Pest Management

Remotely Sensing Arthropod and Nutrient Stressed Plants: A Case Study With Nitrogen and Cotton Aphid (Hemiptera: Aphididae)

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ABSTRACT Remote sensing can be used in combination with ground sampling to detect aphid- (*Aphis gossypii* Glover) infested cotton (*Gossypium hirsutum* L.). Changes in wavelengths in the near-infrared (NIR) have proven useful for such detection, but these changes can be confused with other factors stressing plants, such as water deficiency and nutrient status. This study was designed to test the utility of this technology to distinguish between two factors stressing plants: nitrogen deficiency and aphids. Field plots were created by applying varying rates of nitrogen to cotton at different dates in the growing season in 2003 and 2004. Subplots were created by applying disruptive insecticides, which increased aphid populations in a portion of the subplots. Airplane and satellite remote sensing data in 2003 and 2004 were supplemented with ground sampling of aphid populations in both years. Insecticide application, nitrogen application rate and date influenced aphid abundance. Cotton with higher aphid populations could be distinguished from cotton with natural aphid infestations independent of plant nitrogen status using a NIR wavelength in 2003 and a proprietary 2004 index. Complex distinctions among varying nitrogen treatments and aphid abundance were not possible using this data. In the future, possible confounding factors should be investigated from the perspective of their change on crop physiology before remote sensing can be used in an integrated pest management (IPM) program.

KEY WORDS arthropod damage, multispectral, hyperspectral

Remote sensing is a precision technology with origins in the defense and aerospace sectors used to acquire information about objects without direct contact. This information can be processed and combined into indices, which are useful because they can convert multidimensional data into a single dimension. Remote sensors can detect information over large areas and in wavelengths outside those that humans can perceive and are sometimes used to detect arthropod pests, pest damage, and/or other stressors in agricultural systems.

Airborne sensors are used on a limited basis in agriculture to gather reflectance data from soil and vegetation. One reason for this limitation in pest management arises from the difficultly encountered when assigning unique spectral characteristics to specific plant stressors. For example, one study could distinguish water deficiency from other agronomic factors (Lelong et al. 1998), but another could not because of the variability of different agronomic factors (Tilling et al. 2007). The number of ground-truth measurements needed may prove too excessive for practical spectral distinction of arthropod stress effects on plants to other stress factors. In the study of Lelong et al. (1998), 12 ground-truth measurement variables were needed to accomplish this. This may be one of the most serious barriers to discovering a spectral response pattern unique to pests and the plants on which they feed, if such unique characteristics exist.

Cotton aphids (*Aphis gossypii* Glover) have emerged as a severe midseason pest of Acala cotton (*Gossypium hirsutum* L.) in the San Joaquin Valley (SJV) of California over the last 15 yr. High cotton aphid populations during the midseason can lower yields because phloem-feeding aphids remove sugary contents that would have been allocated to the reproductive structures of the plant. Spider mites (*Tetranychus* sp.) are also pest of cotton and, in contrast to aphids, pierce cotton cells with stylets and remove the chloroplasts. Spider mite-damaged cotton proceeds from small brown spots on leaves to complete bronzing of the leaves. In the SJV, both spider mite and aphid-damaged cotton have lower near-infrared reflectance (NIR) values than uninfested cotton, but spider-mite damaged cotton has not been distinguished from aphid-damaged cotton using remote sensing methods alone (Reisig and Godfrey 2006). In addition, NIR reflectance decreases when plants senesce or when canopy cover decreases (Richardson et al. 1975, Summy et al. 1997, Datt 1998, Brewster et al. 1999). Hence, other factors that stress cotton may be confused with arthropod-infested cotton. The likelihood of false negatives or positives for the subject in

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question mandates that ground-truthing remain within a remote sensing program to distinguish crop stressors (Lelong 1998).

Cotton physiologically responds to aphids and changes in photosynthetic and transpiration rates caused by feeding have been documented (Shannag et al. 1998). Theoretically, these changes should be detectible with remote sensing. However, the physiological changes in the plant to insect feeding are complex, even for a single compound, such as a pigment or groups of pigments. For example, in wheat, *Diuraphis noxia* Mordvilko and *Schizaphis graminum* Rondani cause chlorophyll loss in *Triticum aestivum* L. leaves as they feed (Riedell and Blackmer 1999, Ni et al. 2002, Mirik et al. 2007), but undamaged leaves from the same plant may compensate for these pigment losses (Ni et al. 2001, 2002; Wang et al. 2004). Furthermore, the degree of chlorophyll loss is dependent on the aphid species and genotype of *T. aestivum*, because different chlorophyll degradation pathways are activated within the plant through feeding (Ni et al. 2001, 2002; Wang et al. 2004).

