Legume-supported cropping systems for Europe

Legume Futures Report 1.3

Novel feed and non-food uses of legumes

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Legume Futures

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FOREWORD

The legume family, *Fabaceae*, is one of the largest in the plant kingdom. Almost all species in the family form symbioses with bacteria in the family *Rhizobiaceae*, leading to biological nitrogen fixation. While some other species scattered through the plant kingdom also fix nitrogen through symbioses, the legumes are the largest group and the most useful in agriculture.

This capacity for nitrogen fixation has several impacts. It means that the plants can grow in nitrogen deficient soils and at the same time produce protein-rich plant material, particularly protein rich seeds. This high protein content and production, which is intrinsic to legumes, determines much of the role of legumes not only in general human and animal nutrition, but also their suitability for novel feed uses and uses in the non-food sector. Biological nitrogen fixation is a characteristic of pioneer plants and so gives rise to another potential use of legumes in the bioremediation or colonization of soils otherwise unsuited for agriculture.

Legumes are also, compared with cereals, rich in a range of secondary plant compounds. Legumes have evolved mechanisms to produce and concentrate these compounds to protect against pest and disease attack. The bioactivity of these compounds opens up non-food opportunities which are specific to legumes.

This report also looks at non-traditional feed uses, such as whole-crop silage and fish feeds, examines some industrial uses of legumes in the bio-based economy, and concludes with a catalogue of recent demonstrations of the activities of bioactive compounds derived from legumes. A comprehensive gathering of such data would require hundreds of pages and thousands of references, and this document is intended to introduce the reader to the literature and present some of the more interesting highlights that are relevant in the context of European agriculture.

Fred Stoddard,

Helsinki, Finland, October 2013

INTRODUCTION

This report considers novel uses of legumes in the animal feed and non-food industries. If we are to consider "novel" uses, first we need to define "traditional" uses. With regard to animal feed, traditional uses include the grazing of pastures or saving of hay from grass mixed with legumes including clovers and alfalfa. Other traditional uses include usage of soya bean meal as a protein supplement for poultry, pigs and ruminants, and most uses of dried pea, faba bean or lupins for the same purpose. Lupins, being a fairly recently developed set of crops, may be considered novel in some regions and markets. Less traditional uses of legumes include using whole-crop grain legumes for silage, feeding lupin seeds to non-ruminants, and any use of plant proteins for feeding fish.

Among non-food uses, green manuring is too familiar to need mention here. The capacity of legume-rhizobium symbioses to fix atmospheric dinitrogen leads to potential impacts in bioenergy and biorefining that are discussed below. Legumes contain many secondary compounds that both contain nitrogen and protect the plant from herbivores seeking nutrition. Some of these compounds have been used in traditional medicine, and modern science has validated some of these properties. Other medicinal, biocidal or growth-promoting uses have been discovered.

There is a huge and developing literature on the non-traditional usage of legumes in foods, such as the substitution of other legume "milks" for soya milk, itself a substitution for milk from cows. While this literature is not addressed directly here, we touch on peptides and some other bioactive compounds that would most likely be consumed in a food matrix.

Three reviews have provided good examples and starting points for this review. Morris (1997) reviewed industrial and pharmaceutical legumes, and updated the review in 2003, with a preference for species adapted to growing in the United States. Howieson et al. (2008) reviewed a selected range of uses of legumes, focusing on human-health benefits, anthelminthics for ruminants, aquaculture, and deep rooting for access to water and nutrients.

NOVEL USES IN ANIMAL FEED

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There is a small but positive literature on ensiling of grain legumes and on their use as protein supplements for ruminants. Legumes have a low content of water-soluble carbohydrates and a high buffering capacity, so require wilting or treatment with additives such as formic acid or lactic acid bacteria in order to ensile satisfactorily (Pursiainen & Tuori 2008). Given these restrictions, silage can be made from whole-crop pea (Pursiainen & Tuori 2008, Borreani et al. 2009), faba bean (Pursiainen & Tuori 2008, Borreani et al. 2009, Pakarinen et al. 2011), white lupin (Fraser et al. 2005a, Pakarinen et al. 2011), narrow-leafed lupin (Fraser et al. 2005b) and yellow lupin (Serrano 1989). Results are often improved by mixing a cereal with the legume, e.g., durum wheat - faba bean (Mariotti et al. 2012). Soya bean meal as a protein supplement can be replaced in dairy or beef cattle feeding by seed or meal of pea (Brunschwig & Lamy 2003), faba bean (Moss et al. 2000), white lupin (Froidmont & Bartiaux-Thill 2004), narrow-leafed lupin (Eriksson 2010, Niwinska & Andrzejewski 2011) and yellow lupin (Marley et al. 2008). The choice of legume for the supplement depends on the background silage, as the slow breakdown of lupins makes them more suitable with grass silage, while pea and faba bean are more suitable with maize silage (Wilkins & Jones 2000).

The presence of anti-nutritional factors in grain legumes has more effect on their use for monogastrics than on that for ruminants. Trypsin inhibitors, vicine-convicine, tannins and alkaloids are implicated as limiting factors, depending on the animal and the feed. Nevertheless, reports can be found on the successful use of almost every grain legume species for feeding pigs, broiler hens, laying hens, and turkeys. Laying hens are notoriously sensitive to the vicine-convicine of faba bean, and broilers also benefit from the absence of these anti-s (Vilarino et al. 2009). Normal-vicine faba bean was acceptable at up to 31% in a mixed feed for broilers (Laudadio et al. 2011) and white lupin was acceptable up to 24% (Laudadio & Tufarelli 2011). Field pea, in contrast, could be included up to 50% of the feed of laying hens (Fru-Nji et al. 2007). The low digestibility of the storage galactan in lupin seeds reduces their value in monogastric feeds. Supplementing a feed based on yellow lupin (Olkowski et al. 2010) or narrow-leafed lupin (Steenfeldt et al. 2003) with a glycanase mixture resulted in significantly improved performance of broilers. Pea was more digestible than faba bean and narrow-leafed lupin in turkey diets (Palander et al. 2006). Pea and faba bean are equally suitable for grower and finisher pigs (Smith et al. 2013), and low tannin content provides a valuable increase in the apparent metabolizable energy of the legume (Crépon et al. 2010). The standard ileal digestibility of protein from narrow-leafed and yellow lupin was as good as that of soy bean meal, while those of pea and faba bean were significantly lower (Jezierny et al. 2011).

