

Towards automatic phenotyping of sunflower for their resistance to *Orobanche Cumana* at early stages of the interaction

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Context and objectives



New races of *O. cumana* have emerged these last years, overcoming some sunflower resistances



need of **new resistance sources**



Identifying and characterizing new resistance genes or QTL require **phenotyping tools**



The **rhizotron** system used in INRA Toulouse allows to grow *Orobanche*-inoculated sunflower plants, and screen for resistances at **early stage of the interaction**

Louarn et al., 2016, Frontiers in Plant Science



Objectives:

1-Optimization of the rhizotron culture system

2-Automatization of imaging and image analysis of rhizotron-grown *O. cumana* inoculated sunflower roots for rapid screening of resistances.

1 - The rhizotron culture system

★ 5 days old sunflower plantlets,

Race Bourret (France)

Genotype 2603



1-Optimisation of the rhizotron culture system

- ★ Test the effect of **Phosphate concentration** in the nutrient solution
½ Long Ashton with: Low Phosphate (**LowP**): **7,5 μM P** / High Phosphate (**HighP**): **750 μM P**
- ★ Test the effect of **pH** in the nutrient solution (½ Long Ashton, LowP and HighP)
pH = 3-4-5-6-7 (8-9-10 under progress)

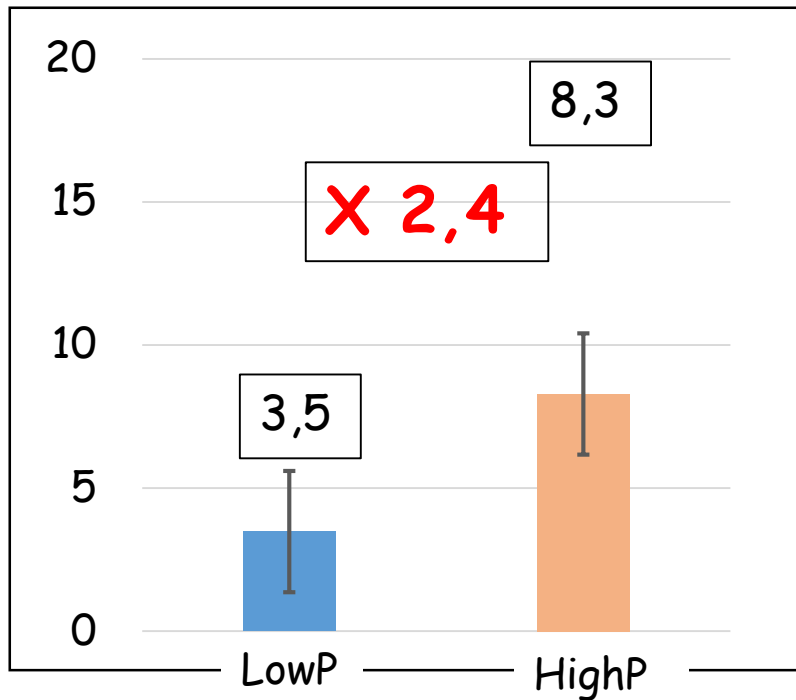


High Phosphate concentration in the nutrient solution increases significantly the tubercle number.

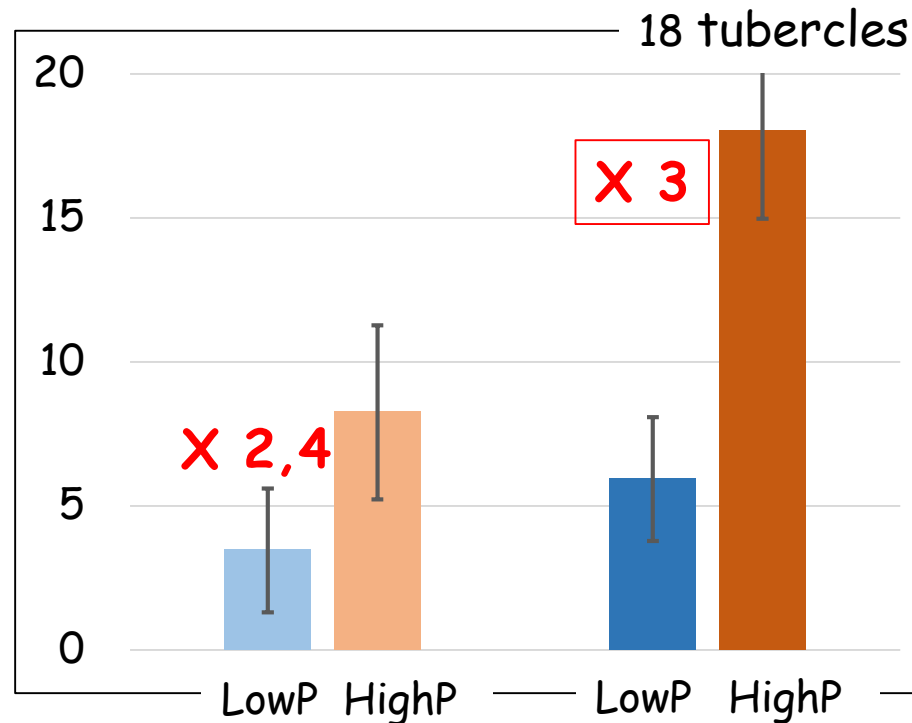
★ Test the effect of **Phosphate concentration** in the nutrient solution

