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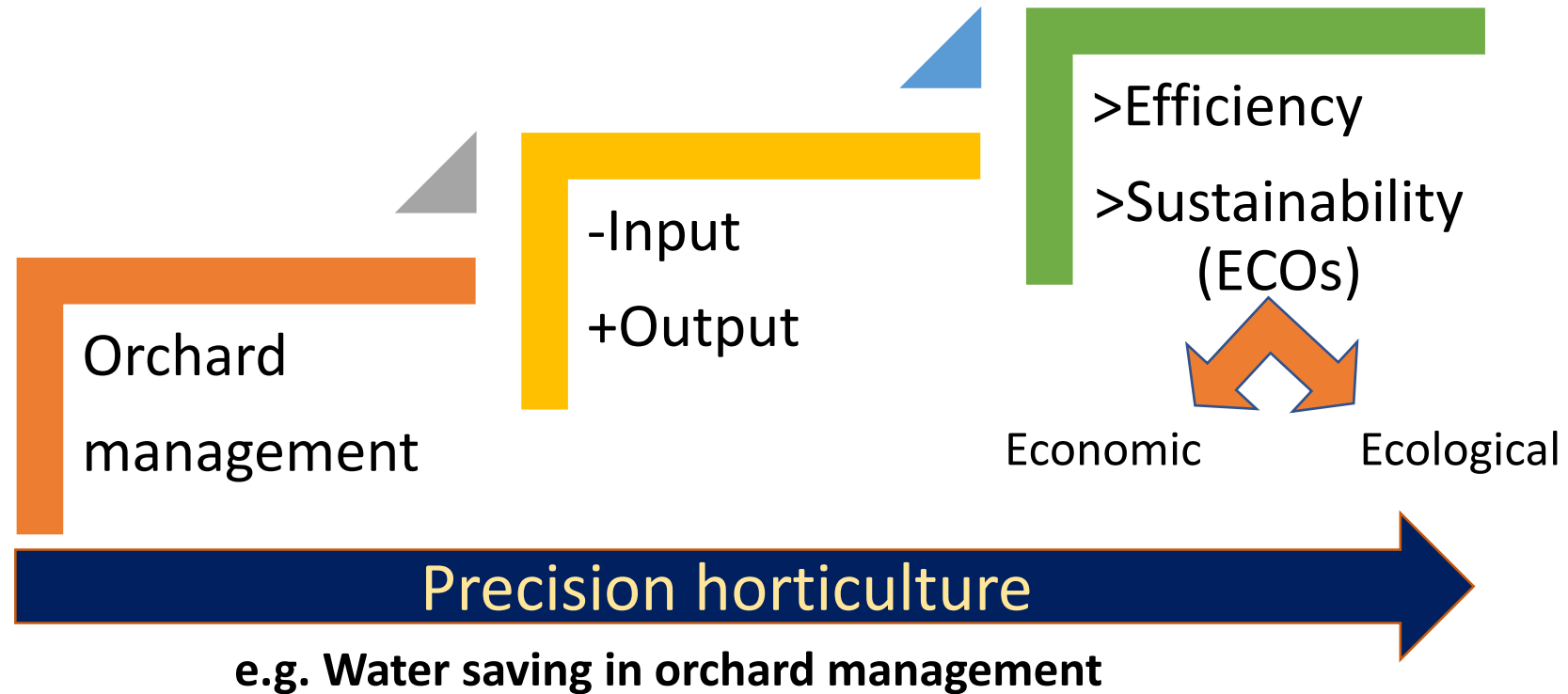
In-field automatable tools for the determination of plant physiological responses and fruit quality parameters in 'September Bright' nectarines subjected to deficit irrigation strategies



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# Background



*Estimate correctly when and how much water to provide with irrigation based on plant water status, rather than on soil water content/status.*

*Are there any methods for continuous determination of tree water status?*

## HYPOTHESES

- Continuous fruit growth rates and leaf turgor pressure dynamics change under different irrigation treatments in nectarines.
- Deficit irrigation applied at different fruit growth stages differently affect tree physiology

## OBJECTIVES

- Find out the most sensitive continuous indicator of water deficit.
- Test portable, non-destructive devices for in-field determination of leaf and fruit composition

## RESEARCH QUESTIONS

- Are fruit growth and leaf turgor pressure related to each other?
- Can we associate fruit growth and leaf turgor pressure to midday stem water potential ( $\Psi_{\text{stem}}$ )?
- Are near-infrared (NIR) and fluorescence spectroscopy suitable for in-field non-destructive determination of fruit and leaf composition (e.g. sugars, dry matter and flavonoids)?

# Materials & methods

- Open Tatura system
- Drip irrigation

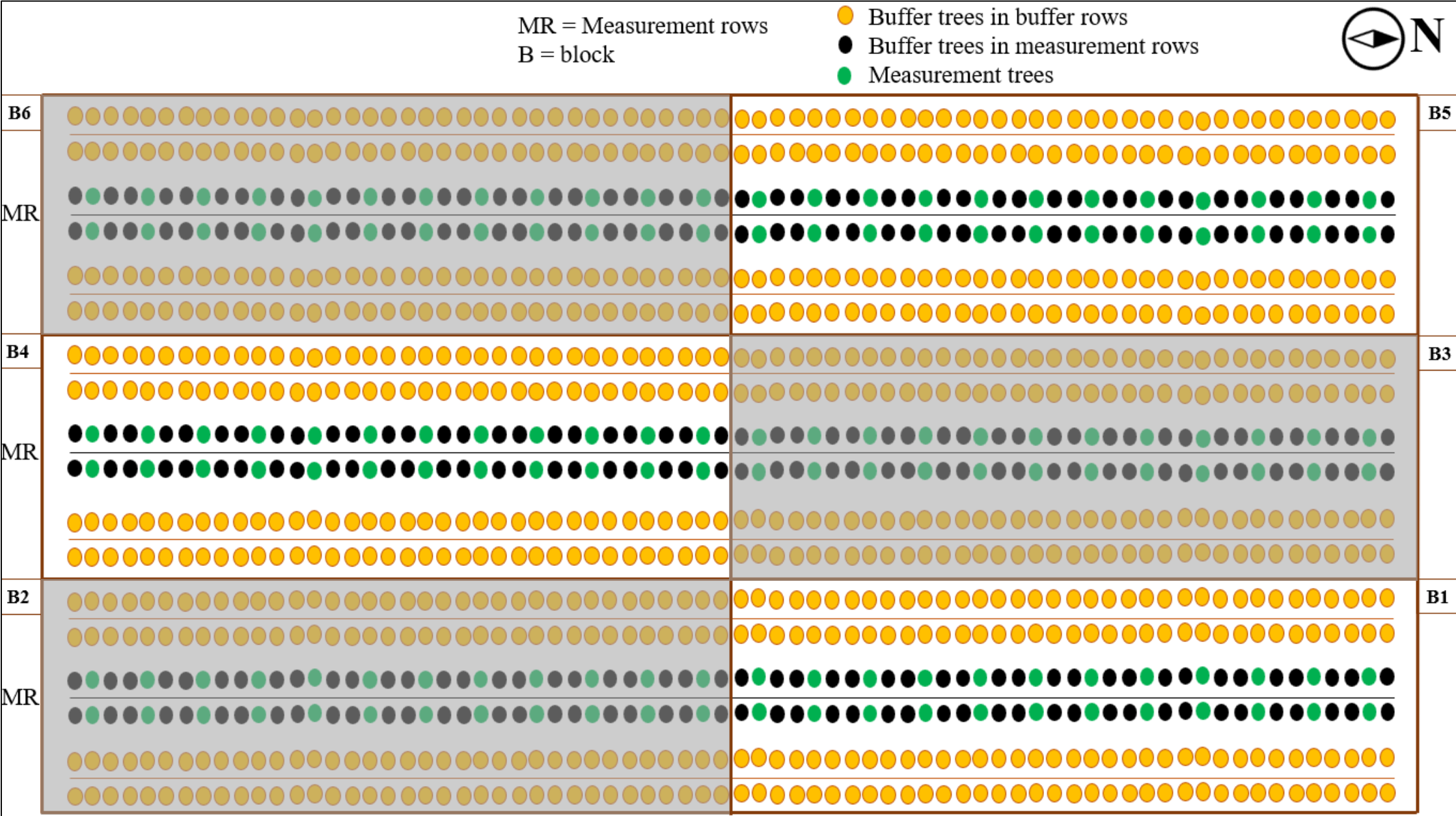


- 144 measurement trees Planting density: 2200 trees/ha
- 4-years-old trees
- 'September Bright' nectarines grafted on 'Elberta' rootstock
- 6 randomized blocks



# Randomized block design

- 6 blocks
    - 12 irrigation treatments
      - 2 Canopy orientation treatments
- 144 measurement trees



# Full factorial design (12x2)

## IRRIGATION TREATMENTS

- 12 treatments

| Irrigation treatments | Fruit growth stages          |            |            |            |
|-----------------------|------------------------------|------------|------------|------------|
|                       | I                            | II         | IIIa       | IIIb       |
|                       | 0% of ETc                    | 0% of ETc  | 0% of ETc  | 0% of ETc  |
|                       | 20% of ETc                   | 20% of ETc | 20% of ETc | 20% of ETc |
|                       | 40% of ETc                   | 40% of ETc | 40% of ETc | n.a.       |
|                       | Full irrigation: 100% of ETc |            |            |            |

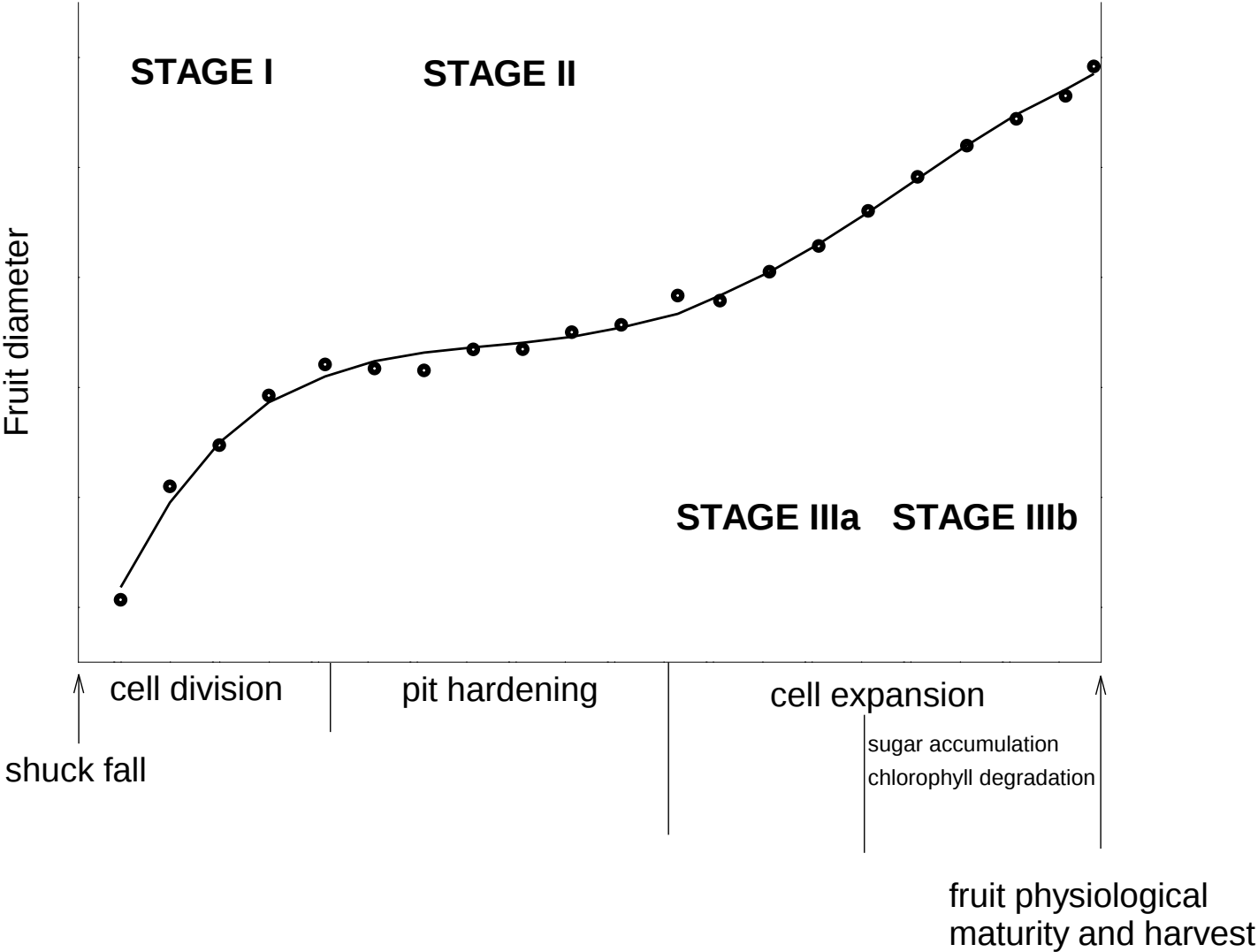
## CANOPY ORIENTATION TREATMENTS

- 2 treatments (72 West & 72 East trees)



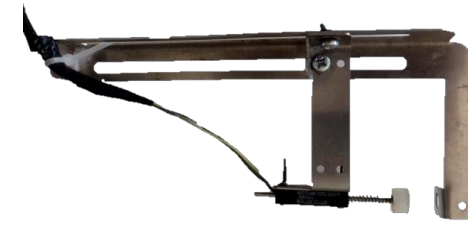
Multiple measurements were taken over time:

- Throughout the day (daily curves)
- At weekly intervals
- At growth stage intervals



