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Dep. Biologia
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Changes of metabolism in *Hypericum perforatum* cells after elicitation with *Colletotrichum gloeosporioides*

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Hypericum perforatum

- important medicinal plant
- antidepressive, antiviral, anticancer activities
- economic importance

High demand of biomass

- 👉 ecological problems (wild harvesting)
- 👉 phytochemical variability



Solution: cultivation

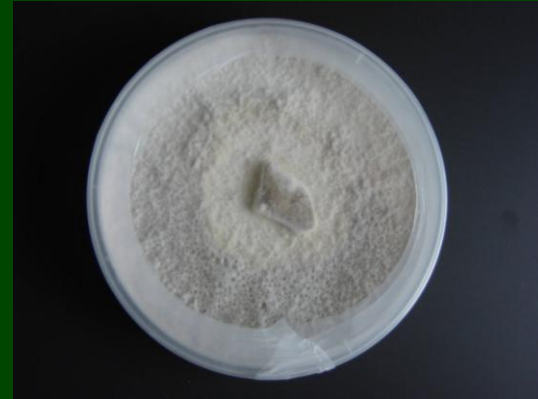
- 👍 biomass consistence

Major problem

- 👉 anthracnose (*Colletotrichum gloeosporioides*)

Colletotrichum gloeosporioides

- common plant pathogen
- host-pathogen model
- major pathogen of *H. perforatum* L.



In vitro cultures of *H. perforatum* L.

- easy to manipulate
- closed system



H. perforatum
suspension cultures

Induction (5th day of culture)

Methyl jasmonate (MeJ) or
salicylic acid (SA)

Elicitation (6th day of culture)

Autoclaved *C. gloeosporioides*
biomass suspension

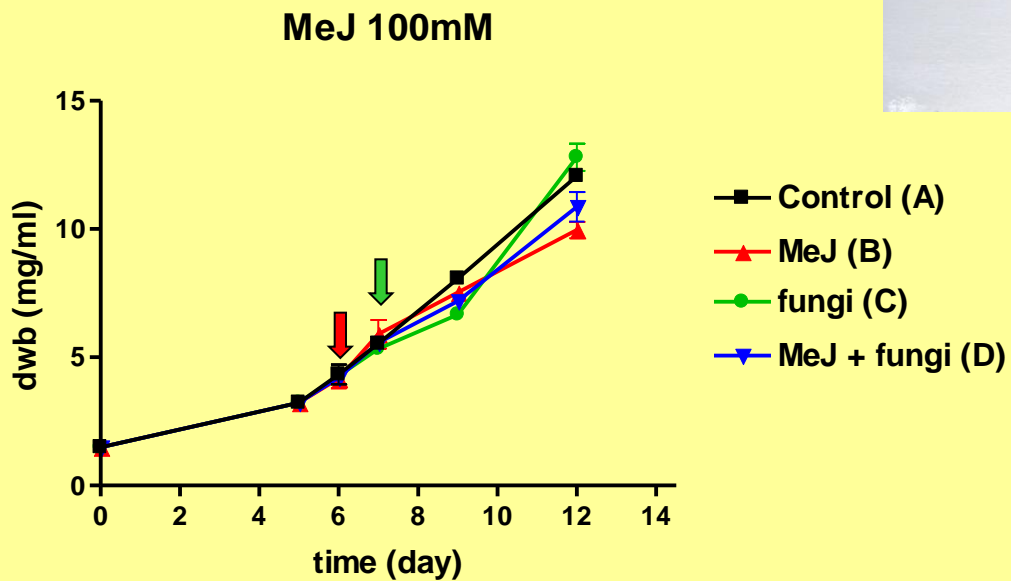
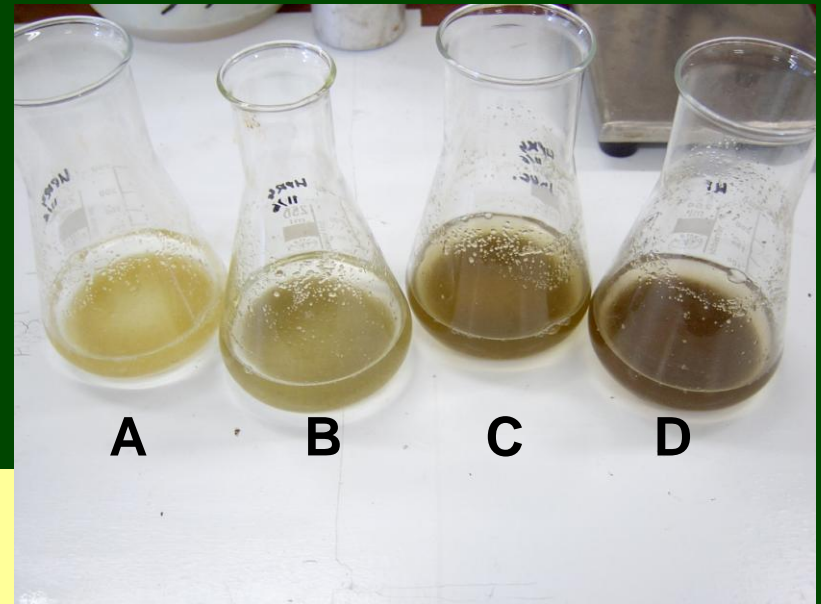
Analysis

