Accelerated biodegradation of 2,4-D in legume rhizospheres: elucidation of the mechanisms responsible

Liz Shaw

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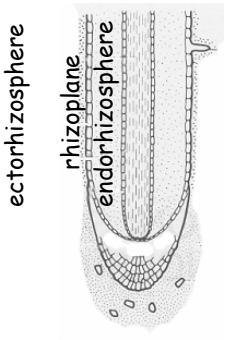
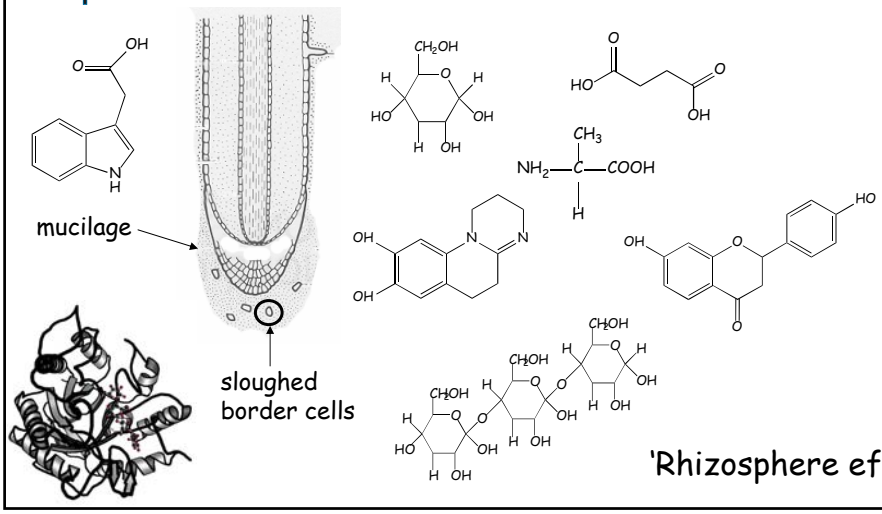
The rhizosphere



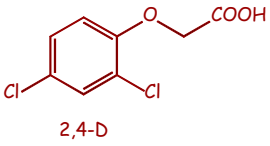
- Distinctive soil-plant root environment
- Enriched in organic carbon due to rhizodeposition
- As much as 40% of carbon that is photosynthetically fixed is translocated below ground

Rhizodeposit composition

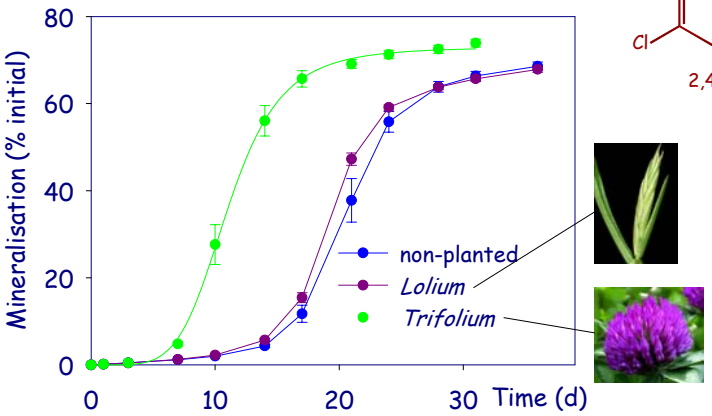


'Rhizosphere effect'

Enhanced biodegradation of 2,4-dichloro-phenoxyacetic acid in rhizosphere soil of red clover



2,4-D



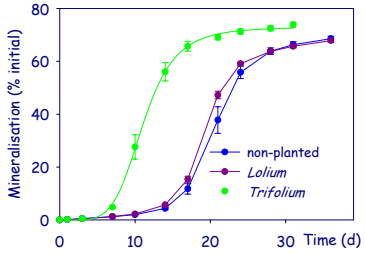
Shaw & Burns (2005) *Soil Biol. Biochem.* **37**, 995

Summary of research reporting a 'rhizosphere effect' on the biodegradation of pesticides

