LegumePlus
Marie Curie Initial Training Network

University of Reading

Current Research on the Potential of Bioactive Legumes for Animal Nutrition and Sustainability

Professor Irene Mueller-Harvey

Overview

- Introduction: the projects, and the plants
- The chemistry of tannins
- Agronomy and genetics
- Action against parasitic nematodes
- Nutrient use, environment and food quality
- Looking to the future

Introduction: the projects and the plants

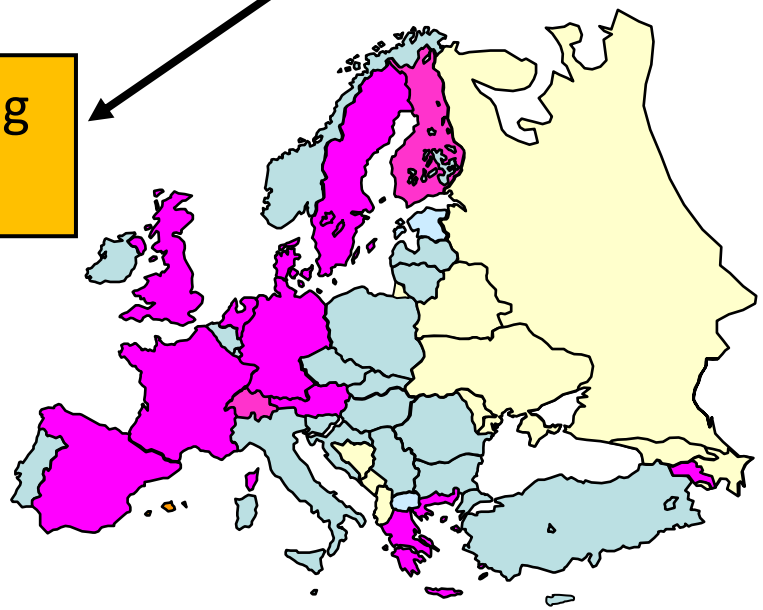
Sainfoin collection, Germplasm screening, Agronomic evaluations

Nutritional & environmental effects

Chemical composition

Anti-parasitic properties

→ Plant screening tools



17 partners in 13 countries
30 fellows (= PhD students)

HEALTHYHAY
Marie Curie Research Training Network

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I Mueller-Harvey

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L Falco Sales, M Martin Lorenzo
A Ramsay, H Ropiak, C Fryganas

Turun yliopisto
University of Turku

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NIAB
National Institute of Agricultural Botany

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COTSWOLD Grass Seeds DIRECT

I Wilkinson, P Totterdell

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L Skot

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Agroscope

A Luescher, R Koelliker, C Malisch, K Kempf
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WAGENINGEN UR
For quality of life

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J Aufrere, V Niderkorn, K Theodoridou, G Copani
H Hoste, F Manolaraki, J Quijada, B Azuhnwi

DEMETER

S Sotiraki,
T Saratsis

UNIVERSITY OF COPENHAGEN
Research

S Thamsborg, AR Williams,
A Novobilský, O Desrues

DairyNZ

G Waghorn

Examples of bioactive legumes

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	% tannins (g/100 g plant)
Dorycnium rectum	15 - 20
Sericea lespedeza (*Lespedeza cuneata*)	6 – 13
Big trefoil (*Lotus pedunculatus*)	5 - 10
Sulla (*Hedysarum coronarium*)	2 - 12
Sainfoin (*Onobrychis viciifolia*)	1 – 8
Birdsfoot trefoil (*Lotus corniculatus*)	2 – 5

→ Can control parasitic worms
→ But tannin concentrations vary

Mueller-Harvey 2006

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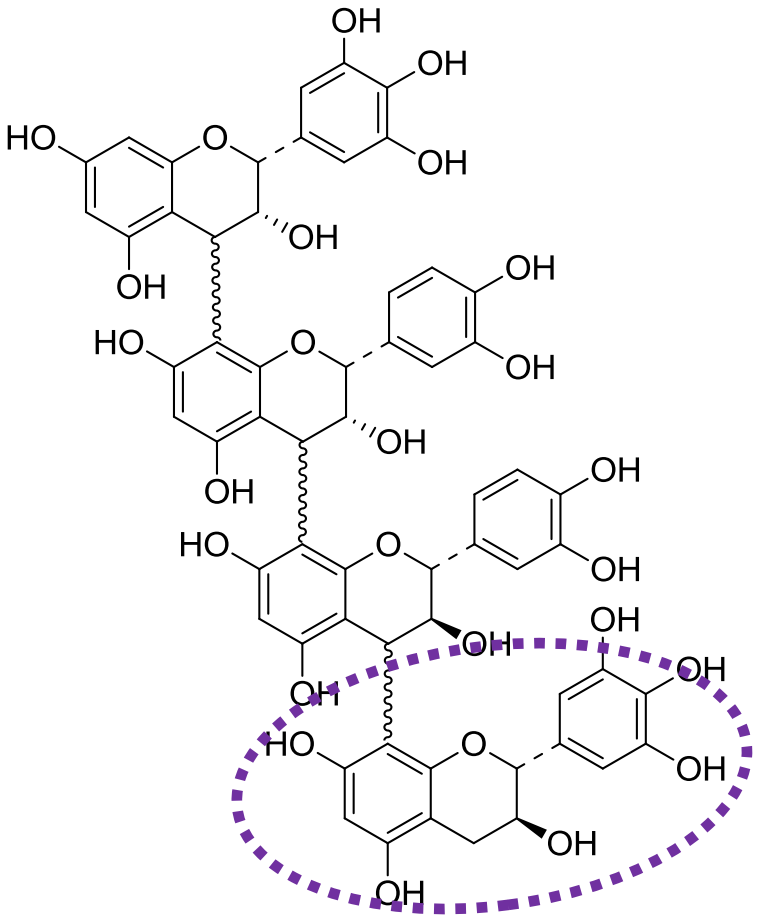
The chemistry of tannins

Bioactive ingredients = tannins



Mueller-Harvey 2006; Waghorn 2008

Bioactive ingredients = tannins

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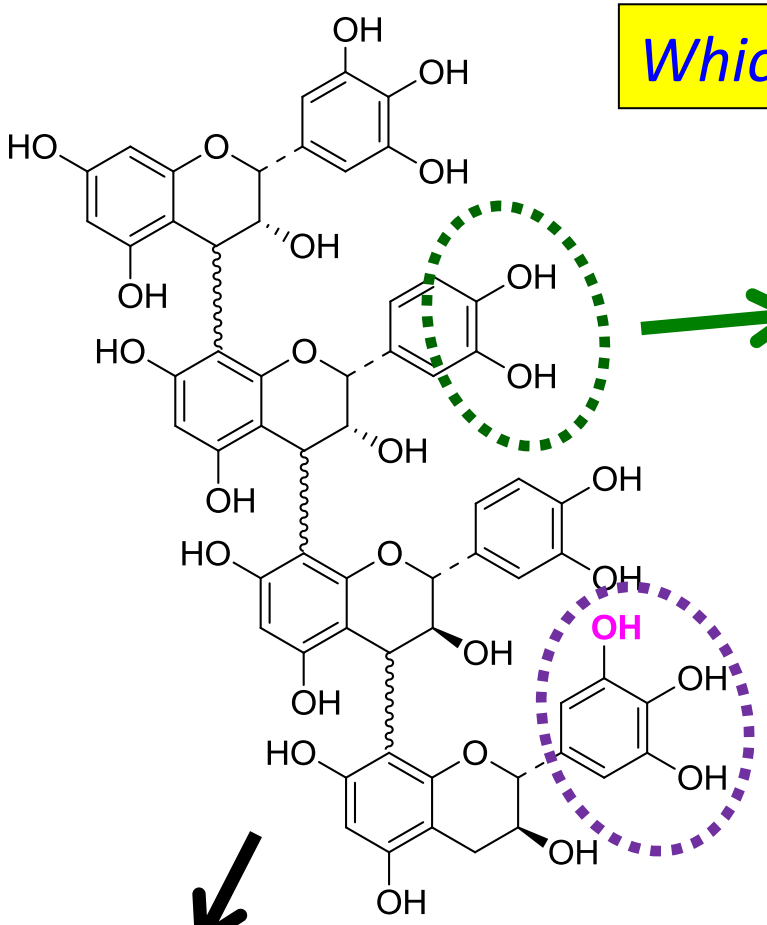