# Equipment used for field measurements

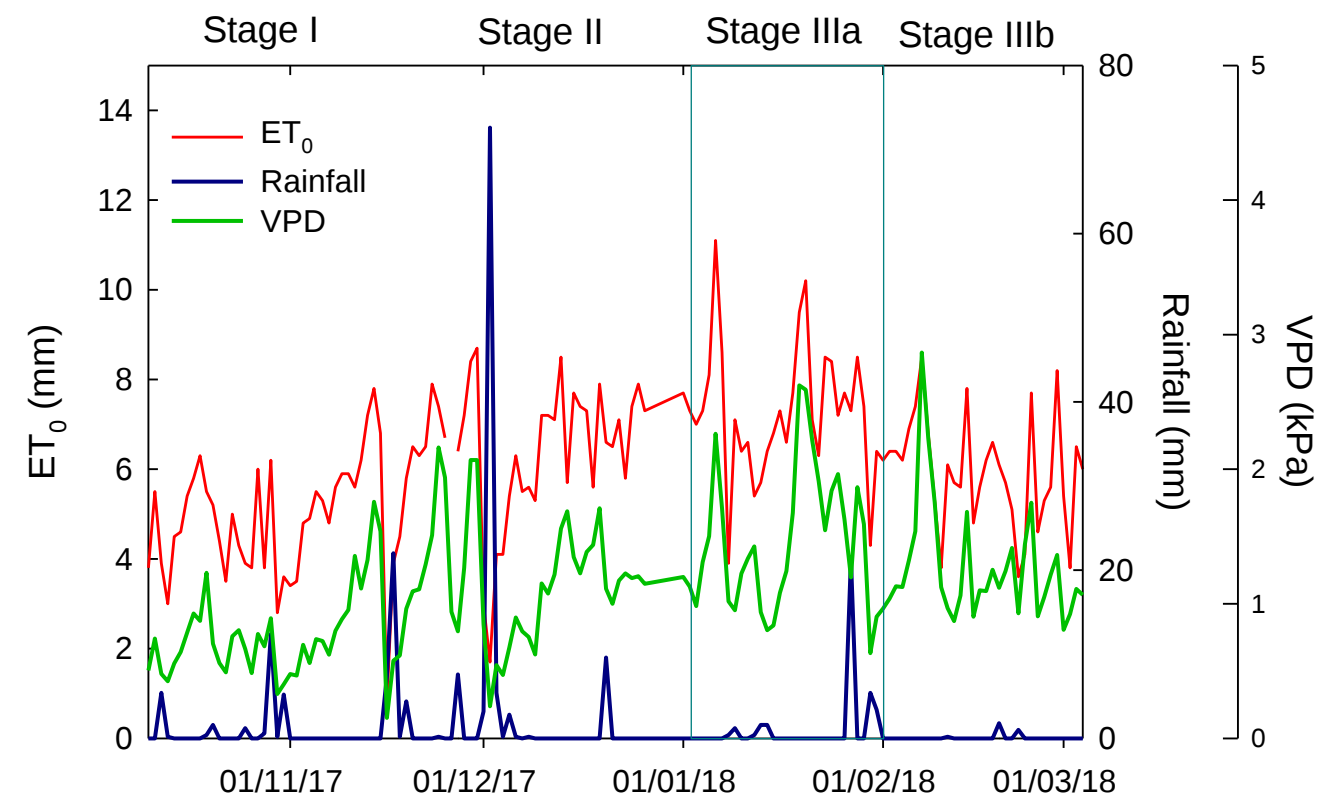
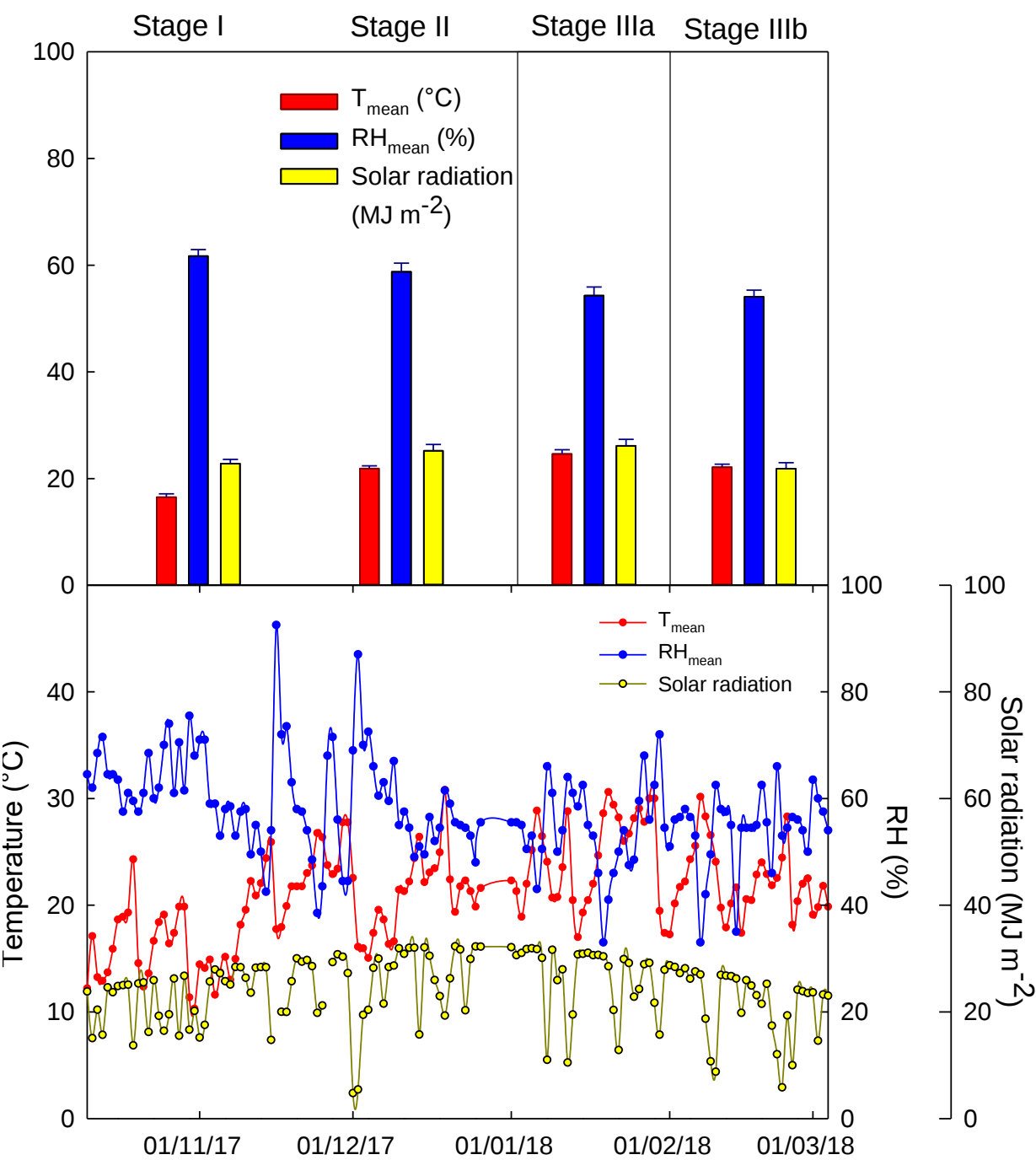
- **Fruit gauges** for continuous measurements of fruit growth
- **Leaf patch clamp pressure (LPCP) probes** for continuous measurements of leaf turgor pressure
- **Calibit** (digital calliper) for fruit diameter measurements
- **DeltaT AP4 dynamic porometer** for leaf stomatal conductance ( $g_s$ )
- **Light trolley** and ceptometer for canopy light interception
- **LICOR 6400** for photosynthesis and leaf fluorescence
- **SPAD meter** (SPAD index) for determination of chlorophyll content
- **Pressure chamber** for determination of stem water potential
- **DA-meter** (IAD index) for determination of chlorophyll degradation
- **Felix F-750 NIR portable device** for determination of SSC and DM
- **Multiplex Force-A fluorometer** for determination of flavonoids



# Results (presented in red)

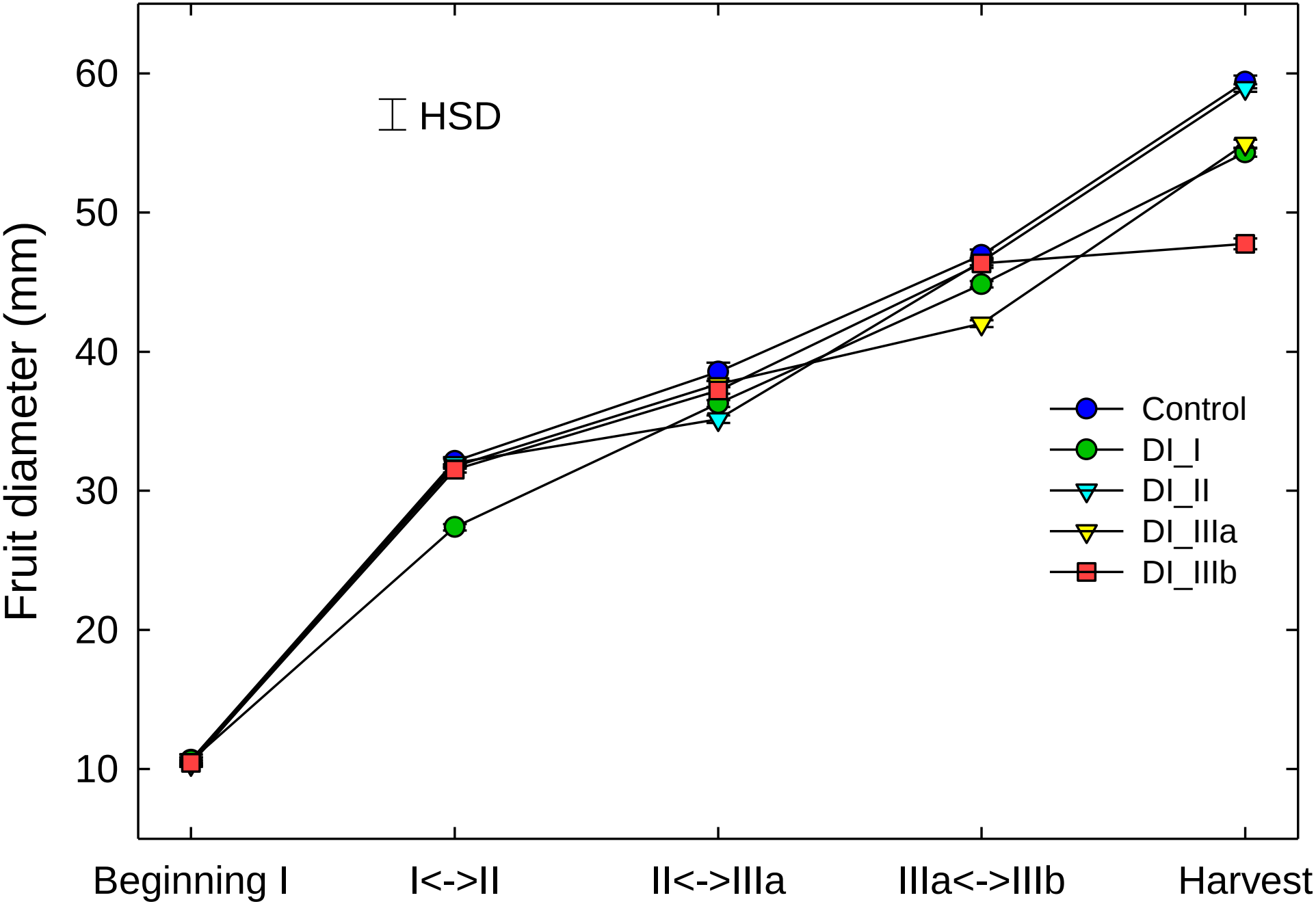
- Multiple Vs single winter buds – Influence of previous year irrigation treatments
- Multiple Vs single spring fruitlets – Influence of previous year irrigation treatments
- **Fruit diameter**
- **Effective area of shade (estimate of tree vigour and light interception)**
- **Stomatal conductance ( $g_s$ )**
- Trunk cross-sectional area (TCSA)
- Leaf photosynthetic activity ( $P_n$ )
- **Efficiency of PSII ( $\Phi_{PSII}$ )**
- Leaf relative water content (RWC)
- **Stem water potential ( $\Psi_{\text{stem}}$ )**
- Leaf water potential ( $\Psi_{\text{leaf}}$ )
- **SPAD index**
- Yield, fruit weight, crop load and flesh firmness
- IAD index
- **Anthocyanin and flavonol indices in fruit and leaves**
- **SSC and DM**
- Starch and sugars in wood
- **Leaf turgor pressure dynamics**
- **Fruit growth dynamics**

# Weather and irrigation

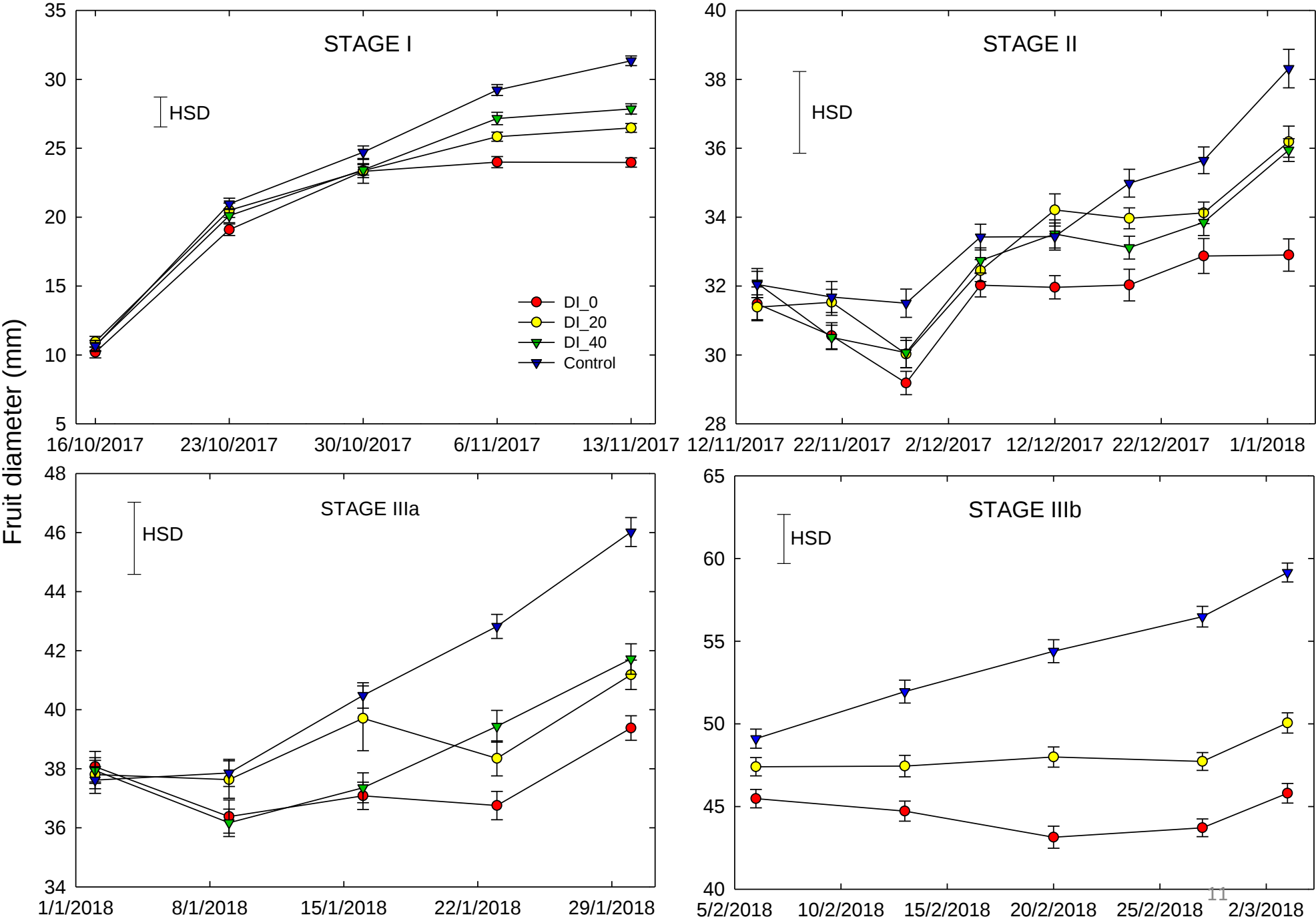


|            | Rainfall (mm) | Full irrigation (100% of $ET_c$ , mm) | Rainfall + full irrigation (mm) |
|------------|---------------|---------------------------------------|---------------------------------|
| Stage I    | 64            | 73                                    | 137                             |
| Stage II   | 141           | 78                                    | 219                             |
| Stage IIIa | 35            | 81                                    | 116                             |
| Stage IIIb | 3             | 83                                    | 86                              |
| Total      | 243           | 315                                   | 559                             |

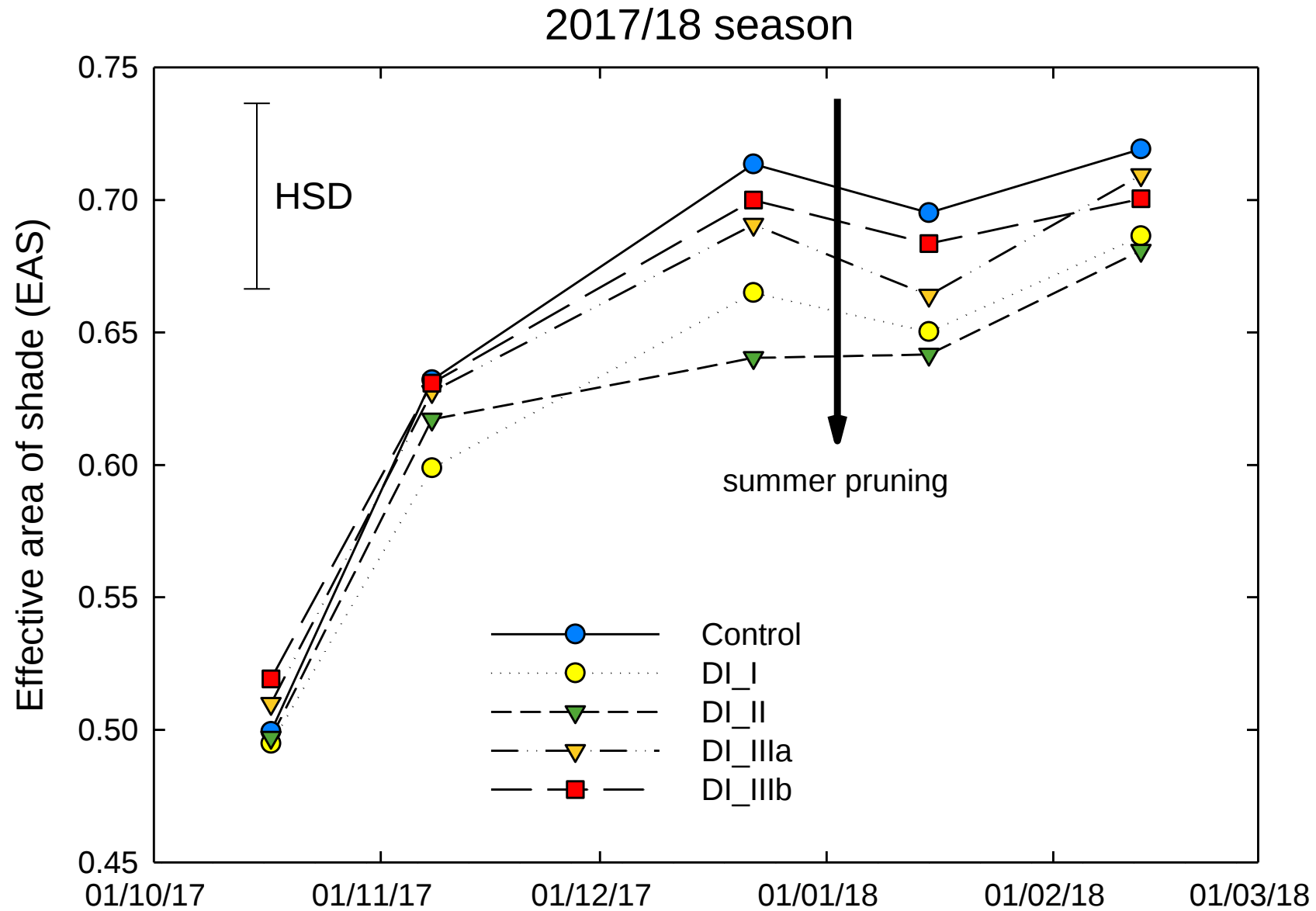
Fruit diameter  
(irrigation  
treatments  
aggregated by  
stage)



