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METABOLOMIC STUDIES FOR THE INTERACTION *GLYCINE MAX-FUSARIUM TUCUMANIAE*

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Sudden-death syndrome (SDS) of soybean can be caused in Argentina by 4 different *Fusarium* species: *F. brasiliense, F. crassistipitatum, F. tucumaniae* and *F. virguliforme*. *Fusarium tucumaniae* and *F. virguliforme* are the primary etiological agents of soybean SDS in Argentina and United States, respectively. The resistance mechanism to these pathogens in soybean is complex. Metabolomic technology was explored to phenotype resistance to *F. tucumaniae*. Two soybean cultivars (CV) with contrasting levels of resistance to SDS were inoculated with an isolate of *F. tucumaniae* grown on sorghum grain, using the layer method. Uninoculated controls were included for both genotypes. Plants were grown for 7, 10, 14 and 25 days. Four independent biological replicates were harvested at indicated times. Foliar disease, root rot incidence and severity, shoot height, shoot and root weights were rated at each time. Areas under disease severity progress curves were also calculated 25 days after inoculation (DAI). All data were subjected to statistical analysis. Means were compared by least significant differences ($p<0.05$). The resistant cultivar showed lower foliar disease and root rot incidence and severity, higher plant height, and root and shoot weights, than the susceptible cultivar. Uninoculated controls remained healthy. Experiments were extended to root metabolite profiling by gas chromatography mass spectrometry (GC-MS). Metabolite levels were normalized to the ribitol internal standard. Compounds were putatively identified by comparison of their retention index and mass spectrum with those present in the commercial mass spectra library NIST. Data from two biological replicates from tolerant and susceptible CVs obtained at 7, 10, 14 and 25 DAI were evaluated by principal components (PC) and principal coordinates analysis (ACoP). Analyses were either performed for individuals or pools. Results obtained allow us to identify and monitor the relative levels of 30 metabolites, including amino acids, organic acids, soluble sugars, secondary metabolites, inorganic and nitrogen compounds. These metabolites were more abundant at 7 and 10 DAI. The first two PCs (PC1 and PC2) explained 76.97 %, 84.88% and 79.11% of the variance of the susceptible, tolerant and pooled CV profiles, respectively. As these values are lower than expected (> 90%), the first four PCs had to be considered. In the susceptible CV, inorganic phosphate and sucrose showed the highest weight in PC1 and PC2, respectively. However, in the tolerant CV, inositol and sucrose were the most important variables in PC1 and malonic acid, citric acid, inorganic phosphate and myoinositol heavily contributed to PC2. When these data were pooled and analyzed, inorganic phosphate and sucrose were associated with PC2 but no variables could be identified in PC1. In accordance with PC analysis, the first two ACoP factors (F1 and F2) explained the 63.90%, 67% and 43.34% of the variance of the susceptible, tolerant and pooled CV profiles, respectively. Four DAI metabolic profiles could be clearly separated in the tolerant CV, but 7 and 10 DAI remained close together in the susceptible CV and in the pool analyses.

METABOLOMIC STUDIES FOR THE INTERACTION *Glycine max-Fusarium tucumaniae*

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Introduction

Sudden death syndrome causal agents

- ***F. tucumaniae***
- ***F. virguliforme***

} are the primary etiological agents of SDS in Argentina and United States

- *F. brasiliense*
- *F. crassistipitatum*

➢SDS has increased in prevalence and severity resulting in significant yield losses to Argentinean soybean farmers.

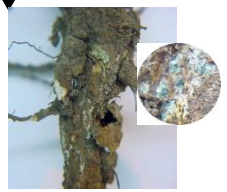
➢Yield losses vary considerably depending on several factors, but yield losses up to 41% were estimated in a trial conducted during the 2010/2011 growing season.

➢The use of resistant cultivars is one of the main measures for managing SDS. The resistance mechanism to these pathogens in soybean is complex.

➢Screening for SDS resistance has been conducted under field conditions, both in natural and artificially infested soil. Even when cultivars are screened in artificially infested soil, disease development is unpredictable due to the sensitivity of symptomology to environmental factors.

Isolating and identification of sudden death syndrome-causing *Fusarium* species

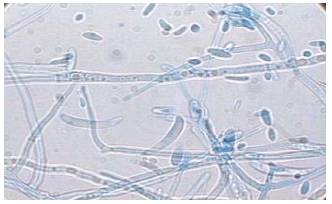
Soybean plant exhibiting typical SDS symptoms



Isolating (two protocoles):

*Desinfecting tissues

*Direct macroconidia transferring



F. solani

F. solani (clade 3), Macroconidia and microconidia abundant on long conidiophores (x1000).

Determine growth rate on PDAS:

1) Fast growing → Discard those colonies

2) Slow growing (≤2 cm in 4 days) → Keep those colonies

Identification

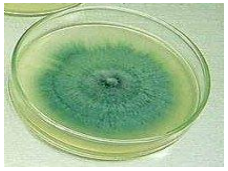
**** Colour***

**** Conidia***

Colour of colonies:

1) yellow

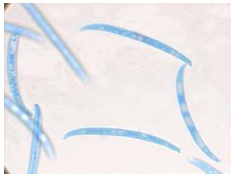
2) blue-green

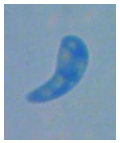


F. crassistipitatum

F. tucumaniae
F. virguliforme
F. brasiliense

Microconidia are rarely observed





F. tucumaniae produces macroconidia longer and slender than *F. virguliforme*, this produces comma-shaped microconidia.

F. brasiliense
Macroconidia shorter and wider than *F. tucumanaie.*

Pathogenicity test

Symptoms of Sudden Death Syndrome

Foliar symptoms of SDS.
©Lisandro Lenzi INTA-EEA Marcos Juárez (Córdoba).

Root rot with blue sporulation.

Field symptoms of Sudden Death Syndrome

Main objective

Metabolomic technology was explored to phenotype resistance to *F. tucumaniae*.

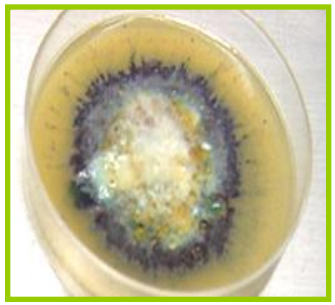
Specific objectives

1. **To identify which metabolites are produced during the host-pathogen interaction *Glycine max-F. tucumaniae*, in susceptible and resistant genotypes;**
2. **To determine the starting point of this response and the ocurrence of metabolic changes during the experiment, on roots and shoots;**
3. **To compare the metabolic profiles in these tissues and id the metabolic pathway of each one.**

Materials and methods

This study was conducted to detect and quantify metabolomic changes and identify potential biomarkers associated with this interaction.