Which tannins are best for what?

Procyanidin tannins
(***amino acid absorption*** ↑)

Prodelphinidin tannins
(***parasitic worms*** ↓)

Larger tannins
(***parasitic larvae*** ↓)

Mueller-Harvey 2006; Waghorn 2008

Which bioactive legumes and *where*?

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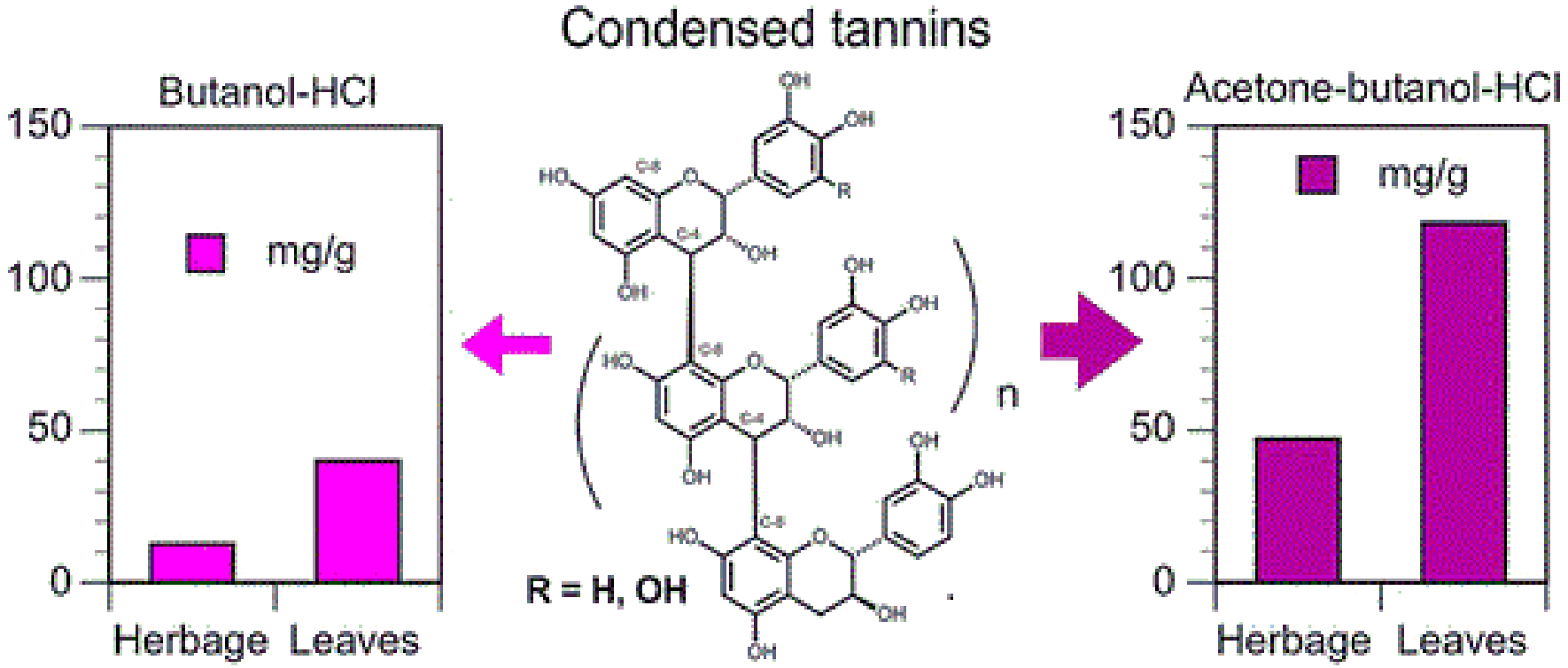
	Red clover	Birdsfoot trefoil	Sainfoin	Alfalfa
EU latitudes	35° - 64°	35° - 56°	35° - 54°	40 - 60°
Soil pH:				
Tolerance	**4.5 – 8.5**	**5.5 – 7.5**	**6.0 – 8.9**	**5.5 – 8.5**
Optimum	**6.0 – 7.5**	**6.0 – 6.5**	**6.5 – 8.0**	**6.5 – 8.0**
Yield	**Good**	Fair	**Medium**	**High**
Establishment	Easy	Moderate	**Difficult**	Moderate
Persistence	Poor	Medium	**Good**	Med-Poor
Tolerance to:				
Water logging	Fair	**Good**	**Poor**	**Poor**
Drought	**Poor**	Medium-poor	**Good**	**Good**
Anthelmintic	**No**	Low	**Yes**	**No**
Anti-bloating	**No**	**Yes**	**Yes**	**No**
Oestrogenic	**Yes**	**No**	**No**	**No**
Bioactives	**PPO**	**Tannins**	**Tannins**	**No**

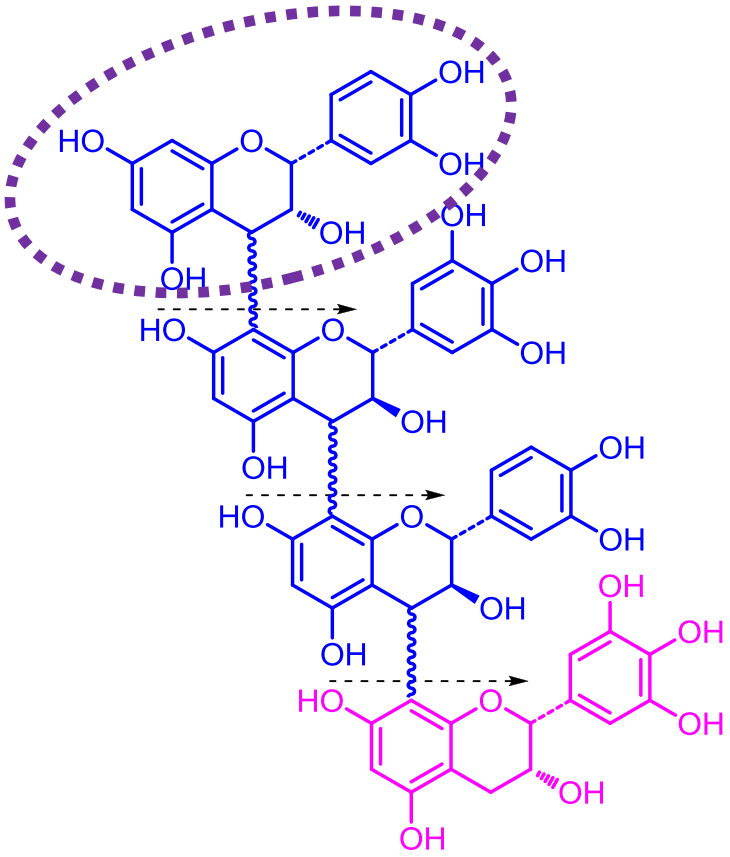
Results from 'LegumePlus': Advances in tannin analysis tools - A new HCl-butanol assay