½ Long Ashton with:

- Low Phosphate (LowP): 7,5 μM P
- High Phosphate (HighP): 750 μM P

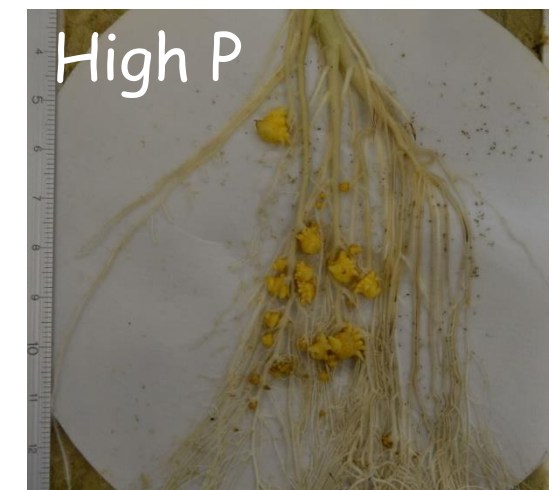
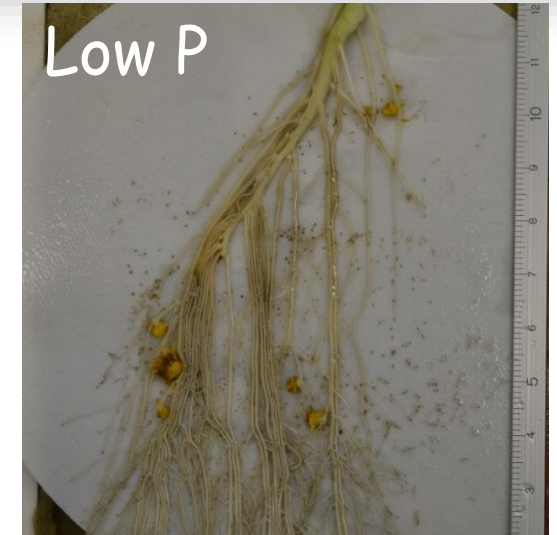


