Phenotyping the Response of an Apple Tree Hybrid Population to Soil Water Constraint under Field Conditions: New Insights Brought by High Resolution Imaging

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Context

- *Global changes → new traits for fruit breeding*
- *Transpiration behavior of apple → genetic variability of response to a water constraint*
- *High-throughput genetic/genomic data → high-throughput phenotyping methodology*

Estimation of leaf transpiration by thermal infrared:

o Usually used for irrigation scheduling *via* water stress indices: Crop Water Stress Index (CWSI)
(Idso et al., 1981 ; Jackson et al.,1981)

- based on difference between leaf surface and air T° (T_s-T_a) and its variation
- continuous cover
- semi-arid and arid conditions

o CWSI also used as indicator of plant water status in annual crops

- g_s estimation through I_g index : $I_g = (T_{dry} - T_{leaf}) / (T_{leaf} - T_{wet})$ *(Jones et al., 1999, 2009)*
- mapping Ψ_{leaf} from CWSI *(Alchantis et al., 2010)*

Moran *et al.* (1994): Water Deficit Index (WDI), through adaptation of CWSI
WDI → based on relation between (T_s -T_a) and the NDVI vegetation index
o applicable to discontinuous cover
o empirical estimation : $WDI_e = (T_s - T_{min}) / (T_{max} - T_{min}) = 1 – Et_{act} / Et_{max}$ *(Clarke, 1997)*

Use of multispectral imaging for phenotyping an apple hybrid population (mature trees) under water constraint applied in field conditions

Study based on:

- Airborne high-resolution image acquisition in RGB, NIR and TIR *(Lebourgeois et al., 2008 ; Avion Jaune®)*
- Estimation of tree transpiration rate through foliage temperature
- Vegetation index: adapted T° (TIR image) at the nature of transpiring surface
- Applying WDI at tree scale, sensitivity of this index to genotypic differences

Hypotheses :

→ **Relevant and sensible method for characterizing the stomatal response of hybrids to water stress**

→ **Ability to reveal apple genetic variability for this trait (disentangling isohydric *vs* anisohydric behaviors)**

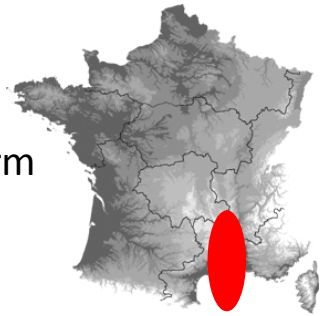
(e.g. grapevine: Schulze, 2003 ; Soar et al., 2006)

Experimental approach: field set-up

1. Location

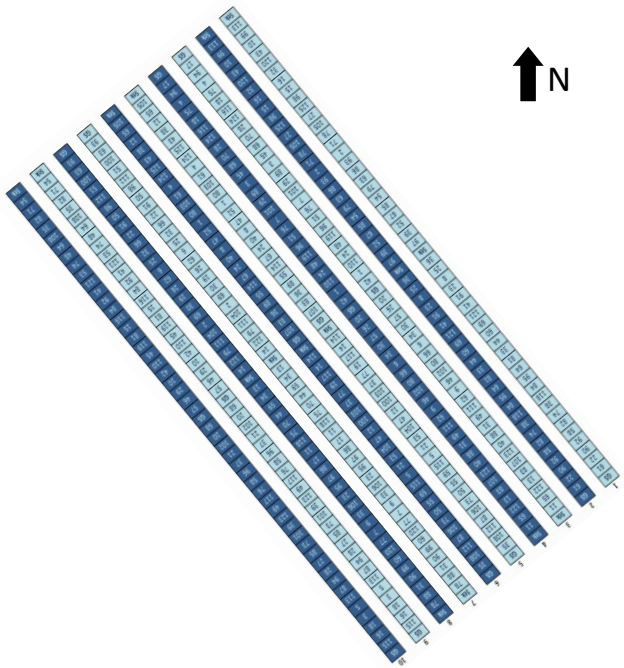
INRA-Diaphen Melgueil exp^{l} farm

(43°36’ N, 3°58’ E)