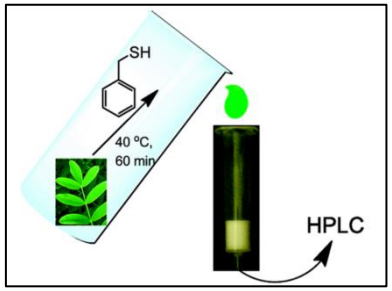


- Acetone: >2-fold tannin yield

Grabber *et al* 2013

Tannin analysis by thiolysis-HPLC



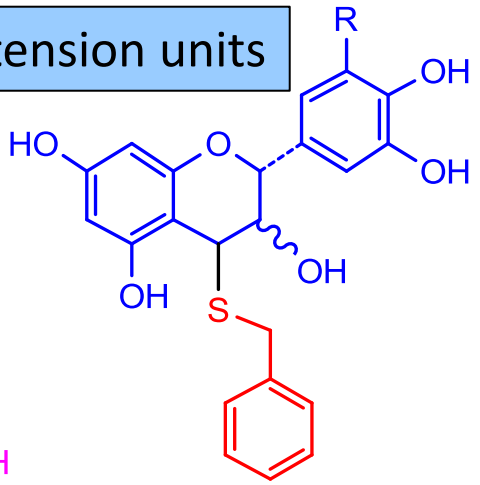


HCl

benzyl mercaptan

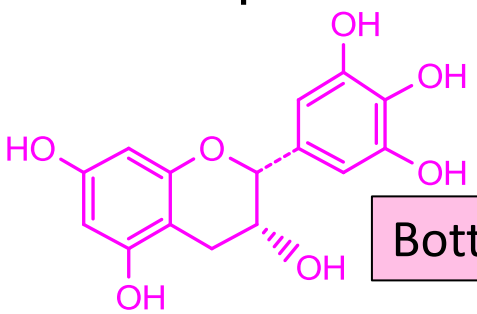
Top extension units

3 X



+

1 X



Bottom, terminal unit

- Thiolysis-HPLC for
  Tannin content, tannin size & subunit composition
  in plants or extracts

NIRS analysis for tannin contents

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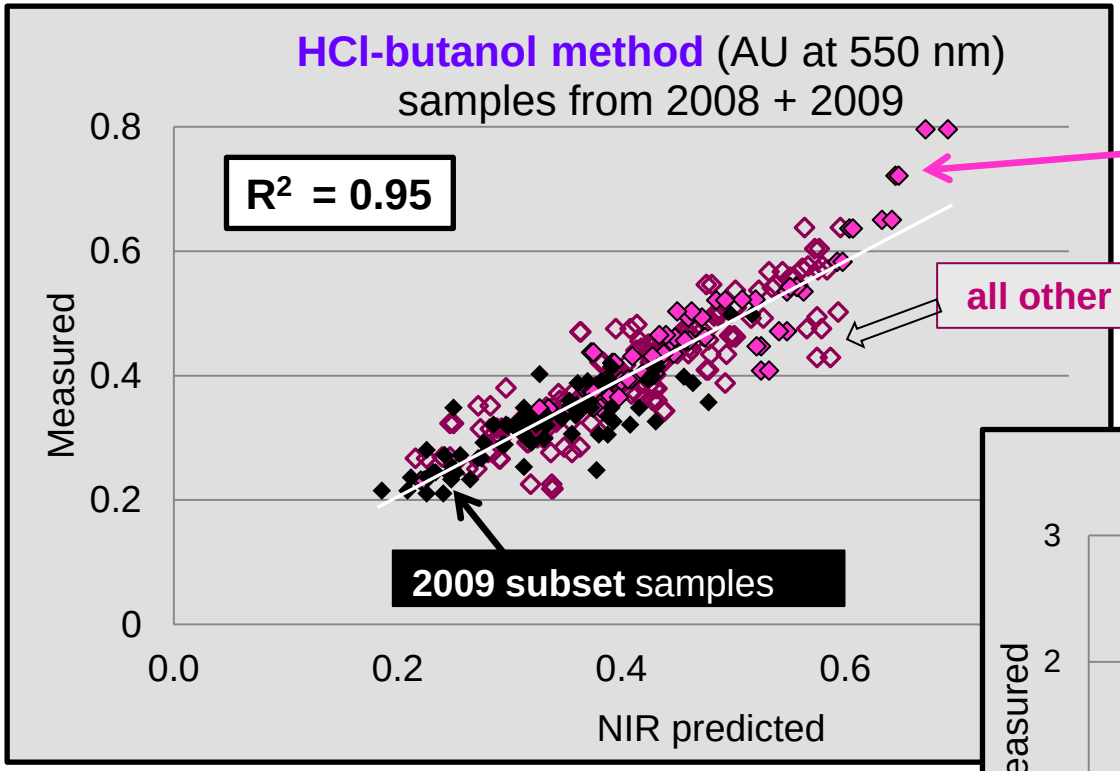