20 days



20 days

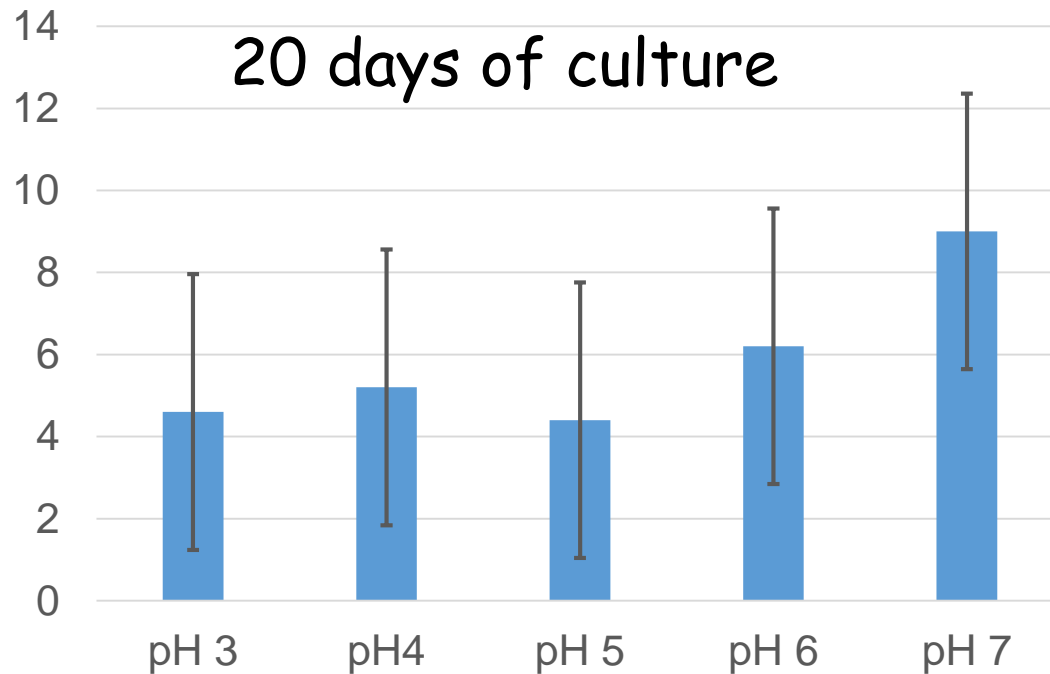
28 days



The pH of the nutrient solution (between 3 to 7) has no significant effect on the tubercle number in rhizotron



Test the effect of **pH** in the nutrient solution ($\frac{1}{2}$ Long Ashton, LowP and HighP)
pH = 3-4-5-6-7



Conclusions I

★ Nutrient solution of the rhizotron culture system is being optimized using **high phosphate** solution. These results suggest that:



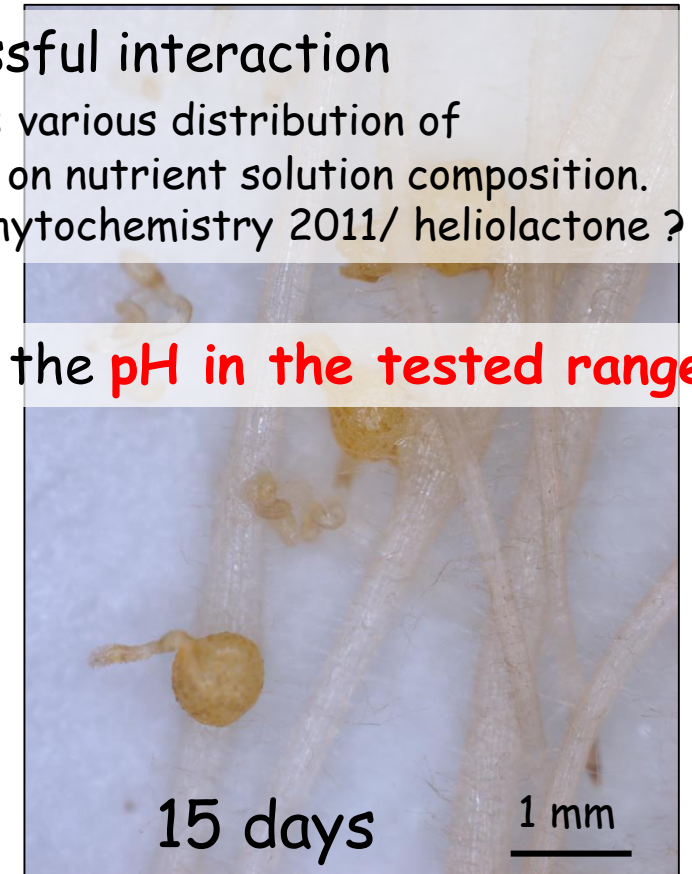
-tubercles can be detected earlier than following 21 days of culture



-Low Phosphate concentration is not essential for successful interaction

in line with the results of Ueno et al. (Phytochemistry, 2014): various distribution of germination stimulants in sunflower root exudates depending on nutrient solution composition. Dehydrocostus lactone (ind. of P concentration; Joël et al., Phytochemistry 2011/ heliolactone ?