HPLC-DAD-MS analysis of phenols

Cell viability (*tripan blue*)

AOS measurement (*DCFH-DA*)

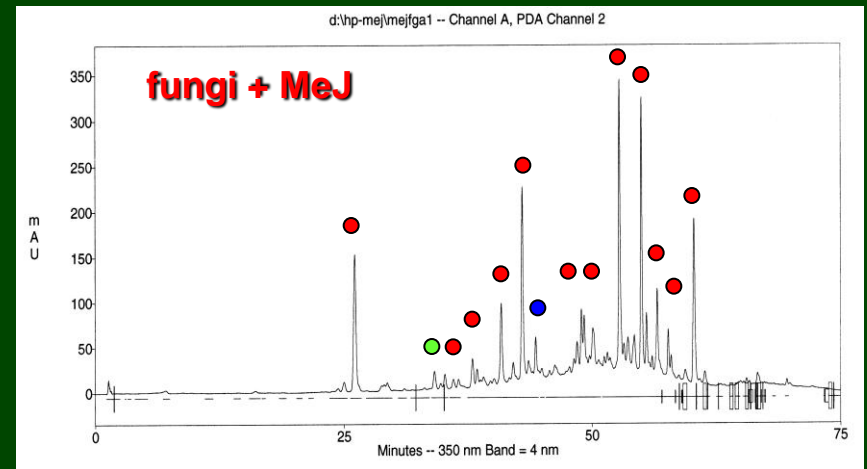
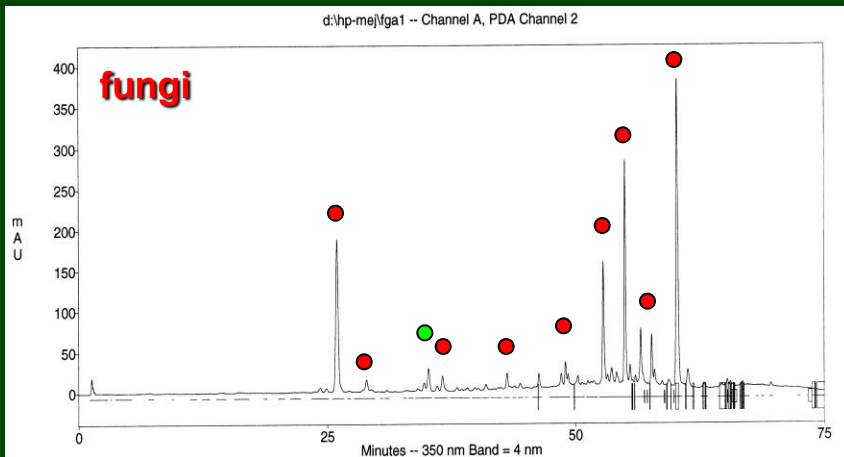
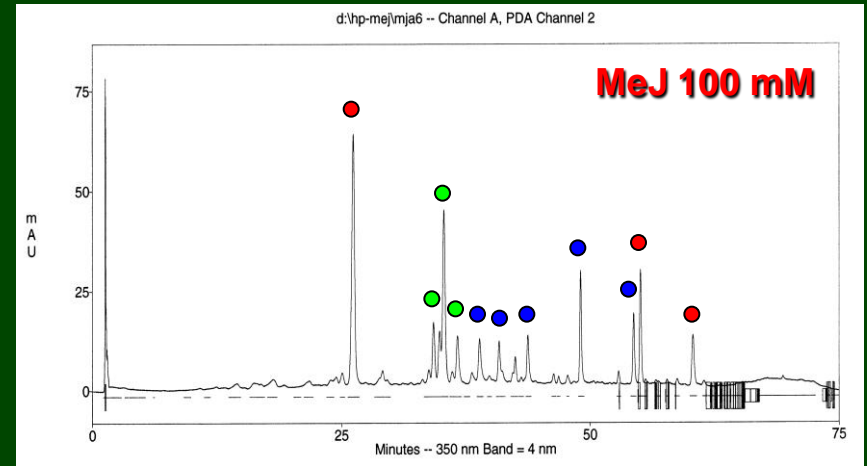
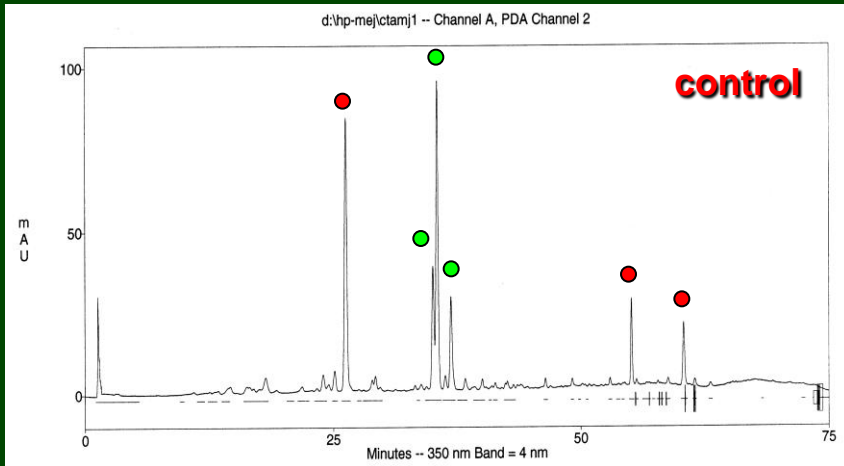
MeJ/fungi elicitation – culture changes