Sorghum grain



Layer method

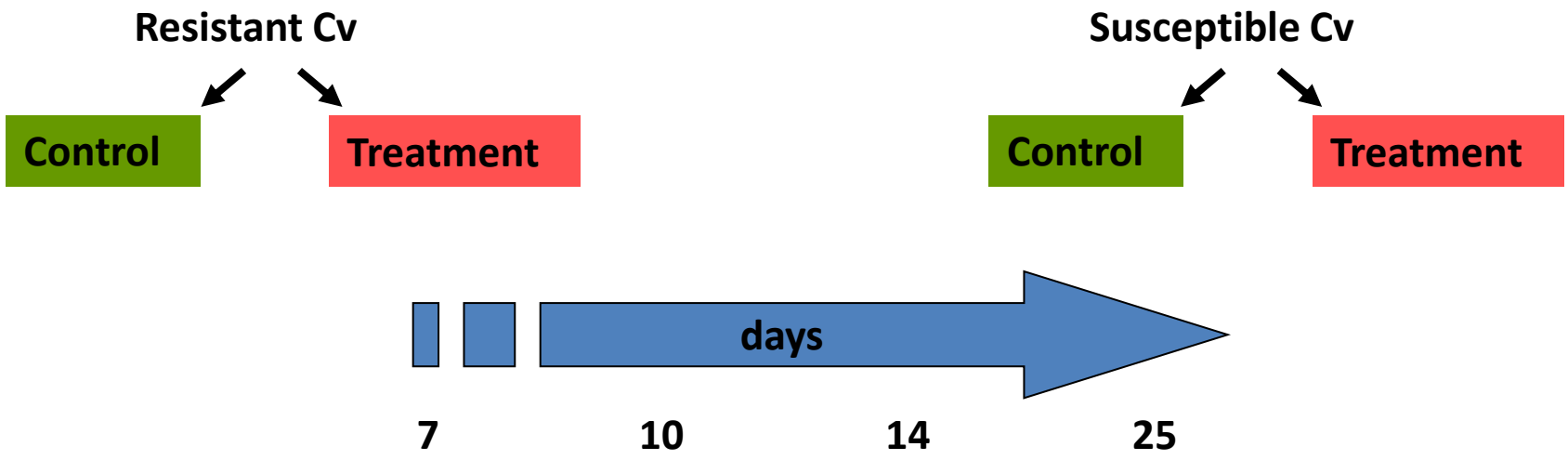
5 seeds/ pot

Susceptible

Resistant

Disease rating

Root and shoot metabolite profiling by GC-MS (gas chromatography mass spectrometry)



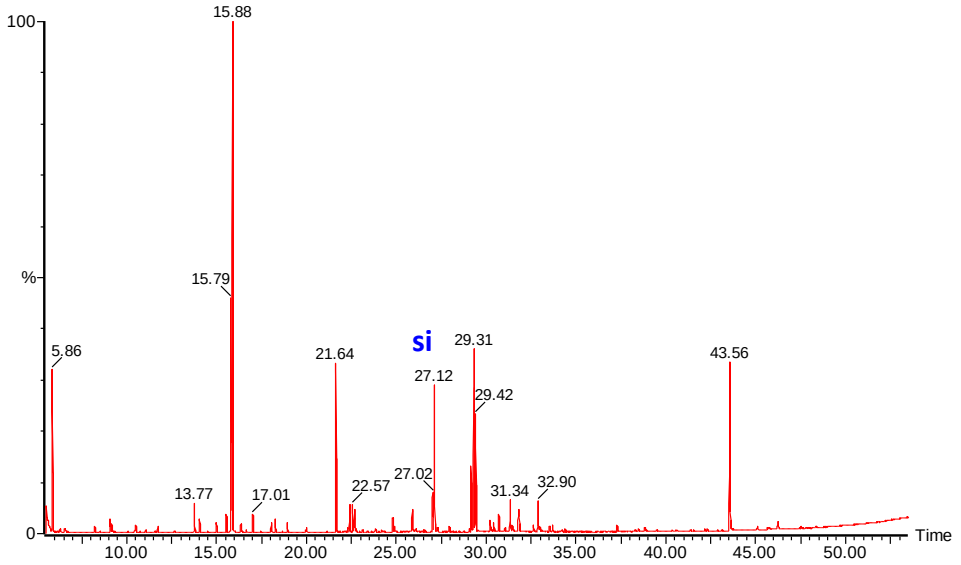
Foliar disease, root rot incidence and severity, shoot height, shoot and root weights were rated at each time. Areas under disease severity progress curves were also calculated 25 days after inoculation. All data were subjected to statistical analysis. Means were compared by least significant differences ($p<0.05$).