★ The *O.cumana*-sunflower interaction was not dependent upon the **pH in the tested range**



II-The Raspberry imaging system

Raspberry Pi: a nano-computer



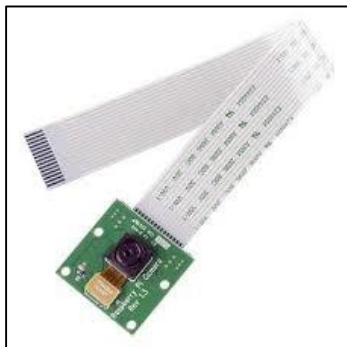
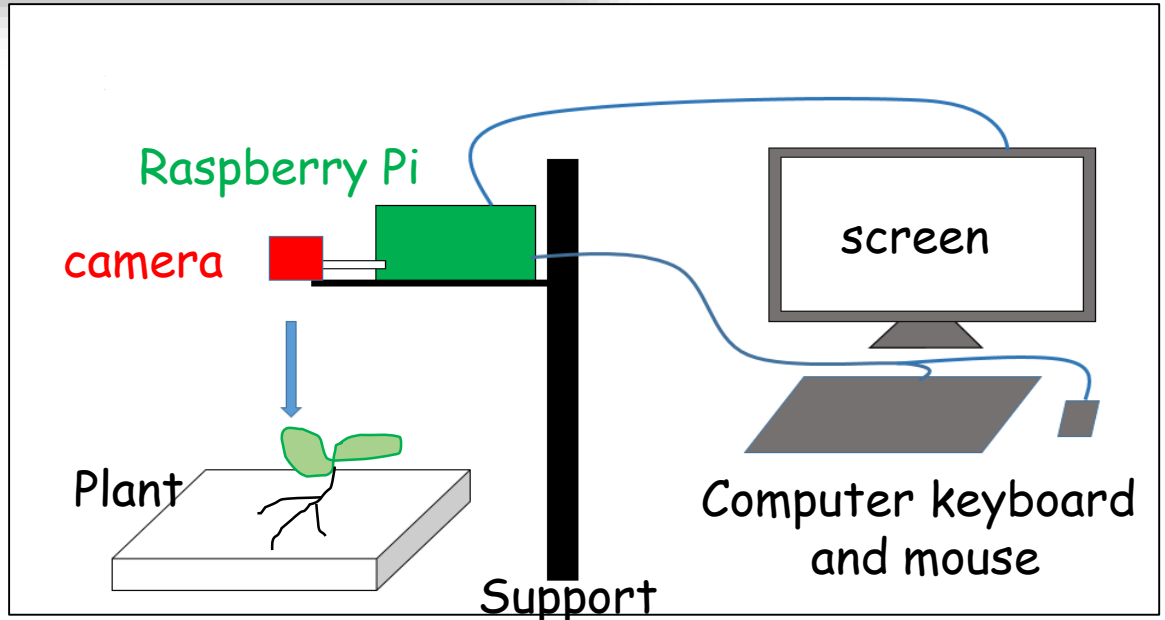
1 USB Port

