in: Juliane Bendig und Georg Bareth (Hrsg.) (2014): Proceedings of the Workshop on UAV-basaed Remote Sensing Methods for Monitoring Vegetation. - Kölner Geographische Arbeiten, **94**. Köln: 7-14

HIGH-RESOLUTION HYPERSPECTRAL AND THERMAL IMAGERY ACQUIRED FROM UAV PLATFORMS FOR EARLY DETECTION OF VERTICILLIUM WILT USING FLUORESCENCE, TEMPERATURE AND NARROW-BAND INDICES

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Abstract

Verticillium wilt (VW) caused by the soil-borne fungus *Verticillium dahliae* Kleb, is the most limiting disease in all traditional olive-growing regions worldwide. This pathogen colonizes the vascular system of plants, blocking water flow and eventually inducing water stress. The present study explored the use of high-resolution thermal imagery, chlorophyll fluorescence, structural and physiological indices (xanthophyll, chlorophyll a+b, carotenoids and B/G/R indices) calculated from multispectral and hyperspectral imagery as early indicators of water stress caused by VW infection and severity. The study was conducted in two olive orchards naturally infected with *V. dahliae*. Time series of airborne thermal, multispectral and hyperspectral imagery were conducted with 2-m and 5-m wingspan electric Unmanned Aerial Vehicles (UAVs) in three consecutive years and related to VW severity at the time of the flights. Concurrently to the airborne campaigns, field measurements conducted at leaf and tree crown levels showed a significant increase in crown temperature (Tc) minus air temperature (Ta) and a decrease in leaf stomatal conductance (G) across VW severity levels, identifying VW-infected trees at early stages of the disease. At leaf level, the reduction in G caused by VW infection was associated with a significant increase in the Photochemical Reflectance Index (PRI_{570}) and a decrease in chlorophyll fluorescence. The airborne flights enabled the early detection of VW by using canopy-level image-derived airborne Tc-Ta, Crop Water Stress Index (CWSI) calculated from the thermal imagery, blue / green / red ratios (B/BG/BR indices) and chlorophyll fluorescence, confirming the results obtained in the field. Airborne Tc-Ta showed rising temperatures with a significant increase of ~2K at low VW severity levels. Early stages of disease development could be differentiated based on CWSI increase as VW developed, obtaining a strong correlation with G (R^2=0.83, P<0.001). Likewise, the canopy-level chlorophyll fluorescence dropped at high VW severity levels, showing a significant increase as disease progressed at early VW severity levels. These results demonstrate the viability of early detection of *V. dahliae* infection and discrimination of VW severity levels using remote sensing. Indicators based on crown temperature, CWSI, and visible ratios B/BG/BR as well as fluorescence were effective in detecting VW at early stages of disease development. In affected plants, the structural indices, PRI, chlorophyll and carotenoid indices, and the R/G ratio were good indicators to assess the damage caused by the disease.

Keywords: stress detection, hyperspectral, thermal, fluorescence, high resolution, UAV, vegetation indices, *Verticillium dahliae*.

1. Introduction

VW of olive (*Olea europea* L.) trees, caused by the soil-borne fungus *Verticillium dahliae* Kleb, is the most limiting disease of this crop in all traditional olive-growing regions worldwide (Jiménez-Díaz et al. 2012). This pathogen infects the plant by the root and colonizes its vascular system, blocking water flow and eventually inducing water stress (Van Alfen 1989). New remote sensing methods based on high-resolution hyperspectral and thermal imagery have demonstrated the potential for nutrient and water stress detection, and photosynthetic rate assessment by the detection of the chlorophyll fluorescence emission (R_{760} at O_2-band) (Zarco-Tejada et al. 2009, 2012), the estimation of the vegetation temperature as an indicator of transpiration and stomatal conductance (Berni et al. 2009a; 2009b; Zarco-Tejada et al. 2012) and narrow-band spectral indices. These indices are related with the xanthophyll cycle pigments (PRI) (Suárez et al. 2008, 2009, 2010) and the estimation of chlorophyll and carotenoid content. The hypothesis is that thermal and hyperspectral indices acquired from the airborne imagery are sensitive to physiological changes induced by the infection and colonization by *V. dahliae*. The main objective of this research was to detect these physiological changes using high-resolution thermal imagery and physiological indices calculated from multispectral and hyperspectral imagery as indicators of VW infection and severity in olive orchards.