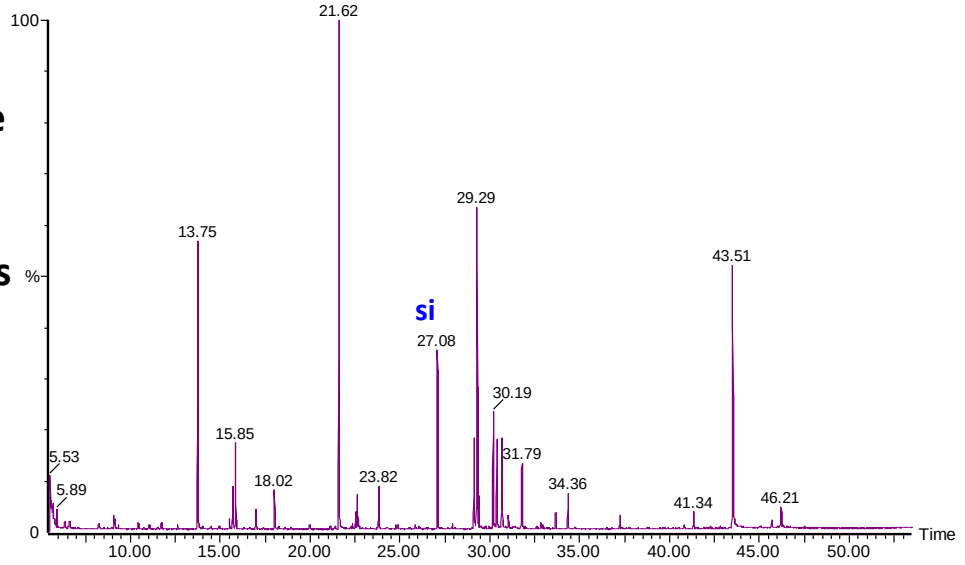
Shoots

Roots

2 BR

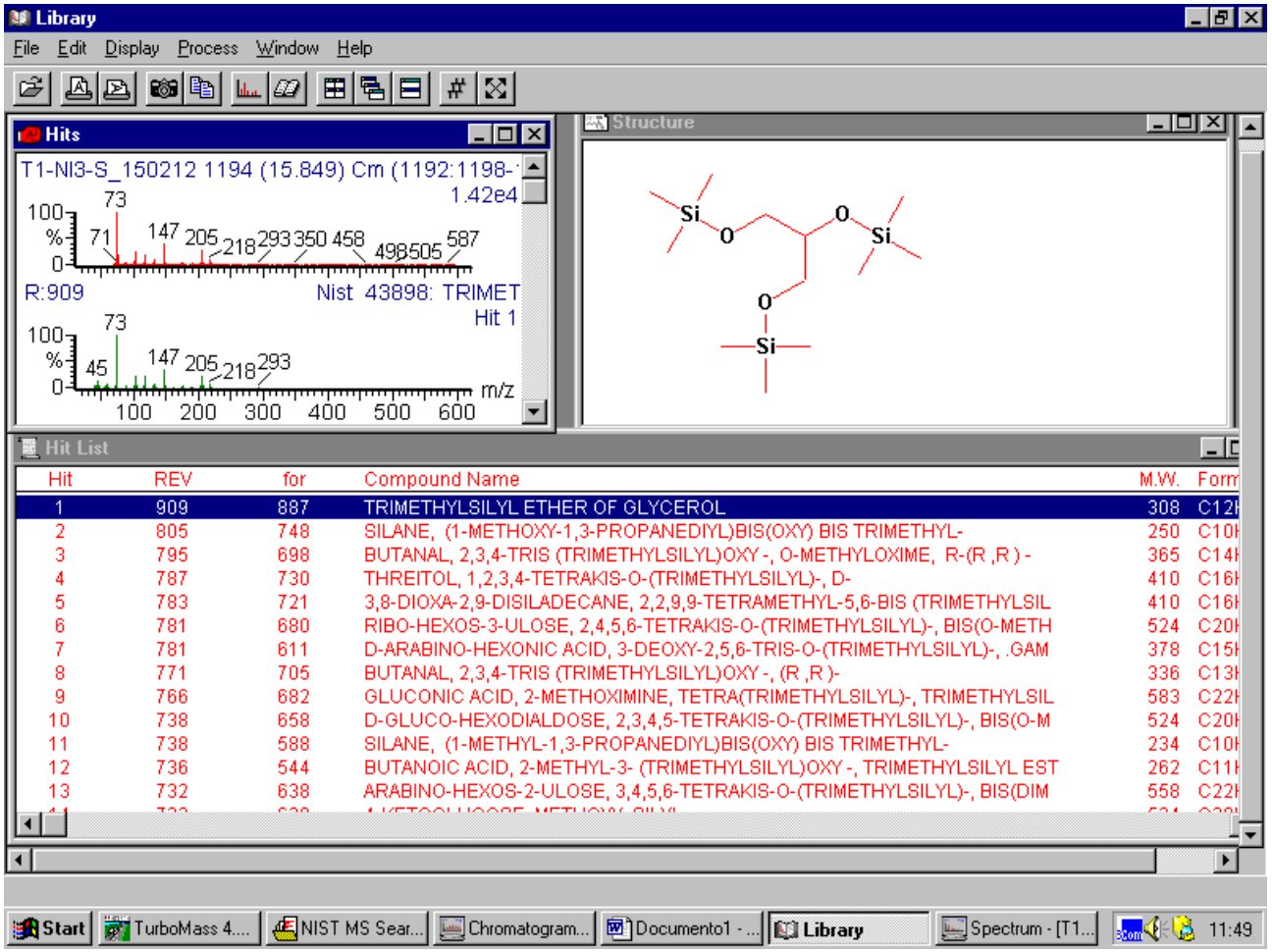
Analysis by GC-MS
(4 technical replicates)

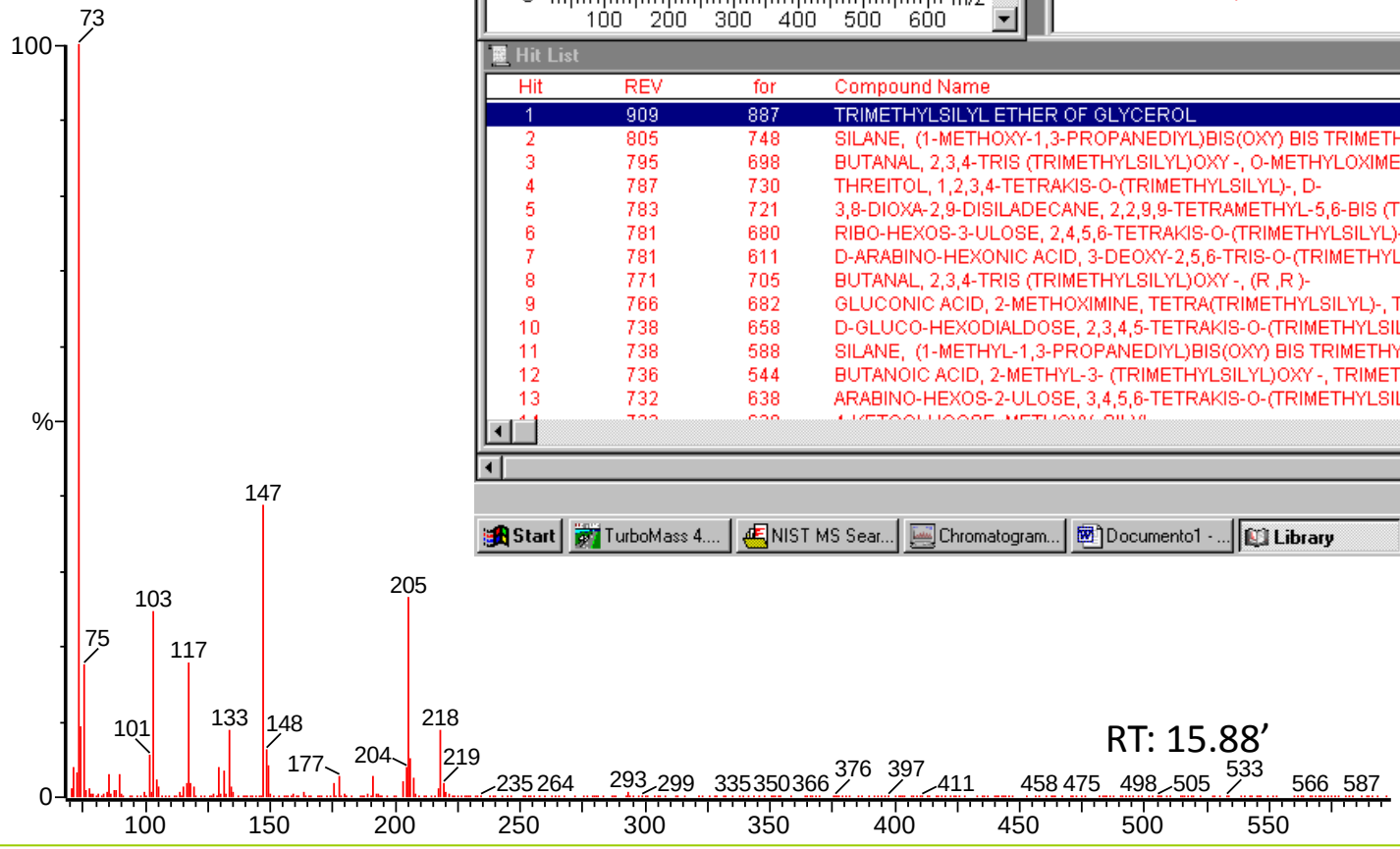




Root metabolite profiling by GC-MS. Metabolite levels were normalized to the ribitol internal standard. Data from two BR were evaluated by principal components and principal coordinates analysis.

Compounds were identified by comparison of their retention index and mass spectrum with those present in the commercial mass spectra library NIST.