Reference	Pesticide	Plant species
Boyle and Shann, 1995 Boyle and Shann, 1998	**Phenoxyacetic acid**: 2,4-D and 2,4,5-T	Red clover, daisy fleabane, barnyard grass, fall panicum, early goldenrod, chicory, timothy grass, green foxtail, sunflower
Kruger et al., 1997 Marchand et al., 2002 Pervovitch et al., 1996 Piutti et al., 2002 Singh et al., 2004	**Triazine**: Atrazine and simazine	Summer cypress, maize, pennisetum
Hoagland et al. 1997	**Chloroacetanilide**: Metolachlor and alachlor	Maize
Zablotowicz et al., 1997	**Diphenolic ether**: Acifluorfen	Soybean

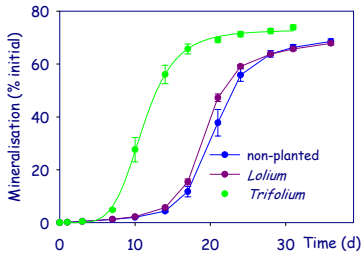
- research is descriptive, not mechanistic

Overall aim: to investigate mechanisms of *Trifolium* rhizosphere enhanced 2,4-D biodegradation



?

Overall aim: to investigate mechanisms of *Trifolium* rhizosphere enhanced 2,4-D biodegradation

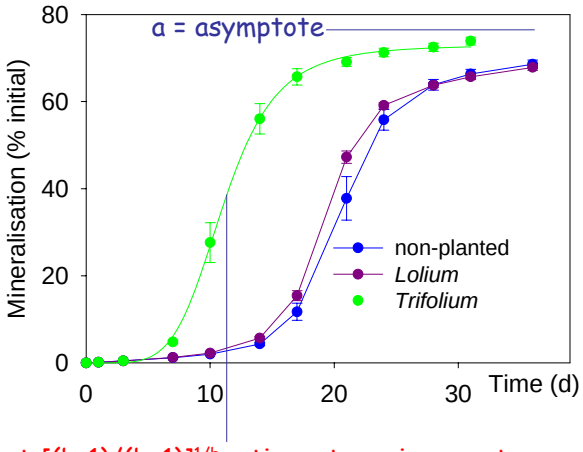


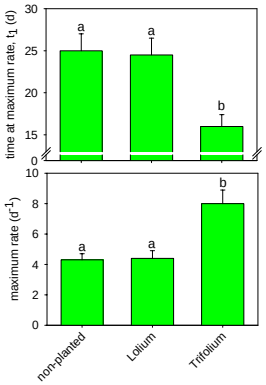
Functional engineering of rhizosphere for benefit (i.e. rhizoremediation)

- Specific objectives:
  1. To investigate the impact of plant and soil properties on the enhanced biodegradation
  2. To compare the number and diversity of 2,4-D degradative microorganisms in *Trifolium*, *Lolium* and non-planted systems

Treatment of mineralization data

$Y = a[1+(t/t_0)^b]^{-1}$





$t_1 = t_0[(b-1)/(b+1)]^{1/b}$ = time at maximum rate

Maximum rate = $(-a.b)/(4t_1)$

Specific objectives:

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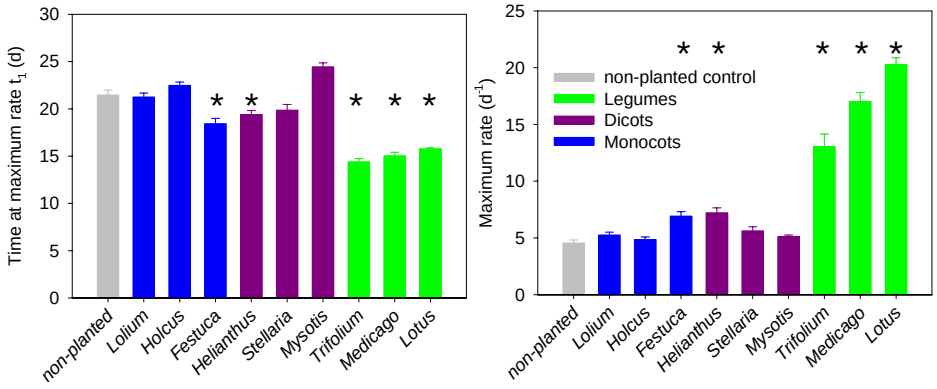
The rhizosphere effect has legume specificity



