Early infestations by arthropod pests induce unique changes in plant compositional traits and leaf reflectance

Christian Nansen,* Machiko Murdock, Rachel Purington and Stuart Marshall

Abstract

BACKGROUND: With steadily growing interest in the use of remote-sensing technologies to detect and diagnose pest infestations in crops, it is important to investigate and characterize possible associations between crop leaf reflectance and unique pest-induced changes in plant compositional traits. Accordingly, we compiled plant compositional traits from chrysanthemum and gerbera plants in four treatments: non-infested, or infested with mites, thrips or whiteflies, and we acquired hyperspectral leaf reflectance data from the same plants over time (0–14 days).

RESULTS: Plant compositional traits changed significantly in response to arthropod infestations, and individual chrysanthemum and gerbera plants were classified with 78% and 80% accuracy, respectively. Based on leaf reflectance, individual plants from the four treatments were classified with moderate accuracy levels of 76% (gerbera) and 73% (chrysanthemum) but with a clear distinction between non-infested and infested plants. Accurate and consistent diagnosis of biotic stressors was not achieved.

CONCLUSION: To our knowledge, this is the first study in which infestations by multiple economically important arthropod pests are directly compared and associated with leaf reflectance responses and changes in plant compositional traits. It is important to highlight that imposed stress levels were low, period of infestation was short, and hyperspectral remote-sensing data were acquired at four time points with analyses based on large data sets (3826 leaf reflectance profiles for chrysanthemum and 4041 for gerbera). This study provides novel insight into crop responses to different biotic stressors and into possible associations between plant compositional traits and hyperspectral leaf reflectance data acquired from crop leaves.

Keywords: arthropod pest management; remote sensing; image analysis; precision agriculture; plant stress

1 INTRODUCTION

Accurate and practically feasible detection and diagnosis of emerging (low-density) infestations by arthropod pest species is an important challenge in effective and sustainable pest management.1 Many decisions related to a given pest management strategy hinge on details directly linked to which pest species is causing crop stress. For instance, different arthropod pest species infesting the same crop: (i) often have different label restrictions on dosage; (ii) may show considerably different levels of susceptibility; (iii) often have species-specific seasonal and diurnal activity patterns, which can greatly influence when to apply pesticides most effectively; (iv) have different complexes of natural enemies, which often require special considerations to maximize their relative ability to complement and synergize other pest management practices; and (v) have unique growth population dynamics and dispersal propensities (which are likely to be uniquely influenced by environmental conditions, such as weather and crop management practices), so the risk of a pest outbreak ultimately leading to significant crop damage/loss is highly species-specific. These are just some of the reasons why identification of the causal pest species is considered critically important when developing effective and sustainable arthropod pest management strategies.2,3

Most, if not all, crop production systems are potentially threatened by more than one species of arthropod pests. Seasonal variation in the occurrence/abundance of a given arthropod pest, type of feeding and oviposition symptoms, and severity of damage to the most affected part of the crop may be used in combination to narrow down or specifically identify the causal arthropod pest agent. Moreover, skilled/trained personnel walk along rows of crops or aisles inside greenhouses and perform visual inspection to detect and diagnose signs and symptoms of crop stress, and this is a key element of effective pest management.4

* Correspondence to: C Nansen, Department of Entomology and Nematology, University of California Davis, Davis, CA, USA. E-mail: chrnansen@ucdavis.edu

© 2021 The Authors. Pest Management Science published by John Wiley & Sons Ltd on behalf of Society of Chemical Industry.
However, even though different arthropod pests elicit different crop damage symptoms, visual crop inspection is labor intensive and therefore costly, and requires significant labor when performed in large-scale commercial operations. Furthermore, in some cases, accurate detection and diagnosis of emerging arthropod pest outbreaks can be problematic because abiotic and some biotic stressors can cause similar signs and/or symptoms, which may be challenging to distinguish.5–8 Although visual inspection remains a critical component of effective pest management, a number of factors, including labor shortages and operational costs, are driving a growing demand for the automation of agricultural practices, particularly crop monitoring and the detection and diagnosis of arthropod pest outbreaks.9,10

Remote-sensing technologies, especially imaging systems, have been investigated as ways to perform accurate and reliable crop monitoring and have also been integrated more generally into the development of precision agriculture management practices.11–16 In particular, due to the importance of spectral resolution, hyperspectral remote-sensing technologies are being highlighted for their potential to improve the management of agricultural and forestry resources.15 A key driver behind this research interest in remote-sensing technologies is that across virtually all cropping systems, review articles provide unequivocal support for claims about leaf reflectance changes in response to biotic stressors.8,17–21 However, the vast majority of studies into remote sensing to detect biotic stressors are based on one crop and one biotic stressor. Thus for most biotic stressors, it is not known to what extent the effect of a particular stressor on crop phenology can be differentiated from other biotic stressors and from abiotic stressors.

Despite the considerable potential associated with hyperspectral remote sensing, it is important to also highlight some of the main challenges. For instance, hyperspectral remote-sensing technologies are still expensive (and therefore potentially cost-prohibitive) and often require specially trained personnel to effectively operate systems and acquire high-quality data. However, applied and fundamental research into hyperspectral remote-sensing technologies may lead to the construction of simpler sensors to acquire signals in a few spectral bands in which crop leaf reflectance values have been identified as strong and reliable indicators of crop stress. For hyperspectral remote-sensing technologies to become widely adopted, it is equally important to mention challenges related to the calibration of sensor hardware (signal-to-noise ratio of data collected with different systems) and spectral data calibration (for comparison of data collected at different time points and under different abiotic conditions).15,17,22–26 Inconsistencies and stochastic noise in the calibration of both sensor hardware and spectral data adversely affect the performance (accuracy and robustness) of classification algorithms, so studies are needed in which the overall sensitivity of classification algorithms to stochastic reflectance noise is quantified experimentally.27 In addition, it is important to carefully examine underlying associations between leaf reflectance and plant physiology/metabolism as ways to potentially describe likely effects of biotic stress, and this was investigated in this study.