2 Ethernet Port

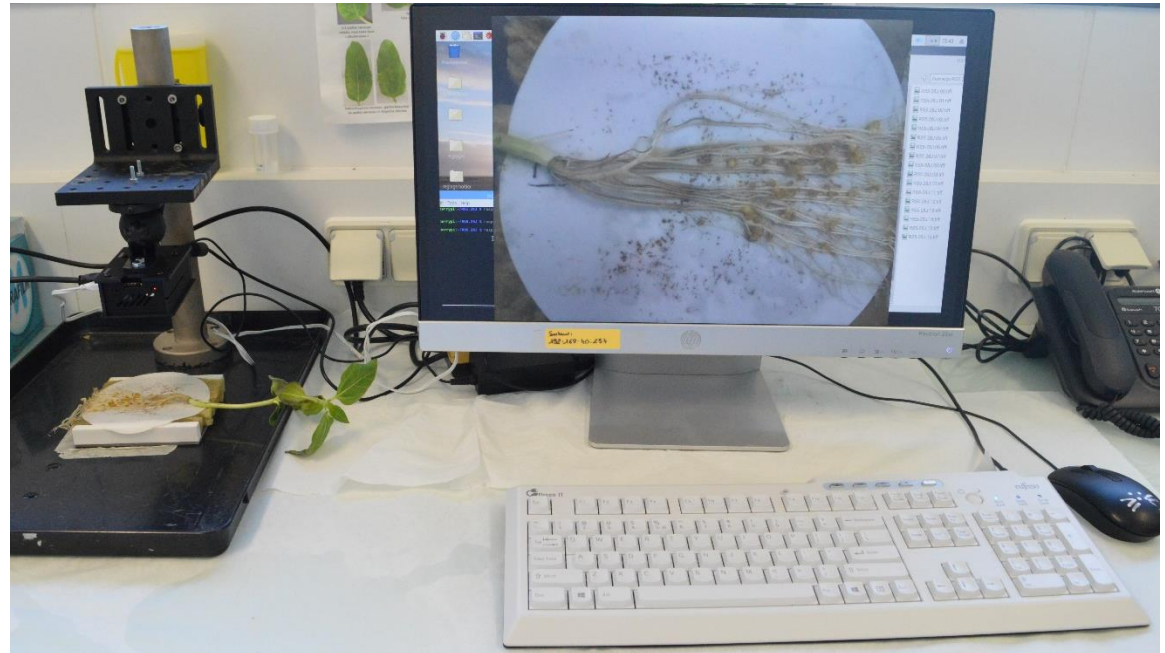
3 Camera plug

4 HDMI Port

5 Power supply



camera



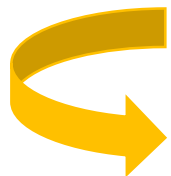
Comparison of Single Lens Reflex camera versus Raspberry-camera images



6016 x 4000 pixels



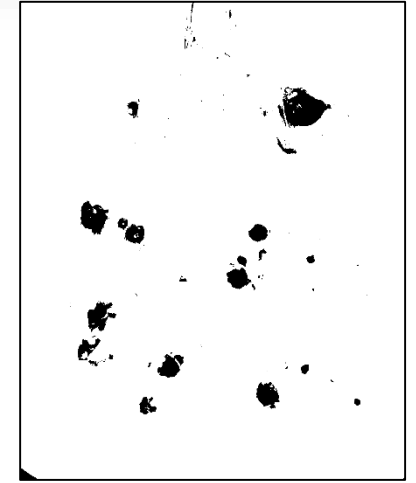
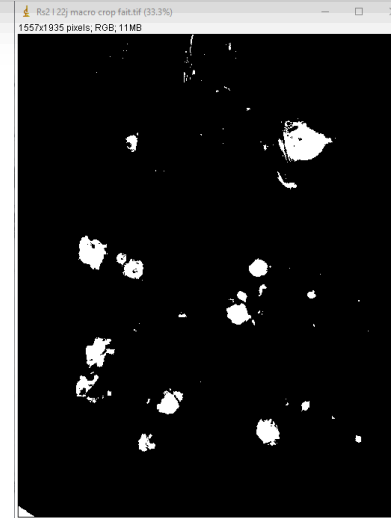
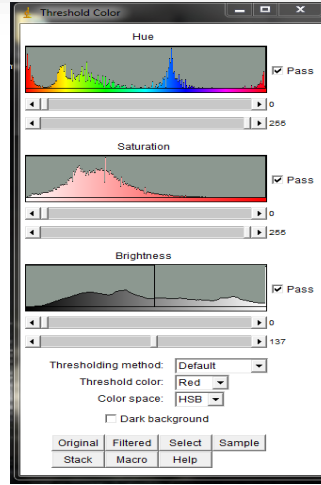
3280 x 2464 pixels



High quality images obtained with both camera, even if less definition (pixels) in Raspberry-camera images

Automatic counting of tubercules using Image J

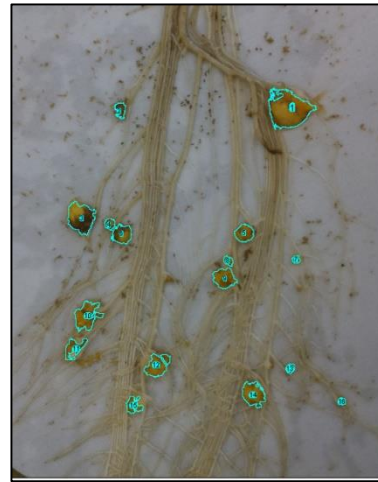
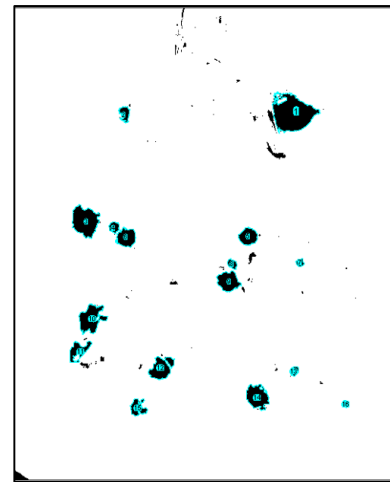
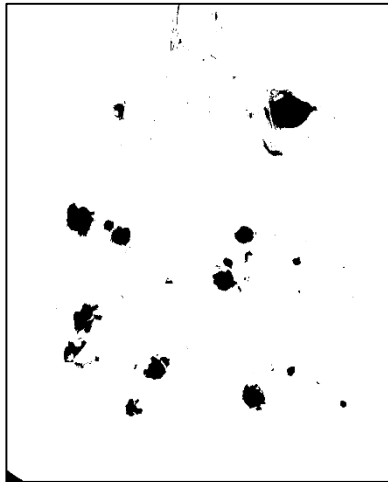
Image acquisition
(Raspberry camera)