↓ - addition of MeJ (100 mM)

↓ - addition of fungi

MeJ/fungi elicitation – HPLC profiles



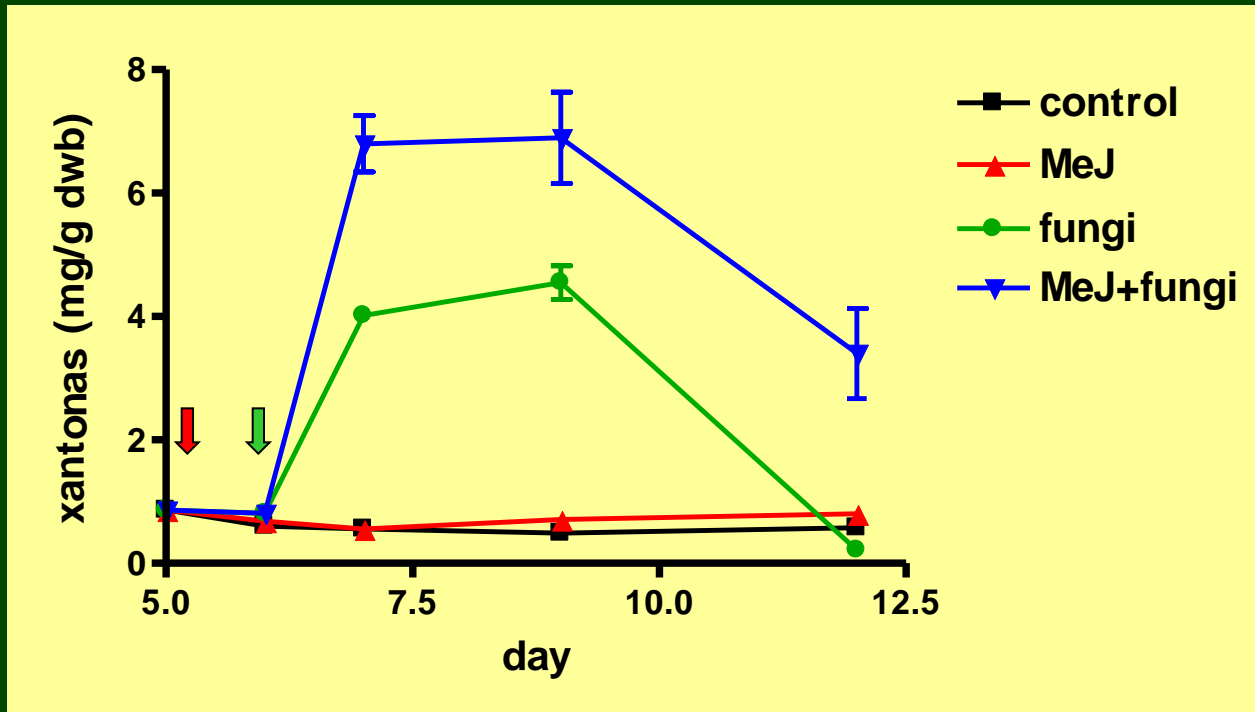
1,3,6,7 xanthones derivatives - ●

quercetin derivatives - ●

luteolin derivatives - ●

MeJ/fungi elicitation – phenolic changes

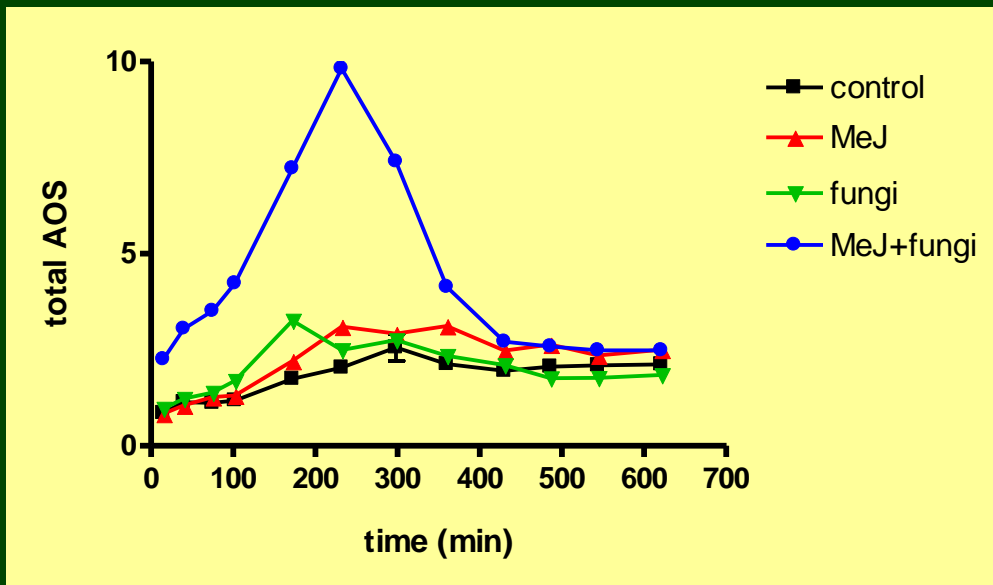
Xanthone accumulation



↓ - addition of MeJ (100 mM)