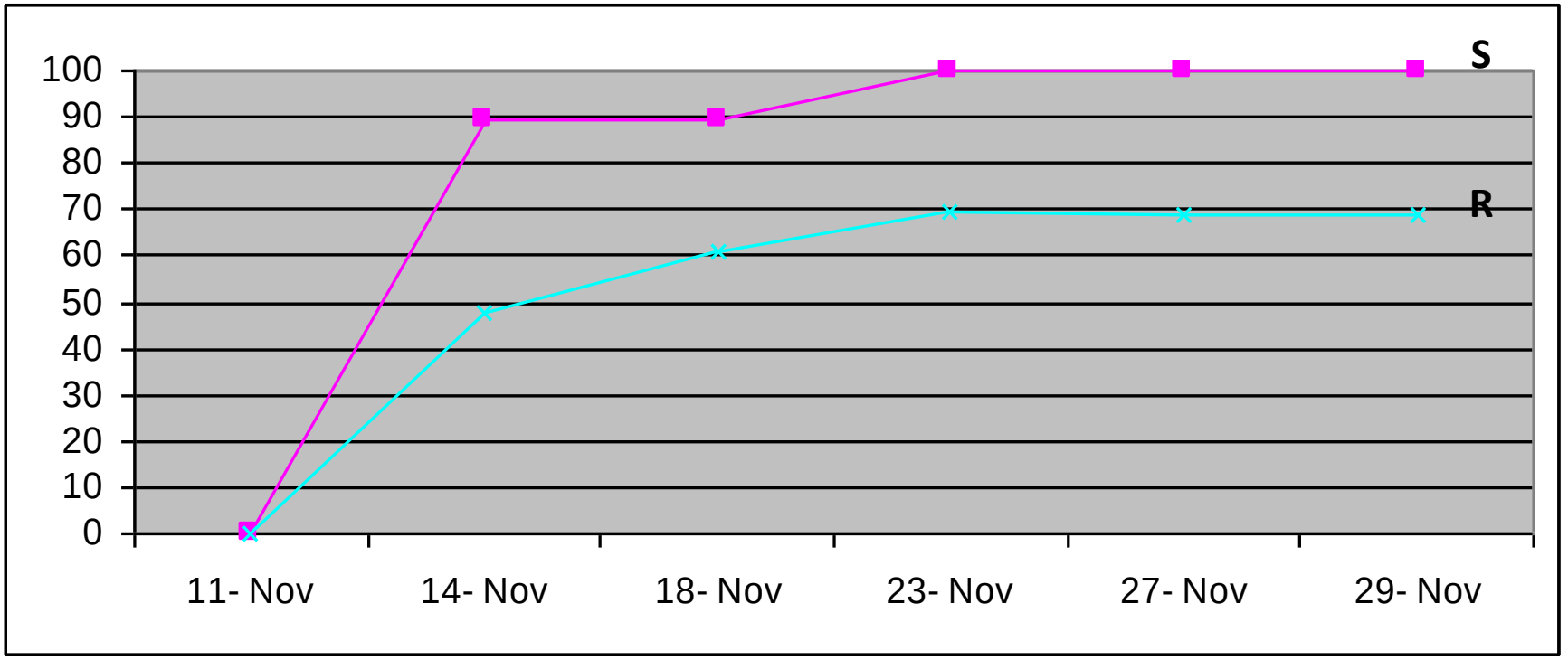




Results

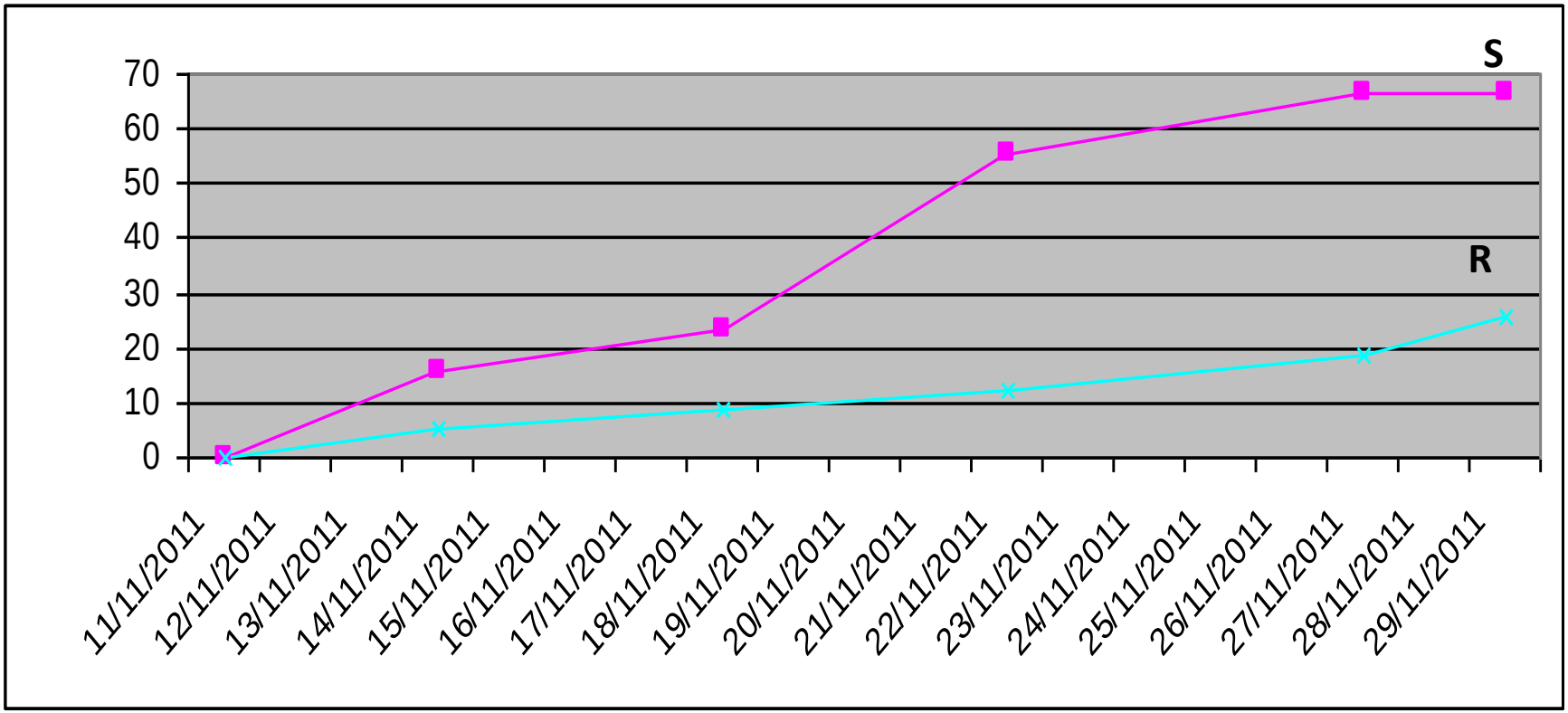
✓ **The resistant cultivar showed lower incidence than the susceptible cultivar.**

Incidence (% plants with foliar symptoms)



✓**The resistant cultivar showed lower foliar disease severity than the susceptible cultivar.**