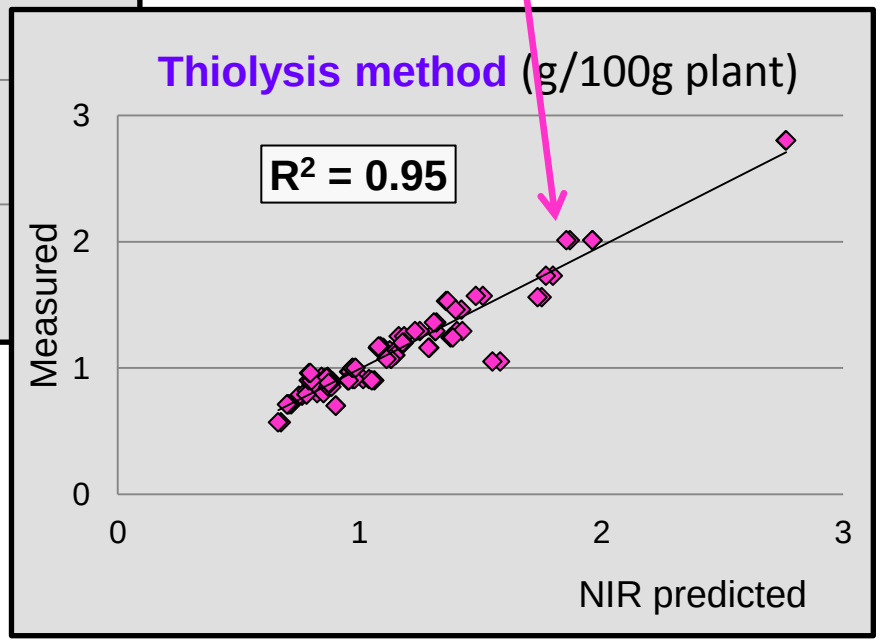

➢ NIRS for predicting tannin content

Martin-Lorenzo *et al* 2010; Gea *et al* 2011; Stringano *et al* 2011

Development of robust tannin methods: NMR

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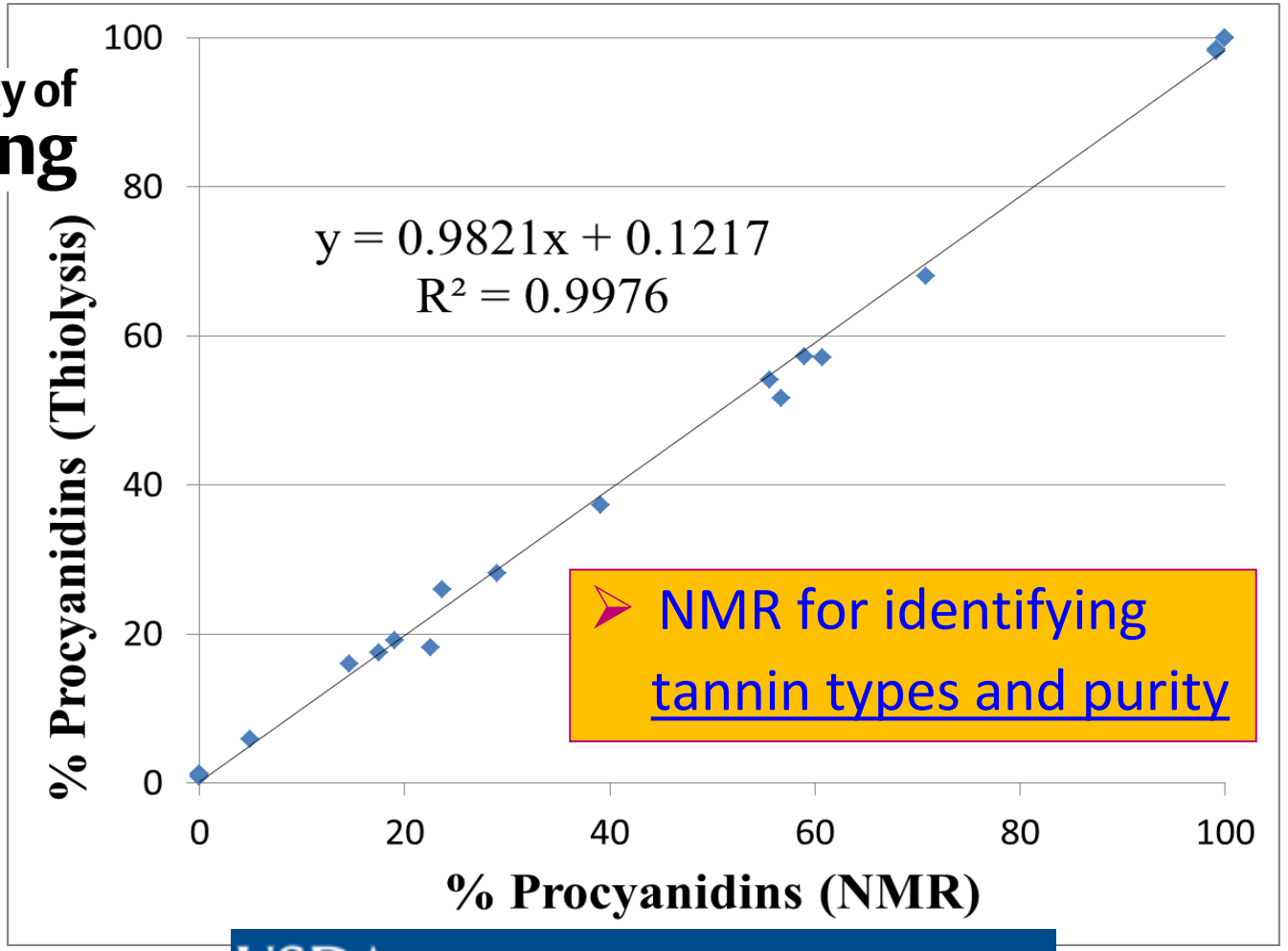

- NMR for identifying tannin types and purity

USDA United States Department of Agriculture

Zeller *et al* 2015

Thiolysis *vs* UPLC-MS/MS analysis

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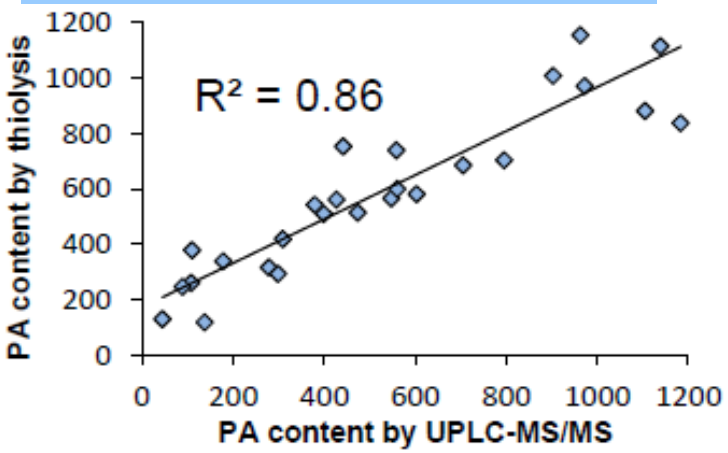
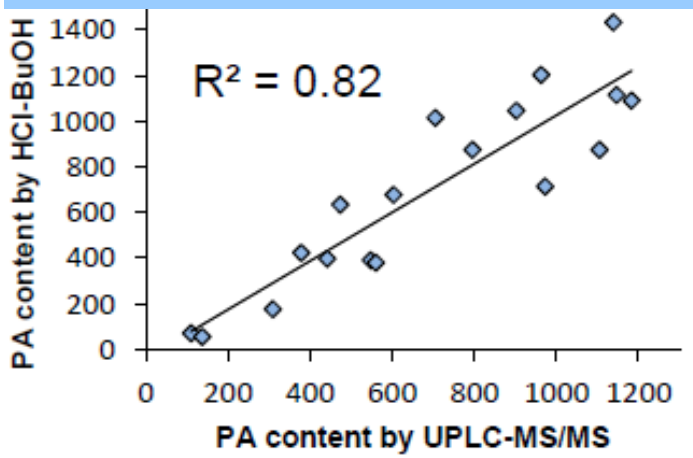
% Tannins by thiolysis

