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Detection of Stress Induced by Soybean Aphid (Hemiptera: Aphididae) Using Multispectral Imagery from Unmanned Aerial Vehicles

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Abstract

Soybean aphid, Aphis glycines Matsumura (Hemiptera: Aphididae), is a common pest of soybean, Glycine max (L.) Merrill (Fabales: Fabaceae), in North America requiring frequent scouting as part of an integrated pest management plan. Current scouting methods are time consuming and provide incomplete coverage of soybean. Unmanned aerial vehicles (UAVs) are capable of collecting high-resolution imagery that offer more detailed coverage in agricultural fields than traditional scouting methods. Recently, it was documented that changes to the spectral reflectance of soybean canopies caused by aphid-induced stress could be detected from groundbased sensors; however, it remained unknown whether these changes could also be detected from UAV-based sensors. Small-plot trials were conducted in 2017 and 2018 where cages were used to manipulate aphid populations. Additional open-field trials were conducted in 2018 where insecticides were used to create a gradient of aphid pressure. Whole-plant soybean aphid densities were recorded along with UAV-based multispectral imagery. Simple linear regressions were used to determine whether UAV-based multispectral reflectance was associated with aphid populations. Our findings indicate that near-infrared reflectance decreased with increasing soybean aphid populations in caged trials when cumulative aphid days surpassed the economic injury level, and in open-field trials when soybean aphid populations were above the economic threshold. These findings provide the first documentation of soybean aphid-induced stress being detected from UAV-based multispectral imagery and advance the use of UAVs for remote scouting of soybean aphid and other field crop pests.

Key words: remote sensing, reflectance, unmanned aerial vehicle, multispectral, crop scouting

Soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), is currently the most widely grown field crop in the United States, with 36.5 million hectares producing over 120 million metric tons of grain in 2017 (USDA-NASS 2018). The United States continues to lead the world in soybean production and the north-central United States accounts for over 75% of the nation's production (USDA-NASS 2018). Over the past two decades, however, there has been a dramatic change in soybean production in the north-central United States, due to an invasive species, the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). Prior to the invasion by soybean aphid in 2000, there were few insects reaching levels causing economic injury to soybean and fewer than 0.1% of soybean fields in the north-central United States were sprayed with insecticide (Ragsdale et al. 2011, Hodgson et al. 2012). However, by 2006, there was more than

a 130-fold increase of insecticide applications to soybean in the region (Ragsdale et al. 2011, Hodgson et al. 2012). This increase was largely due to the soybean aphid's ability to rapidly reproduce and reduce soybean yields (Beckendorf et al. 2008, Ragsdale et al. 2011).

Soybean aphid damages soybean by inserting piercing-sucking mouthparts into the phloem of the plants to extract photosynthate (Tilmon et al. 2011). This feeding can decrease yield through plant stunting, decreased leaf area, reduced pod and seed number, decreased seed weight and oil concentrations, and even plant death (Hill et al. 2004, Mensah et al. 2005, Ragsdale et al. 2007, Beckendorf et al. 2008). Furthermore, soybean aphids excrete honeydew on leaf surfaces while feeding, which can promote the growth of sooty mold (Tilmon et al. 2011) and further reduce yields by inhibiting photosynthesis (Hill et al. 2010). Soybean aphid infestations left untreated have been documented to reduce soybean yields by more than 40% (Ragsdale et al. 2007). Soybean aphid is considered the most economically important insect pest of soybean in the north-central United States (Ragsdale et al. 2007, Hurley and Mitchell 2017) and considerable research has been performed to develop cost-effective management strategies for soybean aphid (Hodgson et al. 2004, 2007, 2012; Ragsdale et al. 2007; Ragsdale et al. 2011).

Current management recommendations involve routine scouting of soybean fields to monitor soybean aphid populations (Hodgson et al. 2004). Routine scouting is needed because widespread outbreaks of soybean aphid are erratic, and the timing of colonization can fluctuate (Hodgson et al. 2012). When aphid populations reach an economic threshold of 250 aphids per plant, chemical control is recommended to prevent aphids from reaching the economic injury level of ~674 aphids per plant (Ragsdale et al. 2007, Koch et al. 2016). While many farmers follow these recommendations, some farmers are reluctant to adopt these practices because the scouting process can be arduous and time consuming (Bueno et al. 2011). A more efficient binomial sampling plan was established in 2004, but further testing of this method found that correct management decisions were attained only 79% of the time, the other 21% of the time decisions to apply insecticide were made before aphid populations reached the economic threshold (Hodgson et al. 2004, 2007; Ragsdale et al. 2011). Furthermore, current scouting practices do not provide complete coverage of a field creating the potential to miss areas heavily infested with soybean aphid. The difficulty associated with counting aphids within a large field of densely planted soybean and the lack of coverage provided by current scouting methods has led some farmers to use prophylactic applications of insecticides rather than base chemical treatment on estimates of aphid populations in the field (Olson et al. 2008). This prophylactic method of control can increase production costs and risk for development of insecticide resistance, and be detrimental to nontarget organisms and water quality (Song and Swinton 2009, Bueno et al. 2011, Koch et al. 2016). Incorporating remote sensing offers the potential to improve management of soybean aphid by decreasing the effort and cost of scouting while increasing field coverage, which may increase adoption of management practices based on estimates of in-field pest abundance and thereby decrease unnecessary pesticide applications.