2. Field set-up

- **488 apple trees**
- **122 hybrids** (Starkrimson * Granny Smith progeny) / M9 roostock
- **2 parents**
- **10 rows**
- **2 seasonal water treatments : S, NS** (Stressed, Non Stressed)
- **2 tree replicates per genotype & treatment**



Not irrigated during summer (S)

Always irrigated (NS)

3. Orchard management

Micro-meteorology

- Global radiation, direct & diffuse PPFD,
- Soil & air T°, air RH
- Wind speed
- Rainfall

Soil

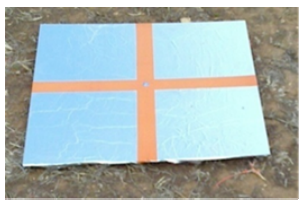
- Soil water content (Sentek^{TM} capacitive sensors)
- Soil water potential (Watermark^{TM} probes)

1. 2010 campaign image acquisition

Acquisition system:
- Visible (RGB)
- Near Infrared (NIR)
- Thermal Infrared (TIR)

Soil device:
- 9 geolocation targets (image superposition)
- 1 « hot » target
- 1 « cold » target

] Atmospheric correction



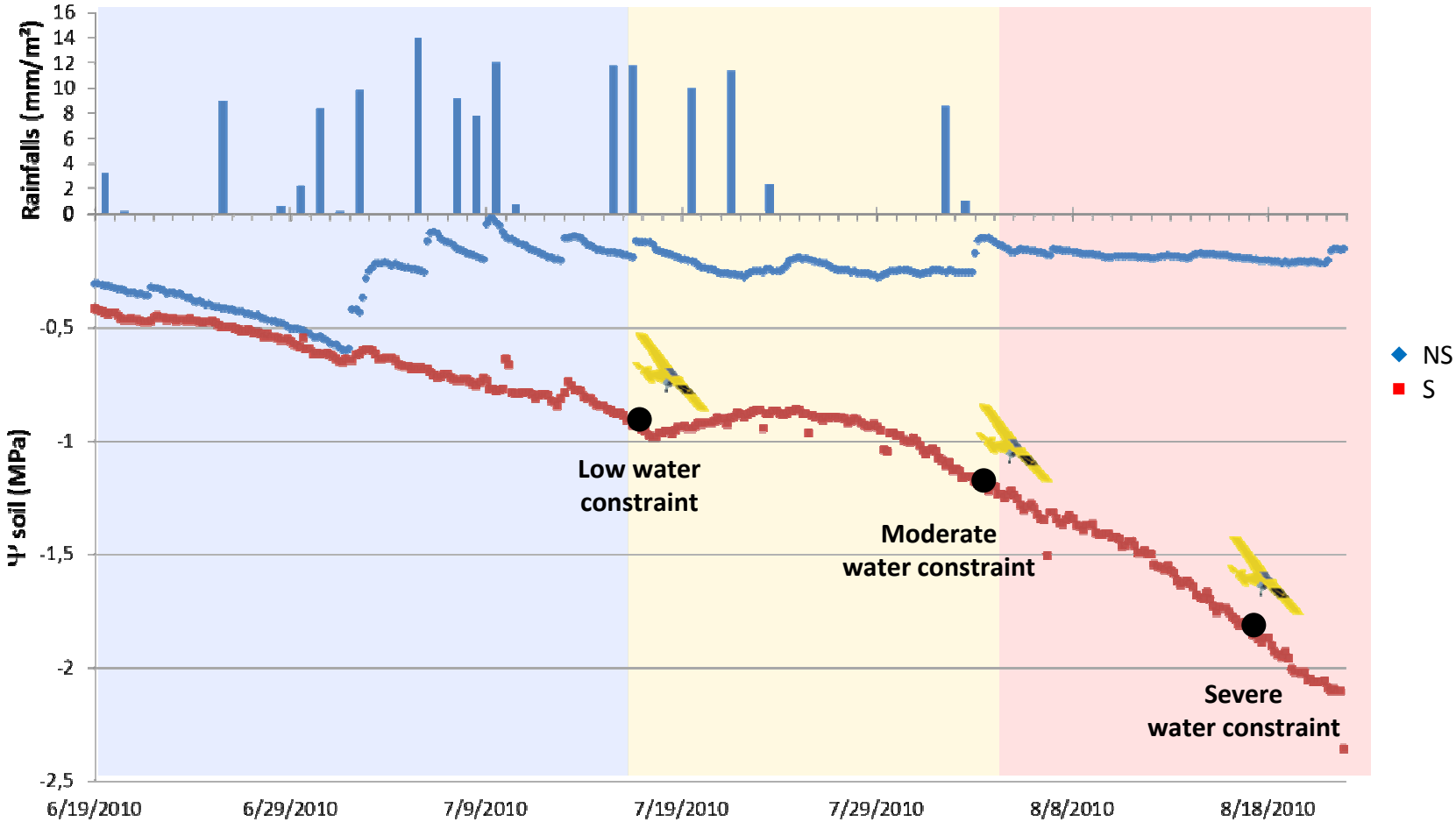
Aluminium targets (for TIR images geolocation)