Wavelengths and indices sensitive to chlorophyll *a* and *b* and the byproducts of chlorophyll degradation (anthocyanins and carotenoids) have been explored in various plants (Chappelle et al. 1992, Datt 1998, Blackburn 2007, Carroll et al. 2008). Indices sensitive to anthocyanins and carotenoids often focus on wavelengths in the green, red, and near-infrared portions of the spectrum (Gitelson and Merzlyak 2004, Gitelson et al. 2009). Anthocyanin indices are highly correlated with anthocyanin plant levels, but the magnitude and direction of the response, as examined thorough reflectance indices, is plant species-dependent (Gitelson et al. 2009). Moreover, the bounds of these indices are most likely species dependent, unlike an index such as the normalized difference vegetative index (Rouse et al. 1974), which ranges from −1 to 1. Carroll et al. (2008) suggested that the amalgamation of such indices combined with agronomic information might be used to distinguish insect stress from other types of stressors. Some of the NIR wavelengths are sensitive to multiple factors involving crop health. As a result, remote sensing–derived indices that are specifically sensitive to physiological changes within the plant should be explored (Lelong et al. 1998, Blackburn 2007, Carroll et al. 2008).

SJV growers increased nitrogen inputs in Acala cotton from ≈110 kg N/ha during the late 1980s to a peak at ≈200 kg N/ha in the mid-1990s (Cisneros and Godfrey 2001). Cisneros and Godfrey (2001) concluded that the high nitrogen inputs were an important factor contributing to high aphid population. Cotton with high levels of nitrogen was also positively correlated with high aphid populations in the Rolling Plains of Texas (Slosser et al. 1997). Nitrogen inputs in the SJV stabilized to between 150 and 200 kg N/ha from the late 1990s to 2007 (USDA 2008) but still remained high compared with the 1980s. Certain vegetation indices are correlated with nitrogen levels in SJV cotton fields, especially those using wavelengths in the green band (≈550 nm) and the modified chlorophyll absorption index (Daughtery et al. 2000, Ojala et al. 2004), and remote sensing could be used to detect areas in fields that have plants with high nitrogen levels. If areas of plants with high levels of nitrogen can be identified, they may have higher aphid population levels.

This study was initiated by experimentally creating areas of cotton with varying levels of nitrogen and aphid populations. Although wavelengths in the green band are associated with nitrogen levels in cotton (Daughtery et al. 2000, Ojala et al. 2004), only wavelengths and indices in the NIR will be tested. NIR wavelengths are associated with aphid-infested cotton (Reisig and Godfrey 2006, 2007), and the goal was to distinguish aphid-infested cotton under varying levels of plant health, mediated by nitrogen level, using airborne remote sensing. Indices used by Carroll et al. (2008) that are sensitive to anthocyanins and/or carotenoids will also be explored. This paper is a case study to examine whether a single agronomic stress factor (nitrogen fertilization) and a single insect stress (aphid) to the cotton plant can be distinguished using remote sensing.

Materials and Methods

Field Preparation. Acala cotton was planted at the University of California Cotton Research and Extension Center near Shafter. Different amounts of nitrogen (urea-ammonium nitrate, 32% N) were shanked into the soil on 13 June, 4 July, 25 July, and 16 August 2003 (112 and 224 kg/ha) and 24 June, 8 July, and 26 July 2004 (90 and 270 kg/ha). A single tractor passed across all plots on these dates, regardless of whether or not nitrogen was applied. Twenty-four plots (≈15.2 m long by ≈8.1 m wide [eight rows]) were arranged in a randomized factorial (nitrogen rate and application date) block design, with three replications per treatment in 2003. The design was slightly unbalanced in 2004 with 40 plots and three to four replications per treatment. In early June of both years, 3 m of plants were removed down each row between plots for delineation. No plants were removed across rows.

Aphids naturally populated the fields. At the onset of infestation, plots were randomly split into subplots (≈15.2 m long by ≈ 4.1 m wide [four rows]) and treated with a bifenthrin (Capture 2 EC at 16.9 g [AI]/ha; FMC, Philadelphia, PA) application on 14 August 2003 (Slosser et al. 2001). The purpose of this application (the subplot factor) was to flare aphid levels in one half of the plot while leaving the other half of the plot with natural aphid levels. Similarly, plots were randomly split into subplots in 2004 on 26 July, but lambda-cyhalothrin (Warrior at 3.8 g [AI]/ha; Syngenta Crop Protection, Greensboro, NC) was substituted for bifenthrin to increase aphid populations over the previous year (Kerns and Stewart 2000, Slosser et al. 2001). All plots were treated with buprofezin (Courier at 48.8 g [AI]/ha; Nichino America, Wilmington, DE) on 26 July 2004 to manage *Bemisia argentifolii* Bellows and Perring populations. For all insecticide applications, a single tractor pass was made over each plot, regardless of treatment.