Fruit diameter  
(in-stage  
weekly trends)



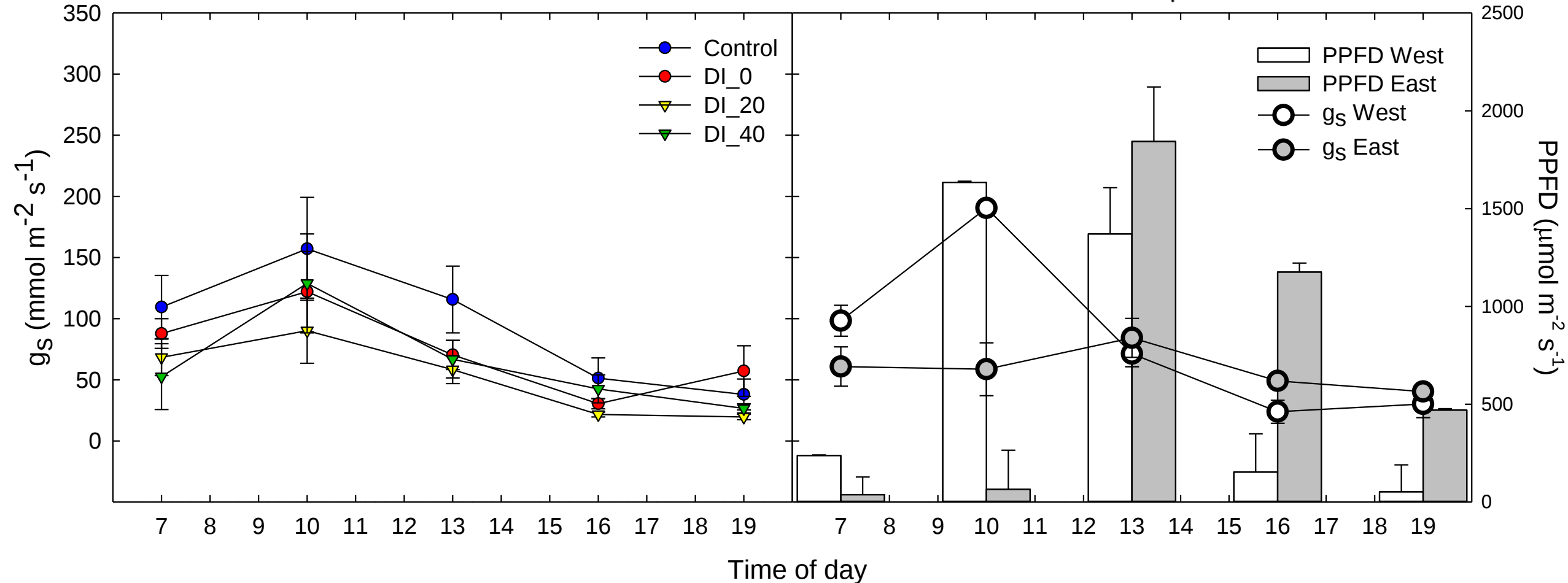
# Tree vigour and light interception (Effective area of shade, EAS)



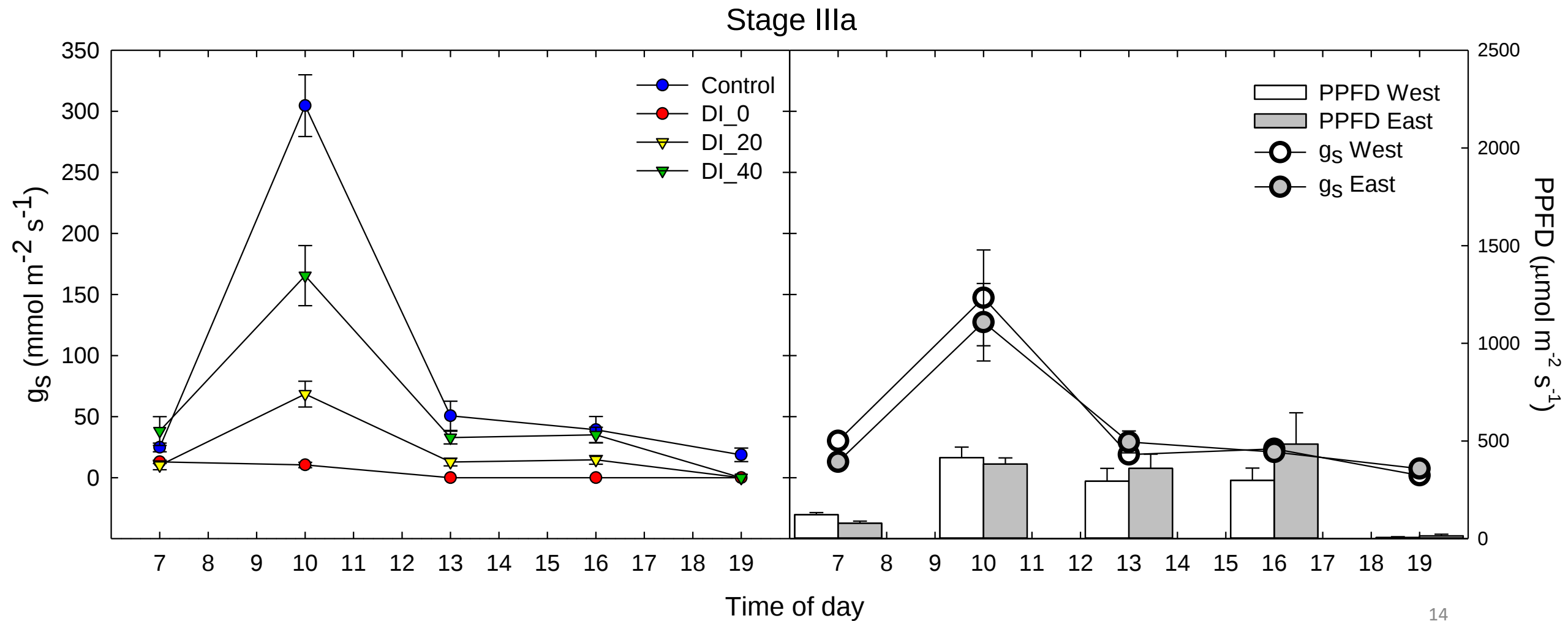
# Diurnal stomatal conductance ( $g_s$ )



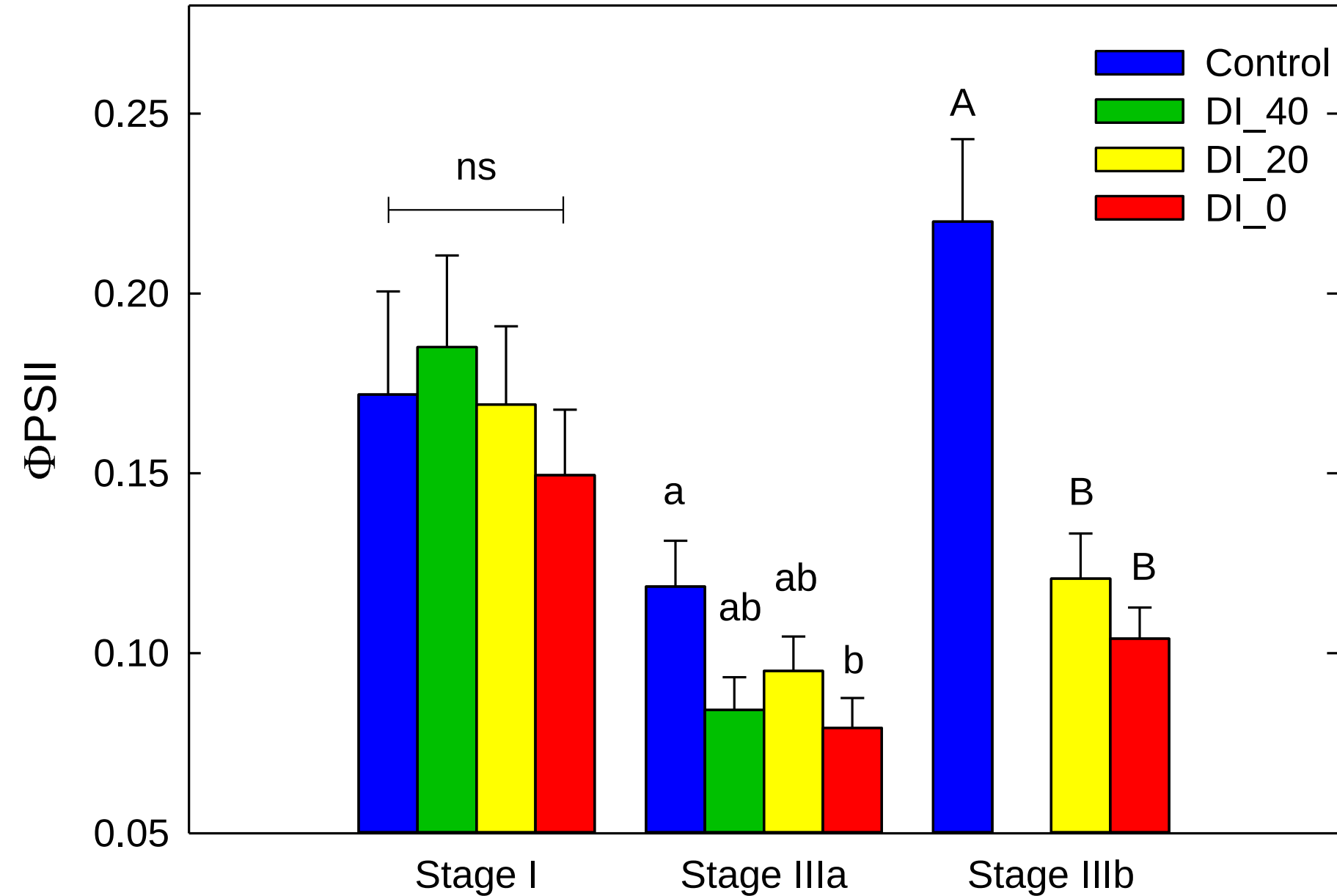
Stage II



# Diurnal stomatal conductance ( $g_s$ )

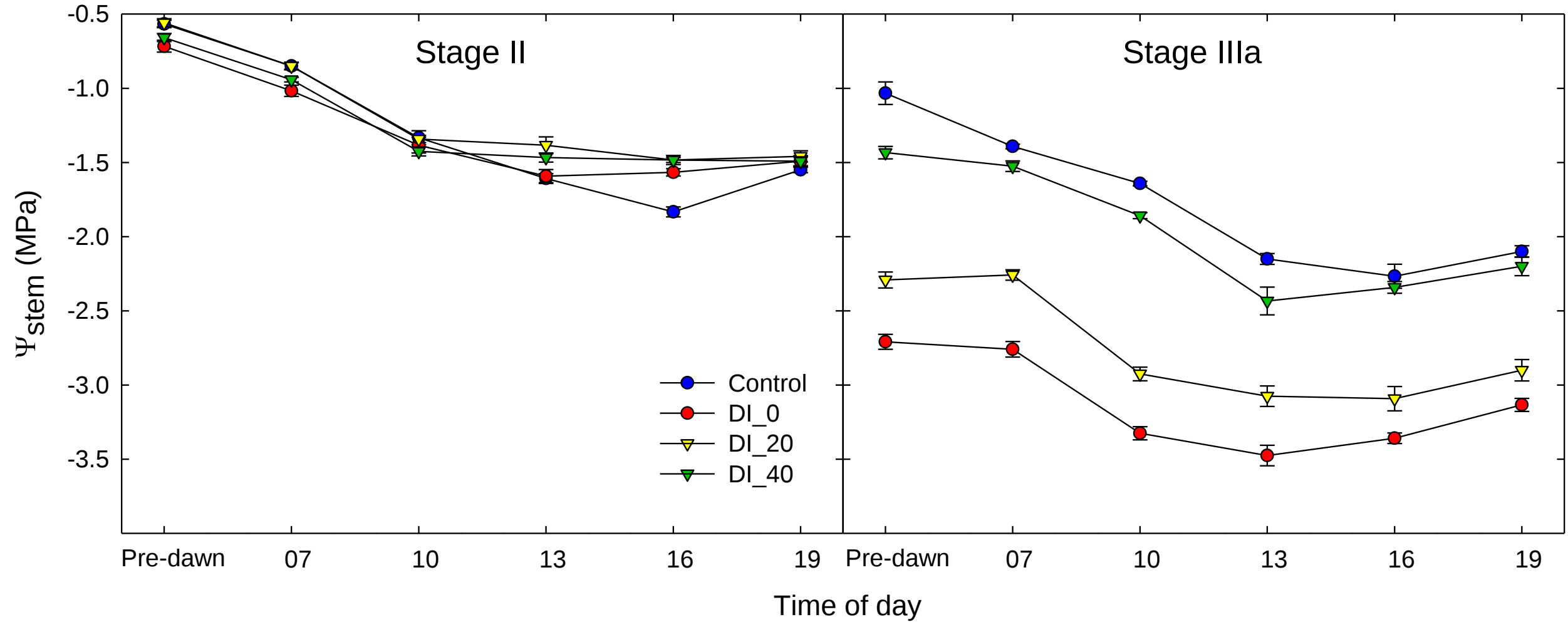


# Efficiency of PSII ( $\Phi$ PSII)



ANOVA and mean separation by Tukey's multiple comparison test  
Different letter represent significant differences for  $p < 0.05$