Thus European cool-season grain legumes can be used to replace part or all of the imported soya bean meal used in feeding poultry, pigs and ruminants, at least on an experimental scale. Amino acid balance, ileal digestibility for monogastrics, rumen stability for ruminants, apparent metabolisable energy, and the restrictive effects of the individual antinutritional factors of each cultivar, all need to be taken into account. Some of these limiting factors can be amended through breeding, others through post-harvest treatment, and others by using additives or other means of balancing the rest of the feed formulation.

LEGUMES IN FEEDS FOR FISH AND CRUSTACEANS

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Aquacultural production is an important economic activity in the EU, with a total value estimated at €2,800 million, representing 7.6% of global aquaculture value and 4.6 % of global tonnage. This represents almost a doubling in output since the late 1980s (Tacon, 1997). The enterprises driving this production vary in scale from cottage industry to large multinational operations, although 90 % are small-to-medium enterprises (SMEs) (Varadi et al., 2000).

The farming of fish and shellfish for food supplies approximately 50 % (by mass) of all the fish and shellfish consumed worldwide (National Research Council, 2011), and the bulk of this production originates from the highly commercialised sector that has transformed the practice of aquaculture largely through the use of modern science and engineering. Commercial aquaculture production is a high-value activity and wholly dependent on the use of manufactured feed. The sourcing of the components of this feed is of primary consideration to achieve efficient production while minimising environmental impacts. The increased output of the commercial aquaculture sector with reduced environmental impacts has been achieved by reducing the use of wild fish resources in feed and increasing feed conversion efficiency. In particular, the amount of high-quality edible fish produced per unit of fish used in fish-feed has increased.

Aquaculture also provides rural employment and alleviates rural poverty, and sustainability of production is now considered a more desirable objective than volume of production (De Silva 2001, Foresight 2011). Moreover, as the world's population is projected to be 9 billion by 2050 (United Nations, 2009), there is doubt in equal measures about the ability of both traditional cottage and commercial aquaculture systems to meet increasing global demand for food. Although the aquaculture industry produces a vast array of fin fish, molluscs and crustaceans by catch and managed activities, two groups of farmed species, namely shrimp or prawns (Penaeidae) and the salmon family (Salmonidae), offer opportunities for increased economic potential and enhanced food security, due to their relatively high market value and potential for vertical business integration (Swick & Cremer, 2001). Most importantly, although they are omnivorous to carnivorous, research has shown that other sources of protein and oil can substitute for the fish products normally (and in natural ecosystems) used for their nutrition (Trushinski et al., 2006). Vegetarian fish, such as carp, may be able to make even better use of plant-based feeds. A successful future for European aquaculture industries appears to rest on an abundant, affordable and, most importantly, sustainable supply of alternative to fish meal (FM) in aquaculture feeds. The ingredients that can substitute for fish meal may be sourced from land animals, microorganisms (algae, yeast and bacteria) and plant sources such as oilseeds, cereals and grain legumes (Gatlin et al., 2007). The feed is generally formulated into pellets that are sprinkled onto the surface of the water, from which the fish rapidly snatch it. Cohesion of the pellets is essential, so components such as starch that gelatinizes in the pellet extruder, and wheat gluten are commonly used (Brinker & Friedrich, 2012).

Increasing the amount of fish produced per unit of fish used in feed is central to developments in the nutrition of fish. This requires increasing the plant-based components of fish feed while not compromising fish growth, health and product quality. However, there have been few empirical determinations of the nutrient requirements of fish species farmed using plant-based feeds (National Research Council, 2011). Plant feedstuffs for aquaculture tend to provide relatively low levels of essential amino acids, particularly methionine, tryptophan and histidine, but these can be supplemented by inexpensive, synthetic amino acids (Dias et al., 2005). Higher plants also provide low levels of the bioactive long-chain, polyunsaturated (omega-3) fatty acids that are produced by marine algae. Hence, fish meal and fish oil are still important components of many aquaculture feeds, but research on plant oils is moving rapidly and plant sources of long-chain, polyunsaturated fatty acids are being developed to deliver quantities at the commercial scale necessary to meet the demands of the aquaculture industry (Miller et al., 2008; Naylor et al., 2009).

Initial success using faba bean and narrow-leafed lupin as aquaculture feedstocks, and the increased global demand for fish produced in Europe, may help to develop the market for locally produced grain legumes in Europe. Some large commercial fish farms (such as EWOS, UK) have committed to avoid purchasing soya bean grown in previously forested areas of the tropics. In experiments, soya bean meal had a negative and dose-dependent effect on fish digestive systems attributed to anti-nutritional factors (ANFs), with a consequent loss of fish health, meat quality and yield (Krogdahl et al., 2003). Thus heat treatments to denature the ANFs are usually necessary when soya bean meal is to be used for fish feed, and the economic and environmental costs of fish meal, soya bean meal, and alternative grain legume protein sources need to be taken into the overall equation.

As shown below, faba bean and narrow-leafed lupin are both particularly well suited for use in salmon feed, and dehulled, protein enriched meal could successfully displace other vegetable protein sources, such as those based on soya bean. Formulation of fish feed is as complex and precise as that of other animal feeds, and in experiments, feeds under comparison are formulated to be as isoenergetic and isonitrogenous as possible.

Grain legumes contain anti-nutritional factors that affect different animal systems, including proanthocyanidins and condensed tannins , along with alpha-galactosides, lectins, saponins, phytic acid, and trypsin inhibitors. In faba bean, there are also the pyrimidine glycosides vicine and convicine and the free amino acid L-DOPA (L-3,4-dihydroxyphenylalanine), while lupins contain alkaloids.