Figure 1 illustrates two basic hypotheses that underpin the use of leaf reflectance data in the detection and diagnosis of biotic stressors in crops. Hypothesis 1 is that infestation by a given arthropod pest (or any other abiotic or biotic stressor) is associated with unique changes in plant compositional traits (that is, phytochemicals, other organic compounds, element composition, and physiological variables associated with photosynthesis). Hypothesis 2 is that changes in plant compositional traits are associated with detectable and unique features in leaf reflectance profiles. Considering the potential and importance of developing accurate and reliable remote-sensing technologies to automate crop management, there are surprisingly few studies in which the authors have combined analyses of plant compositional traits and leaf reflectance data. Such studies, especially those describing and comparing plant compositional trait responses to different stressors, are needed to further support the underlying hypothesis of arthropod pests being associated with species-specific plant responses that can be detected and diagnosed based on analyses of leaf reflectance. In support of both hypotheses, Carter and Knapp28 concluded that plants tend to show a quasi-universal increase in leaf reflectance in spectral bands near 700 nm in response to a wide range of abiotic and biotic stressors. In addition, these authors demonstrated experimentally that an increase in leaf reflectance was associated with a reduction in chlorophyll concentration.28 This excellent study28 provided important insight into the specific significance of leaf reflectance in spectral bands near 700 nm, but practical applications of reflectance-based detection and diagnostics of stressors will be limited unless unique reflectance features can be associated with specific abiotic and biotic stressors. Under low/emerging pest pressure, changes in plant compositional traits and/or reflectance values in individual spectral bands may be quite subtle and therefore non-significant if examined individually. However, when examined based on a multivariate approach, a specific arthropod infestation may be associated with a detectable and significant stress response because ratios among plant compositional traits change. Recently, Ribeiro et al.29 examined the extent to which the phytochemical composition of maize plants (Zea mays L.) was affected by experimental infestation with green belly stink bug, Dichelops melacanthus (Dallas) (Hemiptera: Pentatomidae). The authors concluded that multivariate analysis of phytochemicals provided a stronger indication of the plant stress response to herbivory than analyses of individual phytochemicals. In addition, they provided support for the claim that changes in leaf reflectance features can be used as indicators of subtle biotic plant stress levels.

This study is composed of two separate but highly complementary parts that address each of the two hypotheses in Figure 1. “Plant compositional traits” refer to the combination of leaf element composition (N, S, P, K, Mg, Ca, Na, B, Zn, Mn, Fe, Cu, and Al) and photosynthetic activity (photosynthesis, stomatal conductance, transpiration rate, and intercellular CO2) and were collected from two ornamental plant species, Chrysanthemum L. (Asteraceae) (chrysanthemum) and (Gerbera jamesonii) (Asteraceae) (gerbera). Individual plants were subjected to one of four experimental treatments: (i) control (non-infested); or plants infested with (ii) two-spotted spider mites, Tetranynchus urticae Koch (Acari: Tetranychidae), referred to as mites; (iii) western flower thrips, Frankliniella occidentalis (Thysanoptera: Thripidae), referred to as thrips; or (iv) silverleaf whiteflies, Bemisia tabaci (Hemiptera: Aleyrodidae), referred to as whiteflies. Plant compositional traits were collected at baseline (prior to arthropod infestation) and after 2 weeks of arthropod-induced stress. We determined the extent to which plant compositional traits could be used to classify individual chrysanthemum and gerbera plants from the different treatments. In the second part of the study, we acquired hyperspectral leaf reflectance data from the same chrysanthemum and gerbera plants at four time points: 0 (before infestation), and 3, 7, and 14 days after arthropod infestation. To our knowledge, this is the first study in which infestations by multiple economically important arthropod...
pests are directly compared and associated with leaf reflectance responses and changes in plant compositional traits.

2 MATERIALS AND METHODS

2.1 Plants and arthropod pests

All plants were grown in individual pots inside screen cages (BugDorm-2120F insect-rearing tents: width = 60 cm, depth = 60 cm, and height = 60 cm; BioQuip Products) at temperature-controlled greenhouse facilities at the University of California, Davis. Abiotic conditions inside the screen cages were: 25–30°C (average = 27.8°C) and 40–50% relative humidity (RH; average = 46.2%). To trigger/elicit flowering, chrysanthemum plants were maintained in a blacked-out greenhouse (black and white panda film with black side facing inwards into the greenhouse) with artificial lighting (1000 W high-pressure sodium artificial lighting with a 12:12 h light/dark photoperiod). Chrysanthemum plants were obtained from Gro-Link Plant Co. They were planted in soilless media (UC Agronomy Mix) in 4-inch pots and were continuously supplied with fertilizer (UC Davis modified Hoagland’s solution). One week before arthropod infestations, chrysanthemum plants were trimmed for standardizing purposes, so that each plant contained one stem with one flower bud. Gerbera plants were obtained from Dümmen Orange and planted in soilless media (UC Agronomy Mix) in 6.5-inch pots with continuously supplied fertilization (UC Davis modified Hoagland’s solution) through drip irrigation (ppm): N = 131.5, P = 40.5, K = 180.0, Ca = 101.0, Mg = 52.0, S = 685.1, Fe = 1.5, Cu = 0.1, Mn = 0.3, Mo = 0.1, and Zn = 0.1. Drip irrigation was delivered to individual pots as two separate irrigation events of 1 min each and 8 h apart (2 × 35 ml = 70 ml per day).

Arthropod pests used in experimental infestations were obtained from continuous colonies at UC Davis: two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetanychidae), referred to as mites; (ii) 15 adult female two-spotted spider mites, *T. urticae* Koch; (iii) 20 adult silverleaf whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), referred to as whiteflies; and (iv) 20 adult western flower thrips, *F. occidentalis* (Thysanoptera: Thripidae), referred to as thrips. Whitefly adults were removed from cages after 2 days of infestation. After 14 days, arthropod infestations were verified on all plants. Plants were discarded if they had been accidentally infested, and infested plants without confirmed presence of infestation were also discarded. It is important to highlight that the selected infestation levels were quite low and that arthropod pests were only allowed 14 days to establish and impose crop stress. After 14 days of arthropod infestation, none of the plants showed any clearly visual signs of infestation, so this study was performed with plants that were subjected to subtle biotic stress levels.

