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Spectral monitoring of two-spotted spider mite damage to pepper leaves

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Two-spotted spider mites (TSSM; *Tetranychus urticae* Koch) cause significant damage to crops and yields, in the field as well as in greenhouses. By feeding, TSSM destroy chloroplast-containing cells; this damage can be spectrally detected in the reflectance of the visible and near-infrared regions. This study focuses on hyperspectral reflectance data of greenhouse pepper (*Capsicum annuum*) leaves, obtained by integrated sphere. The reflectance data were transformed into vegetation indices allowing early TSSM damage detection by separation between leaf damage levels. One-way analysis of variance of coupled damage levels was applied to each of the vegetation indices. We concluded that early identification of TSSM greenhouse pepper leaf damage can be obtained by multispectral means. Furthermore, the proposed methods may identify the damage on the upper side of the leaves although the TSSM feed on the underside of leaves.

1. Introduction

Two-spotted spider mites (TSSM; *Tetranychus urticae* Koch) are polyphagous and feed on the underside of leaves, piercing the chloroplast-containing cells. By damaging the chloroplasts, leaf chlorophyll content is decreased. The amount and rate of change of the chlorophyll depends on TSSM density and duration of feeding (Alatawi *et al.* 2007). A positive exponential correlation was found between TSSM population density (Nihoul *et al.* 1991) or cumulative density and leaf damage (Alatawi *et al.* 2007). TSSM prefer young leaves and their population on young leaves in the upper part of the plant exhibits the potential of spreading (Nihoul *et al.* 1991). Therefore, it is hypothesized that, under optimal plant growth conditions, as is possible in greenhouses, spectral monitoring of the upper leaves can lead to TSSM damage identification even in early stages of infestation.

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Damage to leaves as done by TSSM can be spectrally detected by the visible (VIS) and near-infrared (NIR) regions (Buschmann and Nagel 1993, Hatfield *et al.* 2008). Therefore, remote sensing tools and techniques are potentially useful for monitoring TSSM damage distribution (Reisig and Godfrey 2007). Electromagnetic spectral monitoring of plant stress by hyperspectral band formation is a common remotely sensed application, but the separation between specific sources of stress by spectral data is not common (Pinter *et al.* 2003). Although it was not possible to find a unique spectral signature for TSSM or aphid-stressed cotton at the leaf scale (Reisig and Godfrey 2007), Yang *et al.* (2009) reported the ability to separate between greenbug (*Toxoptera graminum*) and Russian wheat aphid (*Diuraphis naxia*) damage to wheat at the canopy scale by multispectral hand-held radiometer. Since remote sensing for entomological applications is in the exploratory phase, small-scale studies using integrated spheres for sub-leaf analysis are useful as a first step towards large-scale applications by ground, airborne or space-borne sensors on the canopy or sub-field level (Reisig and Godfrey 2007). Therefore, if we assume that optimal growing conditions are available for plants in a greenhouse, the main stress sources to identify will be insects or diseases.

Since spider mites can rapidly develop resistance to acaricides (Dekeyser 2005) other control methods besides applying acaricides over the whole greenhouse or field should be considered. Another way to deal with pests is by releasing their natural enemies in order to suppress the pests, mainly in greenhouses (Legowski 1966). It is most desirable to detect insect damage as early as possible in order to allow corrective action to be efficient (Fraulo *et al.* 2009). Early identification of TSSM damage and implementation of treatment, according to need, should minimize the damage to yield and the amount of natural enemies to be bought and released (Alatawi *et al.* 2007) and, by that, allowing potential higher profit to the growers.

The current study is aimed at detecting early TSSM damage by spectral separation between damage levels in greenhouse pepper leaves, as a first step towards multispectral sensor for TSSM damage detection of pepper plants in greenhouses.