No nitrogen samples were taken in 2003, but the field was planted in 2002 with a small-grain crop to homogenize and deplete residual soil nitrogen. To assure that the field was nitrogen-depleted in 2004, residual soil nitrogen content was quantified by collecting random soil samples within the field in early summer, 2004. Sixteen samples were taken by homogenizing soil at each individual sample location, taken at a depth of ≈75 cm. These 16 soil samples were pulverized through a 2-mm sieve, oven dried for 30 min, and immediately sent to the University of California Division of Agriculture and Natural Resources Analytical Laboratory for analysis. Total carbon and nitrogen were assessed using the Carlo Erba Combustion Method (AOAC International 1997).

Relative nutrient uptake in the plants was analyzed at three key points in the growing season in 2004. Petiole samples were taken from the fifth mainstem node leaf, counted from the top of the plant, on 5, 20, and 27 August. Nutrients were analyzed by the University of California Division of Agriculture and Natural Resources Analytical Laboratory. Total extractable nitrogen (NO_3-N) and nitrogen in ammonium (NH_4-N) were analyzed as described by Carlson et al. (1990), total extractable soluble potassium (K) following Johnson and Ulrich (1959), and extractable phosphate (PO_4-P), as detailed by Prokopy (1995).

Data Collection: Ground-Truthing. Aphids were sampled at approximately weekly intervals within the subplots in 2003 by collecting 10-leaf samples (fifth mainstem node leaf from the top of the plant) per subplot. Twenty-leaf samples (fifth mainstem node leaf from the top) were collected in 2004, making the analysis more robust by increasing the power of obtaining a true measurement of population density. Aphids were counted in the laboratory under 50× magnification, and accumulated aphid-days were calculated for the period after treatment (Ruppel 1983). Yield data for 2003 and 2004 were collected as detailed by Reisig and Godfrey (2006).

Remote Sensing: Airplane Data 2003. Remote sensing data were collected on 20 August 2003 and 11 September 2003. The data were the same images used by Reisig and Godfrey (2006) and were obtained from by Opto-Knowledge Systems (OKSI, Torrance, CA) using an airplane-mounted multispectral and hyperspectral camera. Images were calibrated by OKSI for individual flights using panels colored with nonspecular paint. This finish created a dull surface. A different shade was used for each panel: white, gray, and black. Each panel was 10 by 10 m so that it included several image pixels. The entire spectrum of reflectance was measured on the ground by OKSI. Radiance measured by the camera systems was converted to reflectance using this information and a variety of multiplicative factors: illumination, transmission of objects, atmospheric water vapor, responsivity of the sensor, etc. Wind caused turbulence in the airplane on 11 September 2003, which affected the image. This image was orthorectified in a geometric correction using data from plane-mounted gyros and a global positioning system (GPS) receiver, as well as other data such as altitude.

Table 1. Vegetation indices and near-infrared wavelength tested, 2003

Vegetation index	Equation	Reference
Anthocyanin reflectance index (ARI)[a]	ARI = R_{559} − R_{705} × R_{782}	(Gitelson and Merzlyak 2004)
Carotenoid reflectance index, green (CRI_{GREEN})[a]	CRI_{GREEN} = R_{511} − R_{559} × R_{782}	(Gitelson and Merzlyak 2004)
Carotenoid reflectance index, red (CRI_{RED})[a]	CRI_{RED} = R_{511} − R_{705} × R_{782}	(Gitelson and Merzlyak 2004)
	≈850 nm = R_{850}	

[a] These indices were used exclusively from a hyperspectral image taken on 11 Sept. 2003.

ENVI (Research Systems, Boulder, CO) software and a geographic information systems (GIS)-based approach were used to draw regions of interests around the subplots. Subplot length could be distinguished on the image using soil borders between subplots. Subplot width was determined by identifying corners of the experiment and identifying the width of each plot using the measurement tool within ENVI. Regions of interest used for data analysis were at least 1 pixel from the calculated plot edge and thus represented the interior of each subplot. Twenty pixels (22 m^2) were sampled from each subplot from the multispectral image on 20 August. Sixteen to 26 pixels (18–29 m^2) per subplot were sampled in 2003 from the multispectral image on 11 September. Twenty-four pixels (26 m^2) were sampled from each subplot from the hyperspectral image on 20 August. Twenty-two to 30 pixels (24–33 m^2) per subplot were sampled in 2003 from the hyperspectral image on 11 September.