2.2 Plant compositional traits related to arthropod infestations

In total, we acquired plant compositional trait data from 108 plant samples (Table 1). Because plant compositional trait analyses required destructive sampling of leaf materials, baseline samples represented leaf samples collected immediately prior to infestation and were obtained from plants not being infested with arthropods. Additionally, we collected leaf samples from the four treatments after 2 weeks of arthropod infestation. In data analyses, baseline and control data were grouped into one treatment (control). Leaf material from individual plants was collected, dried for 48 h at 70°C, and subsequently ground to a fine powder and subjected to analyses of element composition of leaf samples (N, S, P, K, Mg, Ca, Na, B, Zn, Mn, Fe, and Cu); element composition analyses were performed by a commercial laboratory (https://algreatlakes.com/). Four photosynthetic activity parameters, photosynthesis (mol CO₂ m⁻² s⁻¹), stomatal conductance (mol H₂O m⁻² s⁻¹), transpiration rate (mmol H₂O m⁻² s⁻¹) and intercellular CO₂ (µmol CO₂ mol air⁻¹), were measured on individual plants with a LiCOR 6400XT (https://www.licor.com) portable photosynthesis system both before and after arthropod infestations. Photosynthetic activity parameters were taken from leaves in the middle of the canopy, with a controlled CO₂ supply (400 µl) and flow (400 µm). Photosynthetically active radiation (PAR) was set to 1000 µmol m⁻² s⁻¹, which is considered the approximate light saturation point.²⁰

2.3 Leaf reflectance to detect and diagnose pest-induced plant stress responses

We acquired hyperspectral imaging data from individual chrysanthemum and gerbera plants at 0 (baseline, before infestation), 3, 7, and 14 days after arthropod infestation. In total,
144 (chrysanthemum) and 137 (gerbera) hyperspectral images were acquired. Hyperspectral imaging was performed inside a temperature- and humidity-controlled dark-room (20–23°C and 50–75% RH). We used a push-broom hyperspectral camera (PIKA L; www.resonon.com), which was mounted on a customized robotic rail system with the lens approximately 1.5 m above the plant canopy. The customized hyperspectral imaging system is shown in Figure 2(a), and representative images of chrysanthemum and gerbera plants are shown (Figure 2b,c). Hyperspectral images were acquired with the spatial resolution of about 9 pixel mm\(^{-2}\) under artificial lighting (12 and 15 W and 12 V halogen light bulbs on either side of the lens). Hyperspectral imaging data from individual plants comprise data in 150 spectral bands from 380 to 1015 nm (spectral resolution = 4.2 nm). A piece of white Teflon was used for white calibration, and both dark and white calibrations were performed immediately prior to each imaging event to obtain relative reflectance. To smooth hyperspectral data and reduce the size of data files from each plant, hyperspectral image files were subjected to 15 × 15 pixel (spatial) binning (Figure 2d,e). After 15 × 15 spatial binning, we used the method described by Nguyen and Nansen\(^{31}\) and deployed a NDVI-related radiometric filter to include only binned pixels within a specific band ratio. Moreover, the following band ratio was calculated: \(\frac{R_{750} - R_{705}}{R_{750} + R_{705}}\), and only pixels with a band ratio above 0.50 and below 0.90 were included. After selection, an average of 28.3 (chrysanthemum) and 28.1 (gerbera) binned pixels were used for data analyses of leaf reflectance from each species (chrysanthemum = 3826 binned pixels and gerbera = 4041 binned pixels).

### 2.4 Statistical analyses

All data processing and analyses were conducted in R v.3.6.1 (R Foundation for Statistical Computing). Regarding plant compositional traits, three separate analyses were performed for each ornamental plant species and both plant compositional traits and leaf reflectance data: principal component analysis (PCA), analysis of variance (ANOVA), and support vector machine classification. In all analyses, the objective was to compare plants among the four treatments. We used the “devtools” library to perform PCA, and dichotomous dummy variables accounting for

| Table 1. Ornamental crop plant samples included in this study |
|---------------------------------------------------------------|
| **Crop plant species** | **Baseline** | **Control** | **Mites** | **Thrips** | **Whiteflies** | **Grand total** |
|------------------------|-------------|-------------|-----------|------------|---------------|-----------------|
| Gerbera                | 6           | 14          | 5         | 10         | 5             | 40              |
| Chrysanthemum          | 10          | 22          | 12        | 12         | 12            | 68              |
| Total                  | 16          | 36          | 17        | 22         | 17            | 108             |

Plant compositional traits [N, S, P, K, Mg, Ca, Na, B, Zn, Mn, Fe, and Cu] and photosynthetic activity [photosynthesis (mol CO\(_2\) m\(^{-2}\) s\(^{-1}\)), stomatal conductance (mol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), transpiration rate (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)), intercellular CO\(_2\) (μmol CO\(_2\) mol air\(^{-1}\))] were obtained from 108 individual crop plant samples from two ornamental crops (chrysanthemum and gerbera) in five treatment classes: baseline (before infestation), control (non-infested), or infested with two-spotted spider mites (mites), western flower thrips (thrips), or silverleaf whiteflies (whiteflies).
treatments (control, mites, whiteflies, and thrips) were included. The "multcomp" library was used to perform ANOVAs of individual plant compositional traits, and post-hoc Tukey pairwise comparisons were performed when significant treatment effects were detected. Regarding selection of algorithm to classify samples from different treatments based on either plant compositional traits or leaf reflectance profiles, an initial analysis was based on leaf reflectance data from gerbera plants and consisted of direct performance comparison of linear discriminant analysis ("MASS" and "caret" libraries), random forest (library randomForest), and support vector machine (library e1071). The following Cohen's kappa coefficients were obtained: linear discriminant analysis = 0.556, random forest = 0.651, support vector machine = 0.715. Consequently, support vector machine-based classification of both plant compositional traits and leaf reflectance data was performed.

Regarding reflectance data acquired from chrysanthemum and gerbera plants 0, 3, 7, and 14 days after arthropod infestation, because baseline hyperspectral images (0 days after infestation) were acquired immediately prior to arthropod infestations, these data were all considered control treatment. In ANOVAs, we generated average reflectance profiles per plant and analyzed treatment effects in all 150 spectral bands. Post-hoc Tukey comparisons were performed when significant treatment effects were detected. Each of the two PCAs (one for each ornamental plant species) was based on reflectance values in all 150 spectral bands in which observations were equal to the number of binned pixels (chrysanthemum = 3826 binned pixels and gerbera = 4041 binned pixels). We included dichotomous variables accounting for each of the four treatments, and a variable, "Time", accounting for days of infestation (0, 3, 7, and 14) was also included. For each ornamental plant species, we performed support vector machine classification in which treatment was used as a

![Figure 3](image-url)

**Figure 3.** Principal component analyses (PCA) of plant compositional traits from chrysanthemum and gerbera plants. PCA were performed based on 68 chrysanthemum samples (a) and 40 gerbera samples (b). Dichotomous dummy variables denoted treatment classes (control, mites, whiteflies, and thrips).

