The ABSTRESS project - Work Package 1 : Define and establish the experimental conditions for investigating plant response to drought and Fusarium stress

Christophe Salon

Work Package #1 : Define and establish the experimental conditions for investigating plant response to drought and Fusarium stress

INRA, CSIC, FERA, ARTERA

Christophe Salon, Carmen Bianco, Adrian Charlton, Roberto Defez, Mike Dickinson, Rebecca Iglesias, Christian Jeudy, Tracy Lawson, Jack Mathews, Ulrike Mathesius, Phil Mullineaux, Marion Prudent, Nicolas Raspail, Diego Rubiales.

Objectives

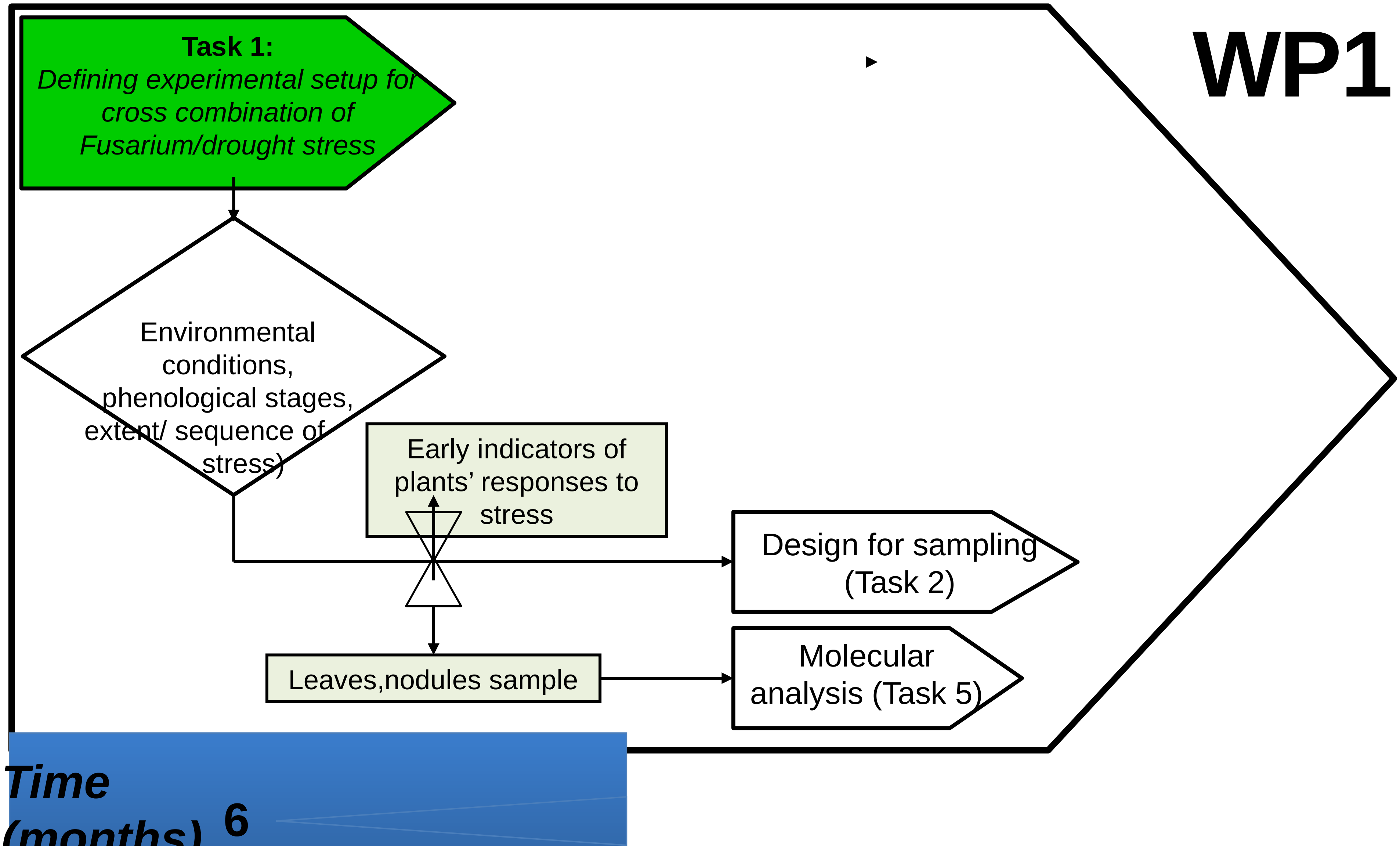
- **Protocols** for investigating resistance mechanisms to drought and Fusarium,
- **Produce material** for the consortium,
- **Characterize** plant/pathogen interactions under drought stress and compare with optimum environmental conditions in a factorial design,
- **Give guidance** to identified plant phenotypic traits for selecting genotypes having enhanced resistance to drought and Fusarium.

Tasks

Task No.	Activities	Inst. Responsible for Task	Person(s) Responsible for Task
T1.1	Defining experimental setup for cross combination of *Fusarium*/drought stress	INRA/CSIC	

Description of the work

M. Prudent and C. Salon, INRA + CSIC/E

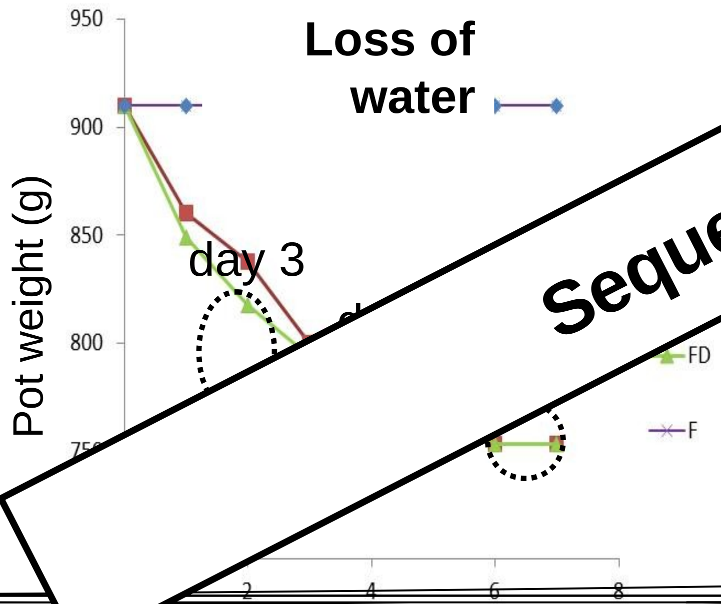


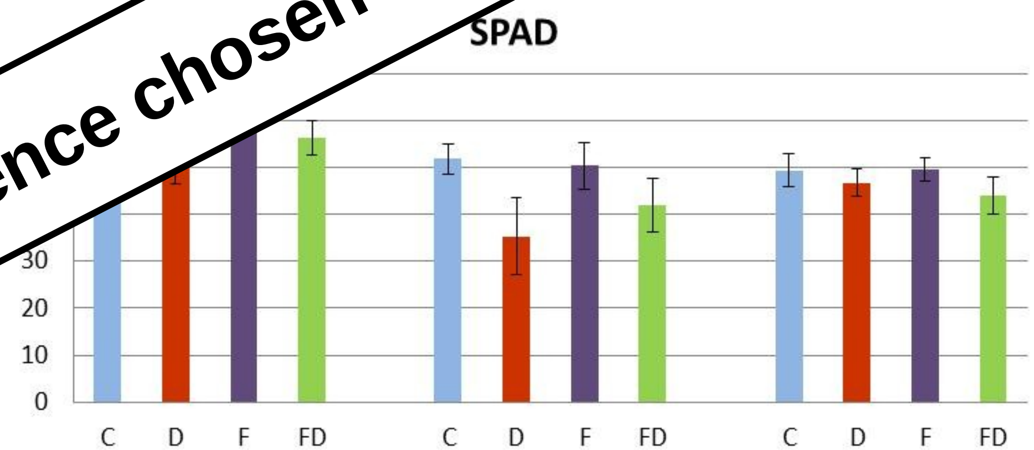
Set up: PEA example

Pilot on Cameor

Sowing → 8 d → Fusarium inoculation (~ 2.5 leaves) → 3 d → Water stress imposition (~ 4 leaves) → 15 d

Sequence chosen

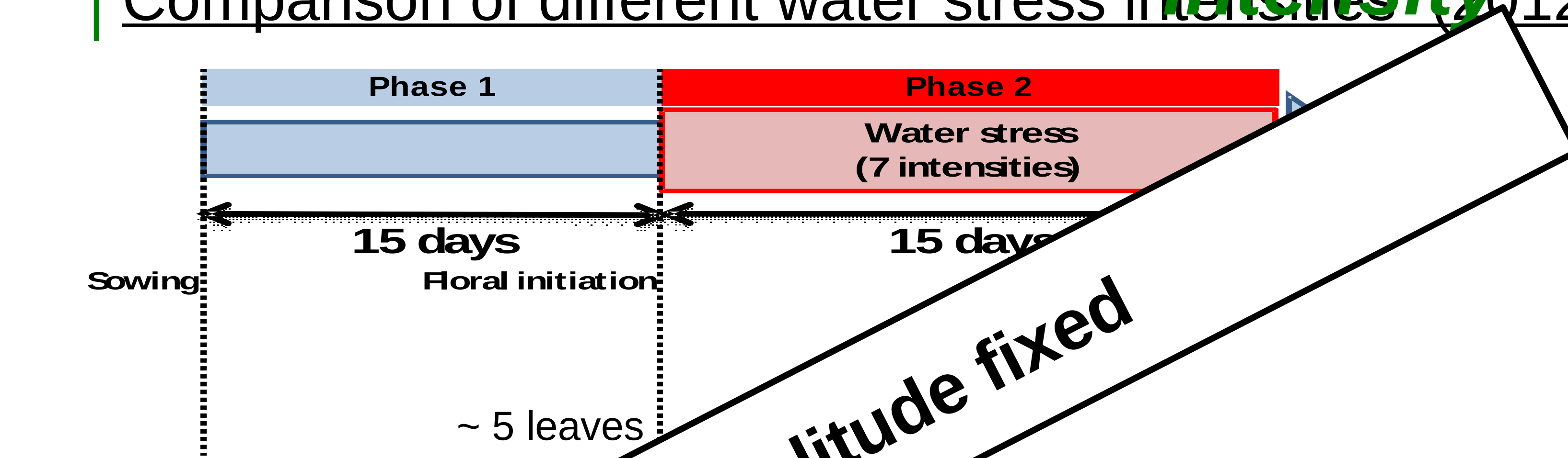


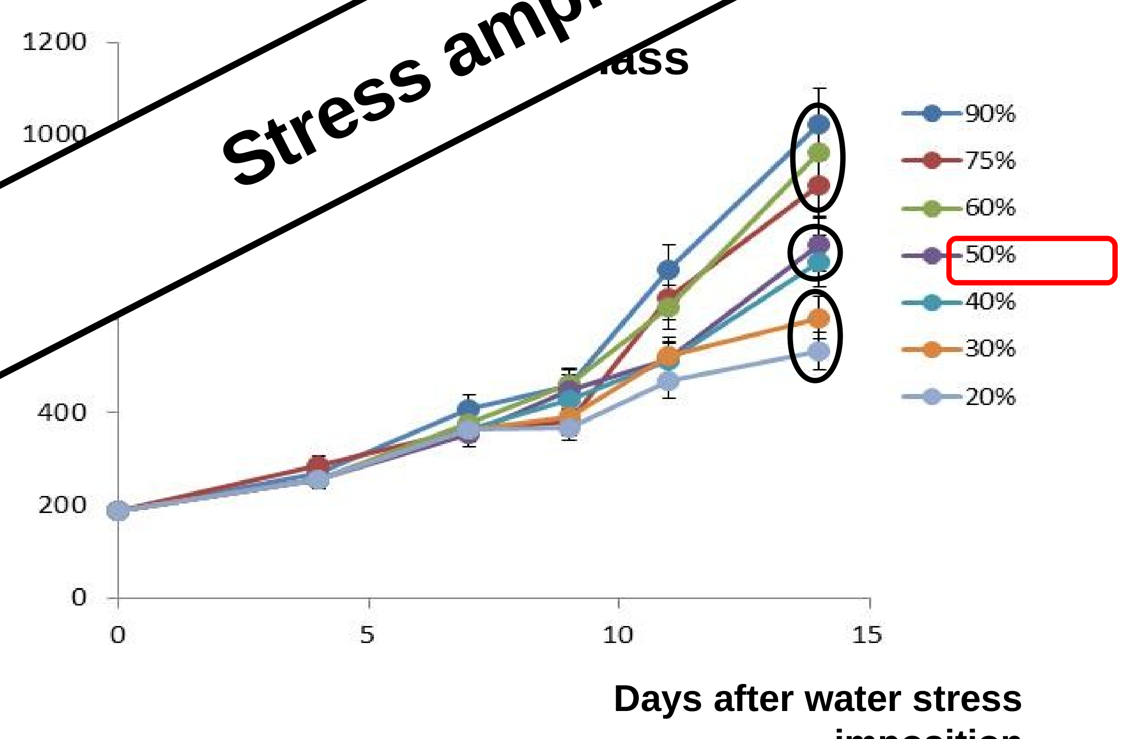


+ 3 volumes of Fusarium inoculation tested: 10, 20, 30, 50 mL

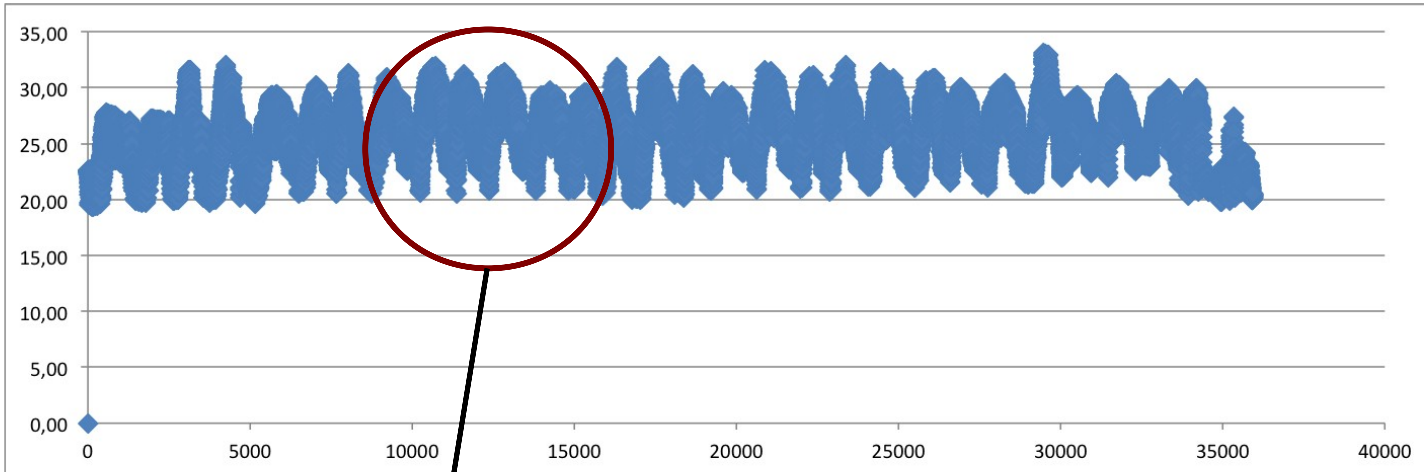
Set up: PEA example *Stress intensity*

Comparison of different water stress intensities (2012) :