The proanthocyanidins and condensed tannins that reduce the efficiency of the digestion of proteins by animals (Griffiths 1986; Griffiths and Jones 2006) are in the seed coat, and hence removed in the dehulling and air-classification processes that are expected to be used in making protein concentrates for fish feed (see below). Most cultivars of narrow-leafed lupin are low in tannin, and two zero-tannin genes (zt1 and zt2) are used in faba bean breeding programmes (Crépon et al., 2010). Of the remaining anti-nutritional factors in faba bean, vicine and convicine are associated with protein bodies and are likely to have elevated concentration in purified protein extracts (Olsen & Andersen, 1978). Their aglycones, divicine and isouramil, cause oxidative stress in rats, laying hens, and glucose-6-phosphate dehydrogenase deficient humans (Arese et al. 1981), and are considered the most likely of the faba bean anti-nutritional factors to affect fish. A gene for low vicine-convicine content, zvc, has been identified and used in faba bean breeding programmes (Crépon et al., 2010), so it is likely that this factor will cease to be a significant problem in the near future. ANFs are among the natural defence mechanisms of plants, and they are varied in chemistry and in mode of action. Their content can often be reduced by breeding in the long term and by processing in the short term, although the processing is generally expensive and complete removal is seldom commercially feasible. More significant is the fact that salmonid growth and feed efficiency tends to be higher on moderate levels of several plant proteins than on a single, perhaps because in this way no single ANF is limiting. This observation is of critical importance for feed formulations, which demand that that many new plant protein sources have a role alongside those already used in the blend. A complex blend of several legume components replaced 66% of the dietary animal protein without significantly reducing growth or quality of the rainbow trout (Gomes et al. 1995).

There are several times more publications on the use of lupins than that of faba bean in feeds for salmonids. A feed based on soya bean (plant protein replacing 20% of animal protein) was significantly worse for Atlantic salmon, *Salmo salar*, than other nine feeds, and it was associated with symptoms of enteritis, whereas feeds based on pea or dehulled faba bean gave the best results (Aslaksen et al. 2007). The low cellulose content of the dehulled narrow-leafed lupin was associated with high lipid digestibility (Aslaksen et al. 2007). Narrow-leafed lupin kernel meal showed no negative effects on the growth of rainbow trout at the maximum experimental level of 30% of the diet (Glencross et al. 2008). Protein concentrate from narrow-leafed lupin resulted in a higher feed conversion ratio than the equivalent from pea (Zhang et al. 2012). Removal of the oligosaccharides is a necessary step in preparing digestible feeds from narrow-leafed lupin (Glencross et al. 2003).

Replacing animal protein with plant protein results in friability of the fish faeces, and addition of guar gum, extracted from another legume, the guar bean (*Cyamopsis tetragonoloba*), to the feed of rainbow trout allowed the faeces to retain their stability and sink to the bottom of the tank, maintaining system hygiene (Brinker & Friedrich 2012).

Given that sustainably produced plant-derived feedstuffs are available, their suitability to underpin the production of fish species beyond salmonids will also be important, since commercially successful farms for non-salmonids such as turbot, eel, European sea-bass and gilthead sea-bream exist in Europe. There is also considerable additional commercial opportunity to develop the use of grain legume protein in feeds for a wide variety of farmed marine and freshwater species. It is also pertinent to note that carp raised in the earthen ponds that dominate the aquaculture production of Eastern and Central Europe (5% of EU aquaculture output: Varadi et al., 2000) are herbivorous or omnivorous species and easily assimilate protein from grain legumes, including pea, soya bean (Davies & Gouveia 2010), white lupin, yellow lupin and faba bean (Mazurkiewicz 2009), with an optimum protein composition around 30% plant protein and 70% animal protein. European sea bass (Dicentrarchus labrax) showed little difficulty with diets where almost all fish meal was replaced with a mixed plant-based diet containing maize gluten, wheat gluten, soya bean meal and rapeseed meal (Kaushik et al. 2004).

Lupin kernel meal has been widely tested as a feed component for the black tiger shrimp, *Penaeus monodon*, and the Pacific white shrimp, *Litopenaeus vannamei*. In most studies, the kernels have been dehulled and defatted before use. Alkaloids at normal levels caused a brief interruption in feeding by black tiger shrimp, without long-term detriment (Smith et al. 2007a). Depending on the lupin and the shrimp species, the shrimp grew well with up to 40% (Smith et al. 2007b), 41.5% (Smith et al. 2007a), or 50% (Molina-Poveda 2013) of the protein being plant-derived.

The high concentration of protein in aquaculture feeds (with a weighted average of ca. 35 % for salmon), cannot be achieved by most legume grains without further processing. While the main energy component of legume grains is lipid, non-starch polysaccharide (in lupins), or starch, for those legume grains containing starch (such as faba beans), air classification (AC; also known as “air fractionation” or “protein enrichment”) may be applied (Shapiro and Galperin, 2005). AC allows the dry separation of particles from finely ground flours according to their size, shape, density and, hence, flow behaviour in an air-stream (Boye et al. 2010; Pelgrom et al. 2013). The fine grinding can be achieved by well controlled pin milling and jet milling methods. After the fine grinding, the protein bodies of the seeds can be detached from the starch granules. Then the protein-rich fraction containing mainly fine particles that fly faster is separated from the starch-rich fraction containing mainly coarse particles by a stream of air.

As compared to the more traditional protein-starch separation method, wet fractionation, AC is more cost effective (saving energy, water and time), hygienic and ecological. Moreover, AC keeps the processed starch and protein in a more native form without reducing their functionality. The wet-process method is dependent on the solubility difference between protein and starch. A large amount of water is used in wet processing for starch production from pulses such as mung bean and faba bean. The process takes a long time and uses considerable energy input during the washing, delivering and precipitating (centrifuging) steps. In addition, the collection of protein from the wet process needs acid for protein precipitation and further inputs of energy for centrifugation and drying. The heat-drying step in wet-processing of legume protein may denature the proteins and reduce their rehydration capability. As a result, in practice, it is considered difficult to use the protein-containing "wastewater" from wet-processing of legume starch for feed and food (Ishida et al. 2012).

