost trifoliates (i.e., newly developed trifoliates) (Sims and Gamon 2002, Guendouz et al. 2012).

Soybean aphid feeding affected soybean spectral reflectance. The observed decreases in NIR reflectance and NDVI with increasing CAD suggest that cumulative injury by soybean aphids could be predicted from spectral reflectance of the soybean canopy. The magnitude of changes in spectral reflectance values caused by soybean aphid was similar to changes caused by aphid species in other crops (Riedell and Blackmer 1999, Reisig and Godfrey 2006, Mirik et al. 2007). For example, on 28 August 2013, canopy-level NIR reflectance of plants undergoing an aphid infestation of 5,500 CAD [i.e., economic injury level, Ragsdale et al. (2007)] would show 13% lower NIR

reflectance than plants with no aphids ( $NIR = 0.926 - 1.75 \times 10^{-6} \times CAD$ ) (Table 1.1). Future research is needed to determine if the effect of soybean aphid on canopy spectral reflectance, detected in this study using a ground spectroradiometer, can also be detected from airborne and spaceborne remote sensing platforms. Further work is also needed to distinguish spectral changes induced by soybean aphid from confounding factors such as other herbivores, nutritional deficiencies, diseases, interaction with cultivar, and water stress.

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## 1.7. Tables

**Table 1.1.** Model estimates of simple linear regressions of the effect of cumulative aphid-days (CAD) on canopy-level spectral reflectance of soybean plants at 680 nm, 800 nm and Normalized Difference Vegetation Index (NDVI) in Rosemount, MN, 2013 and 2014.

Canopy reflectance	Sample date	Intercept	Slope	F-value	df <sub>model,error</sub>	R <sup>2</sup> (%)	P-value
RED (680 nm)	21-Aug-13	0.023	$3.58 \times 10^{-6}$	0.383	1,22	1.7	0.542
	28-Aug-13	0.022	$6.84 \times 10^{-7}$	1.452	1,22	6.2	0.241
	30-Jul-14	0.025	$-3.92 \times 10^{-7}$	1.344	1,19	6.6	0.261
	5-Aug-14	0.021	$6.65 \times 10^{-8}$	0.248	1,19	1.3	0.624
NIR (800 nm)	21-Aug-13	0.541	$1.18 \times 10^{-5}$	0.028	1,22	0.1	0.869
	28-Aug-13	0.500	$-1.23 \times 10^{-5}$	9.663	1,22	30.5	0.005*
	30-Jul-14	0.587	$-4.11 \times 10^{-6}$	0.738	1,19	3.7	0.401
	5-Aug-14	0.539	$-8.00 \times 10^{-6}$	9.694	1,19	33.8	0.006*
NDVI $\left(\frac{NIR - RED}{NIR + RED}\right)$	21-Aug-13	0.922	$-1.02 \times 10^{-5}$	0.735	1,22	3.2	0.401
	28-Aug-13	0.918	$-5.83 \times 10^{-6}$	15.897	1,22	41.9	0.001*
	30-Jul-14	0.918	$6.91 \times 10^{-7}$	0.488	1,19	2.5	0.493
	5-Aug-14	0.926	$-1.75 \times 10^{-6}$	40.066	1,19	67.8	<0.001*

\* Indicate significant effect of CAD on canopy reflectance ( $\alpha = 0.05$ ). Otherwise, CAD had no effect on canopy reflectance.

**Table 1.2.** Model estimates of simple linear regressions of the effect of cumulative aphid-days (CAD) on leaf-level spectral reflectance of middle trifoliates of soybean plants at 680 nm, 800 nm and Normalized Difference Vegetation Index (NDVI) in Rosemount, MN, 2013 and 2014.

Reflectance of middle trifoliates	Sample date	Intercept	Slope	F-value	df <sub>model,error</sub>	R <sup>2</sup> (%)	P-value
RED (680 nm)	21-Aug-13	0.047	$-9.92 \times 10^{-7}$	1.186	1,22	5.1	0.288
	28-Aug-13	0.051	$1.37 \times 10^{-7}$	1.149	1,22	5.0	0.295
	30-Jul-14	0.053	$4.05 \times 10^{-7}$	1.083	1,19	5.4	0.311
	5-Aug-14	0.053	$-2.58 \times 10^{-7}$	7.788	1,19	29.1	0.012*
NIR (800 nm)	21-Aug-13	0.531	$-1.38 \times 10^{-5}$	4.544	1,22	17.1	0.044*
	28-Aug-13	0.525	$-7.09 \times 10^{-6}$	103.268	1,22	82.4	<0.001*
	30-Jul-14	0.541	$-3.25 \times 10^{-7}$	0.014	1,19	0.1	0.908
	5-Aug-14	0.493	$-3.18 \times 10^{-6}$	17.127	1,19	47.4	0.001*
NDVI $\left(\frac{NIR - RED}{NIR + RED}\right)$	21-Aug-13	0.836	$-7.76 \times 10^{-7}$	0.086	1,22	0.4	0.772
	28-Aug-13	0.823	$-3.02 \times 10^{-6}$	51.593	1,22	70.1	<0.001*
	30-Jul-14	0.821	$-1.27 \times 10^{-6}$	1.582	1,19	7.7	0.224
	5-Aug-14	0.805	$-2.89 \times 10^{-7}$	0.874	1,19	4.4	0.362

\* Indicate significant effect of CAD on leaf-level reflectance of middle trifoliates ( $\alpha = 0.05$ ).

**Table 1.3.** Model estimates of simple linear regressions of the effect of cumulative aphid-days (CAD) on leaf-level spectral reflectance of uppermost trifoliates of soybean plants at 680 nm, 800 nm and Normalized Difference Vegetation Index (NDVI) in Rosemount, MN, 2013 and 2014.

Reflectance of uppermost trifoliates	Sample date	Intercept	Slope	F-value	df <sub>model,error</sub>	R <sup>2</sup> (%)	P-value
RED (680 nm)	21-Aug-13	0.047	$2.37 \times 10^{-7}$	0.034	1,22	0.2	0.856
	28-Aug-13	0.050	$1.03 \times 10^{-7}$	0.660	1,22	2.9	0.425
	30-Jul-14	0.057	$1.16 \times 10^{-7}$	0.130	1,19	0.7	0.722
	5-Aug-14	0.055	$-3.51 \times 10^{-7}$	14.338	1,19	43.0	0.001*
NIR (800 nm)	21-Aug-13	0.515	$-1.50 \times 10^{-6}$	0.079	1,22	0.4	0.781
	28-Aug-13	0.520	$-7.59 \times 10^{-6}$	78.398	1,22	78.1	<0.001*
	30-Jul-14	0.491	$4.38 \times 10^{-6}$	3.118	1,19	14.1	0.093
	5-Aug-14	0.475	$-3.80 \times 10^{-7}$	0.554	1,19	2.8	0.466
NDVI $\left(\frac{NIR - RED}{NIR + RED}\right)$	21-Aug-13	0.834	$-1.20 \times 10^{-6}$	0.092	1,22	0.4	0.764
	28-Aug-13	0.825	$-3.15 \times 10^{-6}$	96.958	1,22	81.5	<0.001*
	30-Jul-14	0.792	$1.19 \times 10^{-6}$	2.166	1,19	10.2	0.157
	5-Aug-14	0.792	$1.07 \times 10^{-6}$	19.156	1,19	50.2	<0.001*

\* Indicate significant effect of CAD on leaf-level reflectance of uppermost trifoliates ( $\alpha = 0.05$ ).

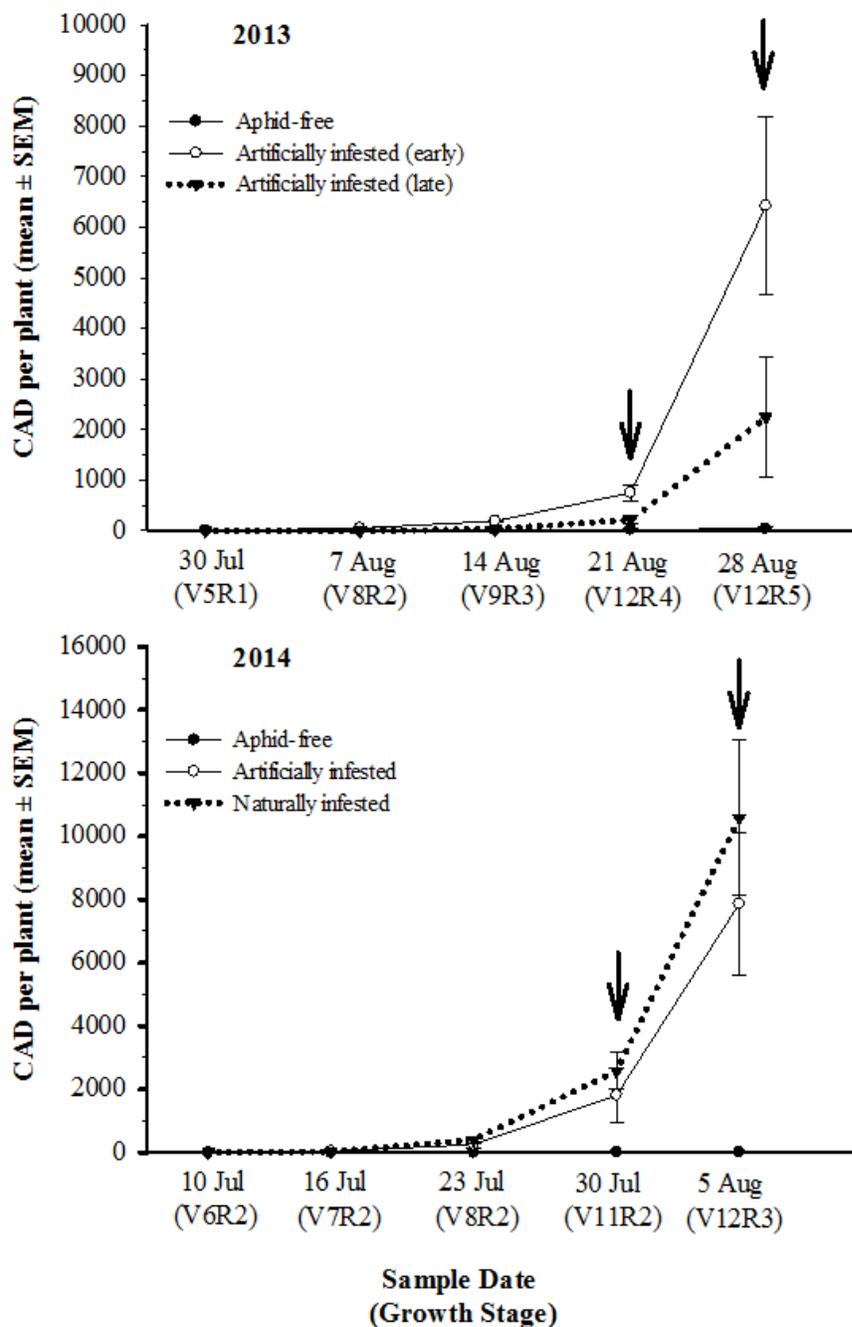
**Table 1.4.** Model estimates of simple linear regressions of the effect of cumulative aphid-days (CAD) on indexed chlorophyll content of middle and uppermost soybean trifoliates in Rosemount, MN, 2013 and 2014.

