POST-HARVEST DETOXIFICATION.
The Key To Alternative *Vicia* Grain Legumes?

Enneking, D. *

Waite Agricultural Research Institute, University of Adelaide, Glen Osmond 5064, South Australia
* Present Address: Center for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia, Nedlands 6009, Western Australia, enneking@cyllene.uwa.edu.au

Vicia sativa, *V. narbonensis*, *V. ervilia* and *V. articulata* (syn. *V. monantha*) are well suited for Mediterranean dryland agriculture. The use of these species as grain legumes has been largely restricted to the supplemental feeding of ruminants and draft animals, and in times of famine, this use has sometimes been extended to human consumption.

We have studied the unpalatability of *V. villosa* and *V. narbonensis* grain, using a porcine feed-intake bioassay. Two different antifeedant principles were isolated. The seed of *V. villosa* contains the toxic arginine analogue, canavanine[1] and that of *V. narbonensis* the dipeptide g-glutamyl-S-ethenyl-cysteine[2], which incidentally is analogous to the flavour precursor, g-glutamyl-S-prop-1-enyl-cysteine present in chives (*Allium schoenoprasum*)[3].

V. villosa is not a grain legume, but a forage crop. Other species, such as *V. articulata* and *V. ervilia* which do also contain canavanine in their seeds are used as grain legumes for supplemental feeding of ruminants. Their use as food for monogastric animals and humans is limited by the presence of canavanine.

V. sativa contains the favism toxin, vicine, as well as the neurotoxic and antinutritional cyanoamino acids, ß-cyanoalanine and g-glutamyl-ß-cyanoalanine. The antinutritional non-protein amino acids in *V. sativa* and *V. narbonensis* can be inactivated by mild acid hydrolysis[2]. Under alkaline conditions, canavanine degrades to deamino-canavanine which is inactive in the bioassay[1] Thus, in principle, the major undesirable non protein amino acids in *Vicia* spp. seeds, can be inactivated by either acidic or alkaline chemical processes.

In view of the biological functions for these factors in the ecology of *Vicia* spp., it appears that post-harvest detoxification could well be the best long-term option for the sutainable development of these crops.

Traditional utilisation practices and preparative methods for *Vicia* grain should now be assessed in detail for their effectiveness in minimising the ingestion of these factors whilst preserving their nutritive properties. Innovative application of this knowledge can reasonably be expected to provide the basis for the wider utilisation of *Vicia* spp. as grain legumes.

1. Enneking, D. Giles, L. C., Tate, M. E., Davies, R. L. (1993). L-canavanine: a natural feed- intake inhibitor for pigs (isolation, identification and significance) *J. Sci. Food Agric.* **61**, 315-325
2. Enneking, D. 1993, The biological chemistry of *Vicia* toxins. Ph. D. thesis. University of Adelaide, South Australia
3. Mattikala, E J. & Virtanen, A. I. (1962). A new g-glutamylpeptide, g-L-glutamyl-S-(prop-1-enyl)-L-cysteine, in the seeds of chives (*Allium schoenoprasum*). *Acta Chem. Scand.* **16**, 2461-2462

Enneking, D. (1995) Post-harvest detoxification: the key to alternative Vicia grain legumes? In : Yusuf, H. K. M. and Lambein, F., Eds. Lathyrus sativus and Human Lathyrism: Progress and Prospects. Dhaka: University of Dhaka; 1995; pp. 85-92.

POST-HARVEST DETOXIFICATION. The Key To Alternative *Vicia* Grain Legumes?

Enneking, Dirk*

Waite Agricultural Research Institute, University of Adelaide, Glen Osmond 5064, South Australia
* Present Address: Centre for Legumes in Mediterranean Agriculture (CLIMA), University of Western Australia, Nedlands 6009, Western Australia

Summary:

Feed inhibitory substances were isolated from the seeds of *V. villosa* and *V. narbonensis* using a porcine feed-intake bioassay. *V. villosa* was found to contain the toxic arginine analogue, canavanine and *V. narbonensis* the dipeptide γ-glutamyl-S-ethenyl-cysteine, which is analogous to the flavour precursor, γ-glutamyl-S-prop-1-enyl-cysteine, present in the seeds of chives (*Allium schoenoprasum*). Under alkaline conditions, canavanine degrades to deaminocanavanine which is inactive in the bioassay, whilst γ-glutamyl-S-ethenyl-cysteine is inactivated by mild acid hydrolysis. In principle, the major undesirable non protein amino acids and peptides in *Vicia* spp. seeds, can be inactivated by either acidic or alkaline chemical processes.