3 ULA flights
- July 16: low water constraint
- August 3: moderate water constraint
- August 17: severe water constraint

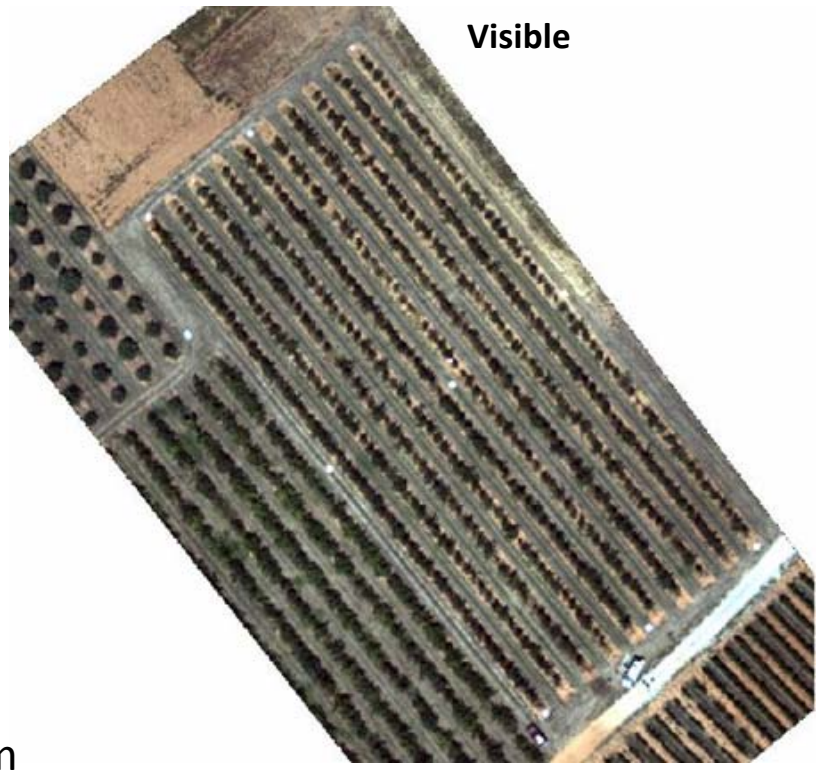
] 11h30-12h00 / 10h-11h

Airborne image acquisition between 350 and 480m altitude

Experimental approach: water stress scheduling



1. Acquisition of 5 channels

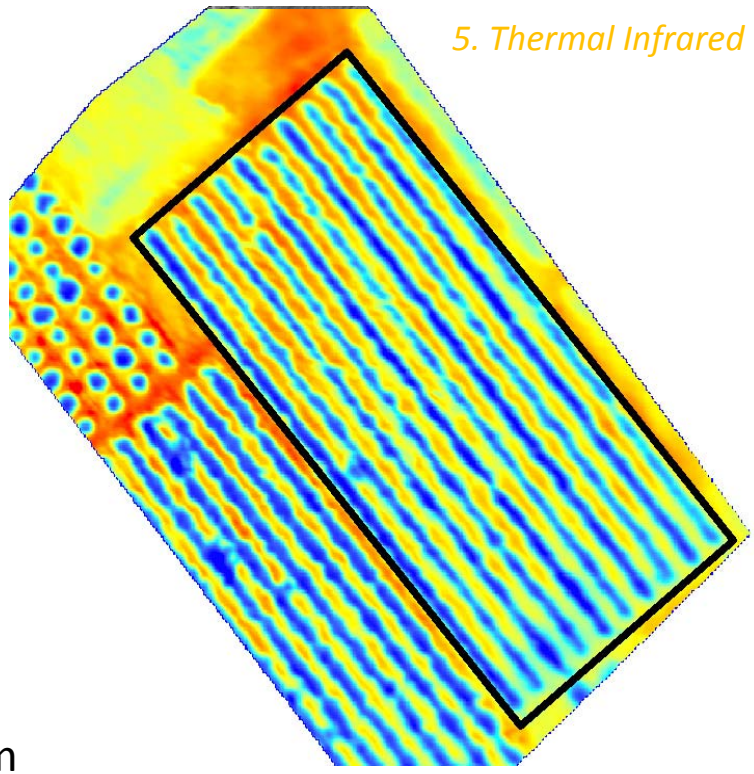


Visible: - blue: ~450 nm