↓ - addition of fungi

MeJ/fungi elicitation – oxidative burst

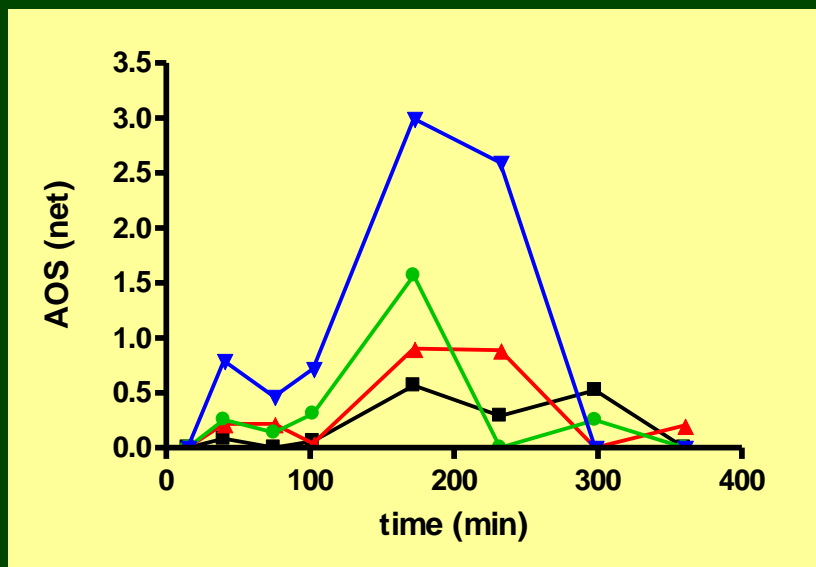


DCFH-DA

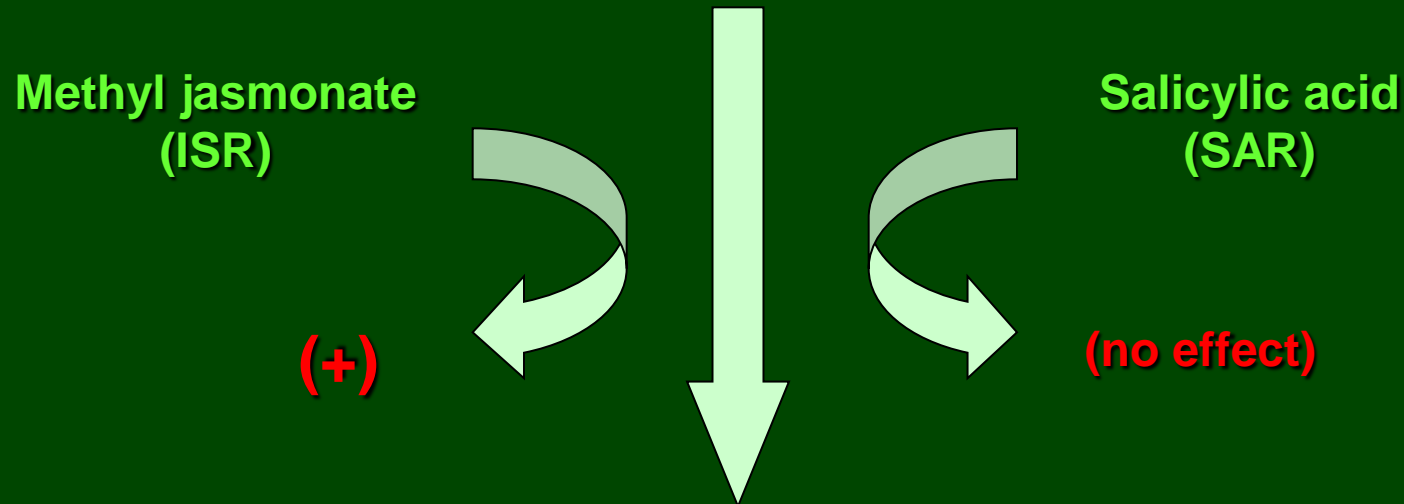
(dichlorodihydrofluorescein diacetate)

fluorescence

$(A_{n+1} - A_n)$