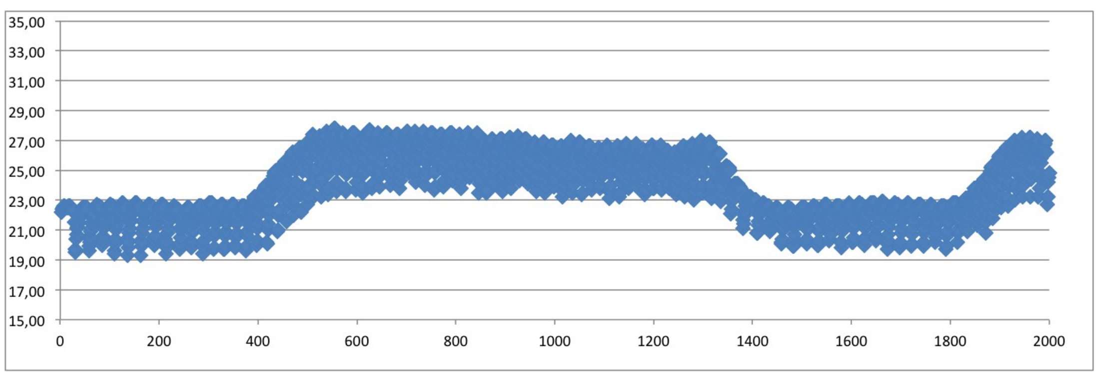




Set up: greenhouse conditions

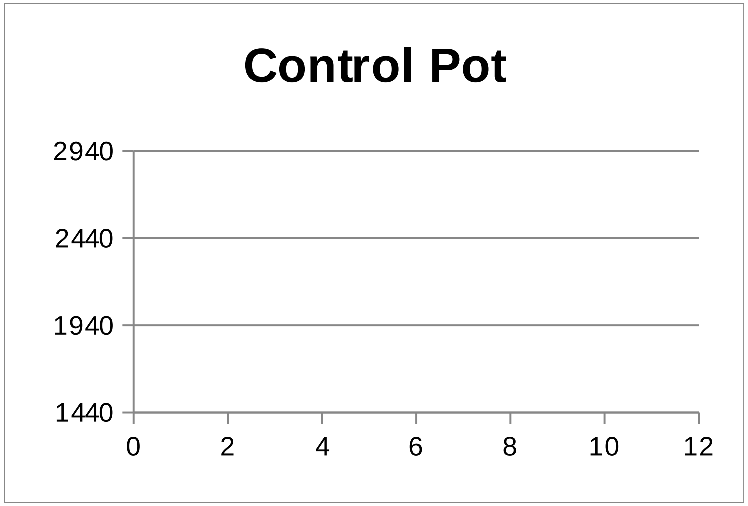


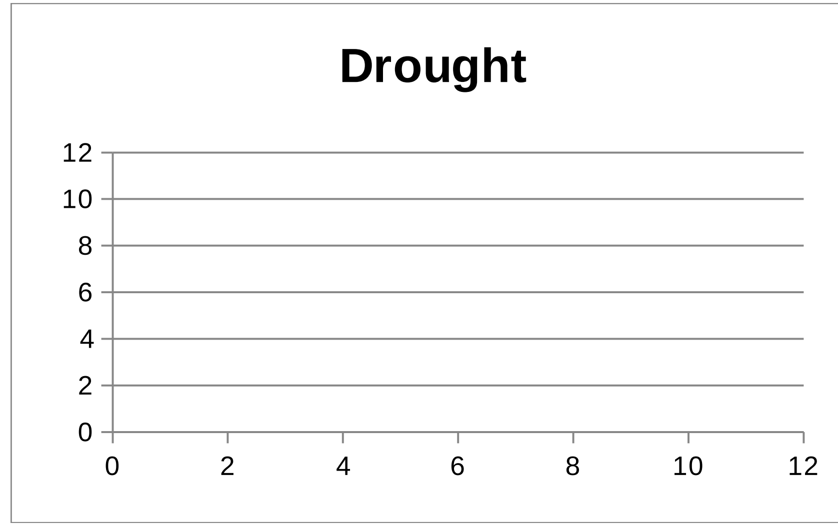
…and daily



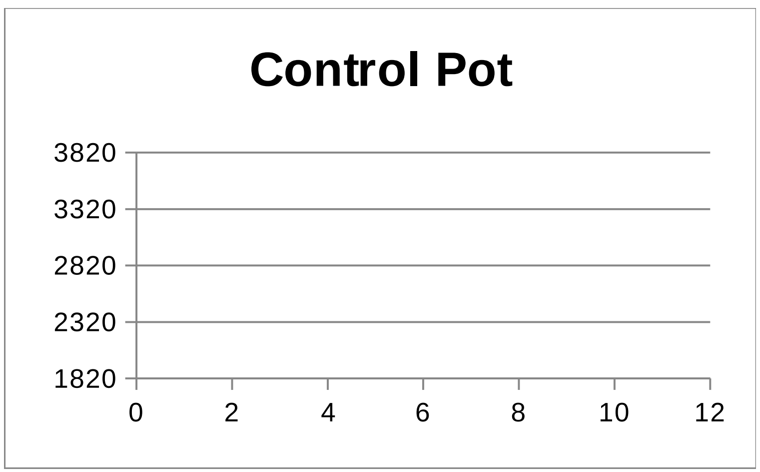
Plant watering verified

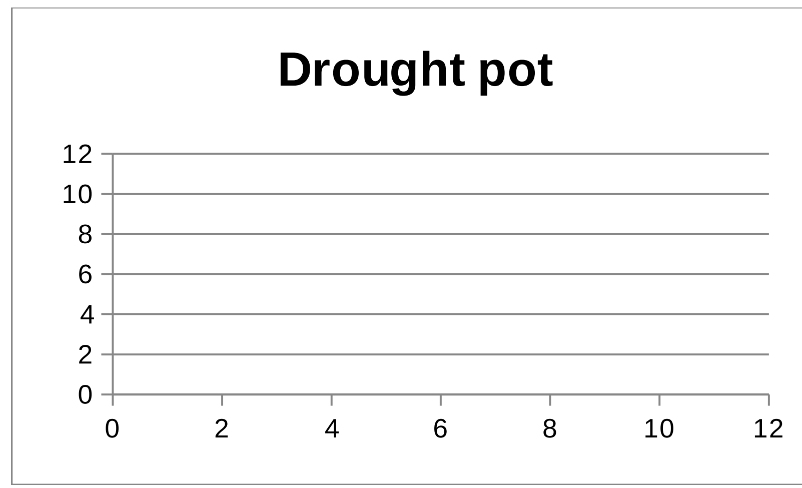
BOM





BOP





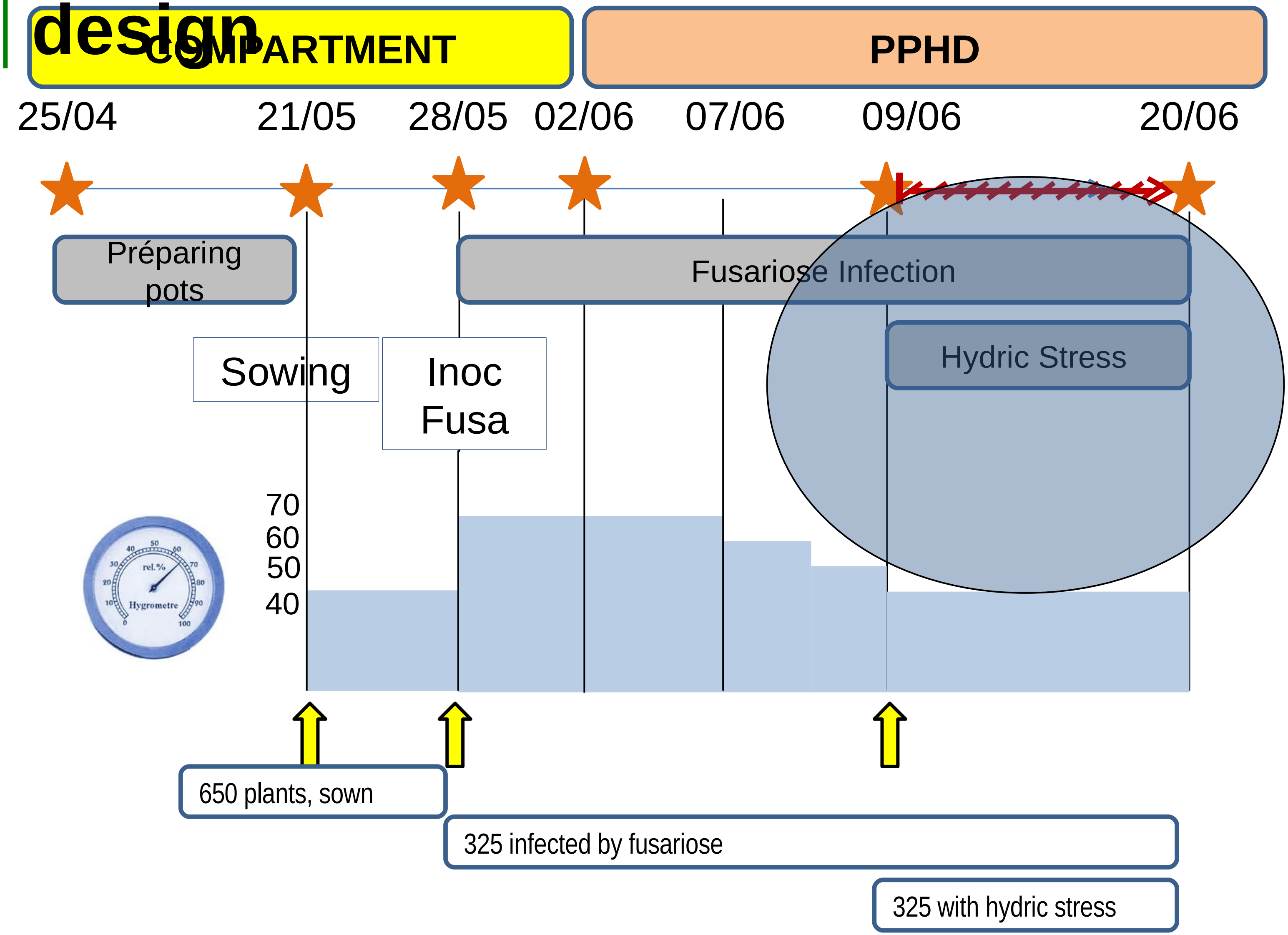
- ✓ **Automatic**

Lessons from the « pilots »

- Transferring protocols devised for the infection of plants with *Fusarium* in highly controlled environments: challenging in the local conditions in Dijon.
- Drought conditions, with fluctuations in the internal environment of the platform: challenging conditions in which to undertake controlled experiments.
- This added complexity for example the interpretation of physiological data
- This ensures that findings are likely to be more relevant to those obtained in field trials than anticipated.

  - **Relevant transferable protocols for scaling up combined stresses in the large phenotyping platform**

=> Large scaling experiment design



perlite : biot sand B4, 3 :1

28°C day, 24°C night;

16 hours

Detail

Drought period

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Day of week

INRA SCIENCE & IMPACT — 5 plants * 4 treatments+ 29 plants (12/rhizotrons + 15N/marquage)

- Poro et Psycro
- DM (Shoots, roots, nod)
- Nb nod
- Surface foliaire
- Phénotypage
- Fluxomique

University of Essex — 8 plants* 4 treatments

- Gaz exchange

- Harvest(shoot et roots)
- Freezer storage

Detail

Drought period

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Day of week

INRA SCIENCE & IMPACT 5 plants * 4 treatments+ 29 plants (12/rhizotrons + 15N/marquage)

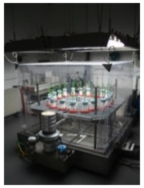
- Poro et Psycro
- DM (Shoots, roots, nod)
- Nb nod
- Surface foliaire
- Phénotypage
- Fluxomique

INRA SCIENCE & IMPACT

*3 plants * 4 treatments*

- Visible cabin
- Rhizotron et rhizocab

- 13C et 15N labeling



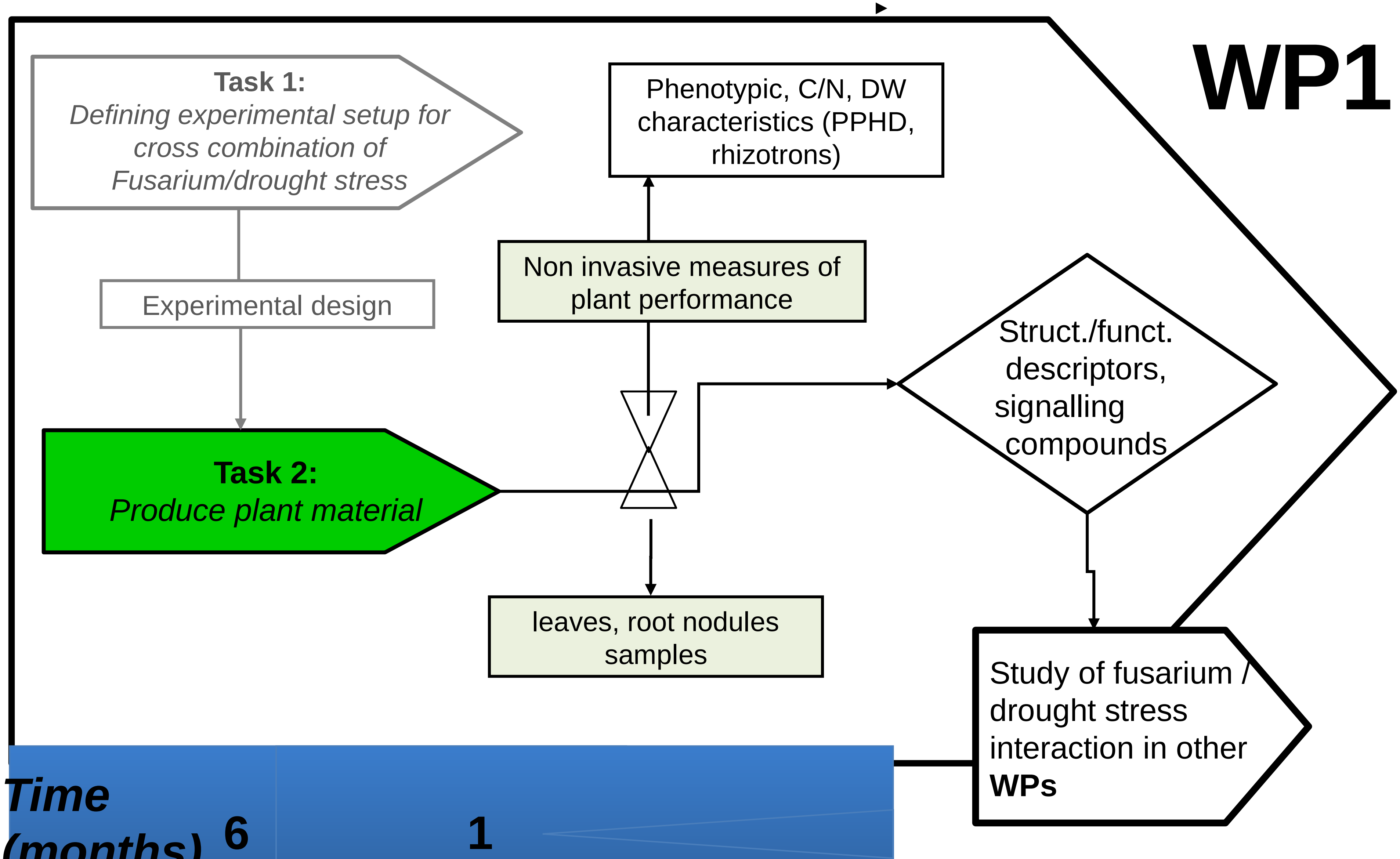
*3 plants * 4 treatments*

Tasks

Task No.	Activities	Inst. Responsible for Task	Person(s) Responsible for Task
T1.1	Defining experimental setup for cross combination of *Fusarium*/drought stress	INRA/CSIC	

Description of the work

C. Salon, INRA.



Tasks

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T1.1	Defining experimental setup for cross combination of *Fusarium*/drought stress	INRA/CSIC	

Description of the work

M. Prudent and C. Salon, INRA

WP1

Task 1:
Defining experimental setup for cross combination of Fusarium/drought stress

Task 2:
Produce plant material

Task 3:
Analyzing data

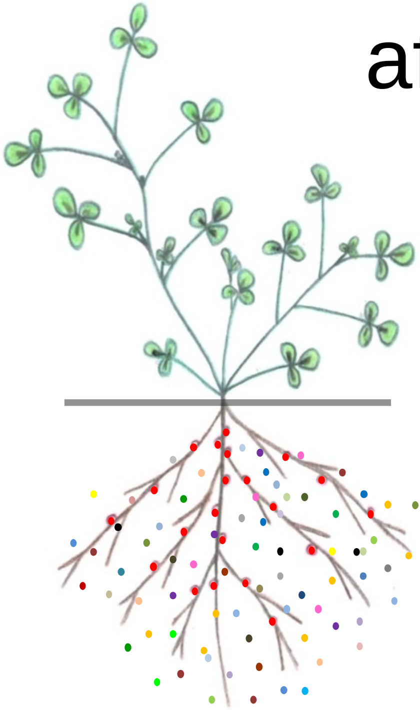
Struct./funct/ descriptors

Key process, involved in structural or functional changes

Study of fusarium/drought stress interaction in other **WPs**

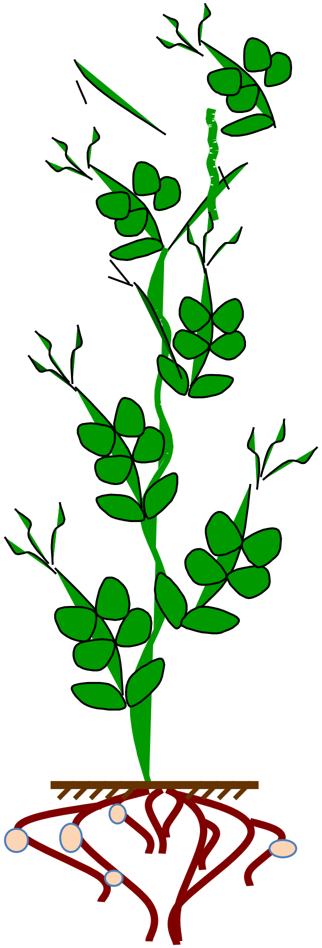
Time (months)	6	1	1	2

Some results: medicago and pea



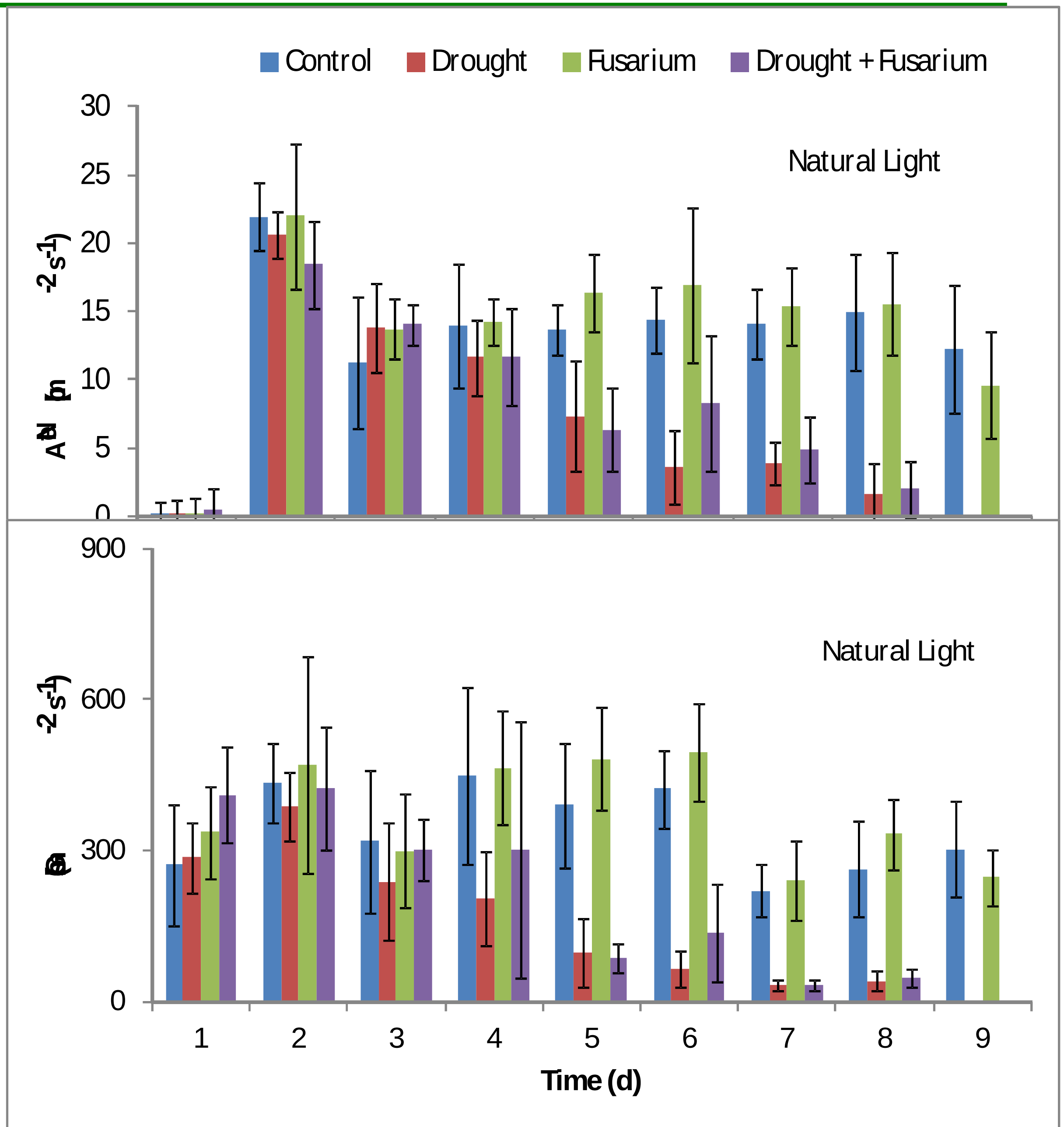
How did treatments affect after 12 days:

- development,
- growth: carbon acquisition, partitioning



Physiological data (Pre-pilot)

University of Essex



IRGA measurements (Essex)