AC has been used for many food and feed purposes in cereal and legume processing, for example, starch purification (Vasanthan & Bhatty 1995, Létang et al. 2001), protein concentration (Gunawardena et al. 2010) and fibre enrichment (Ferrari et al. 2009). The efficiency of AC depends on the nature of the grains and some parameters of the process. First, lipid removal is normally favourable for the protein, fibre and starch separation in lipid-containing materials such as oats (Sibakov et al. 2010) and rapeseed meal (King & Dietz 1987), and hence probably for separating protein from fibre in lupins, but is unnecessary for legumes like faba bean that contain little lipid. Second, lower seed moisture content results in a higher protein-starch separation efficiency of faba bean and field pea (Tyler & Panchuk 1982). Third, the milling should not be too coarse, too fine or break the starch granules in order to achieve good protein-starch separation (Boye et al. 2010, Pelgrom et al. 2013). In addition, the parameter settings of the air-classifier such as wheel speed and airflow also have to be optimised for an acceptable separation of protein from starch. Separation may be improved by re-milling and reclassification of the coarse (starch-rich) fraction, after which the resultant second fine (protein-rich) fraction is combined with the first protein fraction. Multiple passes give increased protein concentration in the fine fraction but with decreasing benefits with each pass (Wu & Nichols, 2005). The efficiency of AC should be comprehensively evaluated, as a process producing a higher yield of a fraction may result in a lower purity (for example, protein concentration in protein fraction) (Pelgrom et al., 2013). Moreover, on the same raw material, higher efficiency of protein separation (the percentage of the flour protein recovered in the protein fraction) may be associated with lower efficiency of starch separation (Tyler & Panchuk, 1982). Protein separation efficiency by AC was up to 68.0% in pea and up to 69.7% in faba bean (Tyler & Panchuk 1982). Since then, technology has advanced, and protein separation efficiency was up to 76.8 % in a pea AC study (Pelgrom et al. 2013). The presence of some starch is beneficial to the formation of the feed pellets under heat extrusion, and faba bean starch expands well on gelatinisation, so wheat or other cereal starch can be removed from the formulation.

It is clear that intensive European aquaculture will benefit from a reduced reliance on soy bean and marine-based protein sources for environmental, nutritional and supply reasons. The use of de-hulled faba beans and lupins can provide a protein source that meets this need in a sustainable manner. Air classification is an appropriate method for enhancing the protein content of the faba bean component of the feed mixture.

NON-FOOD USES OF LEGUMES

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One of the main reasons for using bioenergy is the reduction of greenhouse-gas emissions. The manufacture of synthetic nitrogen fertilizer relies heavily on fossil fuel as a source of energy input, and the use of synthetic and organic nitrogen fertilizer leads to the release of nitrous oxide, a potent greenhouse gas. Hence, the use of nitrogen fertilizer in bioenergy cropping is generally minimized, and legumes have a potential role (Stoddard 2008).

First-generation biofuels

The first-generation biofuels are made using simple technologies in order to replace fossil fuels. Starch from cereals and sugars from sugar crops are fermented to bioethanol to replace or substitute for petrol (gasoline). Seed oil can be converted to biodiesel by transesterification, in which the bond of the fatty acid with a glycerol residue is replaced by one with a methanol residue. Biomethane, produced by digestion of a wide range of organic substraits, is sometimes put into this category, but its production is based more on biomass, so it is handled in the next section of this chapter.

Legume starch can be converted to bioethanol in the same way as cereal starch, but since starchy legumes generally yield much less than cereals and their starch content is lower, it is highly unlikely that this will ever be economic or sustainable.

An early life-cycle analysis of bioenergy production showed that the nitrogen-fixation capacity of soya bean gave it a significant advantage over other oilseeds (Hill et al. 2006). Nevertheless, the use of soya oil for bioenergy is as questionable as the use of any other food or feed material, and other oils have been sought. For the semi-arid zone, two woody species contend: *Jatropha curcas* L. (Euphorbiaceae) and the Indian native *Pongamia pinnata* (L.) Pierre (sometimes put in genus *Millettia*) (Fabaceae). The toxicity of *Jatropha* (it is known in English as vomit nut) has inspired remarkably heated debate about its appropriateness as a crop for poor farmers, and *Pongamia* grows in similar environments, fixes its own nitrogen, and yields more oil per hectare without toxicity problems (Scott et al. 2008, Biswas et al. 2011). Karanjin, a furanoflavonoid reputed to have pesticidal properties, can be extracted from the oil as a value-added compound (Vismaya et al. 2010). The oil-free meal contains anti-nutritional factors that impede its use as an animal feed (Vinay & Sindhu Kanya 2008), but it can be put through a methane digester to recover further bioenergy, and the nitrogen- and mineral-rich residue used as a fertilizer.

Biomass production

The current focus on biomass is largely for energy purposes. Whole crop digestable biomass can be digested to methane, in a basically low-technology process, replacing natural gas. The lignocellulosic (woody) fraction can be combusted for heat and electricity, or converted to cellulosic ethanol for transport fuel. A further option is pyrolysis to synthesis gas (that can be polymerized over catalysts to form liquid fuels) and biochar (a fine charcoal that is used either as a solid fuel or a non-biodegradable, carbon-sequestering soil amendment).

Trees
The wood of *Acacia* species and the North American native *Robinia pseudoacacia* L. (black locust) has long been used as fuel, and ways to convert it into modern biofuels are under investigation. Life-cycle analysis showed that *Robinia* in the Po valley of Italy was superior to *Eucalyptus globulus* and hybrid poplar in Galicia, Spain, for the production of cellulosic bioethanol, largely because of its nitrogen autonomy (Gonzalez-Garcia et al. 2012). Short-rotation coppicing enhances harvestable yield of *Robinia* (Grunewald et al. 2009), and there is already a large literature on managing the crop in this way.

Black locust survives temperatures as low as -40°C in Canada, so it has widespread potential in Europe. Its main drawback is the production of suckers, giving it potential as an invasive alien. At lower latitudes, in Greece, *Acacia cyanophylla* produced more biomass at lower cost per tonne than *Eucalyptus camaldulensis*, *Populus nigra*, and *Arundo donax* (Tzanakakis et al. 2012). The wood of many acacias, as well as *Robinia*, is dense, very durable, and used for such purposes as fence posts, while that of the Australian native *A. melanoxylon* is highly prized for cabinet-making.

Grass-legume intercrops
Many perennial grasses have shown potential as bioenergy crops, but require some nitrogen fertilization, so the scope for using a legume intercrop has been investigated. Results are often disappointing, for a number of reasons. Crop mixtures are quite difficult to design, as they require compatibility and complementarity in resource acquisition both above and below ground, and in phenology and growth cycles. Furthermore, perennial rhizomatous grasses such as miscanthus (*Miscanthus* x *giganteus*) that are harvested as dry lignocellulose in the spring conserve much of their nitrogen in the soil-plant system, with the result that fertiliser nitrogen requirements are low.