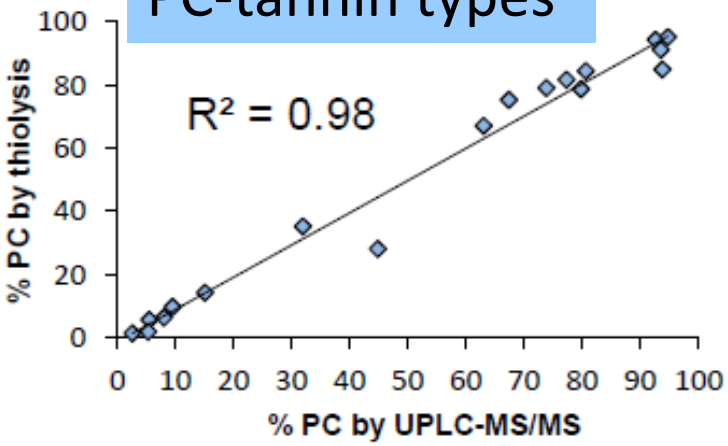


% Tannins by HCl-butanol



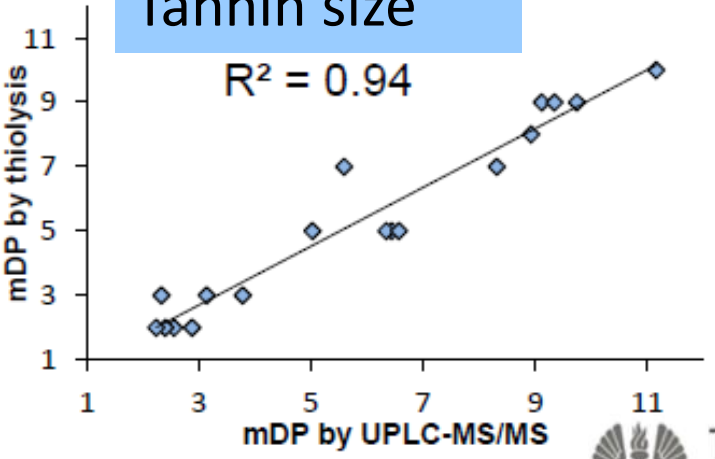
(C)

PC-tannin types



(D)

Tannin size



Thiolysis = days; Mass Spec = minutes

➢ Plant breeders need fast tools

Turun yliopisto
University of Turku

Engström *et al* 2014

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Agronomy and genetics

Results from 'HealthyHay' project

- Sainfoin taxonomy needs revision
- Large variation:
  - Agronomic characteristics, growth habit
  - Tannin contents, tannin structures, seasonal changes
  - Anthelmintic (deworming) effects
  - Probably also nutritional effects (contradictions in literature)
- Problems: Weeds

→ 30 promising accessions for Southern England

→ 3 promising accessions for Northern Spain

Hayot Carbonero et al 2012; Demdoum 2012; Stringano et al 2012

Summary of 'LegumePlus' work: Agronomy

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Agroscope

INRA
Institut National de la Recherche Agronomique

Switzerland, France:

Co-cultivation of sainfoin with companion species:

- Less weeds, better establishment, higher mixture yield
- Harvested biomass has better nutritive value

Switzerland: Drought stress:

- Some accessions performed very well

Lethbridge, Canada:

- Co-cultivation of alfalfa with new sainfoin populations (to tackle pasture bloat)

Malisch *et al* 2014; Copani *et al* 2014

Summary of 'LegumePlus' work: Molecular markers for sainfoin

- Identified novel SNP, SSR and SRAP marker resources
- Data to be deposited in Open Access Database

  → Ability to characterise genetic diversity of sainfoin

  → Tools for sainfoin breeding

Sainfoin collection: tannin variation

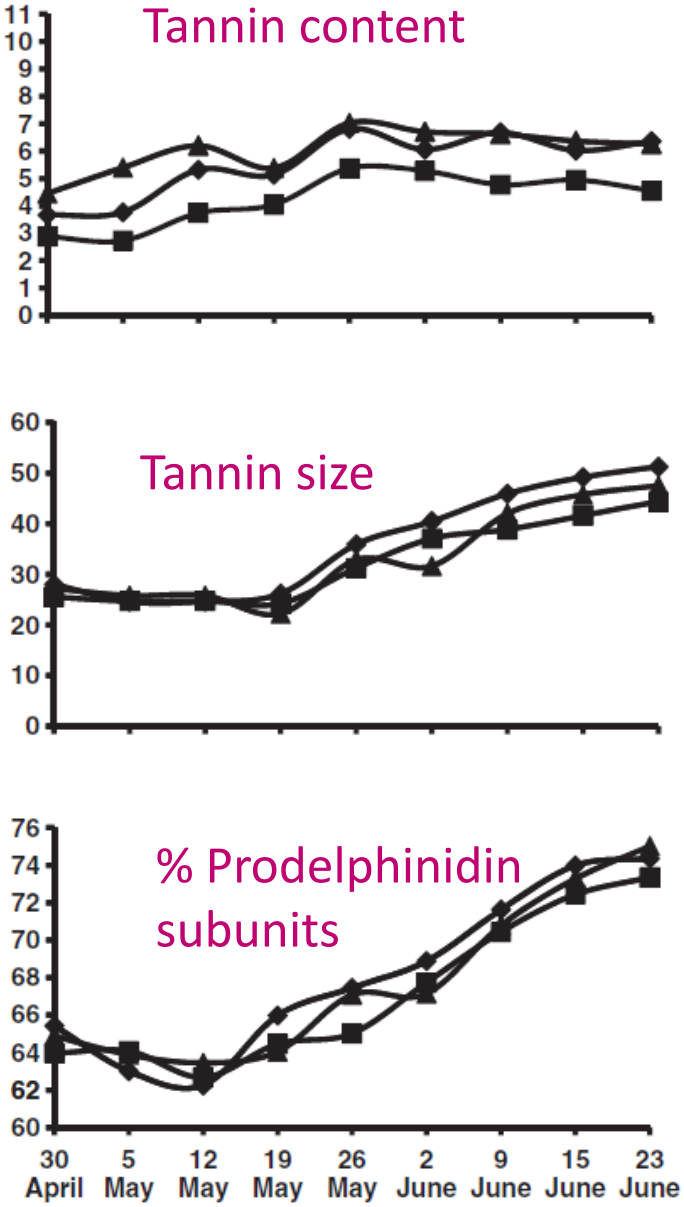
Accession	Origin	Tannin content (g/100 DM)	Tannin size (mDP)	% Prodelphinidins (3 OH groups)	% *Trans* flavanols
Hampshire Common	**UK**	1.1	**12**	68	20
247	Morocco	**0.6**	25	83	27
CPI 63820	Spain	1.1	18	**53**	**17**
CPI 63767	USA	0.9	**84**	83	30
Wkt 10	Turkey	**2.8**	18	**95**	**32**

Stringano et al. 2012; Hayot Carbonero 2011

Tannins also change with:

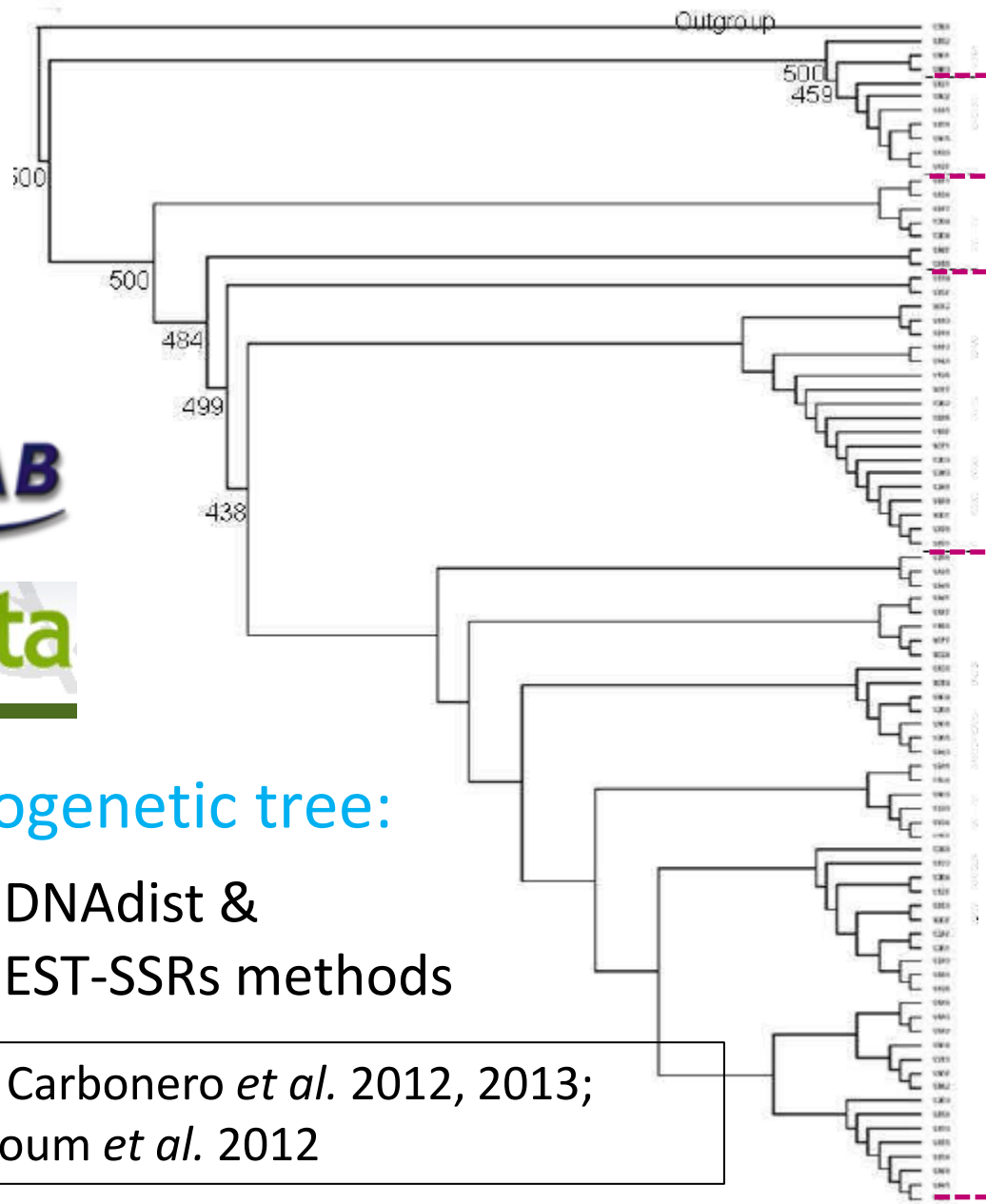
- Season (in France)
- Plant part

	g CT / 100 g DM	CT size (mDP)	PD %
Leaves	**5.0**	**37**	**80**
Stems	4.3	25	54



Theodoridou *et al* 2011; Azuhnwi *et al* 2013

DNA sequences & molecular markers



Heliobrychis

Hymenobrychis

Lophobrychis

Onobrychis viciifolia
Western Europe

Onobrychis viciifolia
Rest of world:
Turkey, Poland, Czech Rep, Romania, Armenia, China, Morocco, USA

Phylogenetic tree:

DNAdist &
EST-SSRs methods

Hayot Carbonero *et al.* 2012, 2013;
Demdoum *et al.* 2012

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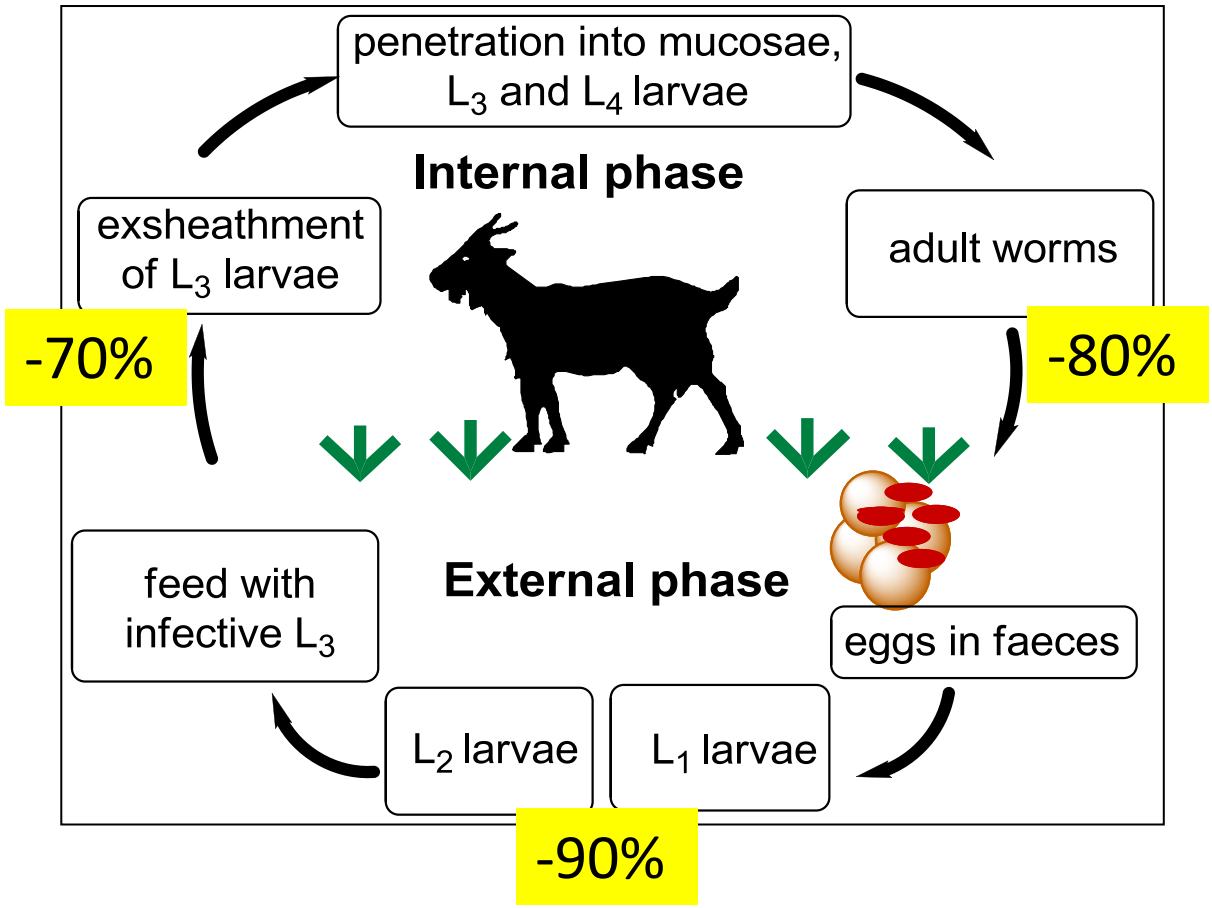
Action against parasitic nematodes