Shaw and Burns (2005) *Environ. Microbiol.* **7**, 191

Specific objectives:

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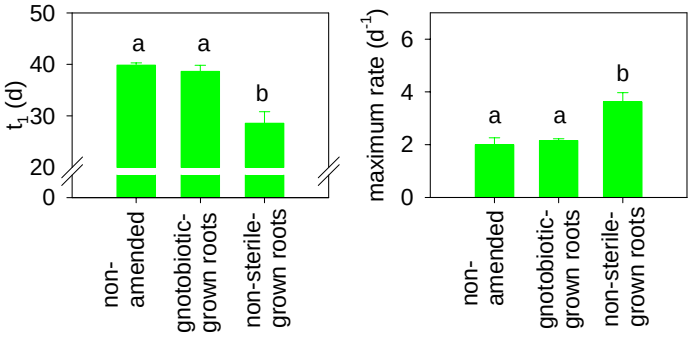
Production of the active component is dependent on the presence of microorganisms



Shaw and Burns (2005) *Environ. Microbiol.* **7**, 191.

Specific objectives:

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The production of the rhizodeposit component that stimulates 2,4-D mineralization:

- Has specificity to legumes
- Is dependent on presence of microorganisms
- Is dependent on plant age

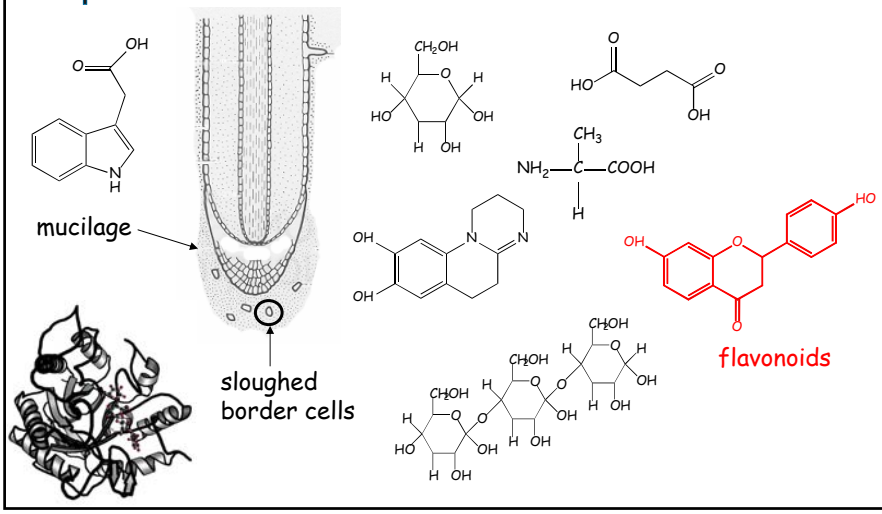
Specific objectives:

1. To investigate the impact of plant and soil properties on the enhanced biodegradation
2. To compare the number and diversity of 2,4-D degradative microorganisms in *Trifolium*, *Lolium* and non-planted systems

The production of the rhizodeposit component that stimulates 2,4-D mineralization:

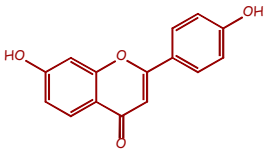
- Has specificity to legumes
- Is dependent on presence of microorganisms
  - Implies plant-microbe signalling
- Is dependent on plant age

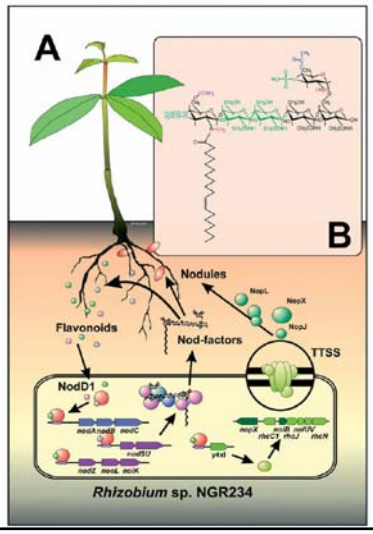
Rhizodeposit composition



Role of flavonoids: nod factor inducers

- E.g. 7,4'-dihydroxyflavone





from Broughton *et al.* (2003) *Plant and Soil* **252**, 129

The mechanism?

