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Contribution of airborne remote sensing to high-throughput phenotyping of a hybrid apple population in response to soil water constraints

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Abstract. In this study, we developed a remote sensing approach, normally applied to management of crop irrigation, with the purpose of phenotyping a plant population in the field. The experiment was performed on 'Starkrimson' x 'Granny Smith' apple progeny (122 hybrids, 4 replicates) at adult stage, cultivated in field on Diaphen platform (INRA Montpellier, France). Airborne images acquisition in RGB and NIR bands permit to compute the Normalized Difference Vegetation Index (NDVI), while differences between tree canopy surface measured by thermal IR imaging (T_s) and air temperature (T_a) made it possible to calculate water stress indices. The Water Deficit Index (WDI), derived from the Crop Water Stress Index (CWSI), was considered for its applicability to discontinuous plant cover. WDI was computed on each individual tree, and focused on its central zone (60 cm diameter buffer). The first results showed significant genotypic effects and drought effects for indices during imposed summer water shortage, without interaction between them. Moreover, this first experiment permits to determine field measurements that are necessary to validate the method, linking image interpretation and tree water status variables.

Keywords. *Malus domestica* Borkh – Genetic variability – Water stress – Thermal imaging – Airborne images acquisition.

Contribution de la télédétection aérienne au phénotypage à haut débit pour une population de pommiers hybrides en réponse aux contraintes hydriques du sol

Résumé. *Dans le cadre de cette étude, nous avons développé une approche de télédétection, normalement appliquée à la gestion de l'irrigation des cultures, afin de phénotyper une population végétale aux champs. L'expérience a été effectuée sur une descendance de pommiers 'Starkrimson' x 'Granny Smith' (122 hybrides, 4 répétitions) au stade adulte, cultivés aux champs sur plate-forme Diaphen (INRA Montpellier, France). L'acquisition d'images aériennes dans les bandes RGB et NIR a permis de calculer l'Indice de Végétation Normalisé (NDVI), tandis que les différences entre la surface de la canopée des arbres mesurée par images thermiques IR (Ts) et la température de l'air (Ta) ont permis de calculer les indices de stress hydrique. L'Indice de Déficit Hydrique (WDI), dérivé de l'Indice de Stress Hydrique des Cultures (CWSI), a été considéré en raison de son applicabilité à un couvert végétal discontinu. Le WDI a été calculé pour chaque arbre individuel, en se focalisant sur sa zone centrale (avec une zone-tampon de 60 cm de diamètre). Les premiers résultats montrent des effets génotypiques significatifs et des effets de la sécheresse pour les indices pendant la pénurie d'eau imposée en été, sans interaction entre eux. De plus, cette première expérience permet de déterminer les mesures de terrain qui sont nécessaires pour valider la méthode, mettant en rapport l'interprétation des images et les variables liées à l'état hydrique des arbres.*

Mots-clés. Malus domestica *Borkh – Variabilité génétique – Stress hydrique – Imagerie thermique – Acquisition d'images aériennes.*

I – Introduction

Thermal imaging is generally used for water status monitoring and irrigation scheduling on annual crops. Leaf temperature is an indicator of water status and permits an estimation of stomatal conductance (Jones *et al.,* 1999). Water stress indices like CWSI have been developed since some years (Idso *et al.,* 1981; Jackson *et al.,* 1981). They were designed for application to continuous cover, and revealed a particular interest in semi-arid and arid conditions, where active transpiration increases leaf to air temperature differences. Moran *et al.* (1994) proposed and developed an extension of CWSI to the partially covering crops, with the Water Deficit Index (WDI).

Taking into account the current high-throughput genotyping possibilities, it appears necessary to develop tools and approaches which could permit in parallel high-throughput phenotyping of plant traits (Berger *et al.,* 2010). For perennial crops such as fruit trees, measurements performed in controlled conditions at juvenile stage do not permit a straightforward prediction of mature tree behavior in field. Some leaf traits like stomatal conductance can be positively affected by the fruit acting as a sink, or be negatively affected by higher vapor pressure deficits which generally prevail. Moreover in the context of global change, the current breeding traits adopted in fruit trees such as fruit quality, pest and disease resistances, or regularity of yield, are not fully satisfying sustainable production objectives (Laurens *et al.,* 2000). New breeding traits could be proposed and implemented, consisting in better adaptation to water stress and/or better water use efficiency (Condon *et al.*, 2004; Regnard *et al.*, 2008).