- green: ~550 nm
- red (R): ~670 nm

Near Infrared (NIR): ~745nm

Thermal Infrared (TIR): 8-14µm

1. Acquisition of 5 channels



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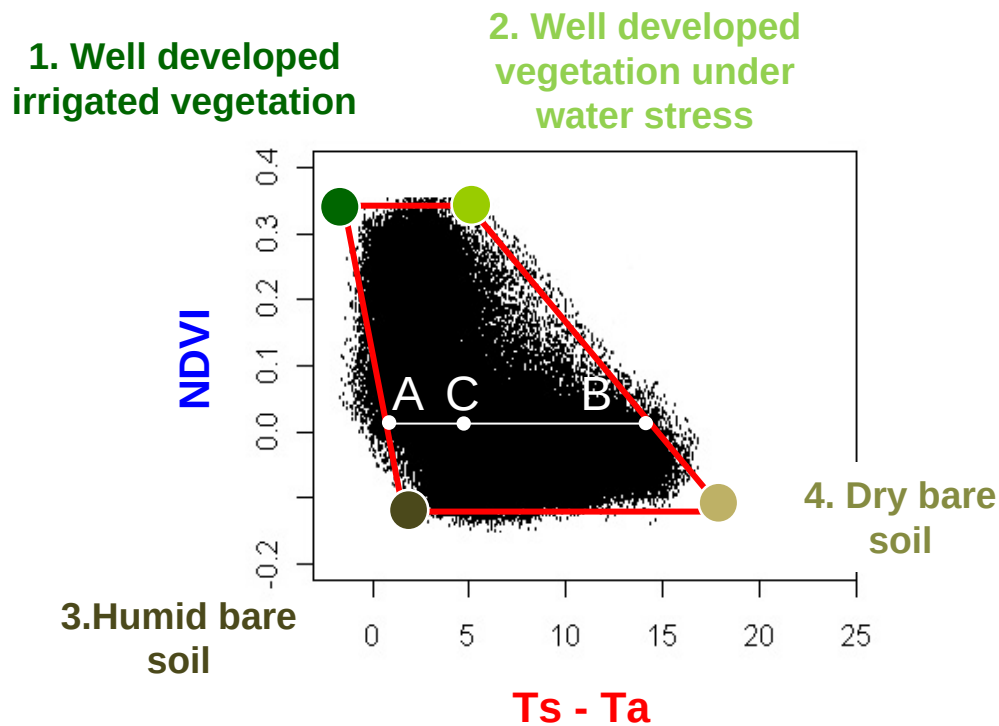
2. Water Deficit Index Computation

- **NDVI** = (NIR – R) / (NIR + R)
- (T_s - T_a): difference between surface and air temperature
- pixels plotted according to NDVI and T_s - T_a