- The stimulatory rhizodeposit (isoflavonoid?) component is acting as:
  - A significant additional growth substrate for 2,4-D degraders
  - An inducer of 2,4-D catabolism

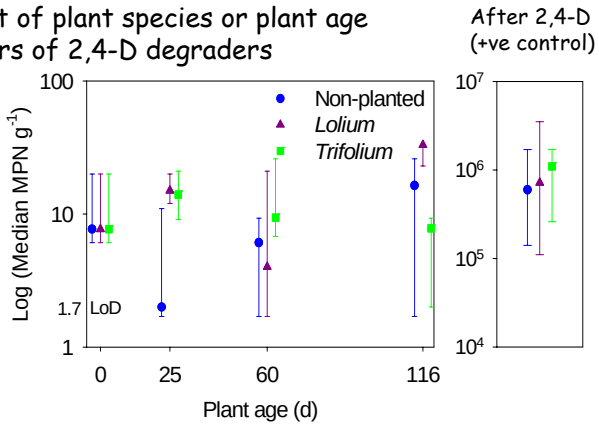
Is the stimulatory rhizodeposit enhancing 2,4-D mineralization by acting as a growth substrate?

- If this was the case, would expect:
  - Increased numbers of 2,4-D degraders in the rhizosphere of *Trifolium* compared to *Lolium* or non-planted **pristine** soil
  - Rhizosphere-dependent shift in diversity of 2,4-D degrading microorganisms

Specific objectives:

1. To investigate the impact of plant and soil properties on the enhanced biodegradation
2. To compare the number and diversity of 2,4-D degradative microorganisms in *Trifolium*, *Lolium* and non-planted systems

No effect of plant species or plant age on numbers of 2,4-D degraders



Shaw and Burns (2004) *Appl. Env. Microbiol.* **70**, 4766

Specific objectives:

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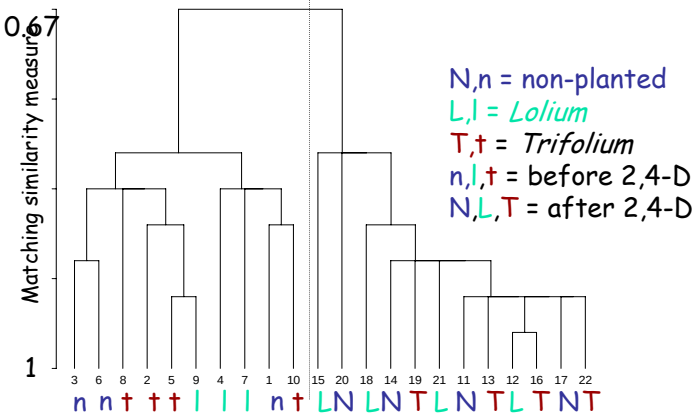
2. To compare the number and diversity of 2,4-D degradative microorganisms in *Trifolium*, *Lolium* and non-planted systems

No effect of plant species on 2,4-D degrader diversity:

Hierarchical cluster analysis of SSCP profiles: *tfdA*α from soil



Shaw and Burns (2004) *Appl. Env. Microbiol.* **70**, 4766

The mechanism?