Position <sup>a</sup>	Sample date	Intercept	Slope	F-value	df <sub>model,error</sub>	R <sup>2</sup> (%)	P-value
Middle	30/Jul/2014	48.91	$-2.32 \times 10^{-4}$	3.22	1,19	14.48	0.089
	5/Aug/2014	50.11	$-1.23 \times 10^{-4}$	7.46	1,19	28.18	0.013*
Uppermost	21/Aug/2013	43.65	$-1.12 \times 10^{-4}$	0.01	1,22	0.03	0.935
	28/Aug/2013	48.06	$1.84 \times 10^{-5}$	0.05	1,22	0.22	0.829
	30/Jul/2014	32.69	$-5.77 \times 10^{-4}$	3.69	1,19	16.28	0.070
	5/Aug/2014	29.99	$3.10 \times 10^{-4}$	6.78	1,19	26.30	0.017*

<sup>a</sup>Vertical position of the trifoliolate within a plant where chlorophyll meter (Spad-502 DL Plus, Minolta, Japan) was positioned to record relative chlorophyll content.

\* Indicate significant effect of CAD on relative chlorophyll content ( $\alpha = 0.05$ ).

## 1.8. Figures



**Fig. 1.1.** Soybean aphid abundance over time [cumulative aphid-days (CAD)] in three treatments used to create differential aphid densities in soybean plots at Rosemount, MN in 2013 and 2014. Arrows denote sample dates which soybean reflectance measurements were recorded.

## 2. Chapter II. Optimizing Band Selection for Spectral Detection of *Aphis glycines* Matsumura in Soybean

### 2.1. Summary

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an important insect pest of soybean in the north-central United States and southern Canada. This study used hyperspectral data and simulated wide-band sensors to estimate *A. glycines* abundance using soybean canopy reflectance. Field trials were conducted in 2013 and 2014. *A. glycines* populations were manipulated using a combination of artificial infestations at different plant growth stages, naturally occurring aphids, and disruptive insecticide applications. Fine-mesh cage plots were arranged in a randomized complete block design with 7–8 replications. Ground-based soybean canopy reflectance and whole-plant densities of *A. glycines* were recorded at approximately weekly intervals. Simple, quadratic, and cubic regression models were used to predict cumulative aphid-days (CAD) based on plant reflectance. Akaike's Information Criteria (AIC) was used to rank and select the most appropriate models with least complexity or equivalently the highest information gain. Lower AIC values were considered to provide better models. Cubic regression models showed AIC values similar or lower than quadratic and simple linear regression models. The subset of narrowband wavelengths that optimized estimation of CAD was similar to that obtained using simulated wide-band sensors. Increasing sensor bandwidth corresponded to larger AIC values (worse models). Canopy reflectance at 750-940 nm was associated with CAD. The smallest AIC values were observed with narrow- and wide-band sensors centered at 780 nm. Our results hold promise to characterize and identify soybean stress due to *A. glycines* injury. The

information provided may help with the design of commercial-grade sensors for detection of *A. glycines*.

## 2.2. Introduction

Soybean, *Glycine max* (L.) Merrill is an important crop to feed animals and supply derivative products, including that of vegetable oil, soy milk, and gluten-free flour, to a broad range of global markets (Wilson 2008). In the north-central United States, where 70% of the US soybean is produced annually (USDA-NASS 2016), soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the primary insect pest decreasing soybean yield (Ragsdale et al. 2011). *A. glycines* injury can be characterized by stunted plants, leaf discoloration, plant death, and facilitated disease infections (Hill et al. 2001, Mensah et al. 2005), but morpho-physiological changes can occur in the absence of visible symptoms (Macedo et al. 2003, Beckendorf et al. 2008, Grisham et al. 2010).

Cumulative aphid-days (i.e., CAD) is an index of aphid abundance over time that has been effectively used to predict aphid-induced crop damage (DiFonzo et al. 1995, Kieckhefer et al. 1995, Legg and Brewer 1995, Reisig and Godfrey 2010). In soybean, CAD has been applied for making management decisions about foliar insecticides to prevent *A. glycines* populations from reaching the economic injury level of about 5,500 CAD (Ragsdale et al. 2007, Koch et al. 2016) and estimating season-long populations in soybean research [e.g., Seagraves and Lundgren (2012), Wiarda and Fehr (2012), Tran et al. (2016)]. Accurate estimation of CAD requires frequent (i.e., weekly or biweekly)

direct counts of insects on plants (Hodgson, Sisson, et al. 2012) (Hodgson, Sisson, et al. 2012). However, growers' perception about time involved in direct insect counts may contribute to lower adoption of traditional pest scouting methods (Olson et al. 2008) and may be associated with the use of prophylactic insecticide applications in soybean.

Remote sensing of plant spectral (light-derived) responses to aphid feeding is a promising alternative to estimate injury without direct insect counts and, thus, increase adoption and efficiency of scouting programs (Hatfield et al. 2008, Mirik, Ansley, Michels Jr., et al. 2012, Reynolds et al. 2012). Soybean morpho-physiological responses to *A. glycines* include changes in photosynthetic pigments, gas exchange, photosynthetic rate, rubisco activity, and structure of leaf mesophyll (Macedo et al. 2003, Diaz-Montano et al. 2007, Pierson et al. 2011), which may affect plant reflectance at visible (i.e., red, green, blue) and near-infrared wavelengths (Riedell and Blackmer 1999, Carter and Knapp 2001, Blackburn 2007). Alves et al. (2015) showed that canopy reflectance of soybean plants at 680 and 800 nm (i.e., a red and near-infrared wavelength, respectively) was affected by CAD. Although these two narrowband wavelengths are commonly associated with plant responses to insect injury (Gamon and Surfus 1999, Richardson et al. 2002), exploring other spectral bands may improve the detection of insect injury using canopy reflectance (Nansen et al. 2009, Singh et al. 2009).

Selection of an optimal subset of spectral bands is an important step to reduce calculation complexity of hyperspectral data containing reflectance measures from thousands of contiguous narrowband wavelengths (Thenkabail, Enclona, Ashton, and Meer 2004). Spectral bands specifically selected for detection of *A. glycines* may also

optimize the use of remote sensing for early detection of this stressor (Shibayama et al. 1993) and may assist in the design of multispectral sensors capable of differentiating among stressors (Mahlein et al. 2012). Whereas multispectral data may be insufficient for characterizing pest-specific effects because of the few wide-bands, hyperspectral data can provide very fine spectral resolution and hundreds or thousands of spectral bands for optimizing pest detection (Carter and Miller 1994, P. M. Mehl et al. 2002).

In entomological research, simple linear regression models have been used for modeling the relationship between the intensity or amount of injury and plant reflectance [e.g., Board et al. (2007), Mirik et al. (2007), (2012), Prabhakar et al. (2011)]. This simplistic approach assumes that the spectral response of the crop to insect injury is characterized by a linear relationship, in which each unit of injury has the same magnitude of effect on plant reflectance (Pedigo and Rice 2006, Peña and Slate 2006). For some crop-insect interactions, however, the effect of increasing injury on plant morpho-physiology may be better characterized by curvilinear instead of linear relationships, which can consequently change the relationship between injury and plant reflectance (Madden 1983, Buntin 2000, Reynolds et al. 2012).

Quadratic and cubic regressions produce curvilinear models that have been popular for describing crop responses to stressors, but curvilinear models have been ignored in the data analysis of plant reflectance in entomological research. By focusing solely on simple linear models, hyperspectral data analyses may obscure the actual wavelengths that best predict injury using plant reflectance. Therefore, a better approach to evaluate the performance of wavelengths to predict insect injury without penalizing

them by lack of fit may require not only simple linear models, but also quadratic and cubic regression models (Blackburn and Steele 1999, Bauerle et al. 2004). When none of these regression models fit well to the injury effect, more sophisticated statistical approaches such as principal component, neural networks, fuzzy, and partial least-squares regression analysis may be employed (Warner and Shank 1997, Kimes et al. 1998, Pinter Jr et al. 2003). Akaike's Information Criteria (AIC) can be used to account for the complexity of the models and identify optimal and parsimonious models (Akaike 1974). AIC has been almost universally accepted in some areas of statistics because it indicates models with least complexity and equivalently highest information gain (Bozdogan 1987), which is not possible by using the commonly used coefficient of determination ( $R^2$ ). Competing models differing by 2 or less AIC units are generally assumed to be comparable (Burnham and Anderson 2003).

Our objectives were: 1) compare linear, quadratic, and cubic regression models to estimate *A. glycines* abundance using soybean canopy reflectance, 2) rank narrowband wavelengths that best estimate CAD, and 3) select simulated multispectral sensors with different bandwidth and band centers. With the recent advancements of customized multispectral sensors, the information of bandwidth and band centers may optimize the spectral detection of *A. glycines* using commercial-grade sensors. Our results can advance the use of remote sensing for detection of *A. glycines* in soybean.

### **2.3. Materials and Methods**

Trials were conducted at the University of Minnesota Outreach Research and Education (UMore) Park in Rosemount, MN, in 2013 and 2014. Broadly adapted soybean varieties were seeded on 8 June 2013 (variety: ‘Pioneer 91Y92’, Pioneer Hi-Bred International Inc., Johnston, IA, USA) and 27 May 2014 (variety: ‘Pioneer P19T01R’, Pioneer Hi-Bred International Inc., Johnston, IA, USA). Each field was sown with  $\approx 495,000$  soybean seeds per hectare and 0.17 m row spacing. Fertilizers were not applied and weed management was performed according to standard production practices (Egel et al. 2012). Plots (1 $\times$ 1 m) were separated from neighboring plots by 3-m bare-soil alleys. Plots were enclosed by fine-mesh PVC-framed cages (1 $\times$ 1 $\times$ 1 m, 0.02 cm mesh size, 100% polyester, Quest Outfitters Inc., Sarasota, FL, USA) at the V3 growth stage (Fehr and Caviness 1977). Twenty-four plots in 2013 and twenty-one plots in 2014 were arranged in randomized complete block designs with eight replications per treatment in 2013 and seven replications per treatment in 2014. To establish differential *A. glycines* populations in 2013, artificial aphid infestations and insecticides applications were combined in three treatments: treatment 1) no artificial infestation + insecticide application [11.35 g a.i. per hectare of  $\lambda$ -cyhalothrin (Warrior II with Zeon technology®, Syngenta Crop Protection Inc., Greensboro, NC, USA)] if ten or more aphids were found in a plot; treatment 2) infestation of 140 aphids per plot at the V3 growth stage (i.e., early infestation) + no insecticide; and, treatment 3) infestation of 250 aphids per plot at the V6 growth stage (i.e., late infestation) + no insecticide. The aforementioned treatments were modified in 2014 to utilize the natural infestation recorded prior establishment of the trial: treatment 1) no artificial infestation + insecticide application [11.35 g a.i. per

hectare of  $\lambda$ -cyhalothrin (Warrior II with Zeon technology®, Syngenta Crop Protection Inc., Greensboro, NC, USA)] if ten or more aphids were found in a plot; treatment 2) infestation of 150 aphids per plot at the V3 growth stage to supplement resident aphids naturally occurring prior infestation + no insecticide; and treatment 3) resident aphid populations naturally occurring + no insecticide. Artificial infestations used mixed-age (i.e., adults + nymphs) wingless *A. glycines* obtained from a laboratory colony maintained at the University of Minnesota.