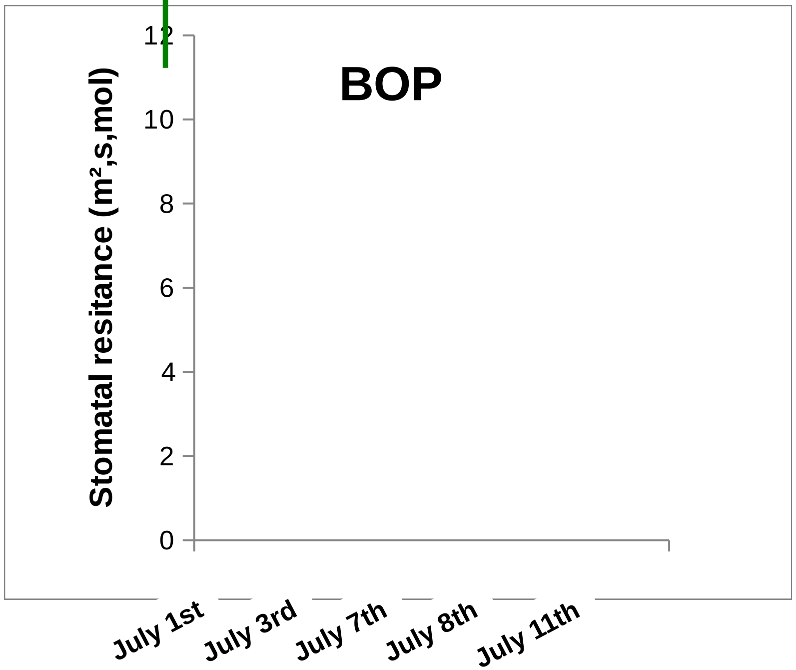
University of Essex

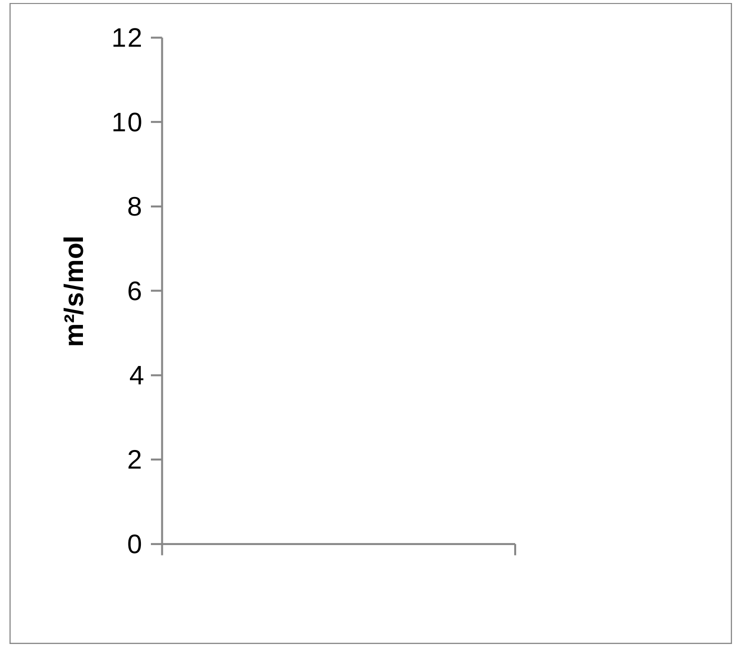
Instantaneous (snapshot) readings of Assimilation rate (Anet) and stomatal conductance (gs) under natural light and saturating light. Two leaves
(youngest fully expanded) per plant and five reps per treatment.

·Example from Pre-pilot.

All IRGA measurements were re-calculated to correct for area. All measurement taken between 9 am -12pm

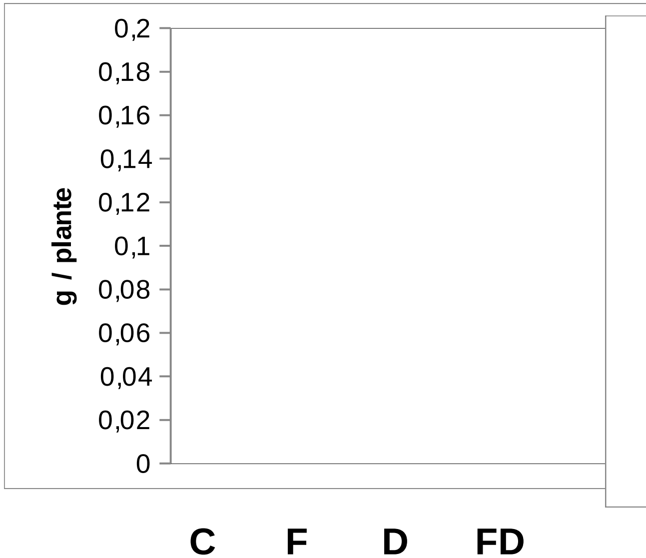
Stomatal resistance



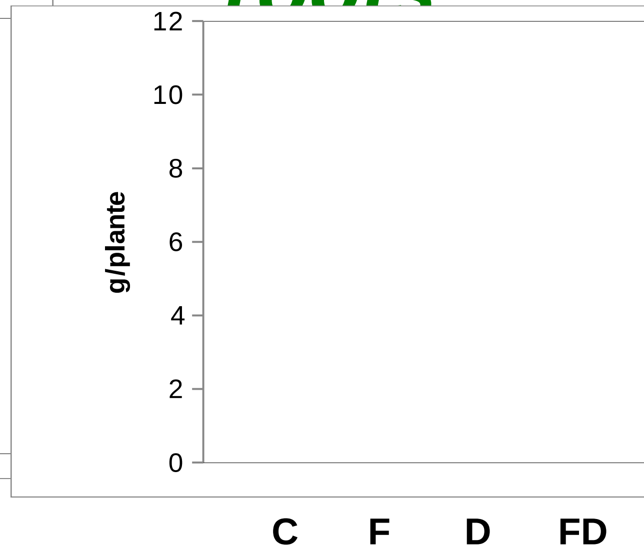


- Drought increases stomatal resistance.
- Fusarium:
  - amplified the drought stress of plants for both BOM and BOP
  - had no effect when plants were well watered for pea and a detrimental effect similar to water stress alone for Medicago plants

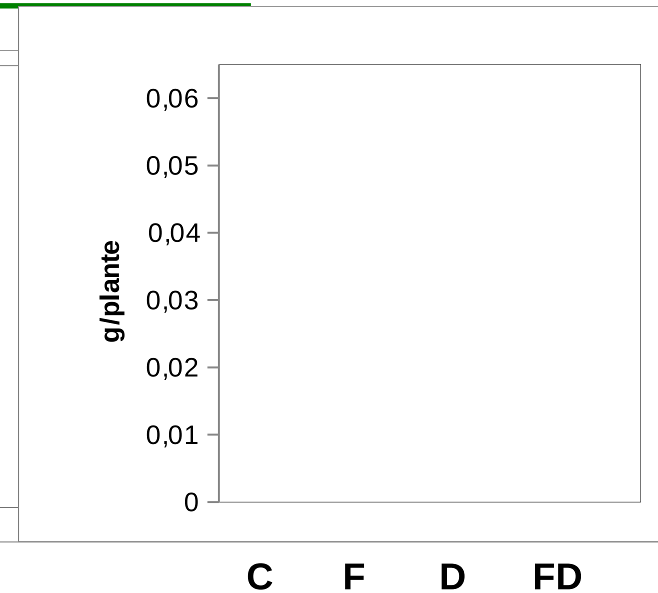
BOMedicago *Growth: plant, shoots, roots*



Plant DM

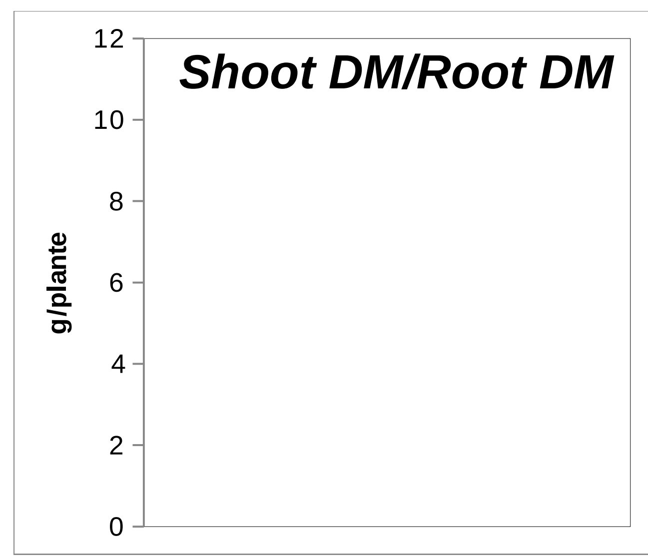


Shoot DM



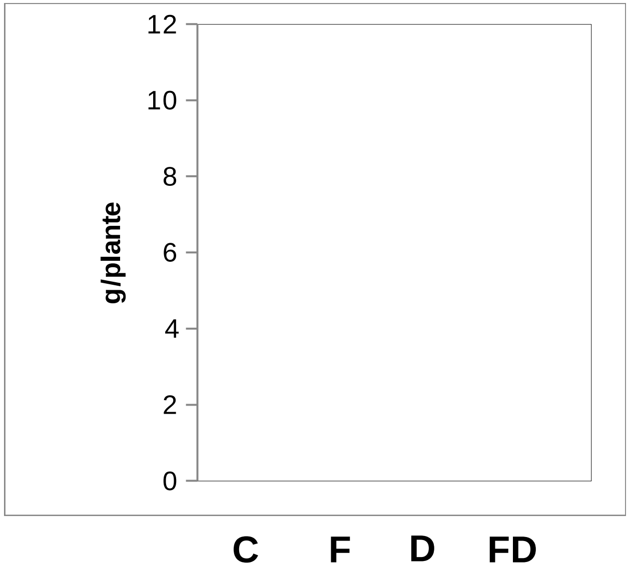
Root DM

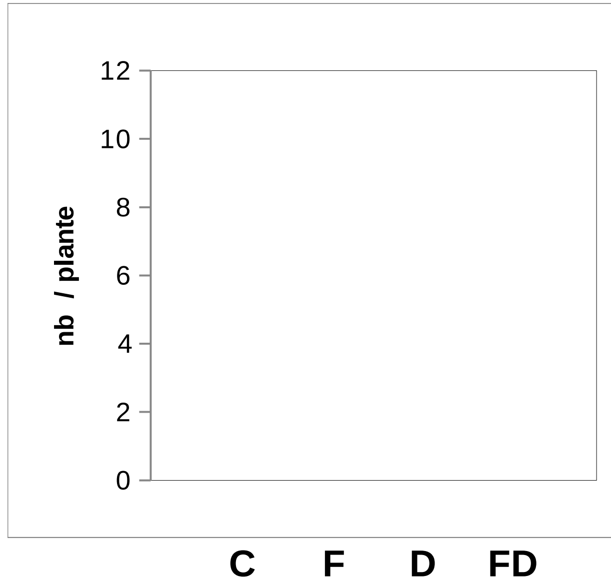
- **Stress reduce plant growth**
- **Shoot biomass mostly impacted.**
- **Shoot over root biomass ratio decreased for water stressed. Already reported in previous report (M18) and for various species.**
- **Fusarium seemed to counteract the drought stress effect (reducing impact on shoots mostly and also roots): explanations ?**

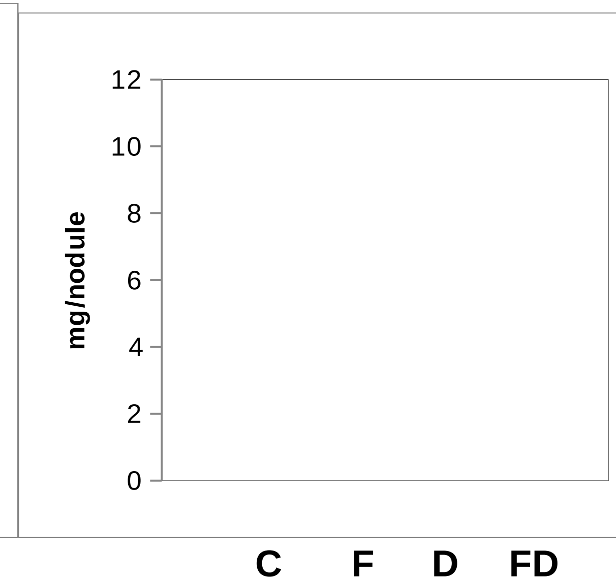


C: Control; F: Fusarium infected; D: Drought applied; FD: both

BOMedicago *Nodules*

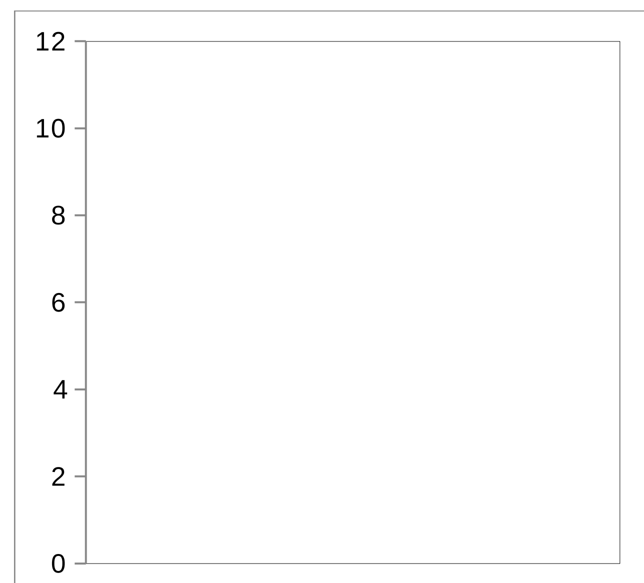






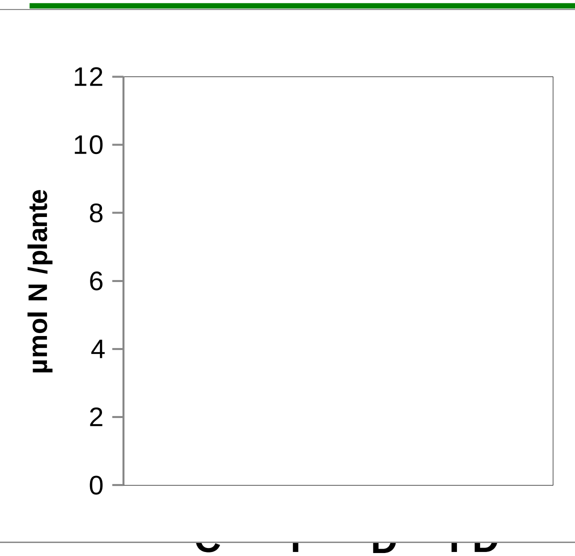
Total Nodule DM = Nodule number X Mean nodule weigth

- **Stress reduce nodule DM (F effect ?)… …either nodule number and/or ind. weigth.**
- **Fusarium leads to bigger nodules in absence of water stress and smaller with drought (?).**
- **Nodules represent a lower part of nodulated roots under stress**
- ***similar as in M18***

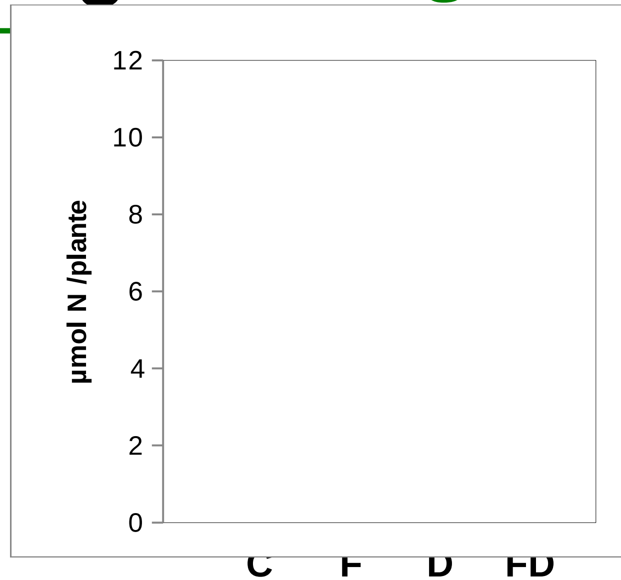


C: Control; F: Fusarium infected; D: Drought applied; FD: both

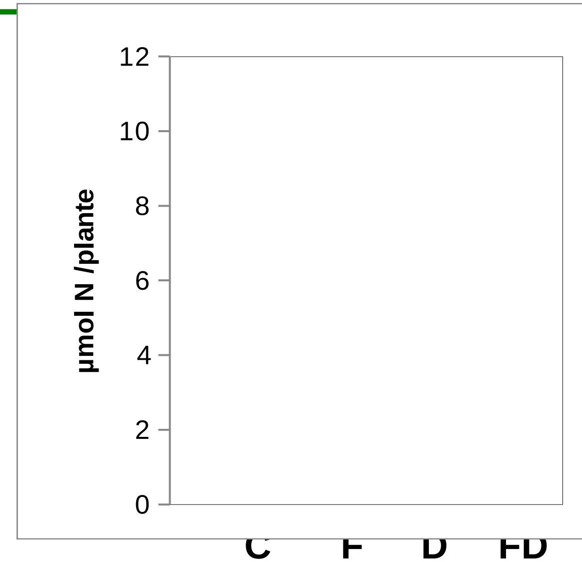
BOMedicago *Nitrogen content*



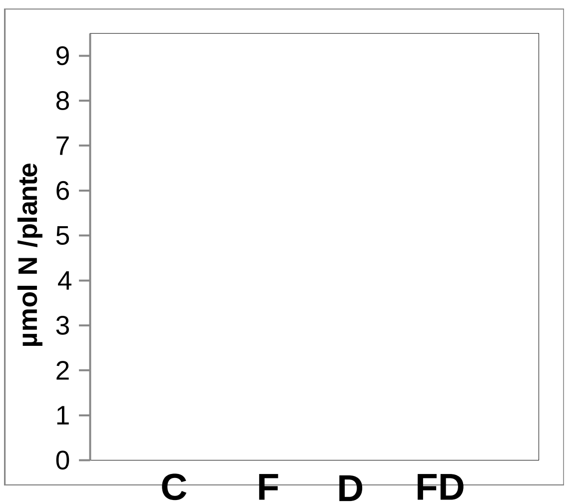
Plant N



Shoot N



Root N

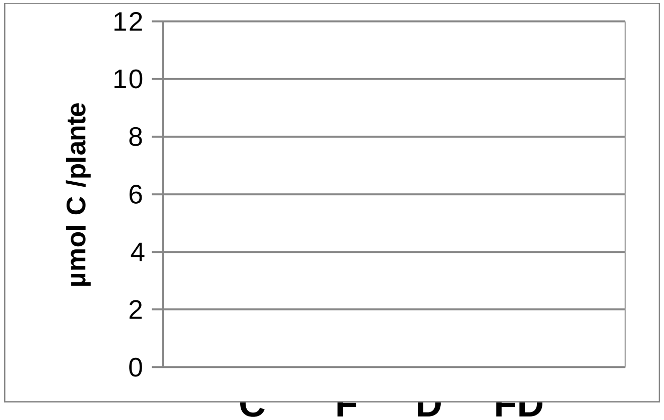


Nodule N

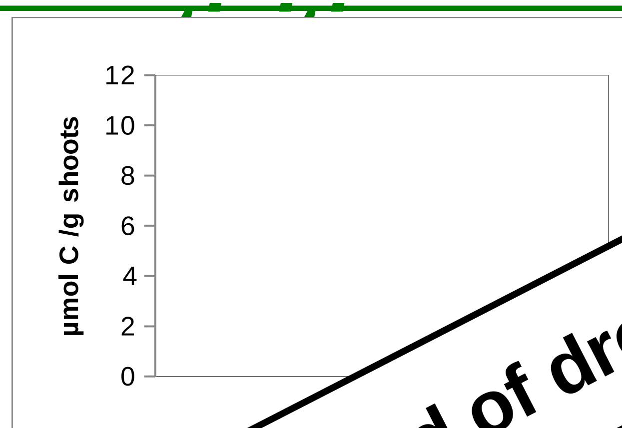
- **Compartments [N] :**
  - **N status slightly affected, a slight reduction of root N content.**
  - ***OK with previous data***
- **Fusarium did not affect N content of plants parts.**