Foliar disease severity



AUDPCs Susceptible 492

Resistant 158

10 days after-inoculation

Resistant

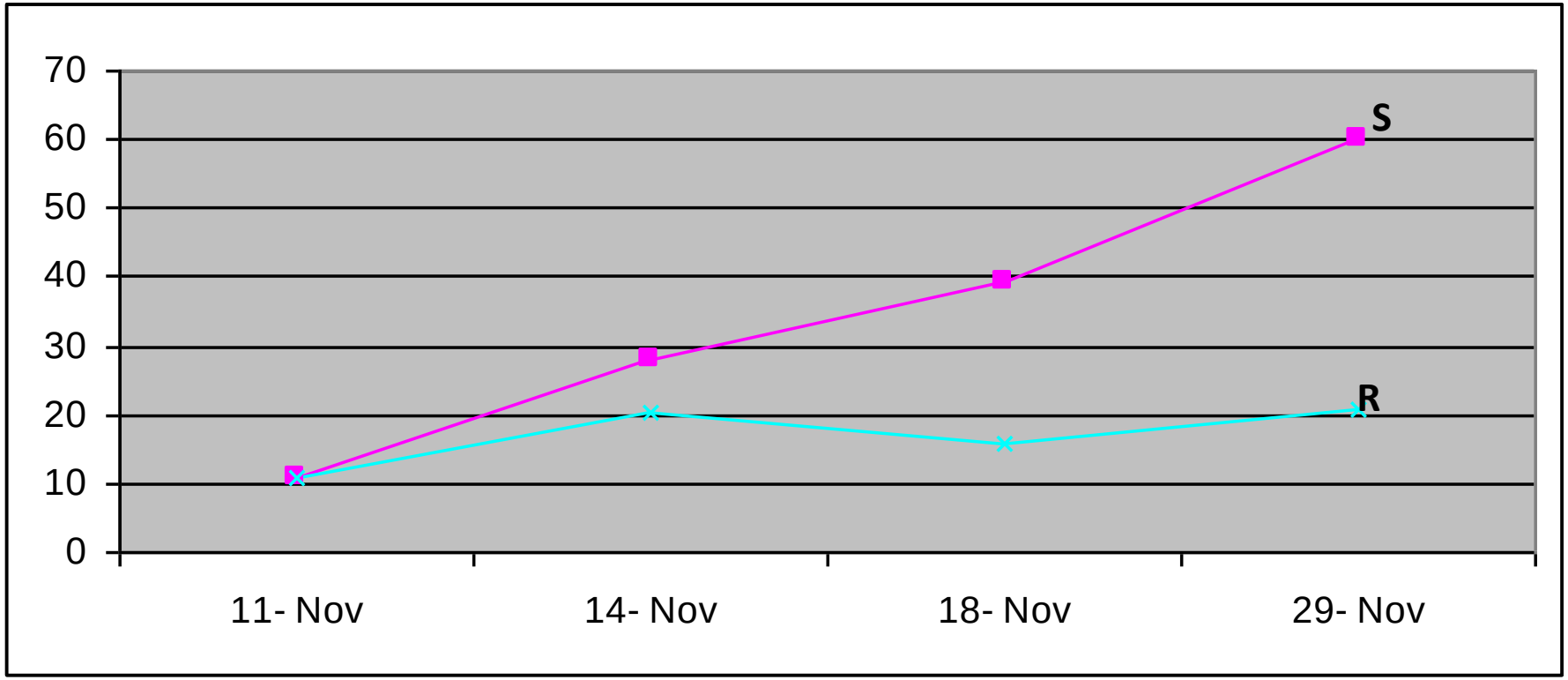
Susceptible

Control

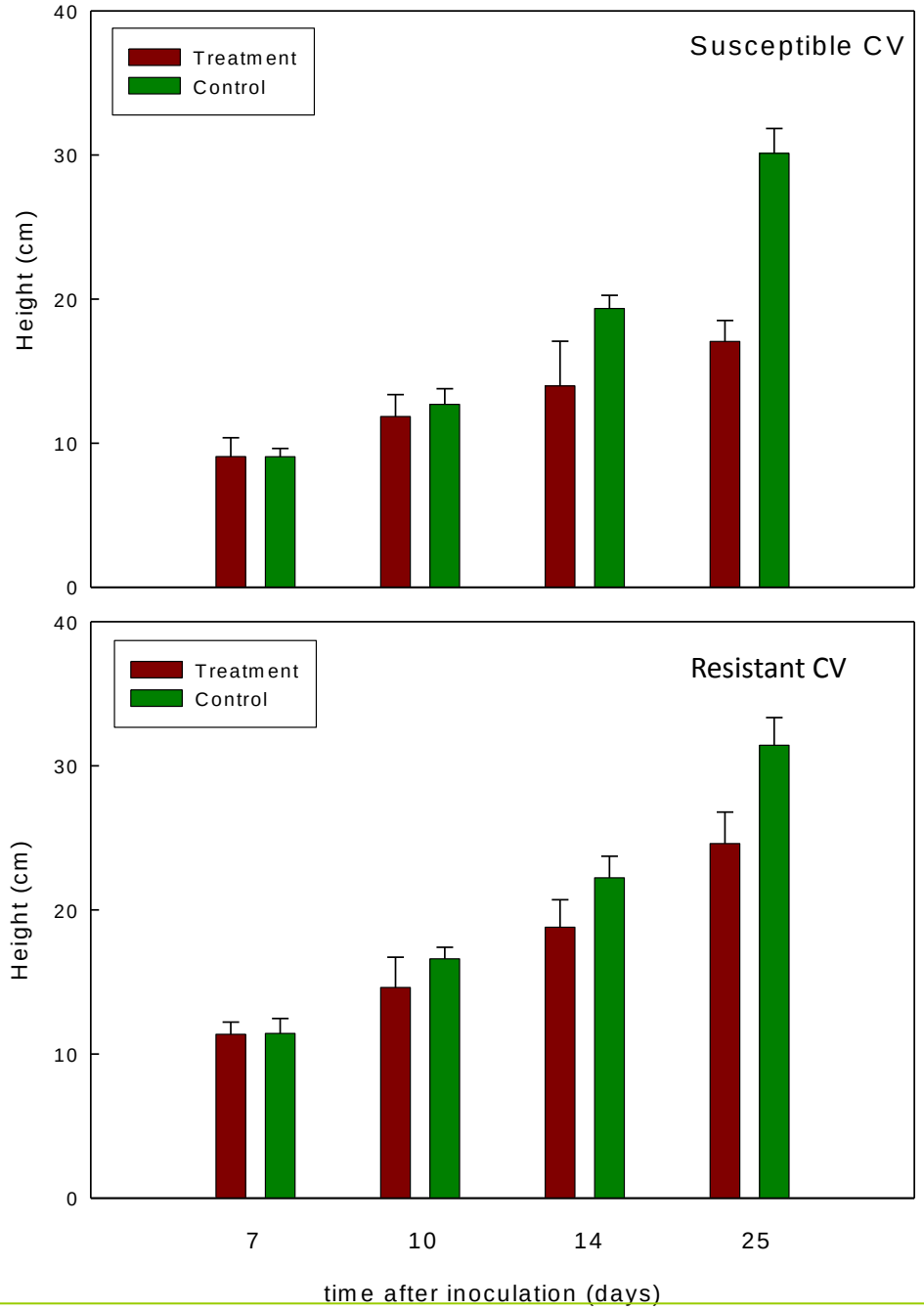
Treatment

✓**The resistant cultivar showed lower root rot severity than the susceptible cultivar. Uninoculated controls remained healthy.**