On these bases, our study consisted in applying multispectral imaging to phenotyping plant responses to water stress. It was performed on an apple hybrid population where ecophysiological measurements are time consuming and produce variable results, along with the atmospheric variations affecting the crop (e.g. air temperature, wind speed, solar irradiance). Our work hypotheses were (i) that use of very high resolution imaging at tree scale (airborne image acquisition in RGB, NIR and TIR) will be a relevant method for phenotyping leaf traits at plant canopy scale – the whole population being considered at the same time; and (ii) that high resolution imaging and use of water stress indices will constitute a relevant and sensible method for discriminating plant stomatal response to water stress.

II – Material and methods

1. Location, field set-up and environmental measurements

The study was performed on an apple orchard located at Melgueil experimental farm and belonging to INRA Diaphen platform (Mauguio, 43°36'35N, 3°58'52 E). The plant material consisted in an apple progeny of 122 hybrids ('Starkrimson' x 'Granny Smith') repeated 4 times. Trees were grafted on M9 rootstock, and distributed along 10 rows within the plot, with 5 rows supporting a summer drought treatment: stressed trees (no irrigation, S), while 5 rows were well watered (not stressed trees, NS). The S and NS rows were alternated within the experimental set-up (Fig. 1). For normally watered trees, irrigation was scheduled according to soil water potential, with a microsprayer system located in the row, in line with professional practice. Environmental conditions were monitored by meteorological sensors. Global and photosynthetically active solar radiation, soil and air temperatures, air humidity, wind speed and precipitations were measured, averaged and stored by a CR10X data logger. Soil water status was monitored with capacitive and tensiometric probes.

2. Airborne image acquisition, field measurement

Image acquisition system was composed of two digital cameras Canon EOS 400D (10.1 Megapixel CMOS sensor) each equipped with an objective focal length of 35mm, and one thermal camera FLIR B20HS (320x240 matrix) for the acquisition of TIR images (8.5 to 14.0 µm). One digital camera acquired visible images in Red, Green and Blue bands (RGB) while the second was modified according to Lebourgeois *et al.* (2008) to acquire pictures in the Near Infrared (NIR). The 2010 airborne campaign comprised four ultra-light aircraft flights planned during summer 2010. One flight

(July 16) was scheduled before the application of water stress, two flights during the mid-summer soil water stress (August 3 and 17), and the last one after normal irrigation retrieval, on September 14. Images were taken between 9:00 and 11:00 (solar time) at 300 to 680 m elevation. Pictures taken at 300 m elevation showed a 5cm resolution in RGB and NIR, and 30 cm in TIR.

Nine aluminum targets were distributed within the experimental field for image geolocation (Fig. 1). Moreover, temperature measurement of cold and hot reference surfaces were performed with a thermal infrared thermoradiometer KT19 (Heitronics®) during each airborne acquisition.

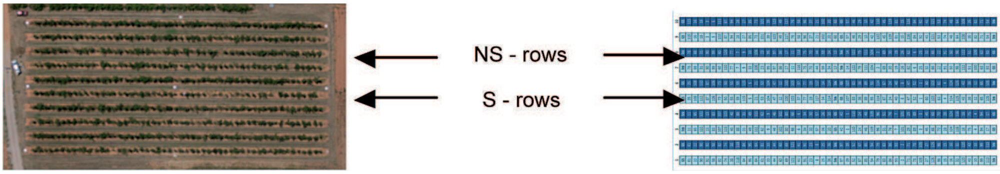

Fig. 1. RGB image of experimental field (ca. 6000 m², left), and field plan (right). Aluminium targets (9 white points) placed at field periphery and middle were DGPS localised for accurate image geolocation. On plan, NS rows are colored in dark blue and S rows colored in clear blue.

3. Determination of WDI with vegetation index (NDVI) and temperature differences between leaf surface (T_s) and air (T_a)

For image transformation we used Erdas Imagine® software.

The Normalized Difference Vegetation Index (NDVI) was computed according to Rouse *et al.* (1973) from red (R, extracted from RGB matrix) and near infrared (NIR) bands:

$$NDVI = (NIR-R) / (NIR+R)$$

T_s-T_a value was obtained by difference between the surface temperature of vegetal cover (T_s) and the air temperature (T_a) acquired by meteorological data logger. T_s at tree level was estimated from thermal values measured at aircraft level (TIR images) corrected by the atmospheric interference, which was itself measured by the temperature difference between soil reference surfaces (KT19 measurements) and corresponding temperatures measured at aircraft level.

Within each tree a central buffer zone of 60 cm diameter was delimited to compute average values of NDVI and T_s-T_a values.