C: Control; F: Fusarium infected; D: Drought applied; FD: both

BOMedicago *Fluxo: N and C specific*



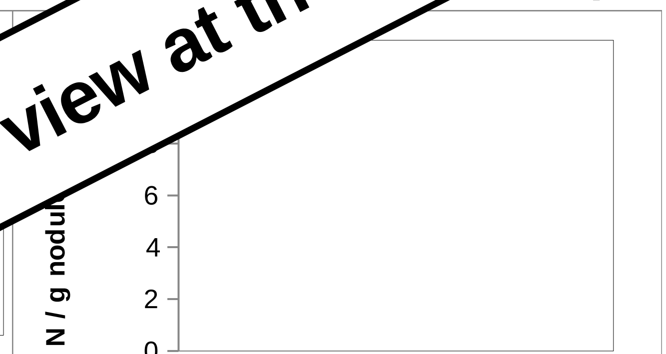
Daily C incorporation



ncorporation/shoot DM

A detailed view at the end of drought

N incorporation

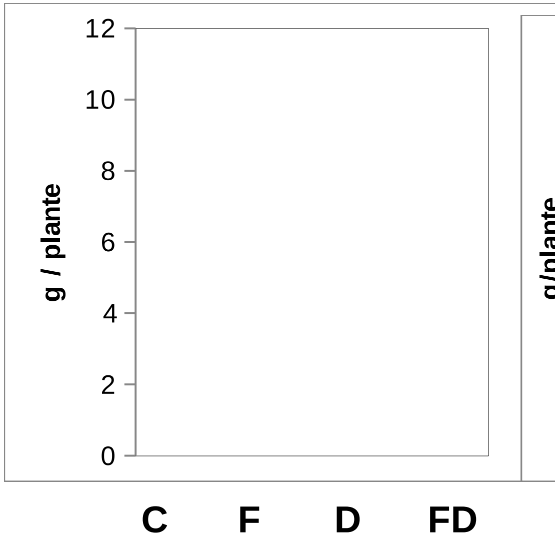


Nodule SA = N incorporation/nodule DM

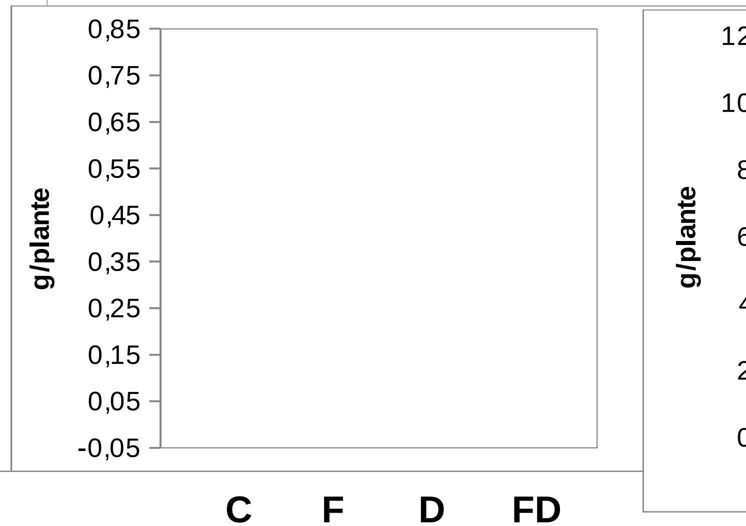
- Stems (photosynthesizing) acquired more carbon than leaves
- Water stress affects plant C/N allocation, mostly to shoots, stems and nodules, not to root (constant)
- No effect of Fusarium on C and N acquisition
- Leaf SA reduced, Nodules SA maintained by drought

BOPea

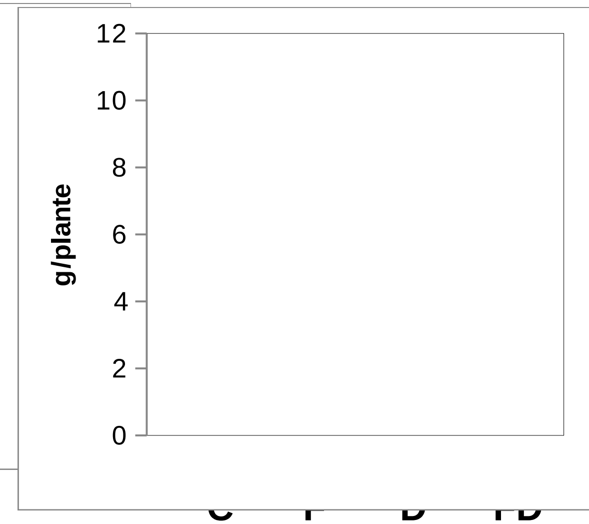
Growth: plant, shoots, roots



Plant DM



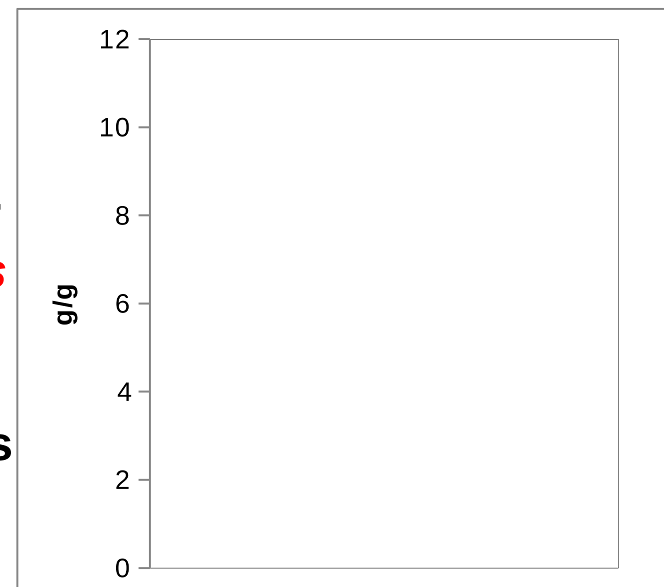
Shoot DM



Root DM

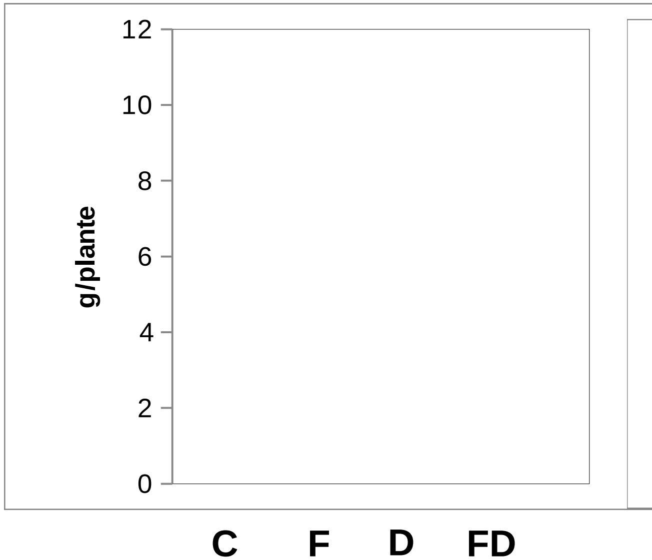
- **Drought reduces slightly plant growth**
  - **Shoot biomass mostly impacted, roots maintained.**
  - **Shoot over root biomass ratio decreased for water stressed. *Already reported in previous report (M18) and for various species*.**
- **Fusarium (again) seems to counteract the drought stress effect (reducing impact on shoots mostly and also roots).**

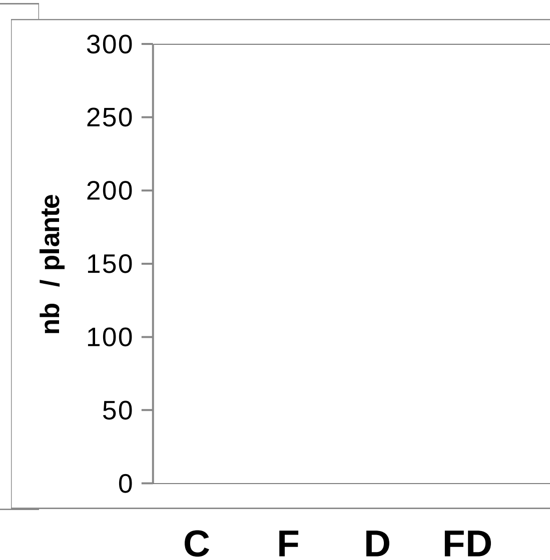
Data similar as in M18's dynamic

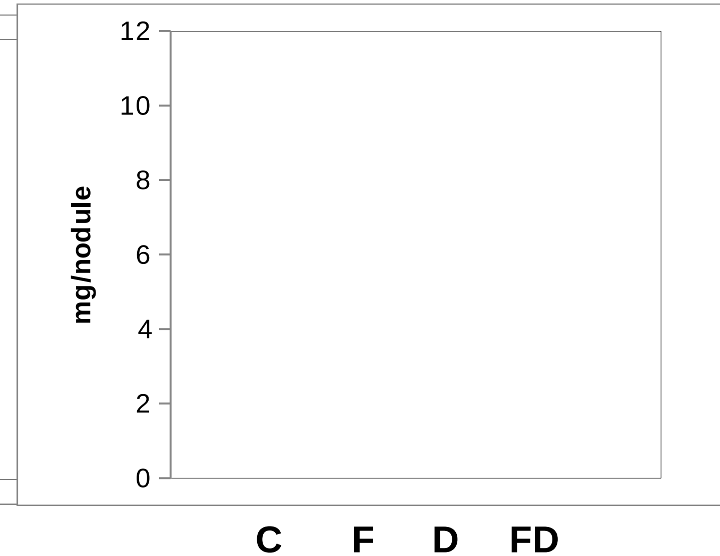


- C: Control; F: Fusarium infected; D: Drought applied; FD: both

BOPea *Nodules*

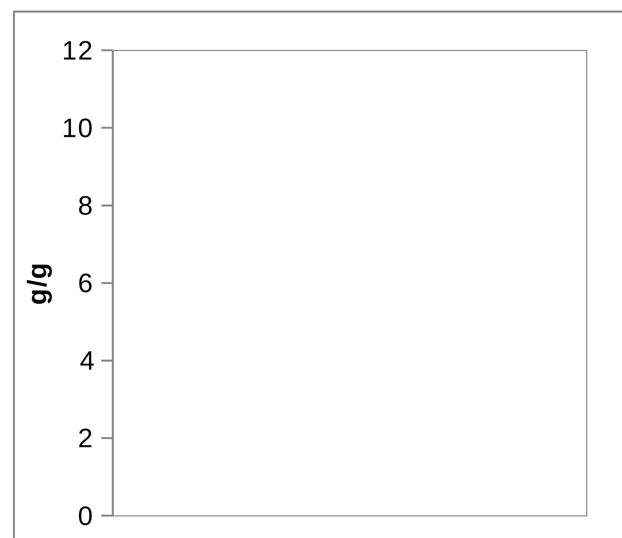






Total Nodule DM = Nodule number X Mean nodule weigth

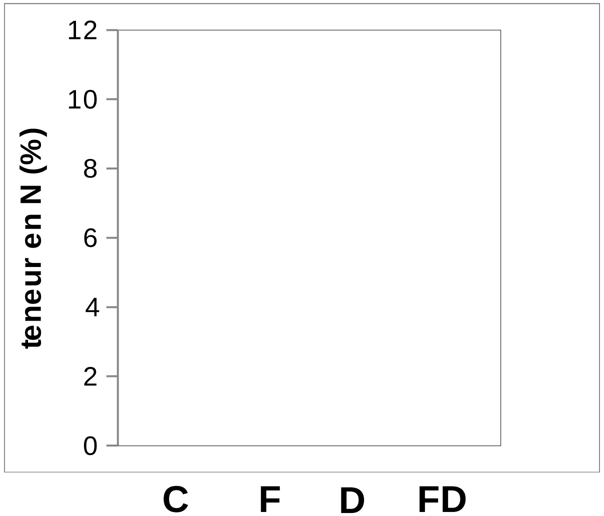
- **Drought**
  - **reduces nodule weigth and number with fusarium.**
- **Fusarium leads to bigger nodules with or without water stress**
- **Nodules = a lower part of nodulated roots under drought stress**
- ***Similar as in M18 and for Mt experiment***



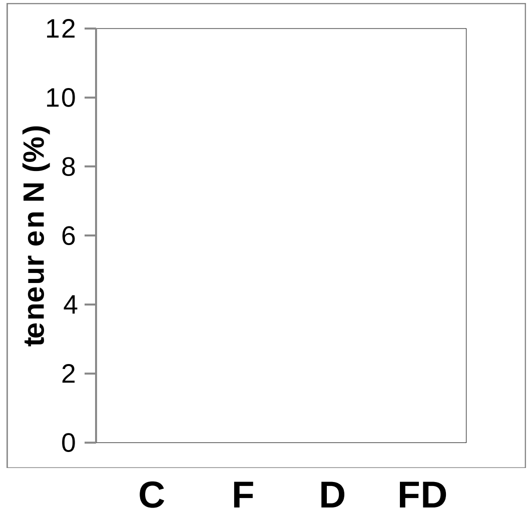
C: Control; F: Fusarium infected; D: Drought applied; FD: both

BOPea

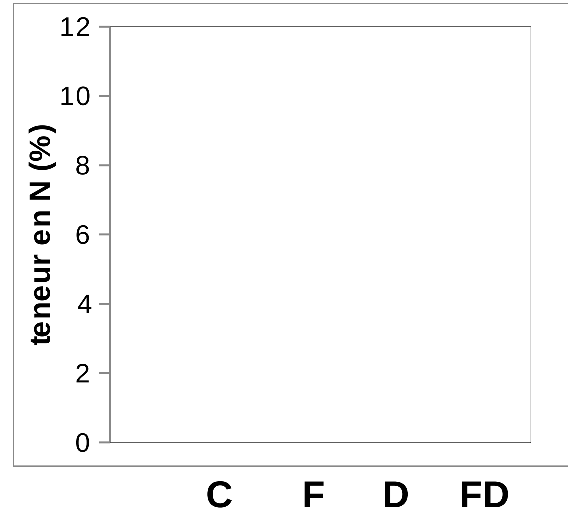
Nitrogen content



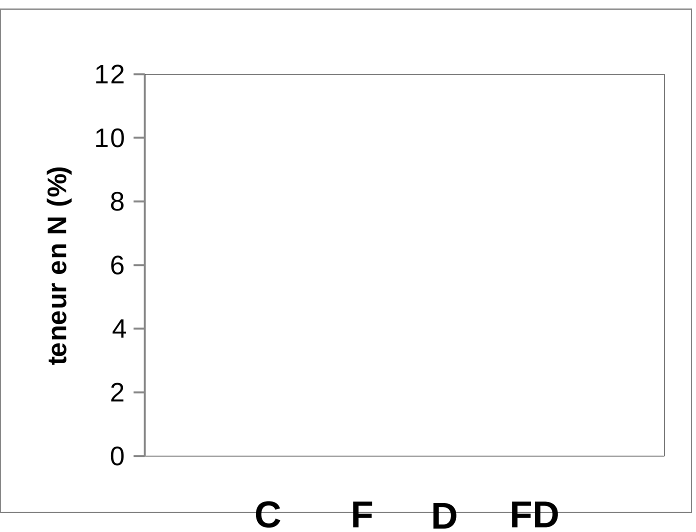
Plant N



Shoot N



Root N



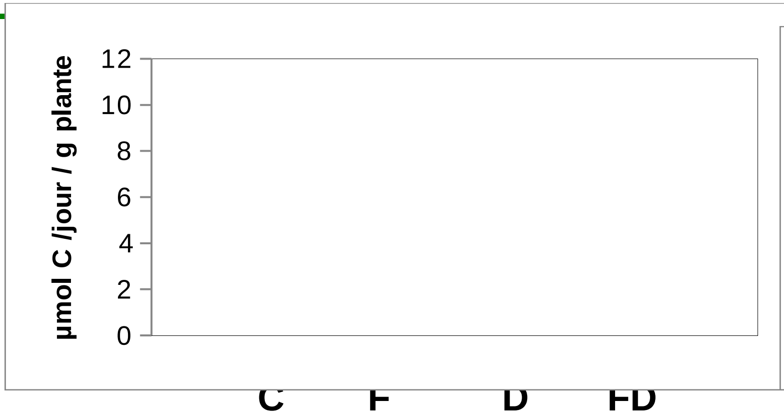
Nodule N

- **Drought**
  - **Plant N decreased by 20%**
  - **Mostly shoots**
  - **Nodules slightly affected**
- **Fusarium did not affect N content of plants parts.**

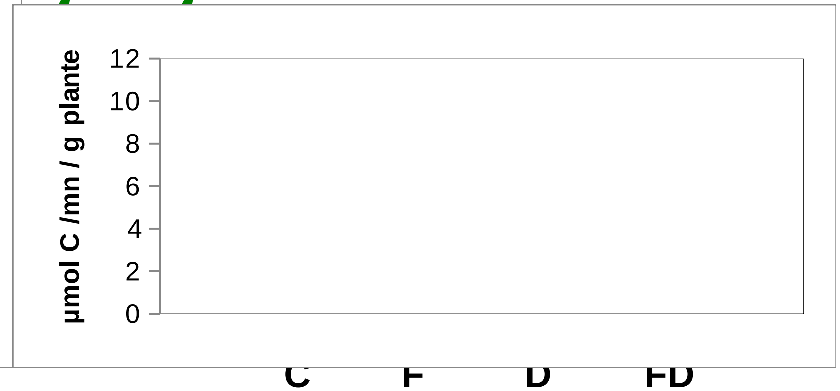
C: Control; F: Fusarium infected; D: Drought applied; FD: both