In middle latitudes of North America, yields of switchgrass (*Panicum virgatum*) were not significantly affected by selected legume intercrops, but nitrogen fertilisation was greatly reduced or eliminated (Wang et al. 2010). In lower latitudes of North America, intercropping of alfalfa (*Medicago sativa* L.) with the switchgrass improved overall yield to that of a highly fertilized grass control at a low-fertility site , but did not affect economic yield at a high-fertility site (Butler et al. 2013). At high latitudes in Europe, reed canary grass (*Phalaris arundinacea*) requires annual N fertilization, and we have preliminary evidence that this can be met by mixed cropping with *Galega orientalis* (Legume Futures project, in progress). Thus there is clear potential to replace nitrogen fertilization of some energy grasses by a nitrogen-fixing legume intercrop, and this contributes positively to the greenhouse gas mitigation effect of the system.

Biorefining

Biorefining offers a way for combining feed and bioenergy production. Alfalfa leaves or leaf protein can be used for livestock feed and the lignified stems as biofuel (González-García et al. 2010, Kamm et al. 2010). Similar suggestions have been proposed for clover-grass or clover-cereal mixtures (Thomsen & Hauggaard-Nielsen 2008). In such systems, the technical product can be polylactate for biodegradable plastics, instead of bioethanol or similar biofuel. The area was recently and comprehensively reviewed (Jensen et al. 2012). A narrowly defined study based on energy and exergy relationships suggested that legumes have no merit in energy crop production, owing to their lower yields, but its authors acknowledged that environmental impacts were not considered, and the only legumes considered among the 12 bioenergy crops were soya bean and alfalfa (Brehmer et al. 2008). The dry matter yields of alfalfa used in their calculations were remarkably low, at 4.5 t/ha. Thus, the Brehmer et al. study is useful insofar as it raises questions that should be addressed, but its conclusions are based on an imperfect data set.

Other industrial uses of woody legumes include fibre and pulp. Sunnhemp (*Crotalaria juncea*) is so-called because of its technological similarity to hemp. It produces long fibres that can be used in similar ways to hemp or jute (Ingle & Doke 2006). Leucaena (*Leucaena leucocephala*) is often used to provide forage in subtropical areas, but its stems also produce good quality fibre for pulping (Díaz et al. 2007).

Tree legumes that are grown for one purpose may be further exploited for other uses. *Acacia senegal* is grown primarily for the production of gum arabic, but it produces an annual crop of seeds with an oil concentration of around 10% (Nehdi et al. 2012). The oil has a high content of oleic and linoleic acids, making it suitable for some food and industrial purposes when the relatively high content of free fatty acids is removed (Nehdi et al. 2012), and the seeds have a protein content up to 39% (Balogun & Fetuga 1986), although the prevalence of antinutritional factors precludes their use as feed. Seed oils of another source of gum arabic, *Acacia arabica,* contain industrially important cyclopropene and epoxy fatty acids (Hosamani et al. 2002). Similarly, black locust trees produce an abundance of seeds that have potential in the biorefining chain or at least as a feedstock for the methane digester.

Phytoremediation

Phytoremediation is bioremediation with plants, that is, the use of plants and their associated microorganisms to amend polluted or contaminated environments. Other physical or chemical methods of remediation are based on moving the contamination to another location or replacing one difficult chemical with another. Phytoremediation, in contrast, is an environmentally friendly technology, requires minimal resources, preserves natural soil properties, acquires its energy (mainly) from sunlight, achieves high levels of microbial biomass and is low in cost, but is usually slow. Use of contaminated ground for production of food or feed is usually undesirable, but there are few concerns about its use for bioenergy or other industrial purposes. By using polluted soils, which simultaneously undergo bioremediation, for bioenergy or industrial cropping, considerable environmentally excellent results of importance both for nature and society can be achieved.

There is a large literature on the application of legumes to bioremediation of contamination by heavy metals and petroleum products. Bioremediation of oil contamination is based on the enhancement of living conditions for soil microorganisms by exudates and breakdown products of plant roots. The oil raises the C:N ratio of the soil, so legumes are more likely to be successful as they require little soil nitrogen. Polycyclic aromatic hydrocarbons (PAHs) are highly toxic, low mobility, durable residues of oil pollution so their degradation is a high priority. Numerous studies have shown that the presence of plants results in faster degradation of oil residues than in bare soil. Plant cover also reduces erosion and legumes improve soil fertility.

Many papers report the detrimental effects of oil pollution, including PAHs, on germination, shoot dry weight and root dry weight of specific plant species. Only a few of these go further and examine the detailed effects on other important root traits such as root length and surface area. Useful variations in these aspects of root structure were shown in 13 grasses and 8 legumes grown in nitrogen-amended, oil-contaminated soil (Kirkpatrick et al., 2006).

The plant has little direct effect on the degradation of petroleum products. Rather, it is the soil-borne microbes that do the degradation, and their community composition is affected by the plant and its associated rhizosphere bacteria, including rhizobia. In a pot experiment, alfalfa was marginally better than oilseed rape or perennial ryegrass at promoting the degradation of pyrene, a PAH (D'Ovidio et al. 2013). None of the three species took up detectable quantities of pyrene. The combination of the perennial legume *Galega orientalis* and its nitrogen-fixing rhizobia *Rhizobium galegae* has shown potential for oil bioremediation purposes (Suominen et al., 2000; Lindström et al., 2003). The combination is tolerant of oil and in pot experiments, promoted the degradation of motor oil (Jussila et al., 2006; Kaksonen et al., 2006). Field experiments to test the effect of *Galega* on oil-contaminated ground are in progress in the Legume Futures project. Faba bean has been shown effective in promoting the degradation of crude oil in Kuwaiti sands (Radwan et al., 2000).

The presence of oil contamination apparently enhances the release of root exudates from the plants that in turn enhance the growth of certain soil bacteria. In a split-root experiment, where half of the soil was PAH-contaminated and the other half not, the composition of the soil microbial community was altered in the uncontaminated soil, but only in the legume treatments, not in the grasses (Kawasaki et al. 2012). In contrast, in a Chinese experiment, the grass tall fescue promoted PAH degradation more than alfalfa, but the intercrop was more efficient than either sole crop (Sun et al. 2011). The legume does not need to be alive to affect PAH degradation. Pea straw was much more effective than wheat straw at promoting PAH degradation, as it promoted the growth of a wide range of soil microbes in a pot experiment (Shahsavari et al. 2013).