Introduction

Recent agronomic research has singled out some vetches (*Vicia* species) as potential alternative grain legume crops for cultivation in dry areas with Mediterranean-type climates (Saxena *et al.*, 1993). *Vicia sativa* L., *V. narbonensis* L., *V. ervilia* (L.) Willd. and *V. articulata* Horn. (syn. *V. monantha* Desf. non Retz) are ancient grain and forage crops of the Mediterranean and South-West Asia (Zohary and Hopf, 1988; Enneking, 1994). Use of these species as grain legumes has been largely restricted to the supplemental feeding of ruminants and draft animals. In times of famine they have also been used for human consumption (Enneking, 1994). Problems with the feeding of *Vicia* grain to monogastric animals have been repeatedly encountered and these effects can be rationalised with an understanding of *Vicia* seed toxicity which has been the subject of our studies (Enneking *et al.*, 1993; Enneking, 1994).

The aim of this paper is to present recent results from studies of the seed unpalatability factors present in *V. villosa* (Enneking *et al.*, 1993) and *V. narbonensis* (Enneking, 1994) and to summarise the current knowledge about the ecological function of these compounds in *Vicia* species and possible strategies for their management.

Materials & Methods

A porcine feed-intake bioassay has been used to identify feed intake inhibitors from *V. villosa* (Enneking *et al.*, 1993) and *V. narbonensis* grains (Enneking, 1994). Fractions were separated from 30 % aqueous ethanol extracts using ultrafiltration and ion-exchange chromatography. In addition, degradative treatments such as peroxidation and wet autoclaving of the milled grains were tested for their effect on porcine feed intake. The test diets were fed for 4 days to individually caged young pigs (20-40 kg) after an initial pre-treatment period during which the control diet was fed (8% soybean as the legume component for the *V. villosa* experiments, and 35% peas for the *V. narbonensis* trials). The results are expressed as the dimensionless feed intake ratio (treatment intake/pre-treatment intake).

Enneking, D. (1995) Post-harvest detoxification: the key to alternative Vicia grain legumes? In : Yusuf, H. K. M. and Lambein, F., Eds. Lathyrus sativus and Human Lathyrism: Progress and Prospects. Dhaka: University of Dhaka; 1995; pp. 85-92.

Capillary Zone Electrophoresis Analysis of aqueous 60 % v/v ethanol extracts (adjusted to pH 7.5) was carried out under free zone mode in 20 mmol Na_3PO_4, pH 7.5, 20 kV using a 48.5 cm x 50 µ capillary (effective length 40 cm) with detection and quantitation at λ_1 200 nm and λ_2 222 nm against a pure standard of γ-glutamyl-S-ethenyl-cysteine.

Results

Fig 1. A comparison of the pig-feed inhibitory activity of diets containing 8% *V. villosa* seed meal or 12.5% and 25% of *V. narbonensis* seed meal