- The stimulatory rhizodeposit component is acting as:
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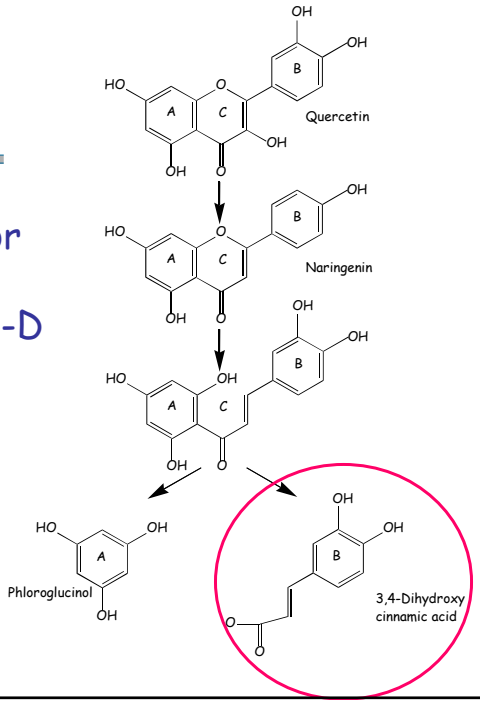
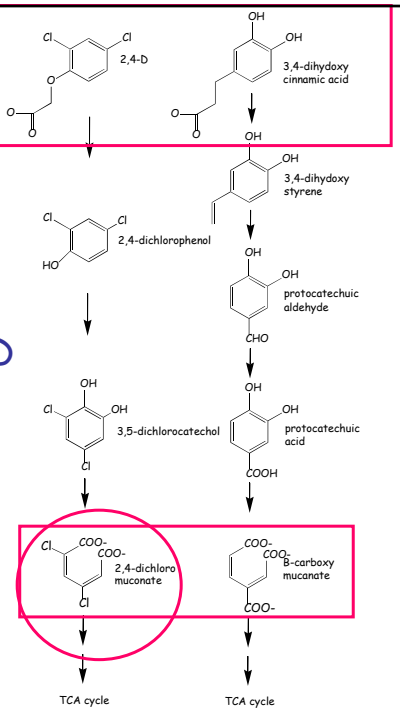
Suggested mechanism for the role of flavonoids as inducers in enhanced 2,4-D mineralization (1)



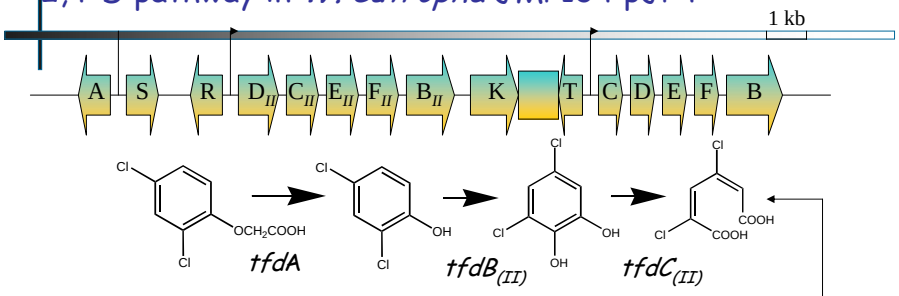
TfdA can accept hydroxy cinnamic acids as substrates[1]

Suggested mechanism for the role of flavonoids as inducers in enhanced 2,4-D mineralization (2)



[1] Dunning Hotopp and Hausinger, 2001 *J. Mol. Catal. B* 15: 155

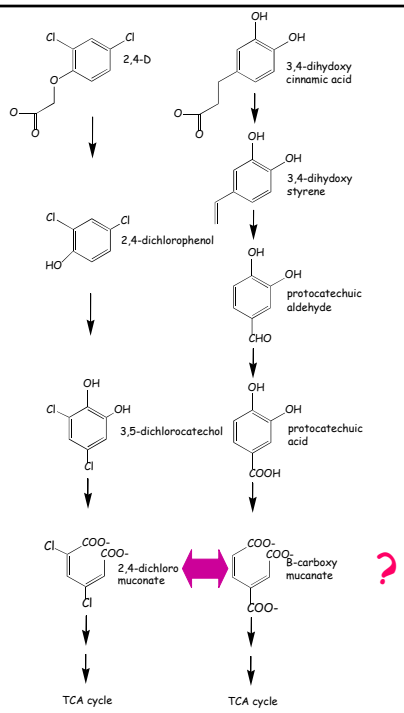
2,4-D pathway in *W. eutropha* JMP134 pJP4



*tfd*R gene product activates expression and the effector molecule for TfdR is 2,4-dichloro-cis,cis-muconate.