1-crop image

2-color threshold

3-binary mask



| File | Edit | Font | Results |
|------|-------|--------|---------|
| | Area | XStart | YStart |
| 1 | 19321 | 1081 | 344 |
| 2 | 1842 | 467 | 402 |
| 3 | 946 | 1046 | 416 |
| 4 | 1421 | 1038 | 548 |
| 5 | 10183 | 269 | 805 |
| 6 | 1401 | 409 | 876 |
| 7 | 4058 | 961 | 898 |
| 8 | 4710 | 451 | 899 |
| 9 | 647 | 984 | 1001 |
| 10 | 793 | 1181 | 1026 |
| 11 | 1136 | 888 | 1028 |
| 12 | 5619 | 893 | 1078 |
| 13 | 7918 | 314 | 1213 |
| 14 | 3766 | 309 | 1365 |
| 15 | 6257 | 590 | 1429 |
| 16 | 970 | 1150 | 1471 |
| 17 | 6761 | 1009 | 1542 |
| 18 | 2916 | 500 | 1602 |
| 19 | 543 | 1353 | 1608 |

4-fill holes

5-analyse particles
(size over 500 pixels)

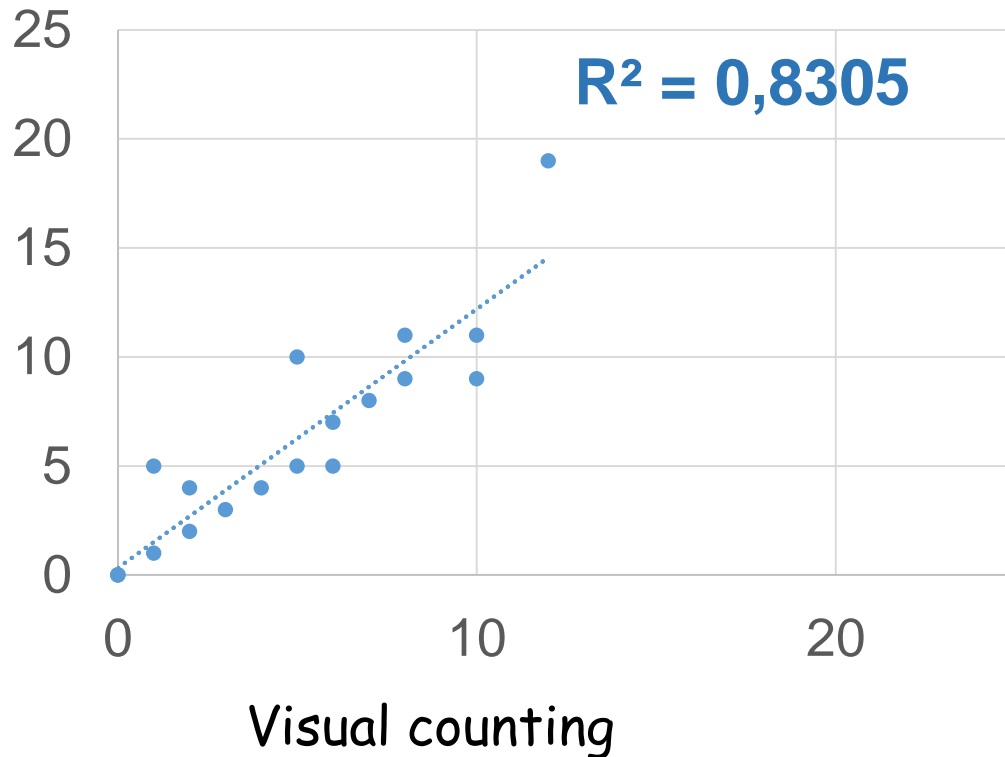
Tubercles are
counted and measured
semi-automatically