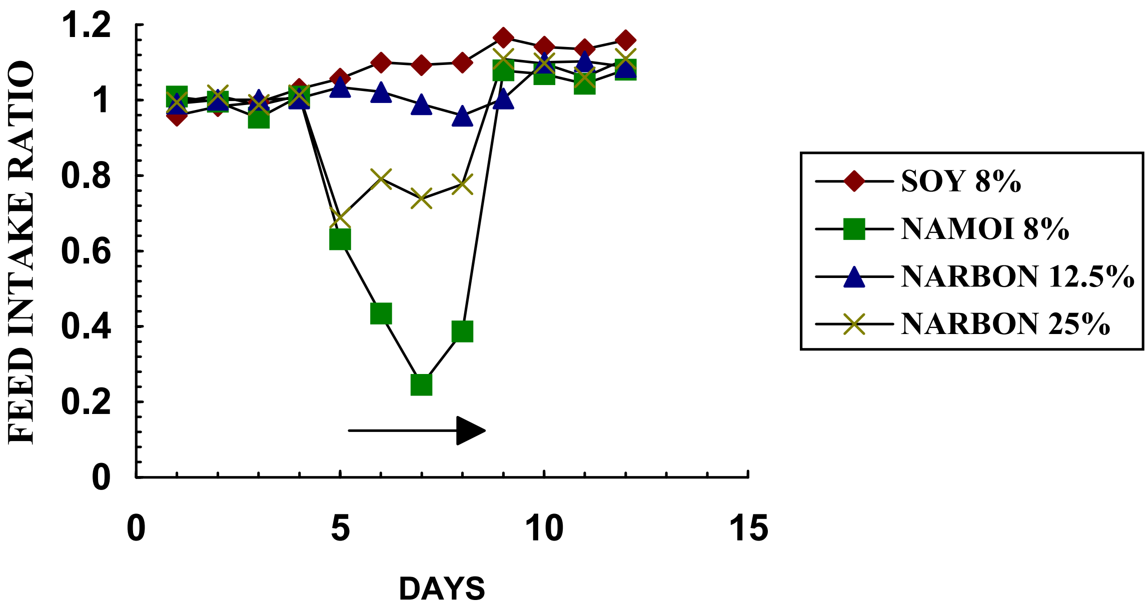


Fig 1. shows that inclusion of *V. villosa* or *V. narbonensi*s seed meal during the treatment period (indicated by an arrow) leads to a reduction in porcine feed intake. The feed-inhibitory activity of diets containing 35% V. *narbonensis* seed meal is markedly lower than those with a 8% *V. villosa* content. After a series of fractionations and testing of the resulting fractions by bioassay, the feed-inhibitory activity of *V. villosa* seed was located in the basic cationic fraction, the major component of which was found to be L-canavanine, a toxic arginine analogue. This compound was then isolated and fed at a concentration equivalent to that found in the positive *V. villosa* control diet. The resultant feed inhibitory response was statistically indistinguishable ($P < 0.01$) from the positive control. Thus, it could be demonstrated that canavanine is the major porcine feed-inhibitory factor present in the seeds of *V. villosa*. Under alkaline conditions, canavanine degrades to deaminocanavanine which is inactive in the bioassay (Enneking *et al.*, 1993, and references therein).

Fig. 2 The relationship between porcine feed intake and the concentration of γ-glutamyl-S-ethenyl-cysteine in different feeds tested for their palatability

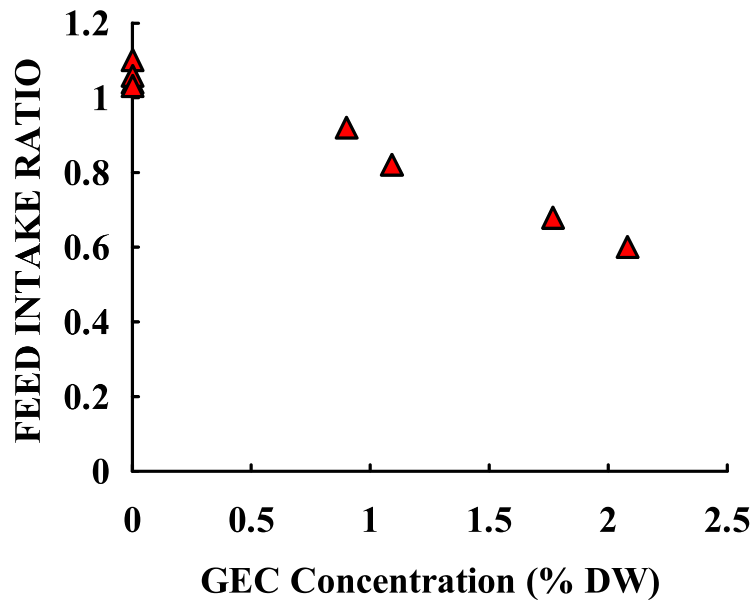
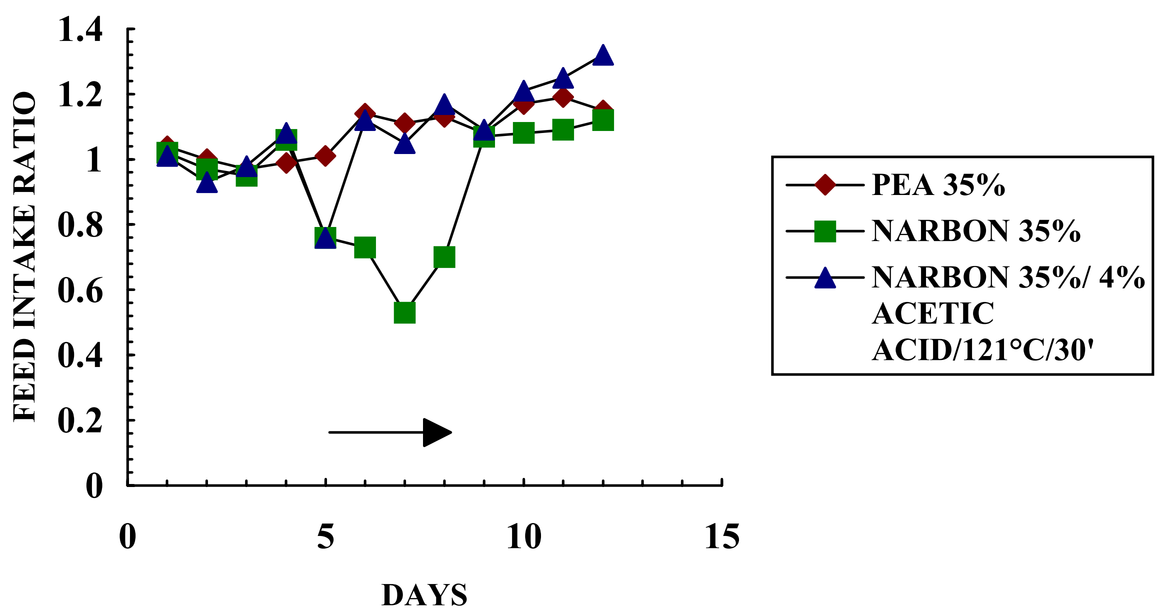