Heavy metal and metalloid contamination can be bioremediated in several ways. The plant may accumulate the contaminant, allowing it to be taken away and safely disposed (phytoextraction or hyperaccumulation); it may be physiologically tolerant of the contaminant and grow regardless of its presence; or it may exclude the contaminant, so the crop product may be safely used. Again, the role of the soil microbiological community is critical, but there is very little literature to indicate any particular effect of legumes on it, in contrast to the oil-contamination literature. Inoculation of yellow lupin with a heavy metal-tolerant strain of Serratia rhizosphere bacteria reduced translocation of the heavy metals to the shoot and improved overall plant growth (El-Aafi et al. 2012). Tolerance to heavy metal contamination is necessary in rhizobia used in such conditions, and as might be expected, can be found when it is sought (Nonnoi et al. 2012).

BIOACTIVE COMPOUNDS FROM LEGUMES

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Legumes protect themselves from oxidative stresses and herbivores with a range of secondary compounds, including alkaloids, saponins and isoflavonoids. These have found antibiotic and health-promotive uses (Table 2). In some cases, analysis has not proceeded beyond a crude aqueous or solvent extract, but in many cases the specific active compound has been identified and tested. It is notable that the same compound can have several activities. The list presented in the table focuses on reports in refereed journals. Many of these reports also list species that were not affected by the treatment, but they are not included in Table 2 because of the space required.

Pueraria is a traditional Chinese medicinal plant (Gegen and Fengen in Chinese, kudzu in English), *Astragalus* species are medicinals in many cultures from Turkey to China (Huangqi in Chinese) and *Glycyrrhiza* species have been used as medicinals all over Eurasia (Gancao in Chinese, licorice in English). Modern science has tested and validated some of these properties and demonstrated their biochemical basis. Puerarin, the distinctive isoflavonoid glycoside of *Pueraria* has the glycoside C-linked rather than O-linked, so it does not get deglycosylated in the digestive tract, and it stays soluble unlike other isoflavones such as daidzein and genistein, accounting for some of the differences in its effectiveness (Cho et al. 2012). A comprehensive catalogue of the cardiovascular, cerebrovascular and other pharmacological activities of *Pueraria* and its components is provided by Wong et al. (2011). Licorice isoflavonoids and saponins have been found useful against oral and vaginal pathogens, confirming some of their traditional uses.

Anticancer activities of the indolizidine alkaloids swainsonine and castanospermine were identified in the 1990s (Molyneux et al. 2007), but there are few recent articles on these issues. Swainsonine is now known to be produced by endophytic fungi rather than the host plant (Braun et al. 2003, Cook et al. 2013).

The genus *Crotalaria* belongs to the Genisteae alliance clade (*Genisteae, Crotalarieae, Thermopsideae, Podylarieae, Liparieae,* and part of *Sophoreae*), characterized (unlike other legumes) by the production of alkaloids, generally quinolizidine alkaloids, except *Crotalaria* and some *Lotononis* spp. that produce pyrrolizidine ones. The remaining *Lotononis* spp. produce quinolizidine alkaloids. It appears that no legume is able to produce both types of alkaloids.

Numerous *Crotalaria* species are used as green manure all around the world. *Crotalaria* produce various active natural substances. More than 20 species are known for their toxicity to ruminants (Watt & Breyer-Bradwijk, 1962 ; Verdcourt &Trump, 1969). Neal et al. (1935) purified an alkaloid (monocrotalin) from *C. spectabilis*. Later Kingsbury (1964) showed that monocrotaline was responsible for crotalism, a lethal illness affecting the nervous system, lung and liver. Since then, more than 50 pyrrolizidine alkaloids have been isolated from 45 *Crotalaria* species. (Mears & Mabry 1971; Kinghorn & Smolenski 1981; Polhill 1982). Further *Crotalaria* plants produce more biologically active molecules like flavonoids (Rao & Rao 1985; Yadava & Singh 1992; Wanjala & Majinda 1999) and chalcones (Yang et al. 1998; Kumar et al. 1999).

Alkaloid production is reputed to be the mechanism underlying the useful capacity of some *Crotalaria* species to control root-knot nematode populations (7 species), root-lesion nematodes *Pratylenchus* (2 species), *Rotylenchulus* (2 species), *Helicotylenchus* and *Radopholus*. Many field experiments in several tropical and sub-tropical countries have shown the resistance of *Crotalaria* to root-knot and other nematodes parasitizing crop plants, and that introduction of these resistant *Crotalaria* species into crop rotations resulted in significant decreases of some nematode populations in soils. In Brazil, *Crotalaria spectabilis* strongly reduced populations of *Meloidogyne incognita* (Huang et al. 1981). In India, *Crotalaria juncea* is recommended for management of *Pratylenchus zeae* in sugarcane plantations (Sundararraj & Mehta 1990). In Florida (USA), yields of eggplant and squash were enhanced after crop rotation with *Crotalaria spectabilis* had reduced *Meloidogyne arenaria* populations (McSorley et al. 1994). The development of *Meloidogyne incognita* larvae inoculated to *Crotalaria spectabilis* stopped at the 3rd stage (Sano et al. 1983). Aqueous extracts of *Crotalaria spectabilis* roots inhibited development of stage L2 larvae of *Meloidogyne incognita* (Subramaniyan & Vadivelu 1990).

Jourand et al. (2004) tested an extract of *Crotalaria grantiana* on tomato plants infested by *Meloidogyne incognita*. The biological activity was nematostatic: nematodes were not killed but were completely paralysed in a 1 mg/ml (w/v) extract, and the effect was reversible, as the paralysed juveniles recovered mobility in water and were able to infest a susceptible tomato plant. Freeze-dried aqueous extract from *C. grantiana* leaves added to a sterile sandy substrate at 1 mg/ml protected susceptible tomato plants from *M. incognita* infestation. This suggests a promising use of *C. grantiana* both as green manure and a natural alternative to synthetic chemicals in nematode population control, especially in integrated or organic pest management for vegetable crops of tropical and temperate areas. In a second step, these authors tested the sensitivity of 3 species of *Meloidogyne* to leaf and root extracts of 15 West African species of *Crotalaria*. In some species, both extracts were equally active, and in others, one was more active than the other, while one species had no detectable activity on the nematodes. Considering the greater biomass contribution of the leaves and stems compared to the roots when the plants are used as green manure, *C. barkae*, *C. grantiana*, *C. pallida* and *C. podocarpa* were considered the most efficient species, whatever the targeted species of *Meloidogyne*.