| Response variable | Chrysanthemum | Gerbera | F-value |
|-------------------|---------------|---------|---------|
| Photosynthesis    | A A B B       | A AB B A | 10.09*** |
| Transpiration     | A B B C       | A B B A | 22.97*** |
| Intercellular CO₂ | A A A B       | NS NS NS NS | 12.92*** |
| Conductance       | A A A B       | A B B A | 5.03** |
| Fe                | A B AB AB     | AB AB B | 3.14* |
| K                 | A B B B       | NS NS NS NS | 8.03*** |
| P                 | A ABC C AB    | NS NS NS NS | 8.05*** |
| Mn                | NS NS NS NS NS | 0.03 | 4.39** |
| Cu                | NS NS NS NS NS | 2.72 | 4.19** |
| Mg                | NS NS NS NS NS | 0.53 | 1.92 |
| S                 | NS NS NS NS NS | 1.6 | 2.06 |
| Na                | NS NS NS NS NS | 0.81 | 1.03 |
| Zn                | NS NS NS NS NS | 2.16 | 1.22 |
| Al                | NS NS NS NS NS | 4.21** | 0.58 |
|                   |               | NS NS NS NS NS | 1.92 |
|                   |               | NS NS NS NS NS | 2.06 |
|                   |               | NS NS NS NS NS | 1.03 |
|                   |               | NS NS NS NS NS | 1.22 |
|                   |               | NS NS NS NS NS | 1.09 |

Analysis of variance was used to compare selected average plant compositional traits for control (non-infested) and infested (combining data from plants infested with two-spotted spider mites, western flower thrips, or silverleaf whiteflies). 'Infested/Control' denotes the relative response to infestation. F-value is the statistical result from average comparisons, with * being significant at the 0.05-level, *** being significant at the 0.001 level. "A", "B", "C", and "D" denote statistical differences among treatments. "NS" denote non-statistical difference. "**,***, and ****" denote statistical differences at the 0.05, 0.01, and <0.001 levels, respectively.
categorical response variable and reflectance values in the 150 spectral bands from binned pixels were used as explanatory variables. Accuracy of support vector machine classification was based on kappa values and tenfold validation. Each pixel was classified independently of other pixels from the same plant, so individual plants were assigned to the treatment, which had the highest number of pixels. As an example, out of 21 pixels from a chrysanthemum plant, 11 were classified as control, two as mites, seven as thrips, and one as whiteflies. Thus, the given plant was assigned to the control treatment.

3 RESULTS

3.1 Plant compositional traits and arthropod infestations of chrysanthemum

PCA of plant compositional trait data from chrysanthemum revealed that 45% of the total variance was explained by the two principal axes, PCA1 and PCA2 (Figure 3a). The dummy variable accounting for control plants was positioned leftwards along the principal axis, PCA1, while dummy variables accounting for the three arthropod infestations were positioned rightwards along the principal axis, PCA2. This alignment of treatments suggested that infestation (yes/no) accounted for a considerable portion of the variance in plant compositional traits. In addition, the position of treatment dummy variables along the second axis, PCA2, suggested that leaf samples from plants infested with mites or thrips showed high degree of similarity and were different from those infested with whiteflies. Most of the plant compositional traits were positioned in the left part of Figure 3(a) and were therefore positively associated with leaf reflectance and arthropod infestations. In ANOVAs, the four variables associated with photosynthesis (photosynthesis, stomatal conductance, transpiration rate, and intercellular CO₂) responded significantly to treatment effects, and all four were significantly different between control and whitefly-infested plant samples (Table 2). Three elements (Fe, K, and P) also showed a significant response, but each varied in their relative treatment response. Support vector machine classification of samples from chrysanthemum plants was associated with an overall accuracy (based on tenfold validation) of 78% and kappa = 0.93. (Table 3).

3.2 Plant compositional traits and arthropod infestations of gerbera

A PCA of gerbera data revealed that about 44% of the total variance was explained by the two principal axes, PCA1 and PCA2 (Figure 3b). The dummy variable accounting for control plants was placed downwards along the second axis, PCA2, while dummy variables accounting for infestations with mites and thrips were positioned upwards along the principal axis, PCA2. As seen in the analysis of chrysanthemum data, plant compositional traits showed a high degree of disassociation between control plants and those infested with mites and thrips. Most of the plant compositional traits were positioned in the lower part of Figure 3(b) and were therefore positively associated with leaf samples from control plants. Another similarity between PCA of chrysanthemum and gerbera data was that the dummy variable accounting for whitefly infestations was somewhat disassociated from the other three treatments, which is an indication of whitefly infestation inducing a somewhat different stress response from mite and thrips infestations. Three of the four photosynthesis variables responded significantly to treatment effects and were significantly different between control and whitefly-infested plant samples (Table 2). Two elements (Fe and Mn) responded significantly but each varied in their relative treatment response. Support vector machine classification of samples from gerbera plants was associated with an overall accuracy (based on tenfold validation) of 80% and kappa = 0.96. (Table 3).

3.3 Chrysanthemum leaf reflectance and arthropod infestations

Average leaf reflectance curves from chrysanthemum plants across treatments at the four time points showed a high degree of consistency 3–14 days after infestation (Figure 4a). In addition, baseline average leaf reflectance in spectral bands from 710 to 1015 nm was markedly higher than at the other time points. A decrease in leaf reflectance could be attributed to maturation/darkening of leaves, and a distinct temporal leaf reflectance trend is important because it may mask or reduce the ability to detect possible treatment effects. Despite the temporal trend shown in Figure 4(a), and the fact that baseline and control data were grouped together into one class, ANOVAs of average reflectance profiles from chrysanthemum plants showed significant treatment responses in individual spectral bands from 380 to 500 nm, 640 to 695 nm, and 705 to 1015 nm with multiple relative peaks in F-values (Figure 4b). Pairwise Tukey analyses of average reflectance in three peak spectral bands (485, 674, and 984 nm) showed consistent significant difference between control and biotic stressor treatments but no significant differences among biotic stressor treatments (Table 4). In Figure 4(b), it is clearly seen how leaf reflectance responses to mites and thrips were similar and generally induced an increase in leaf reflectance. It is also seen that whitefly induced a decrease in leaf reflectance in

| TABLE 3. Confusion matrices of support vector machine classification of plant compositional traits from chrysanthemum and gerbera plants |

| Actual treatment | Chrysanthemum | Gerbera |
|------------------|---------------|---------|
|                  | Control | Mites | Thrips | Whiteflies | Control | Mites | Thrips | Whiteflies |
| Control          | 32      | 0     | 0      | 0          | 20      | 0     | 0      | 0          |
| Mites            | 0       | 11    | 1      | 0          | 0       | 5     | 0      | 0          |
| Thrips           | 2       | 0     | 10     | 0          | 0       | 1     | 9      | 0          |
| Whiteflies       | 0       | 0     | 0      | 12         | 0       | 0     | 0      | 5          |

Plant compositional traits from 68 chrysanthemum samples or 40 gerbera samples were used to perform support vector machine classifications. Values in bold denote accurate predictions/assignments to treatments. Regarding chrysanthemum samples, overall accuracy based on tenfold validation = 78%, and kappa = 0.93. Regarding gerbera samples, overall accuracy based on tenfold validation = 80%, and kappa = 0.96.
spectral bands from 380 to 500 nm and near 680 nm. Importantly, relative difference (biotic stressor /control) values varied between 0.95 and 1.05 (red horizontal line in Figure 4b), which suggests a stress response within 5% and therefore emphasizes that leaf reflectance data were acquired during early infestations.