Remote sensing has been used to provide valuable insight into crop management for over 50 yr (Hatfield et al. 2008). Remote sensing for agriculture passively obtains information about within field variability in the health of a crop by relating electromagnetic, or spectral, reflectance to plant biological components and physiology, such as foliar pigment content, cellular structure, water content, as well as canopy coverage and architecture (Pinter et al. 2003). One of the most commonly used spectral indices for remote sensing in agriculture is the normalized difference vegetation index (NDVI). The NDVI is particularly helpful because it combines red reflectance with near-infrared reflectance (NIR). Red reflectance is an indicator of chlorophyll content of the plant canopy and active photosynthesis; and NIR provides information about the cellular structure and intracellular air spaces within leaves, overall canopy coverage, and above ground biomass (Hatfield et al. 2008). When these wavelengths are combined in an index, like NDVI, it provides a measure of overall plant health and has frequently been correlated with crop yield (Ma et al. 2001).

In soybean, different forms of crop stress, such as nutrient deficiencies (Milton et al. 1991, Bai et al. 2018), soybean cyst nematode, *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae) (Nutter et al. 2002, Bajwa et al. 2017), soybean sudden death syndrome, *Fusarium virguliforme* O'Donnell & Aoki (Hypocreales:

Nectriaceae) (Bajwa et al. 2017, Hatton et al. 2017, Hatton 2018, Herrmann et al. 2018, Menke 2018), weed pressure (Koger et al. 2003, Chang et al. 2004, Henry et al. 2004, Gray et al. 2009), and drought stress (Pinter et al. 2003, Jackson et al. 2004, O'Shaughnessy et al. 2011) affect the spectral reflectance of the plants, which can be detected through remote sensing. The biophysical principles behind remote sensing have remained generally consistent over the past 50 yr; however, the technology used to record this information has not. Previously, remote sensing in agriculture used either ground-based systems, which are often restricted by small mapping swaths and limited transportability, or satellites and piloted aircraft which have been expensive, low-resolution, and limited by atmospheric conditions and orbital periods. (Lelong et al. 2008, Zhang and Kovacs 2012). More recently, however, unmanned aerial vehicles (UAVs) equipped with ultra-high spatial resolution multispectral sensors have become increasingly available to consumers and promise lowcost near real-time image acquisition for use in agricultural applications (Nebiker et al. 2008).

Recently, it was reported ground-based remote sensing of soybean was capable of detecting stress to plants caused by soybean aphid (Alves et al. 2015, 2019). However, it remains unknown if the stress caused by soybean aphid to soybean can be detected from UAV-based sensors. Therefore, the goal of this research was to determine whether soybean aphid-induced stress can be detected from UAV-based multispectral sensors. The results of this research will help to identify how UAV-based remote sensing can be incorporated into current crop scouting practices to improve scouting efficiency and adoption of IPM strategies.

Materials and Methods

Caged-Plot Experiments

Research trials were conducted in 2017 and 2018 at both the University of Minnesota Outreach, Research, and Education Park in Rosemount, MN (44° 44' 1.2804" N, 93° 5' 4.2288" W) and at the Iowa State University Northern Research Farm in Kanawha, IA (42° 55′ 51.3408″ N, 93° 47′ 32.4168″ W) (Table 1). Twentyfour plots were established at each location in both years. Each plot was created by planting soybean in two 2.5-m-long rows with 76.2cm row spacing at a seeding rate of 345,000 seeds per hectare. Of these 24 plots, a subset of 12 plots were selected at each location that were not inoculated with pathogens at the time of planting. In Rosemount, soybean variety MN1410R2F5-121 was planted on 8 May 2017 and 10 May 2018. In Kanawha, soybean variety Syngenta S24-K2 was planted on 24 April 2017 and 18 May 2018. No fertilizer was applied at either location and weeds were managed by applying pre-emergent herbicide followed by hand weeding after growth stage VE (Fehr and Caviness 1977). At growth stage V3, plants were carefully inspected for soybean aphids, any aphids found were recorded and removed from plants either by hand or with an insecticide treatment, then PVC frames $(1.5 \times 2.5 \text{ m})$ were placed over each plot and covered with NO-SEE-UM mesh cages (Quest Outfitters, Sarasota, FL) to prevent aphid colonization. In Rosemount in both 2017 and 2018, soybean aphids infested the plots before cages were placed in the field. To remove aphids in 2017, all plots were sprayed with λ -cyhalothrin (116 ml product per ha, Warrior II with Zeon Technology, Syngenta, Greensboro, NC) on 15 June and again with a formulated mixture of λ -cyhalothrin and thiamethoxam (328 ml product per ha, Endigo ZC, Syngenta, Greensboro, NC) on 27 June. To remove aphids in 2018, all plots were sprayed with a formulated mixture of λ -cyhalothrin and thiamethoxam (328 ml product per ha, Endigo ZC, Syngenta) on

Experiments	Location	Treatments	Sample Size	Year	Sample Dates
Caged-Plot Trials	Rosemount, MN	Aphid infested vs. uninfested	<i>n</i> = 12	2017	8 and 22 Aug.
	Rosemount, MN	Aphid infested vs. uninfested	<i>n</i> = 11	2018	8 and 13 Aug.
	Kanawha, IA	Aphid infested vs. uninfested	<i>n</i> = 12	2018	10 and 22 Aug.
Open-Field Trials	Rosemount, MN Plot 1	Treated with insecticide vs. Untreated	<i>n</i> = 16	2018	15 and 22 Aug.
	Rosemount, MN Plot 2	Treated with insecticide vs. Untreated	<i>n</i> = 16	2018	15 and 22 Aug.

 Table 1. Description of experimental details used to determine whether soybean aphid-induced stress could be detected with UAV-based remote sensing

1 June. Cages were inspected weekly to ensure plants stayed free of aphids until the planned infestation.

The 12 plots at each location were divided into two treatments, aphid-free and aphid-infested, in a completely randomized design. In both 2017 and 2018, the aphid-infested treatment was infested with soybean aphids at growth stage R3. In 2017, the infestation procedure consisted of infesting each plot with 200 mixed-stage aphids (i.e., nymphs + wingless adults) on 17 July in both locations. Due to poor aphid establishment in infested cages in Kanawha in 2017, each infested plot in 2018 received 400 mixed-stage aphids on 16 July in Rosemount and 18 July in Kanawha.