### **2.3.1. Aphid Sampling and Spectral Measurements**

Aphid sampling occurred weekly from 30 July to 28 August 2013 and from 10 July to 5 August 2014 using non-destructive, visual, whole-plant inspection. The number of plants sampled per plot was adjusted based on the mean aphid densities of the infested treatments recorded in the preceding week (Seagraves and Lundgren 2012). Twenty randomly-selected plants were sampled until 80% of the plants were infested with at least one aphid. Ten randomly-selected plants were sampled when 80-99% of the plants were infested and five randomly-selected plants were sampled when all plants were infested.

Relative reflectance (i.e., ultraviolet, visible and infrared wavelengths) of the soybean canopies was measured using a handheld spectroradiometer (FieldSpec® 4 Hi-Res spectroradiometer, ASD Inc., Boulder, CO, USA) with a pistol grip assembly (A145653, ASD Inc., Boulder, USA) on 28 August 2013 (i.e., 81 days after planting) and 5 August 2014 (i.e., 70 days after planting). The pistol grip was held at a 45-degree angle at a height of  $\approx 0.6$  m above the canopy. Spectral measurements occurred between 10:00 am and 2:00 pm (i.e., near solar zenith) and with more than 70% cloudless skies.

### 2.3.2. Data Analysis

Mean aphid densities per plot were converted into cumulative aphid-days (CAD) using the formula proposed by Ruppel (1983) and adapted for aphids by Hanafi et al. (1989):  $\sum_{i=1}^n [(x_i + x_{i-1})/2] \times (t_i - t_{i-1})$ , where  $n$  is the number of sample dates,  $x_i$  is the mean number of aphids per plant on sample date  $i$ , and  $(t_i - t_{i-1})$  is the number of days between two consecutive sample dates. Canopy reflectance with no derivative was processed using ViewSpec Pro Version 6.2 (ASD Inc. 2008). The reflectance values of 701 narrowband (i.e., 1 nm) wavelengths between 400 and 1,100 nm were selected to represent the spectral ranges predominantly affected by pest injury (Daughtry et al. 2000, Zhao et al. 2007). Simple, quadratic, and cubic regression models were used to predict CAD using plant spectral reflectance at each individual narrowband wavelength (R Core Team 2016). Relative reflectance at a specific wavelength and block effect were specified as explanatory variables. Narrowband wavelength models were compared graphically and then analytically using Akaike's Information Criteria (AIC) (Akaike 1974) and coefficient of variation (R-square). Significant differences between competing models were indicated by a difference of 2 or more AIC units (Burnham and Anderson 2003). Among the simple, quadratic, and cubic models, the one with the smallest AIC value was selected to be the most parsimonious model for each wavelength. AIC was also used to rank the wavelengths that best predicted CAD. Narrowband wavelengths with lower AIC values were considered to provide higher information gain to estimate CAD and, consequently, optimize detection of *A. glycines* injury. Slopes of the selected

parsimonious models were tested against zero using t-test. Significant p-values ( $\alpha = 0.05$ ) indicated association of soybean plant reflectance at a specific narrowband wavelength with CAD. Residual plots were individually inspected by using a generic plot command in R (R Core Team 2016). AIC values of the three regression models of each wavelength (701 wavelengths  $\times$  3 models = 2,103 AIC values per trial) and R-squares were plotted to identify the regression models and spectral regions that optimized CAD estimation.

To simulate wide-band sensors, simple arithmetic mean was used to average the reflectance values of contiguous narrowband wavelengths centered every 10 nm (e.g., 405, 415, 425, ..., 1,090 nm) and with different band half-widths in increments of 5 nm (i.e., 5, 10, ..., 350). The final 2,450 simulated wide-band sensors were restricted to 400 and 1,100 nm (e.g., band center  $\pm$  half-width:  $405 \pm 5$ ,  $405 \pm 10$ ,  $415 \pm 15$ , ...,  $750 \pm 350$  nm). Statistical analysis was similar to that of narrowband wavelengths. Simple, quadratic, and cubic regression models were used to predict CAD using mean reflectance values of each wide-band sensor and block effects as explanatory variables (R Core Team 2016). AIC was used to select the most parsimonious models and rank the simulated wide-band sensors. Simulated wide-band sensors with lower AIC values were considered to provide higher information gain to predict CAD and, consequently, optimize detection of *A. glycines* injury. Residual plots were individually inspected by using generic plot command in R (R Core Team 2016). 3D-plots were created to identify the regression models and spectral regions that optimized CAD estimation.

Forward model selection was adapted from Blanchet et al. (2008) to remove redundancy and select optimized subset of wide-bands for CAD estimation. Forward

model selection was carried out with only wide-bands identified by the previous regression models to be significantly associated with CAD. The first variable added to the model was the wide-band with the lowest AIC value in each year (Bozdogan 1987, Tanaka 1993). Additional variables were selected by adding the remaining wide-bands one each time. Additional variables were kept in the model if they were statistically different from the simpler model without them, compared by chi-square test (Vuong 1989), and slopes were statistically different from zero using t-test ( $\alpha = 0.05$ ). Forward model selection stopped when additional variables did not significantly improve the quality of the model (i.e., no more significant chi-square and slopes).

## **2.4. Results**

Symptoms of disease infection, sooty mold, nutritional deficiency, and insecticide phytotoxicity were not observed during the experiments. *A. glycines* pressure ranged from 0 to 14,612 CAD (experiment-wide mean  $\pm$  SEM:  $2,903 \pm 873$  CAD) at the time of the spectral measurements on 28 Aug 2013 and from 0 to 21,005 CAD (experiment-wide mean  $\pm$  SEM:  $4,630.97 \pm 683.70$  CAD) at the time of the spectral measurements on 5 Aug 2014. The  $\lambda$ -cyhalothrin insecticide used in our study was considered to have negligible effects on soybean spectral bands associated with CAD in our study (Alves et al. in preparation).

### **2.4.1. Performance of Narrowband Wavelengths**

In 2013, canopy reflectance was not associated with CAD at wavelengths within 400–750, 947–1,056 and 1,088–1,100 nm ( $P > 0.05$ , Fig. 2.1a), but was significantly

associated with CAD at wavelengths within 751–946 and 1,057–1,087 ( $P \leq 0.05$ , Fig. 2.1a). The narrowband wavelengths around 785 nm had the lowest AIC values (Fig. 2.1a). Cubic regression models of all narrowband wavelengths associated with CAD showed lower AIC values than simple and quadratic regression models (Fig. 2.1a).

In 2014, canopy reflectance was not associated with CAD at wavelengths within 400–744 and 939–1,100 nm ( $P > 0.05$ , Fig. 2.1b), but was significantly associated with CAD at wavelengths within 745–938 nm ( $P \leq 0.05$ , Fig. 2.1b). The narrowband wavelengths around 785 nm had the lowest AIC values (Fig. 2.1b). Simple linear regressions of all narrowband wavelengths associated with CAD showed lower AIC values than simple and quadratic regression models (Fig. 2.1b).

Within the spectral range associated with CAD, longer wavelengths had higher AIC values in both years (Figs. 2.1a and b). Near-infrared wavelengths at 750–930 nm typically had lower AIC values than narrowband wavelengths at 931–1,100 nm (Figs. 2.1a and b). Within the spectral range significantly associated with CAD, R-squares varied between 0.61–0.70 and 0.33–0.44 in 2013 and 2014, respectively (Figs. 2.1c and d). R-square and AIC provided similar, clear differences among regression models in 2013 (i.e., lower AIC values corresponded to higher R-square). However, AIC values and R-square provided contrasting comparisons among regression models in 2014. In this year, simple linear regression models had lower AIC values, but their R-square was not visibly higher than that of quadratic and cubic regression models.

#### **2.4.2. Simulated Wide-Band Sensors**

The spectral ranges that optimized *A. glycines* detection using wide-band sensors (Fig. 2.3, Table 2.1) were similar to that obtained using narrowband wavelengths (Fig. 2.2). Some combinations of simulated wide-band sensors provided AIC values (Fig. 2.3) lower than models using soybean canopy reflectance of narrowband wavelengths (Fig. 2.2). Independently of sensor bandwidth, soybean canopy reflectance of wide-band sensors was significantly associated with CAD if sensors were centered at infrared wavelengths (740–1,100 nm) ( $P \leq 0.05$ , Fig. 2.3). A few sensors centered at 600–740 nm were also associated with CAD, but only at wider bandwidths (Fig. 2.3). The smallest AIC values among simulated sensors with the same bandwidth were observed around 780 nm (Fig. 2.3). Wide-band sensors centered at near-infrared wavelengths at 740–930 nm typically had lower AIC values than wide-band sensors centered at 931–1,100 nm (Fig. 2.3). In addition, increasing bandwidth corresponded to increasing AIC values, independently of the centered wavelength (Fig. 2.3).

### 2.4.3. Optimized Subsets of Wide-Bands

As typical of forward model selection, optimized subsets of wide-bands provided lower AIC values and higher R-square (Table 2.1) than single-band models (Figs. 2.1 and 3). For models considering simulated wide-bands with half-width between 5 and 350 nm, optimized subsets of wide-bands were centered at  $780 \pm 5$  and  $690 \pm 160$  nm ( $R^2 = 0.83$ ) in the trial conducted in 2013 and  $780 \pm 5$ ,  $720 \pm 50$  and  $1010 \pm 5$  nm ( $R^2 = 0.94$ ) in the trial conducted in 2014 (Table 2.1). For models considering only very wide bands between 100 and 350 nm, optimized subsets of wide-bands were centered at  $850 \pm 100$ ,  $690 \pm 160$ ,  $770 \pm 160$ , and  $760 \pm 315$  nm ( $R^2 = 0.91$ ) in the trial conducted in 2013 and

850 ± 100, 650 ± 140, and 650 ± 145 nm ( $R^2 = 0.92$ ) in the trial conducted in 2014 (Table 2.1). Within each trial, the lowest AIC values were obtained from simulated wide-bands with half-width of 100–350 nm in 2013 and 50–350 nm in 2014 (Table 2.1). The first optimized band of all subsets was selected from the wide-band pool that had the minimum half-width allowed. For instance, the first optimized band for the half-width of 50–350 nm was 810 ± 50 nm in both years. The second optimized band was 690 ± 160 nm for all subsets in 2013 and 720 ± 50 nm for most of the subsets in 2014 (Table 2.1). Model selection in 2014 provided subsets with lower AIC values and higher R-squares than in 2013, but typically required more wide-bands.