2. Methodology

Ninety seven samples of seemingly damaged and undamaged leaves were randomly collected in a greenhouse of Kibbutz Nirim (31°20′N; 34°24′E) in the northwest Negev region, Israel. The leaves were collected from the canopy top of 6-month-old pepper plants, put in plastic bags inside a thermally isolated case cooled by ice with no direct contact with the plastic bags and immediately transported to the laboratory. Identification of the damage along with its level was assessed by a pest control advisor. Four levels of damage were identified (see figure 1): no damage (ND) (25 samples); light damage (LD) (26 samples); medium damage (MD) (23 samples); and high damage (HD) (23 samples).

The leaf samples were spectrally examined by a Licor, 1800–12s, external integrating sphere connected by optic fibre to Analytical Spectral Devices (ASD) FieldSpec Pro FR spectrometer (Analytical Spectral Devices Inc., Boulder, CO, USA) with a spectral range of 350–2500 nm in 1 nm intervals. The ASD was programmed to average 40 readings to create each spectrum. The spectral measurements were obtained at the Remote Sensing Laboratory in the Jacob Blaustein Institutes for Desert Research, Ben-Gurion University, Sede-Boker Campus, within 4 hours after leaving the greenhouse. Reflectance and transmittance measurements were obtained for each leaf. The reflectance measurements were obtained for the upper side of the

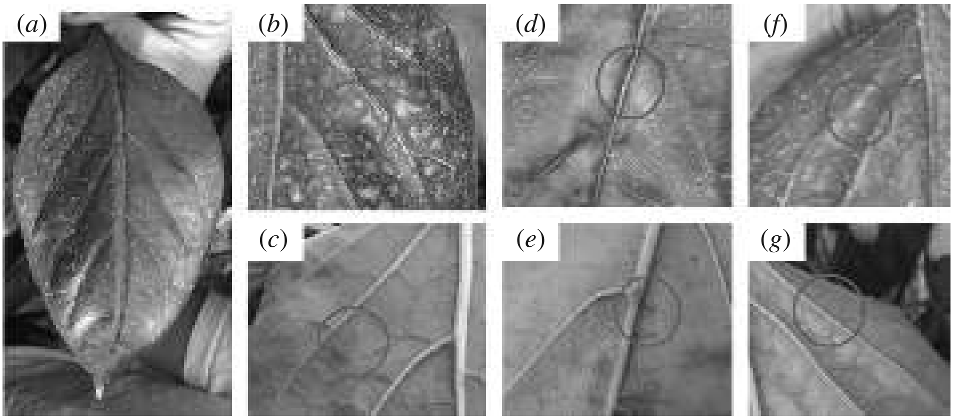

Figure 1. Leaves from the four damage levels: (*a*) upper side of no damage (ND); (*b*) upper side of low damage (LD); (*c*) underside of LD; (*d*) upper side of medium damage (MD); (*e*) underside of MD; (*f*) upper side of high damage (HD); and (*g*) underside of HD. The amount of TSSM on the underside of the leaf does not represent the severity of the damage. Red circles indicate the same spot on the upper and under sides of the same leaf. Note the TSSM activity (web and individuals) on the underside of the leaves.

leaf. The spectral data were preprocessed to 2 nm intervals in the range of 400–1000 nm and each spectrum was related to the damage level of the leaf. The spectral data were resampled to several broad bands as presented in table 1. The reflectance values of these bands were used to calculate several vegetation indices as presented in table 2, with the exception of the red-edge inflection point (REIP) that was

Table 1. Spectral bands' centres and widths.

Band	Centre (nm)	Range (nm)
Blue (B)	490	470–510
Green (G)	560	540–580
Red (R)	666	656–676
Red-edge (RE)	715	710–720
Near infrared (NIR)	790	770–810

Table 2. Equations of the vegetation indices.