These infestations were accomplished by pinning leaf cuttings, each with 25 mixed-stage soybean aphids, to the abaxial side of the uppermost fully expanded trifoliate of plants within the caged plots. These infested leaf cuttings were evenly spaced throughout the plot. In 2017, each infested plot received 8 leaf cuttings and in 2018 each infested plot received 16 leaf cuttings. Soybean aphids were taken from a laboratory colony to infest cages at both locations in 2017 and in Kanawha in 2018. Several caged plots in Rosemount in 2018 were blown open during a storm on 17 June, and consequently three plots were naturally infested with soybean aphid before the intentional infestation. While two of these plots had fewer than 60 aphids per plant on 9 July, one plot exceeded the economic threshold (i.e., 250 aphids per plant) and was removed from the experiment, resulting in 6 aphid-infested plots and 5 aphid-free plots in Rosemount in 2018. Aphids from the plot that was removed from the experiment were used to artificially infest the remaining aphid-infested plots at that location in 2018.

Before and after infestation, aphid densities for each plot were estimated weekly from 6 June to 23 August 2017 in Rosemount and 13 June to 23 August 2017 in Kanawha. In 2018, aphid densities for each plot were estimated weekly from 25 June to 13 August 2018 in Rosemount and 5 July to 22 August 2018 in Kanawha. In order to assess the aphid populations in each plot, the fine-mesh cages were temporarily removed to allow counting and were replaced after the counts were recorded. The two rows of each plot were visually divided into four evenly spaced sections and one plant was randomly selected from each section of row, for a total of eight plants per plot. The number of aphids on each of these plants was assessed by nondestructive, whole-plant counts. Caution was taken to minimize the risk of inadvertent transfer of aphids to aphid-free plots.

Open-Field Experiments

Open-field experiments were conducted in 2018 at the University of Minnesota Outreach, Research, and Education Park in Rosemount, MN (Table 1). A commercial field was planted on 17 May 2018 with soybean variety Asgrow-AG1435 at a seeding rate of 368,000 per hectare and 76.2-cm row spacing. No fertilizer was applied, and weeds were managed by applying labeled rates of pre-emergent herbicide on 21 May 2018, and postemergent herbicide on 22 June 2018. Any weeds found within the sample area after postemergent herbicide application were removed by hand to ensure there were no weeds present during image acquisition. Two 0.4-hectare plots were established by tilling a 1.5-m alley around uniform areas within the field on 13 August. When soybean aphid densities reached an average of 250 aphids per plant, a strip measuring 15.9-m by 63.6-m in each of these 0.4-hectare plots was sprayed with a formulated mixture of λ -cyhalothrin and thiamethoxam (328 ml product per ha, Endigo, Syngenta) to create different levels of aphids within each plot.

A 16-cell grid was created within each 0.4-hectare plot with each cell measuring 15.9×15.9 m. Within each cell, aphid densities were estimated from 8 to 10 plants selected in a stratified random method on 15 and 22 August 2018. The number of aphids on each plant were assessed by destructive, whole-plant counts. The global positioning system (GPS) coordinates of each plant counted for aphids was recorded with a handheld GPS unit (GPSmap 62s, Garmin Ltd, Olathe, KS) with a GPS signal accuracy of less than 3 m.

Spectral Reflectance Measurements

Canopy spectral reflectance measurements were recorded weekly from caged plots at both locations between 8 and 22 August in 2017 and 2018. Canopy spectral measurements were recorded from 0.4-hectare open plots on 15 and 22 August 2018. Imagery was recorded with a nadir-facing multispectral camera (Quad Multispectral Sensor, Sentera Inc., Minneapolis, MN) mounted on an UAV (Solo, 3DR, Berkeley, CA). The multispectral camera was attached to the UAV via a vibration plate to minimize distortion in the imagery caused by UAV movement. The multispectral camera was equipped with a standard red, green, and blue color light sensor (1.2MP CMOS RGB), and customized to include a narrowband red sensor (1.2MP CMOS Mono 675 ± 12.5 nm), a narrowband nearinfrared sensor (1.2MP CMOS Mono 775 ± 12.5 nm), and a broadband near-infrared sensor (1.2MP CMOS Mono 825 ± 100 nm). These customized bands were selected based on previous groundbased remote sensing work for soybean aphid, and preliminary analysis of band simulation and optimization for soybean aphid (Alves

et al. 2015, 2019). All sensors had global shutter, a lens focal length of 6.05 mm, and were set to autoexposure during image capture. Flights were automated using the open-source software, Mission Planner (available and maintained ardupilot.org), and performed in a cross-grid pattern with 80% forward overlap and 70% sidelap, at an altitude of 50 m in 2017 (3.2-cm Pixel GSD), and at 40 m in 2018 (2.5-cm Pixel GSD) on both the caged and open plots following recommendations included in the Sentera documentation packet (Quad Multispectral Sensor Documentation Packet, Sentera Inc.).

Imagery was recorded within 2.5 h of solar noon to minimize the effect of solar angle and shadowing on the crop canopy. Furthermore, all imagery was recorded after canopy closure to minimize the effect of bare ground and shadowing within the spectral measurements. All caged plots and open fields were also scouted prior to image acquisition to ensure there were no other confounding factors within the sampled areas such as disease, drought stress, nutrient deficiency, or other common stressors.

In an attempt to minimize atmospheric effects on the recorded imagery, flights were only flown when light conditions were uniform, such as cloudless days or at times when no visible clouds were moving between the sun and the crop canopy. To avoid potential damage to plants or handling effects, imagery was recorded immediately after the mesh cages were removed from the caged-plots and before performing aphid counts and all aphid counts in the open fields were recorded either the day before or the day after UAV-based spectral measurements were taken. Reference panels with known reflectance properties were placed in the field prior to each flight to aid in converting camera Digital Number (DN) values to relative reflectance through the empirical line method (Smith and Milton 1999).