PCA of reflectance values in all spectral bands from 3826 binned pixels showed that two principal axes, PCA1 and PCA2, explained about 82% of the total variance (Figure 5a). The 150 spectral bands are shown as black dots, and were located in the left part along the negative side of PCA1, whereas biotic stressor and time were located in the right part along the positive side of PCA1. Thus, reflectance values in spectral bands decreased in response to biotic stress and over time. Of the three biotic stressor treatments, whitefly treatment was positioned furthest from control and closest to the variable time, which suggests that this stressor caused the most distinct reflectance response and showed the strongest response over time.

Based on tenfold validation, support vector machine classification of binned pixels from chrysanthemum plants was associated with an overall accuracy of 70% and kappa = 0.65, and it is seen that the data set (due to all binned pixels from baseline) was heavily skewed towards the control treatment (Table 5). We examined the classification of pixels from each plant and assigned each plant to the treatment with the highest number of pixels. Using this approach, the overall accuracy of classification of individual plants was 76%. Because of the data bias towards control plants, it is not surprising that plants from this category were classified with the highest level of accuracy (92%). In addition, fewer than 10% of plants from any of the three biotic stress treatments were mis-classified as control. Examination of mis-classification
responses in individual spectral bands near 550 nm and 715 nm
tance pro
average re
that can adversely affect the ability to accurately classify plants
chrysanthemum plants, but is still important to highlight as a factor
Thus, the temporal trend was different from that observed in chry-
700 to 1015 nm was apparent 14 days after infestation (Figure 4c).
3.4 Gerbera leaf reflectance and arthropod infestations

Across treatments, average reflectance profiles were similar
0–7 days after infestation, but an increase in spectral bands from
700 to 1015 nm was apparent 14 days after infestation (Figure 4c).
Thus, the temporal trend was different from that observed in chry-
santhemum plants, but is still important to highlight as a factor
that can adversely affect the ability to accurately classify plants
based on average reflectance profiles. ANOVAs of average reflect-
ance profiles from gerbera plants showed significant treatment
responses in individual spectral bands near 550 nm and 715 nm
(Figure 4d). We performed pairwise Tukey analyses for spectral
bands representing these regional peaks (543 and 716 nm) and
showed that average reflectance values varied significantly
between control plants and those subjected to either mites or
thrips infestations, whereas whitefly infestation did not induce a
significant stress response (Table 4). Except for spectral bands
from 400 to 500 nm, relative difference (biotic stressor/control)
varied between 0.95 and 1.05 (red horizontal line in Figure 4d),
which suggests a stress response within 5% and therefore emph-
izes that leaf reflectance data were acquired during early
infestations.

In PCA of 2824 binned pixels, the two principal axes, PCA1 and
PCA2, explained approximately 74% of the total variance, and
we identified the following trends (Figure 5b). Control treatment
was located downwards along the negative portion of PCA2,
whereas time and thrips and whiteflies were positioned upwards
along the positive portion of PCA2. Thus, thrips treatment showed
the strongest change in spectral reflectance over time, and of the
three biotic stressors, was the treatment that was most distinct
from control. Mite treatment was positioned leftwards along the
negative portion of PCA1, whereas all spectral bands were posi-
tioned rightwards along the positive portion of PCA1. This
position of dots representing spectral bands indicated that,
compared with the other three treatments, mite infestation caused a general decrease in spectral reflectance.

Support vector machine classification of binned pixels from gerbera plants into treatments, based on tenfold validation, was associated with an overall accuracy of 76% and kappa = 0.71 (Table 6). Similar to leaf reflectance data from chrysanthemum plants, this data set from gerbera plants was also heavily biased towards control plants. Overall, classification of individual plants was associated with an accuracy of 73%. Control plants were classified with 89% accuracy, those from mite- and thrips-infested plants were classified with approximately 68% accuracy, and binned pixels from whitefly-infested plants were classified with only 39% accuracy. Plants infested with mites or thrips were not misclassified as infested with whiteflies, and plants infested with mites or thrips were misclassified as each other (meaning, they were highly similar in terms of leaf reflectance). In fact, if classification of plants infested with mites or thrips were combined, then accuracies would exceed 95%. Main results from analyses of leaf reflectance from gerbera plants were that: (i) biotic stressors caused a marked and detectable change (control versus biotic stress treatments) in leaf reflectance; (ii) plants infested with mites or thrips showed a high degree of similarity in terms of leaf reflectance; and (iii) diagnosis of whitefly infestation was associated with low classification accuracies (39%) and therefore did not support the second study hypothesis.

4 DISCUSSION

A steadily growing number of studies describing the potential of remote-sensing technologies in a wide range of agricultural applications supports the claim that, in combination with robotics, these technologies may lead to profound change and possibly revolutionize food production systems. However, it is important to emphasize that widespread adoption of remote-sensing technologies for the early detection and diagnosis of biotic stressors face several key challenges, including: (i) low and inconsistent signal-to-stochastic noise ratio in some portions of the radiometric spectrum; (ii) spectral calibration of sensor hardware to allow accurate comparison of remote-sensing data acquired with different instruments; and (iii) consistent conversion of acquired remote-sensing data into reflectance (spectral calibration), and it is of paramount importance to identify and thoroughly examine assumptions underpinning the successful and reliable deployment of remote-sensing technologies. We examined two hypotheses that are directly linked to the use of remote-sensing technologies to detect and diagnose biotic stressors in crop plants.