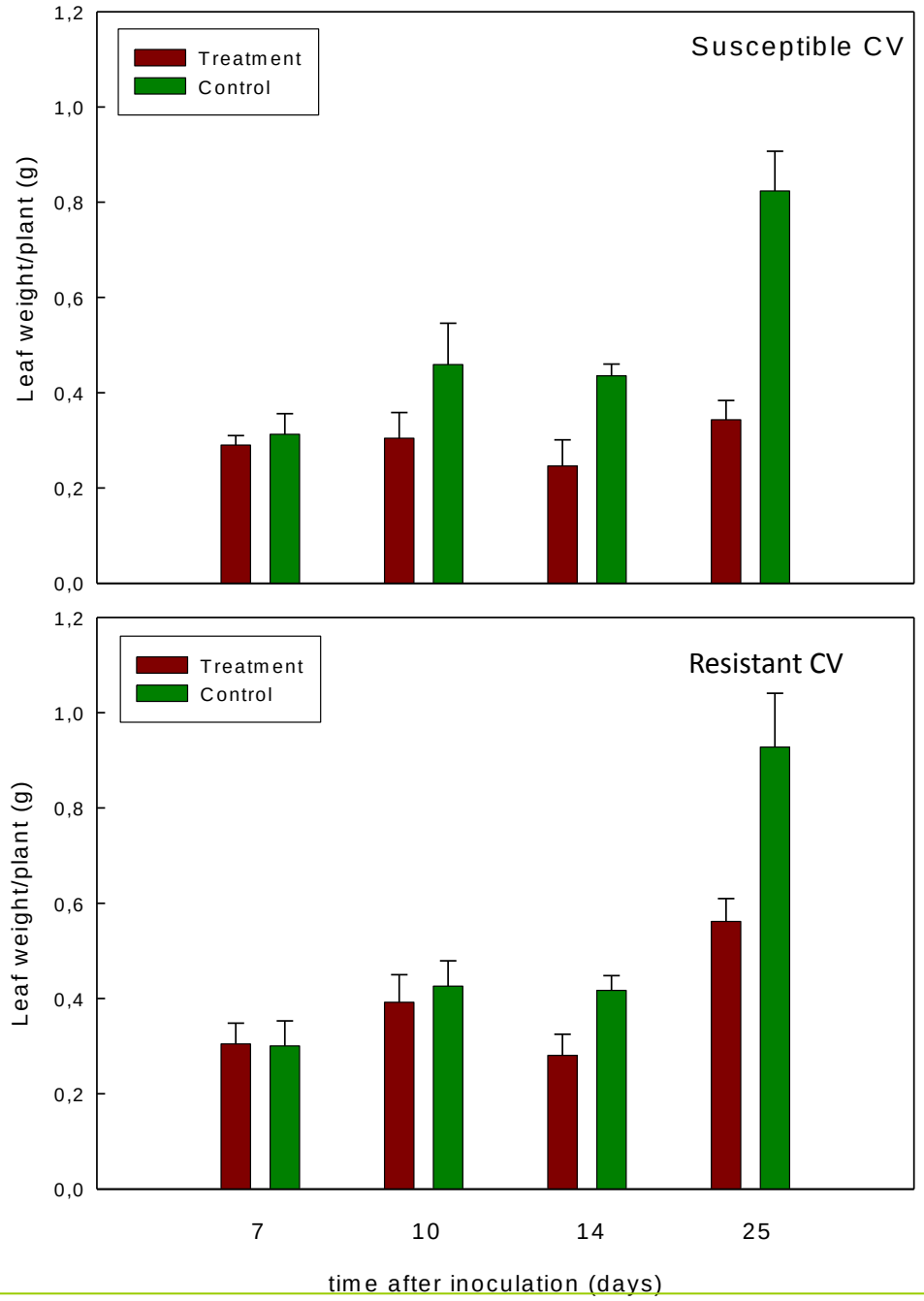
Root rot severity



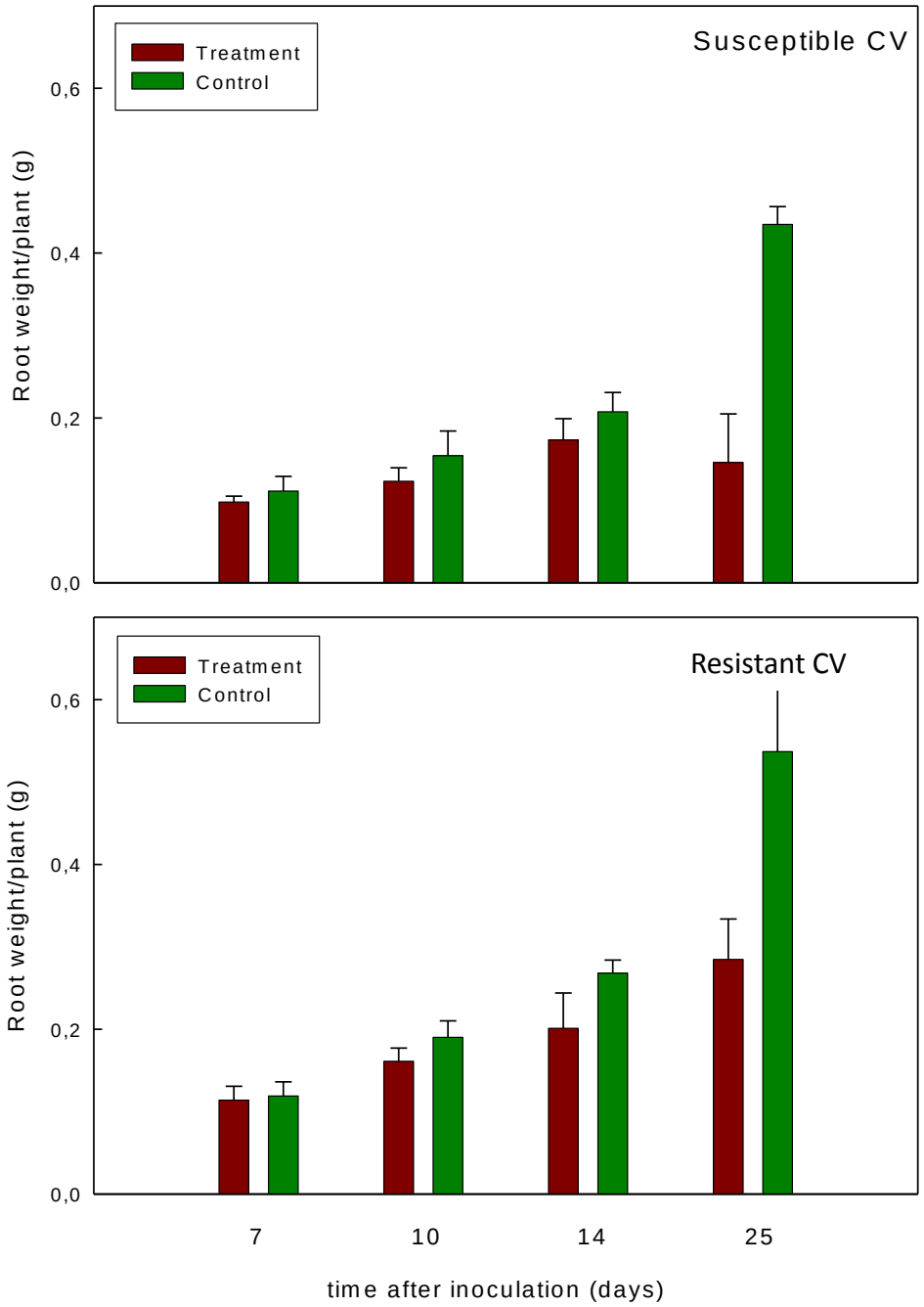
Plant height



Shoot weight



Root weight

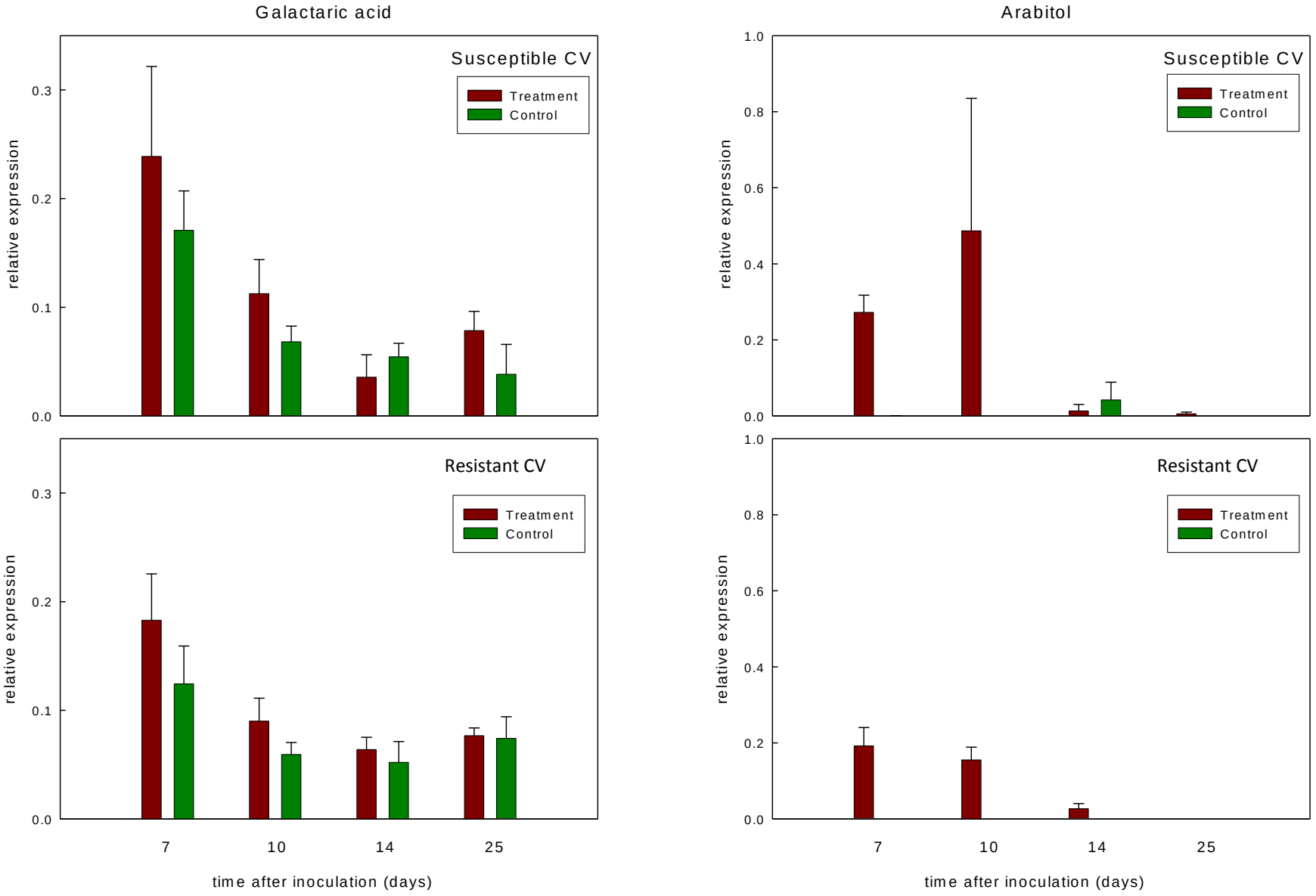

Susceptible CV
Treatment
Control