Remote Sensing: Satellite Data 2004. Remote sensing data were collected on 25 August 2004 and were the same images used by Reisig and Godfrey (2006). DigitalGlobe (Longmont, CO) calibrates to reflectance from the earth's surface using objects with known units of reflectance and a radiative transfer model with sensitivities for atmospheric scattering and absorption. The frequency of calibration was unknown, because AgriDataSensing (Fresno, CA) provided proprietary reflectance data and indices. Data from the images were retrieved by ENVI processing, as described above. Four pixels per subplot were sampled from the 2004 panchromatic image (31 m^2), and data from these same regions of interest were analyzed from the remaining 2004 images.

Statistical Analyses. An NIR reflectance band and several indices were considered from the 2003 airborne data (Table 1). Band and index choice from 2003 and 2004 flight data were based positive results from previously published data for detection of cotton infested with aphid (Reisig and Godfrey 2006). Several indices were analyzed that had not previously been explored for detection of cotton infested with aphids and that were sensitive to physiological changes within plant leaves (Gitelson and Merzlyak 2004) for 2003 data. For both airplane and satellite data, all wavelengths and indices were individually analyzed using mixed model analysis of variance (ANOVA; $P < 0.05$; PROC MIXED; SAS Institute, 2003) as a factorial split plot design. Yield data from both years were analyzed using the same two-way mixed-model ANOVA. Random effects included those with blocks (block × nitrogen application rate [N]), block × date of nitrogen applications [date]), whereas the remaining factors were fixed: N, date, N × date, subplots (aphids), N × aphids, date × aphids, and N × date × aphids.

In 2004, a single plot, located on a field edge, had extremely high aphid populations. By the time the remote sensing data were taken, these cotton plants were defoliated. Hence, this observation was dropped from the analysis as an outlier that had unrealistic influence on the treatment.

Each nutrient from the petiole analyses was analyzed using a mixed-model repeated-measures ANOVA model ($P < 0.05$; PROC MIXED), using each day that petioles were collected and the REPEATED statement. Random effects included those with blocks (block × nitrogen levels [N], block × date of nitrogen applications [date]), whereas the remaining factors were fixed: N, date, N × date, subplots (aphids), N × aphids, date × aphids, and N × date × aphids.

Denominator degrees of freedom were calculated following the methods of Kenward and Roger (1997) in all analyses. Tukey's honesty significant difference procedure was used for mean separation in the above analyses. After running the analyses, pairwise comparisons for treatments with the same nitrogen rate (N × aphids or N × date × aphids) and application time (date × aphids or N × date × aphids) were analyzed to determine whether remote sensing images could separate cotton infested with aphids (insecticide-treated) from relatively uninfested cotton (untreated; i.e., aphids at natural levels). If this succeeded, other comparisons were analyzed to determine whether nitrogen treatments could be distinguished among varying levels of aphid infested cotton. Data presented are untransformed arithmetic means and SEs.

Results

2003 Ground-Truthing Data. Aphid populations fluctuated in the bifenthrin-treated subplots and peaked on 3 September at 21 aphids per leaf. This was more than twice the number of aphids of the previous week, when there was an average of 10 aphids per leaf in these subplots. Populations were suppressed by natural enemies and averaged five aphids per leaf on 11 September. Aphid abundance in untreated subplots was more constant, with an average of 12 aphids per leaf on 27 August and 13 aphids per leaf on 3 September. However, by 11 September, abundance decreased to an average of four aphids per leaf (Fig. 1).

Aphid levels were generally higher when higher levels of nitrogen were applied and when it was applied earlier. On 27 August, plots with nitrogen applied on 13 June averaged 16 aphids per leaf, plots with nitrogen applied on 4 July averaged 10 aphids per leaf, plots with nitrogen applied on 25 July averaged 8 aphids per leaf, and plots with nitrogen applied on 16 August averaged 10 aphids per leaf. On 3 September, plots with 112 kg N/ha averaged 14 aphids per leaf, whereas plots with 224 kg N/ha averaged 20 aphids per leaf. By 11 September, abundance equilibrated among treatments with three to five aphids per leaf (Fig. 1). Yield differences were not statistically significant for any factor in the analysis. Overall seed cotton mean yield was 802 kg/ha.

Airplane Data—Multispectral: 20 August and 11 September. There were no factors with significant differences for any wavelength or index.

Airplane Data—Hyperspectral: 20 August. The hyperspectral reflectance image for this date was unavailable.