2. Material and methods

2.1 Study site description

Two experimental areas were selected in Andalusia, in southern Spain, to account for differences in weather conditions, crop age and tree crown size, olive cultivars with different reactions to VW and VW incidence and severity.

The first study site was located in Castro del Rio (Cordoba province) in a 7-ha commercial orchard planted in 2001 with the olive cultivar (cv.) Picual at a spacing of 6 x 4 m. The initial VW incidence, calculated as the percentage of VW symptomatic trees, was estimated in 12%. The second study site was located in Utrera (Seville province), in a 10-ha commercial orchard planted in 2006 with cv. Arbequina. The olive trees were planted at a spacing of 6 x 3 m. VW incidence was estimated in 30%. Both orchards were drip irrigated and managed using no-tillage practices; weed control was achieved with herbicide treatments between rows.

2.2 Verticillium wilt assessment

Incidence and severity of VW symptoms were assessed in both plots in coincidence with the airborne campaigns. Severity of the disease (DS) was assessed by visual observation of foliar symptoms in each individual tree and assessment on a 0 to 4 rating scale according to percentage of foliage with disease symptoms, where: 0 = 0%, 0.2 and 0.5 = initial symptoms, 1 = 1 to 33%, 2 = 34 to 66%, 3 = 67 to 100%, and 4 = dead plant. Severity of disease symptoms were grouped in asymptomatic (DS=0), initial ($0.2 \leq DS \leq 0.5$), low ($1 \leq DS \leq 1.5$), moderate ($2 \leq DS \leq 2.5$) and severe ($3 \leq DS \leq 3.5$) disease symptoms.

2.3 Field measurements

Leaf and near-canopy field measurements were conducted in the olive orchard located in Castro del Rio (Cordoba) during the summer of 2011 to estimate: i) the diurnal variation of crown temperature (Tc-Ta) and stomatal conductance (G) in trees covering a gradient in severity levels; and ii) the variation at midday along the VW severity levels of Tc-Ta, leaf steady-state chlorophyll fluorescence (Fs), leaf Photochemical Reflectance Index (PRI) (Gamon et al. 1992) and leaf G.

Eight trees showing different VW severity levels were selected to record pure crown temperature (Tc) from 7:00 to 17:00 GMT at 5-minute intervals in two data loggers (model CR10X, Campbell Sci., Logan, UT, USA) with infrared temperature (IRT) sensors (22º half-angle FOV) (model IRR-P, Apogee, Logan, UT, USA) placed 1 m above trees. Air temperature (Ta) and relative humidity (RH) were measured above the canopy (approx. 6 m above the ground) with a portable weather station (Model WXT510, Vaisala, Finland). In each of the eight monitored trees, leaf G was measured from 7:00 to 17:00 GMT at 2-hour intervals with a leaf porometer (model SC-1, Decagon Devices, Washington, DC, USA) to monitor the diurnal variation of G for the different VW severity levels. A total of five illuminated leaves per tree were measured at each time interval.

In addition, 25 trees covering a gradient in severity levels from asymptomatic to severely affected trees were chosen to monitor the variation of Tc-Ta, leaf Fs, leaf PRI_{570} and leaf G between 10:00 and 13:00 GMT. Tc-Ta was measured with IRT sensors placed above tree crowns. Leaf Fs and PRI_{570} were measured in 25 illuminated leaves per tree using the PAM-2100 Pulse-Amplitude Modulated Fluorometer (Heinz Walz GMBH, Effeltrich, Germany) and a PlantPen instrument custom designed to measure the R_{531} and R_{570} bands (Photon System Instrument, Brno, Czech Republic), respectively. Leaf G was measured in five illuminated leaves per tree with the leaf porometer previously used.

2.4 Airborne campaigns

Imagery was acquired from both experimental sites in three consecutive years using narrow-band multispectral, hyperspectral and thermal cameras. The multispectral and thermal cameras were used in airborne campaigns conducted twice per crop season in spring and summer of 2009 and 2010 at 10:30 and 12:00 GMT, respectively. In addition, the thermal camera was flown twice in June 2011. Hyperspectral and thermal images were acquired from the Castro del Rio site on 23 June 2011 at 9:00 GMT using a micro-hyperspectral imager concurrently with the thermal camera operated previously.