Root weight/plant (g)
0,0
0,2
0,4
0,6
Resistant CV
Treatment
Control
Root weight/plant (g)
0,0
0,2
0,4
0,6
7
10
14
25
time after inoculation (days)

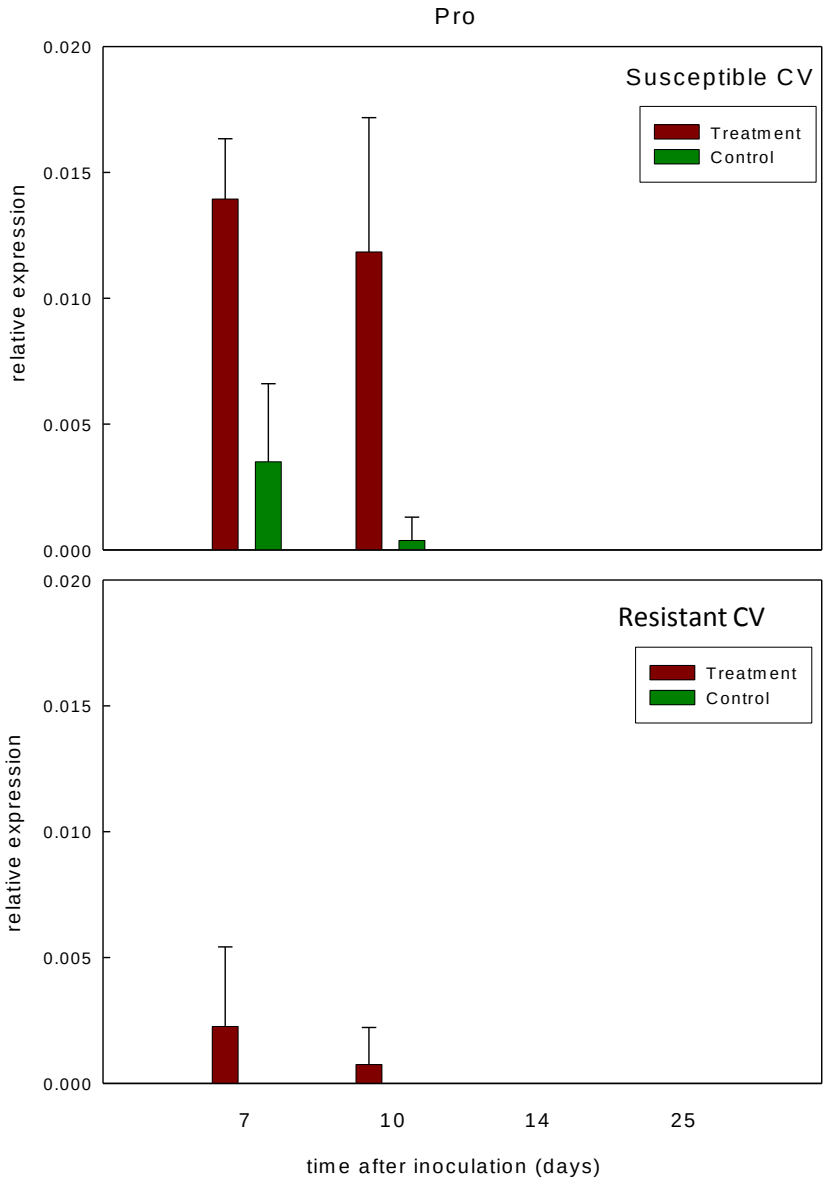
30 metabolites were identified and quantified