BOPea

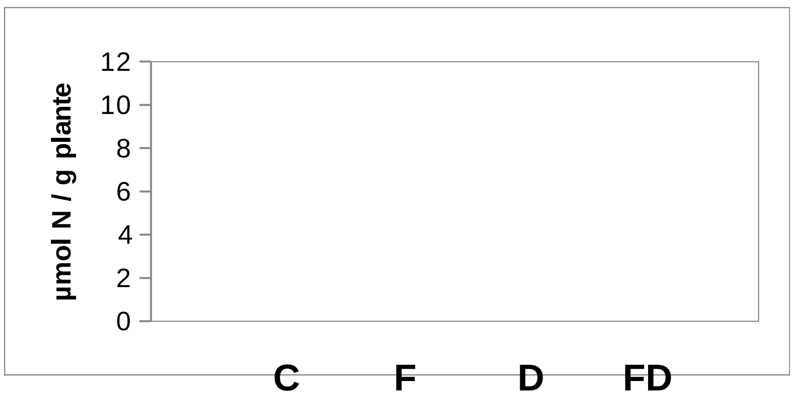
Fluxo: N and C specific



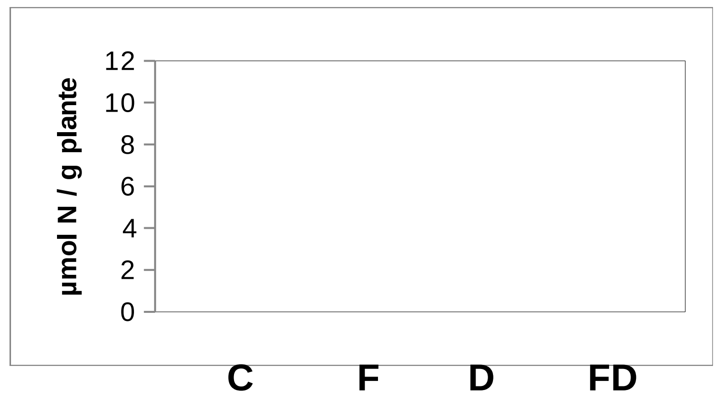
Daily C incorporation



Leaf SA = C incorporation/shoot DM



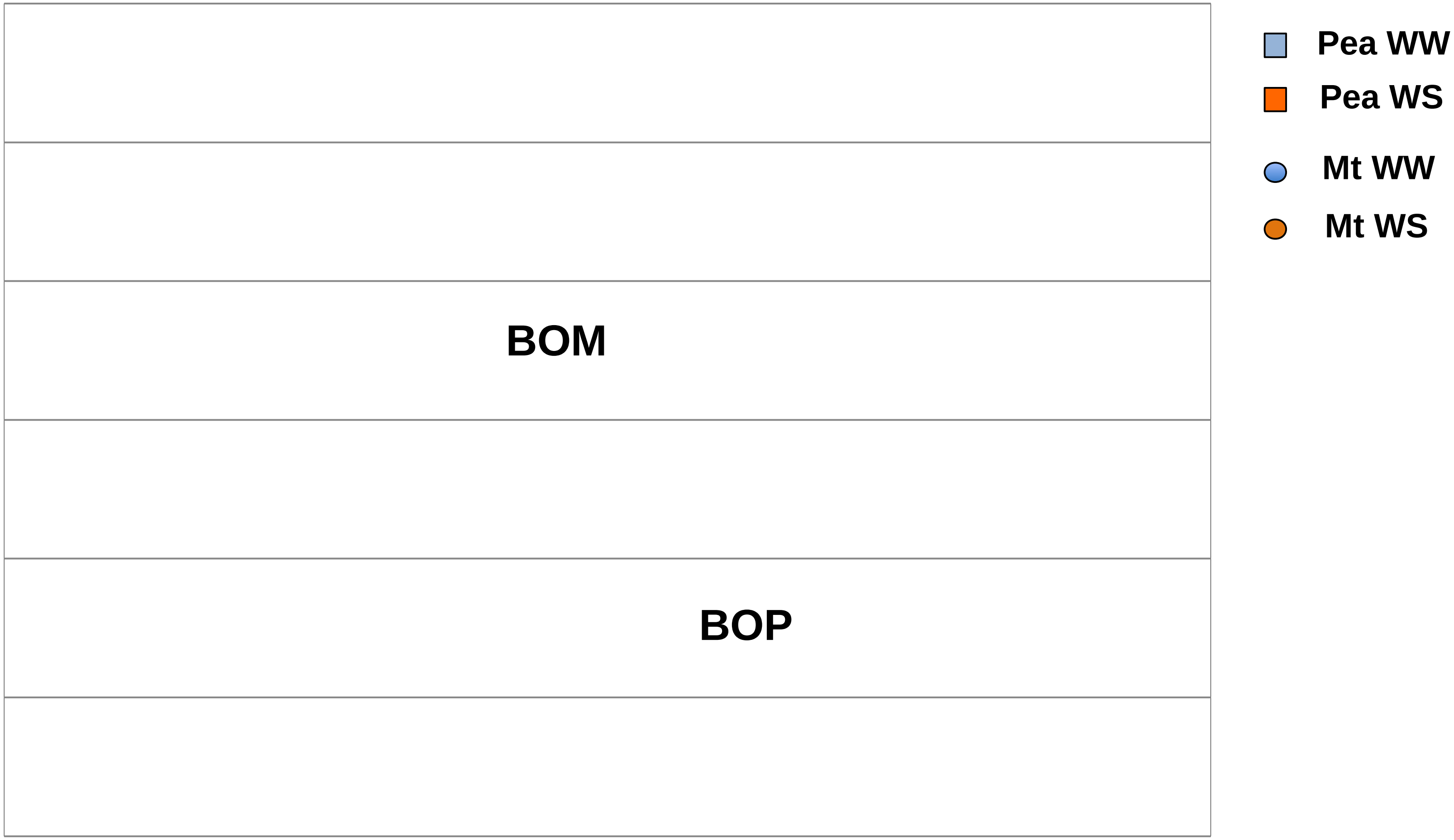
Daily N incorporation



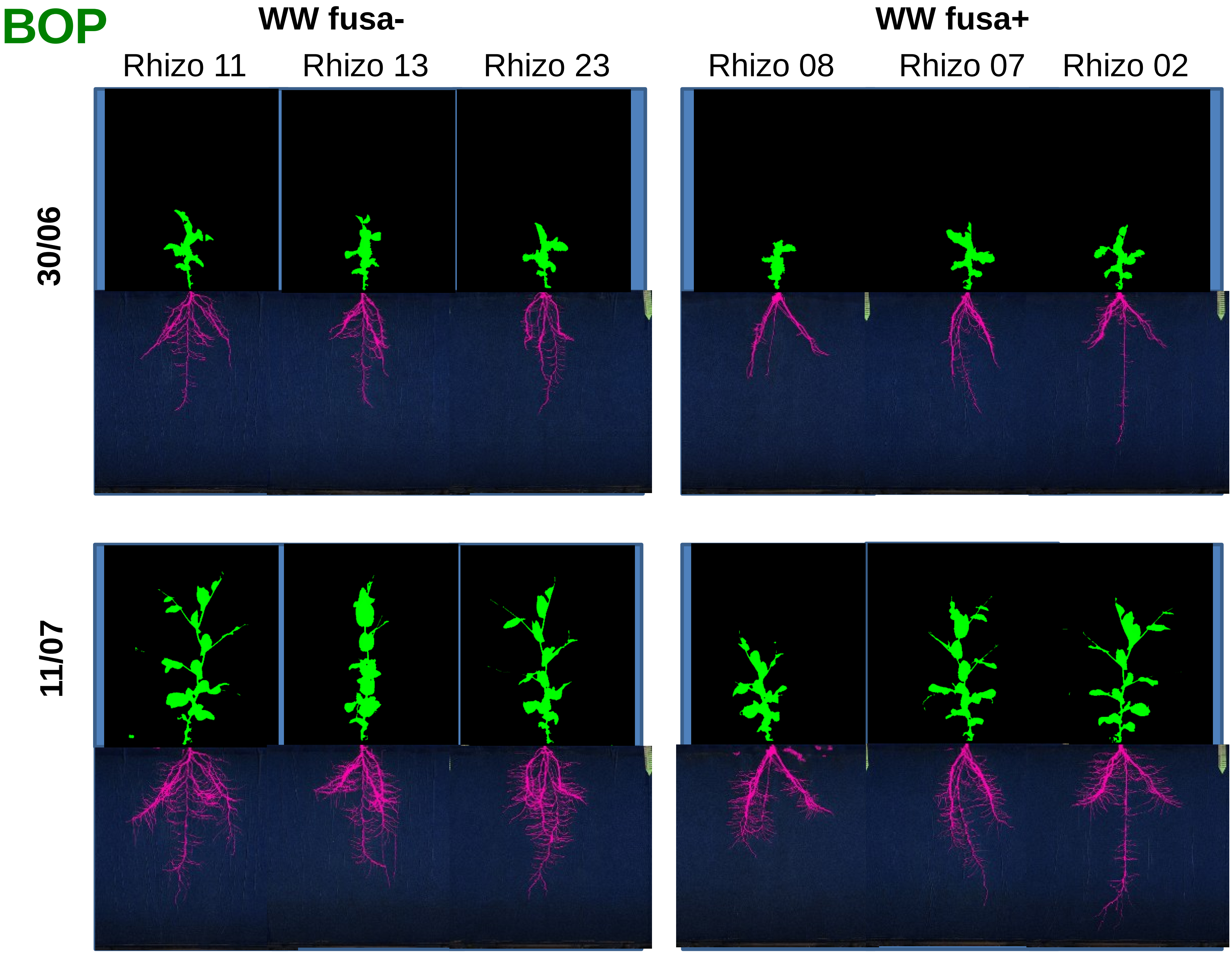
Nodule SA = N incorporation/nodule DM

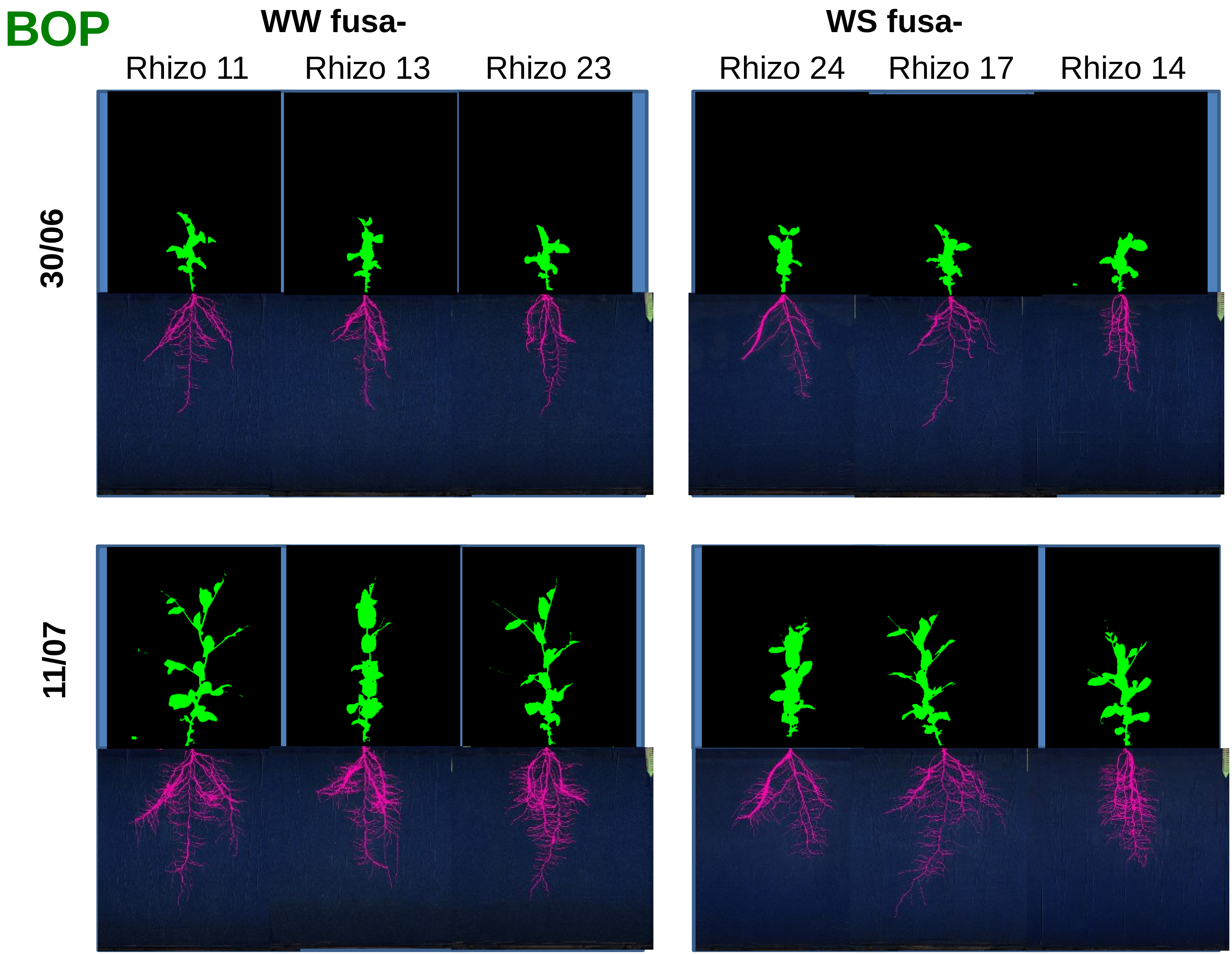
- **Pea leaves main sink for new C**
- **Water stress affects plant C and N allocation, mostly to shoots, stems and nodules, not to root (maintained)*, like Mt***
- **No effect of Fusarium on C and N acquisition**
- **Both leaf SA and Nodules SA reduced by drought, reinforced by total nodule biomass decrease**
- **As the decrease in nodule biomass was rel. less important than that of total N plant incorporation a greater decrease of nodule specific activity is observed/expected.**

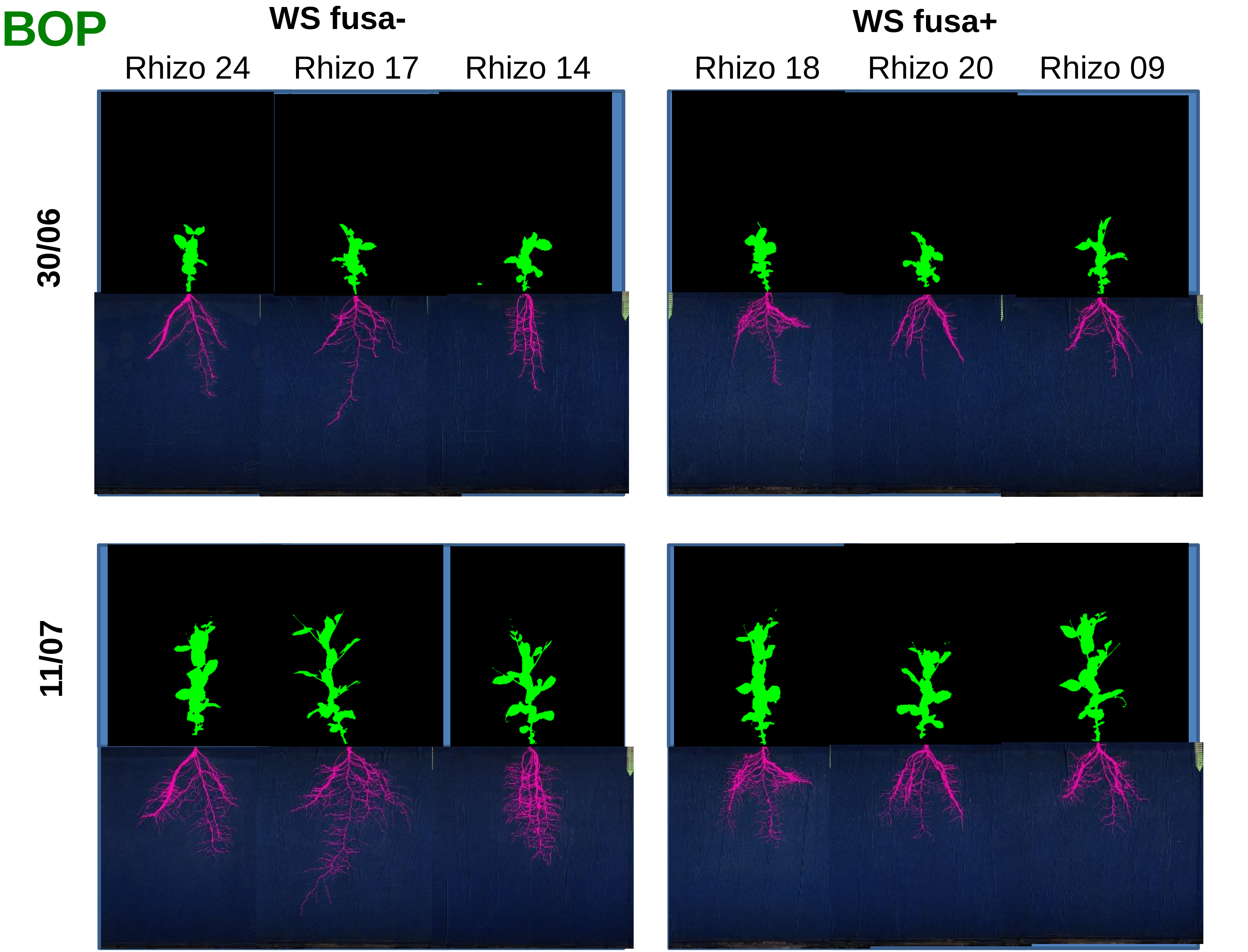
BOPea and BOM



- C and N flows tightly linked, plant impacted by water stress have lower C and N incorporations (as seen before)
- Slopes indicate the “reactivity” of species: Mt less reactive to drought
- Higher efficiency of Mt : smaller nodules and less numerous nodules) ?
  - Mt nodules have twice SA than pea nodules


BOP
WW fusa-
WW fusa+
Rhizo 11
Rhizo 13
Rhizo 23
Rhizo 08
Rhizo 07
Rhizo 02
30/06
11/07


BOP
WW fusa-
WS fusa-
Rhizo 11
Rhizo 13
Rhizo 23
Rhizo 24
Rhizo 17
Rhizo 14
30/06
11/07