The flights were conducted with two different unmanned aerial vehicles (UAVs) operated by the Spanish Laboratory for Research Methods in Quantitative Remote Sensing (Quantalab, IAS-CSIC, Spain) (Berni et al. 2009b; Zarco-Tejada et al. 2008; 2012). The UAV used for the multispectral and thermal acquisition had a 2-m wingspan for a fixed-wing platform at 5.8 kg take-off weight (TOW) (mX-SIGHT, UAV Services and Systems, Germany) capable of 1-hour endurance. Hyperspectral images were acquired with a larger UAV with a 5-m wingspan for a fixed-wing platform having 13.5 kg take-off weight (TOW) (Viewer, ELIMCO, Seville, Spain) capable of 3-hour endurance. This larger platform was required when operating the micro-hyperspectral imager due to the heavier payload.

The multispectral sensor consisted of a 6-band multispectral camera (MCA-6, Tetracam, Inc., California, USA) flying at 250 m above ground level (AGL) yielding a spatial resolution of 20 cm (Berni et al. 2009b; Zarco-Tejada et al. 2009). The bandsets used in each study site included centered wavelengths at 450, 490, 530, 570, 670 and 800 nm. The micro-hyperspectral imager (Micro-Hyperspec VNIR model, Headwall Photonics, MA, USA) was flown in the spectral mode with 260 bands. The hyperspectral images were obtained at 53 x 42 cm resolution, resampled to 40 cm for a flight conducted at 550 m AGL (Zarco-Tejada et al. 2012). The multispectral and hperspectral images were radiometrically calibrated and atmospheric corrected following the procedure explained in Berni et al. (2009b) and Zarco-Tejada et al. (2012), respectively.

The thermal camera (MIRICLE 307, Thermoteknix Systems Ltd, Cambridge, UK) was flown at altitudes ranging between 150 m and 250 m AGL in 2009 and 2010, and at 550 m AGL when flown together with the micro-hyperspectral imager in 2011. The camera was radiometrically calibrated following the procedure explained in Berni et al. (2009b). Thermal images were acquired at 20 cm pixel resolution.

3. Results and discussion

3.1 Field measurements results

Diurnal crown measurements of temperature (Tc-Ta) revealed that the maximum differences between VW severity levels occurred at midday, showing a rise in Tc-Ta as VW severity level increased (Calderón et al. 2013). In fact, Tc-Ta values were able to discriminate asymptomatic trees from those affected at early (DS≤1.5) stages of the disease development, showing temperature differences close to 2K. The diurnal leaf G data showed a decrease in G values as disease severity level increased (Calderón et al. 2013). These results are in agreement with Berni et al. (2009b) and Sepulcre-Cantó et al. (2006), who assessed the relationship between G and water stress levels due to deficit irrigation practices in olive trees. Our results revealed that G was able to discriminate between healthy asymptomatic trees and those at early stages of disease development, which had G values at least 300-500 mmol/m^2/s lower than those of healthy trees.

Data on Tc-Ta, leaf G, leaf PRI_{570} and leaf Fs acquired at midday were analyzed in trees with different VW severity levels (Calderón et al. 2013). Tc-Ta measured in VW affected trees was significantly (P<0.05) higher than that measured in asymptomatic trees. By contrast, G showed a negative trend as VW severity increased, showing significant (P<0.05) changes from asymptomatic trees at those showing moderate and severe symptoms. Moreover, PRI_{570} was significantly lowest (P<0.05) in asymptomatic trees, and increased steadily with the increase in VW severity. This result is consistent with that obtained by Suárez et al. (2008; 2009) in water-stressed trees. Leaf chlorophyll fluorescence measurements of Fs showed a downward trend as VW severity level increased as previously found for trees under water stress (Pérez-Priego et al. 2005; Zarco-Tejada et al. 2009; 2012).

3.2 Airborne hyperspectral, multispectral and thermal imagery results

Tc-Ta extracted from the airborne thermal imagery tended to increase as VW severity level increased (Fig. 1). Symptomatic trees showed significantly (P<0.05) higher Tc-Ta values than asymptomatic trees at any disease severity level at Castro del Rio, or at low or higher disease severity at Utrera.