Image Processing

Before images were processed, each image was visually inspected for quality ensuring no hotspots or banding was observed in the imagery. Imagery was then normalized for autoexposure, stitched into orthomosaics, and converted to relative reflectance. Prior to correction for autoexposure, images were cast from 8-bit depth to 16-bit depth in MATLAB Image Processing Toolbox (MathWorks Inc., Massachusetts) to avoid saturation during autoexposure correction. Images were then corrected for exposure time, digital gain, and analog gain using;

 $Normalized DN = \frac{DN}{(Exposure Time * Digital Gain * 2^{Analog Gain})}$

(Quad Multispectral Sensor Documentation Packet, Sentera Inc.).

The images were then stitched using Pix4Dmapper (Pix4D SA, Lausanne, Switzerland) using the camera model parameters recommended within the Sentera documentation packet, and the Sentera template for processing options (Quad Multispectral Sensor Documentation Packet, Sentera Inc.). Coefficients from a linear fit between observed DN and known reflectance of the reference panels were used with the empirical line method for reflectance calibration of the Pix4D generated orthomosaics (Smith and Milton 1999).

Analyses

For the caged-plot experiment, plot reflectance values were extracted from the stitched orthomosaics using ImageJ (Version 1.52k, National Institutes of Health, Bethesda, MD). The area of reflectance extracted from each plot was centered over the middle of the two rows and was the same size for each plot, ensuring not to include pixels of the cage frame around each plot. The aphid counts were converted to cumulative aphid days (CAD), which is a measure of the cumulative aphid stress caused to the plants over time. CAD was calculated following the methods proposed by Ruppel (1983) and adapted for aphids by Hanafi et al. (1989).

We selected narrowband NIR (775 ± 12.5 nm), narrowband red (675 ± 12.5 nm), and the vegetation index NDVI for analyses as these have previously been identified as affected by soybean aphid-induced stress in ground samples (Alves et al. 2015). NDVI was calculated as NDVI=[(NIR-red)/(NIR+red)] (Rouse Jr. et al. 1973). Simple linear regression followed by analysis of variance (ANOVA) (R Development Core Team 2013) was used to determine whether CAD had an effect ($\alpha = 0.05$) on aerially measured spectral reflectance (Alves et al. 2015, 2019). Visual assessment of residual plots indicated log10 transformation of CAD was required to meet statistical assumptions for linear regression analysis.

For the open-field experiments, the cells in each 0.4-hectare plot consisted of 4 cells over the insecticide-treated area, and 12 cells over the untreated area. Only the narrowband NIR (775 \pm 12.5 nm) sensor was used for analysis of the open-field experiments, because it was found to be the optimal band for detecting aphid-induced stress in previous research and was unlikely to be affected by insecticide treatments (Alves et al. 2017, 2019). Reflectance data within each cell were equally cropped on all sides to avoid any edge effects around the borders of the 0.4-hectare plot and between the insecticide-treated area and untreated area. Reflectance values were extracted in R (raster package, Hijmans 2017, R Development Core Team 2013). Only aphid counts within the cells after cropping were averaged to obtain a single average value of aphids per plant for each cell. Simple linear regression followed by ANOVA (R Development Core Team 2013) was used to determine whether average number of aphids per plant had an effect ($\alpha = 0.05$) on spectral reflectance values. CAD was not used for this experiment because aphid counts were taken on too few sample dates. Residual plots were visually inspected to ensure assumptions were met for linear regression analysis.

Results

Caged Plots

On 8 and 22 August 2017 in Rosemount, 8 and 13 August 2018 in Rosemount and 22 August 2018 in Kanawha, aphids surpassed the economic injury level (>6,500 CAD) within the infested cages (Ragsdale et al. 2007). However, on 10 August 2018 at the time spectral measurements were taken in Kanawha, aphids had not reached the economic injury level. Across sites and years, red reflectance was not associated with CAD (Table 2), except for 13 August 2018 in Rosemount where red reflectance increased with increasing CAD (Table 2). On all dates where soybean aphid populations reached the economic injury level, NIR reflectance decreased with increasing CAD (Table 2). On 10 August 2018 in Kanawha, which was before soybean aphid reached the economic injury level, NIR reflectance was not associated with CAD (Table 2). When CAD reached the economic injury level there were also decreases in NDVI values with increasing CAD (Table 2), except in Kanawha on 22 August 2018 where there was a marginal decrease in NDVI values with increasing CAD (Table 2). On 10 August 2018 in Kanawha, there was no association between NDVI and CAD.

Open-Field Experiments

Plot 1 had 347 ± 69 (mean \pm SEM) aphids per plant in the treated areas and 861 ± 42 aphids per plant in the untreated areas on 15 August 2018. On this date, NIR reflectance decreased by 0.5%