Indices	References
$\mathrm{NDVI} = \frac{\rho_{NIR} - \rho_R}{\rho_{NIR} + \rho_R}$	Tucker (1979)
$\mathrm{GNDVI} = \frac{\rho_{NIR} - \rho_G}{\rho_{NIR} + \rho_G}$	Gitelson *et al.* (1996)
$\mathrm{REGNDVI} = \frac{\rho_{RE} - \rho_G}{\rho_{RE} + \rho_G}$	Current study
$\mathrm{REBNDVI} = \frac{\rho_{RE} - \rho_B}{\rho_{RE} + \rho_B}$	Current study
$\mathrm{NRENDVI} = \frac{\rho_{NIR} - \rho_{RE}}{\rho_{NIR} + \rho_{RE}}$	Current study
$\mathrm{REIP} = 700 + 40\left\{\frac{[(\rho_{670}+\rho_{780})/2]-\rho_{700}}{\rho_{740}-\rho_{700}}\right\}$	Guyot and Baret (1988)

Notes: The indices abbreviations are mentioned in the text. ρ stands for reflectance value of either band, as defined in table 1, or wavelength (in nm).

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calculated by narrow bands with 2 nm intervals. The Normalized Difference Vegetation Index (NDVI) and the Green NDVI (GNDVI) concepts are the bases for three, proposed, wide bands calculated indices: Red-Edge GNDVI (REGNDVI); Red-Edge Blue NDVI (REBNDVI); and Near-infrared Red-Edge NDVI (NRENDVI).

In order to determine whether spectral data can provide significant separation between damage levels, one-way analysis of variance (ANOVA) was applied for each of the indices for all damage levels together as well as for paired damage levels; since there are four damage levels all six possible couplings were analysed. The ANOVA data analysis was executed by Statistica v.9 software (StatSoft®, Tulsa, OK, USA). The ANOVA results were not subject to any kind of adjustment for multiple comparisons.

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3. Results and discussion

Figure 2 shows that the HD level has the highest reflectance in the green region and the lowest in the NIR region, whereas ND exhibits the opposite. These results are in agreement with the findings presented by Mirik *et al.* (2006) concerning greenbug-infested wheat canopy. Moreover, the reflectance level of the ND in the 550–650 nm region is remarkably separated from the other damage levels and therefore might allow early identification of infestation. Similarly the NIR region, with reflectance values in wavelengths longer than 760 nm, can be applied for HD identification. These differences in VIS and NIR reflectance are assumed to originate by TSSM activity resulting in pigments content changes and leaf structure distraction, respectively. Figure 2 also shows variation among the four levels of damage corresponding to reflectance values in two spectral regions, in the 550–650 nm and in the red-edge (RE) slope at 710–720 nm. Therefore, the RE band was comprised in the proposed indices for further analysis. For exploring possibilities of spectral identification or separation of damage levels, NDVI, GNDVI and REIP were chosen and the other three new indices were formulated; see table 2. The averaged value of each of the indices for each of the damage levels is presented in figure 3. Based on gradients assessment between the four damage levels for each index, the GNDVI shows the highest sensitivity to differences between

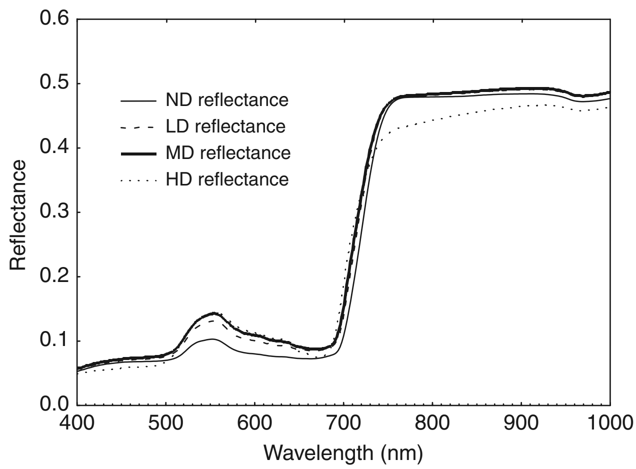

Figure 2. Averaged reflectance values of the four damage levels.