- Scatterplot delimited by quantile regression

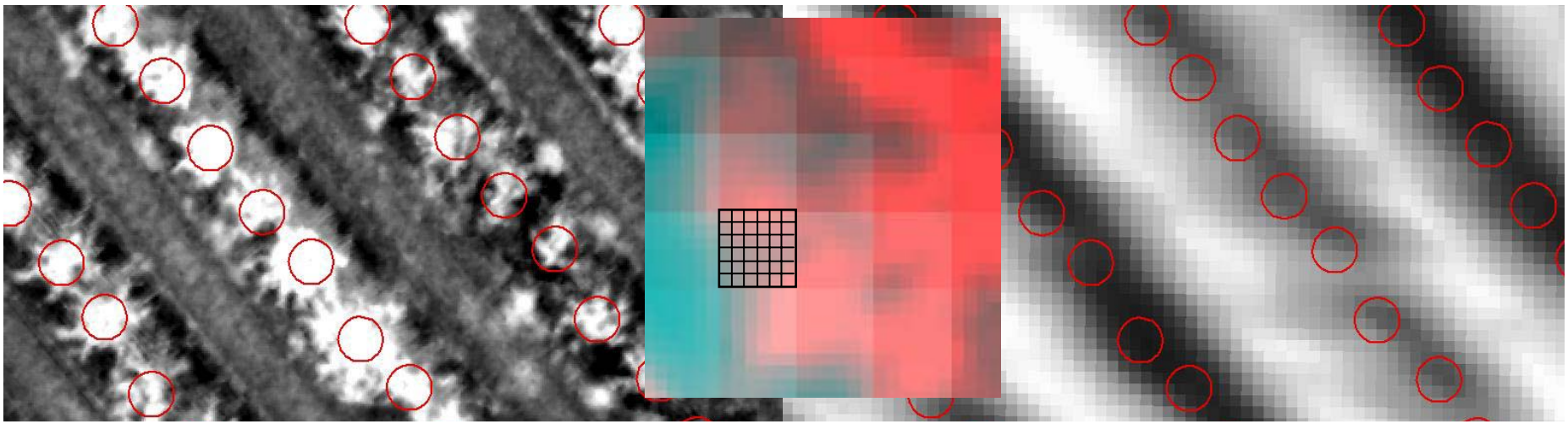
- Equations of the 2 oblique lines permit computation of WDI

$$WDI = \frac{(T_s - T_a) - (T_s - T_a)_{min}}{(T_s - T_a)_{max} - (T_s - T_a)_{min}} = 1 - \frac{ET_{act}}{ET_{max}} = \frac{AC}{AB}$$

WDI index varies from 0 (well-watered tree) to 1 (severely stressed)