## 2.5. Discussion

Over the last four decades, development of ground-, air-, and space-based remote sensing instruments has provided a unique opportunity to detect insect injury with very fine spectral and temporal resolutions (Pohl and Van Genderen 1998, Wang et al. 2006). Optimizing selection of spectral bands for detection of *A. glycines* in soybean may increase the efficiency of integrated pest management programs through remote scouting (Pinter Jr et al. 2003). This study demonstrated that narrowband and simulated wide-band sensors were sufficient to estimate *A. glycines* abundance over time (i.e., CAD).

Cubic regression models were more parsimonious or did not differ within 2 AIC units from simple and quadratic regression models (Fig. 2.1) (Bozdogan 1987, Arnold 2010). The curvilinear relationship at several wavelengths indicated that insect-induced changes on soybean spectral reflectance depended on the amount of *A. glycines* injury

that had accumulated. Spectral responses depending on the magnitude of insect effects may suggest that plant morpho-physiology may also respond curvilinearly to increasing densities of *A. glycines* (Madden 1983, Pedigo and Rice 2006, Reynolds et al. 2012), which may be associated with some degree of tolerance to *A. glycines* injury (Palmer 2015, Kucharik et al. 2016). Therefore, the use of simple linear regression models alone, which has been a common practice in the entomological literature on remote sensing, would have provided misleading interpretations of spectral bands that optimize *A. glycines* detection because of the lack of fit at some wavelengths.

Spectral detection of *A. glycines* using soybean canopy reflectance of narrowband or simulated wide-band sensors depended on the choice of band center and bandwidth. Wider bands penalized insect detection (Fig. 2.3) and increased the number of required spectral bands independently of the center band (Table 2.1). Wide-band sensors were significantly associated with *A. glycines* abundance (Fig. 2.3) when the band center was located around the narrowband wavelengths that were also associated with *A. glycines* abundance (Fig. 2.1). Narrowband wavelengths and simulated wide-band sensors centered at the beginning of the near-infrared spectral range (i.e., 750–940 nm) were determined to be the most effective for detection of *A. glycines*. Similar near-infrared wavelengths were also associated with injury of other aphid species attacking wheat (Riedell and Blackmer 1999). This effect on near-infrared wavelengths may have indicated that injury of *A. glycines* predominantly affected leaf ultrastructure or canopy architecture (Knippling 1970, Grant 1987). We speculated that these potential changes in leaf ultrastructure may be associated with strengthening of leaf cell walls, plant

production of secondary metabolites, saliva discharge, or cell punctures during probing activities, but unlikely to be caused by cell death (Bradley et al. 1992, Li et al. 2008).

Spectral detection of *A. glycines* was not possible using canopy reflectance at wavelengths of the visible spectrum (i.e., 400–600 nm) (Figs. 2.1 and 2.3), which may have indicated that its injury had no detectable effects on leaf pigment content (Sims and Gamon 2002, Gitelson et al. 2003). Wavelengths that were weakly or not affected by *A. glycines* may be used to detect other insect pests (e.g., Board et al. 2007), disease infections (e.g., Carter 1993, Das et al. 2013), drought stress (e.g., Inoue and Peñuelas 2006, Rhee et al. 2010), weeds (e.g., Koger et al. 2003, Gray et al. 2009), and nutritional deficiencies (e.g., Milton et al. 1991, Rogovska and Blackmer 2009).

The simulation approach undertaken to identify combinations of narrowband wavelengths into wide-band sensors yielded significant associations between soybean canopy reflectance and *A. glycines* abundance (Fig. 2.3). Simulated sensors with band centers at 785 nm optimized the detection of *A. glycines* (Figs. 2.1 and 2.3), but single-band models and subsets of optimized wide-bands centered at other parts of the near-infrared spectral range also provided precise estimates of *A. glycines* abundance. Our study using ground-based spectroradiometers in a controlled field environment likely had more detection power and less confounding effects (e.g., other pests and atmospheric conditions) than using unmanned aerial vehicles or satellites for data collection in commercially cultivated soybean fields. Therefore, validation under field conditions and with different platforms will be required to test the performance of the customized multispectral sensors that optimized *A. glycines* detection. It may also be inevitable to

consider the availability of sensors and the quality of their spectral data before extrapolating our results. Narrowband wavelengths and wide-bands with small bandwidths (e.g.,  $\pm 5\text{--}10$  nm) captured enough electromagnetic radiation at the near-infrared spectral range to estimate *A. glycines* in our study, but may be insufficient in large scale applications (REF).

This study showed that hyperspectral data can reveal spectral bands that optimize *A. glycines* detection. Our approach simulating wide-bands and using curvilinear regression models to analyze hyperspectral data may improve researchers' ability to select spectral regions and facilitate the design of sensors for detection of other stressors.

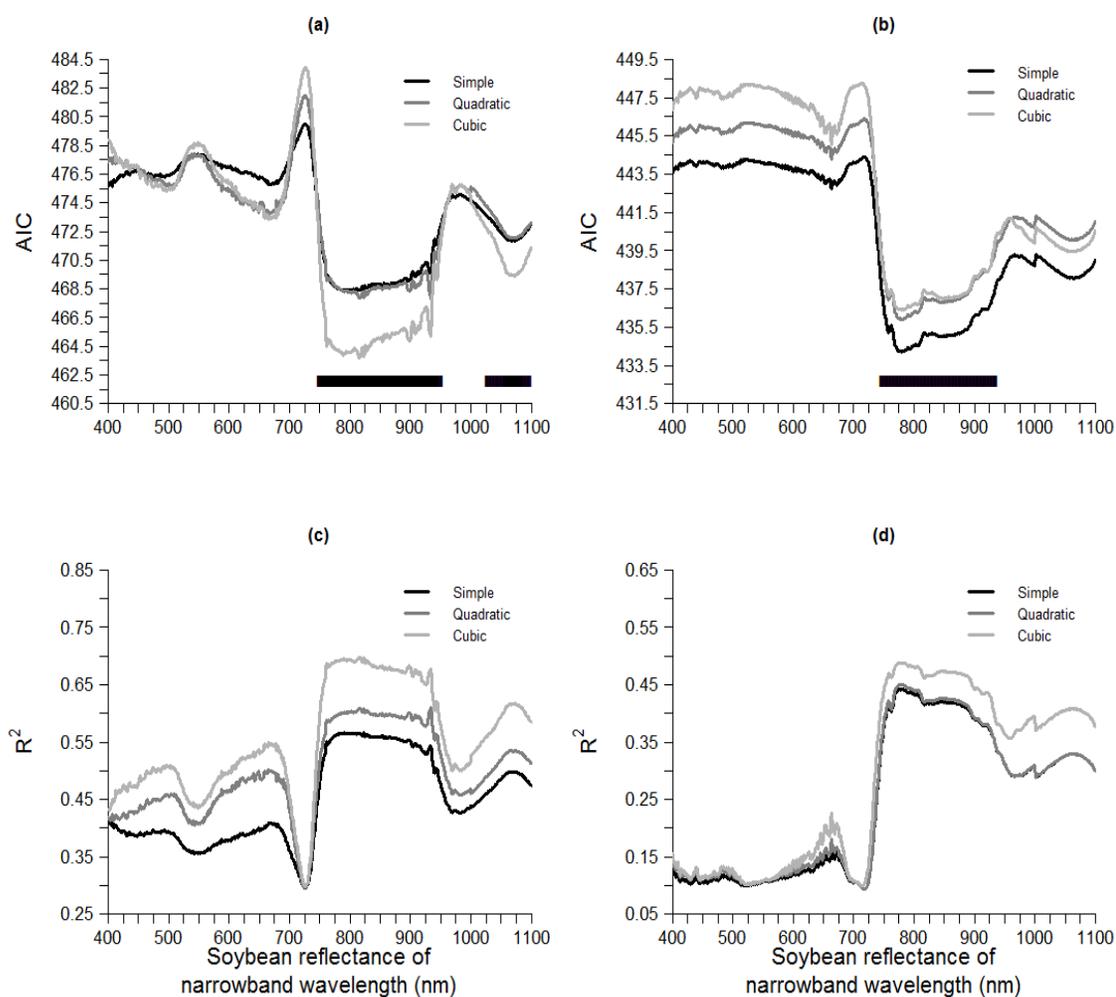
## 2.6. Tables

**Table 2.1.** Subsets of wide-bands identified to optimize detection of *Aphis glycines* using soybean canopy reflectance in the trials conducted in 2013 and 2014. Optimized wide-bands were selected by forward model selection among simulated sensors with different bandwidth and center bands.

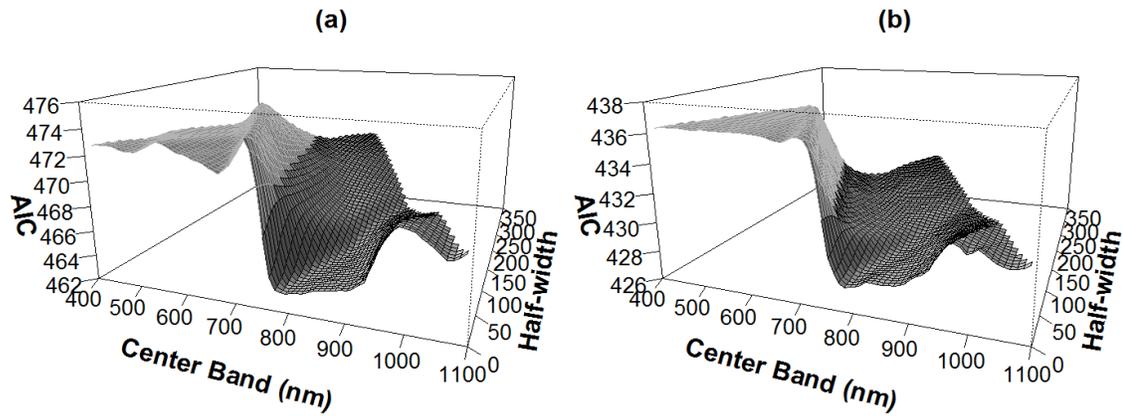
Trial	Half-width (nm)	Optimized Wide-Bands	Anova	AIC
2013	5-350	780 ± 5 and 690 ± 160	F <sub>9,14</sub> = 7.60; P = 0.004; R <sup>2</sup> = 0.83	447.87
	10-350	780 ± 10 and 690 ± 160	F <sub>9,14</sub> = 7.62; P = 0.005; R <sup>2</sup> = 0.83	447.81
	20-350	780 ± 20 and 690 ± 160	F <sub>9,14</sub> = 7.78; P = 0.004; R <sup>2</sup> = 0.83	447.40
	50-350	810 ± 50, 690 ± 160, and 730 ± 145	F <sub>10,13</sub> = 9.37; P < 0.001; R <sup>2</sup> = 0.87	441.88
	100-350	850 ± 100, 690 ± 160, 770 ± 160, and 760 ± 315	F <sub>11,12</sub> = 11.30; P = 0.001; R <sup>2</sup> = 0.91	436.10 <sup>a</sup>
2014	5-350	780 ± 5, 720 ± 50 and 1010 ± 5	F <sub>9,11</sub> = 19.02; P < 0.001; R <sup>2</sup> = 0.94	391.50
	10-350	780 ± 10, 720 ± 50 and 1010 ± 10	F <sub>9,11</sub> = 18.87; P < 0.001; R <sup>2</sup> = 0.94	391.66
	20-350	780 ± 20, 720 ± 50, and 980 ± 30	F <sub>9,11</sub> = 15.90; P < 0.001; R <sup>2</sup> = 0.93	395.02
	50-350	810 ± 50, 720 ± 50, 1010 ± 75, and 750 ± 75	F <sub>10,10</sub> = 25.57; P < 0.001; R <sup>2</sup> = 0.96	383.57 <sup>a</sup>
	100-350	850 ± 100, 650 ± 140, and 650 ± 145	F <sub>9,11</sub> = 14.87; P < 0.001; R <sup>2</sup> = 0.92	396.32

<sup>a</sup> Combination of wide-bands that provided the lowest AIC value within the same year.