Suggested mechanism for the role of flavonoids in enhanced 2,4-D mineralization (2)

Involvement of flavonoids as inducers requires further testing

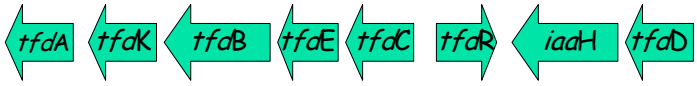


Further research to investigate the role of isoflavonoids and inducers

- Establish the role of isoflavonoids
  - Spiking experiments with commercially available isoflavonoids
  - Use of legume mutants (e.g. *Medicago truncatula*) deficient in isoflavonoid biosynthesis
- Establish whether rhizodeposits (isoflavonoids) act as inducers of the 2,4-D pathway
  - Use of transcriptional fusions to quantify gene expression in model bacterial strains

Role of plant-microbe interactions in the evolution of xenobiotic catabolic pathways

Arrangement of *tfd* genes in plasmid pEST4011 of *Achromobacter xylosoxidans* subsp. *dentrificans* EST4002



From Vedler *et al.* (2004) *J. Bacteriol.* **186**, 7161.

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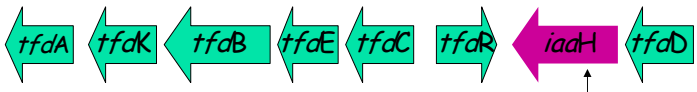


Indole acetamide hydrolase (*Bradyrhizobium japonicum* USDA110)

From Vedler *et al.* (2004) *J. Bacteriol.* **186**, 7161.

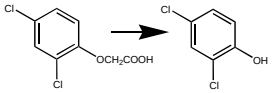
Thank you

NATURAL ENVIRONMENT RESEARCH COUNCIL

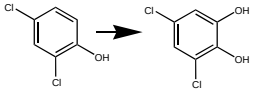
HERBICBIOREM

tfd A, B, and C primer sets

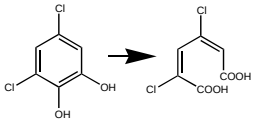
*tfd*A
(Vallaeys et al. (1998) *FEMs Microb. Ecol.* **20**, 163.)

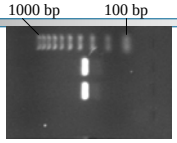


*tfd*B
(This study)

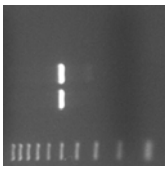


*tfd*C
(Calvaca et al. (1999) *FEMS Micro. Ecol.* **29**, 45.)

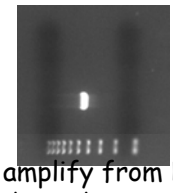




RASC
JMP134
-ve



JMP228 (pEMT8)
Bacillus B11
RASC
JMP134
-ve



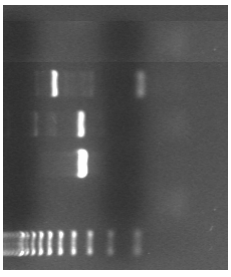
Bacillus B11
JMP 228 (pEMT8)
RASC
JMP134
-ve

Used these primer sets to PCR amplify from DNA extracted directly from soil or from 2,4-D degrading enrichment cultures
NO PRODUCTS

tfdA-like genes in 2,4-D-degrading bacteria belonging to the BANA cluster in α-*Proteobacteria*