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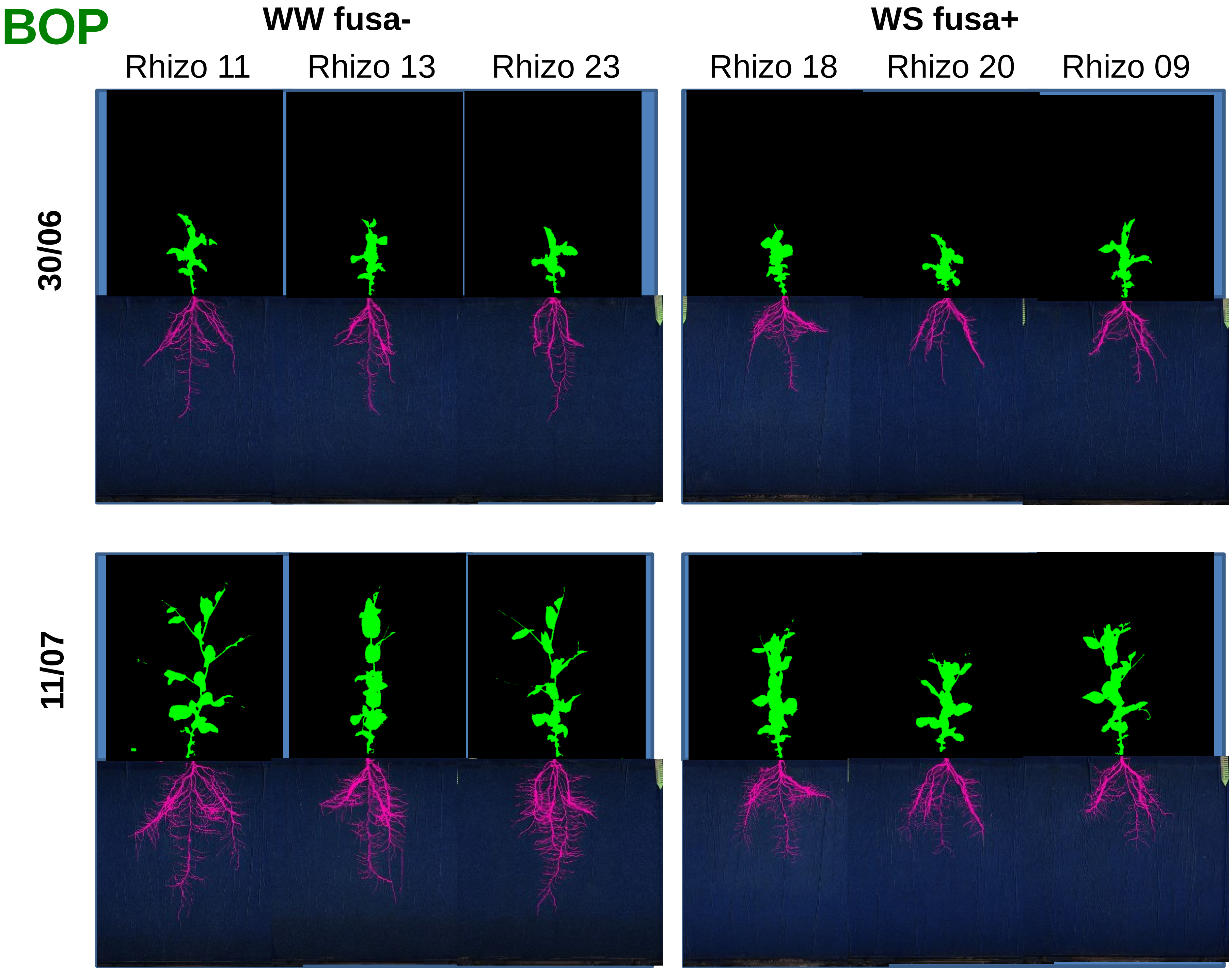
WS fusa+
Rhizo 24
Rhizo 17
Rhizo 14
Rhizo 18
Rhizo 20
Rhizo 09
30/06
11/07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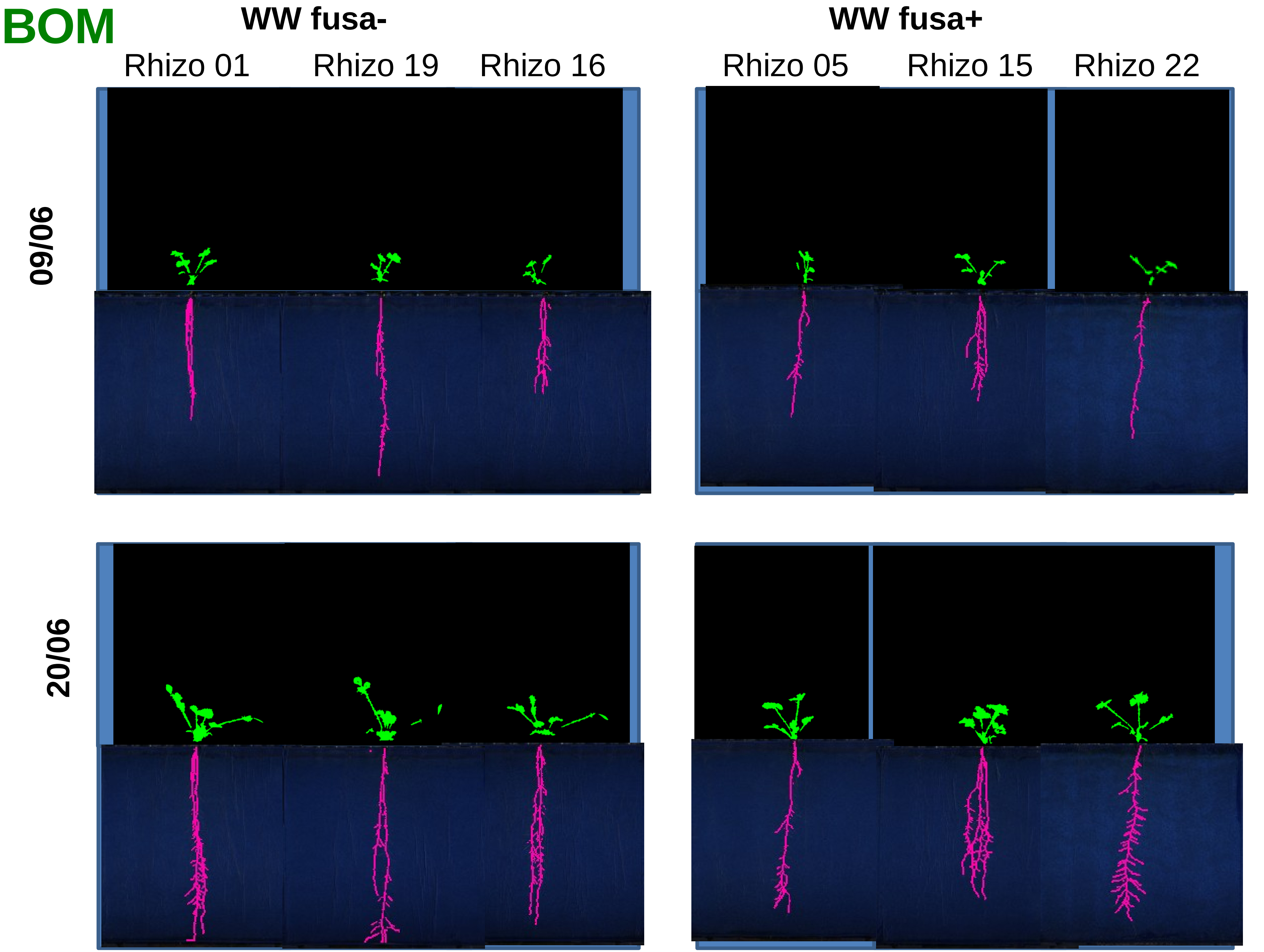
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09/06
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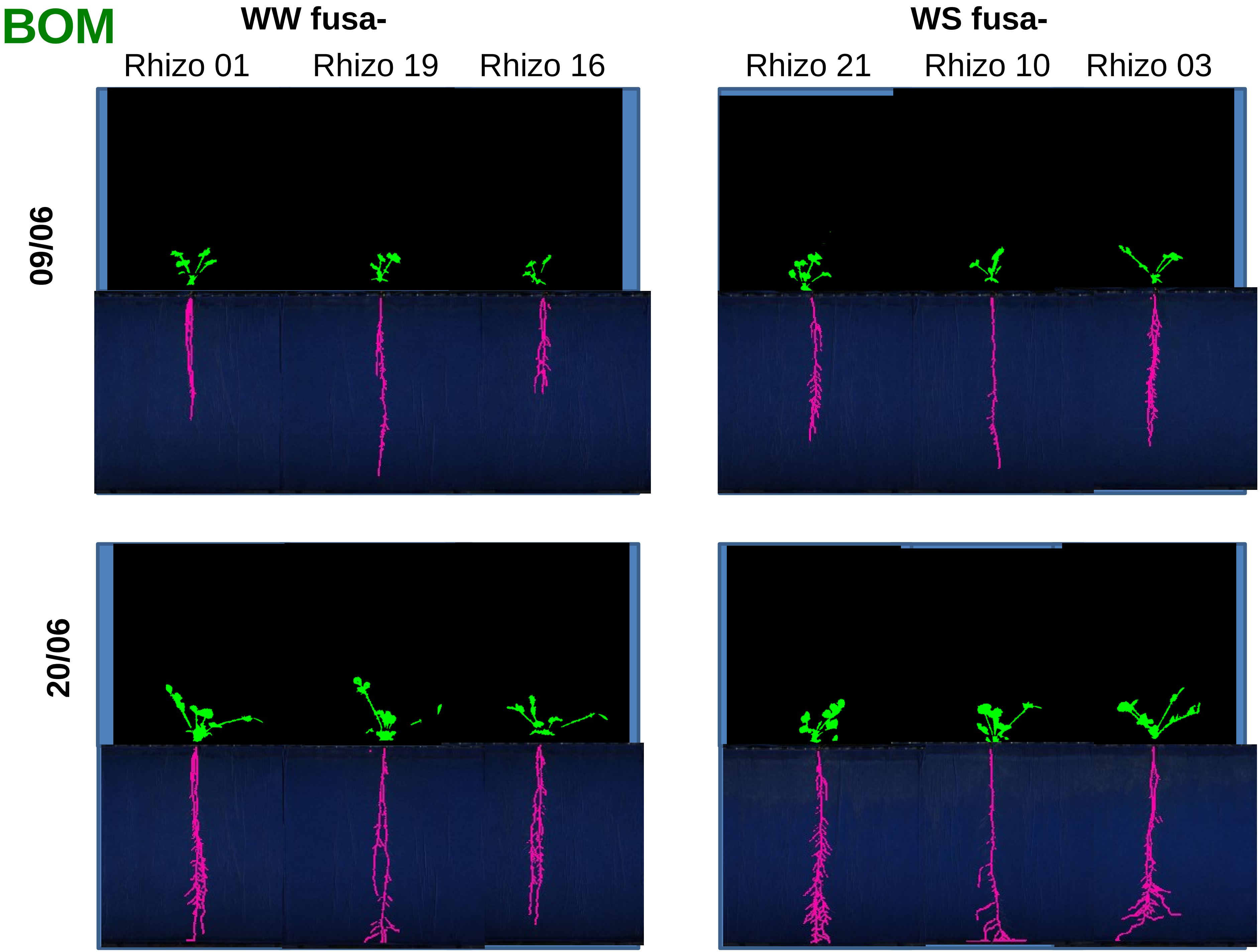
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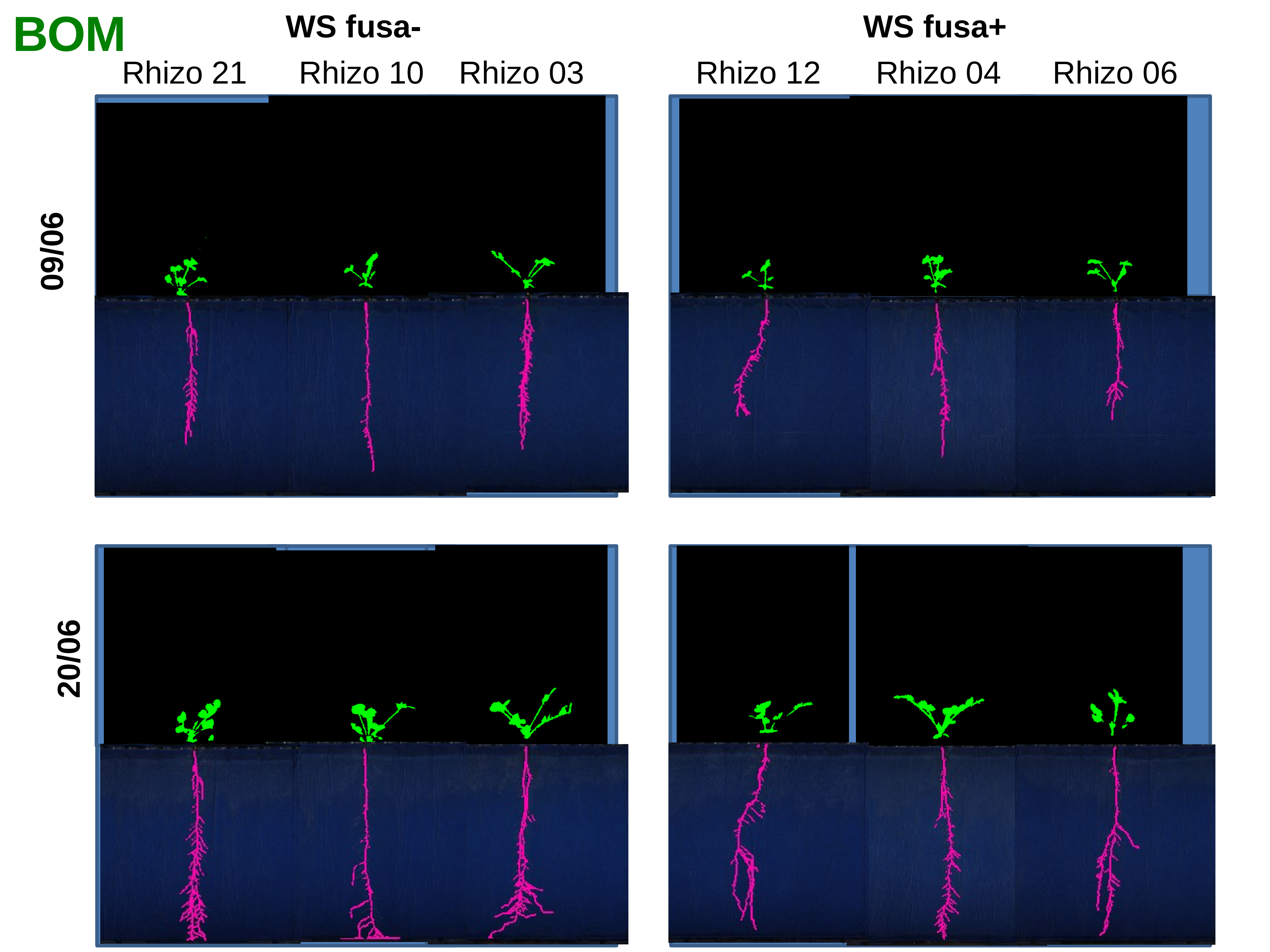
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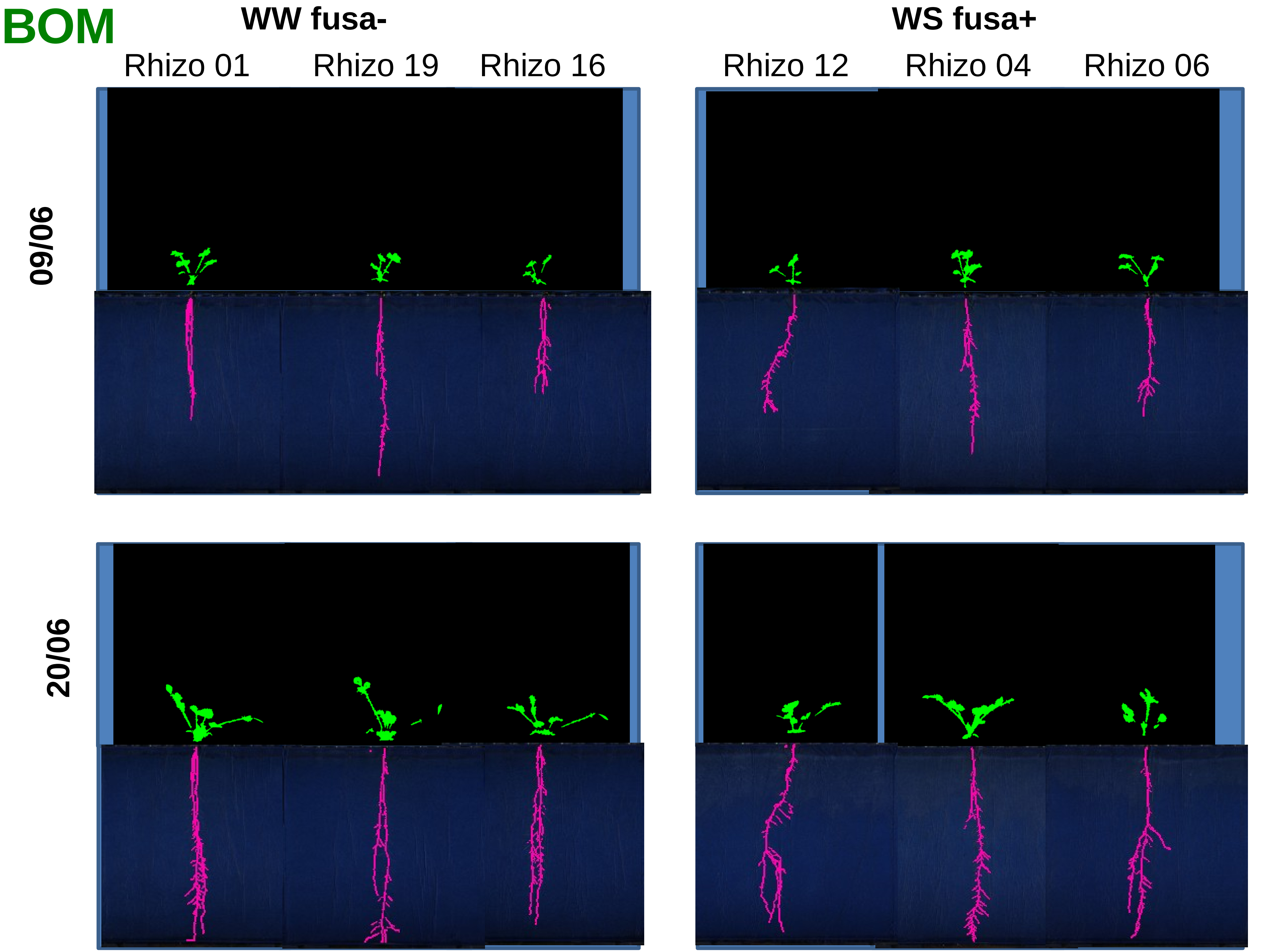
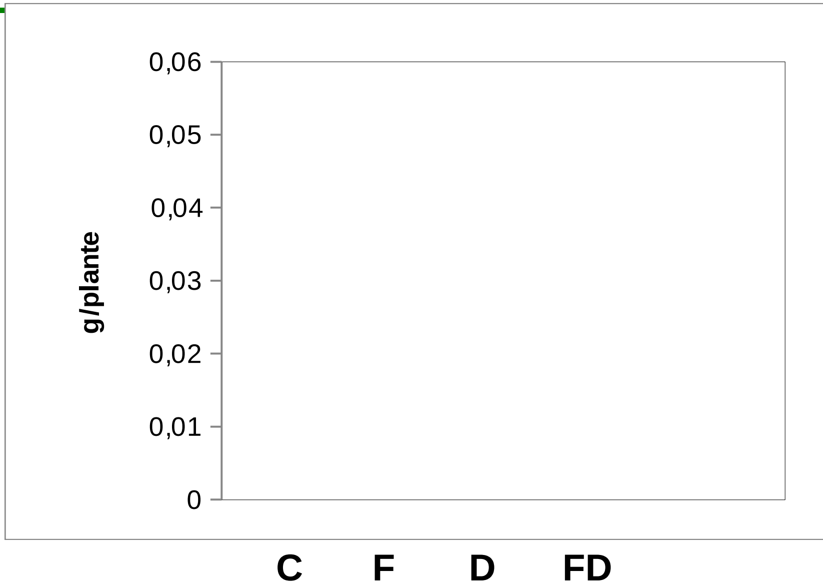
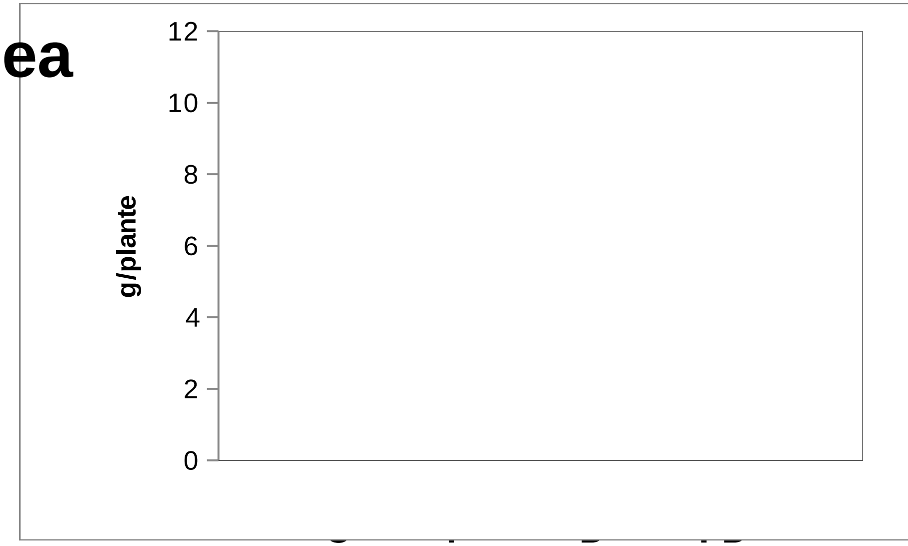