Airplane Data—Hyperspectral: 11 September. There was a significant difference in the two-way interaction of N × aphids (Table 2). In plots with identical nitrogen treatments, untreated subplots could be distinguished from bifenthrin-treated subplots with a single index. Cotton with 224 kg/ha N added had significantly higher $\mathrm{CRI_{RED}}$ values in bifenthrin-applied subplots (−0.0170) compared with untreated subplots (−0.0197).

There were also significant differences among the interaction of date × aphids (Table 2). Subplots with bifenthrin which received nitrogen on 25 July had significantly higher values using the ARI (−0.0121) and $\mathrm{CRI_{RED}}$ (−0.0170) compared with subplots that received the same amount of nitrogen but were untreated (ARI = −0.0141, $\mathrm{CRI_{RED}}$ = −0.0200). Plots with more added nitrogen had higher NIR reflectance values (0.2970) than plots with less nitrogen added (0.2866). Subplots with higher aphid abundance (bifenthrin = 0.2854) had lower NIR reflectance values than plots with lower aphid abundance (untreated = 0.2982). Conversely, subplots with higher aphid abundance (bifenthrin = −0.0052) had higher $\mathrm{CRI_{GREEN}}$ index values than plots with lower aphid abundance (untreated = −0.0057).

2004 Ground-Truthing Data. Low nitrogen levels were detected (<25 kg N/ha-m) in the soil before nitrogen applications in 2004. After nitrogen applications, nitrogen in nitrate (NO_3-N) levels in the petioles were significantly different between the treatments with varying nitrogen application rates (F = 19.52; df = 1,20.2; P = 0.0003). Furthermore, nitrogen in ammonium (NH_4-N) levels were significantly different between lambda-cyhalothrin-treated subplots (aphids flared) and untreated subplots (F = 10.11; df = 1,17.1; P = 0.0055). Potassium (K) and phosphorous in phosphate (PO_4-P) levels were not significantly different. Effects for application dates were not observed.

Lambda-cyhalothrin successfully flared aphid populations (Fig. 2). On 20 August, subplots with lambda-cyhalothrin averaged five aphids per leaf, with 72 accumulated aphid-days, whereas untreated subplots averaged less than one aphid per leaf, with 3 accumulated aphid-days. On 27 August, subplots with lambda-

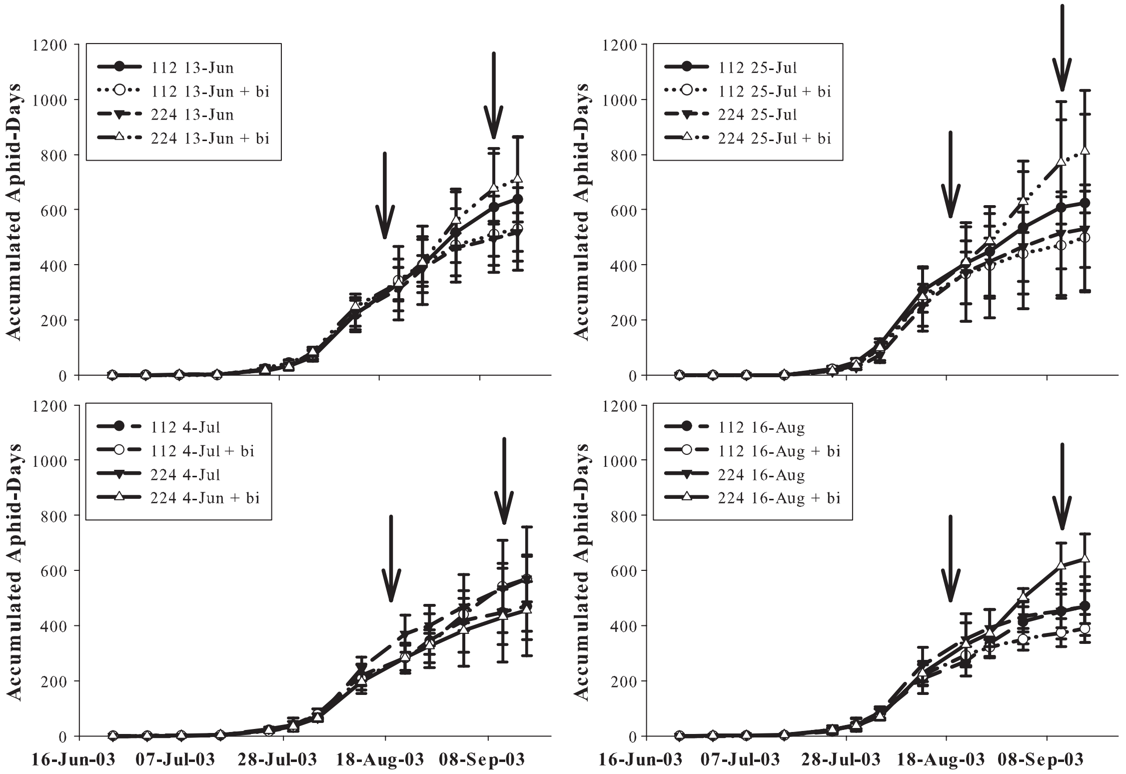

Fig. 1. Mean total accumulated aphids-days over time for kilogram nitrogen per hectare applied on 13 June, 4 and 25 July, and 16 August 2003. Numbers after legend symbol represent kg N/ha and date of nitrogen application. Arrows represent dates of remote sensing data acquisition, and vertical bars represent mean SE. bi, bifenthrin application.