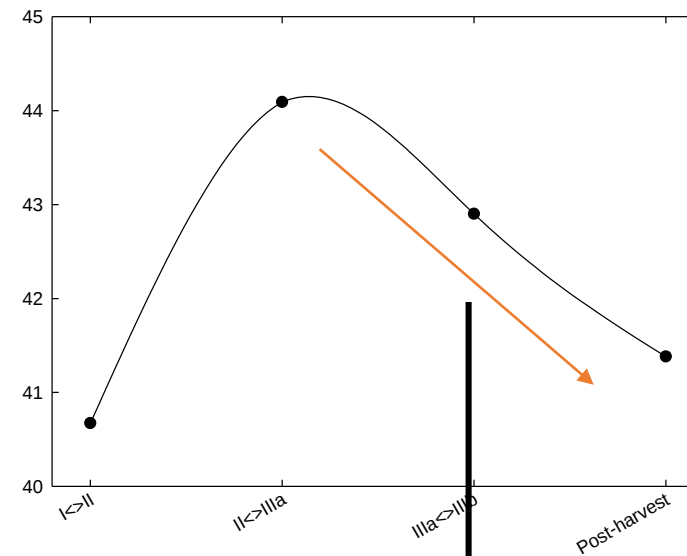
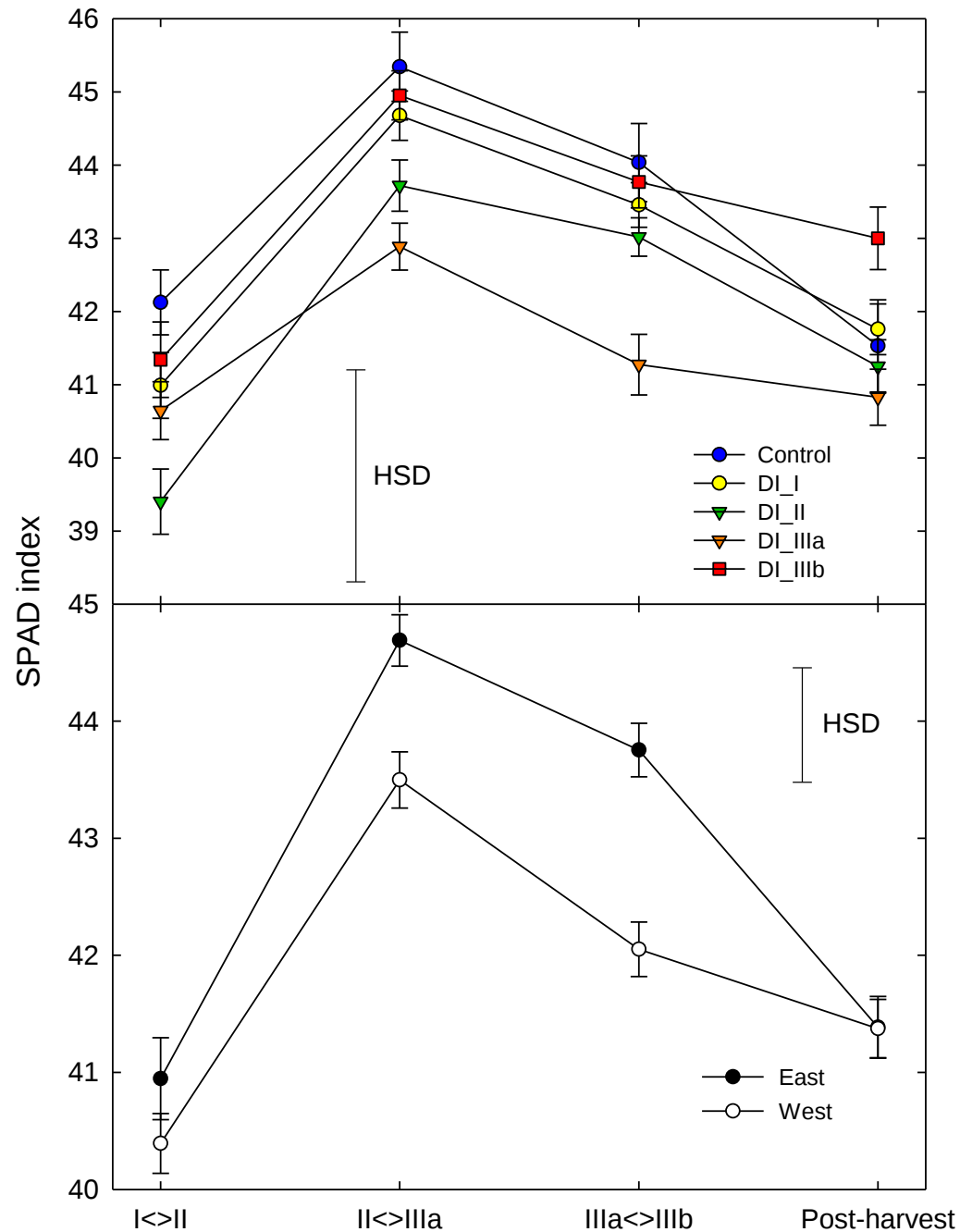
## Daily stem water potential ( $\Psi_{\text{stem}}$ ) curve



Error bars represent standard errors.

N of replicates for treatment = 6

## SPAD index (i.e. leaf chlorophyll content)



### ANOVA:

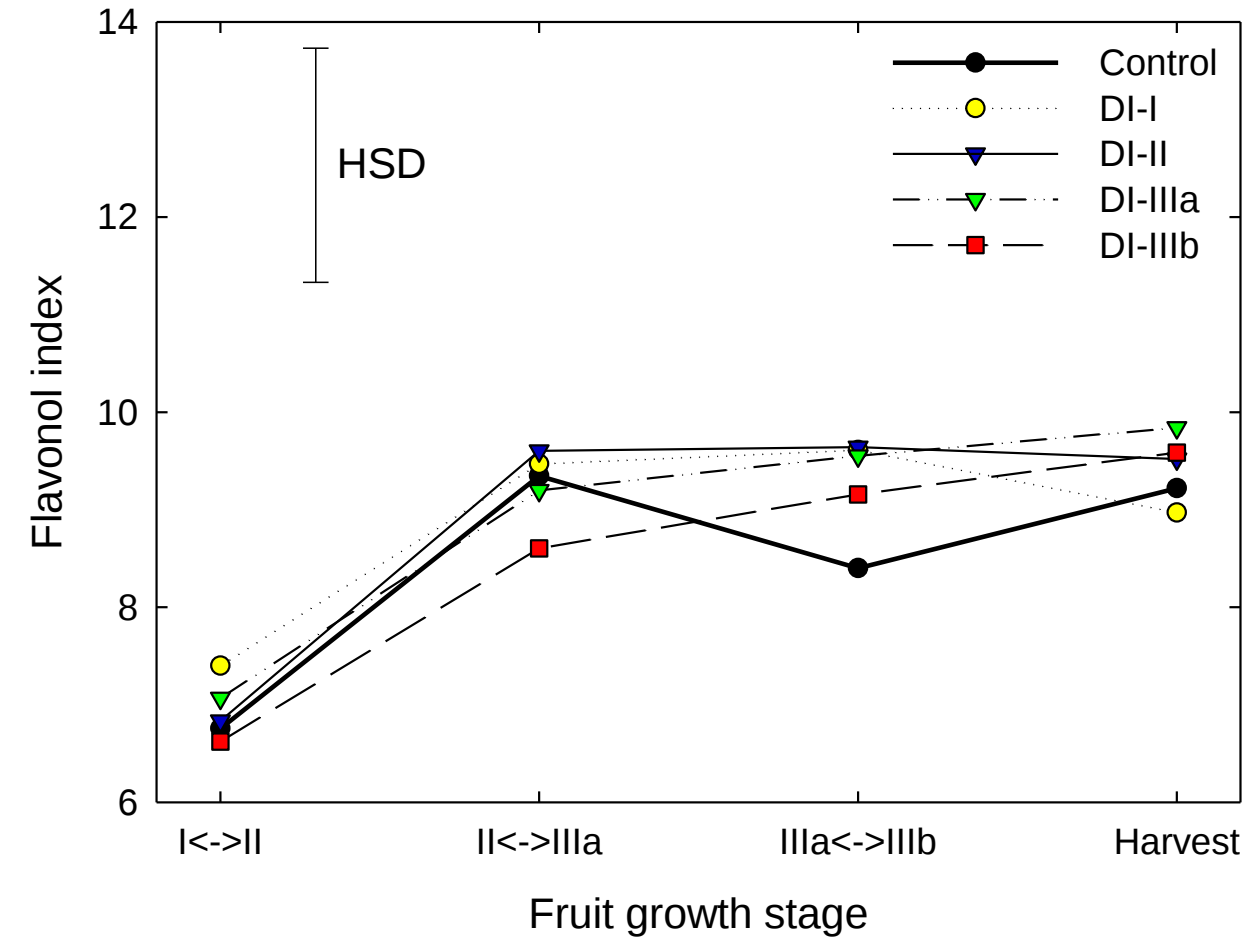
- Irrigation →  $p < 0.001$
- Growth stage →  $p < 0.001$
- Canopy Orientation →  $p < 0.001$
- Irr Treat \* Growth Stage → ns
- Canopy orientation \* growth stage →  $p < 0.05$

Error bars represent standard errors.



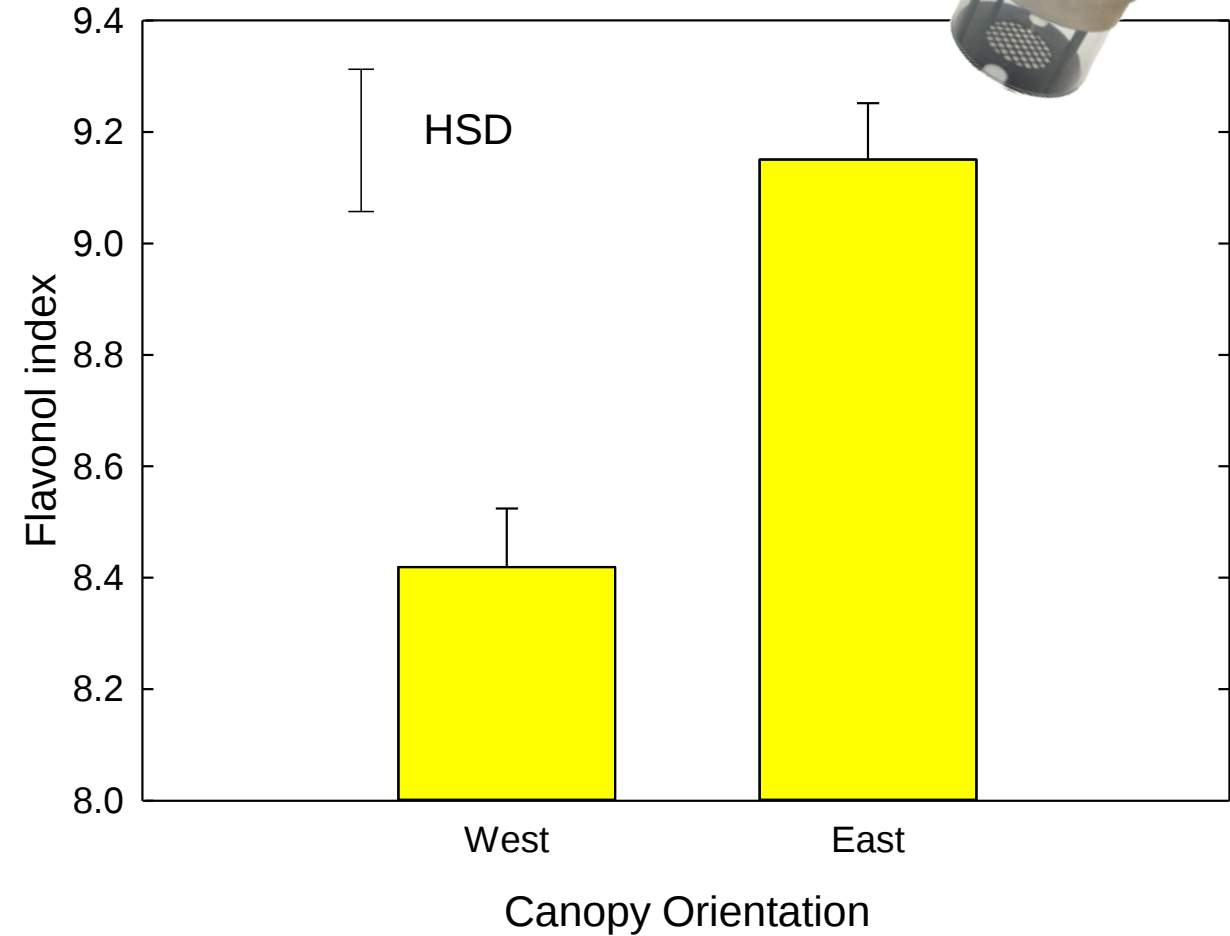
Chlorophyll likely to be converted into fruit secondary metabolites (i.e. anthocyanins, flavonols, etc)

# Fluorometer Multiplex 3 Force A on leaves → flavonol index



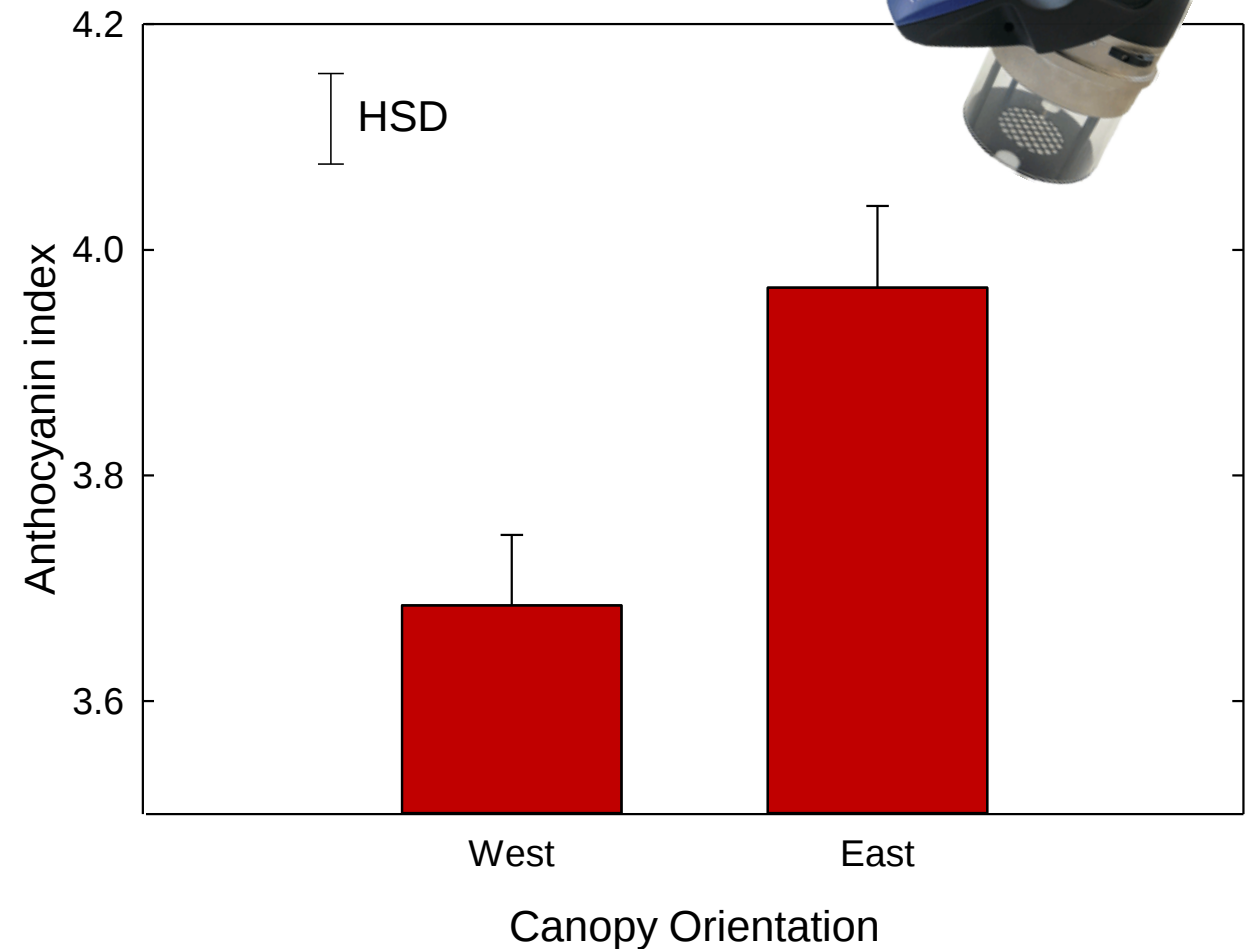
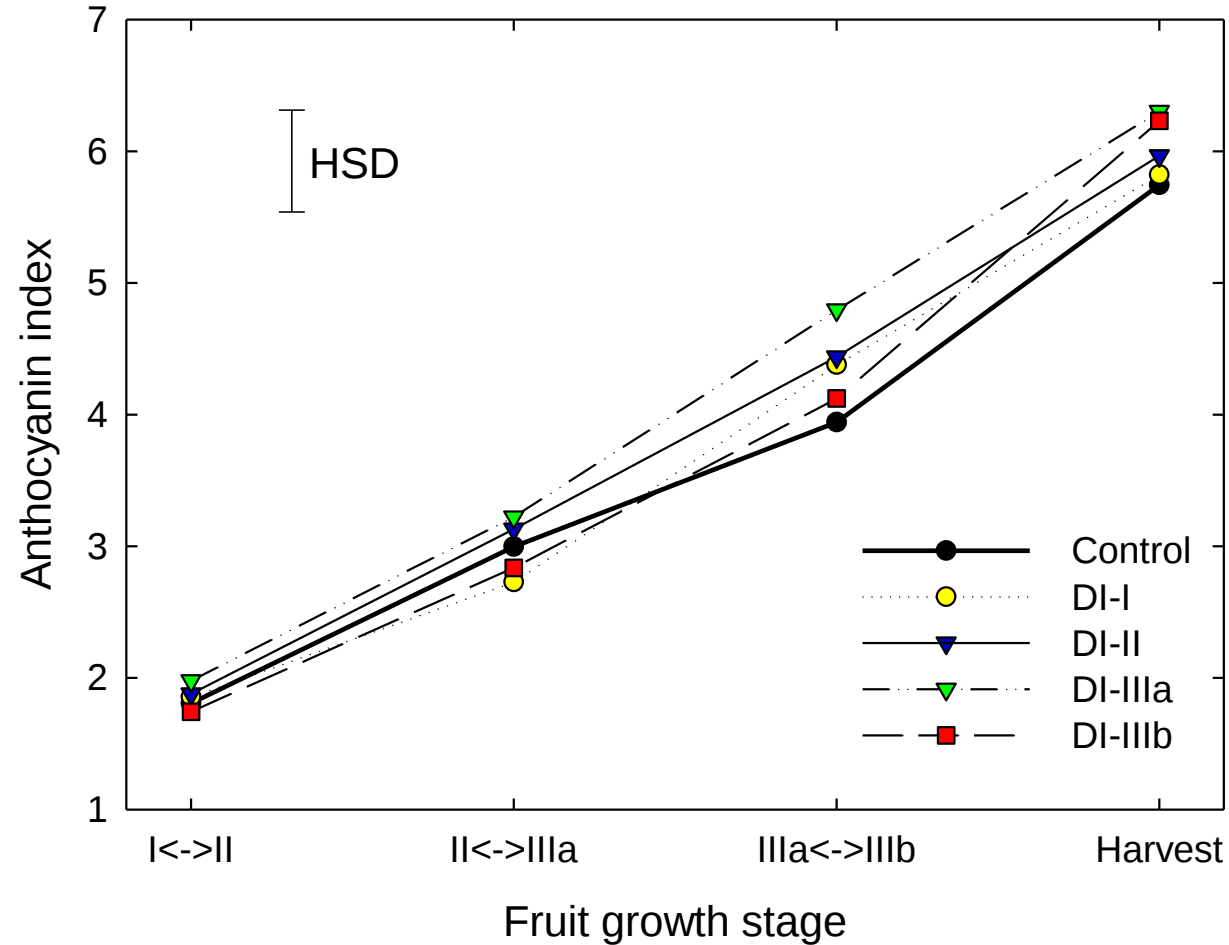
## ANOVA:

- Irrigation Treatment →  $p < 0.001$
- Growth stage →  $p < 0.001$
- Irr Treat \* Growth Stage →  $p < 0.01$



- Canopy orientation →  $p < 0.001$
- Error bars represent standard errors.

# Fluorometer Multiplex 3 Force A on leaves → anthocyanin index

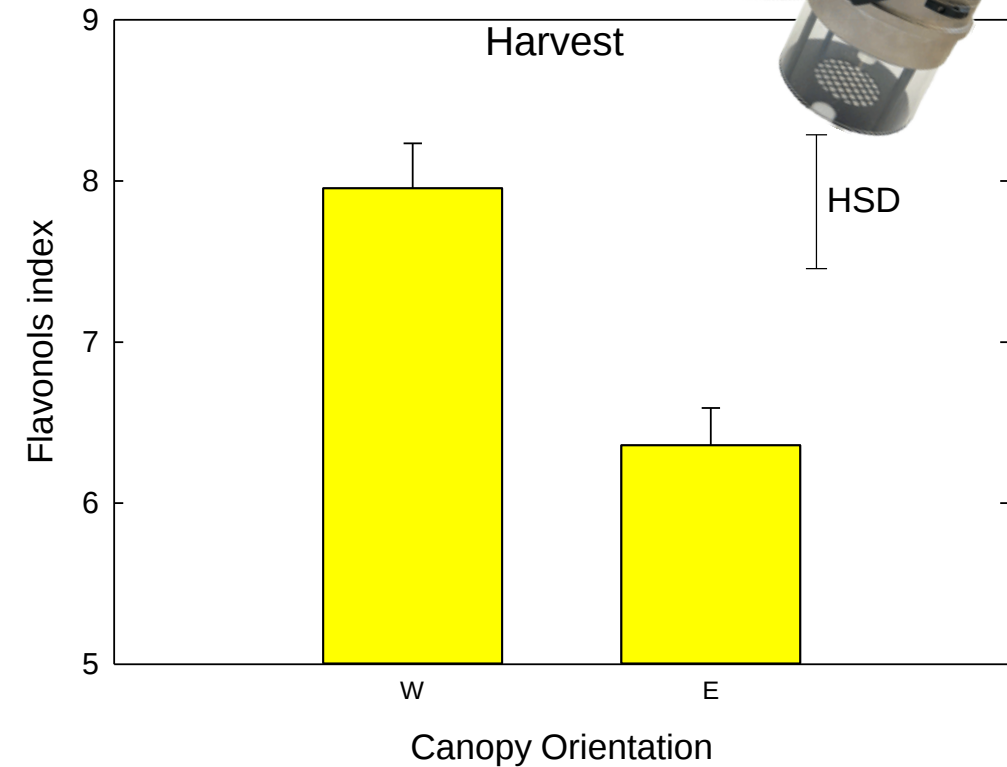
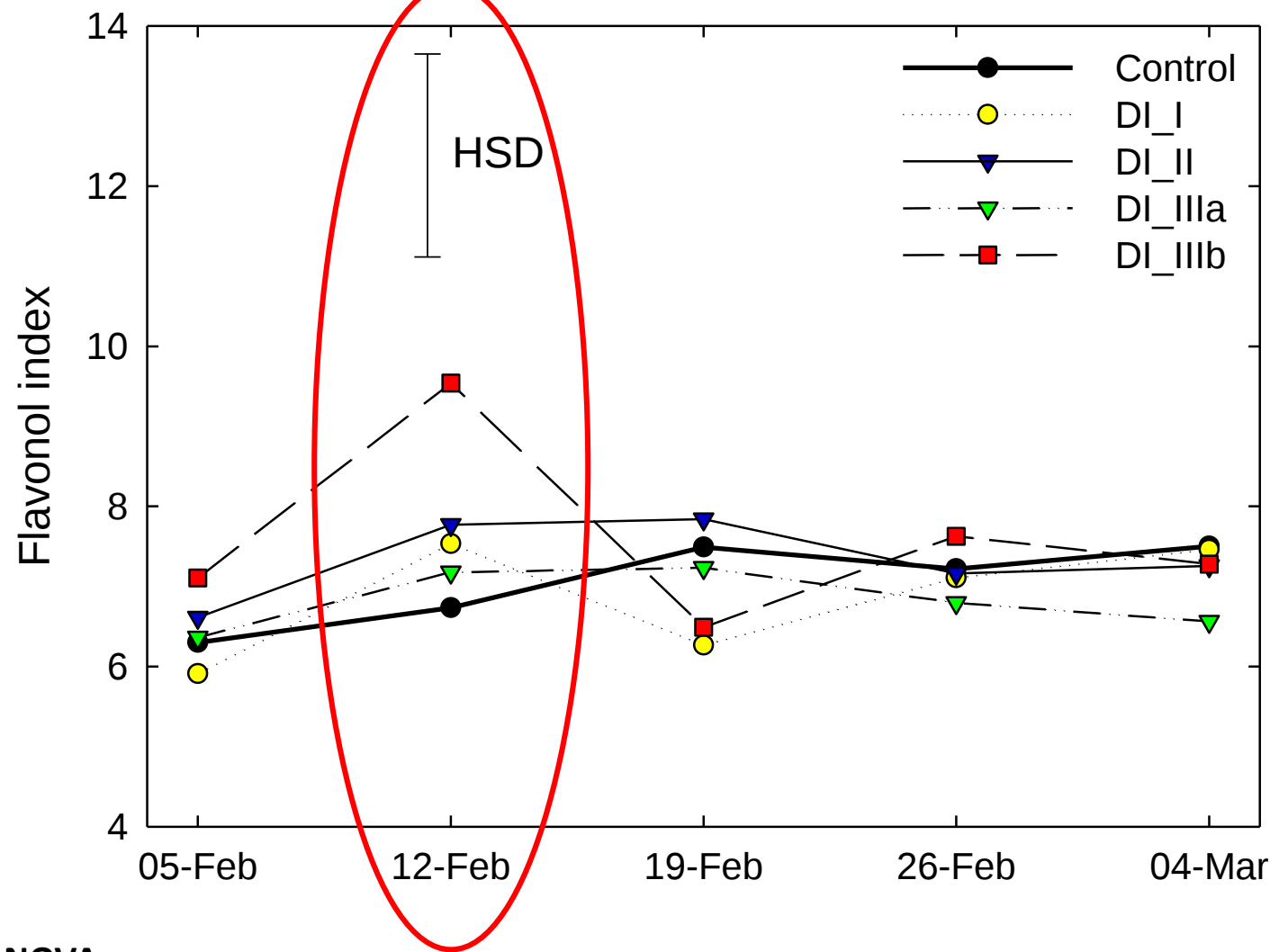


## ANOVA:

- Irrigation Treatment →  $p < 0.001$
- Growth stage →  $p < 0.001$
- Irr Treat \* Growth Stage →  $p < 0.001$

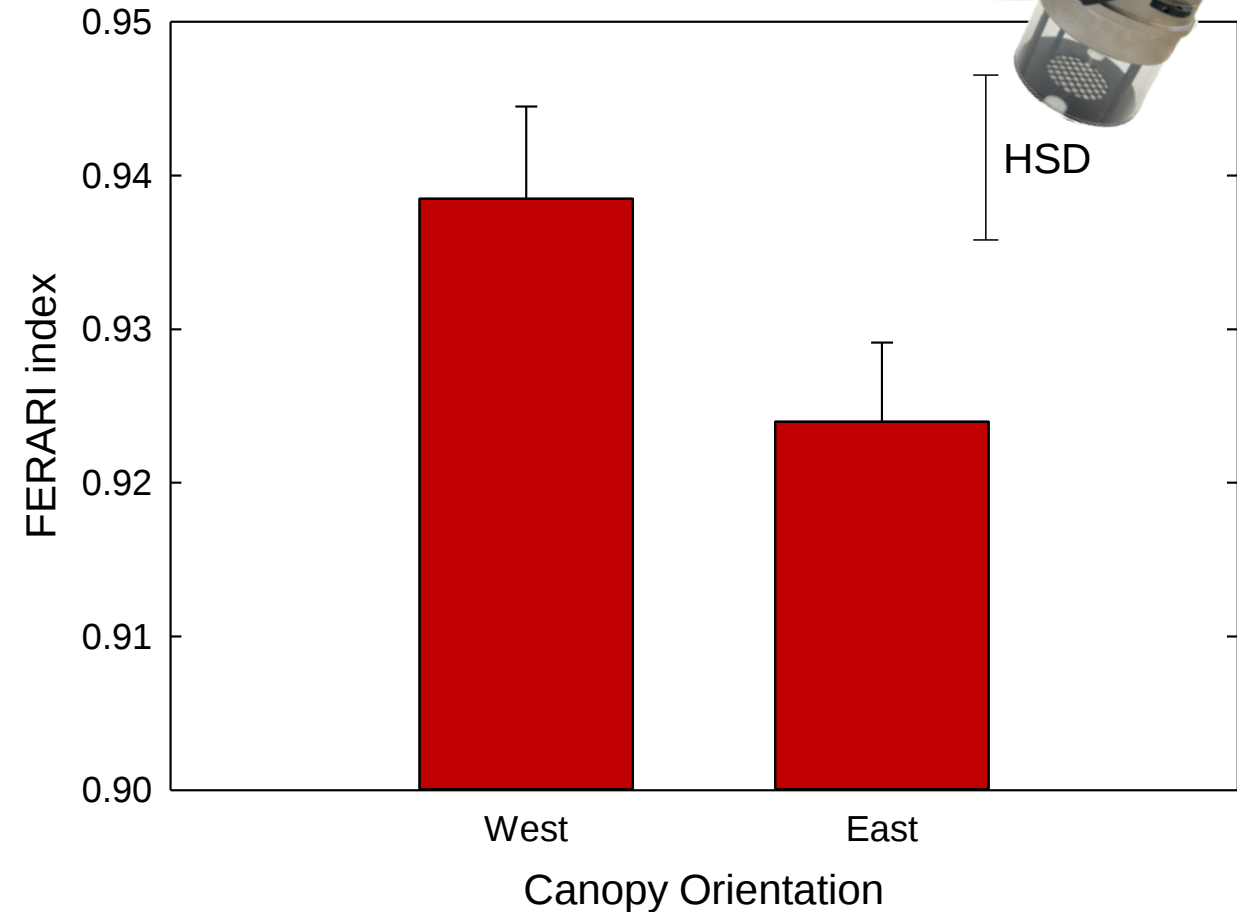
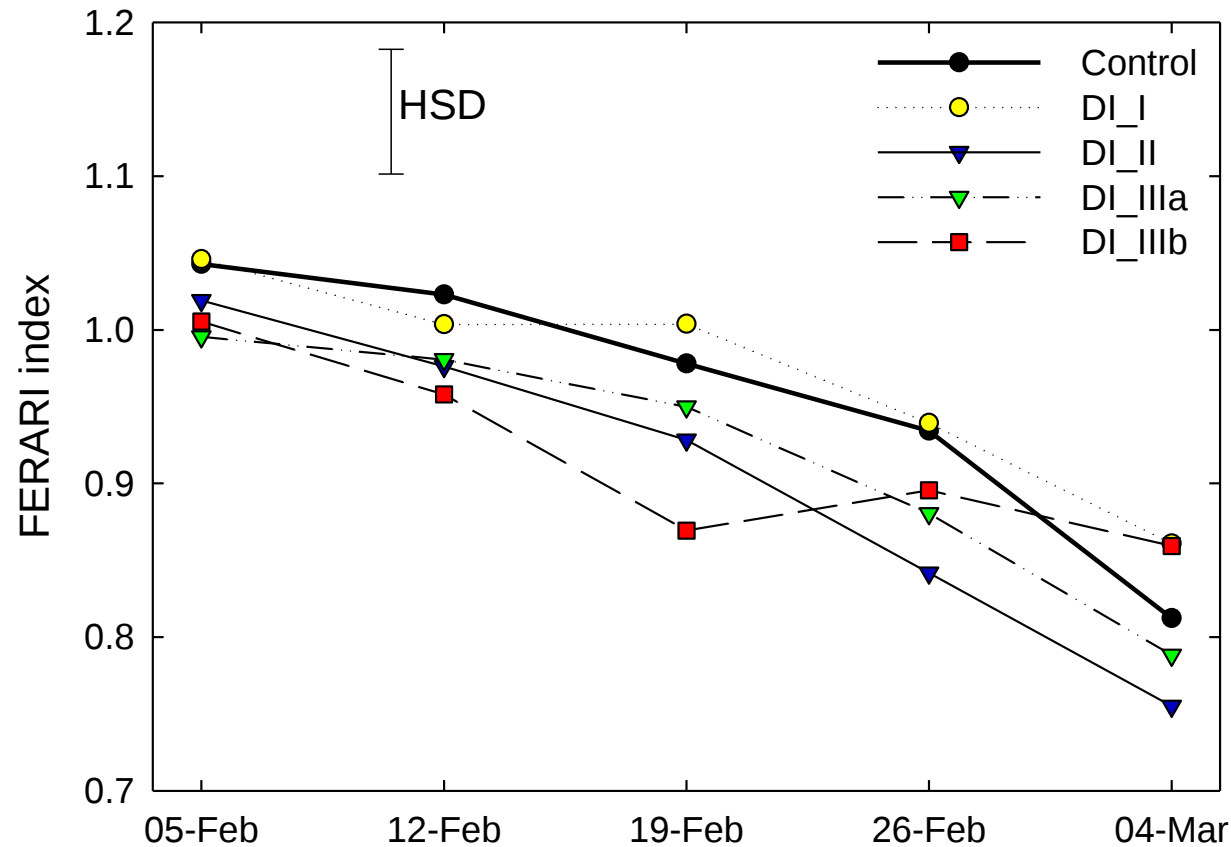
- Canopy orientation →  $p < 0.001$
- Error bars represent standard errors.