The first hypothesis is that infestation by a given arthropod pest is associated with unique changes in plant compositional traits. Analyses of plant compositional traits highlighted similar trends in chrysanthemum and gerbera data. Moreover, arthropod infestation was associated with significant changes in plant compositional traits, especially those directly associated with photosynthesis. We also showed that mite and thrips infestations appear to induce similar plant responses, and plant responses to these two biotic stressors were partially different from those induced by whitefly infestations. Importantly, support vector machine classification based on plant compositional traits classified the four treatments (control, mites, thrips and whiteflies) with 78% (chrysanthemum) and 80% (gerbera) accuracy. Thus, study results provided strong support for the first hypothesis.

The second hypothesis is that changes in plant compositional traits are associated with detectable and unique features in leaf reflectance profiles. Several important results were derived from analyses of leaf reflectance data. First, in both ornamental species, but especially chrysanthemum, we observed considerable temporal trends, which may adversely affect the accuracy of classification algorithms, especially when plants are only exposed to subtle stress responses. Second, in both ornamental species, we observed a distinct difference between control plants and those subjected to biotic stressors, although in terms of leaf reflectance, relative stress responses to biotic stressors were quite different in chrysanthemum and gerbera plants. Third, support vector machine classification of leaf reflectance data from chrysanthemum plants showed that stress induced by mite and whitefly infestations could be diagnosed with moderate accuracy (74% and 67%, respectively), whereas thrips infestation was associated with low (46%) classification accuracy. Finally, support vector machine classification of leaf reflectance data from gerbera plants showed that stress induced by mite and thrips infestations could be diagnosed with high accuracy (97%) if combined, whereas whitefly infestation was associated with low (39%) classification accuracy. Because the current study was performed with quite subtle infestations and pests were only allowed 14 days to impose plant stress, the results presented were only in partial agreement with the second hypothesis.

### TABLE 6. Confusion matrices of support vector machine classification of leaf reflectance data from gerbera plants

| Actual treatment | Binned pixels | Individual plants |
|------------------|--------------|------------------|
|                  | Control      | Mites            | Thrips | Whiteflies | Control | Mites | Thrips | Whiteflies |
| Control          | 2126         | 33               | 38     | 37         | 89%     | 3%    | 5%     | 3%         |
| Mites            | 40           | 305              | 102    | 49         | 4%      | 68%   | 29%    | 0%         |
| Thrips           | 47           | 175              | 540    | 101        | 4%      | 29%   | 68%    | 0%         |
| Whiteflies       | 56           | 36               | 69     | 287        | 13%     | 22%   | 26%    | 39%        |

A total of 4041 binned pixels from chrysanthemum plants were used to perform support vector machine classification. Numbers in bold denote accurate predictions/assignments to treatments. Overall accuracy based on tenfold validation = 76%, and kappa = 0.71. Individual plants were assigned to the treatment that had the highest number of pixels. As an example, of 21 pixels from a chrysanthemum plant, 11 were classified as infested with whiteflies, and 1 as whiteflies. Thus, the given plant was assigned to the control treatment. Percentages of plants assigned to treatments are shown in bold denoting accurate predictions/assignments to treatments. Overall accuracy of classification of individual plants = 73%.
4.1 Plant compositional traits

We are aware of only a few studies in which arthropod infestation has been associated with changes in element composition. Jood et al.\textsuperscript{32} found that infestation of stored grains caused significant increases in grain levels of six elements (P, Fe, Zn, Mn, Ca, and Cu). Fe levels showed significant treatment effects in both chrysanthemum and gerbera plants, whereas P, Mn and Cu levels only showed significant treatment effects in one of the two ornamental species. In chrysanthemum, K levels were negatively influenced by arthropod infestations. Studies involving spider mite infestation of maize plants\textsuperscript{33,34} and aphid infestation of wheat\textsuperscript{34} also showed clear indications of being associated with low K levels in host plants. In a review of over 2000 studies regarding effects of K on pest and disease incidence in plants, Perrenoud\textsuperscript{35} showed that K fertilization reduces pest infestation. A reduction in K levels in plants is believed to cause accumulation of soluble sugars and amino acids and reduce long distance transport of organic nutrients in the xylem and phloem,\textsuperscript{36} which could be interpreted as an increase in plant suitability for herbivores. Amtmann et al.\textsuperscript{36} also mentioned that low K levels may partially impair control of guard cells and therefore increase plant transpiration, so there may be an association between K levels and photosynthetic activity. In chrysanthemum, all four variables (photosynthetic, transpiration, intercellular CO\textsubscript{2}, and stomatal conductance) varied significantly between control and whitefly treatments. Amtmann et al.\textsuperscript{36} described how reduction of K levels in plants may lead to reduced photosynthetic activity due to reduced proton gradients across the thylakoid membrane, which is driving ATP synthesis. A reduction in photosynthesis means an increase in leaf reflectance, so our data from chrysanthemum plants corroborated this physiological explanation. In both ornamental plants species, we found that ANOVAs of plant compositional traits highlighted significant treatment effects, but post-hoc Tukey analyses showed that differences in averages were insufficient to enable clear distinction of individual treatments. However, when using all plant compositional traits as explanatory variables in support vector machine classification, plants from different treatments could be classified with 78\% (chrysanthemum) and 80\% (gerbera) accuracy. Thus, as shown in a similar study of phytohormones,\textsuperscript{29} combinations of plant compositional traits analyzed in a multivariate approach are needed to accurately detect and diagnose biotic stressors, and the results from this study are in agreement with the first hypothesis (Figure 1).

4.2 Leaf reflectance

Analyses of stress responses in individual spectral bands are important for the development of simple sensor technologies, in which reflectance data are only collected in a few selected bands. Significant treatment effects in leaf reflectance from chrysanthemum plants occurred in spectral bands from 380 to 500 nm, 640 to 695 nm, and 705 to 1015 nm, whereas significant treatment effects in leaf reflectance from gerbera plants occurred in spectral bands near 550 nm and 715 nm. Carter and Knapp\textsuperscript{28} concluded that leaf reflectance in spectral bands near 700 nm typically show a strong stress response, and this was also found in this study, with peaks in treatment effects (based on F-values in ANOVAs) at 674 nm (chrysanthemum and 716 nm (gerbera). None of the examined individual spectral bands revealed a significant difference among plants subjected to one of the three biotic stressors. Thus, our results did not provide support for the possibility of using single spectral bands to differentiate among treatments. However, two multivariate approaches, PCA and support vector machine classification, applied to leaf reflectance data from both chrysanthemum and gerbera highlighted partial support for the second study hypothesis. In chrysanthemum, mite infestation caused the strongest stress response, whereas in gerbera plants stress induced by mites and thrips appeared to induce the strongest and similar stress responses.