3. Water Deficit Index Computation :

Each tree delimited by a 60 cm radius buffer zone



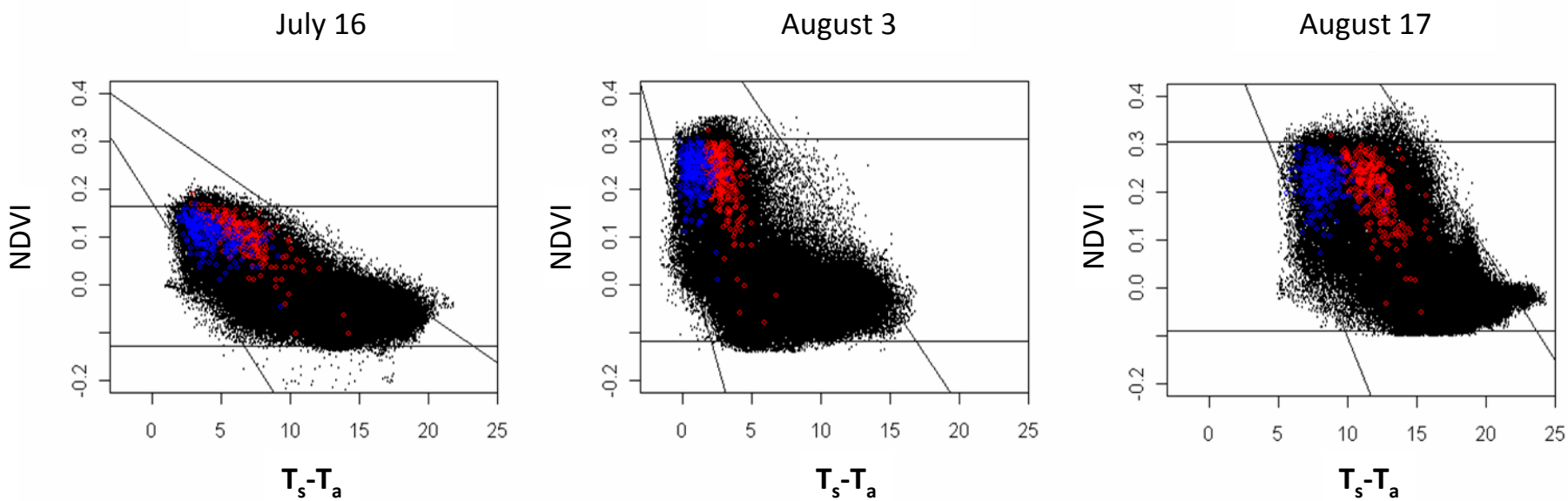
3*3 to 5*5cm resolution for NDVI image

30*30cm resolution for TIR image

Performed on Erdas imagine® software

- **NDVI and T_s - T_a values of each individual pixel → WDI values**
- **avg. NDVI & T_s - T_a values per tree in buffer zone → avg. tree WDI value**

1. NDVI, Ts – Ta and WDI, 3 dates



Effect	NDVI		T_s-T_a		WDI		NDVI		T_s-T_a		WDI		NDVI		T_s-T_a		WDI	
	F	*p-value*	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*	*F*	*p-value*
Genotype	3.6	$< 10^{-15}$	2	$< 10^{-5}$	1.8	$< 10^{-4}$	3.3	$< 10^{-14}$	1.1	n.s.	1.9	$< 10^{-4}$	2.5	$< 10^{-8}$	1.3	0.052	1.5	$< 10^{-2}$
Drought	0.6	n.s.	307	$< 10^{-15}$	506	$< 10^{-15}$	15	$< 10^{-3}$	902	$< 10^{-15}$	775	$< 10^{-15}$	18	$< 10^{-4}$	1739	$< 10^{-15}$	1672	$< 10^{-15}$
G * D	0.9	n.s.	0.5	n.s.	0.5	n.s.	0.9	n.s.	0.6	n.s.	0.5	n.s.	0.8	n.s.	0.6	n.s.	0.6	n.s.

- Genotypic effect on WDI although less effect on T_s - T_a
- Strong drought effect on most stress indices
- Additive effects of Genotype and Drought
- Relationships between soil moisture, tree water status and WDI (not shown)

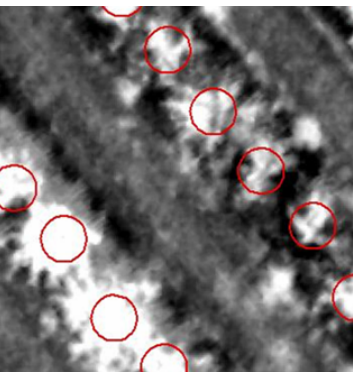
Discussion

1 - Pixel resolution of TIR images

1 pixel in thermal domain # 36 to 100 pixels in visible domain

Resolution to be increased to distinguish well exposed & shadow leaves, soil & branches

Use of Unmanned Aerial Vehicle (UAV) to increase resolution of TIR image (6 to 10 cm)



2 - Delineating most appropriate foliage zone for each tree

60 cm buffer method: more soil “top-viewed” for smaller trees

→ Taking a larger interest zone

→ NDVI value thresholding to disentangle soil *vs* vegetation and delimitate tree foliage

3 - Genetic analysis to be performed :

- Apple tree behavior,
- Genetic determinants of transpiration

Conclusions and prospects

On-going Nicolas Virlet's PhD work (2010-13)

2011 campaign: currently analyzed; image pipeline analysis undertaken

2012 : image and field acquisition just running … with an UAV Oktokopter®

Apple tree behavior under water constraint

- Genotypic differences mostly revealed at the beginning of water stress application
- UAV regular flights → dynamic response of each tree to increasing stress
- Analysis of time series: disentangling isohydric *vs* anisohydric behaviors

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