cyhalothrin averaged 13 aphids per leaf, whereas untreated subplots averaged <1 aphid per leaf. Finally, on 2 September, subplots with lambda-cyhalothrin averaged eight aphids per leaf, whereas untreated subplots averaged less than one aphid per leaf.

The amount of nitrogen that was applied had mixed effects on the aphid populations, but the date that nitrogen was applied influenced aphid populations more consistently. Plots that received 90 kg N/ha averaged four aphids per leaf on 20 August, five aphids per leaf on 27 August, and three aphids per leaf on 2 September. Plots that received 270 kg N/ha averaged three aphids per leaf on 20 August, seven aphids per leaf on 27 August, and five aphids per leaf on 2 September. Plots receiving 90 kg N/ha had lower aphid numbers per leaf than plots receiving 270 kg N/ha near the date that the remote sensing data were taken (25 August). However, plots receiving 90 kg N/ha had nearly identical accumulated aphid-days compared with plots that received 270 kg N/ha. Overall, effects of the application date on aphid abundance were greatest during the time period date that remote sensing data were taken.

Seed cotton yields ranged from 843 kg/ha in plots that received 270 kg N/ha on 8 July to 762 kg/ha in plots that received 90 kg N/ha on 24 June. There were

Table 2. Hyperspectral airplane system data gathered 11 Sept. 2003: ANOVA results

Factors	≈850 nm			ARI			CRI_{GREEN}			CRI_{RED}		
	F	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df
N[a]	2.78	0.1150	16	0.37	0.5512	17.8	0.77	0.3941	15.2	0.62	0.4416	16
Date[b]	1.67	0.2125	16	2.44	0.1144	12	0.34	0.8003	11.3	1.64	0.2190	16
N × date[b]	1.98	0.1573	16	2.60	0.1006	12	0.58	0.6390	11.3	2.00	0.1551	16
Aphids[a]	7.85	0.0128[c]	16	14.46	0.0014[c]	17.5	8.20	0.0194[c]	8.69	14.22	0.0017[c]	16
N × aphids[a]	3.16	0.0946	16	8.83	0.0083[c]	17.5	5.13	0.0507	8.69	8.76	0.0092[c]	16
Date × aphids[b]	0.94	0.4433	16	8.00	0.0050[c]	10.2	1.97	0.2166	6.23	3.76	0.0323[c]	16
N × date × aphids[b]	0.93	0.4474	16	0.98	0.4410	10.2	1.14	0.4041	6.23	1.32	0.3024	16

N, nitrogen rate; Date, nitrogen application date; Aphids, subplots.

[a] Numerator df = 1.

[b] Numerator df = 3.

[c] $P < 0.05$.