Regarding crop leaf response to whitefly infestations, Lu et al.\textsuperscript{37} used a hyperspectral spectrometer from control and infested tomato leaves and highlighted reflectance at 560, 575, and 720 nm as providing indication of yellow leaf curl disease, which is vectored by whiteflies. The authors used their results as support for the claim that spectral indices and multispectral reflectance may provide accurate detection and diagnosis of plant diseases vectored by whiteflies. Gu et al.\textsuperscript{38} used hyperspectral reflectance data in 128 spectral bands to classify tomato plants with/without tomato spotted wilt virus infection in tobacco, and this disease is vectored by thrips. The practical challenge associated with detection of plant diseases vectored by whiteflies and thrips is that once infected plants are essentially lost, as there are no means to reverse or suppress the etiology of these viral infections. Several studies have demonstrated crop leaf reflectance responses to spider mite infestations,\textsuperscript{33,39–44} and in most of these studies spider mite infestation resulted in increases in leaf reflectance. In our analyses of leaf reflectance data, mite infestation caused a relative increase in leaf reflectance in chrysanthemum plants but a relative decrease in gerbera plants. Thus, from comparison of two different crop plant species subjected to the same biotic stressor, our results indicate that plants may respond quite differently to a given stressor.

4.3 Conclusions

Attempts to directly associate individual plant compositional traits, such as K, with the difference between non-infested and infested plants or to a specific arthropod pest would likely ignore and disregard important and complex interactions among physiological plant responses. That is, both uptake and activity of metabolic pathways involving individual plant elements are clearly associated and also intricately linked to overall photosynthetic activity and plant metabolism, so it seems less meaningful to study plant compositional traits individually. Similarly, it may be argued that analyses of individual spectral bands or simple band indices are unlikely to fully characterize responses to a given stressor and even less likely to enable accurate classification of different stressors. Multivariate analyses of plant compositional traits provided highly accurate (tenfold validation results exceeding 80\%) classification of plants, but element composition analyses require destructive sampling of leaf material and are labor-intensive and time-consuming. In addition, if commercial laboratories are involved, costs may become a challenge. Thus, there are important reasons for continued research into technologies, such as remote sensing, to detect and diagnose arthropod infestations. Leaf reflectance data from both chrysanthemum and gerbera highlighted that control plants were distinctly different from those subjected to biotic stressors. Even though each leaf reflectance data set included a considerable temporal trend, individual plants from the four treatments were classified with an overall accuracy of 76–77\%. Although this accuracy may seem low compared with other published studies on detection and diagnosis of biotic crop stressors, it is important to highlight that imposed stress levels were quite low, period of infestation was short, and different stressors were expected to induce similar stress responses and therefore be challenging to distinguish. Results

Pest Manag Sci 2021; 77: 5158–5169 © 2021 The Authors.

www.soci.org
from this study highlighted that different crop plant species may respond differently to biotic stressors. Furthermore, different plant species may also respond differently to environmental conditions and to time of growth. Thus, it may be challenging to successfully apply a classification algorithm developed on data from one plant to classify individual plants from another species (or even different crop variety of the same species). Successful use of leaf reflectance-based detection and diagnosis of crop stressors will therefore likely require the development of crop/variety-specific classification algorithms. Finally, our data suggest that it may only be possible to accurately detect and diagnose biotic stressors once the stress induces leaf reflectance responses beyond 5%. Further studies are needed to provide improved insight into the complex interactions between crops—stressors—plant compositional traits—leaf reflectance, as this study highlighted both general trends and unique differences.

**ACKNOWLEDGMENTS**

We wish to thank Grolink Plant Company LLC for supplying chrysanthemum plants. This study was sponsored through a combination of funding by the U.S. Department of Agriculture’s (USDA) Agricultural Marketing Service through grant 18-00001-056-SC as well as partial funding from the American Floral Endowment, the Glocckner Foundation, and USDA/ARS Floriculture, Nursery Research Initiative.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