H. perforatum cultures vs *C. gloeosporioides*



Induction of the xanthone accumulation – phytoalexins ?

Induction of an oxidative burst – incompatible interaction ?

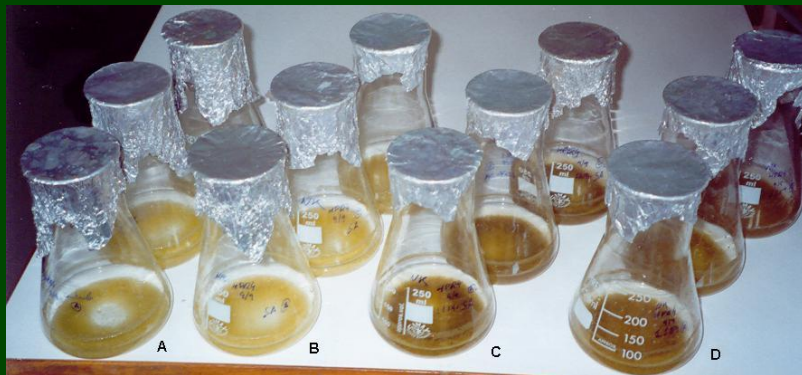
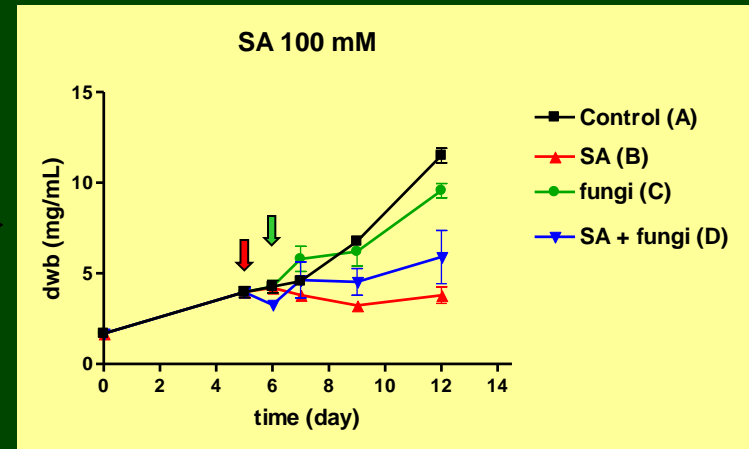
Acknowledgements