# Multiplex 3 Force A fluorometer on fruit → Flavonol index



- ANOVA:
- Merged Irrigation Treatment (by fruit growth stage) →  $p < 0.01$
  - Date →  $p < 0.001$
  - Irrigation Treatment \* Date →  $p < 0.05$
  - Canopy orientation →  $p < 0.001$

# Fluorometer Multiplex 3 Force A on fruit → fluorescence excitation ratio anthocyanin relative index (FERARI)



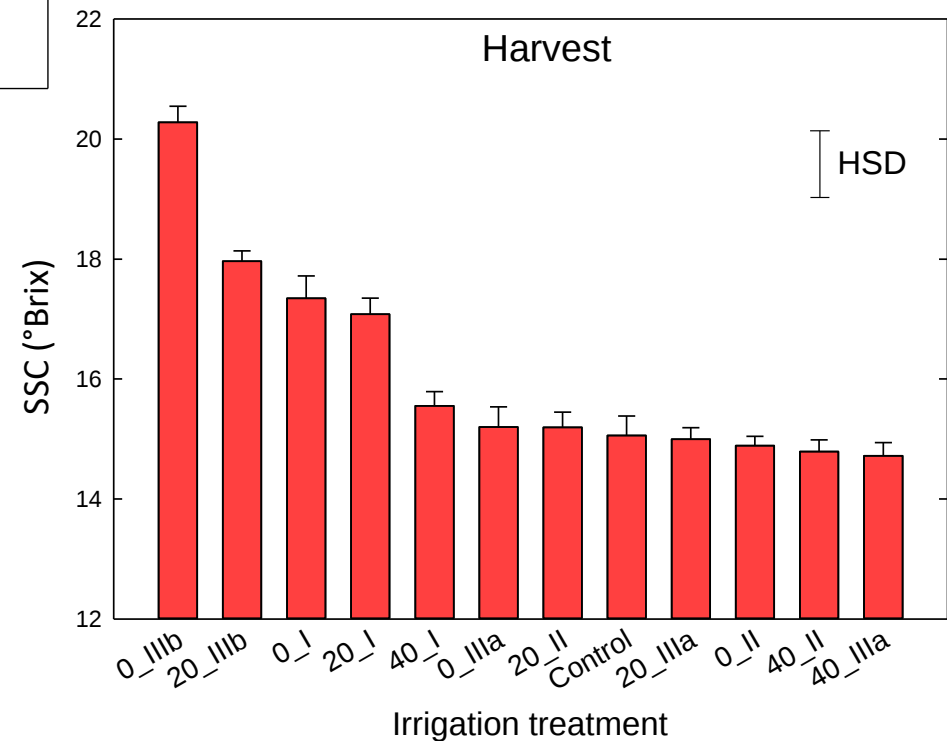
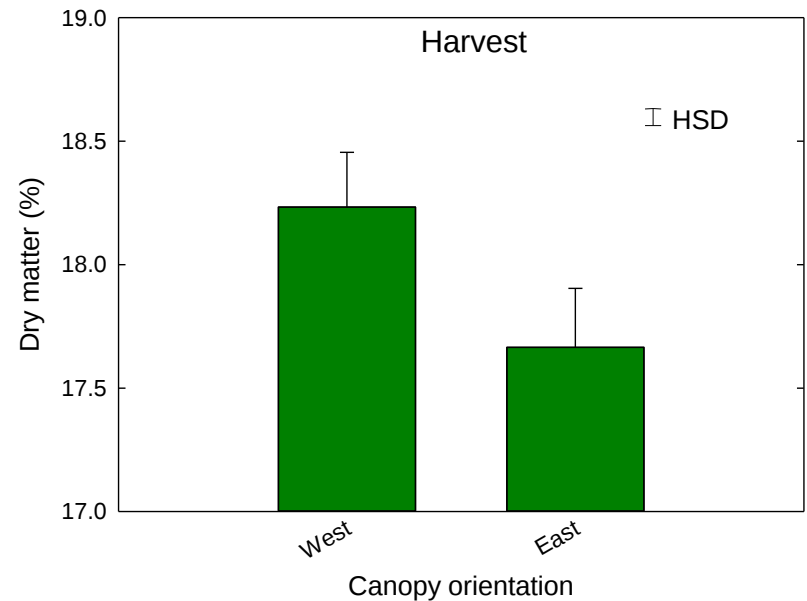
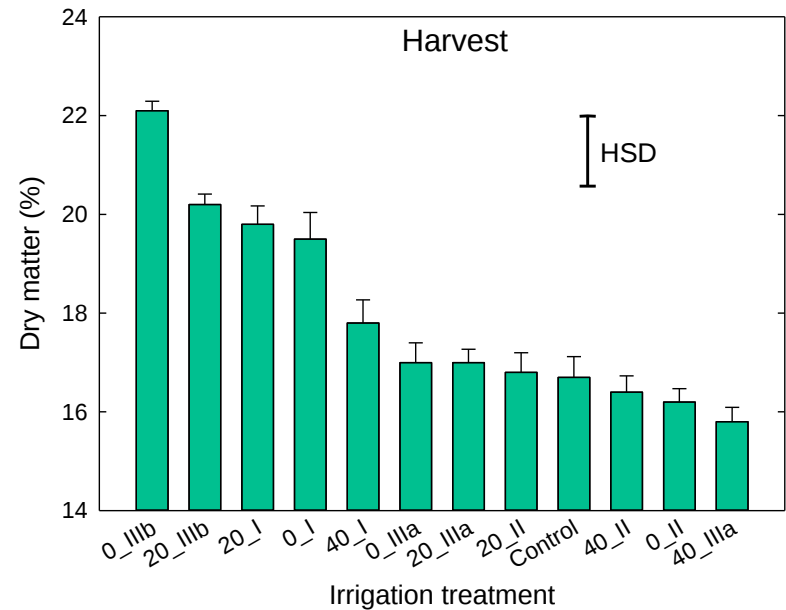
## ANOVA:

- Date →  $p < 0.001$
- Irrigation Treatment →  $p < 0.001$
- Canopy orientation →  $p < 0.001$
- Irrigation Treatment \* Date →  $p < 0.001$

- Canopy orientation →  $p < 0.001$

Despite at harvest no significant differences

# Felix F-750 → Dry matter and SSC



Error bars represent standard errors.

# Fruit growth and leaf turgor pressure indices

## FRUIT

Fruit gauge output is a signal in mV

### 1. Fruit diameter z-scores:

$$z = (x - \mu) / \sigma$$

### 2. Fruit relative growth rate (RGR, $\mu\text{m mm}^{-1} \text{min}^{-1}$ ):

$$\text{RGR} = (\ln \text{Diameter}_2 - \ln \text{Diameter}_1) / (t_2 - t_1)$$

## LEAVES

LPCP probe output is an inverted value of leaf turgor pressure ( $p_p$ )

### 1. $p_p$ z-scores:

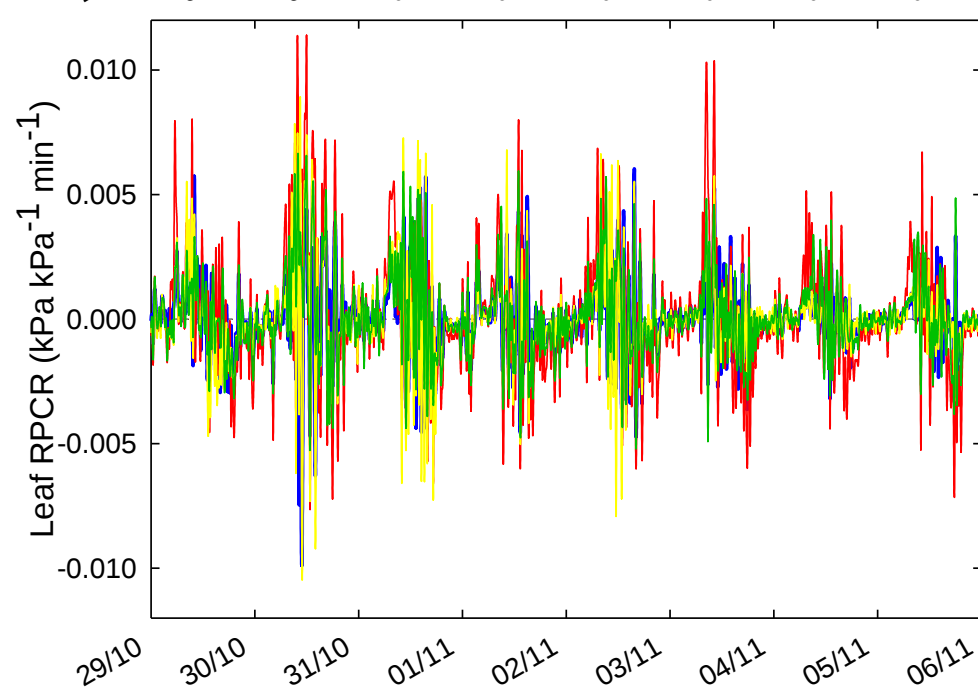
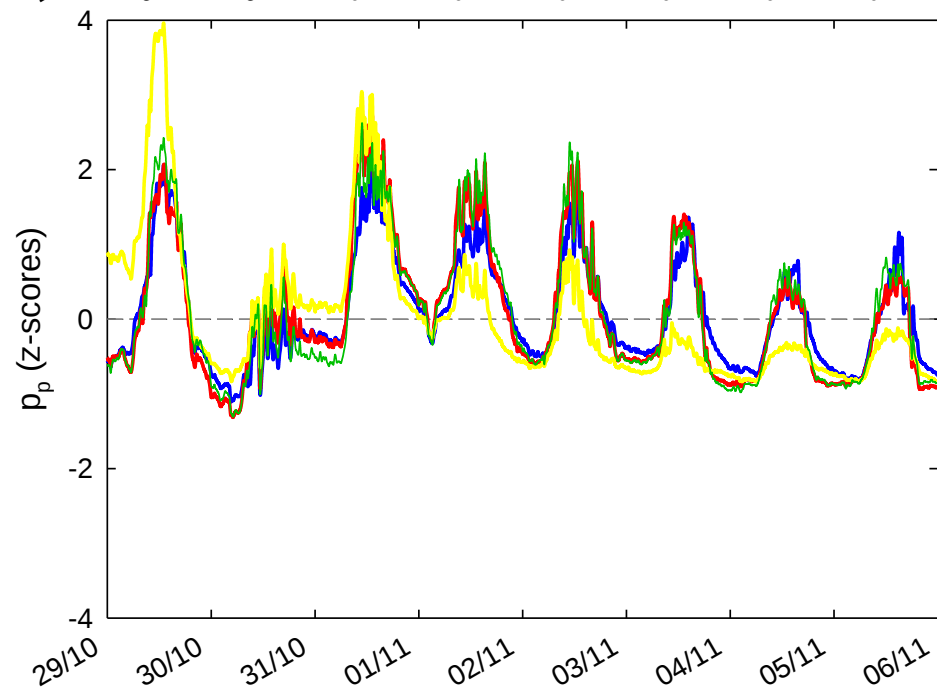
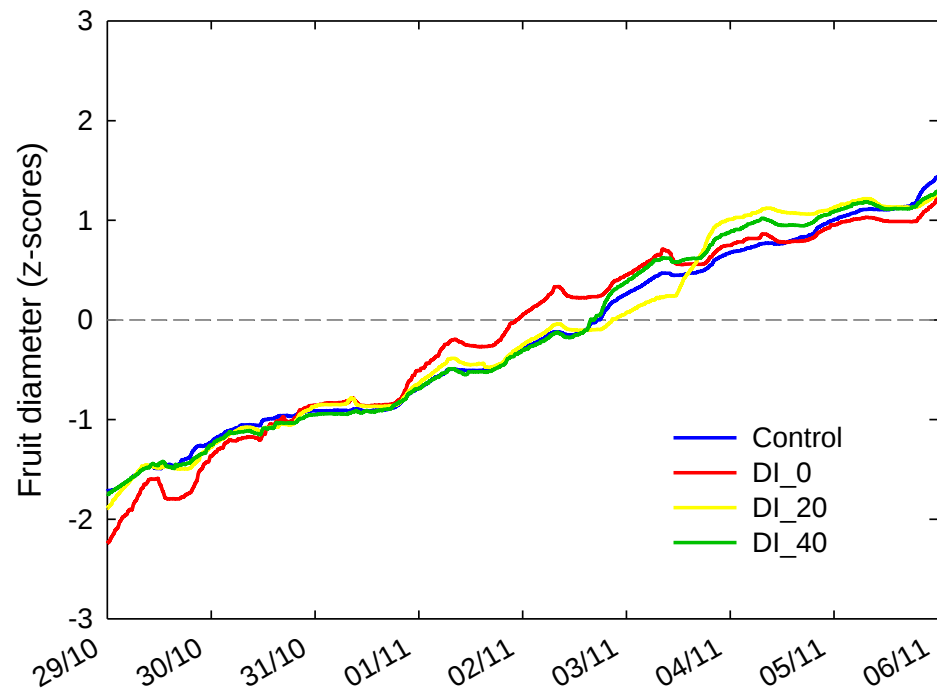
$$z = (x - \mu) / \sigma$$