## 2.7. Figures



**Fig. 2.1.** Akaike's Information Criteria (AIC) and coefficients of variation ( $R^2$ ) of regression models used to predict cumulative aphid-days (CAD) using soybean reflectance at 701 narrowband wavelengths recorded in field trials conducted in 2013 (a and c) and 2014 (b and d). Black bars (I) at the bottom of the upper graphs indicate 1-nm wavelength bands that had a significant association with CAD (i.e., slopes of regression were different from zero at  $\alpha = 0.05$ ). 1-nm wavelength bands not showing black vertical bars were not associated with CAD ( $P > 0.05$ ).



**Fig. 2.2.** Akaike's Information Criteria (AIC) of regression models used to predict cumulative aphid-days (CAD) using soybean reflectance of simulated wide-band sensors with different bandwidth and center bands in trials conducted in 2013 (a) and 2014 (b). Darker areas indicate models that plant reflectance of wide-band sensors was significantly associated with CAD ( $\alpha = 0.05$ ).

### 3. Chapter III. Potential Confounding Effects of Foliar Insecticides on Spectral Detection of Soybean Pests

#### 3.1. Summary

Spectral reflectance data obtained using space-, aerial-, and ground-based sensors promises to increase the efficiency and adoption of crop scouting for agricultural pests. However, it is unknown if the spectral data used for research purposes and detection of plant stress are also affected by foliar insecticides applied for pest management. Our study assessed the effects of foliar insecticides on leaf reflectance of soybean *Glycine max* (L.) Merr. Two-year field trial and a greenhouse trial were conducted using randomized complete block and completely randomized designs, respectively. Treatments consisted of an untreated check, a new systemic insecticide (sulfoxaflor), and two representatives of the most common insecticide classes used for soybean pest management in the northcentral U.S. ( $\lambda$ -cyhalothrin and chlorpyrifos). Insecticides were applied at labeled rates recommended for controlling soybean aphid (*Aphis glycines* Matsumura). Leaf-level reflectance was measured using a ground-based spectroradiometer. Evaluations were made at 3 h and every other day up to 11 d after spray. Sulfoxaflor affected leaf reflectance at some red and blue wavelengths, but had no effect at near-infrared or green wavelengths. Chlorpyrifos affected leaf reflectance at some green, red, and near-infrared wavelengths, but had no effect at blue wavelengths.  $\lambda$ -cyhalothrin had the least effect on spectral reflectance among the insecticides, with changes to only a few near-infrared wavelengths. Our results contribute to the understanding of the potential effects of leaf surface residues and plant physiological responses to insecticide applications. Understanding of pesticide-induced changes in

plant reflectance is crucial for development of pest management programs using spectral data.

### 3.2. Introduction

Soybean [*Glycine max* (L.) Merr.] is the most economically important oilseed crop cultivated around the world (Wilcox 2004, Hartman et al. 2011). Soybean seed is a key component of livestock feed and, consequently, production of animal-derived protein for human consumption (Boland et al. 2013). The combined effects of human population growth and increasing demand for animal-derived protein have required growers to practice an intensive production system with little tolerance for crop losses (Oerke 2006, Heaton et al. 2013). In the north central United States, where the soybean aphid [*Aphis glycines* Matsumura (Hemiptera: Aphididae)] has been the primary insect pest since its invasion in 2000 (Ragsdale et al. 2011), timely applications of broad-spectrum foliar insecticides are routinely required for preventing economic yield losses (Hodgson, McCornack, et al. 2012). Pyrethroids (e.g.,  $\lambda$ -cyhalothrin) and organophosphates (e.g., chlorpyrifos) are the most common insecticide classes used to control soybean aphids (Johnson et al. 2009). A potential alternative to these broad-spectrum insecticides for soybean aphid management is a novel active ingredient referred to as sulfoxaflor within the sulfoxamine insecticide class (Tran et al. 2016). Sulfoxaflor has a mode of action similar to that of the neonicotinoid insecticide class and is less harmful to natural enemies than broad-spectrum insecticides (Sparks et al. 2013).

Determination of timing for applying insecticides typically relies on traditional ground-based scouting of the crop (e.g., direct insect counts) (Pedigo et al., 1986; Radcliffe et al., 2009). However, perception of growers about the time involved and inconvenience of direct insect counts can limit adoption of threshold-based pest management recommendations (Hodgson et al. 2004, Olson et al. 2008, Bueno et al. 2011). Recent research aimed at alternative methods to increase scouting efficiency has shown that technological innovations, such as unmanned aerial systems (*aka* UAS or drones) and satellites, can provide reliable low-cost detection of pest injury (Zhang and Kovacs 2012, Gómez-Candón et al. 2014), which could increase adoption of scouting and threshold-based pest management recommendations.

Spectral detection of insect injury depends on pest-induced changes in plant morpho-physiology (Knipling 1970, Nansen and Elliott 2016). Foliar insecticides used to create gradients of pest pressure in research trials or to control pest populations in agricultural production have been assumed to have negligible effects on plant spectral reflectance (e.g., Luedeling et al., 2009; Reisig and Godfrey, 2010; Alves et al., 2015). However, some studies have established that pesticides can affect crop morpho-physiology similar to pest injury (Toscano et al. 1982, Jones et al. 1986, Bashir et al. 2014). This pesticide effect may confound the use of plant spectral responses for pest detection after insecticide applications. Although the labeled rates of pesticides generally have minimum direct effect on crop yield (Saladin and Clement 2005), they may cause subtle changes in plant responses including changes in chlorophyll content, photosynthetic rate, intercellular air spaces, biomass, and water use efficiency (Lapre et

al. 1982, Grossmann and Retzlaff 1997, Grossmann, K.; Kwiatkowski, J.; Caspar 1999, Haile et al. 2000, Swoboda and Pedersen 2009, Bashir et al. 2014). Furthermore, previous studies have indicated that pesticide residues may change the spectral energy reflected and absorbed by plants (Makio et al. 2007, Nansen et al. 2010, Sánchez et al. 2010).

Changes to plant spectral responses due to foliar insecticide applications may pose a challenge for using spectral data for crop scouting after insecticide applications. This study was undertaken to determine whether insecticides intended for soybean pest management in the northcentral US can affect spectral responses of soybean leaves over time. We hypothesized that foliar insecticides may affect soybean spectral reflectance due to residues on leaf surfaces and/or systemic effects altering plant morpho-physiology. The former may have more immediate implications after insecticide applications, while the latter may have more extended implications for the use of spectral data for pest detection. Our study contributes to the understanding of insecticide-induced effects on plants and the development of wavelength-specific systems used for detection of insect injury.

### **3.3. Materials and Methods**

#### **3.3.1. Greenhouse Study**

Twenty plastic pots (10 × 10 × 10 cm) were filled with potting soil (Sunshine<sup>®</sup> MVP, Sun Gro Horticulture Inc., Bellevue, WA). One soybean seed (variety: 'P19T01R', Pioneer Hi-Bred International Inc., Johnston, IA, USA) was sown per pot on 30 May 2014. Potted plants were kept in the greenhouse facilities of the University of Minnesota,

Saint Paul, MN. Artificial lighting supplemented natural light with plants grown under a photoperiod of 16:8 (L:D) h (LU400/H/ECO bulbs, GE Lighting Solutions LLC, Cleveland, OH, USA). Air temperature and relative humidity were recorded using a data logger (HOBO U23 Pro v2 data logger, Onset Computer Corporation, Cape Cod, MA, USA). No supplemental fertilizer was applied. Potted plants were arranged in a completely randomized design with five replications per treatment. The treatments were an untreated check (i.e., control) and three insecticides applied at the high label rates recommended for controlling soybean aphid:  $\lambda$ -cyhalothrin (Warrior II with Zeon technology<sup>®</sup>, Syngenta Crop Protection Inc., Greensboro, NC, USA) at 0.12 l of product/ha, chlorpyrifos (Lorsban<sup>™</sup> 500 EC, Dow AgroSciences LLC, Indianapolis, IN, USA) at 2.34 l of product/ha, and sulfoxaflor (Transform<sup>®</sup> WG, Dow AgroSciences LLC, Indianapolis, IN, USA) at 69.19 g of product/ha. Insecticides were applied on 17 June when plants had two fully-expanded trifoliates [i.e., V2 growth stage based on Fehr and Caviness (1977)]. Insecticides were delivered at 187 l/ha and 276 kpa using a motorized spray chamber with a flat-fan nozzle (XR-8002, Teejet Technologies LLC, Springfield, IL, USA) with no screen. Plants were returned to the greenhouse benches after spraying. Spectral reflectance of the adaxial leaf surfaces were measured pre-spray on 17 June and at 3 h, 1, 2, 3, 7, and 10 d after spray (i.e., 17, 18, 19, 20, 24, and 27 June, respectively). An average of ten reflectance measurements was obtained from the middle leaflet of each trifoliolate present at the time of the insecticide applications. Relative reflectance of individual leaflets from 350 to 2500 nm (i.e., ultraviolet, visible, and infrared spectral ranges) was measured using a self-illuminated handheld spectroradiometer (FieldSpec 4

Hi-Res spectroradiometer, ASD Inc., Boulder, CO, USA). Insect densities, plant diseases and deficiencies, and insecticide phytotoxicity were monitored throughout the duration of the trial. Mean temperature ( $\pm$  SEM) was  $24.37 \pm 0.26$  °C and mean relative humidity ( $\pm$  SEM) was  $70.92 \pm 1.97$  % over the period of evaluations.