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Wavelength/Index	Site	Date	Intercept	Slope	F-value _{df}	P-value	R^2
Red (675 ± 12.5 nm)	Rosemount, MN	8 Aug. 2017	2.927	0.013	0.008(1,10)	0.784	0.008
	Rosemount, MN	22 Aug. 2017	1.755	0.053	$2.755_{(1,10)}$	0.128	0.216
	Rosemount, MN	8 Aug. 2018	2.068	0.089	2.010(1,9)	0.190	0.183
	Rosemount, MN	13 Aug. 2018	0.996	0.175	6.812	0.028*	0.431
	Kanawha, IA	10 Aug. 2018	1.767	-0.010	$0.217_{(1.10)}$	0.652	0.021
	Kanawha, IA	22 Aug. 2018	1.663	7.83×10^{-5}	$0.000_{(1.10)}$	0.997	0.000
NIR (775 ± 12.5 nm)	Rosemount, MN	8 Aug. 2017	73.588	-3.144	10.900(1,10)	0.008*	0.522
	Rosemount, MN	22 Aug. 2017	51.955	-4.331	5.791 _(1,10)	0.037*	0.367
	Rosemount, MN	8 Aug. 2018	71.727	-7.680	$21.800_{(1.9)}$	0.001*	0.708
	Rosemount, MN	13 Aug. 2018	89.487	-11.504	45.250 _(1,9)	< 0.001*	0.834
	Kanawha, IA	10 Aug. 2018	49.253	-0.234	0.186(1,10)	0.676	0.018
	Kanawha, IA	22 Aug. 2018	39.918	-0.980	6.496 _(1,10)	0.029*	0.394
NDVI	Rosemount, MN	8 Aug. 2017	0.925	-5.30×10^{-3}	5.400(1,10)	0.042*	0.351
$\left(\frac{NIR Red}{NIR \ + \ Red}\right)$	Rosemount, MN	22 Aug. 2017	0.968	-0.023	7.373	0.022*	0.424
	Rosemount, MN	8 Aug. 2018	0.982	-0.026	17.550(1,10)	0.002*	0.661
	Rosemount, MN	13 Aug. 2018	1.045	-0.033	$26.770_{(1.9)}$	< 0.001*	0.748
	Kanawha, IA	10 Aug. 2018	0.930	1.58×10^{-5}	$0.000^{(1,9)}_{(1,10)}$	0.92	0.000
	Kanawha, IA	22 Aug. 2018	0.920	-0.002	4.389(1,10)	0.063	0.305

Table 2. Model estimates from simple linear regressions for the effect of log10-transformed CAD on soybean canopy red reflectance, NIR, and NDVI recorded from an UAV from caged-plots in Rosemount, MN, 2017, and Rosemount, MN and Kanawha, IA, 2018

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

Table 3. Model estimates from simple linear regressions for the effect of soybean aphid density (aphids per plant) on soybean canopy NIR
recorded by UAV from open fields in Rosemount, MN, 2018

Wavelength	Site	Date	Intercept	Slope	<i>F</i> -value _{df}	P-value	\mathbb{R}^2
NIR	Plot 1	15 Aug. 2018	58.286	-0.005	11.929(1.14)	0.004*	0.460
775 ± 12.5 nm	Plot 1	22 Aug. 2018	55.562	-0.011	78.900	< 0.001*	0.849
	Plot 2	15 Aug. 2018	54.570	-0.003	$3.723_{(1,14)}^{(1,14)}$	0.074	0.210
	Plot 2	22 Aug. 2018	60.945	-0.010	$10.668_{(1,14)}^{(1,14)}$	0.006*	0.432

*Indicates a significant effect of soybean aphid density on soybean canopy reflectance ($\alpha = 0.05$). Otherwise, soybean aphid density had no significant effect on reflectance.

per 100 aphids per plant (Table 3). On 22 August 2018 in plot 1, the treated area had 81 ± 27 aphids per plant on average, while the untreated area had 798 \pm 31 aphids per plant. On this date, NIR reflectance decreased by 1.0% per 100 aphids per plant (Table 3).

In plot 2, the treated portion of the plot had 226 ± 53 aphids per plant and the untreated portion had 665 ± 82 aphids per plant on average on 15 August 2018. On this date, NIR reflectance decreased marginally by 0.3% per 100 aphids per plant (Table 3). On 22 August 2018 in plot 2, the treated portion of the plot had 119 ± 24 aphids per plant, and the untreated portion of the plot had 400 ± 41 aphids per plant. On this date, NIR reflectance decreased by 1.1% per 100 aphids per plant (Table 3).

Discussion

The use of UAVs for agriculture has immense potential to improve decision making for crop management by providing high temporal and spatial resolution information on soils, crop nutrients, pests, moisture, and yield (Canis 2015). Previous research showed the potential for use of remote sensing for soybean aphid through ground-based research (Alves et al. 2015, 2019). The findings from the present experiments provide the first documentation of UAV-based remote detection of soybean aphid-induced stress in soybean. Further work is needed to determine whether ground-based remote

sensing for other pests and cropping systems may also extend to UAV-based approaches.

These findings advance the use of UAVs and remote sensing as actionable tools for scouting soybean aphid. On caged soybean plants, soybean aphid caused significant decreases in NIR reflectance and NDVI, but there were generally no changes to red reflectance, except for in the caged-plot experiment on 13 August 2018. These results, showing a decrease in NIR reflectance and NDVI caused by soybean aphid-induced stress, agree with previous ground-based remote sensing work on soybean aphid (Alves et al. 2015, 2019) and are consistent with remote sensing findings of other species of aphids and other hemipterans in grain crops (Mirik et al. 2007; Elliott et al. 2007, 2009, 2015; Prabhakar et al. 2011, 2013). When significant relationships were detected between CAD and reflectance, aphid populations were relatively high (i.e., above EIL). Because significant associations in the caged experiment were only observed when aphid populations exceeded the EIL, using linear regressions of spectral data for detecting soybean aphid-induced stress may not identify stress early enough to make actionable decisions and prevent economic injury. Further work is required to determine whether actionable decisions can be made from this research, specifically, it needs to be determined if these measured changes in reflectance can be used to classify aphid pressure as above or below treatment thresholds and differentiate aphid-induced stress from other stressors. Currently, these findings suggest that remote sensing may aid in

conventional scouting by directing the field scout to areas with decreased NIR reflectance.