Agronomic trials in tropical or mediterranean conditions show that the use of *Crotalaria* plants as green manure can significantly reduce the dominant population of nematodes and effectively shield sensitive vegetable crops (Legume Futures project, in progress). Proliferation of nematodes in agricultural soils is a major problem worldwide, together with the high toxicity of the corresponding chemical treatments. Introducing *Crotalaria* plants having both capacities of symbiotic nitrogen fixation and nematode population control may be promising in agriculture (Wang et al., 2002, 2003).

CONCLUSIONS

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In addition to their vital role in sustainable agriculture, legumes have properties that make them valuable in animal feeding, industry, and medicine, in ways that are sometimes surprising and unexpected. Further uses are, no doubt, under development, and current uses can still be optimized by breeding, manufacture, or formulation.

Forage legumes, traditionally used only for feeding ruminants, have new roles as providers of leaf protein for feeding other animals, nitrogen for agroecosystems, and lignocellulose for biomass uses, in biorefining systems. Grain legumes can be used as whole-crop silage for ruminants given appropriate management, and protein fractions from them can be used as sustainable fish feeds, as long as the formulation takes care of amino acid, mineral and vitamin composition. Legumes have a vital role in bioremediation of oil-contaminated ground, where their independence of soil nitrogen is essential, but no greater role than any other plant in remediation of heavy metal contamination. The role of legumes in bioenergy production remains somewhat ambiguous. They have a clear role in providing nitrogen in some systems, but in many other situations, the nitrogen can come from methane digestate, or sewage sludge that cannot be used for food or feed production because of heavy metal or microbiological concerns.

Isoflavones, alkaloids, and other secondary compounds produced by legumes have proven to have a number of potential economic uses, including in the health industry. The effect of Crotalaria alkaloids, and some other legume chemicals, on nematodes has great implications for sustainable agricultural systems. Similarly, the suppression of the growth of malaria mosquitoes is an exciting outcome. The economics of some of these uses remain to be demonstrated, as do their effectiveness in the field rather than in the laboratory.

Table 2. Activities, source species, and effects of bioactive compounds from selected legumes.

Activity	Legume species	Target species	Active compounds	Reference
Antibacterial	*Piptadeniastrum africanum*	*Staphylococcus aureus*, *Streptomyces mutans*	Tannins	Brusotti et al. 2013
Antibacterial	*Glycyrrhiza uralensis*	*Streptomyces mutans*	Pterocarpene: Glycyrrhizol A; Isoflavonoid: 6,8 diisoprenyl-5,7,4'-trihydroxyisoflavone	He et al. 2006
Antibacterial	*Glycyrriza uralensis* and *G. glabra*	*Streptomyces mutans*, *Porphyromonas gingivalis*	Saponin: glycyrrhizin	Messier et al., 2012 (review)
Antibacterial	*Glycyrriza uralensis*	*Streptomyces mutans*, *Porphyromonas gingivalis* (*in vitro*)	Isoflavonoids: licoricidin, licorisoflavan A	Gafner et al. 2011
Antibacterial	*Crotalaria pallida*	*Escherichia coli*, *Proteus* sp., *Ralstonia solanaceum*, *Erwinia* sp. *in vitro*	Peptide *Cp*-AMP from seed protein	Pelegrini et al. 2009
Antibacterial	*Ceratonia siliqua*	*Listeria monocytogenes in vitro*	Total phenolics from leaves	Aissani et al. 2012
Antibacterial	*Lupinus albus, L. angustifolius, L. hispanicus, L. luteus, L. mutabilis*	*Pseudomonas syringae, P. putida, Erwinia carotovora, in vitro*	Quinolizidine alkaloids: lupinine	de la Vega et al. 1996
Antifungal	*Piptadeniastrum africanum*	*Pyricularia grisea*	Saponins	Brusotti et al. 2013
Antifungal	*Glycyrriza uralensis and G. glabra*	*Candida albicans*	Isoflavonoid: glabridin; Saponin: 18-β-glycyrrhetinic acid; Chalcone: licochalcone	Messier et al., 2012 (review)

Activity	Legume species	Target species	Active compounds	Reference
Antifungal	*Glycyrrhiza species* (probably *G. glabra*)	*Candida albicans*	Isoflavonoid: glabridin; Chalcone: licochalcone	Messier & Grenier 2011
Antifungal	*Glycyrriza glabra*	*Candida albicans* (in vitro)	Saponin: 18-β-glycyrrhetinic acid	Pellati et al. 2009
Antifungal	*Glycyrriza glabra*	*Candida albicans* drug-resistant mutants (*in vitro*), other filamentous fungi	Isoflavonoid: glabridin	Fatima et al. 2009
Antifungal	*Crotalaria pallida*	*Fusarium oxysporum, Rhizoctonia solani in vitro*	Peptide *Cp*-AMP from seed protein	Pelegrini et al. 2009
Antifungal	*Astragalus verus*	*Trichophytum verrucosum*, *in vitro* and *in vivo* on guinea pig	Aqueous extract	Mikaeili et al. 2012
Antifungal	*Astragalus membranaceus*	*Trichoderma viride*, *Botrytis cinerea, Fusarium oxysporum, F. solani, in vitro*	Chitinase	Kopparapu et al. 2011
Antifungal	*Astragalus mongholicus (A. membranaceus* var. *mongholicus)*	*Botrytis cinerea, Fusarium oxysporum, Colletotrichum* sp.	Galactose-recognizing lectin	Yan et al. 2005
Antifungal	*Astragalus verrucosus*	*Aspergillus niger, Botrytis cinerea*	Butanolic extract; saponin Astraverrucin II	Pistelli et al. 2002
Antifungal	*Caesalpinia cacalaco*	*Colletotrichum lindemuthianum* on common bean: fungistatic	Phenolics	Veloz-Garcia et al 2010
Antifungal	*Prosopis alba, P. kuntzei, P. nigra, P. ruscifolia*	*Coriolus versicolor* wood-rot fungus	Tannin: (-)-mesquitol	Pizzo et al. 2011