### **3.3.2. Field Study**

A field study was conducted at the Minnesota Agricultural Experiment Station, Saint Paul, MN, in 2014 and at the University of Minnesota Rosemount Research and Outreach Center, Rosemount, MN, in 2015. Soybean variety ‘IA3027RA1’ (Iowa State University Research Foundation, Ames, IA, USA) known to be resistant to soybean aphid (Hill et al. 2006, Varenhorst et al. 2015, Ajayi-Oyetunde et al. 2016) was planted on 24 June 2014 and 24 June 2015. This aphid-resistant variety was selected to prevent potential confounding effects of soybean aphids on plant reflectance (Alves et al. 2015). Plot size was 2-m long  $\times$  2.3-m (3 rows) wide in 2014 and 3-m long  $\times$  2.3-m (3 rows) wide in 2015. In both years, seeding rate was  $\sim$ 370,660 seeds/ha, row spacing was 0.76 m, plots within blocks were separated by 2.4 m, and blocks were separated by 3 m of bare soil. Weed management was performed by hand weeding to prevent potential confounding effects of herbicides on plant responses. Plots were arranged in a randomized complete block design with five replications of four treatments. The treatments were the same as those used in the greenhouse trial: an untreated check,  $\lambda$ -cyhalothrin (0.12 l of product/ha), chlorpyrifos (2.34 l of product /ha), and sulfoxaflor (69.19 g of product/ha). Plants had four fully-expanded trifoliates when insecticides were

applied on 24 July 2014 and five-fully expanded trifoliate when insecticides were applied on 27 July 2015 [i.e., V4 and V5 growth stages, respectively, based on Fehr and Caviness (1977)]. Insecticides were delivered at 187 l/ha and 276 kpa using a CO<sub>2</sub>-pressurized backpack sprayer. The backpack sprayer was mounted with a 1.5-m spray boom and flat-fan nozzles (XR-8002, Teejet Technologies LLC, Springfield, IL, USA) with no screen. In 2014, the spectral reflectance of the adaxial leaf surfaces was measured pre-spray on 22 July and at 3 h, 1, 4, 7, and 11 d after spray (i.e., 24, 25, 28, 31 July, and 4 August, respectively). In 2015, the spectral reflectance of the adaxial leaf surfaces was measured at 3 h, 2, 3, and 8 d after spray (i.e., 27, 29, 30 July, and 4 August, respectively). Spectral measurements were taken from three randomly-selected plants per plot using self-illuminated handheld spectroradiometers in 2014 (FieldSpec 4 Hi-Res spectroradiometer) and 2015 (QualitySpec Trek spectroradiometer, ASD Inc., Boulder, CO, USA). An average of ten spectral measurements was obtained from the middle leaflet of each trifoliate present at the time of the insecticide applications. Air temperature, relative humidity, and rainfall were retrieved from the nearest National Weather Service weather station <http://www.ncdc.noaa.gov/>. Insect densities and injury, disease infection, nutritional deficiency, and insecticide phytotoxicity were monitored throughout the duration of the trials. In 2014, mean temperature ( $\pm$  SEM) was  $22.29 \pm 0.37$  °C over the evaluation period and rainfall of 10.16, 2.03, and 0.25 mm occurred at 1, 9, and 10 d after spray, respectively. In 2015, mean temperature ( $\pm$  SEM) was  $22.28 \pm 0.65$  °C over the evaluation period and rainfall of 33.02 mm occurred at 1 d after spray.

### 3.3.3. Data Analysis

Relative reflectance values, interpolated to 1 nm intervals (i.e., narrowband), were processed using ViewSpec Pro Version 6.2(ASD Inc. 2008). The 701 wavelengths between 400 and 1100 nm were selected to represent the spectral range predominantly affected by pest injury (Knipling 1970, Daughtry et al. 2000, Zhao et al. 2007). Linear mixed-effects models (lmer) were specified using the lme4 package (Bates et al. 2014) in the R environment (R Core Team 2016). Trials were analyzed separately (i.e., not combined in the same model). The  $\log_{10}(x)$ -transformation of the relative reflectance of the narrowband wavelengths was the response variable of the linear mixed-effects models – a total of 701 models per trial. A similar approach to analyze hyperspectral data was used by Mariotti et al., (1996), Thenkabail et al., (2004), Zhao et al., (2007), and Reisig and Godfrey (2007, 2010). For greenhouse and field trials, terms were included in the model as fixed effects to represent the variability due to the insecticide treatments, evaluation time periods, and their two-way interaction. For the greenhouse trial, the nested effects of trifoliates in plants (i.e., 1 | plant/trifoliates) were specified as random effects. For the field trials, the nested effects of blocks, plots, plants and trifoliates (i.e., 1 | Block/Plot/Plant/Trifoliolate) were specified as random effects. The optimizer of the lmer models was the Nelder Mead optimizer and the estimates of the parameters were obtained by maximum likelihood estimation. A two-sided Dunnett's mean separation test was used for comparing the reflectance values between treated and untreated plants ( $\alpha = 0.05$ ) (Dunnet 1955, Potter et al. 1990, Formusoh et al. 1992). Dunnett's confidence limits of

the logarithmic differences between each insecticide treatment and the untreated check were created using a generic plot command (R Core Team 2016). Residuals and quantile-quantile plots were individually analyzed and did not show evidence to reject homoscedasticity or normality assumptions.

### **3.4. Results**

There were no visible symptoms of disease infection or nutritional deficiency in the plants of the greenhouse or field trials. Insect densities and injury were not observed in the plants of the greenhouse trial. In the field trials, insect densities and injury were well below the economic injury level (Ragsdale et al. 2007, Hodgson, Sisson, et al. 2012). Furthermore, exploratory analyses revealed that leaf spectral response at pre-spray evaluation dates did not differ significantly ( $P > 0.05$ ) among treatments (data not shown).

#### **3.4.1. Greenhouse Study**

Insecticide effects on soybean reflectance depended on the insecticide and time after spray. At 3 h after spray, sulfoxaflor increased leaf spectral reflectance at three red wavelengths (682–684 nm), but had no effect at the other 698 wavelengths (400–681 and 685–1100 nm) (Fig. 3.1a). At 10 d after spray, sulfoxaflor decreased leaf spectral reflectance at 14 red wavelengths (662–671, 673, 678, 681, and 686–687 nm), but had no effect at the other 687 wavelengths (400–661, 672, 674–677, 679–680, 682–685, and 688–1100 nm) (Fig. 3.1f). Sulfoxaflor also had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) at 1, 2, 3, and 7 d after spray (Figs. 3.1b–e).  $\lambda$ -

cyhalothrin and chlorpyrifos had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) at 3 h, 1, 2, 3, 7, and 10 d after spray (Figs. 3.1g–l and m–r).

### 3.4.2. Field Study

Insecticide effects on soybean reflectance depended on the insecticide and time after spray. At 1 d after spray, sulfoxaflor decreased leaf spectral reflectance at 28 blue wavelengths (400–413, 416–422, 428–433 and 435 nm), but had no effect at the other 673 wavelengths (414–415, 423–427, 434, 436–1100 nm) (Fig. 3.2b). Sulfoxaflor had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) at 3 h, 4, 7, and 11 d after spray (Figs. 3.2a and c–e). At 11 d after spray,  $\lambda$ -cyhalothrin increased leaf spectral reflectance at 13 near-infrared wavelengths (712–724 nm), but had no effect at the other 688 wavelengths (400–711 and 725–1100 nm) (Fig. 3.2j).  $\lambda$ -cyhalothrin and chlorpyrifos had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) at 3 h, 1, 4, and 7 d after spray (Figs. 3.2f–i and k–n). Chlorpyrifos also had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) at 11 d after spray (Fig. 3.2o).

Sulfoxaflor had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) at 3 h, 2, 3, and 8 d after spray (Figs. 3.3a–d). At 3 h after spray,  $\lambda$ -cyhalothrin decreased leaf spectral reflectance at 45 blue wavelengths (400–405, 407–433, 436–439, 443–444, 446, 449–452, and 454 nm), but had no effect at the other 656 wavelengths (406, 434–435, 440–442, 445, 447–448, 453, and 455–1100 nm) (Fig. 3.3e).  $\lambda$ -cyhalothrin had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100

nm) recorded at 2, 3, and 8 d after spray (Figs. 3.3f–h). Chlorpyrifos decreased leaf spectral reflectance at 60 blue wavelengths (400–457, 459–460 nm), but had no effect at the other 641 wavelengths (458, 461–1100 nm) at 3 h after spray (Fig. 3.3i). At 2 d after spray, chlorpyrifos had no effect on leaf spectral reflectance at all 701 wavelengths (400–1100 nm) (Fig. 3.3j). At 3 d after spray, chlorpyrifos increased leaf spectral reflectance at 17 red and near-infrared wavelengths (695–711 nm), but had no effect at the other 684 wavelengths (400–694 and 712–1100 nm) (Fig. 3.3k). At 8 d after spray, chlorpyrifos increased leaf spectral reflectance at 164 green, red, and near-infrared wavelengths (517–650 and 689–718 nm), but had no effect at the other 537 wavelengths (400–516, 651–688, and 719–1100 nm) (Fig. 3.3l).

### **3.5. Discussion**

Previous studies have shown that insecticide residues on food, feed, and plant surfaces can significantly affect spectral data (Makio et al. 2007, Nansen et al. 2010, Sánchez et al. 2010). However, potential confounding effects of foliar insecticides on the use of spectral data for detection of plant stressors, such as insects, have been assumed to be negligible (Luedeling et al. 2009, Reisig and Godfrey 2010, Alves et al. 2015). The results presented here indicate that commonly-used foliar insecticides may have implications for subsequent use of spectral data for pest detection in soybean.

Changes to leaf spectral reflectance that occurred within hours after insecticide application were attributed to effects of residues on leaf surfaces. Although morpho-physiological responses of soybean plants to insecticides are not well understood, related

studies with other crops and stressors suggested that plant responses are detected after 24–48 h from initiation of the stress (Oosterhuis et al. 1990, Haile et al. 1999). Therefore, the significant effects observed at 3 h after spray were unlikely to be caused by plant morpho-physiological responses. In general, leaf surface residues affected plant reflectance at few wavelengths within the visible spectral range shortly after application. For example, sulfoxaflor increased leaf reflectance at a small portion of wavelengths in the red spectral range in the greenhouse trial at 3 h after spray (Fig. 3.1a), but this effect lost significance and disappeared in the following six days (i.e., up to 7 d after spray) (Figs. 3.1b–e). Under field conditions, sulfoxaflor leaf surface residues had no effect on leaf reflectance in both field trials (Figs. 3.2a and 3.3a). We speculated that photodegradation of insecticide molecules under direct sunlight exposure may be responsible for the differing results between greenhouse and field conditions (Katagi 2004). There were also instances that the residues of  $\lambda$ -cyhalothrin and chlorpyrifos did not affect soybean leaf reflectance in the greenhouse (Figs. 3.1f and k), but decreased leaf reflectance in the field 2015 (Figs. 3.3e and i). Therefore, the effects of residues of foliar insecticides on plant surfaces is complex and may depend on factors other than sunlight [e.g., temperature (Gonias et al. 2008) and leaf ultrastructure (Hull 1970)].