The CWSI estimated from the high-resolution airborne thermal imagery acquired on 2 June 2011 in the Castro del Rio site as described in Berni et al. (2009a) showed significantly (P<0.05) lower values for asymptomatic trees (Calderón et al. 2013). CWSI derived from the thermal imagery on 15 June 2011 decreased linearly and significantly as a function of leaf G (R^2=0.83; P<0.001) (Calderón et al. 2013). These results indicate that the

CWSI obtained from high spatial resolution thermal imagery can be used to detect the lower transpiration rates induced by *V. dahliae* infection, as could be expected according to the previous results in Berni et al. (2009a), showing the usefulness of the CWSI as a water stress indicator.

The effects of VW on the canopy structure were captured by structural indices such as the NDVI, RDVI, OSAVI, TVI, MTVI, SR and MSR. Moderate and severe VW symptoms induced significantly (*P*<0.05) lower values of NDVI, OSAVI, SR and MSR indices than those estimated in asymptomatic trees (Tab. 1). Furthermore, TVI and MTVI showed an increase (*P*<0.05) in trees showing initial symptoms (Tab. 1). These results demonstrate the consistency of structural indices as VW damage indicators due to the expected effects on crown density at moderate or advanced stages of disease development.

The xanthophyll indices were calculated based on PRI formulations using bands R_{570} (PRI_{570}) and R_{515} (PRI_{515}) as references. The PRI_{515} index was more sensitive to VW than PRI_{570}, as showed significantly (P<0.05) lower values when trees were affected by moderate or severe symptoms (Tab. 1). This result confirms those obtained by Hernández-Clemente et al. (2011) in forest canopies and those of Stagakis et al. (2012) in orange and mandarin orchards, which demonstrated the robustness of the PRI_{515} to structural effects.

The chlorophyll fluorescence signal estimated with the FLD method showed a significant (*P*<0.05) increase at initial and low stages of disease symptom severity, slightly decreasing to the severe VW severity level (Tab. 1). This result may indicate that the photosynthetic apparatus of the plant remains undamaged being able to dissipate the excess of energy by fluorescence that could not be maintained when the reduction in photosynthesis