The use of insecticides or cages to manipulate pest populations in remote sensing experiments may incorporate potential confounding factors by affecting the relevance of the results to production conditions. In a recent experiment, Alves et al. (2017) found that certain insecticides can have an effect on leaf-level reflectance in the visible portion of the spectrum, but they did not find an effect of insecticides on NIR reflectance (Alves et al. 2017); therefore, insecticide impacts on the NIR spectral measures in the present studies were assumed to be minimal. Furthermore, the pattern of aphid-induced change in NIR reflectance found in open plots also held for caged plots, which suggests the measured effects on NIR reflectance were the result of soybean aphid-induced stress and are robust to these experimental manipulations.

The increase in red reflectance seen in caged plots on 13 August 2018 contrasts previous findings for ground-based remote sensing of soybean aphid, which showed either no change or a decrease in red reflectance for leaf-level measurements (Alves et al. 2015). In soybean and other plants, an increase in red reflectance is commonly associated with reductions in chlorophyll (Chappelle et al. 1992, Gitelson et al. 2003). Soybean aphid feeding may cause a reduction in chlorophyll content of soybean (Diaz-Montano et al. 2007), which could explain the increase in red reflectance exhibited on 13 August 2018. However, chlorophyll measurements were not recorded in the present study.

Sooty mold is a common sign associated with soybean aphid infestations in the field (Koch et al. 2016). There were a number of cages and spots within field plots in which sooty mold started to develop on the honey-dew-coated leaves. Previously it has been documented in greenhouse experiments that citrus leaves covered in sooty mold showed an increase in red reflectance (Summy and Little 2008). The presence of sooty mold is another potential explanation for the significant increase in red reflectance seen on 13 August 2018, when many of the aphid-infested plots had sooty mold from aphid populations above economic injury level.

Our experiments showed that the decrease in NDVI values caused by soybean aphid-induced stress was largely driven by decreased NIR reflectance. This suggests NIR alone may be suitable for mapping soybean aphid-induced stress in soybean fields. Many other causes of soybean stress have been documented to affect NIR reflectance and the visible spectrum (Vigier et al. 2004, Gazala et al. 2013, Bajwa et al. 2017). Soybean aphid generally did not affect the red portion of the spectrum, so there may be potential for the differentiation of soybean aphid-induced stress from other forms of stress in soybean by using combinations of wavelengths as has been attempted in several cropping systems (Yuan et al. 2014, Bajwa et al. 2017). Diseases such as soybean cyst nematode and sudden death syndrome have been documented to affect NIR reflectance and NDVI values in similar ways to soybean aphid-induced stress, but these diseases also sometimes affect the visible spectrum in ways not observed for soybean aphid in this study (Bajwa et al. 2017, Hatton 2018, Herrmann et al. 2018, Menke 2018). The development of tools to differentiate between the spectral response caused by these diseases and soybean aphid-induced stress is ongoing.

More research is necessary in order to develop an actionable management system including remote sensing for soybean aphid. Early uses of remote sensing for scouting soybean aphid will likely rely on spectral data to identify areas with stressed plants followed by ground-truthing, because remote sensing data is often more meaningful when combined with ground data (Casady and Palm 2002; Liaghat and Balasundram 2010). However, there is potential for remote sensing to improve detection of soybean aphid-induced stress and differentiate it from other types of stress encountered within a field.

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References Cited

- Alves, T. M., I. V. Macrae, and R. L. Koch. 2015. Soybean aphid (Hemiptera: Aphididae) affects soybean spectral reflectance. J. Econ. Entomol. 108: 2655–2664.
- Alves, T. M., Z. P. Marston, I. V. MacRae, and R. L. Koch. 2017. Effects of foliar insecticides on leaf-level spectral reflectance of soybean. J. Econ. Entomol. 110: 2436–2442.
- Alves, T. M., R. D. Moon, I. V. MacRae, and R. L. Koch. 2019. Optimizing band selection for spectral detection of *Aphis glycines* Matsumura in soybean. Pest Manag. Sci. 75: 942–949.
- Bai, G., S. Jenkins, W. Yuan, G. L. Graef, and Y. Ge. 2018. Field-based scoring of soybean iron deficiency chlorosis using RGB imaging and statistical learning. Front. Plant Sci. 9: 1002.
- Bajwa, S., J. Rupe, J. Mason, S. G. Bajwa, J. C. Rupe, and J. Mason. 2017. Soybean disease monitoring with leaf reflectance. Remote Sens. 9: 127.
- Beckendorf, E. A., M. A. Catangui, and W. E. Riedell. 2008. Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agron. J. 100: 237–246.
- Bueno, A. de F., M. J. Batistela, R. C. O. de F. Bueno, J. de B. França Neto, M. A. N. Nishikawa, and A. Libério Filho. 2011. Effects of integrated pest management, biological control and prophylactic use of insecticides on the management and sustainability of soybean. Crop Prot. 30: 937–945.
- Canis, B. 2015. Unmanned aircraft systems (UAS): commercial outlook for a new industry. Congressional Research Service (report no. R44192).
- Casady, W. W., and H. L. Palm. 2002. Precision agriculture: remote sensing and ground truthing. University of Missouri Extension, Columbia, MO. https://extension.missouri.edu/p/EQ453 (accessed 30 April 2019).
- Chang, J., S. A. Clay, D. E. Clay, and K. Dalsted. 2004. Detecting weed-free and weed-infested areas of a soybean field using near-infrared spectral data. Weed Sci. 52: 642–648.
- Chappelle, E. W., M. S. Kim, and J. E. McMurtrey. 1992. Ratio analysis of reflectance spectra (RARS): an algorithm for the remote estimation of the concentrations of chlorophyll A, chlorophyll B, and carotenoids in soybean leaves. Remote Sens. Environ. 39: 239–247.
- Diaz-Montano, J., J. C. Reese, W. T. Schapaugh, and L. R. Campbell. 2007. Chlorophyll loss caused by soybean aphid (Hemiptera: Aphididae) feeding on soybean. J. Econ. Entomol. 100: 1657–1662.
- Elliott, N., M. Mirik, Z. Yang, T. Dvorak, M. Rao, J. Michels, T. Walker, V. Catana, M. Phoofolo, K. Giles, and T. Royer. 2007. Airborne multispectral remote sensing of Russian wheat aphid injury to wheat. Southwest. Entomol. 32: 213–219.
- Elliott, N., M. Mirik, Z. Yang, D. Jones, M. Phoofolo, V. Catana, K. Giles, and G. J. Michels. 2009. Airborne remote sensing to detect greenbug stress to wheat. Southwest. Entomol. 34: 205–211.