**REFERENCES**

1. Oerke E-C, Crop losses to pests. *J Agric Sci* 144:31–43 (2006).
2. Pedigo L and Rice M, *Entomology and Pest Management*. Prentice Hall, Waveland Press Inc., IL, USA (2006).
3. Oli DH and Drees BM, Fire ant IPM, in *Integrated Pest Management*, ed. by Radcliffe EB, Hutchinson WD and Cancado RE. Cambridge University Press, Cambridge pp. 390–401 (2009).
4. Sellmer JC,östiguy N, Hoover K and Kelley KM, Assessing the integrated pest management practices of Pennsylvania nursery operations. *HortScience* 39:297–302 (2004).
5. Fageria NK, Baligar VC and Clark RB, Micronutrients in crop production, in *Advances in Agronomy*, ed. by Sparks DL. Academic Press, Cambridge, MA pp. 185–268 (2002).
6. Bergmann W, *Nutritional Disorders of Plants: Visual and Analytical Diagnosis*. Spectrum Akademischer Verlag, Heidelberg, Germany (1992).
7. Bamsey M, Graham T, Thompson C, Berinstein A, Scott A and Dixon M, Ion-specific nutrient management in closed systems: the necessity for ion-selective sensors in terrestrial and space-based agriculture and water management systems. Sensors 12:13349–13392 (2012).
8. Lowe A, Harrison N and French AP, Hyperspectral image analysis techniques for the detection and classification of the early onset of plant disease and stress. *Plant Methods* 13:80 (2017).
9. Jha K, Doshi A, Patel P and Shah M, A comprehensive review on automation in agriculture using artificial intelligence. *Artif Intell Agric* 2:1–12 (2019).
10. Shamshiri RR, Kalantari F, Ting KC, Thorp KR, Hameed IA, Weltzien C et al., Advances in greenhouse automation and controlled environment agriculture: a transition to plant factories and urban agriculture. *Int J Agric Biol Eng* 11:1–22 (2018).
11. Lee WS, Alchanatis V, Yang C, Hirafuji M, Moshoue D and Li C, Sensing technologies for precision specialty crop production. *Comput Electron Agric* 74:23–33 (2010).
12. Zhang C and Kovacs JM, The application of small unmanned aerial systems for precision agriculture: a review. *Precis Agric* 13:693–712 (2012).
13. Zhang C, Walters D and Kovacs JM, Applications of low altitude remote sensing in agriculture upon farmers’ requests – a case study in northeastern Ontario, Canada. *Plos One* 9:e112894 (2014).
14. Anderson K and Gaston JF, Lightweight unmanned aerial vehicles will revolutionize spatial ecology. *Front Ecol Environ* 11:138–146 (2013).
15. Adão T, Hruska J, Pádua L, Bessa J, Peres E, Morais R et al., Hyperspectral imaging: a review on UAV-based sensors, data processing and applications for agriculture and forestry. *Remote Sens (Basel)* 9:1110 (2017).
16. Chilingaryan A, Sukkarieh S and Whelan B, Machine learning approaches for crop yield prediction and nitrogen status estimation in precision agriculture: a review. *Comput Electron Agric* 151:61–69 (2018).
17. Nansen C and Elliott N, Remote sensing and reflectance profiling in entomology. *Annu Rev Entomol* 61:139–158 (2016).
18. Prabhakar M, Prasad YG and MN, Remote sensing of biotic stress in crop plants and its applications for pest management, in *Crop Stress and its Management*: Perspectives and Strategies, ed. by Venkateswarlu B, Shanker AK, Shanker C and Maheswari M. Springer, New York, NY pp. 517–549 (2012).
19. Riley JR, Remote sensing in entomology. *Annu Rev Entomol* 34:247–271 (1989).
20. Behmann J, Mahlein A-K, Rumpf T, Römer C and Plümér L, A review of advanced machine learning methods for the detection of biotic stress in precision crop protection. *Precis Agric* 16:239–260 (2015).
21. Behmann J, Steinrucken J and Plümér L, Detection of early plant stress responses in hyperspectral images. *ISPRS J Photogramm Remote Sens* 93:98–111 (2014).
22. Aasen H, Honkvaaera E, Lucieer A and Zarco-Tejada P, Quantitative remote sensing at ultra-high resolution with uav spectroscopy: a review of sensor technology, measurement procedures, and data correction workflows. *Remote Sens (Basel)* 10:1091 (2018).
23. Honkvaaera E, Arbiol R, Markelin L, Martinez L, Cramer M, Bovet S et al., Digital airborne photogrammetry—a new tool for quantitative remote sensing?—A state-of-the-art review on radiometric aspects of digital photogrammetric images. *Remote Sens (Basel)* 1:577–605 (2009).
24. Schott J, Remote Sensing: The Image Chain Approach. Oxford University Press, New York, NY (2007).
25. Shin J-I, Cho Y-M, Lim P-C, Lee H-M, Ahn H-Y, Park C-W et al., Relative radiometric calibration using tie points and optimal path selection for UAV images. *Remote Sens (Basel)* 12:1726 (2020).
26. Iqbal F, Lucieer A and Barry K, Simplified radiometric calibration for UAS-mounted multispectral sensor. *Eur J Remote Sens* 51:301–313 (2018).
27. Nansen C, Steward AN, Gutierrez TAM, Wintermantel WM, McRoberts N and Gilbertson RL, Proximal remote sensing to differentiate nonviruliferous and viruliferous insect vectors—proof of concept and importance of input data robustness. *Plant Pathol.* 68:746–754 (2019).
28. Carter GA and Knapp AK, Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *Am J Bot.* 88:677–684 (2001).
29. Ribeiro LP, Klock ALS, Filho JAW, Tramontin MA, Trapp MA, Mithöfer A et al., Hyperspectral imaging to characterize plant-plant communication in response to insect herbivory, *Plant Methods* 14:1–11 (2018).
30. Holcomb E, Flore J and Heins R, Photosynthetic response curves for chrysanthemum grown at different PPF levels. *HortScience* 23:206–208 (1988).
31. Nguyen H and Nansen C, Hyperspectral remote sensing to detect leafminer-induced stress in bok choy and spinach according to tiller regime and timing. *Pest Manag Sci.* 76:2208–2212 (2020).
32. Jood S, Kapoor AC and Singh R, Mineral contents of cereal grains as affected by storage and insect infestation. *J Stored Prod Res.* 28:147–151 (1992).
33. Nansen C, Sidumo AJ, Martini X, Stefanova K and Roberts JD, Reflectance-based assessment of spider mite “bio-response” to maize leaves and plant potassium content in different irrigation regimes. * Comput Electron Agric.* 97:21–26 (2013).
34. Lacoste C, Nansen C, Thompson S, Moir-Barnetson L, Mian A et al., Remote sensing of biotic stress in precision crop protection. *Comput Electron Agric.* 72:610–618 (2015).
35. Perrenoud S, *Potassium and Plant Health*. International Potash Institute, Basel (1990).
36. Amtmann A, Troufflard S and Armengaud P, The effect of potassium nutrition on pest and disease resistance in plants. *Physiol Plant* 133:682–691 (2008).
37 Lu J, Zhou M, Gao Y and Jiang H, Using hyperspectral imaging to discriminate yellow leaf curl disease in tomato leaves. *Precis Agric* 19: 379–394 (2018).

38 Gu Q, Sheng L, Zhang T, Lu Y, Zhang Z, Zheng K et al., Early detection of tomato spotted wilt virus infection in tobacco using the hyperspectral imaging technique and machine learning algorithms. *Comput Electron Agric.* 167:105066 (2019).

39 Herrmann I, Berenstein M, Paz-Kagan T, Sade A and Karnieli A, Spectral assessment of two-spotted spider mite damage levels in the leaves of greenhouse-grown pepper and bean. *Biosyst Eng* 157:72–85 (2017).

40 Herrmann I, Berenstein M, Sade A, Karnieli A, Bonfil DJ and Weintraub PG, Spectral monitoring of two-spotted spider mite damage to pepper leaves. *Remote Sens Lett* 3:277–283 (2012).

41 Fraulo AB, Cohen M and Liburd OE, Visible/near infrared reflectance (VNIR) spectroscopy for detecting two spotted spider mite (Acari: Tetranychidae) damage in strawberries. *Environ Entomol* 38:137–142 (2009).

42 Luedeling E, Hale A, Zhang M, Bentley WJ and Dharmasri LC, Remote sensing of spider mite damage in California peach orchards. *Int J Appl Earth Obs* 11:244–255 (2009).

43 Reisig DR and Godfrey LD, Spectral response of cotton aphid- (Homoptera: Aphididae) and spider mite- (Acari: Tetranychidae) infested cotton: controlled studies. *Environ Entomol* 36:1466–1474 (2007).

44 Reisig DD and Godfrey LD, Remote sensing for detection of cotton aphid- (Homoptera: Aphididae) and spider mite- (Acari: Tetranychidae) infested cotton in the San Joaquin Valley. *Environ Entomol* 35: 1635–1646 (2006).