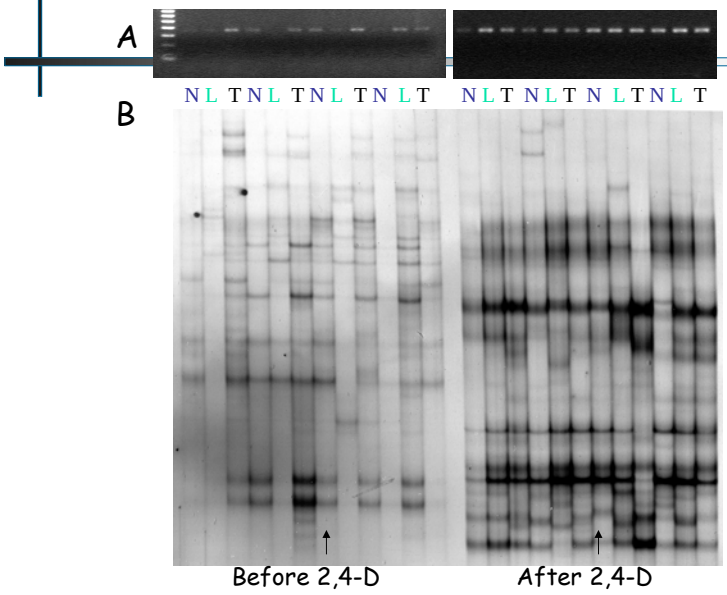
Itoh *et al.* (2002) AEM **68**, 3449.

- Japanese upland soil α-Proteobacterial isolate RD5-C2
- *tfdA*α gene 56-60% homologous to canonical *tfdA* genes
- Designed primers from alignments of *tfdA*α genes in RD5-C2, HW13, BTH and HWK12

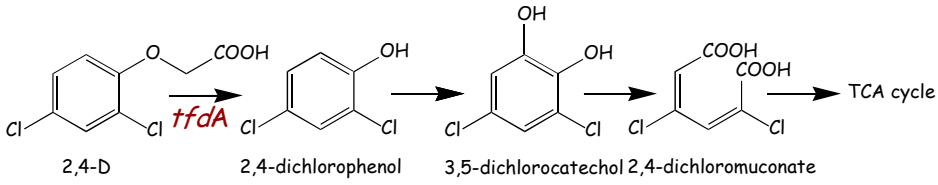


Bacillus B11
RASC
RD5-C2
HW13
-ve
100 bp ladder

PCR amplification (A) and subsequent SSCP analysis (B) of *tfdA*α genes in Sourhope soil before and after 2,4-D application

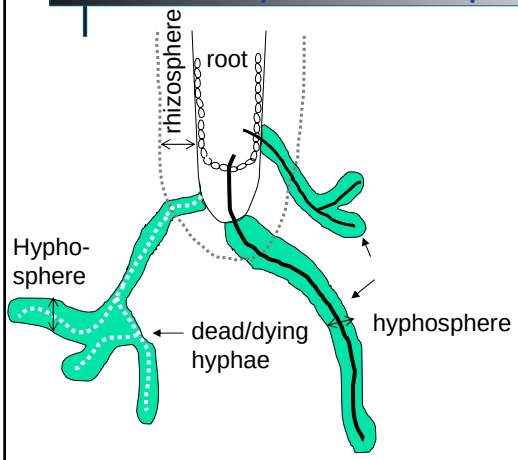


2,4-dichlorophenoxyacetic acid canonical pathway in β-Proteobacteria *Wautersia eutropha* JMP134 pJP4