- Fehr, W. R., and C. E. Caviness. 1977. Stages of soybean development. Spec. Rep. 80. Cooperative Ext. Serv. Iowa State Univ., Ames, IA. pp. 11.
- Gazala, I. F., R. N. Sahoo, R. Pandey, B. Mandal, V. K. Gupta, R. Singh, and P. Sinha. 2013. Spectral reflectance pattern in soybean for assessing yellow mosaic disease. Indian J. Virol. 24: 242–249.
- Gitelson, A. A., Y. Gritz, and M. N. Merzlyak. 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. J. Plant Physiol. 160: 271–282.
- Gray, C. J., D. R. Shaw, and L. M. Bruce. 2009. Utility of hyperspectral reflectance for differentiating soybean (*Glycine max*) and six weed species. Weed Technol. 23: 108–119.
- Hanafi, A., E. B. Radcliffe, and D. W. Ragsdale. 1989. Spread and control of potato leafroll virus in Minnesota. J. Econ. Entomol. 82: 1201–1206.
- Hatfield, J. L., A. A. Gitelson, J. S. Schepers, and C. L. Walthall. 2008. Application of spectral remote sensing for agronomic decisions. Agron. J. 100: S117-S131.
- Hatton, N. M. 2018. Use of small unmanned aerial system for validation of sudden death syndrome in soybean through multispectral and thermal remote sensing. M.S. thesis, Kansas State University, Manhattan, KS.
- Hatton, N. M., E. Menke, A. Sharda, D. Van der Merwe, and W. Schapaugh Jr. 2017. Comparison of aerial and ground remote sensing to quantify sudden death syndrome in soybeans. In 2017 ASABE Annual Invitational Meeting (p.1), 16–19 July 2017, Spokane, WA. American Society of Agricultural and Biological Engineers, St. Joseph, MI.
- Henry, W. B., D. R. Shaw, K. R. Reddy, L. M. Bruce, and H. D. Tamhankar. 2004. Spectral reflectance curves to distinguish soybean from common cocklebur (*Xanthium strumarium*) and sicklepod (*Cassia obtusifolia*) grown with varying soil moisture. Weed Sci. 52: 788–796.
- Herrmann, I., S. Vosberg, P. Ravindran, A. Singh, H.-X. Chang, M. Chilvers, S. Conley, P. Townsend, I. Herrmann, S. K. Vosberg, et al. 2018. Leaf and canopy level detection of *Fusarium virguliforme* (sudden death syndrome) in soybean. Remote Sens. 10: 426.
- Hijmans, R. J. 2017. Raster: geographic data analysis and modeling. R package version 2.6–7. https://CRAN.R-project.org/package=raster/index.html
- Hill, C. B., Y. Li, and G. L. Hartman. 2004. Resistance to the soybean aphid in soybean germplasm. Crop Sci. 44: 98–106.
- Hill, C. B., L. Crull, T. K. Herman, D. J. Voegtlin, and G. L. Hartman. 2010. A new soybean aphid (Hemiptera: Aphididae) biotype identified. J. Econ. Entomol. 103: 509–515.
- Hodgson, E. W., E. C. Burkness, W. D. Hutchison, and D. W. Ragsdale. 2004. Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. J. Econ. Entomol. 97: 2127–2136.
- Hodgson, E., B. McCornack, K. Koch, D. Ragsdale, K. Johnson, M. O'Neal, E. Cullen, H. Kraiss, C. DiFonzo, and L. Behnken. 2007. Field validation of speed scouting for soybean aphid. Crop Manag. 6: 1–8.
- Hodgson, E. W., B. P. McCornack, K. Tilmon, and J. J. Knodel. 2012. Management recommendations for soybean aphid (Hemiptera: Aphididae) in the United States. J. Integr. Pest Manag. 3: E1–E10.
- Hurley, T., and P. Mitchell. 2017. Value of neonicotinoid seed treatments to US soybean farmers. Pest Manag. Sci. 73: 102–112.
- Jackson, T. J., D. Chen, M. Cosh, F. Li, M. Anderson, C. Walthall, P. Doriaswamy, and E. R. Hunt. 2004. Vegetation water content mapping using Landsat data derived normalized difference water index for corn and soybeans. Remote Sens. Environ. 92: 475–482.
- Koch, R. L., B. D. Potter, P. A. Glogoza, E. W. Hodgson, C. H. Krupke, J. F. Tooker, C. D. DiFonzo, A. P. Michel, K. J. Tilmon, T. J. Prochaska, *et al.* 2016. Biology and economics of recommendations for insecticidebased management of soybean aphid. Plant Heal. Prog. 17: 265–269.
- Koger, C. H., L. M. Bruce, D. R. Shaw, and K. N. Reddy. 2003. Wavelet analysis of hyperspectral reflectance data for detecting pitted morningglory (*Ipomoea lacunosa*) in soybean (*Glycine max*). Remote Sens. Environ. 86: 108–119.