Fig 2. shows the relationship between feed intake reduction caused by various diets incorporating *V. narbonensis* seed and fractions thereof and the concentration of γ-glutamyl-S-ethenyl-cysteine, a dipeptide isolated during these studies (Enneking, 1994).

Fig 3. Mild acid hydrolysis improves the palatability of *Vicia narbonensis*



Enneking, D. (1995) Post-harvest detoxification: the key to alternative Vicia grain legumes? In : Yusuf, H. K. M. and Lambein, F., Eds. Lathyrus sativus and Human Lathyrism: Progress and Prospects. Dhaka: University of Dhaka; 1995; pp. 85-92.

Fig 3. shows that the preparation of *V. narbonensis* seeds by soaking overnight in 4% acetic acid followed by autoclaving (121° C, 30 mins) and forced draught drying (17 hrs, 65°C) destroyed the porcine feed-inhibitory activity (treatment period indicated by an arrow).

Discussion

Two different antifeedant principles were isolated from *Vicia* seeds using the porcine feed intake bioassay. The seeds of *V. villosa* contain the toxic arginine analogue, canavanine and those of *V. narbonensis* the dipeptide γ-glutamyl-S-ethenyl-cysteine, which is analogous to the flavour precursor, γ-glutamyl-S-prop-1-enyl-cysteine present in the seeds of chives (*Allium schoenoprasum*) (Mattikala and Virtanen, 1962).

Deaminocanavanine is a well known deamination artifact of canavanine isolations (Rosenthal, 1972) and was also identified during our studies with *V. villosa*. Fractions containing this compound showed no activity in the bioassay (Enneking *et al.*, 1993). The degradation of canavanine to deaminocanavanine under alkaline conditions provides therefore a chemical strategy for the detoxification of this compound and has already been successfully employed for the processing of the canavanine containing seeds of *Canavalia ensiformis* (Obizoba and Obiano, 1988).

γ-Glutamyl-S-ethenyl-cysteine can be rendered inactive by mild acid hydrolysis (Fig. 3) and γ-glutamyl-β-cyanoalanine the major antinutrient, besides vicine, in the seeds of *V. sativa* can also be destroyed by a similar process. It is reasonable to propose that in principle post-harvest detoxification procedures can be developed for these anti-nutritional factors.

With the discovery of γ-glutamyl-S-ethenyl-cysteine in *Vicia* the known low molecular weight toxic principles in *Vicia* seeds can be summarised (table 1).

Table 1 lists the distribution of the five major low molecular weight antinutritive factors which have been identified in seeds of the genus *Vicia* so far. Three major groups of species can be separated by the nature of their seed non-protein amino acids (NPAAs) (Bell and Tirimanna, 1965; Tschiersch and Hanelt, 1967; Bell, 1971). Based on the distribution of the cyanogenic glycoside vicianine and the levels of the pyrimidine glucosides vicine and convicine in some species, two further groups are distinguishable. Vicine and convicine have been identified by Griffiths and Ramsay (1992) in all species examined in their study, however, high levels were restricted to *V. bithynica* and *V. faba*. *V. sativa* seed is also known to contain high levels of vicine (Pitz *et al.*, 1980)

Species of the subgenus *Vicilla*, amongst them *V. ervilia*[1], *V. articulata*, *V. villosa*, *V. benghalensis* and *V. cracca* contain the toxic arginine analogue, canavanine (group 1). Within the subgenus *Vicia*, one group (which includes *V. sativa*) is characterised by the presence of ß-cyanoalanine and γ–glutamyl-ß-cyanoalanine (group 2). Other species of this subgenus, including *V. narbonensis*, are distinguishable by the presence of the hitherto un-identified peptide VA3 (group 3) which has now been identified as γ-glutamyl-S-ethenyl-cysteine. The food legume, *V. faba*, is characterised by the presence of high levels of vicine and convicine.

