ABSTRACT Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the most economically important insect pest of soybean in the north central United States. Scouting-based integrated pest management (IPM) programs could become more efficient and more widely adopted by using plant spectral reflectance to estimate soybean aphid injury. 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 noninfested 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.

RESUMO O pulga˜o-da-soja, *Aphis glycines* Matsumura (Hemiptera: Aphididae), é o inseto-praga mais importante na cultura da soja no Centro Norte dos Estados Unidos. O uso da reflectaˆncia espectral de plantas para estimar populaçã~o~es do pulga˜o-da-soja pode tornar sua amostragem mais eficiente e amplamente adotada. Nosso objetivo foi determinar se o efeito cumulativo da inju´ ria causada pelo pulga˜o-da-soja leva á alterac¸o˜es na reflectaˆncia espectral de plantas de soja. Experimentos de campo foram conduzi- dos em 2013 e 2014 utilizando parcelas isoladas por gaiolas de malha fina. Diferentes nı´veis de inju´ ria for- ram estabelecidos por três tratamentos. O nı´mero de pulgo˜es por planta foi determinado semanalmente. As mediço˜es de reflectaˆncia das plantas ocorreram em duas ocasiões em cada ano. Modelos de regressa˜o linear simples foram usados para determinar o efeito cumulativo da inju´ ria causada pelo pulga˜o-da-soja (CAD) sobre a reflectaˆncia de plantas em comprimentos de onda do vermelho (RED), infravermelho pro´ximo (NIR), indice de vegetac¸a˜o da diferenc¸a normalizada (NDVI) e tambem sobre o conteu´d o de chlorofila. Ao nı´vel de dossel, CAD na˜o teve efeito na reflectaˆncia das plantas no comprimento de onda do RED, mas diminuiu a refletaˆncia no NIR e NDVI. Geralmente, mediço˜es ao nı´vel de dossel e de folhas individuais indicaram semelhanca resposta espectral das plantas de soja ao aumento de CAD. Nosso estudo é a primeira publicac¸a˜o indicando que o uso da reflectaˆncia de plantas de soja tem potencial para detectar a inju´ ria do pulga˜o-da-soja.

KEY WORDS Glycine max, *Aphis glycines*, remote sensing, cumulative aphid-days, reflectance
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 US$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 (Hodgson 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 et al. 2012, Reynolds et al. 2012).

The increasing availability of optical instruments and unmanned aerial systems 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 (RED; 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 Rilpey 1997, Himinima 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 (~680 nm) and NIR (~800 nm) wavelengths in the NDVI equation is usually a better predictor of plant morpho-physiological changes than individual narrow-band 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.

Materials and Methods

Research Plots. Two research trials were conducted at UMore Park, University of Minnesota, Rosemount, MN, in 2013 and 2014. Each trial was seeded with ~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, and 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- by 1-m plots). When plants had three fully expanded trifoliates (V3 growth stage, Fehr and Caviness [1977]), plots (24 plots in 2013 and 21 plots in 2014) were individually enclosed by 1- by 1- by 1-m cages built with PVC tubing and white fine-mesh (0.02 cm mesh size, 100% polyester, Quest Outfitters Inc., Sarasota, FL).

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 λ-cyhalothrin (11.35 g a.i. per ha, Warrior II, Syngenta) at 2-wk intervals or more frequently if ≥10 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 trifoliates; 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 10 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 of five to six plants per plot and secured to the plants with paper clips.

Aphid Assessments. Aphid densities were estimated by nondestructive, 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 <80% of the plants in the aphid-infested treatments. When 80–99% of the plants were infested, a total of 10 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.

Spectral Reflectance Measurements. Plant spectral reflectance was measured using a handheld spectroradiometer immediately after aphid counts (FieldSpec 4 Hi-Res spectroradiometer, ASD Inc., Boulder, CO). 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 2002). Plant reflectance was measured on 21 August and 28 August in 2013 (i.e., 74 and 81 d after planting, respectively), and on 30 July and 5 August in 2014 (i.e., 64 and 70 d after planting, respectively). The spectroradiometer pistol grip assembly (A145653, ASD Inc.) was held at a 45-degree angle from ~0.6 m above the canopy to record canopy-level measurements. The self-illuminated plant probe assembly (AK101800, ASD Inc.) 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 trifoliate and middle trifoliate (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 >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 trifoliate in 2013 and 2014. Chlorophyll was also measured on central leaflets of middle trifoliate in 2014. The chlorophyll meter provides unitless values of indexed chlorophyll content between 0 and 99.9. Lower 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.

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: \( \sum_{i=1}^{n} \frac{(x_i + x_{i-1})}{2} \times (t_i - t_{i-1}) \), 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 2010) 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. 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 trifoliate, and uppermost trifoliate 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 trifoliate. 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 NDVI 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 2010).