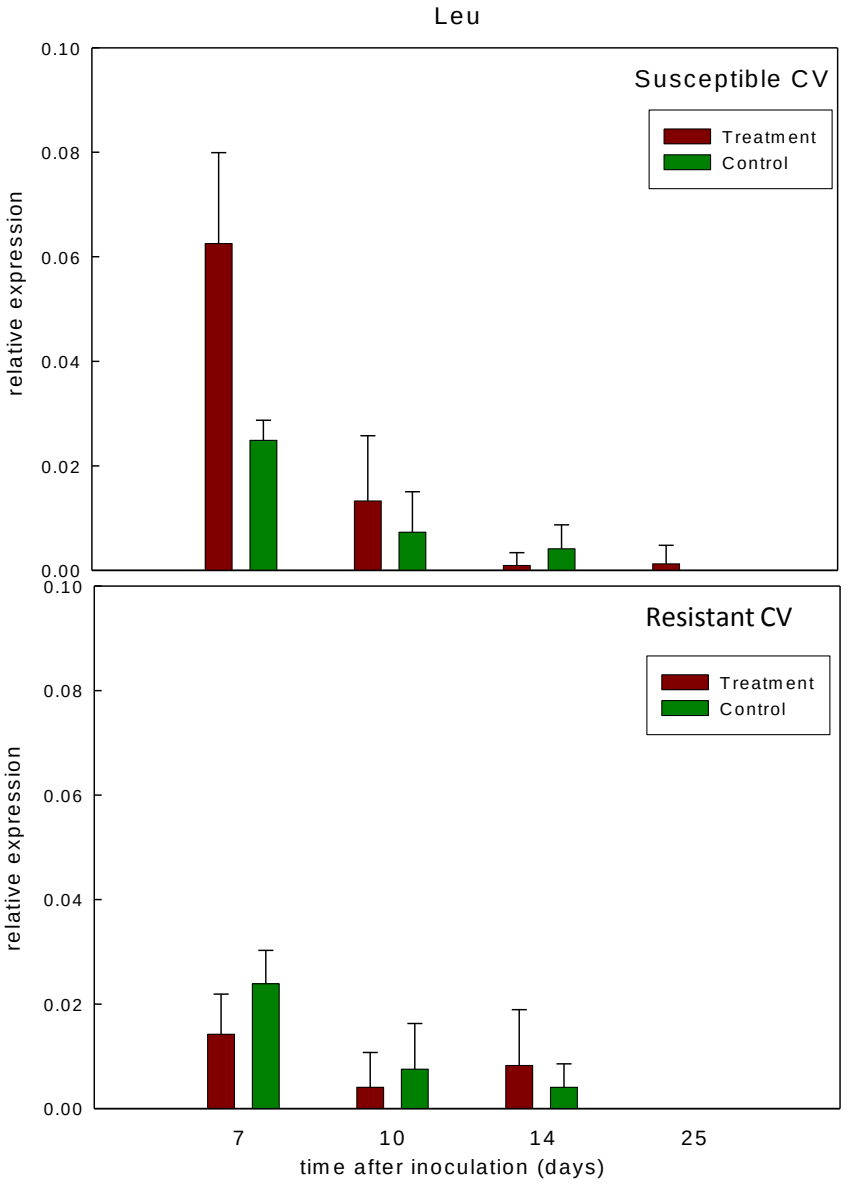
- Sugars (xilulose, glucose, fructose and sucrose)
- Alcohols (arabitol, glycerol, inositol and myoinositol)
- Organic and inorganic acids (lactic, malonic, butanoic, fumaric, succinic, galactaric, citric and phosphoric acids)
- Lipids (palmitic and estearic acids and monoesters with glycerol)
- Aminoacids (Leu, Pro, Gly, Val, Ala, Ser, Asp and Asn)
- Nitrogen compounds (cadaverine, putreanine and urea)

Major differences in sugar, aminoacid and organic and inorganic acid levels were observed at 7 and 10 DAI.

Metabolite Profiling - roots




Pro
Susceptible CV
Treatment
Control
relative expression
0.020
0.015
0.010
0.005
0.000
Resistant CV
Treatment
Control
relative expression
0.020
0.015
0.010
0.005
0.000
7
10
14
25
time after inoculation (days)


Leu
Susceptible CV
Treatment
Control
relative expression
0.10
0.08
0.06
0.04
0.02
0.00
Resistant CV
Treatment
Control
relative expression
0.10
0.08
0.06
0.04
0.02
0.00
7
10
14
25
time after inoculation (days)

Conclusions

- **The resistant cv differed from the susceptible one in all the disease parameters evaluated.**

- **Metabolomics studies were initiated to identify potential early markers of sensitivity to *F. tucumaniae*.**

- **There were no variations in the levels of organic acids, sugars and second messengers in roots due to treatment.**

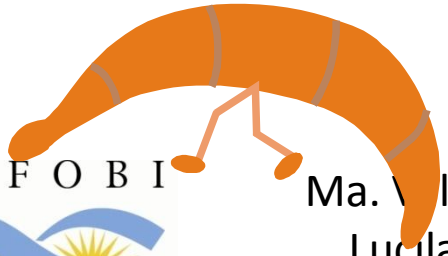
- **However, higher levels of nitrogen compounds, phosphate and glycerol were observed in roots of the susceptible cultivar at the 7^{th} day post-inoculation.**

Further works

- **Metabolic changes in shoots will be also studied.**

- **Considering the observations in roots, we will extend our studies to include the metabolomic changes occurring at earlier stages of the disease (<7 days) and other plant-pathogen interactions.**

Thanks!



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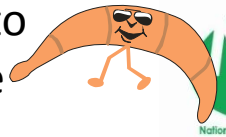


NIAS

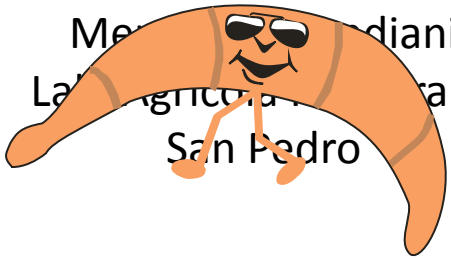
National Institute of Agrobiological Sciences

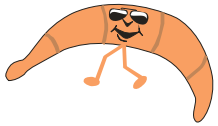
Takayuki Aoki

Me diani

La agricola ... raná

San Pedro





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