Changes to leaf spectral reflectance observed at 1 d or later after insecticide spray may be attributed to morpho-physiological changes on soybean plants. In general, foliar insecticides appear to affect soybean morpho-physiology, but the effects depended on the insecticide, environmental conditions, and time after spray. The physiological processes involved in the interaction between plants and pesticides were reviewed by Mitchell and

Linder (1963), Haynes and Goh (1977), and Wang and Liu (2007). Decreased red reflectance at 10 d after spray in the greenhouse (Fig. 3.1f) and decreased blue reflectance at 1 d after spray in the field 2014 (Fig. 3.2b) may be attributed to potential morpho-physiological responses induced by sulfoxaflor because these significant effects were preceded by non-significant effects at earlier sampling periods. Decreasing plant reflectance of red or blue wavelengths is usually associated with increasing chlorophyll content (Sims and Gamon 2002, Gitelson et al. 2003). The possibility of higher photosynthetic pigment content on plants sprayed with sulfoxaflor relative to the untreated plants may suggest positive plant response to the insecticide spray. However, follow-up research is required to confirm if this is the case. A similar upregulation of plant physiology was observed after application of an insecticide with similar mode of action referred to as neonicotinoid (Gonias et al. 2008, Ford et al. 2010). Such upregulation of plant physiological response is likely a temporary condition that may be weak or of insufficient duration to affect yield (Abdel-Reheem et al. 1991, Haile et al. 1999). The physiological effects of foliar insecticides on plants may be characterized by an initial change in physiology that may affect photosynthetic efficiency and a later recovery period in which insecticide molecules are metabolized and inactivated (Oorschot 1979).

$\lambda$ -cyhalothrin and chlorpyrifos also affected reflectance in a manner suggesting an induction of plant morpho-physiological responses. The increasing near-infrared reflectance induced by  $\lambda$ -cyhalothrin (Fig. 3.2j) may be associated with increased intercellular air spaces caused by pyrethroid insecticides on soybean (Bashir et al. 2014).

Although pyrethroids can also decrease soybean chlorophyll content (Bashir et al. 2014), we did not observe significant effects of  $\lambda$ -cyhalothrin on wavelengths influenced primarily by chlorophyll (i.e., visible wavelengths). Chlorpyrifos did not appear to affect morpho-physiological responses in the greenhouse (Figs. 3.1n–r) or field in 2014 (Figs. 3.2l–o), but may have affected plant morpho-physiology in the field in 2015 because of the increased reflectance of visible and near-infrared wavelengths (Figs. 3.3k and l). Increased red reflectance due to chlorpyrifos application may have resulted from decreased chlorophyll content (Sims and Gamon 2003, Parween et al. 2013). Increased near-infrared reflectance due to chlorpyrifos application may have resulted from changes in leaf ultrastructure (e.g., intercellular air spaces) (Knipling 1970, Grant 1987). Increased near-infrared reflectance may also have resulted from increased production of secondary metabolites after insecticide application (Urlaub et al. 1998, Baranska et al. 2004, Karageorgou and Manetas 2006), but this potential effect of chlorpyrifos on soybean will need further investigation.

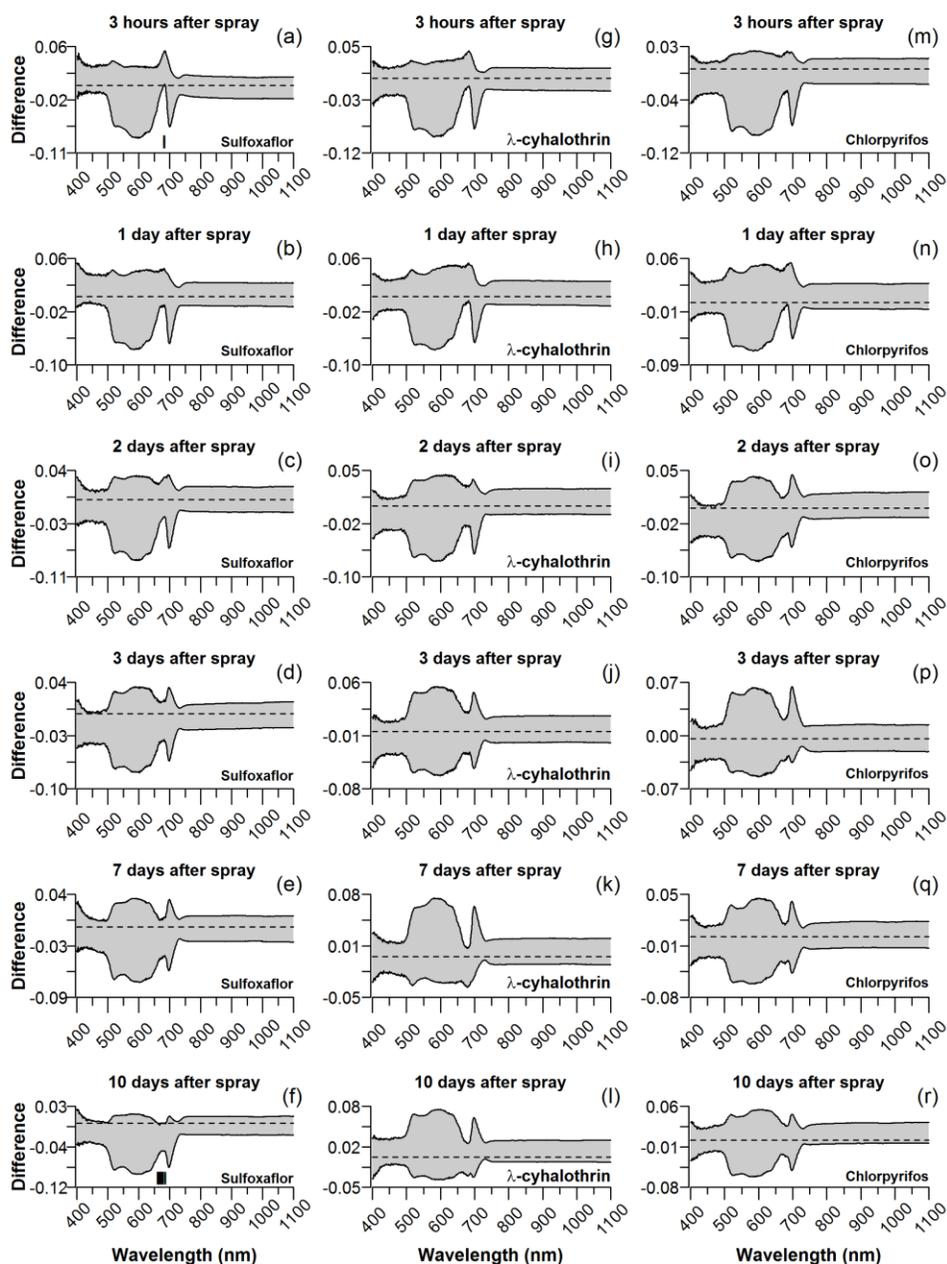
In conclusion, leaf-level reflectance of soybean plants was affected by single applications of foliar insecticides. Immediate effects on reflectance were likely to have been caused by leaf surface residues, while reflectance effects recorded after 1–2 d may have been linked to morpho-physiological effects due to chemical absorption. To prevent the potential confounding effects of leaf surface residues, spectral data could be collected after 1–2 d from an insecticide application. The morpho-physiological effects of foliar insecticides may pose greater limitations to the use of plant spectral data for detection of plant stress because there was no consistent pattern of spectral changes associated with

the insecticides. A possible solution for using spectral data for detection of plant stress after insecticide application is the use of hyperspectral data and selection of wavelengths that optimize the differentiation between insect- and insecticide-induced changes in reflectance. For example, some promising wavelengths to detect soybean aphid (i.e., 680 and 800 nm) (Alves et al. 2015) and *Mungbean yellow mosaic virus* (i.e., 750–1,400 nm) (Gazala et al. 2013) were not affected by the insecticides used in our trials. Further research will be needed to determine if the ground-based, leaf-level effects documented in our study can be detected from other platforms (e.g., UAS and satellites). Caution must be used in extrapolating our conclusions to different platforms because spectral effects may differ between leaf and canopy levels (e.g., Alves et al., 2015). Understanding the effects of pesticide-induced changes on plant reflectance will be crucial for the development of pest management programs using spectral reflectance.

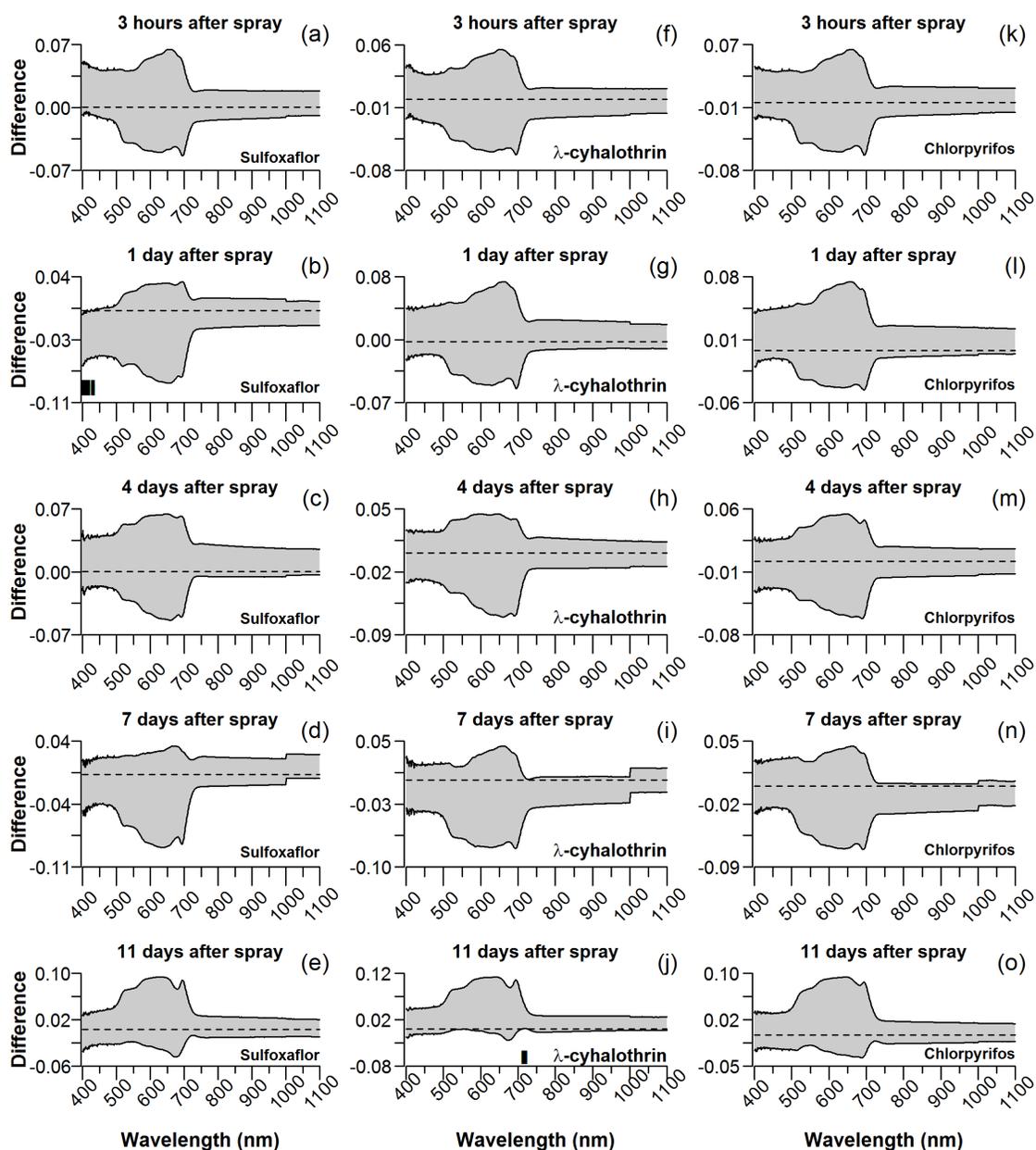
### **3.6. Acknowledgements**

We thank Dr. Fikru Haile for reviewing an earlier version of this chapter and Dr. Brian Aukema for helping with the statistical analysis. We also thank Wally Rich and Daniela Pezzini for helping with data collection and Randy Gettle for technical assistance and provision of the FieldSpec 4 Hi-Res spectroradiometer. This work was supported by the Minnesota Soybean Research and Promotion Council, National Council for Scientific and Technological Development (CNPq/Brazil), and Analytical Spectral Devices (ASD Inc.).