BOM
WW fusa-
WS fusa+
Rhizo 01
Rhizo 19
Rhizo 16
Rhizo 12
Rhizo 04
Rhizo 06
09/06
20/06

BOM and BOP: Roots RhizoTubes vs

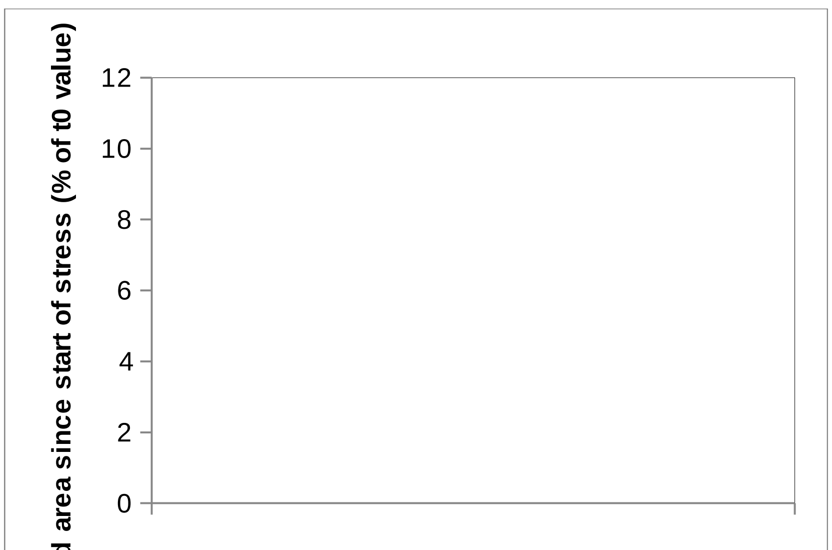
M **Pea**



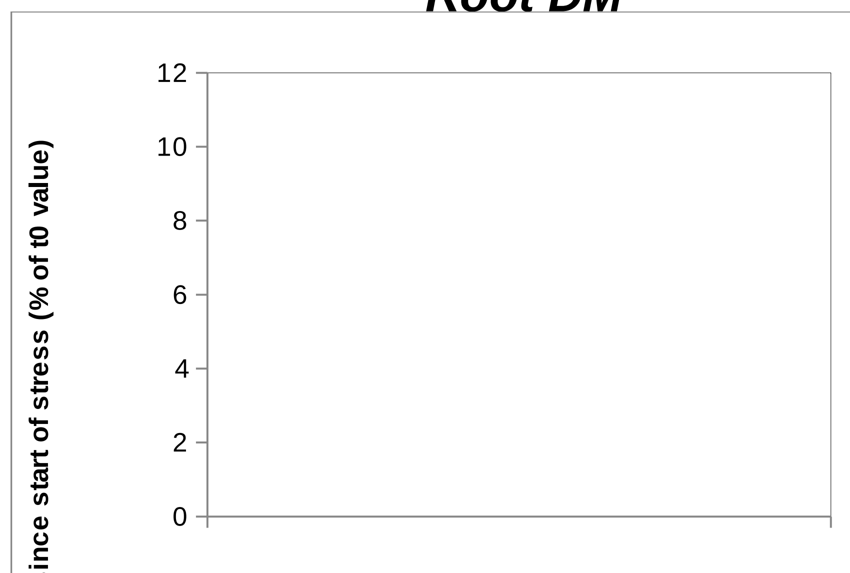
Root DM



Root DM



Root Projected area



Root Projected area

BOPea and BOM: RhizoTubes vs Pots ?

Modulation of biomass allocation to shoot, roots and nodules of pea (Kayanne genotype) and Medicago truncatula plants subjected to a water stress versus well irrigated plants. Results are expressed as the ratio of the difference in biomass of shoots (BMS), roots (BMR) or nodules (BMN) between water stress (WS) and well watered (WW) plants to the biomass of well watered plants (n= 5). As an example for shoots, $(BMS_{WS}-BMS_{WW})*100/BMS_{WW}$.

	Shoots	Roots	Nodules
Pisum sativum			
Pots	-19,9 ± 9,8 (A)	-1,6 ± 14,4. (A)	-41,2 ± 11,9 (A)
RhizoTube	-32,6 ± 13,9 (B)	-7,4 ± 14,2 (B)	-36,9 ± 12,8 (A)
Medicago			
Pots	-26,5 ± 30,0 (A)	-11,5 ± 34,5 (A)	-15,9 ± 46,1 (A)
RhizoTube	-32,4 ± 20,5 (A)	-26,1 ± 23,4 (A)	-27,9 ± 23,3 (A)

Publication submited

- **Same results on another experiment with pots or rhiztrons on pea and medicago: RT are usefull tools**

Tasks

Task No.	Activities	Inst. Responsible for Task	Person(s) Responsible for Task
T1.1	Defining experimental setup for cross combination of *Fusarium*/drought stress	INRA/CSIC	

Description of the work

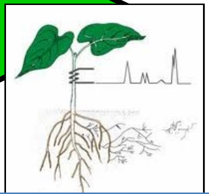
A. Charlton, M. Dickinson, FERA

Task 1:
Defining experimental setup for cross combination of Fusarium/drought stress

Task 2:
Produce plant material

Task 3:
Analyzing data

Task 4:
Metabolomics



Struct./funct/ **metabolomic** descriptors

Key process, involved in structural or functional changes

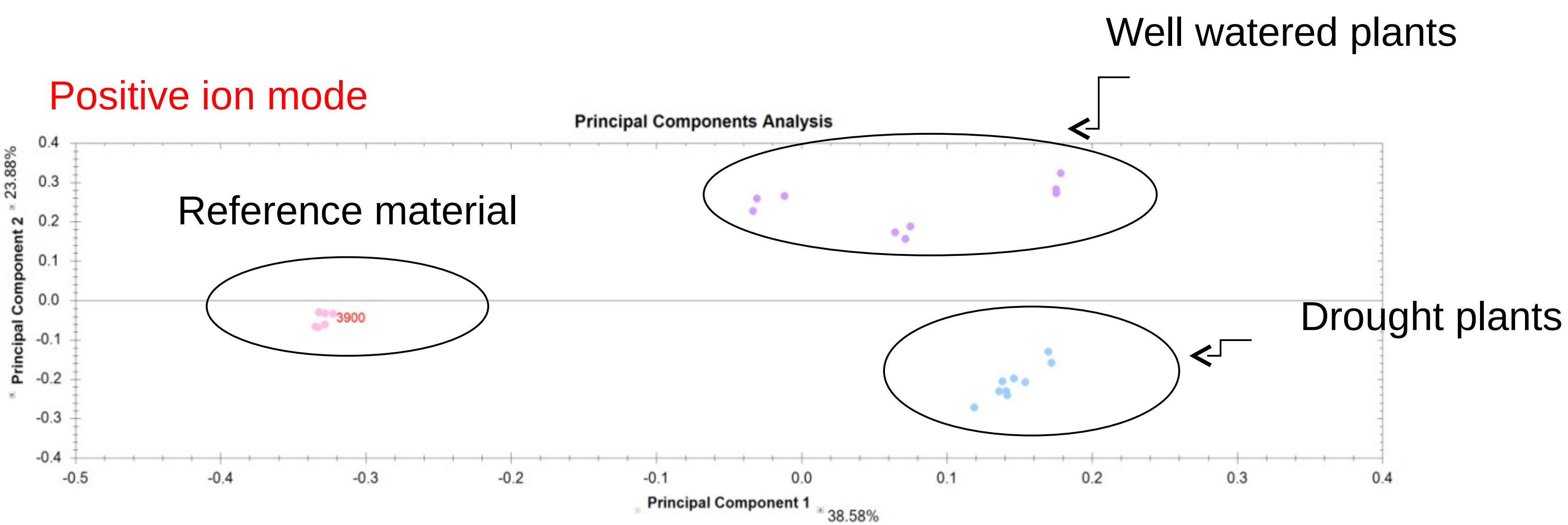
Study of fusarium/drought stress interaction in other **WPs**

Time (months)	6	1	1	2

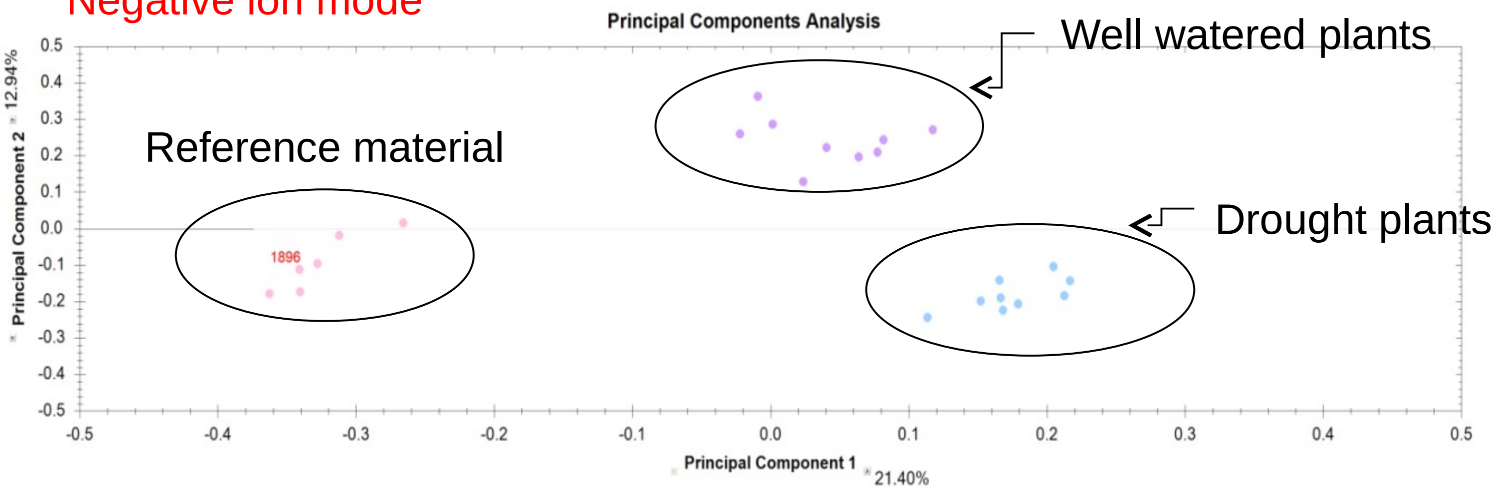
Baseline metabolome data from Medicago /pea

Baseline metabolome data from *Medicago* pilot – LC-HRMS PCA

Positive ion mode



Negative ion mode



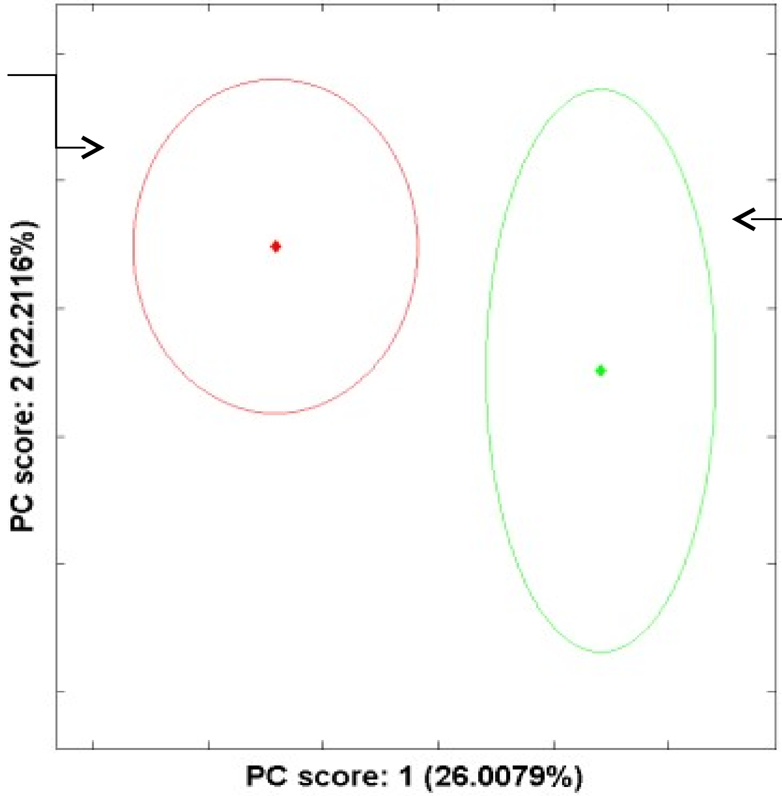
Example compounds signficantly changing in drought stressed ***Medicago*** tentatively identified by LC-HRMS

Tentative compound ID	Levels observed (Up / Down)	Ionisation mode	Mean max fold change
putrescine	↑	Positive	68.4
hesperetin (iii)	↑	Both	39.1
dihydrobiochanin A (ii)	↑	Positive	33.7
glutathione	↓	Positive	31.7
pisatin (i)	↑	Positive	31.0
ascorbate	↓	Negative	29.4
ferreirin (iii)	↑	Both	25.3
4-anisic anhydride (ii)	↑	Both	24.9
dehydroascorbate	↓	Positive	16.3
ermanin (i)	↑	Positive	15.0
proline	↑	Positive	12.9
cirsiliol	↑	Negative	8.2
hematoxylin (iii)	↑	Negative	7.8
histidine	↑	Positive	6.8
inositol (iv)	↓	Positive	6.4
abscisic acid	↑	Negative	5.0
raffinose	↑	Positive	4.6
glucose-6-phosphate	↓	Positive	4.4
hexapyranose (iv)	↑	Positive	3.9
oxylipin-3	↑	Positive	3.5
malic acid	↓	Negative	3.5
asparagine	↑	Positive	3.5
pectolinarigenin (i)	↑	Positive	3.4
gamma-tocopherol	↑	Positive	2.8

Baseline metabolome data from ***Medicago*** pilot – LC-HRMS PCA

Drought plants

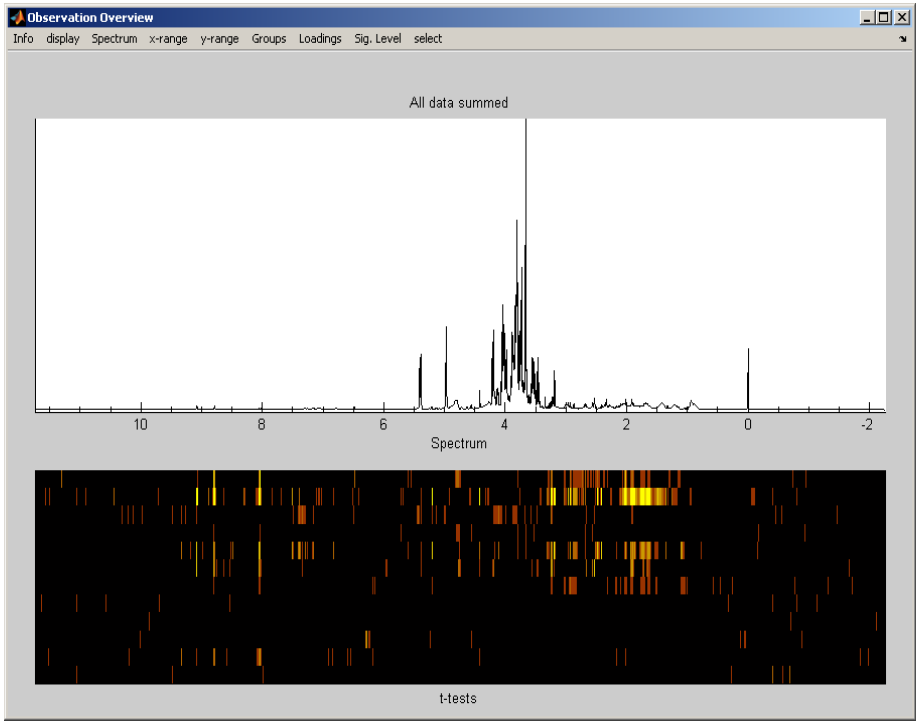
Well watered plants

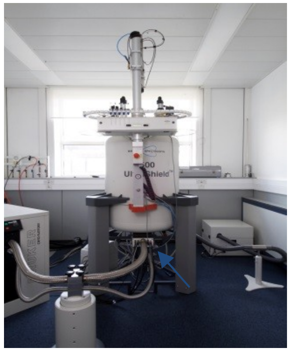


Metabolite variation in drought stressed vs well watered **pea** study: NMR data

- Significant t-test results highlighted by brighter colours

- Clearly metabolic changes associated with drought stress have been detected in the seeds





Metabolite variation in drought stressed vs well watered **pea** study: NMR data

Metabolite	Chemical shift correlations (ppm)
Proline	4.133-63.87; 3.342-48.72; 3.423-48.68; 2.071-31.55; 2.356-31.61; 2.009-26.41.
Leucine	1.712-42.34; 0.969-24.77; 0.957-23.68.
Isoleucine	1.013-17.24; 0.938-13.55.
Valine	3.615-63.25; 2.289-31.84; 1.049-20.64; 0.999-19.37.
Threonine	4.259-68.56; 1.336-21.90.
γ-aminobutyrate	3.012-41.92; 2.311-36.91; 1.904-26.21.
Homoserine (CID:7799)	3.858-56.13; 3.783-61.40; 2.152-34.82; 2.033-34.78.
Myoinositol (CID:892)	4.061-74.95; 3.624-75.24; 3.544-73.89; 3.270-77.14.
Trigonelline (CID:5570)	9.132-148.40; 8.841-147.50; 8.821-148.57; 8.089-130.17; 4.437-50.92.