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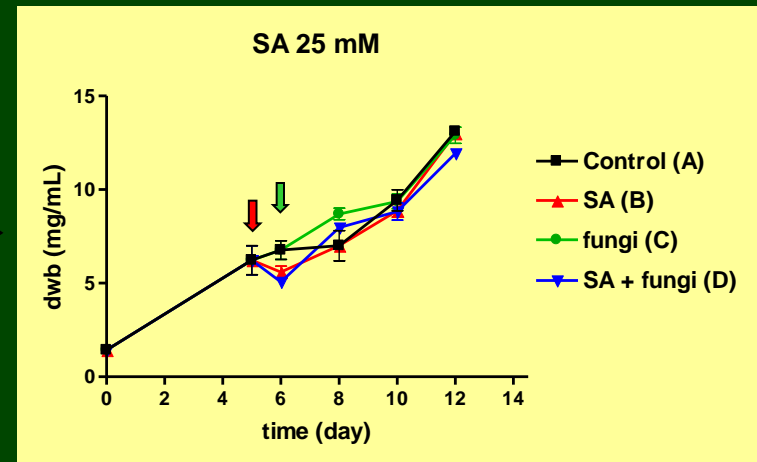
SA/fungi elicitation – culture changes



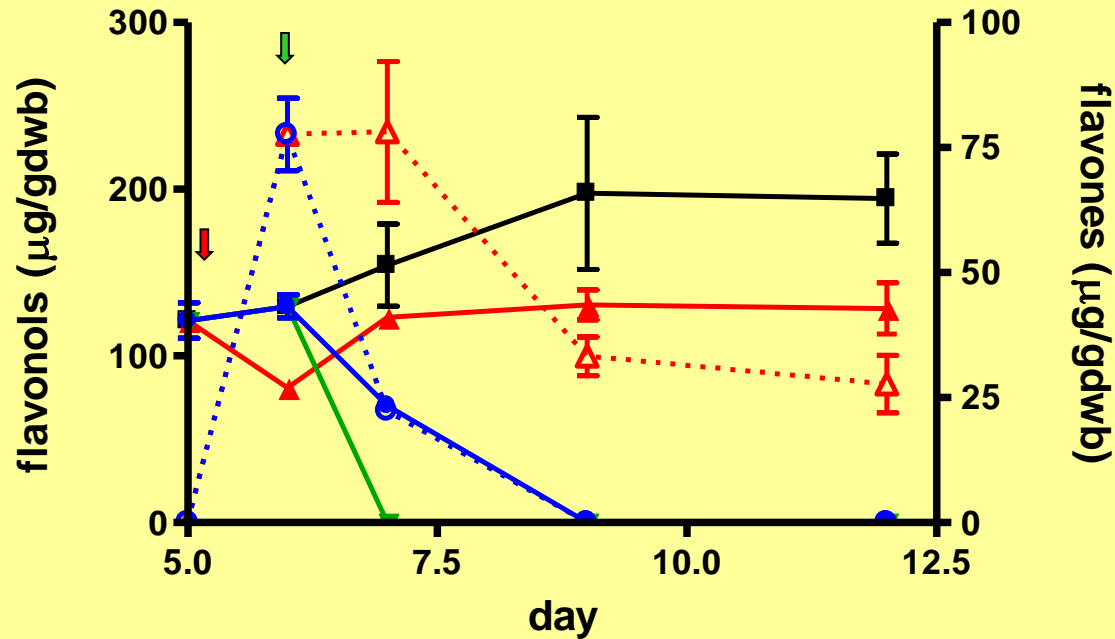
A B D C



A B C D



Flavonoids accumulation



luteolin derivatives – dot line

quercetin derivatives – straight line