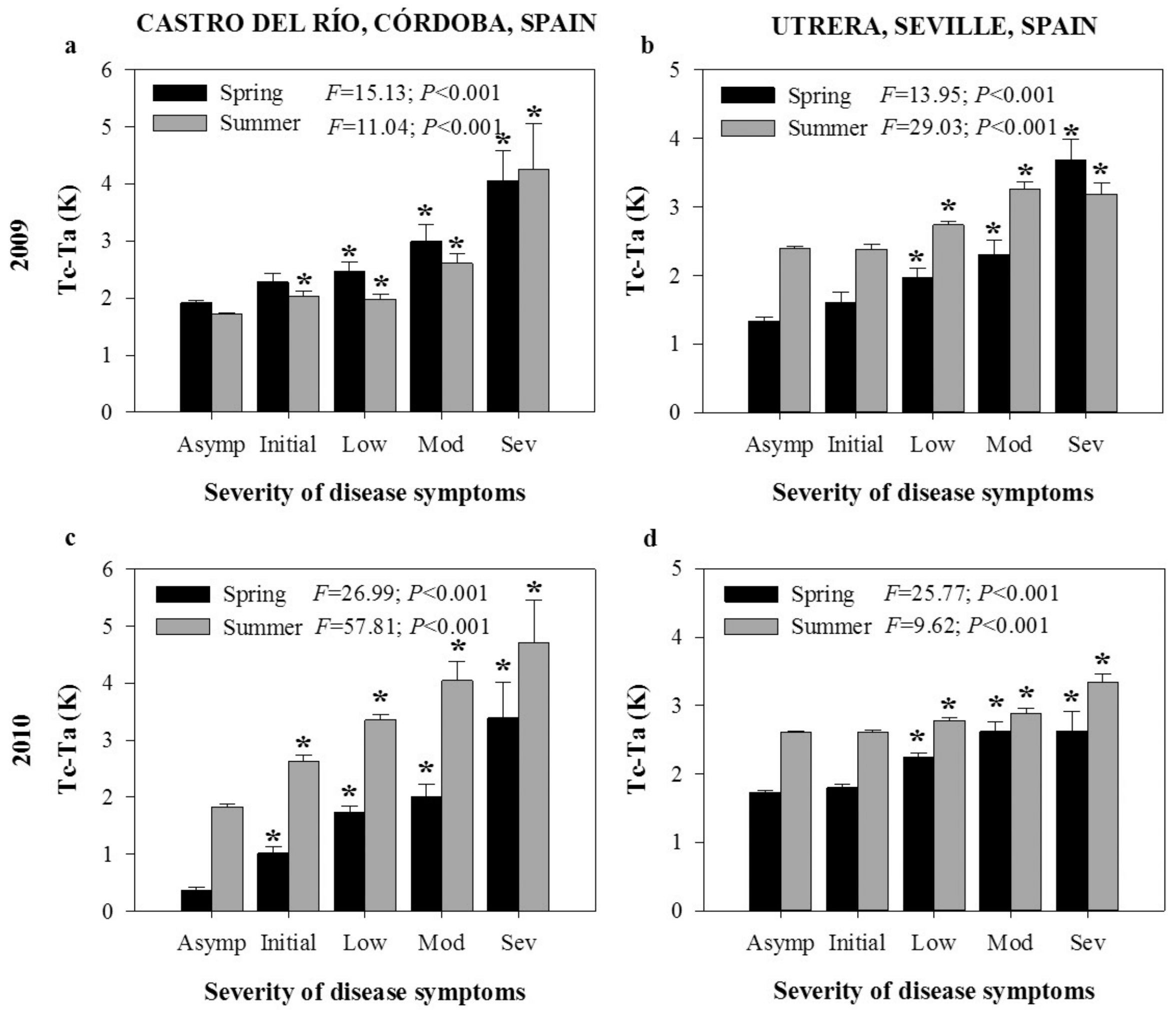

Fig. 1: Mean measurements of crown temperature (Tc-Ta) for the different VW severity levels. Tc-Ta was calculated from thermal imagery obtained in spring and summer of two consecutive years, 2009 (a, b) and 2010 (c, d), for the Castro del Rio (a, c) and Utrera (b, d) study sites. Analysis of variance was conducted and asterisks indicate significant differences from the asymptomatic plants according to Dunnett's two-tailed test at P<0.05. Error bars indicate standard errors.

Vegetation indices	Reference	F[a]	P[a]	Severity of disease[b]			
				I	L	M	S
Structural indices							
NDVI	Rouse et al. (1974)	21.66	<0.001			X	X
RDVI	Rougean & Breon (1995)	9.02	<0.001				X
OSAVI	Rondeaux et al. (1996)	11.52	<0.001			X	X
TVI	Broge & Leblanc (2000)	7.80	<0.001		X		X
MTVI	Haboudane et al. (2004)	7.27	<0.001		X		X
SR	Jordan (1969)	14.35	<0.001			X	X
MSR	Chen (1996)	16.49	<0.001			X	X
Xanthophyll indices							
PRI_{570}	Gamon et al. (1992)	2.98	0.0183				
PRI_{515}	Hernández-Clemente et al. (2011)	11.30	<0.001			X	X
Fluorescence index							
FLD3	Plascyk (1975)	4.66	0.0010	X	X		
Chlorophyll indices							
RedEdge	Zarco-Tejada et al. (2001)	15.95	<0.001			X	X
VOG1	Vogelmann et al. (1993)	22.56	<0.001			X	X
GM1	Gitelson & Merzlyak (1997)	10.99	<0.001			X	X
GM2	Gitelson & Merzlyak (1997)	13.93	<0.001			X	X
PSSRa	Blackburn (1998)	14.70	<0.001			X	X
PSSRb	Blackburn (1998)	14.35	<0.001			X	X
mCAI	Laudien et al. (2003)	5.26	0.0003		X		
TCARI	Haboudane et al. (2002)	6.72	<0.001				X
TCARI/OSAVI	Haboudane et al. (2002)	3.30	0.0105				X
R/G/B indices							
R	Gitelson et al. (2000)	12.27	<0.001				
G	This study	16.51	<0.001				
B	This study	16.01	<0.001	X	X	X	X
BG1	Zarco-Tejada et al. (2005)	6.41	<0.001	X	X		
BG2	Zarco-Tejada et al. (2005)	2.25	0.0611				
BR1	Zarco-Tejada et al. (2012)	12.01	<0.001	X	X	X	
BR2	Zarco-Tejada et al. (2012)	13.68	<0.001		X	X	X
LIC3	Lichtenhaler et al. (1996)	5.72	<0.001				X
Carotenoid indices							
SIPI	Peñuelas et al. (1995)	12.43	<0.001			X	X
PSSRc	Blackburn (1998)	9.73	<0.001			X	X
R_{520}/R_{500}	Zarco-Tejada et al. (2012)	3.67	0.0055				
R_{515}/R_{570}	Zarco-Tejada et al. (2012)	3.08	0.0152				
R_{515}/R_{670}	Zarco-Tejada et al. (2012)	14.06	<0.001				
Plant disease index							
HI	Mahlein et al. (2013)	9.54	<0.001		X	X	X