### 2. Leaf relative pressure change rate (RPCR, $\text{kPa kPa}^{-1} \text{min}^{-1}$ )

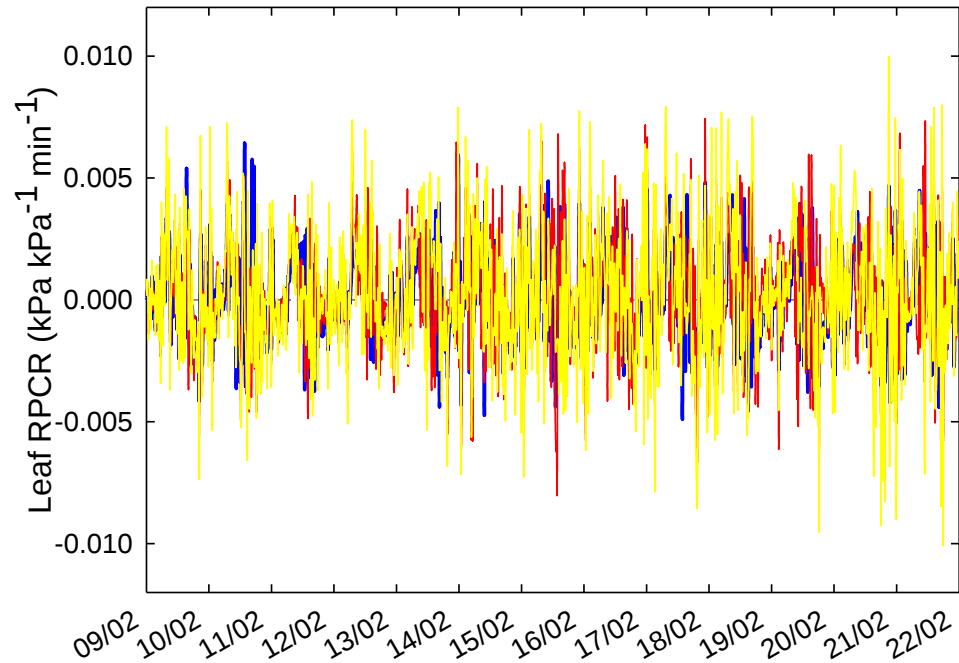
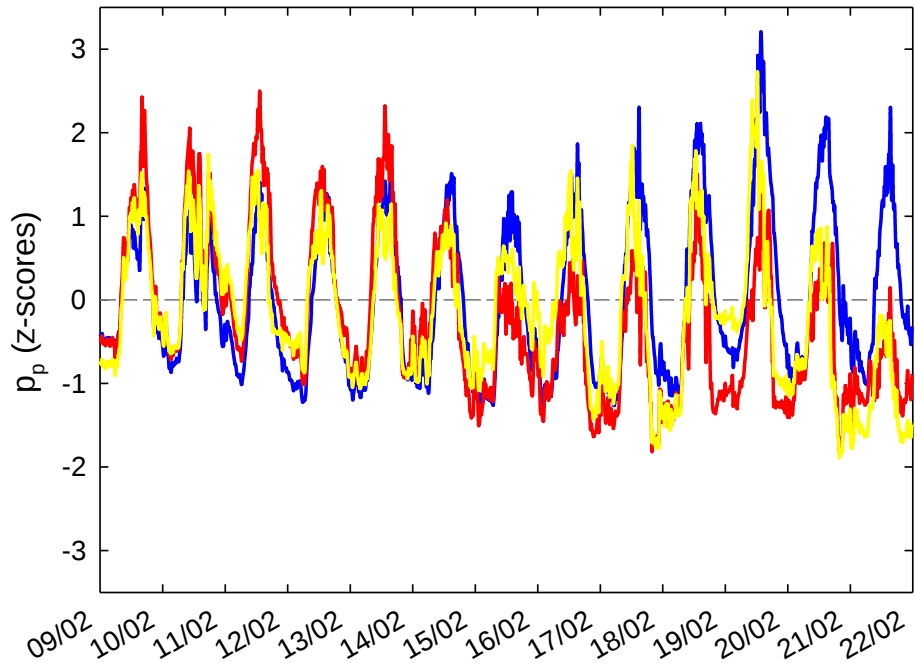
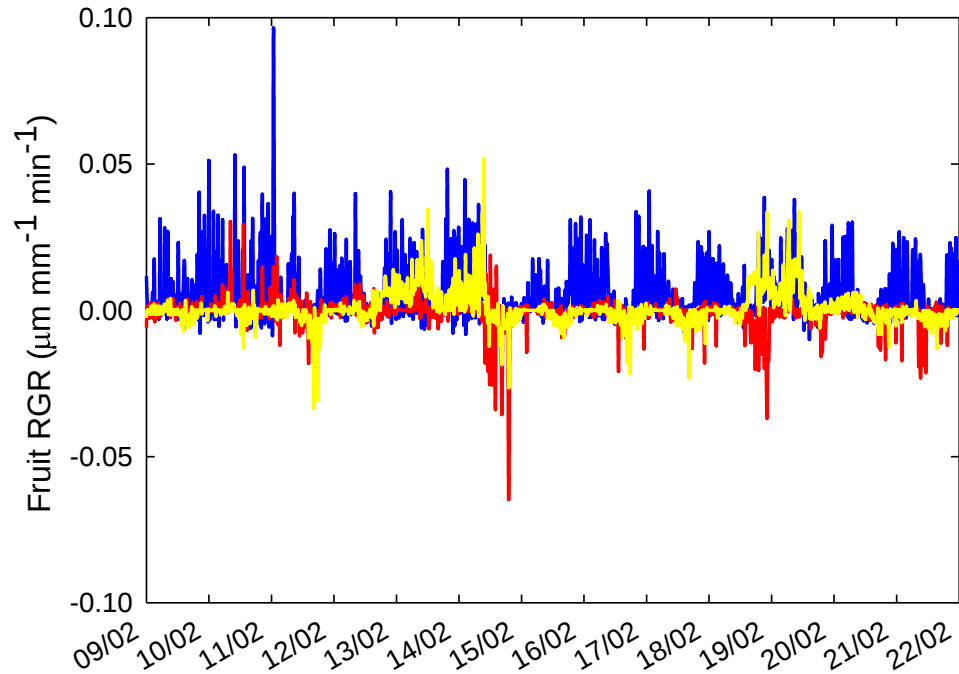
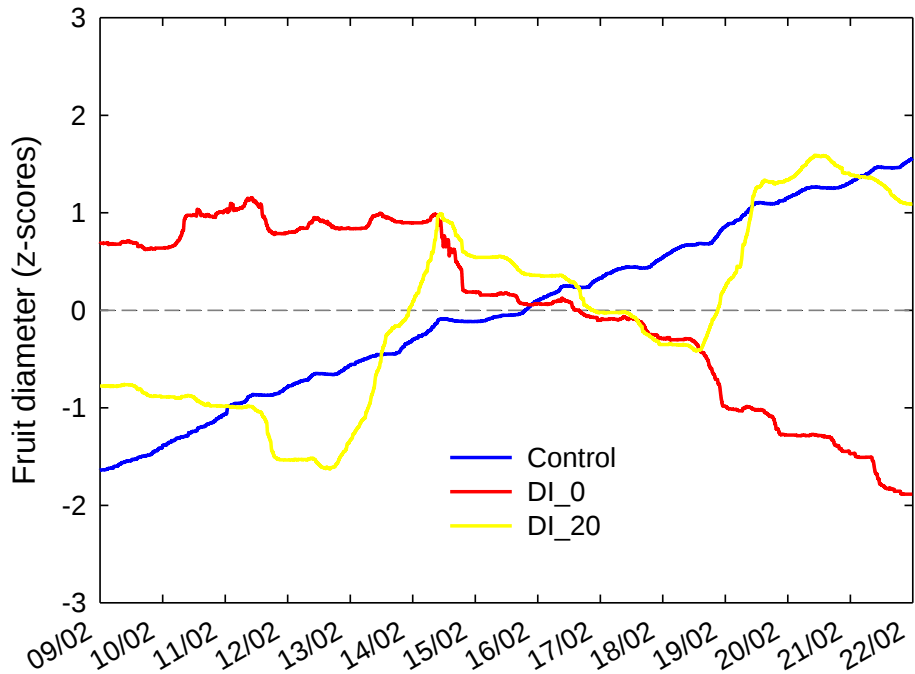
$$\text{RPCR} = (\ln p_{p2} - \ln p_{p1}) / (t_2 - t_1)$$

**RGR and RPCR are 2<sup>nd</sup> derivatives of fruit diameter and  $p_p$ , respectively.**

## Stage I

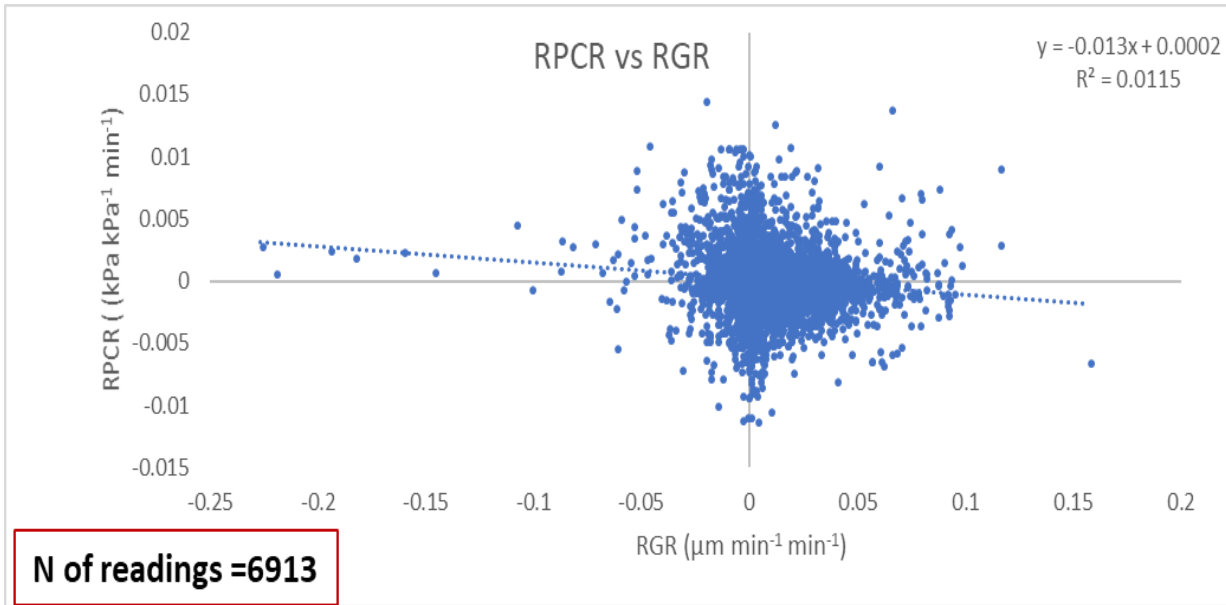


# Stage IIIb

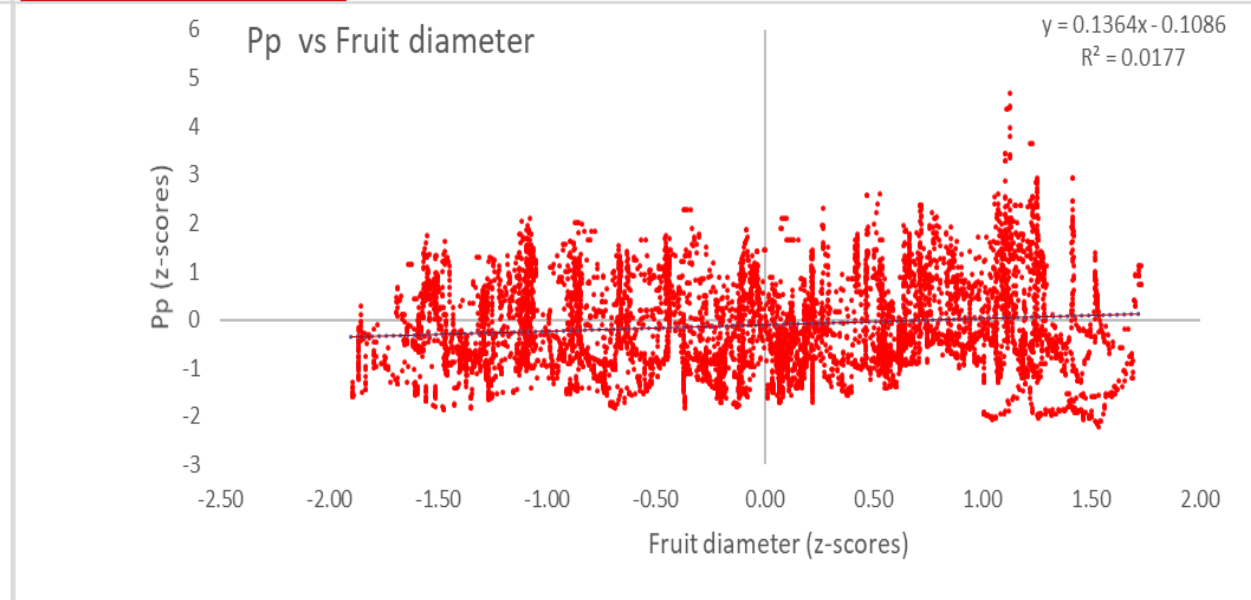
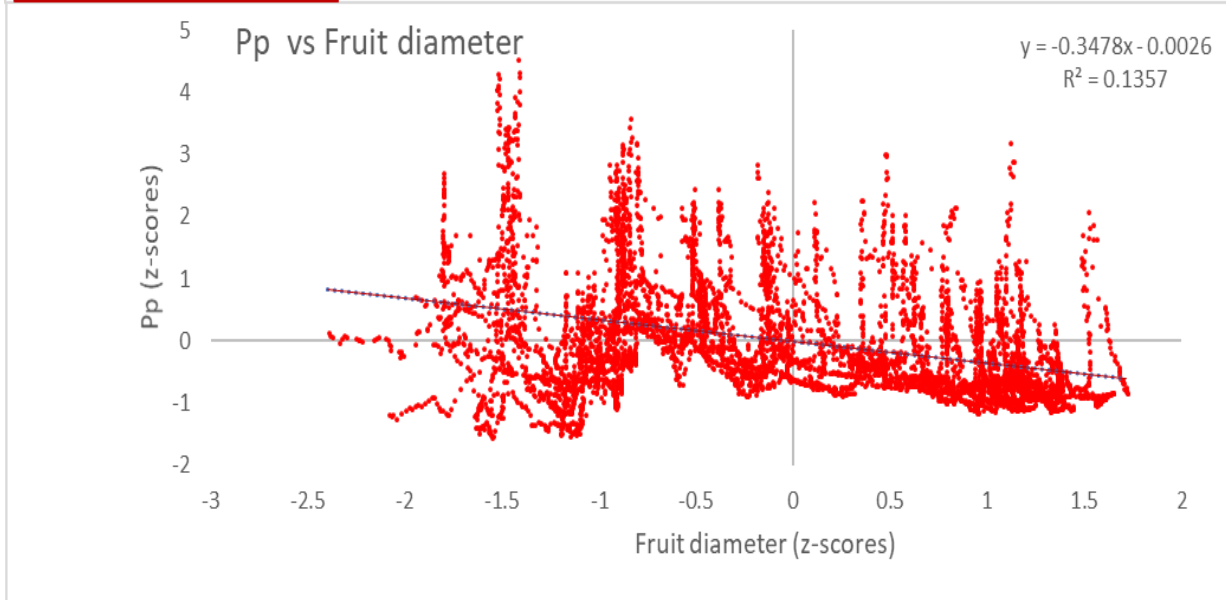
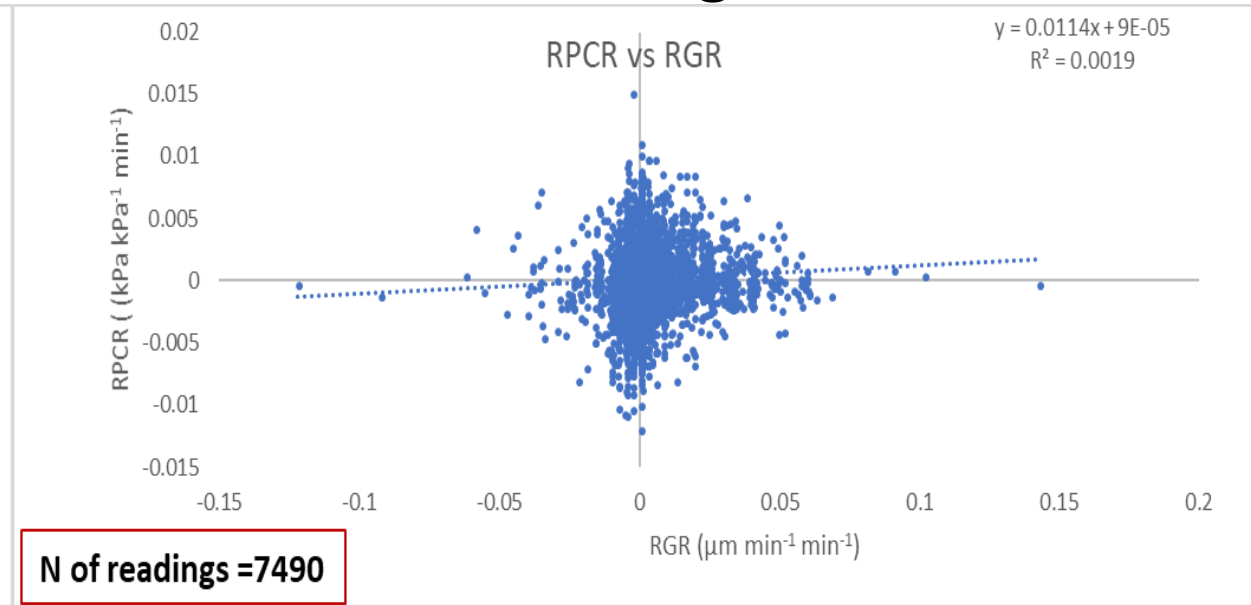