Activity	Legume species	Target species	Active compounds	Reference
Anti-hypertensive	*Pueraria lobata* (in combination with *Salvia miltiorrhiza)*	Spontaneously hypertensive rat *in vivo*, aortic explant *in vitro*	Aqueous extract	Ng et al. 2011
Anti-inflammatory	*Glycyrriza uralensis*	Inflammatory cytokines IL-1β, IL-6, IL-8 and TNF-α	Isoflavonoids: licoricidin, licorisoflavan A	La et al. 2011 (cited by Messier et al. 2012)
Anti-inflammatory	*Astragalus membranaceus*	NO production from stimulated mouse macrophages	Isoflavonoid: Formononetin	Lai et al. 2013
Anti-insect	*Dalbergia oliveri*	*Aedes aegypti*, 3rd instar larvae and pupae, *in vivo*	Isoflavonoids: (+)-medicarpin, formononetin, (+)-violanone	Pluempanupat et al. 2013
Anti-insect	*Sesbania grandiflora*	*Coptotermes formosanus*	Methanolic extract of leaves	Elango et al. 2012
Anti-metabolic syndrome	*Pueraria lobata*	Spontaneously hypertensive rat *in vivo*	Isoflavones	Peng et al. 2009
Anti-metabolic syndrome	*Glycyrrhiza glabra*	Transactivation of peroxisome proliferator-activated receptor γ *in vitro*: antidiabetic	Total DMSO extract	Mueller & Jungbauer 2009
Anti-nematode	*Crotalaria* species	*Meloidogyne incognita* immobilization of adults and reduction of egg hatching; *Rhabditis* sp. repellence	Pyrrolizidine alkaloid Monocrotaline free base and N-oxide	Thoden et al. 2009
Anti-nematode	*Crotalaria juncea*	Various: also questions of human cirrhosis, genotoxicity, cancer & pulmonary hypertension	Dehydropyrrolizidine alkaloids	Colegate et al. 2012

Activity	Legume species	Target species	Active compounds	Reference
Anti-nematode	*Crotalaria spectabilis, C. retusa, Mucuna pruriens*	*Meloidogyne* spp., egg hatching & mobility reduced by exudates *in vitro*; plants are non-host	Uncharacterized root exudates and plant extracts	Osei et al. 2010
Anti-nematode	*Crotalaria juncea*	*Meloidogyne incognita* suppressed, bacterivorous and fungivorous nematode populations promoted in field studies	Unidentified (presumed alkaloids)	Marahatta et al. 2010
Anti-nematode	*Crotalaria juncea*	*Meloidogyne incognita* reproduction reduced, *Rotylenchus reniformis* reproduction halted, pot and field studies	Unidentified (presumed alkaloids)	Robinson & Cook 2001
Anti-oxidant	*Astragalus membranaceus* and *Glycyrrhiza uralensis*	Free-radical scavenging assays and maintenance of viability of H_2O_2-challenged MRC-5 human fetal lung fibroblast cells	Phenolics and flavonoids in combination	Li et al. 2011
Anti-protozoa	*Astragalus lentiginosus, A. mollissimus,* other *Astragalus* spp., *Oxytropis sericea, Swainsona* spp.	*Trypanosoma cruzi* adhesion to host cells	Indolizidine alkaloid: swainsonine	James et al. 2004 (review)
Anti-protozoa	*Castanospermum australe*	*Plasmodium falciparum* adhesion to host cells	Indolizidine alkaloid: castanospermine	James et al. 2004 (review)
Anti-tumor	*Glycyrrhiza species*	Angiogenesis in mouse renal adenocarcinoma	Glycyrrhizic acid	Kim et al. 2013

Activity	Legume species	Target species	Active compounds	Reference
Anti-tumorigenic	*Astragalus membranaceus*	Human colon cancer cells and gastric adeno-carcinoma cells *in vitro*	Total saponins	Auyeung et al. 2012
Antiviral	*Castanospermum australe*	Influenza virus, Human immunodeficiency virus, Dengue fever virus, by prevention of host-virus recognition	Indolizidine alkaloid: Castanospermine	Molyneux et al. 2007(review)
Hepato-protective, antioxidant	*Astragalus corniculatus*	Liver damage from CCl_4 or paracetamol in rats	Purified saponin mixture	Vitcheva et al. 2013
Herbicide	*Glycine max*	*Senna obtusifolia*, *Abutilon theophrasti* in *Lolium perenne* turf	Fatty acid methyl ester, "biodiesel"	Vaughn & Holser 2007
Hypertensive	*Glycyrrhiza* species	Human kidney sodium metabolism	Glycyrrhizic acid, glycyrrhetinic acid	Miettinen et al. 2010
Hypertensive	*Glycyrriza glabra*	Increased expression of mineralocorticoid receptor in rat kidney	Glycyrrhizic acid	Ma et al. 2009
Hypoglycemic	*Lupinus* sp. (probably *L. albus*)	Mouse and human *in vivo*	Storage protein: γ-conglutin	Bertoglio et al. 2011
Insecticide	*Pachyrhizus tuberosus*	Inhibition of electron transport in animal and plant mitochondria	Rotenoids: rotenone, pachyrhizin and erosone from seeds	Ramos-de-la-Peña et al. 2013
Mammalian wound healing	*Astragalus spp.*	Human keratinocyte repair *in vitro*, rat skin *in vivo*	Cycloartane saponins	Sevimli-Gür et al. 2011

Activity	Legume species	Target species	Active compounds	Reference
Neurological disorders	*Astragalus lentiginosus, A. mollissimus,* other *Astragalus* spp., *Oxytropis sericea, Swainsona* spp.	Ruminants: α-mannosidase inhibition	Indolizidine alkaloid: swainsonine	James et al. 2004 (review)
Plant growth promotion (probably by change of soil microflora)	*Lupinus exaltatus*	*Capsicum annuum*, in pots	Total alkaloid extract, applied to soil	Przybylak et al. 2005
Pseudo-oestrogen	*Pueraria lobata*	Dyslipidemia and osteoporosis in mouse models of menopause	Isoflavone: puerarin	Cho et al. 2012
Teratogenesis	*Lupinus formosus, L. arbustus, L. argenteus, L. sulphureus*	Cattle and goats	Piperidine alkaloids	James et al. 2004 (review)
Teratogenesis	*Lupinus albus, L. mutabilis*	Cattle	Quinolizidine alkaloids	James et al. 2004 (review)

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