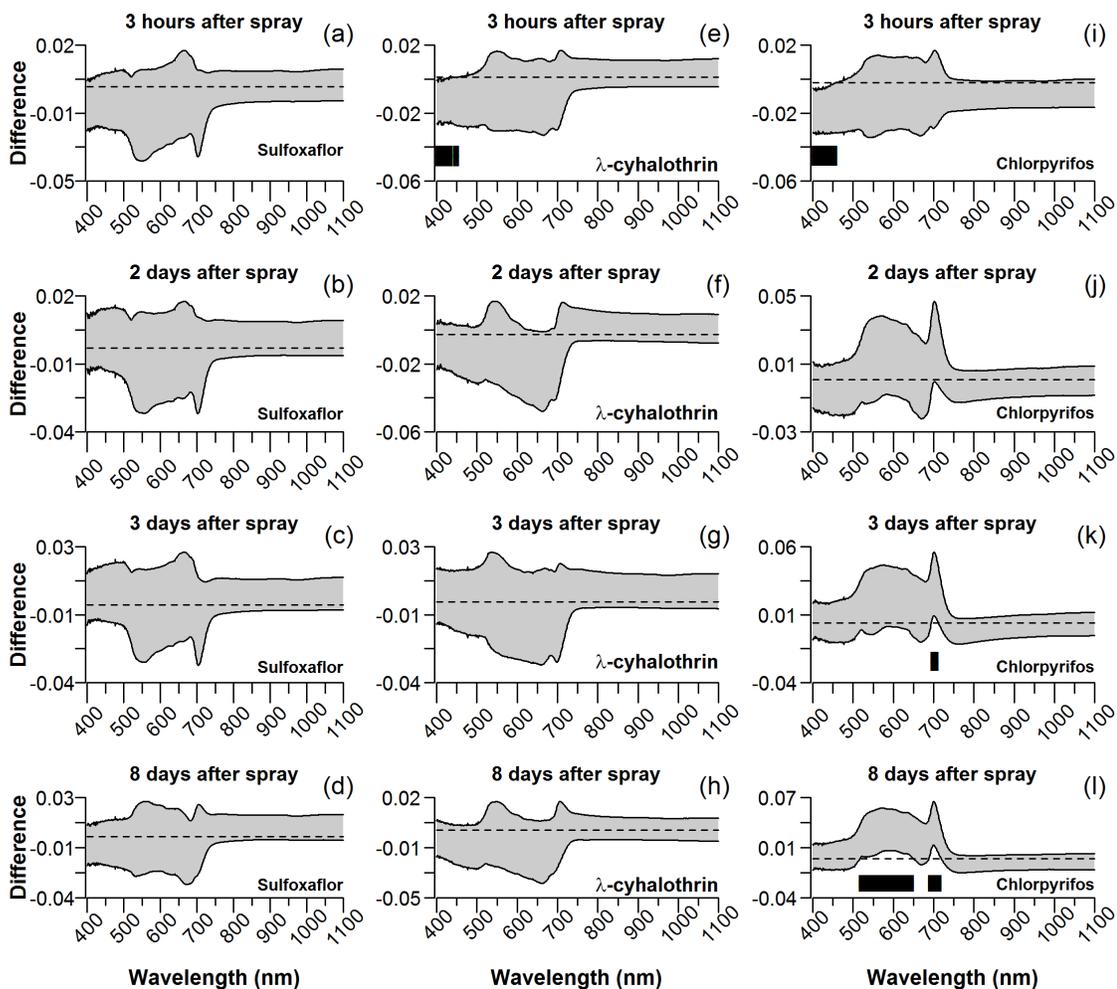
## 3.7. Figures



**Fig. 3.0.1.** Effect of foliar insecticides on soybean reflectance in greenhouse trial. Log<sub>10</sub>-transformed confidence limits of the difference (T-U) between leaf reflectance of insecticide-treated (T) and untreated (U) plants across visible and near-infrared wavelengths recorded over six evaluation periods. Black bars (I) at the bottom of the graphs indicate 1-nm wavelength bands that one application of insecticide treatment had a significant effect on leaf spectral response (i.e., confidence limits are not overlapping zero, Dunnett's test,  $P \leq 0.05$ ). Dashed horizontal lines mark the zero value for the y-axis.



**Fig. 3.0.2.** Effect of foliar insecticides on soybean reflectance in field trial in 2014. Log<sub>10</sub>-transformed confidence limits of the difference (T-U) between leaf reflectance of insecticide-treated (T) and untreated (U) plants across visible and near-infrared wavelengths recorded over six evaluation periods. Black bars (I) at the bottom of the graphs indicate 1-nm wavelength bands that one application of insecticide treatment had a significant effect on leaf spectral response (i.e., confidence limits are not overlapping zero, Dunnett's test,  $P \leq 0.05$ ). Dashed horizontal lines mark the zero value for the y-axis.



**Fig. 3.3.** Effect of foliar insecticides on soybean reflectance in field trial in 2015. Log<sub>10</sub>-transformed confidence limits of the difference (T-U) between leaf reflectance of insecticide-treated (T) and untreated (U) plants across visible and near-infrared wavelengths recorded over six evaluation periods. Black bars (I) at the bottom of the graphs indicate 1-nm wavelength bands that one application of insecticide treatment had a significant effect on leaf spectral response (i.e., confidence limits are not overlapping zero, Dunnett's test,  $P \leq 0.05$ ). Dashed horizontal lines mark the zero value for the y-axis.

#### 4. Dissertation Conclusions

Soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) is the primary insect pest of soybean in the northcentral United States. Soybean aphid may cause stunted plants, leaf discoloration, plant death, and decrease soybean yield by 40%. Sampling plans have been developed for supporting soybean aphid management. However, growers' perception about time involved in direct insect counts has been contributing to a lower adoption of traditional pest scouting methods and may be associated with the use of prophylactic insecticide applications in soybean. Remote sensing of plant spectral (light-derived) responses to soybean aphid feeding is a promising alternative to estimate injury without direct insect counts and, thus, increase adoption and efficiency of scouting programs. This research explored the use of remote sensing of soybean reflectance for detection of soybean aphids and showed that foliar insecticides may have implications for subsequent use of soybean spectral reflectance for pest detection.

Chapter 1 was the first publication showing that feeding from soybean aphid affects soybean spectral reflectance. Using ground-based spectroradiometers at canopy-level, it was found that soybean aphids affected plant reflectance at a narrowband wavelength within the near-infrared spectral range (800 nm), but had no effect at a red narrowband wavelength (680 nm). Soybean aphids also affected a vegetation index referred to as NDVI (i.e., normalized difference vegetation index), which combines the near-infrared and red wavelengths into a value representing photosynthetic pigment content and potential ultrastructure changes in soybean leaves. In general, soybean aphids

induced similar effects on canopy- and leaf-level spectral measurements, but there were a few instances that significant effects at leaf-level were not detected by canopy-level.

Chapter 2 used hyperspectral data and simulated wide-band sensors for detection of soybean aphid. While the first chapter showed that remote sensing is a promising solution based on results from two narrowband wavelengths, the second chapter provided an extensive search for band sensors that could optimize the use of soybean canopy reflectance for soybean aphid detection. Akaike's Information Criteria (AIC) was used to rank and select sensors. Lower AIC values were considered to provide better models. The subset of narrowband wavelengths that optimized estimation of soybean aphid abundance was similar to that obtained using simulated wide-band sensors. Increasing sensor bandwidth corresponded to larger AIC values (worse models). The smallest AIC values (better models) were observed with narrow- and wide-band sensors centered around 780 nm.

Chapter 3 assessed effects of foliar insecticides on spectral response of soybean plants under greenhouse and field conditions. Such effects could potentially confound measures of pest-induced spectral changes. Representatives of the two most commonly used insecticides (i.e., chlorpyrifos and  $\lambda$ -cyhalothrin) and a novel active ingredient referred to as sulfoxaflor affected soybean leaf reflectance.  $\lambda$ -cyhalothrin had the least effect on spectral reflectance and only affected a few near-infrared wavelengths, but sulfoxaflor and chlorpyrifos affected leaf reflectance at several visible and near-infrared wavelengths. I speculated that foliar insecticides had immediate effects via surface residues on plants and delayed effects via morpho-physiological changes induced by the

insecticides. The potential leaf surface residues had transitory effects on soybean reflectance and no consistent pattern of spectral changes was associated with the insecticides.

Overall, my results hold promise to identify and characterize injury of soybean aphid using remote sensing of soybean canopy reflectance. The information provided in this research may help to design optimized sensors for soybean aphid detection and contribute to the understanding of insect- and insecticide-induced effects on plants. It may also improve the current field-wide management tactics by making decisions for pest control when plant spectral reflectance indicates that soybean aphid abundance reached its economic threshold. To incorporate remote sensing into IPM programs, this new scouting method based on plant spectral reflectance will need further research to adjust economic thresholds, application of insecticides with no or short-duration effects on plant spectral data, and better understanding of other plant-pest interactions affecting plant morpho-physiology. It will be important to distinguish spectral changes induced by soybean aphid from other confounding factors such as other herbivores, nutritional deficiencies, diseases, and water stress. Future research will be needed to determine if the ground-based effects documented in our studies can be detected from space- and air-based platforms, such as satellites and unmanned aerial systems. Moreover, advancing our results may contribute to determine where and when insecticides are needed by using the spatial location of soybean spectral responses to soybean aphid infestations. Remote sensing has the potential to expand the use of IPM practices and collaborate to the

mission of feeding an increasing population that has been changing diet habits and will require more production of food.

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