Table 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 NDVI in Rosemount, MN, 2013 and 2014

| Canopy reflectance | Sample date | Intercept | Slope | F-value | df_model_error | R^2 (%) | F-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 July 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^{-5} | 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 | 0.569 |
|                    | 28 Aug. 13  | 0.500     | -1.23 \times 10^{-5} | 9.663 | 1.22 | 30.5 | 0.005* |
|                    | 30 July 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 (NIR - RED)   | 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 July 14  | 0.918     | 6.91 \times 10^{-7} | 0.458 | 1.19 | 2.5 | 0.493 |
|                    | 5 Aug. 14   | 0.926     | -1.75 \times 10^{-6} | 40.066 | 1.19 | 67.5 | <0.001* |

* NDVI, normalized difference vegetation index.

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

Results

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.

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). 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). 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).

Spectral Reflectance of Individual Leaves. On 21 and 28 August 2013 and 30 July 2014, CAD did not affect the RED reflectance of middle trifoliate (Table 2) or uppermost trifoliate (Table 3). However, on 5 August 2014, CAD decreased RED reflectance of middle (Table 2) and uppermost trifoliate (Table 3). CAD decreased NIR reflectance of middle trifoliate 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 trifoliate on 30 July 2014 (Table 2). CAD decreased NIR reflectance of uppermost trifoliate on 28 August 2013 (\( P < 0.001 \)) but did not affect NIR reflectance of uppermost trifoliate on the other three sample dates (Table 3).

CAD decreased NDVI of middle trifoliate on 28 August 2013 (\( P < 0.001 \)), but did not affect NDVI of middle trifoliate on 21 August 2013, 30 July, and 5 August 2014 (Table 2). CAD decreased NDVI of uppermost trifoliate on 28 August 2013 (\( P < 0.001 \)) and increased NDVI of uppermost trifoliate on
Table 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 NDVI in Rosemount, MN, 2013 and 2014

| Reflectance of middle trifoliates | Sample date | Intercept | Slope | F-value | df_model_error | R² (%) | F-value |
|----------------------------------|-------------|-----------|-------|---------|----------------|--------|---------|
| RED (680 nm)                     | 21 Aug. 13  | 0.047     | -9.92×10⁻⁷ | 1.186   | 1.22           | 5.1    | 0.288   |
|                                  | 28 Aug. 13  | 0.051     | 1.37×10⁻⁷  | 1.149   | 1.22           | 5.0    | 0.205   |
|                                  | 30 July 14  | 0.053     | 4.05×10⁻⁷  | 1.083   | 1.19           | 5.4    | 0.311   |
|                                  | 5 Aug. 14   | 0.053     | -2.58×10⁻⁷ | 7.788   | 1.19           | 29.1   | 0.012*  |
| NIR (800 nm)                     | 21 Aug. 13  | 0.531     | -1.38×10⁻⁷ | 4.544   | 1.22           | 17.1   | 0.044*  |
|                                  | 28 Aug. 13  | 0.525     | -7.09×10⁻⁷ | 103.265 | 1.22           | 92.4   | <0.001* |
|                                  | 30 July 14  | 0.541     | -3.25×10⁻⁷ | 0.014   | 1.19           | 0.1    | 0.908   |
|                                  | 5 Aug. 14   | 0.493     | -3.18×10⁻⁶ | 17.127  | 1.19           | 47.4   | 0.001*  |
| NDVI = (NIR - RED) / (NIR + RED) | 21 Aug. 13  | 0.836     | -7.76×10⁻⁷ | 0.086   | 1.22           | 0.4    | 0.727   |
|                                  | 28 Aug. 13  | 0.823     | -3.02×10⁻⁶ | 51.593  | 1.22           | 70.1   | <0.001* |
|                                  | 30 July 14  | 0.821     | -1.27×10⁻⁶ | 1.582   | 1.19           | 7.7    | 0.224   |
|                                  | 5 Aug. 14   | 0.805     | -2.80×10⁻⁷ | 0.574   | 1.19           | 4.4    | 0.362   |

* NDVI, normalized difference vegetation index.

Indicate significant effect of CAD on leaf-level reflectance of middle trifoliates (α = 0.05). Otherwise, CAD had no effect on reflectance of middle trifoliates.

Table 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 NDVI in Rosemount, MN, 2013 and 2014