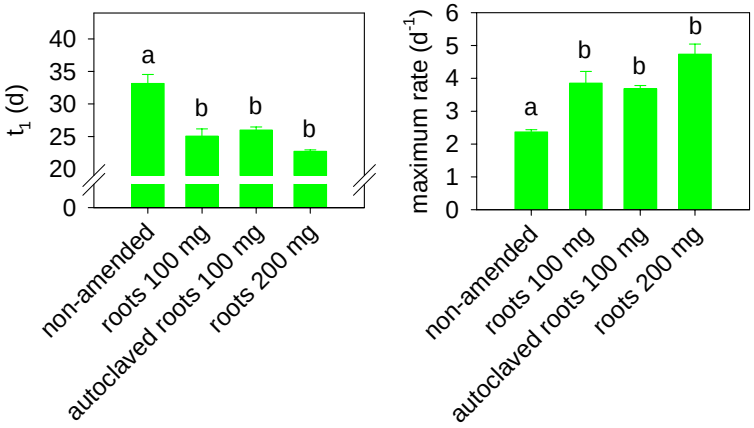


tfdA encodes 2,4-D/α-ketoglutarate dioxygenase

The mycorrhizosphere



Addition of chopped *Trifolium* roots enhances 2,4-D mineralization



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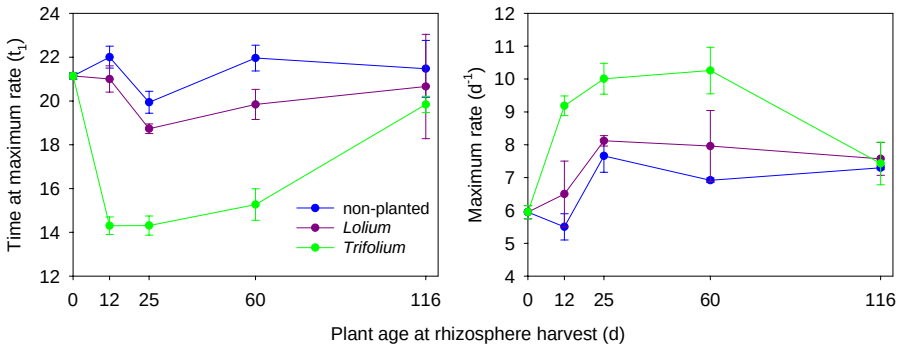
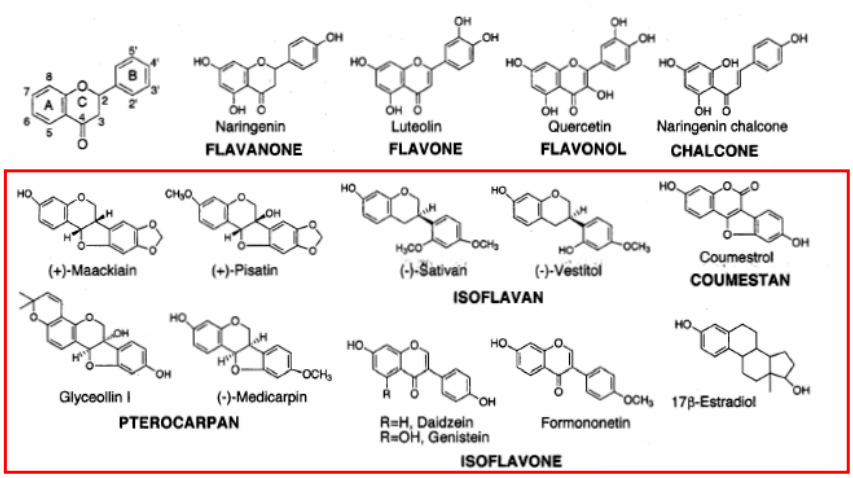
The rhizosphere effect depends on plant species and plant age



Shaw and Burns (2004) *Appl. Env. Microbiol.* **70**, 4766

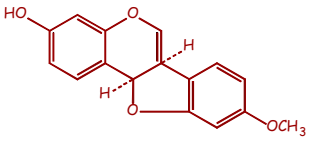
Flavonoids of leguminous plants: a tentative identity for the stimulatory rhizodeposit?



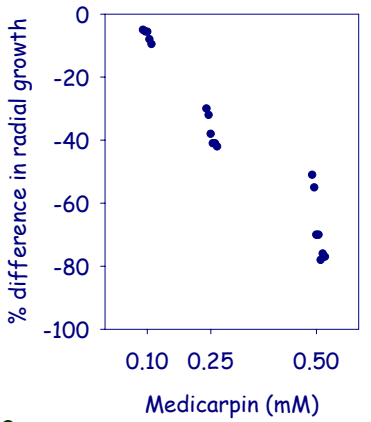
From: Aoki *et al.* (2000) *J. Plant Res.* **113**, 475

Role of flavonoids (2): phytoalexins

- E.g. medicarpin produced by *Medicago* spp.



Antifungal activity of medicarpin on *Nectria haematococca*



Data from Blount *et al.* (1992) *Plant Pathol.* **41**, 333.

Initial mineralisation kinetics are linear