WDI is relative to the Vegetation Index/Temperature (VIT) concept (Moran *et al.*, 1994), which is based on the trapezoidal shape formed by the relationship between (T_s-T_a) and vegetation cover (Fig. 2). Theoretical equations for computation of the trapezoid vertices are given these authors. The ratio between actual evapotranspiration (ET_{actual}) and maximal evapotranspiration (ET_{max}) reflects the water stress intensity, and can be calculated using the following equation:

$$WDI = 1 - [ET_{actual} / ET_{max}] = [(T_s-T_a)_{min} - (T_s-T_a)] / [(T_s-T_a)_{min} - (T_s-T_a)_{max}] = AC / AB$$

Where $(T_s-T_a)_{min}$, $(T_s-T_a)_{max}$, (T_s-T_a) correspond respectively to points A, B and C (Fig. 2).

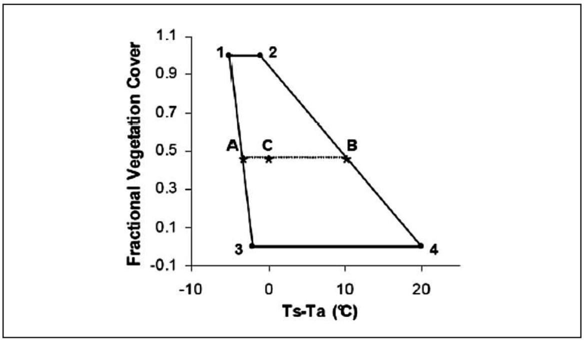

Fig. 2. Illustration of Moran VIT concept and WDI computation. 1: well watered and fully covering vegetation; 2: water stress and fully covering vegetation; 3: saturated bare soil; 4: dry bare soil.

III – Results and discussion

1. Monitoring of soil water stress

Water stress intensity during summer was measured by a series of tensiometric probes located in the middle of the experimental field. For each treatment and at each soil depth, median values of soil water potential (SWP) were considered. Before the summer stress period, we can observe (Fig. 3) a transient decrease of SWP due to irrigation deficit. From May 1, a continuous decrease of SWP was observed for S rows at 60 cm depth (red curve), and the soil drought situation was maintained until end of October, 2 months after irrigation restoring. Considering the tensiometric probe located at tree root depth (30 cm) the soil stress period (pink curve, SWP<0.08 MPa) began concomitantly with the first flight (July-16), and was sharply interrupted by the resumption of irrigation at the end of August. Contrastingly, the SWP values shown by blue curves (NS rows, 30 and 60 cm) were almost always higher than -0.08 MPa (stress threshold) indicating a situation of tree hydric comfort.

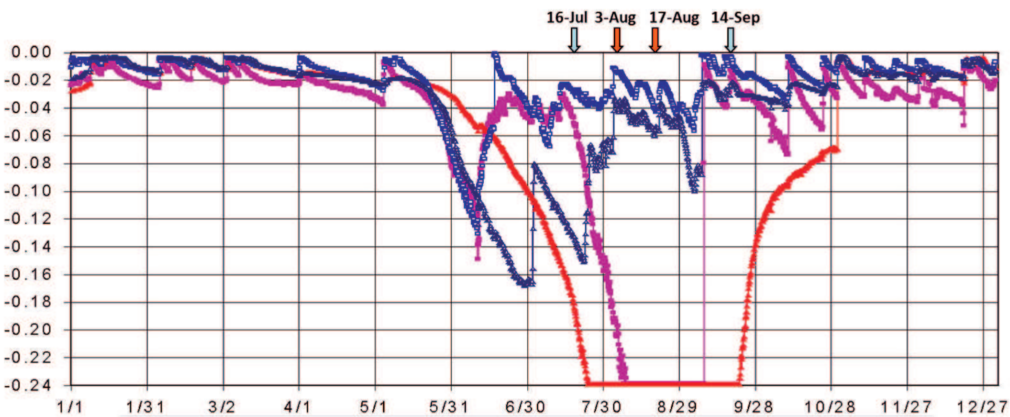

Fig. 3. Monitoring of soil water potential (MPa) in 2010 with tensiometric probes located at two depths (30 cm and 60 cm). The NS treatment is represented by the clear and dark blue curves, for 30 cm and 60 cm respectively, and the S treatment is represented in pink and red, for 30cm and 60cm respectively. Dates of airborne flight image acquisition are indicated by vertical arrows.

2. Water Deficit Index and G, E and GxE effects

The Fig. 4 represents the WDI values computed for each pixel of the experimental plot (dark points), and a series of superimposed blue and red points corresponding to average values of WDI within each individual tree buffer zone, for NS and S trees respectively. Differences between S and NS tree groups, materialised by the distance between blue and red scattered points, were clear except at the last date. From July 16 until August 17, particularly, WDI differences between S and NS trees increased with increasing stress (see F values, ANOVA results, Table 1). After irrigation restoring (September 14), WDI values of S trees did not present any differences with those of NS trees. The transient decrease of SWP, due to a temporary irrigation deficit at the beginning of summer period (Fig. 2) can explain slight but significant differences between WDI values on July 16 (Fig. 4, onset of blue and red point differentiation), before the real stress period after this date.