Comparison visual/ Image J-automatic counting

ImageJ-automatic counting

-median of:
5,5 tubercles/ plant within 3 weeks of culture

-coefficient of correlation R^2 between visual and semi-automatic Image J tubercle counting =
0,83 at 3 weeks of culture



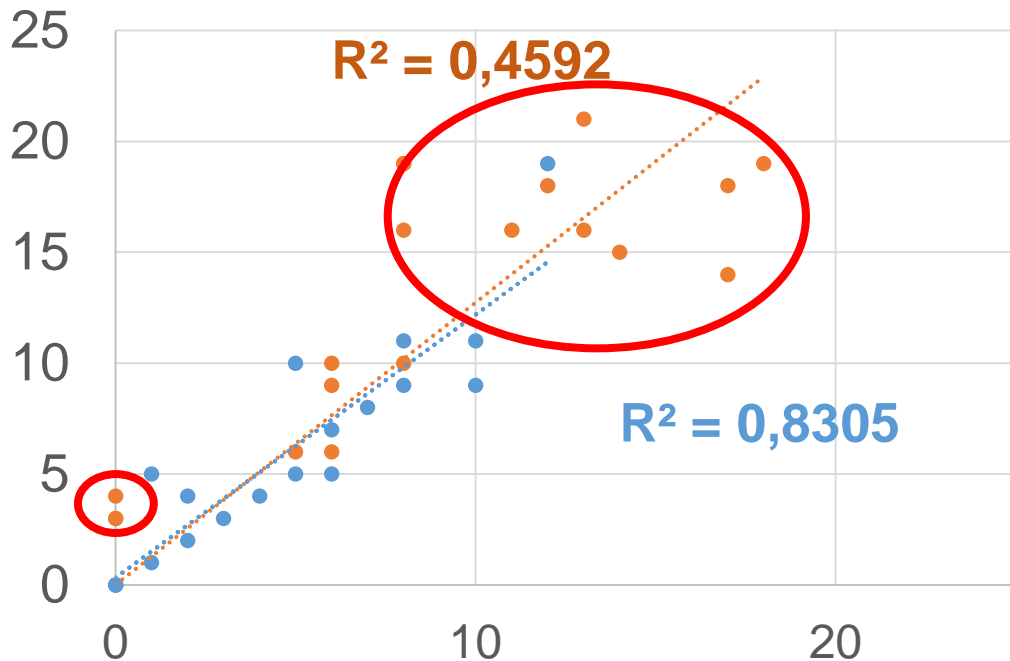
Race Bourret (France)

Genotype 2603

total of 19 rhizotons, 2 experiments
(22/23 days of culture)

Comparison visual/ Image J-automatic counting

ImageJ-automatic counting



Visual counting

total of 19 rhizotons, 2 experiments
(22/23 days of culture
And 28-30 days of culture)

-median of:

5,5 tubercles/ plant within 3 weeks of culture

9,5 tubercles/ plant within 4 weeks of culture

-coefficient of correlation R^2 between visual and semi-automatic Image J tubercle counting =

0,83 at 3 weeks of culture

0,46 at 4 weeks of culture

Very good correlation between visual and ImageJ-semi automatic counting of tubercle numbers in rhizotron culture within **3 weeks of culture**, but need improvements at 4 weeks of culture.

Counting of tubercle numbers can be performed **automatically** by developing a **Macro** using Image J software. This work is under progress.

Conclusions II

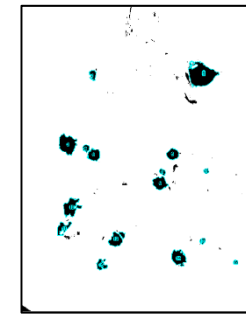
★ We developed a Raspberry-camera system allowing good imaging for Image J-automatic analysis of tubercle-number of rhizotron-grown *Orobanche cumana* inoculated sunflower plants

more than **95 %** of the tubercles can be identified with automatic image analysis.