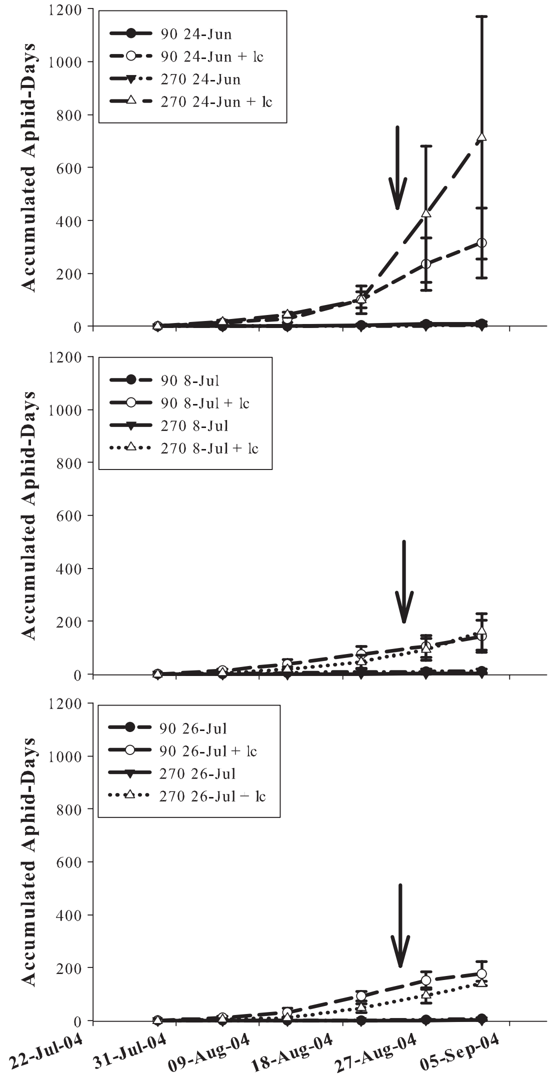

Fig. 2. Mean total accumulated aphids-days over time, 2004 for kilogram nitrogen per hectare applied on 24 June and 8 and 26 July. Numbers after legend symbol represent kg N/ha and date of nitrogen application. Arrow represents date of remote sensing data acquisition, and vertical bars represent mean SE. lc, lambda-cyhalothrin.

no significant differences in yield for any factor, except for the aphid flaring treatment ($F = 5.25$; df = 1,16.7; $P = 0.0353$). Seed cotton yield in subplots treated with lambda-cyhalothrin was 773 kg/ha compared with 830 kg/ha in untreated subplots.

Satellite Data—Multispectral: 25 August. Factors with significant reflectance differences in the images included N × date and aphid flaring (Table 3). All NIR indices could be used to distinguish plots with the highest nitrogen treatment level of 270 kg N/ha. In all indices, when this amount of nitrogen was applied on 24 June, plots had significantly higher values than those with this amount of nitrogen applied on 8 July. Values from a single image (color infrared 4) were significantly lower in lambda cyhalothrin-treated subplots (aphids flared) compared with untreated subplots (Table 3).

Discussion

In both years, aphid-infested cotton was detected independent of the effect of nitrogen. Also, in both years, aphid abundance was affected by insecticide application, nitrogen rate, and date of nitrogen application. However, two separate remote sensing imaging systems were used, mandating an indirect comparison between years. Although data with near-infrared wavelengths were available for both years, direct comparison between years is not possible because different sensor types were used and the method of calibration to reflectance differed (Jackson and Huete 1991). Methods have been developed to compare different sensors with relative ease (Chen et al. 2005, Nelson et al. 2005), but the indices and data received in 2004 were proprietary. This precluded using such methods to provide a direct comparison between data from both year. Finally, direct comparison is impossible because environmental conditions (e.g., field conditions, solar angle) were variable between years. As a result, each experiment (year) was evaluated separately. Similarities will be drawn to distinguish aphid-infested cotton from nitrogen, a probable confounding factor, using remote sensing.

2003 Airplane Data—Multispectral. Cotton with differential aphid populations was indistinguishable using the NIR wavelength (850 nm) from the multispectral camera systems. However, the 850-nm wavelength analyzed using hyperspectral data could be used for this distinction on 20 August and 11 September 2003. Data were collected simultaneously from each sensor and both used the same calibration method. The airplane multispectral system had a range (bandwidth) of ≈40 nm around each band collected, whereas the hyperspectral camera collected a more precise reflectance wavelength (± ≈10 nm) than the multispectral. A similar disparity was observed between the two camera systems when the experiments of Reisig and Godfrey (2006) were analyzed. In addition to differences in band range, nitrogen differences among plots or other unmeasured factors could have also affected this result.

Airplane Data—Hyperspectral. Cotton with differential populations of aphids could be detected using the NIR wavelength and $\text{CRI}_{\text{GREEN}}$ (carotenoid reflectance index, green), regardless of the amount of nitrogen applied. It is unlikely that these differences resulted from the insecticide treatments (Reisig and Godfrey 2006), and they likely resulted from the presence or absence of aphids. Results were less straightforward using the ARI (anthocyanin index), and CRI_{RED} (carotenoid reflectance index, red). With these indices, detection of cotton with differential populations of aphids was influenced by either the amount of nitrogen applied and/or or the date at which nitrogen was applied. The ARI, $\text{CRI}_{\text{GREEN}}$, and CRI_{RED} all focus on information from green and red

Table 3. Multispectral satellite system data gathered 25 Aug. 2004: ANOVA results