[a] *F* statistic and p-value obtained from the standard analysis of variance (ANOVA).
[b] Significant changes in vegetation indices from asymptomatic plants according to Dunnett's two-tailed test at $P<0.05$ are indicated with X for every Verticillium disease severity level.

Tab. 1: Sensitivity of dahlia spectral indices to Verticillium wilt symptoms in olive trees. Vegetation indices were calculated from the dahlia spectral imagery obtained on 23 July 2011 in the Castro del Rio site.

occurred at severely stressed plants, causing a decrease in the chlorophyll fluorescence rate. These results are in agreement with the studies conducted by Pérez-Priego et al. (2005) and Zarco-Tejada et al. (2009; 2012) in citrus and olive orchards under water stress conditions.

The chlorophyll indices TCARI and TCARI/OSAVI showed an upward trend at early stages of the disease, reaching a maximum at the low disease severity level. These results could indicate a decrease in chlorophyll a+b content (Ca+b) at early stages of *dahlia* infection. At advanced stages of the disease, the TCARI and TCARI/OSAVI inverted its trend due to the sharp leaf area index (LAI) drop associated with VW severity, showing significantly ($P<0.05$) lower values at severe disease symptoms (Tab. 1). The chlorophyll red edge index, VOG1, GM1, GM2, PSSRa and PSSRb showed significantly ($P<0.05$) lower values on moderately and severely affected trees compared with values estimated on asymptomatic trees (Tab. 1). The mCAI reached a significantly ($P<0.05$) higher value at trees showing low symptoms level but steadily decreased in trees affected by moderate and severe symptoms, respectively (Tab. 1).

Interestingly, the blue index could discriminate between healthy trees and those affected at any of the disease severity levels that reached significantly lower values ($P<0.05$) (Tab. 1). Similarly, the blue/green ratio BG1 and the blue/red ratios BR1 and BR2 showed downward trends with the increase in disease severity that resulted in significantly ($P>0.05$) lower values at early stages of disease development (Tab. 1). The LIC3 index showed a slightly decrease at early stages of the disease, followed by a significant ($P<0.05$) increase on severely affected trees (Tab. 1). The Greenness, red index and the blue/green ratio BG2 were not able to detect *V. dahlia* infection, since no significant ($P>0.05$) changes were detectable when compared with healthy trees (Tab. 1).

The carotenoid indices SIPI and PSSRc were inversely correlated with disease severity, showing significantly ($P<0.05$) lower values at moderate and severe stages of disease development (Tab. 1). The R_{520}/R_{500}, R_{515}/R_{570} and R_{515}/R_{670} ratios were not useful for the detection of VW as no significant differences were detected between asymptomatic and VW affected trees (Tab. 1).

Finally, the health index (HI) showed lower values ($P<0.05$) as the VW disease severity level increased to low, moderate or severe symptoms, respectively (Tab. 1).

4. Conclusions

This study proved the potentials of detecting early *V. dahliae* infection and discriminating among VW severity levels in olive crops using high-resolution thermal, multispectral and hyperspectral imagery acquired with an unmanned aerial vehicle. Crown temperature, CWSI, B, BG1, BR1 and FLD3 were identified as the best indicators to detect VW at early stages of disease development, while structural indices, PRI_{515}, R/G, HI, and chlorophyll and carotenoid indices proved to be good indicators to detect the presence of moderate to severe damage.

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