An automatic raspberry-camera/image analysis system is being developed combining

1-image acquisition with the raspberry-camera,

2-and a Macro using Image J for automatic counting of tubercle number



| Results | | | |
|---------|-------|--------|---------|
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| 19 | 543 | 1353 | 1608 |

These improvements of culture, imaging and image analysis should facilitate the development of **an automatic phenotyping platform in growth chamber** for screening of *O. Cumana* resistance in sunflower

Thanks to:

Imag 

LIPM-Toulouse
Guillaume Ibarcq
Marie-Claude Boniface
Nicolas Pouilly
Nicolas Langlade
Stéphane Munos

FRAIB-Toulouse
Aurélie Leru

MAS seeds
(Haut Mauco-France)
Aurélie Baussart

Foundings



Additional slides

The rhizotron culture system

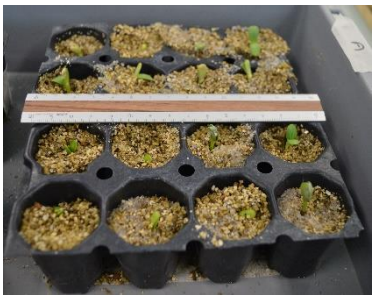
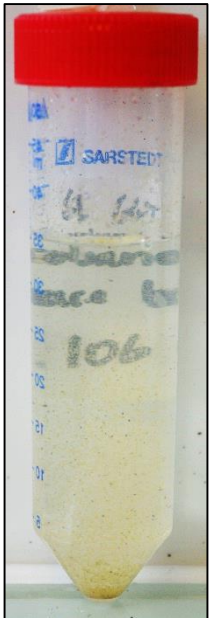
★ 7 day-**conditioned** *O. cumana* seeds (in water- 22°C-dark)

Race Bourret (France)

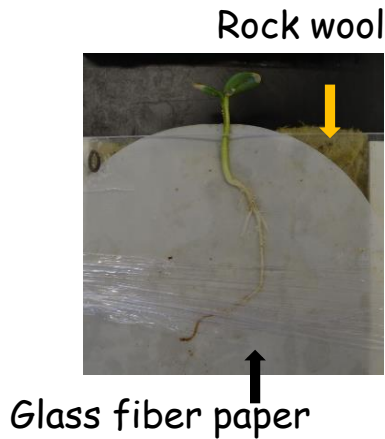
★ **Sterilized** sunflower seeds, **germinated** 2 days at dark, 25°C
Transfer for 3 days in 50/50 sand/vermiculite

Genotype 2603

2 day-germinated
sunflower seeds



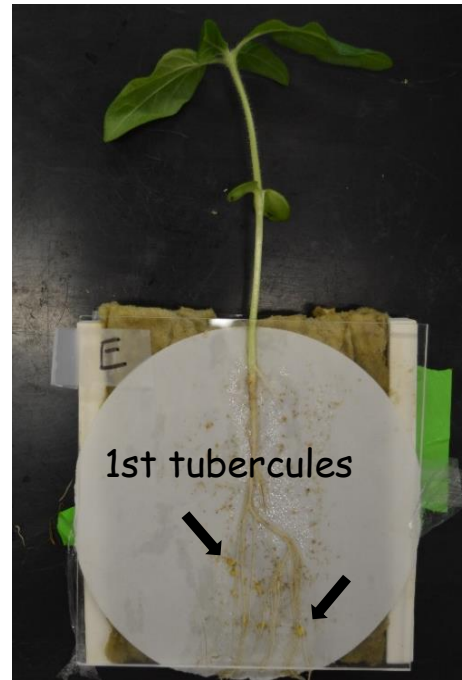
10 mg *O. cumana*
seeds/ rhizotron



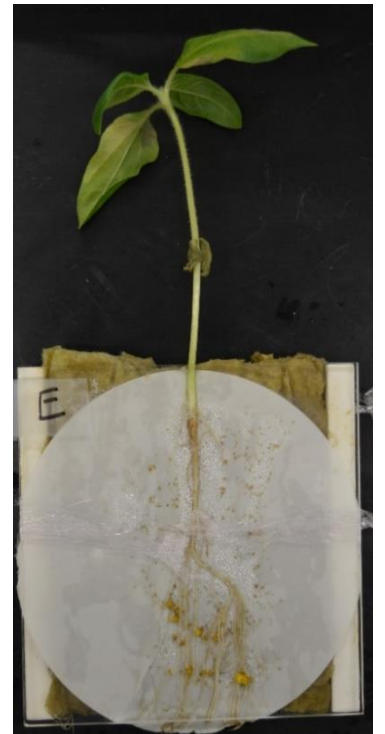
1 day



15 days



21 days



28 days

7 day-
conditioned
O. cumana seeds

+3 days in
sand/vermiculite