Factors	Color infrared $1_{\text{Band 1}}$			Color infrared $2_{\text{Band 1}}$			Color infrared $3_{\text{Band 1}}$			Color infrared $4_{\text{Band 1}}$			Color infrared $4_{\text{Band 3}}$		
	F	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df
N[a]	0.02	0.8863	2.62	0.05	0.8479	1.94	0.04	0.8668	2.29	0.02	0.8977	2.02	0.00	0.9855	27
Date[b]	3.26	0.1345	4.43	2.19	0.2140	4.63	3.11	0.1540	3.97	2.77	0.1690	4.29	0.55	0.5823	27
N × date[b]	4.78	0.0203[c]	19.6	6.42	0.0078[d]	18.1	3.98	0.0362[c]	18.9	4.81	0.0206[c]	18.9	2.55	0.0965	27
Aphids[a]	0.17	0.6849	14.7	0.2	0.6623	12.5	0.13	0.7197	13	0.35	0.5615	13.2	4.29	0.0479[c]	27
N × aphids[a]	1.01	0.3305	14.7	0.74	0.4046	12.5	1.26	0.2828	13	1.29	0.2755	13.2	0.44	0.5109	27
Date × aphids[b]	0.33	0.7271	14.8	1.16	0.3457	12.6	0.66	0.5336	13	0.85	0.4494	13.4	0.56	0.5753	27
N × date × aphids[b]	2.35	0.1303	14.8	1.99	0.1767	12.6	1.14	0.3484	13	1.64	0.2311	13.4	1.37	0.2711	27

[a] Numerator df = 1.
[b] Numerator df = 2.
[c] $P < 0.05$.
[d] $P < 0.001$.
N, nitrogen rate; Date, nitrogen application date; Aphids, subplots.

wavelengths but were not equivalent in their ability to detect aphid-infested cotton in this study.

Both anthocyanins and carotenoids predominate in stressed plants compared with unstressed plants (Matile et al. 1999). Carroll et al. (2008) reported detection of *Ostrinia nubilalis* Hübner-infested corn in 1 yr using the $\text{CRI}_{\text{GREEN}}$ but not the ARI and CRI_{RED}. They identified these indices as less useful for detecting *O. nubilalis* damage in corn and speculated that the pest had not sufficiently degraded chlorophyll in time to affect the anthocyanins and carotenoids using these indices. It is also known that nitrogen levels are highly correlated with chlorophyll levels, with the majority of leaf nitrogen contained in chlorophyll (Yoder and Pettigrew-Crosby 1995). As a result, detecting aphid infestations with both the ARI and CRI_{RED} may be confounded by both aphid and nitrogen effects on plant physiology. Although speculative, the $\text{CRI}_{\text{GREEN}}$ may focus on wavelengths more sensitive to insect-induced changes in plant physiology because it was significant in the study of Carroll et al. (2008) and the study presented here.

2004 Satellite Data—Multispectral. In contrast to the airplane-derived multispectral data, there were significant differences in some factors using satellite-derived multispectral data. A single index, of four, could be used to distinguish between cotton with variable aphid abundance, regardless of the nitrogen applied to the plant. Although the information contained in these indices was proprietary, it was assumed that they focused on wavelengths in the near-infrared, based on names of the indices provided. All indices could be used to distinguish between plots with a high rate of nitrogen applied on 24 June and plots with the same application rate on 8 July, and they most likely documented a unique time in the physiology of the cotton plant. This may have been while, or during the time in which, nitrogen was absorbed from the soil, translocated, and metabolized by the plant. Aphid abundance in 2004 may have been more strongly affected by nitrogen applied in June than later in the year. Aphid levels were comparable to 2003 levels when nitrogen was applied on 24 June 2004. They were lower than those in 2003 when nitrogen was applied in both July and August in 2003. Perhaps plants in 2004 did not uptake nitrogen as well as in 2003 when applied in July and August. However, plant nutrient levels were not measured over time.

Summary. Cotton infested with two different levels of aphid populations could be distinguished in both years, using different sensor systems, regardless of nitrogen levels in the plant. Consistent with the studies of Reisig and Godfrey (2006), wavelengths in the NIR were decreased in cotton with higher relative aphid abundance in 2003. Unfortunately, only a qualitative comparison can be made from the 2004 data, because information from the indices was not available. More complex analysis, such as disentangling the effect of nitrogen level and aphid abundance on the spectral reflectance pattern, was not successful in this study. Entomological studies focusing on the spectral response affected by combinations of various stressors, in concert with arthropod pests, and plant physiological consequences would be useful.

For remote sensing to be useful in applied entomology, it must be able to distinguish arthropod-caused factors in the field from other factors, or it must provide predictability of where an arthropod population is likely to occur (Willers et al. 1999). The experiments presented here provide a case study and show the difficulty of accomplishing this. Further studies may discover an improved relationship proxy for nitrogen levels in plants and aphid abundance levels. Site-specific applications could be used in areas containing plants with high nitrogen levels. Nonetheless, remote sensing data in conjunction with ground sampling must be timely and economically comparable to ground sampling alone for implementation of this technology.

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