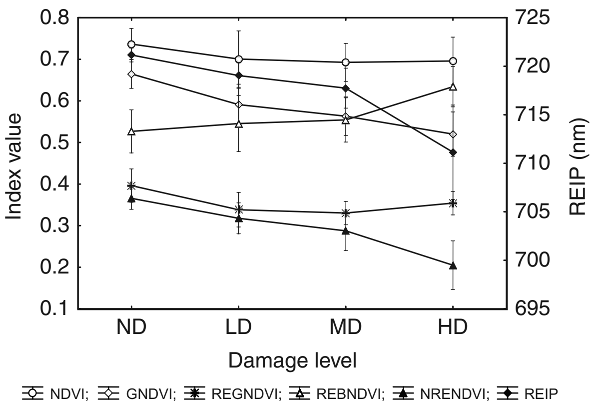

Figure 3. Averaged index values and standard deviations for each of the damage levels.
Note: Since the REIP values are in nanometres and the indices values are unitless, the two axes cannot be scaled.

ND and LD damage levels, and the REBNDVI and NRENDVI are the most sensitive indices to differences between MD and HD damage levels. The REBNDVI shows positive relation to increase in damage level in contrary to the rest of the indices. It is important to mention that since the REIP equation gives the inflection point of the RE slope the values are in nanometre and not unitless as the rest of the indices.

Table 3 presents the ability of each index to separate between the four damage levels by using the one-way ANOVA of the six possible damage levels couplings as well as for the four damage levels together. The GNDVI, NRENDVI and REIP show separation for each of the damage levels. All indices except the REBNDVI can provide early identification of TSSM damage (separating ND from LD; table 3). The REBNDVI can separate the HD from the rest of the damage levels. Among the GNDVI, NRENDVI and REIP that provided consistent separation between increasing damage levels, the GNDVI is a simple index applying commonly used bands; the NRENDVI is also a simple index but its RE band is not very common; and the REIP bands combination

Table 3. The ability of each index to separate between damage levels by one-way ANOVA.

	ND	LD	MD	HD	Four damage levels together
NDVI	a	b	b	b	3.6 ($p = 0.017$)
GNDVI	a	b	c	d	41.6 ($p < 0.0001$)
REGNDVI	a	b, c	c	b, c, d	16.9 ($p < 0.0001$)
REBNDVI	a	a	a	b	16.9 ($p < 0.0001$)
NRENDVI	a	b	c	d	57.5 ($p < 0.0001$)
REIP	a	b	c	d	50.4 ($p < 0.0001$)

Notes: Distinctive letters stand for the ability to significantly ($p < 0.05$) separate between coupled damage levels, whereas the same letter stands for coupled damage levels that are not significantly different (e.g. the NDVI ND is significantly different from LD, MD and HD, whereas LD is not significantly different from MD and HD). The column on the right, entitled 'four damage levels together', presents the F and significance values of one-way ANOVA of the four damage levels together for each index.

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is not common at all. Therefore the GNDVI is considered to be potentially the most efficient one for practical use.

It should be noted that the current study focuses on the possibility of remotely assessing leaf damage as opposed to the usual way of manually scouting to inspect individual leaves for TSSM presence (Fraulo *et al.* 2009). Mirik *et al.* (2006) showed correlation between vegetation indices and greenbug density on wheat in fields and concluded that well-known indices (e.g. NDVI and GNDVI) showed significant and high correlation but only for high densities of greenbugs. Since the density of pests can be logically assumed to be positively related to damage, it might be that the idea of relating spectral properties, such as vegetation indices, to damage levels instead of density can provide early identification of infestation by remote sensing techniques.

4. Conclusions

- Although the TSSM activity is on the underside of leaves, remote sensing spectroscopy is able to monitor the damage by viewing the upper side.
- TSSM damage to greenhouse pepper leaves can be identified early by remote sensing means in the laboratory.
- GNDVI, NRENDVI and REIP can provide consistent separation between increasing damage levels of greenhouse pepper leaves in the laboratory.

The early identification of arthropods is critical and, if early damage to plants can be detected by remote sensing means, then the TSSM infestation can be evaluated manually. After TSSM infestation is identified, the damage level can be potentially monitored in scheduled time intervals by multispectral remote sensing means. This should allow the growers to decide when to control it. In greenhouses with control conditions, a quantitative model can also be considered in contrast to open fields with changing conditions that can lead to a variety of stresses.

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