The problem: parasitic worms & drug resistance



Resistance:

- $$$ billion losses
- Animal welfare
- US, S. Africa: drugs NO longer work

Drugs:

- 99.9 % worms killed

Tannins:

- **Less effective, but enhance innate immunity**

Warmer temperatures → shorter life cycle:

- Climate change → even bigger problem
- **Tannins** interfere with this cycle

Hoste et al. 2012

Tannins can help

Tannins :

- Enhance immunity
- Act as dewormers
- Act as anti-coccidian

Niezen et al 2002; Saratsis et al 2012; Tibe et al 2012

Mechanism:
tannin binding to larvae & worms

Ascaris suum

pig parasite (& human)

- Tannins

Larvae are alive and well

+ Tannins

Larvae are stiff, dead, disintegrating

Williams *et al* 2014

Haemonchus contortus

small ruminant parasite

- Tannins

Worm surface is smooth

+ Tannins

Surface is shrivelled, mouth is blocked

Hoste *et al* 2012

Screening tools:
in vitro anthelmintic assays

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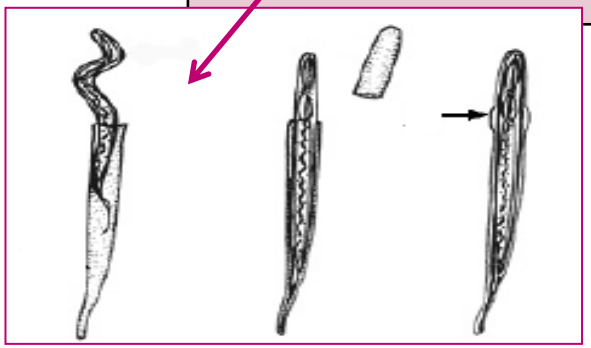
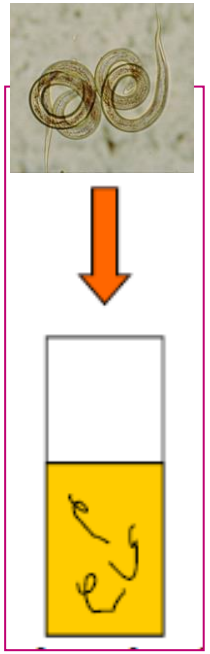
Larval assay	Inhibition caused by sainfoin extracts
Feeding	2 to 50 %
Migration	0 to 48 %
Exsheathment	0 to 82%





Manolaraki 2011; Novobilský et al. 2013; Desrues et al. 2013

Results from 'LegumePlus': Anthelmintic tannin effects

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- Abomasal *vs* intestinal worms: different susceptibilities
- *Haemonchus contortus*, blood sucking – abomasal:
  - controlled by tannins
- *Trichostrongylus colubriformis* – intestinal:
  - controlled by tannins

Results from 'LegumePlus': Tannin-protein interactions

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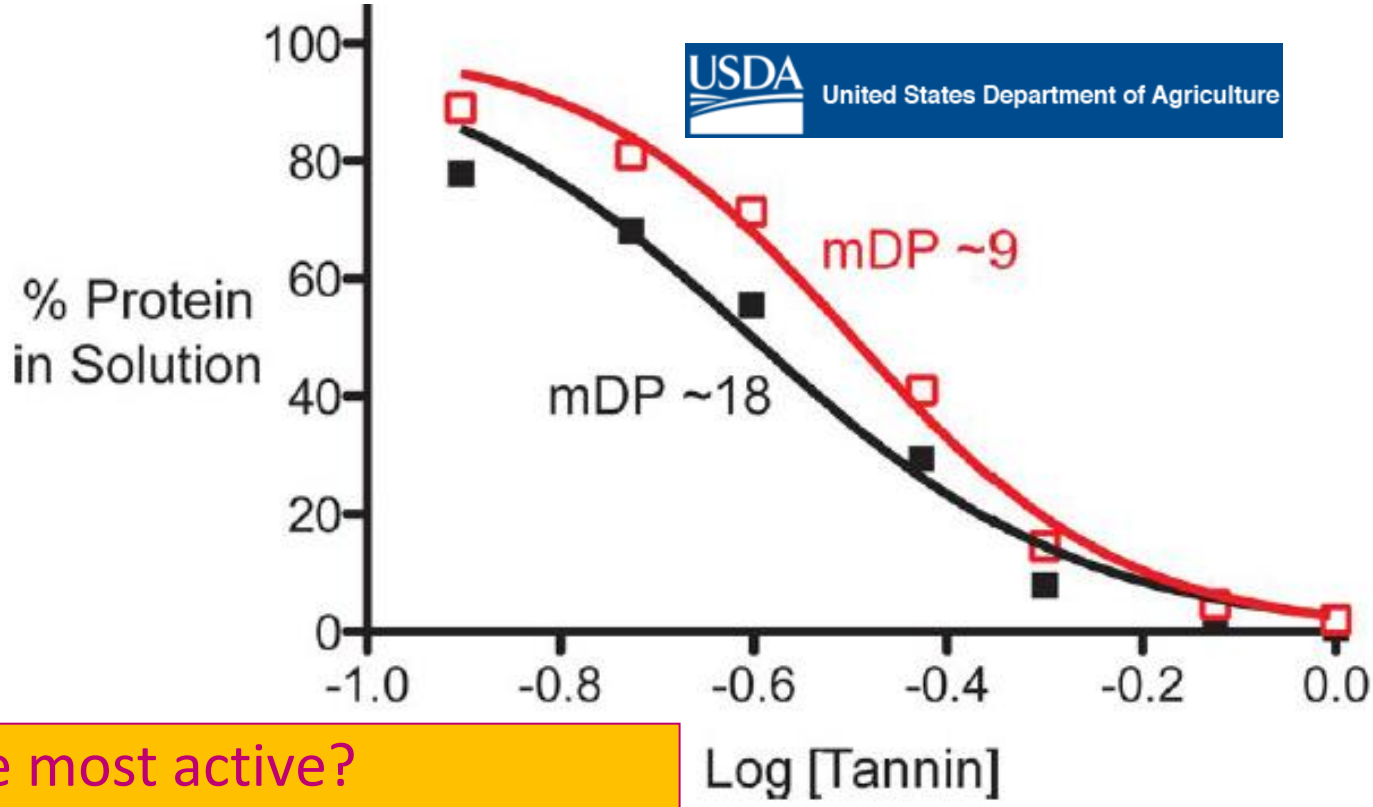

Which tannins are most active?