- Lelong, C. C., P. Burger, G. Jubelin, B. Roux, S. Labbé, and F. Baret. 2008. Assessment of unmanned aerial vehicles imagery for quantitative monitoring of wheat crop in small plots. Sensors (Basel). 8: 3557–3585.
- Liaghat, S., and S. K. Balasundram. 2010. A review: the role of remote sensing in precision agriculture. Am. J. Agric. Biol. Sci. 5: 50–55.
- Ma, B. L., L. M. Dwyer, C. Costa, E. R. Cober, and M. J. Morrison. 2001. Early prediction of soybean yield from canopy reflectance measurements. Agron. J. 93: 1227–1234.
- Menke, E. J. 2018. Using spectral reflectance in soybean breeding: evaluating genotypes for soybean sudden death disease resistance and grain yield. M.S. thesis, Kansas State University, Manhattan, KS.
- Mensah, C., C. DiFonzo, R. L. Nelson, and D. Wang. 2005. Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci. 45: 2228–2233.
- Milton, N. M., B. A. Eiswerth, and C. M. Ager. 1991. Effect of phosphorus deficiency on spectral reflectance and morphology of soybean plants. Remote Sens. Environ. 36: 121–127.
- Mirik, M., G. J. Michels, S. Kassymzhanova-Mirik, and N. C. Elliott. 2007. Reflectance characteristics of Russian wheat aphid (Hemiptera: Aphididae) stress and abundance in winter wheat. Comput. Electron. Agric. 57: 123–134.
- Nebiker, S., A. Annen, M. Scherrer, and D. A. Oesch. 2008. Light-weight multispectral sensor for micro UAV - opportunities for very high resolution airborne remote sensing. Int Arch Photogram Rem Sens Spat. Inf. Sci. 37(B1): 1193–1200.
- Nutter, F. W., G. L. Tylka, J. Guan, A. J. Moreira, C. C. Marett, T. R. Rosburg, J. P. Basart, and C. S. Chong. 2002. Use of remote sensing to detect soybean cyst nematode-induced plant stress. J. Nematol. 34: 222–231.
- Olson, K. D., T. M. Badibanga, and C. DiFonzo. 2008. Farmers' awareness and use of IPM for soybean aphid control: Report of survey results for the 2004, 2005, 2006, and 2007 crop years. Staff Pap. Report. Dep. Appl. Econ., Univ. Minn., St. Paul, MN.
- O'Shaughnessy, S. A., S. R. Evett, P. D. Colaizzi, and T. A. Howell. 2011. Using radiation thermography and thermometry to evaluate crop water stress in soybean and cotton. Agric. Water Manag. 98: 1523–1535.
- Pinter, P. J., Jr., J. L. Hatfield, J. S. Schepers, E. M. Barnes, M. S. Moran, C. S. T. Daughtry, and D. R. Upchurch. 2003. Remote sensing for crop management. Photogramm. Eng. Remote Sens. 69: 647–664.
- Prabhakar, M., Y. G. Prasad, M. Thirupathi, G. Sreedevi, B. Dharajothi, and B. Venkateswarlu. 2011. Use of ground based hyperspectral remote sensing for detection of stress in cotton caused by leafhopper (Hemiptera: Cicadellidae). Comput. Electron. Agric. 79: 189–198.
- Prabhakar, M., Y. G. Prasad, S. Vennila, M. Thirupathi, G. Sreedevi, G. Ramachandra Rao, and B. Venkateswarlu. 2013. Hyperspectral indices for assessing damage by the solenopsis mealybug (Hemiptera: Pseudococcidae) in cotton. Comput. Electron. Agric. 97: 61–70.
- Ragsdale, D. W., B. P. McCornack, R. C. Venette, B. D. Potter, I. V. MacRae, E. W. Hodgson, M. E. O'Neal, K. D. Johnson, R. J. O'Neil, C. D. DiFonzo, *et al.* 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). J. Econ. Entomol. 100: 1258–1267.
- Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011. Ecology and management of the soybean aphid in North America. Annu. Rev. Entomol. 56: 375–399.
- R Development Core Team, R. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/
- Rouse, J. W., Jr., R. H. Haas, D. W. Deering, and J. A. Schell. 1973. Monitoring the vernal advancement and retrogradation (green wave effect) of natural vegetation. Prog. Report no. 7, Texas A&M University, College Station, TX.
- Ruppel, R. F. 1983. Cumulative insect-days as an index of crop protection. J. Econ. Entomol. 76: 375–377.
- Smith, G. M., and E. J. Milton. 1999. The use of the empirical line method to calibrate remotely sensed data to reflectance. Int. J. Remote Sens. 20: 2653–2662.

- Song, F., and S. M. Swinton. 2009. Returns to integrated pest management research and outreach for soybean aphid. J. Econ. Entomol. 102: 2116–2125.
- Summy, K. R., and C. R. Little. 2008. Using color infrared imagery to detect sooty mold and fungal pathogens of glasshouse-propagated plants. HortScience. 43: 1485–1491.
- Tilmon, K. J., E. W. Hodgson, M. E. O'Neal, and D. W. Ragsdale. 2011. Biology of the soybean aphid, *Aphis Glycines* (Hemiptera: Aphididae) in the United States. J. Integr. Pest Manag. 2: 1–7.
- United States Department of Agriculture National Agricultural Statistics Service (USDA-NASS). 2018. Agricultural statistics 2018. https://

www.nass.usda.gov/Publications/Ag_Statistics/2018/Complete %20Publication.pdf (accessed 30 April 2019).

- Vigier, B. J., E. Pattey, and I. B. Strachan. 2004. Narrowband vegetation indexes and detection of disease damage in soybeans. IEEE Geosci. Remote Sens. Lett. 1: 255–259.
- Yuan, L., Y. Huang, R. W. Loraamm, C. Nie, J. Wang, and J. Zhang. 2014. Spectral analysis of winter wheat leaves for detection and differentiation of diseases and insects. F. Crop. Res. 156: 199–207.
- Zhang, C., and J. M. Kovacs. 2012. The application of small unmanned aerial systems for precision agriculture: a review. Precis. Agric. 13: 693–712.