Table 1 presents the results of a two-way ANOVA on WDI values, testing the effects of genotype, soil, and their possible interaction. This analysis reveals significant drought and genotype effects at the three first dates, while no difference was observed on September 14 (not shown). Drought effect was globally prevailing, as shown by *p*-values. On August 17 the genotype effect was less significant than at the two first date, as indicated by a higher *p*-value. This results could result from a lower resolution thermal images, because the airborne acquisition was performed at 680 m instead of 300 m. No interaction was revealed between genotype and drought factors, suggesting either that their effects were purely additive, or that a severe drought situation could mask differential responses of genotype to intermediate soil drought.

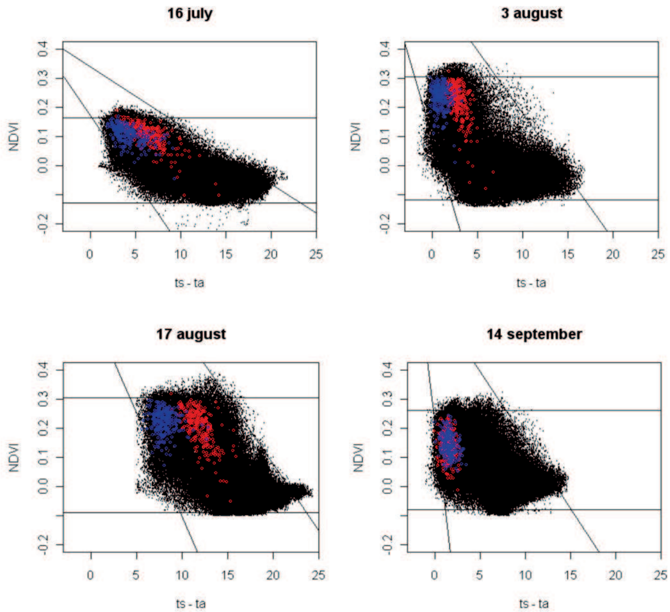

Fig. 4. Graphic representation to WDI values of all field pixels (dark points) and of the apple trees central one. Blue points represent NS trees and red ones S trees. Ts-Ta are expressed in °C.

Table 1. Two-way ANOVA applied to WDI (2010 campaign). *p*-values less than 0.05 show significant effects. No values are presented for Sept.14 because there were no significant effects of both factors

Effect		07-16	08-03	08-17
Genotype	F	1.8	1.9	1.5
	p-value	$<10^{-4}$	$<10^{-4}$	$<10^{-2}$
Drought	F	501	772	1661
	p-value	$<10^{-6}$	$<10^{-6}$	$<10^{-6}$
G * D	F	0.5	0.5	0.6
	p-value	p# 1.0	p# 1.0	p# 1.0

IV – Conclusions

These first results showed that it is possible to reveal the effects of tree genotype and drought by the use of remote sensing tools, thanks to high spatial resolution of airborne images. This result present a "snapshot" dataset, image acquisition are made just at one moment. But for 2010 some supplementary measurements will be necessary to validate the results. We don't have knowledge of each plant water status. Moreover the vegetation index (NDVI) that was used for WDI calculation requires to be assessed in respect to its intrinsic parameters, the leaf area index (LAI) and leaf chlorophyll content (Bégué *et al.*, 2010). These measurements have been taken into account during the 2011 campaign (results currently analyzed). During this 2011 campaign (three airborne imaging flights) water status of some trees was periodically assessed by stem water potential measurements at airborne acquisition dates. In comparison to WDI, our project will be to test and compare the relevancy and the sensitivity of S-SEBI (Simplified Surface Energy Balance Index, Roerink *et al.*, 2000), another water stress index applicable to heterogeneous cover.

On the basis of a study of Möller *et al.* (2007), who used visible and thermal imaging to estimate crop water status, we also plan to acquire more proximal ortho-images of trees and try to assess to which extent airborne acquired images and the resulting vegetation indices are affected by the resolution of TIR images.

Genetic analysis of the hybrid apple population will be realized on the basis of WDI and S-SEBI results, over 2010 and 2011 campaigns, and also through to the analysis of another plant traits, more time-integrative, like fruit carbon isotope discrimination ($\Delta^{13}C$), which is a proxy for water use efficiency (Brendel *et al.*, 2002). Further heritability analysis on functional traits, QTL detection and refined genetics studies related to QTL zones are planned.

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