Tasks

Task No.	Activities	Inst. Responsible for Task	Person(s) Responsible for Task
T1.1	Defining experimental setup for cross combination of *Fusarium*/drought stress	INRA/CSIC	

Description of the work

ABSTRESS
improving the resistance of legume crops to combined Abiotic and Biotic STRESS

R. Defez and C. Bianco, ARTERA

WP1

Task 1:
Defining experimental setup for cross combination of Fusarium/drought stress

Task 2:
Produce plant material

Task 3:
Analyzing data

Task 4:
Metabolomics

Task 5:
Role of symbiotic bacteria

ample,
molec analysis

A ce of
rhi
nod er of
nodu ct on N
fi ate of
sen e of nodule

Impact of mutated genes on performance of symbiotic N fixation in fusarium/drought stress interaction in **WP3**

Time (months)	6	1	1	2	3

The interaction between legumes and rhizobia leads to the development of a nitrogen-fixing symbiosis

M. truncatula

S. meliloti

Root nodules

Sample	Shoot dry wt (mg)	Ratio	Seeds wt (g/plant)	Ratio	Pods wt (g/plant)	Ratio

Several environmental factors can adversely affect the performance of symbiotic nitrogen fixation by legumes. These factors may act at the following levels: survival of rhizobia in the soil, the infection process, nodule growth and nodule function. These factors can also indirectly affect N2 fixing performance through their negative impact on the host plant growth.

wtr – wild type *rhizobium*
GMr – IAA-overproducing *rhizobium*

Journal of Experimental Botany Advance Access published May 12, 2009

Journal of Experimental Botany, Page 1 of 11
doi:10.1093/jxb/erp140

Journal of Experimental Botany
www.jxb.oxfordjournals.org

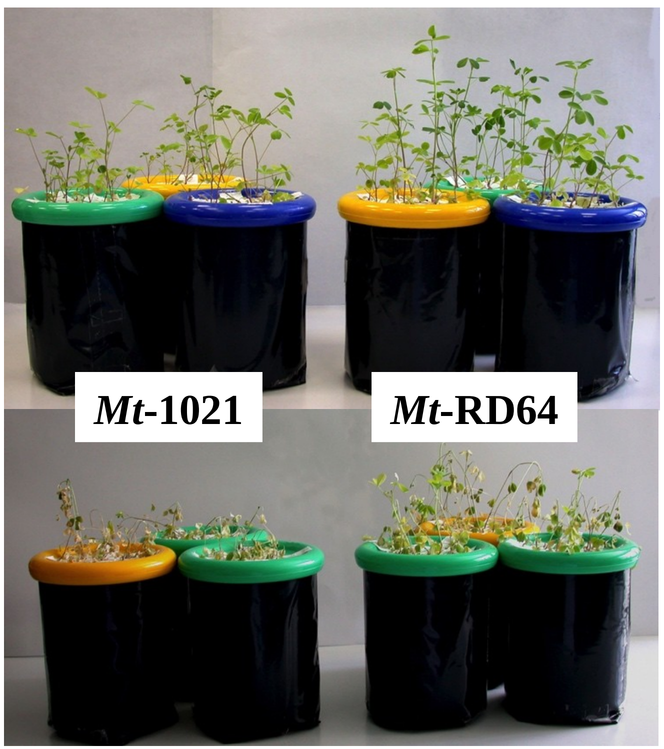
RESEARCH PAPER

Medicago truncatula improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain

Carmen Bianco and Roberto Defez*

Institute of Genetics and Biophysics 'Adriano Buzzati Traverso', via P. Castellino 111, 80131 Naples, Italy

Received 12 March 2009; Revised 3 April 2009; Accepted 6 April 2009



Control **NaCl-treated**

Under salt-stress condition, reduced symptoms of senescence, lower expression of ethylene signalling genes, lower reduction of shoot dry weight, and better nitrogen-fixing capacity have been observed for Medicago plants nodulated by an IAA-overproducing *S. meliloti* strain.

US007846708B2

(12) **United States Patent**
Defez

(10) **Patent No.:** **US 7,846,708 B2**
(45) **Date of Patent:** **Dec. 7, 2010**

(54) **METHOD FOR INCREASING THE SURVIVAL OF BACTERIAL STRAINS OF THE RHIZOBIUM GENUS**

(75) Inventor: **Roberto Defez**, Rome (IT)

(73) Assignee: **Consiglo Nazionale delle Richerch**, Rome (IT)

C12N 5/00 (2006.01)
C12N 5/02 (2006.01)

(52) **U.S. Cl.** **435/243**; 435/244; 435/252.1; 435/410; 435/420; 435/431

(58) **Field of Classification Search** .... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

Applied and Environmental Microbiology, July 2010, p. 4626–4632 Vol. 76, No. 14
0099-2240/10/$12.00 doi:10.1128/AEM.02756-09

Improvement of Phosphate Solubilization and *Medicago* Plant Yield by an Indole-3-Acetic Acid-Overproducing Strain of *Sinorhizobium meliloti*▽†

Carmen Bianco and Roberto Defez*
Institute of Genetics and Biophysics "Adriano Buzzati Traverso," Via P. Castellino 111, 80131 Naples, Italy

Received 13 November 2009/Accepted 18 May 2010

P sufficient P limiting
Mt-1021

P sufficient P limiting
Mt-RD64

Medicago truncatula plants nodulated by an IAA-overproducing *S. meliloti* strain and grown under P-deficient conditions showed significant increases in both shoot and root fresh weights when compared to those nodulated by the wild type strain.

US009157104B2

(12) **United States Patent**
Defez

(10) **Patent No.:** **US 9,157,104 B2**
(45) **Date of Patent:** **Oct. 13, 2015**

(54) **METHOD TO IMPROVE PHOSPHATE SOLUBILIZATION IN PLANTS**

(75) Inventor: **Roberto Defez**, Napoli (IT)

(73) Assignee: **Consiglio Nazionale delle Ricerche**, Rome (RM) (IT)

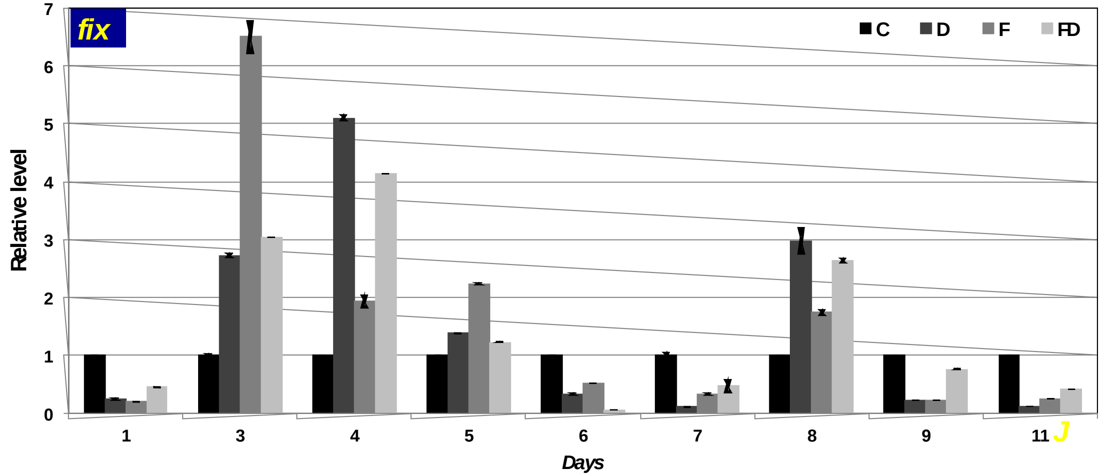
(56) **References Cited**

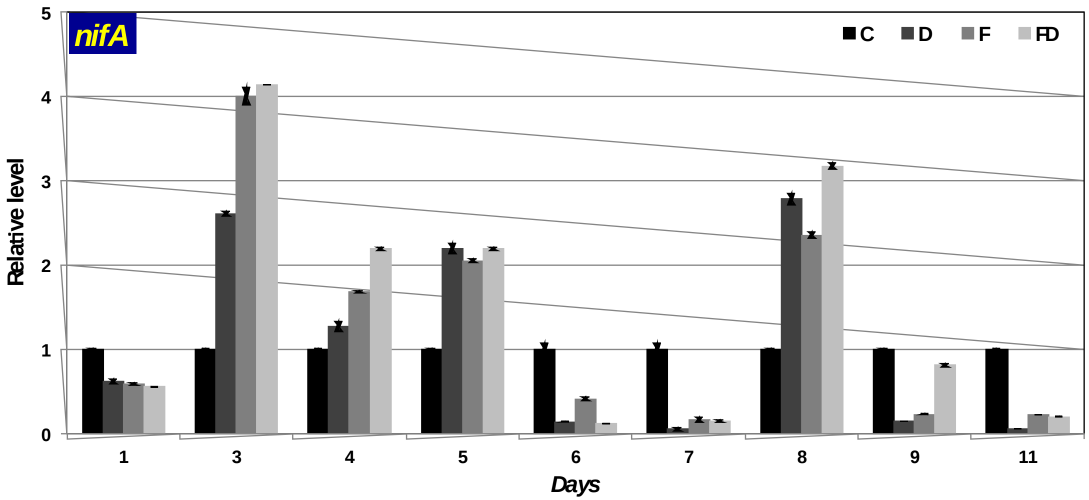
U.S. PATENT DOCUMENTS

7,846,708 B2 * 12/2010 Defez 435/243

FOREIGN PATENT DOCUMENTS

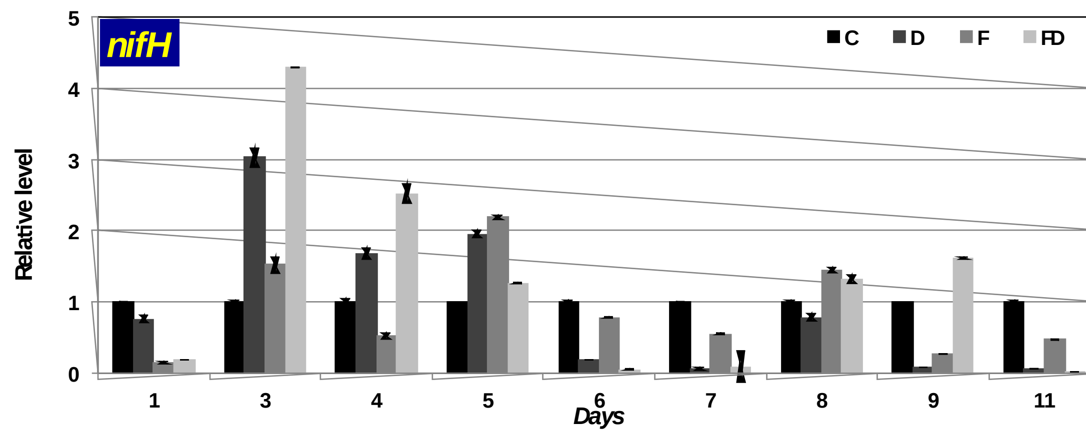
qPCR analysis of nif and fix genes in M. truncatula root nodules during drought stress and Fusarium attack



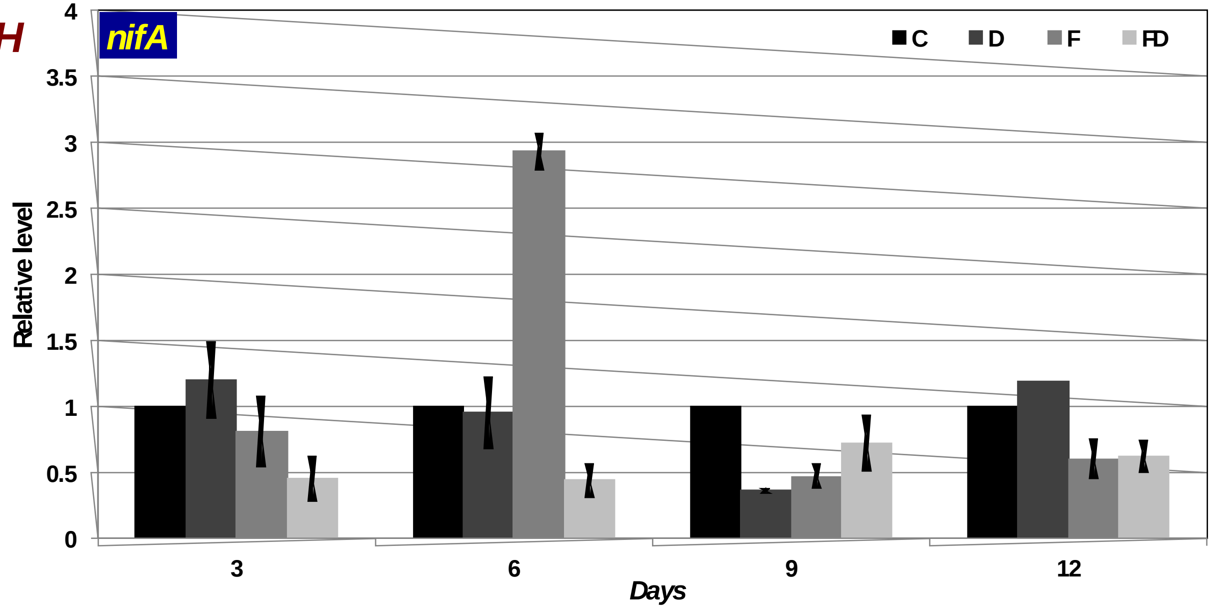


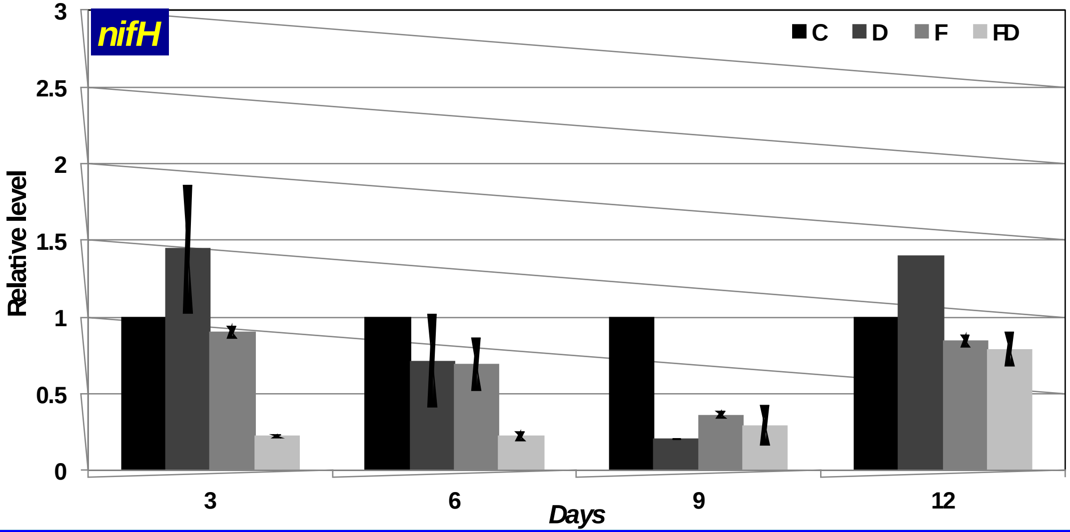
The relative expression levels shown in the Figures are >1 for genes more highly expressed in nodules of D (WS F-), F (WW F+) and FD (WS F+) plants as compared to C (WW F-) control plants. The data reported in the Figure are the means standard deviation of four biological replicates.

The negative effects related to the stress treatments were visible after six days of treatment. Indeed, at this time, all the three genes tested involved in nitrogen fixation were significantly repressed.



qPCR analysis of nifA and nifH genes in Pea root nodules during drought stress and Fusarium attack





The relative expression levels shown in the Figures are >1 for genes more highly expressed in nodules of D (WS F-), F (WW F+) and FD (WS F+) plants as compared to C (WW F-) control plants. The data reported in the Figure are the means standard deviation of four biological replicates.

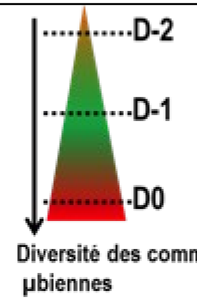
The data clearly show that the singular stress negatively affect the expression of selected genes and that the down-regulation is even more pronounced when double stress (DF) is applied. The down-regulation of N-fixation genes begins as early as the third day of treatment reaching its maximum after 6 day, after which the damage was retained.

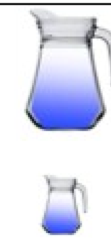
Impact of microbes diversity level

BQR project

- Impact of diversity level of soil microbial communities on pea plant response to water stress

Varying:	level of microbial diversity	Drought extent	Genotype

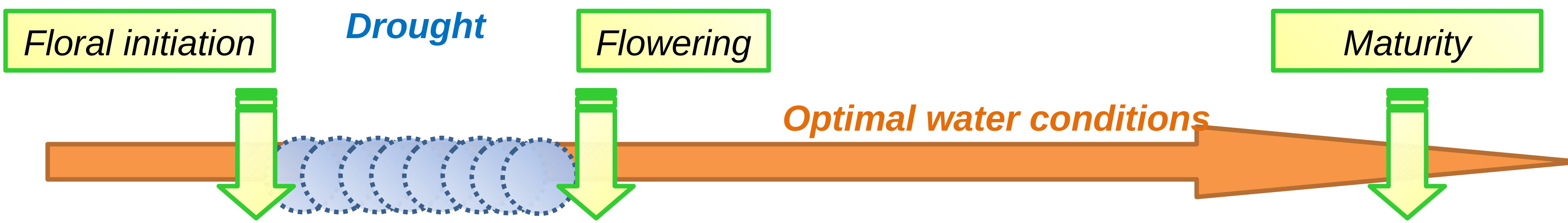




Pea (frisson)

Mutant myc-/nod-

- A higher diversity level of soil microbial communities



- has **no impact on pea drought tolerance…**

… but provides **better pea resilience after stress**

- Similar response with or without symbioses: **non symbiotic communities play a role in this response**

Write an ABSTRESS positioning paper on physiological characterization, 1 for pea and 1 for Mt

Thanks for your attention

Conclusions

- Both integrative and high resolution experiments : **drought greatly decreased carbon incorporation of both Medicago and pea, root biomass less impacted**.
- **Water stress negatively** impacted **nodule number** in **Medicago** while **mean nodule biomass** was targeted in pea.
- No clear trend concerning pathogen attack.
- Labelling experiment shown that N flow was greatly reduced by drought for pea (leaves, stems and nodules), while medicago seemed to be much less impacted for its compartments.

- In pea both i) leaf specific activity and ii) nodule biomass and nodule specific activity were severely decreased by water stress, not by fusarium.
- In Medicago, only leaf specific activity was reduced by water stress.
- A tight carbon/nitrogen relationships was obtained during the labelling experiment:
    - Allows estimating the degree of stress sensed by plants, efficiency to react faced to a stress.

- **Rhizotubes** mimics pot growth
- **Image analysis** is a powerful tool to follow dynamically and automatically, non-destructively shoot and root projected area.