

LegumePlus summary

1. Agronomy and Plant Breeding

- Legumes reduce our dependence on fossil energy and industrial N-fertiliser (Lüscher et al 2014). They also lower the quantities of harmful emissions to the environment (i.e. greenhouse gases such as methane and nitrous oxide). In addition, lower food production costs increase productivity and, importantly, protein self-sufficiency, which is predicted to become a problem in the near future.
- Work on marker development has been undertaken: a framework map has been established, and sequencing completed. We have found marker trait associations for plant height, mass, flowering time and seed yield.
- Sainfoin was found not to show any significant resistance to the weed treatment Glyphosate.

2. Parasitology

- Gastro-intestinal parasites such as nematodes and coccidia are nowadays a world-wide threat to animal production, as many parasites are exhibiting resistance to commercial drugs (Hoste et al 2015). This is akin to the better known threat from anti-microbial resistance reported from bacteria in hospitals.
- *Haemonchus contortus* is highly pathogenic, blood sucking parasite. LegumePlus researchers found that *H. contortus* was more susceptible than *Trichostrongylus colubriformis* to condensed tannins (CTs). The anthelmintic activity against *H. contortus* was associated with the monomeric subunits that give rise to prodelphinidin-type tannins and with tannin polymer size. However, for *T. colubriformis* anthelmintic activity was correlated only with prodelphinidins. These results suggest that CTs have different modes of action against different parasite species (Quijada et al 2015).
- The nematicidal (or anthelmintic) activity of CTs can be significantly enhanced by the addition of quercetin or luteolin, as assessed by the larval exsheathment inhibition assay (LEIA) with *H. contortus* third stage larvae (Klongsiriwet et al 2015).
- CTs may also have promise as an alternative parasite control option for *O. dentatum* in pigs, particularly against adult stages. Moreover, our results demonstrate a varied susceptibility of the different life-cycle stages of the same parasite to CTs, which may offer an insight into the anthelmintic mechanisms of these commonly found plant compounds.
- CTs can have potent, direct anthelmintic effects against another pig parasite, *Ascaris suum*. CTs caused significant damage to the cuticle and digestive tissues of the larvae (Williams et al 2014a,b). The strength of the anthelmintic effect is related to the polymer size of the tannin molecule and their subunit composition. For example, gallocatechin and epigallocatechin subunits within the tannin polymers exerted significant anthelmintic activity, whereas catechin and epicatechin subunits did not.
- Feeding sainfoin significantly reduced the worm burden of *Ostertagia ostertagi* in the abomasum of calves, where CT are thought to be released from complexes with macromolecules due to acidic conditions.
- As well as sainfoin, tannins from plants such as chicory and carob pods have been shown to have strong anti-parasitic effects.

- Sainfoin and sericea lespedeza leaves had more CTs than stems, a slightly higher percentage of PDs, and polymers of larger mean degrees of polymerisation (Mechineni et al 2014).
- Infection with *Eimeria* spp. (coccidian) can be devastating in goats, particularly for young, recently-weaned kids. Dried, pelleted sericea lespedeza has excellent potential as a natural anti-coccidial feed for weaned goats (Kommuru et al 2014).

3. Animal Nutrition and Product Quality

- Silages that contain bioactive legumes are better conserved than pure grass silages. In addition, bioactive legumes are able to preserve protein from degradation during the silage process. Inclusion of bioactive legumes can improve silage quality, and polyphenol oxidase may be more efficient than CTs to improve the nitrogen value of silage (Copani et al 2014)..
- The benefits of including red clover in forage mixtures previously observed on silage quality are also present on ruminal digestion efficiency (Copani et al 2015).
- Sainfoin decreases ruminal protein degradation and metabolic stress by lowering ammonia load. It has an effect on the ruminal community, but it is not clear yet if this is due to a direct effect on the microbes or due to an effect on the nutrient availability.
- Feed intake and milk production were both increased in sainfoin fed animals. Methane production per day and per kg dry matter intake was not affected, but methane produced per kg milk tended to decrease. This suggests that sainfoin fed animals may have a lower environmental footprint.
- Lambs fed sainfoin or birdsfoot trefoil produced meat containing more polyunsaturated fatty acids (PUFA), and less saturated fatty acids (SFA). The meat of lambs fed sainfoin also contained less skatole, and thus had a more appealing flavour than grass-fed lambs.
- Milk from cows fed sainfoin contained less urea, and more of the beneficial C18:3 PUFA.

4. Chemical Analysis

- Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been developed for the qualitative and quantitative analyses of plant tannins (Engström et al 2014). This enables:
 1. quantitation of total tannin content in plant extracts
 2. quantitation of two different tannin types, i.e. total procyanidins (PC) and prodelphinidins (PD) including the procyanidin/prodelphinidin ratio
 3. estimation of the mean degree of polymerisation (mDP) for the tannin polymers
 4. estimation for the first time of 'tannin fingerprints', by studying how the different PC and PD types are distributed along the chromatographic hump
- ¹H-¹³C HSQC NMR spectroscopy appears to be a viable alternative to thioysis for estimating PC/PD and *cis/trans*-flavanol ratios of CT, if precautions are taken to avoid integration of cross-peak contours of contaminants (Zeller et al 2015a).
- Development of a much improved HCl-butanol assay for measuring tannins directly in plant material. This assay can be used for extractable and for non-extractable tannins in the plant residue (Grabber et al 2013).

- In addition, we found that that tannin size influences protein precipitation efficacy (Zeller et al 2015b).

5. Summary & key points:

Partners from Copenhagen University (DK) and IHAP-INRA (FR), Toulouse found that sainfoin and other tannins are useful for antiparasitic effects.

Partners at WUR (NL), ILS (CH) and INRA-Theix (FR) established that sainfoin is useful not only for reducing greenhouse gas emissions but also for improving the quality of meat and milk products from ruminants.

Partners at ISS and Delly Seeds (CH), NIAB and IBERS (UK) discovered sainfoin markers, which will enable development of improved varieties in the future.

Partners at the Universities of Reading (UK) and Turku (FI) developed several novel analytical tools to enable the fast screening of tannins in plant breeding programmes.

Cotswold Seeds is a company taking the lead in the development of sainfoin as a forage, and have surveyed sainfoin use across the UK. The LegumePlus fellows have also gained training in counter-current chromatography (AECS Quikprep), near-IR spectroscopy (NIRS), and in paper and thesis writing (Pete Moore).