- All tannins coat protein surface
- Larger tannins precipitate more protein

→ **Implications for protein digestion & anthelmintic effects ???**

Zeller *et al* 2015;
Dobreva *et al* 2014

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Nutrient use, environment and food quality

Benefits to ruminant nutrition

*Sain(t)*foin = "*Healthy* hay" or "*Holy* hay"

- Non-bloating: suitable for grazing
- Cattle, sheep, dairy goats:
  - Intake (fresh, hay, silage) > alfalfa, white clover, grass
- High level of water-soluble carbohydrates
- More efficient utilisation of energy & protein
- Better growth rates (sheep)

- Legume: requires less nitrogen fertiliser

→ → → **more sustainable farming**

Wang *et al* 2015; Mueller-Harvey 2009

Benefits to ruminant welfare

- Bloat-safe → can be grazed
- 10 to 20% of sainfoin in alfalfa diet → 45 – 93% less bloat

Lethbridge (Canada): New sainfoin populations

- Suitable for co-cultivation with alfalfa
- Rain-fed, irrigated pure stands : better DM yields
- Mixed stands for high-performance grazing and multiple cutting systems

NOT suitable under drought or variable conditions

Wang *et al* 2015

Sainfoin can improve the environment

- Sainfoin: less shedding of food-borne pathogens (*E. coli* O157:H7)
- Sainfoin: less GHG

Wang *et al* 2015

Mechanism: protein degradation in the rumen

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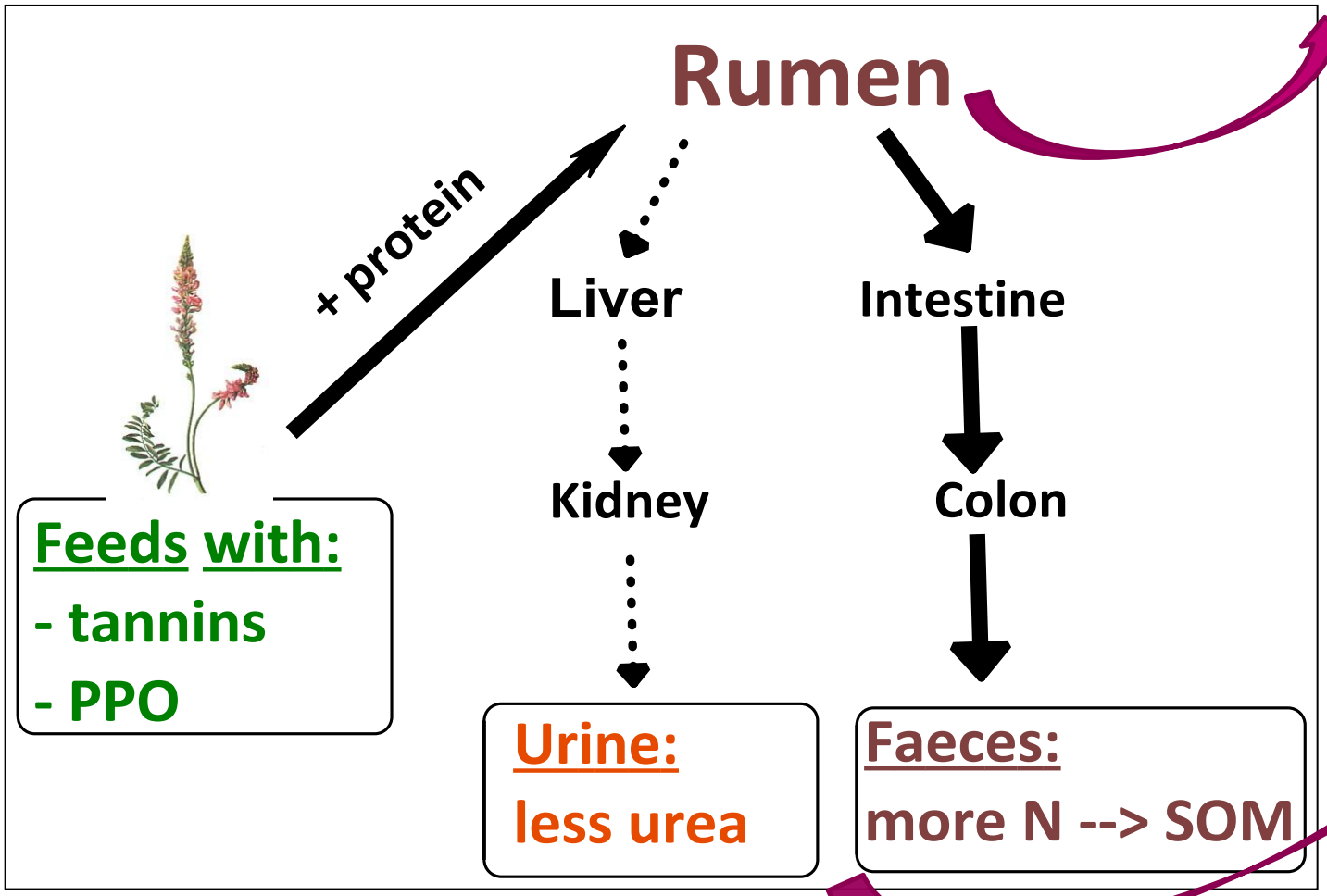

→ Plant tannins & PPO reduce NO_x GHG emissions

Waghorn 2008

Mechanism: protein preservation in silages

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1. High sucrose content → sainfoin is easy to ensile
2. Tannins: reduce proteolysis during fermentation
   - Ensiled sainfoin:
   - Co-ensiling of sainfoin + alfalfa or a grass:

} intact forage protein ↑
} NH_3 ↓

NPN (% of tot N) in silages

No bioactives:	
Alfalfa:	68 – 78 %
Tannins:	
Sainfoin:	23 – 42 %
Big trefoil:	25 – 30 %
Sericea lespedeza:	25 – 55 %
Birdsfoot trefoil:	40 – 75 %
PPO:	
Red clover:	50 – 60 %

Reduction in silages

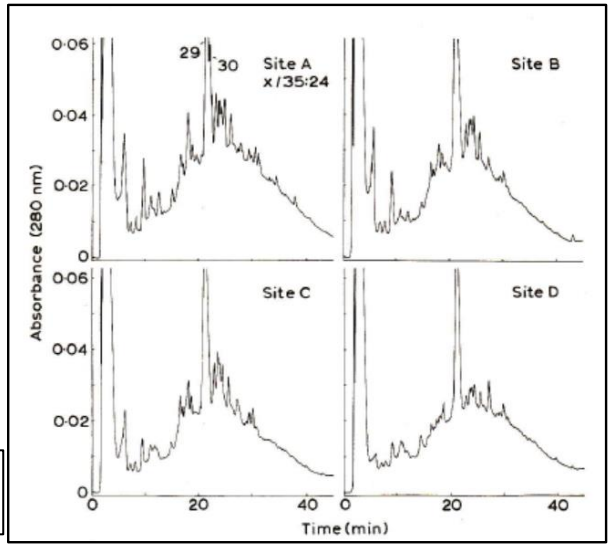
	NPN	NH_3	AA
Alfalfa:	100	100	100 %
Sainfoin:	48	51-77	41-50 %

Copani et al 2014; Lorenz *et al* 2009; Wang et al 2015; Albrecht & Muck 1991

Looking to the future

The next steps in sainfoin research

- Easier establishment: better competition against weeds
- Higher biomass and seed yields
- Identification of optimum tannin content and composition
- Identification of best tannins for nutrition and deworming
- Breeding of varieties with stable & resilient tannin compositions and contents



Mueller-Harvey & Dhanoa 1991; Azuhnwi et al 2013

Further Information

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http://legumeplus.eu

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http://healthyhay.eu

HealthyHay
Marie Curie Research Training Network

http://www.multisward.eu

http://www.legumefutures.eu

http://www.animalchange.eu

http://www.sheepandgoat.com/ACSRPC/Resources/sericea.html

Acknowledging our funders

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