Mycotoxins

Mycotoxins are toxic metabolites produced by certain moulds (fungi) when they grow on decaying vegetation, grains, fruits and nuts.

There are no reports of the Australian Lupin Bean seed being contaminated with the well-known mycotoxins such as aflatoxins, trichothecenes and zearalenone that can occur on other food commodities, including corn, wheat, rice, sorghum, soybean, peanut, spices and tree nuts.

The one exception is the small risk of contamination with the mycotoxin phomopsin.

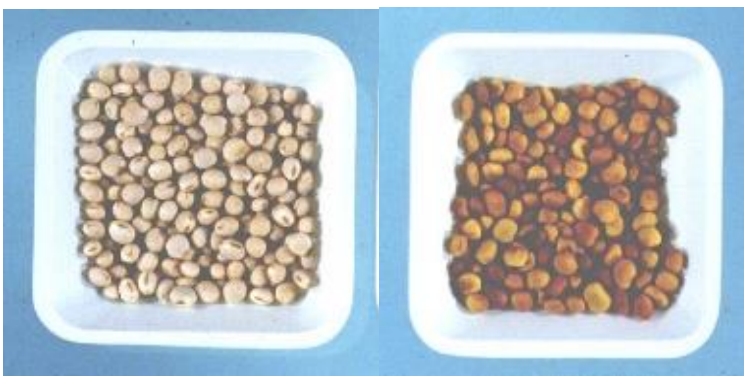
Phomopsin

Phomopsins are a family of cyclic peptide mycotoxins produced by the fungus *Diaporthe toxica* (formerly referred to as *Phomopsis leptostromiformis*). The Australian Sweet Lupin and other lupin species are a host for the fungus. Under wet conditions at crop maturity and post-harvest, infected lupin stems, pods and seeds can develop significant levels of phomopsins. The toxicity risk from phomopsin was first identified in sheep suffering 'lupinosis' - a liver disorder - as a consequence of grazing infected stubble residues. (Culvenor *et al.*, 1977; Williamson *et al.*, 1996).

In the 1980's the Australian Sweet Lupin breeding program successfully produced the first varieties that were resistant to the *Phomopsis* fungus, dramatically reducing the risk of phomopsin contamination and lupinosis (Allen and Cowling 1986).

Seed infection is rare under Western Australian growing conditions, particularly now that *Phomopsis* resistant varieties are grown.

The phomposin contamination of lupin seeds is associated with a distinctive golden-brown discolouration of the seedcoat and a greenish discolouration of the kernel (Petterson and Wood, 1986). This enables the use commercial grain sorting equipment to remove contaminated seed if required. Furthermore, there is a much higher concentration of phomopsin in the seed coat (testa) than in the kernel (cotyledon) of infected seed (Petterson, 1998). Thus the risk of phomopsin contamination is even lower in food products prepared from the lupin kernel.

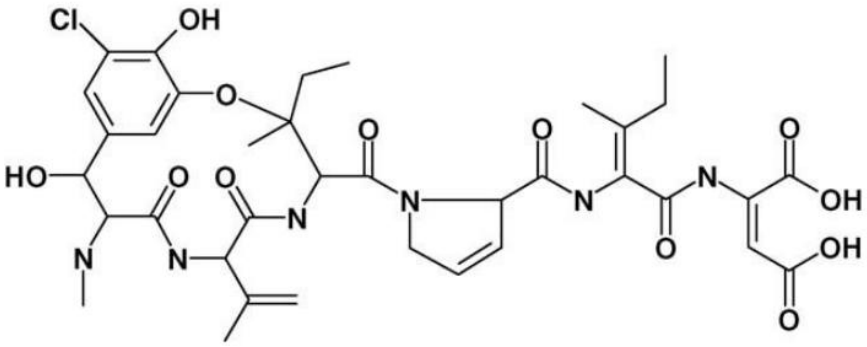


Normal (left) and phomopsin contaminated (right) Australian Lupin Beans

Resistant varieties remain a mainstay of managing the phomopsin risk in Australian Lupin Beans.

Analytical methods

Phomopsin A is the predominant toxic congener ($C_{36}H_{45}Cl\ N_6O_{12}$) a linear hexapeptide modified by an ether bridge to form a 13 membered macrocylic ring (Culvenor et al 1977). Phomopsin B and other analogues are less toxic than phomopsin A.



Molecular structure of phomopsin A
(Reinhard et al., 2006)

An ELISA test was developed by CSIRO in Australia in the early 1990s and has been used to test for phomopsin in food and feed in Australia for many years. Recently access to the specific antibody has been difficult to maintain.

LC-MS/MS detection (deNijs et al. 2013) and more recently stable isotope dilution assay (SIDA) LC-MS/MS using (15)N6 as an isotopically labelled internal standard. has been developed with the ability to detect phomopsin A to a level of 0.5-1 µg/kg in plant and flour matrices (Schloß et al. 2015).

Toxicology

Phomopsin A, is cytotoxic and anti-mitotic molecule, exerting its effect by binding with microtubule sub-unit proteins (tuberin). Other mechanisms include possible impairment of mitochondrial respiration. It is hepatotoxic in all animal species tested at sufficient doses and is also hepatocarcinogenic in rats.

The dose-response information on the severity of toxicities in numerous animal species suggests that human and livestock exposures should be kept as low as possible (European Food Safety Authority, 2012).

Australia and New Zealand have included phomopsin in their mycotoxin regulations, with a limit of 5 µg/kg (5 ppb) in lupin seeds and derived products (FSANZ, 2001).

There are no published records of phomopsin detection in commercially produced lupin foods exceeding this limit.

References

Allen JG and Cowling WA. (1986). Preventing lupinosis with Phomopsis resistant lupins. Journal of Agriculture – WA 27: 89-90.

Culvenor CCJ. et al (1977). Isolation of toxic metabolites of *Phomopsis leptostromiformis* responsible for lupinosis. Aust J Biol Sci 30: 269-277.

de Nijs M., Pereboom-de Fauw D., van Dam R., de Rijk T., van Egmond, H., and Mol, H. (2013). Development and validation of an LC-MS/MS method for the detection of phomopsin A in lupin and lupin-containing retail food samples from the Netherlands. Food Additives & Contaminants 30: 1819-1826.

European Food Safety Authority (2012). https://doi.org/10.2903/j.efsa.2012.2567

FSANZ. (2001). https://www.foodstandards.gov.au/publications/documents/TR1.pdf

Petterson DS. and Wood PMcR. (1986). Phomopsis infection of lupin seed. Journal of Agriculture – WA 27: 53-54.

Reinhard H., Rupp H., Sagar F., and Streule M. (2006). Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing foods. J. Chromatography 1112: 353-360.

Schloß S., Koch M., Rohn S., and Maul R. (2015). Development of a SIDA-LC-MS/MS method for the determination of phomopsin A in legumes. J. Agric Food Chem 63: 10543-10549.

Williamson P. M et al, (1996). *Diaporthe toxica* sp. nov. the cause of lupinosis in sheep. Mycological Research 98: 1364-1368.