| Reflectance of uppermost trifoliates | Sample date | Intercept | Slope | F-value | df_model_error | R² (%) | F-value |
|-------------------------------------|-------------|-----------|-------|---------|----------------|--------|---------|
| RED (680 nm)                        | 21 Aug. 13  | 0.047     | 2.37×10⁻⁷ | 0.034   | 1.22           | 0.2    | 0.556   |
|                                     | 28 Aug. 13  | 0.050     | 1.03×10⁻⁷ | 0.660   | 1.22           | 2.9    | 0.425   |
|                                     | 30 July 14  | 0.057     | 1.16×10⁻⁷ | 0.130   | 1.19           | 0.7    | 0.722   |
|                                     | 5 Aug. 14   | 0.055     | -3.51×10⁻⁷ | 14.338  | 1.19           | 43.0   | 0.001*  |
| NIR (800 nm)                        | 21 Aug. 13  | 0.515     | -1.50×10⁻⁶ | 0.079   | 1.22           | 0.4    | 0.751   |
|                                     | 28 Aug. 13  | 0.520     | -7.59×10⁻⁶ | 78.398  | 1.22           | 78.1   | <0.001* |
|                                     | 30 July 14  | 0.491     | 4.38×10⁻⁶  | 3.118   | 1.19           | 14.1   | 0.093   |
|                                     | 5 Aug. 14   | 0.475     | -3.80×10⁻⁷ | 0.554   | 1.19           | 2.8    | 0.466   |
| NDVI = (NIR - RED) / (NIR + RED)    | 21 Aug. 13  | 0.834     | -1.20×10⁻⁶ | 0.092   | 1.22           | 0.4    | 0.784   |
|                                     | 28 Aug. 13  | 0.825     | -3.15×10⁻⁶ | 96.958  | 1.22           | 81.5   | <0.001* |
|                                     | 30 July 14  | 0.792     | 1.19×10⁻⁶  | 2.166   | 1.19           | 10.2   | 0.157   |
|                                     | 5 Aug. 14   | 0.792     | 1.07×10⁻⁶  | 19.156  | 1.19           | 50.2   | <0.001* |

* NDVI, normalized difference vegetation index.

Indicate significant effect of CAD on leaf-level reflectance of uppermost trifoliates (α = 0.05). Otherwise, CAD had no effect on reflectance of uppermost trifoliates.

5 August 2014 (P < 0.001). CAD had no effect on NDVI of uppermost trifoliates on 21 August 2013 or 30 July 2014 (Table 3).

CAD did not affect indexed chlorophyll content of middle trifoliates on 30 July 2014 (Table 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 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 4).

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 2-yr canopy data revealed that soybean aphid injury can be detected using NDVI and NIR reflectance, but not with RED reflectance (Table 1). Soybean aphid populations in this study (Fig. 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 (F.); 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 2), but canopy-level measurements did not detect this effect on NIR reflectance (Table 1). In another instance, the RED reflectance of both middle and uppermost trifoliates was reduced by CAD (Tables 2 and 3), but canopy-level measurements again did not detect this locally induced effect (Table 1).
However, similar conclusions were obtained by canopy-level and leaf-level measurements of middle and uppermost trifoliate leaves 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 trifoliate leaves (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), two (of four) regression models showed that increasing CAD decreased canopy-level NIR reflectance (Table 1). Decreased NIR reflectance has also been recorded in response to increasing insect injury in other commercial crops (Peñuelas and Filella 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). Better results with NDVI for detection of plant stress were also observed by Peñuelas and Filella (1998) and Yang et al. (2000). 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 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 nonsignificant effect on chlorophyll content on these evaluation dates (Wu et al. 2008, Sánchez et al. 2014). Reduction in chlorophyll content, intercellular CO₂, 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 trifoliate leaves (Table 4). Contradicting the results from the chlorophyll meter, leaf-level measurements indicated that CAD decreased the RED reflectance of middle trifoliate leaves (Table 2), which typically is associated with increased 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 trifoliate leaves (Monje and Bugbee 1992, Karageorgou and Manetas 2006, Manetas 2006, Wu et al. 2007).

Soybean plants surprisingly increased the relative chlorophyll content of uppermost trifoliate leaves on 5 August 2014 (Table 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 trifoliate 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 trifoliate leaves (i.e., newly developed trifoliate; 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

**Table 4. Model estimates of simple linear regressions of the effect of cumulative aphid-days (CAD) on indexed chlorophyll content of middle and uppermost soybean trifoliate leaves in Rosemount, MN, 2013 and 2014**

| Position* | Sample date | Intercept | Slope | F-value | df(model) | Error | R² (%) | P-value |
|-----------|-------------|-----------|-------|---------|-----------|-------|--------|---------|
| Middle    | 30 July 2014| 48.91     | −2.32 × 10⁻⁴ | 3.22 | 1,19 | 14.48 | 0.089 |
|           | 5 Aug. 2014 | 50.11     | −1.23 × 10⁻⁴ | 7.46 | 1,19 | 28.18 | 0.013* |
| Uppermost | 21 Aug. 2013| 43.65     | −1.12 × 10⁻⁴ | 0.01 | 1,22 | 0.03  | 0.935 |
|           | 28 Aug. 2013| 45.06     | 1.84 × 10⁻⁵  | 0.05 | 1,22 | 0.22  | 0.529 |
|           | 30 July 2014| 32.69     | −5.77 × 10⁻⁴ | 3.69 | 1,19 | 16.28 | 0.070 |
|           | 5 Aug. 2014 | 29.99     | 3.10 × 10⁻⁴  | 6.78 | 1,19 | 26.30 | 0.017* |

* Vertical position of the trifoliate 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 (α = 0.05). Otherwise, P-value was not significant (i.e., CAD had no effect on relative chlorophyll content).
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

Fig. 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 on which soybean spectral reflectance measurements were recorded.
reflectance than plants with no aphids \((\text{NIR} = 0.926 - 1.75 \times 10^{-6} \times \text{CAD})\) (Table 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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