# Large regressions (sensor outputs)

## Stage I

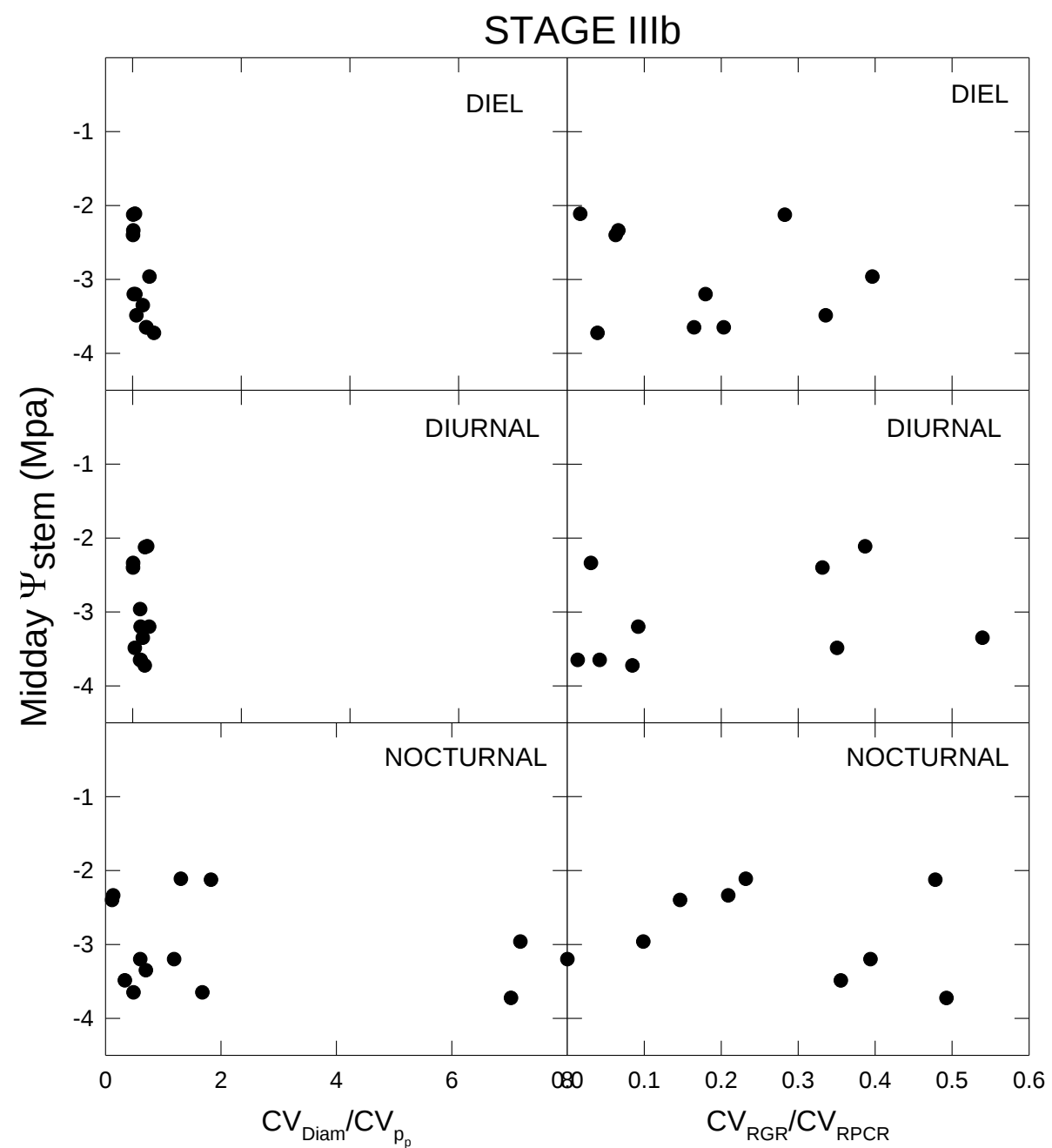
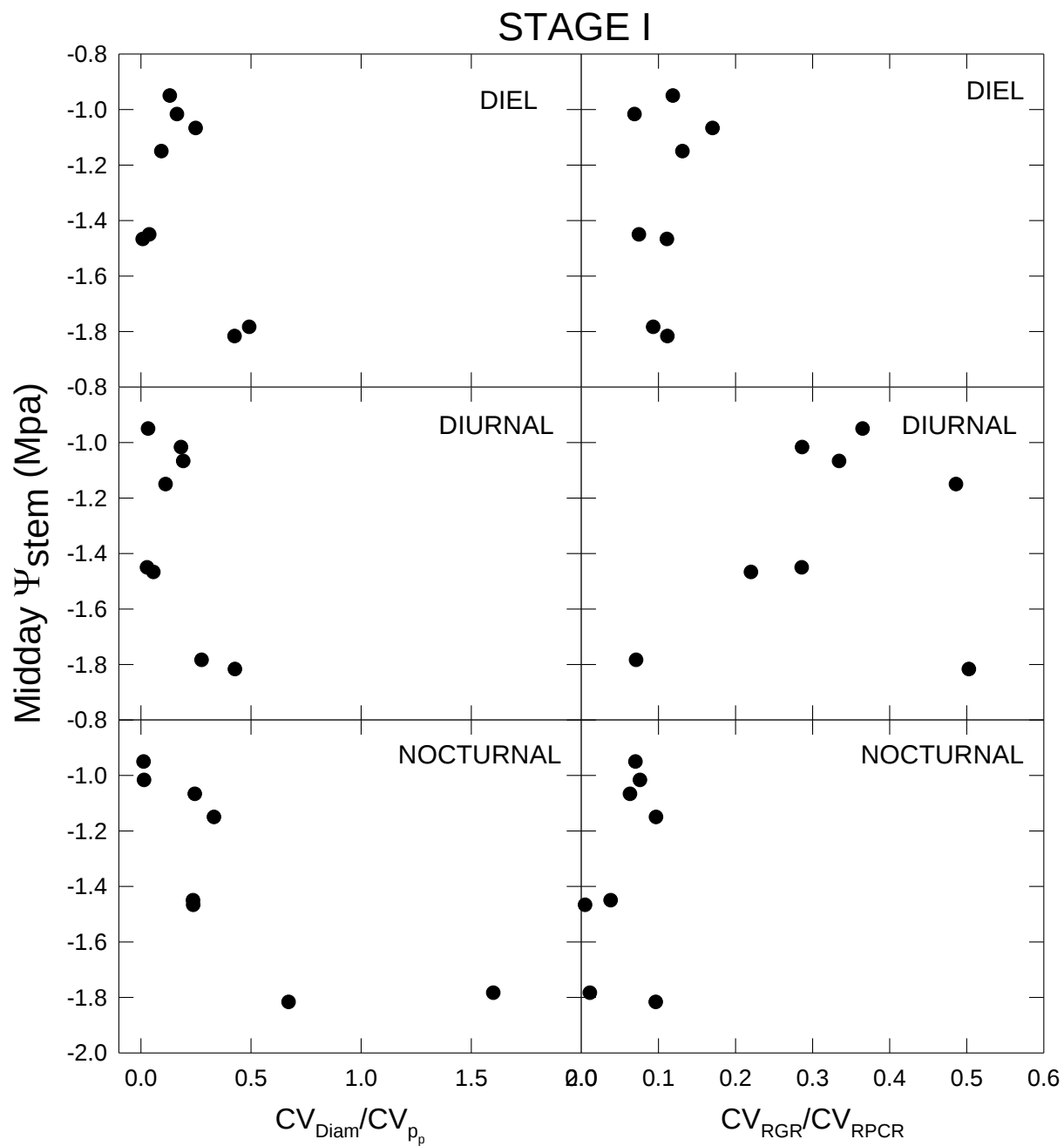


## Stage IIIb



Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{Diam}}/CV_{\text{pp}}$

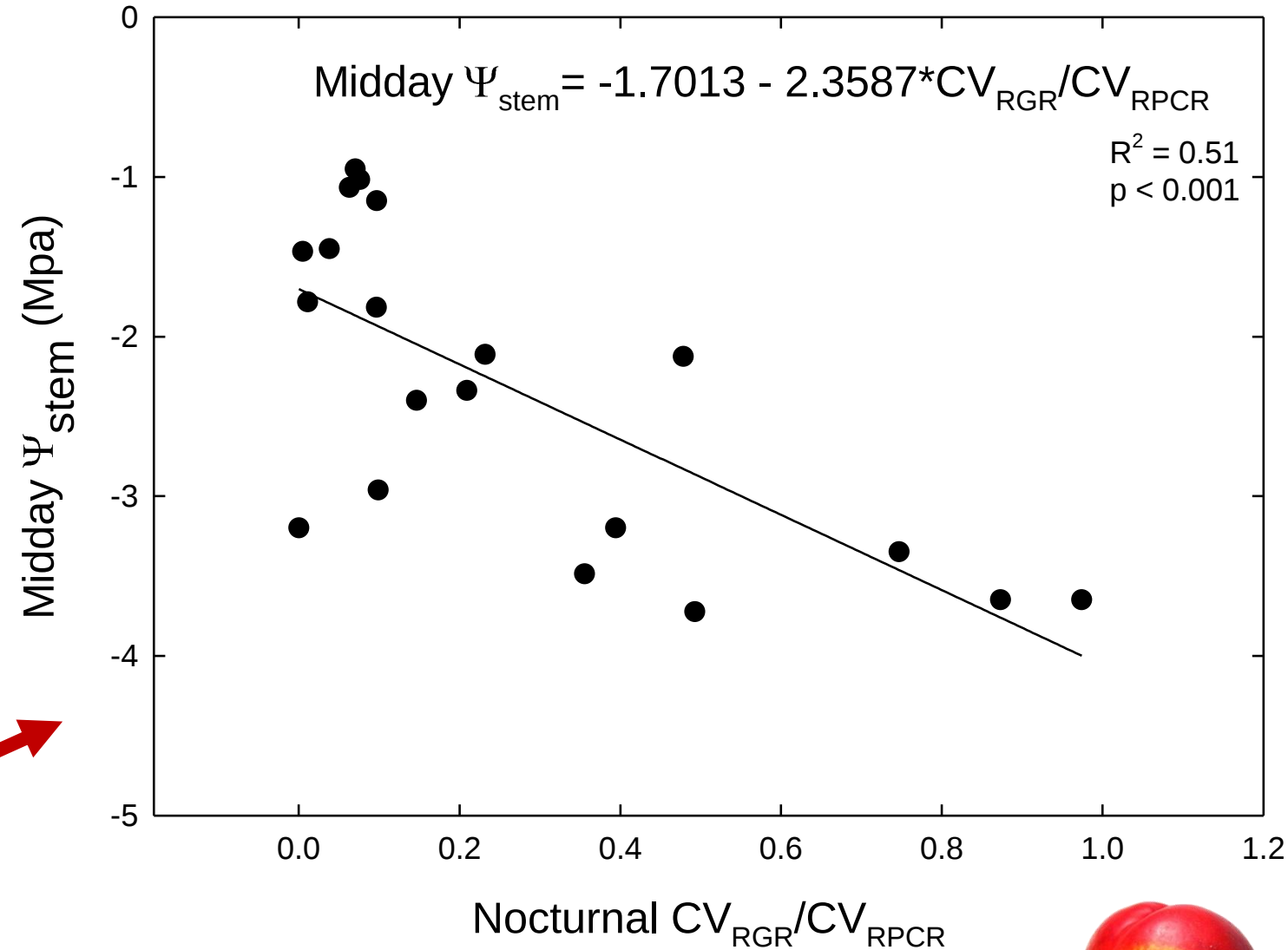
& Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{RGR}}/CV_{\text{RPCR}}$



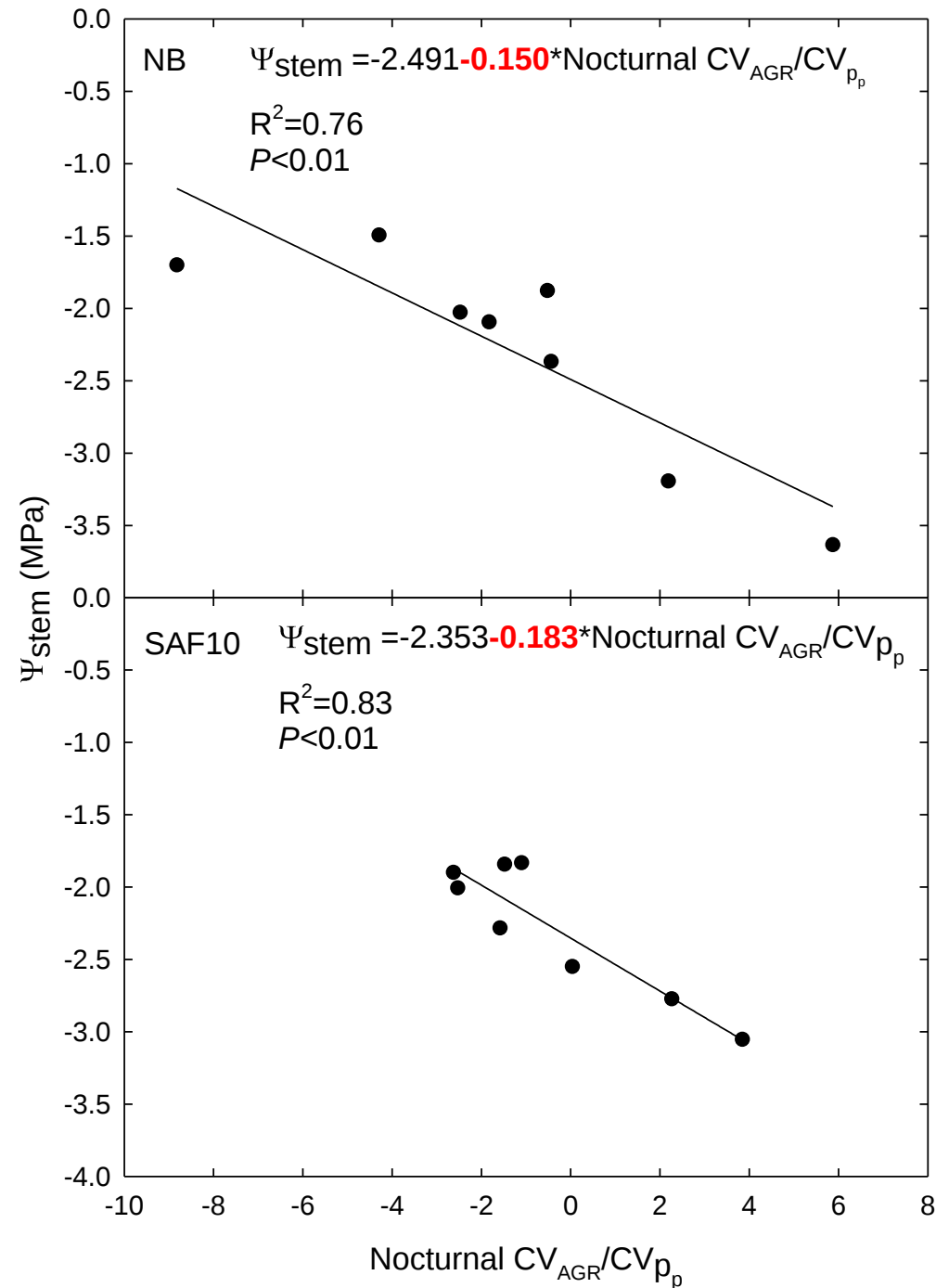
# Stage I and IIIb merged

Several regressions tested:

- Diel
  - Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{Diam}}/CV_{\text{pp}}$
  - Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{RGR}}/CV_{\text{RPCR}}$
- Diurnal
  - Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{Diam}}/CV_{\text{pp}}$
  - Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{RGR}}/CV_{\text{RPCR}}$
- Nocturnal
  - Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{Diam}}/CV_{\text{pp}}$
  - Midday  $\Psi_{\text{stem}}$  Vs  $CV_{\text{RGR}}/CV_{\text{RPCR}}$



# What's going on in olive?



t-test show no significant differences between the slope of the two regressions, implying a genotype-independent relationship

# Conclusions

- A combined index of fruit growth and leaf turgor pressure is a more powerful tool for continuous  $\Psi_{\text{stem}}$  estimation, compared to considering them individually.
- An integrated approach for continuous monitoring of water status in plant organs (e.g. fruit, leaves, trunk, etc.) can be used for remote, automated irrigation management when trees reach well defined  $\Psi_{\text{stem}}$  thresholds (e.g. via I/O Arduino board).
- Fruit quality parameters may be determined by non-destructive field measurements by NIR spectroscopy and fluorescence spectroscopy. These technologies might potentially be automated and installed on trees for regular and real-time determination of desired parameters.

# Acknowledgements

- **Dario and Mark** for supervising me
- **Ian, Lexie, Des, Wendy, Colin, Subhash, Christine, Janine, Maddy, Iris** for scientific and field support
- **James and Andrew** for our fun time (and work) in the orchard
- **Athulya** for being a great hard working student
- **Angela, Cathy, Melly, Andy, Bruce, Megan and Aimee** for support and coffee breaks
- All the other people working at the Tatura Research centre  
&  
my family in Italy...

**GRAZIE!**

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## **Continuous determination of fruit tree water-status by plant-based sensors**

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