The development of efficient screening techniques for the quantitative detection of individual NPAAs is the next logical step for the selection of less toxic and/or more palatable *Vicia* cultivars. Such techniques should be suitable for testing large numbers of samples to facilitate the screening of the available *Vicia* germplasm and material generated through artificial mutagenesis.

---

[1] (Cacho *et al,* 1989) measured 1. 03 and 0.97 g kg^{-1}canavanine in a red and a white seeded *V. ervilia* variety respectively , while Garcia and Ferrando (1989) found levels between 0.005 -2.6 g kg^{-1} (mean values 0.05-0.1 g kg^{-1}) in a spanish collection of *V. ervilia*, with some of the red-seeded varieties having the lowest and the white-seeded some of the highest canavanine concentrations

Table 1. Seed distribution of *Vicia* toxins in important agricultural species

Abbreviations: CN-AA: Cyanoalanine acids e.g. ß-cyanoalanine and γ–glutamyl-ß-cyanoalanine. Relative concentrations (+), (+ +), (+ + +) of γ–glutamyl-ß-cyanoalanine are indicated, all species with this compound had similar concentrations of ß-cyanoalanine (+) ex ref. (1), presence of both recorded by ref. (2); CAN: canavanine (g kg^{-1} Dry Weight (DW) ex ref. (2) , relative concentrations (+ + +) etc. ex ref. (1)); Vicine/Convicine (g kg^{-1} DW, ref. 3; data for concentration ranges: ref. 5; V: presence of vicine identified based on retention time and mass spectrum of TMS derivative, ref. (4)); CN-GLY: cyanogenic glycoside vicianine (µg HCN/g DW, ref. (2))

References: 1. Bell and Tirimanna (1965); 2. Tschiersch and Hanelt (1967); 3. Pitz *et al.* (1980); 4. Yasui *et al.* (1987); 5. Griffiths and Ramsay (1992) 6. Khattab (1988)

SPECIES	CN-AA	CAN	VA_3	Vicine/Convicine	CN-GLY
		subgen. *Vicilla*			
Group 1					
V. tetrasperma (L.) Schreb.		0.6-0.8 (++)	(traces)		
V. articulata Horn.		1.5-2.6 (+ +)			
V. hirsuta (L.) S.F. Gray		0.8-1.7 (+ +)	(+)		
V. ervilia (L.) Willd.		0.2-0.4 (+)			
V. unijuga A. Br.		2.5-3.8			
V. cracca L.		2.1-3.8 (+ +)	(+ +)		
V. villosa Roth		2.5-7.8 (+ +)	(+)		
V. benghalensis L.		1.9-3.4 (+ + +)	(+)		
V. monantha Retz.		1.8-2.4 (+ +)	(+ +)		
		Subgen. *Vicia*			
Group 2					
V. grandiflora Scop.	(+ +) (2)				140-320 (2)
V. sativa L.	(+ +) (2)			0.75/0.08	0-300 (2)
ssp. *nigra* (L.) Ehrh.	(2)				240-990 (2)
ssp. *amphicarpa* (L.) Batt.	(+ +)				
ssp. *macrocarpa* (Mor.) Arcang.	(2)				220-440 (2)
Group 3					
V. narbonensis L.			(++)	0.18-0.62/0.05-0.16	
V. johannis Tamansch.			(?)	0.26-0.35/-	
V. serratifolia Roth			(?)(6)	0.48/-	
V. pannonica Crantz.			(+)		
V. faba L.				4.2-10.8/0.3-5.1	

An important question arising in the context of selection for low-toxin lines is whether the genetic reduction of anti-feedant and anti-nutritional factors is going to have a negative effect on the ecological fitness of the resulting cultivars? In the words of Bell (1977) who reviewed the ecological function of NPAAs, "The development of a toxin-free crop would be totally impractical if the reduction in toxicity to man or domestic animals was accompanied by an equal or greater reduction in toxicity to predatory insects which might destroy the crop before it could be harvested".

Holt and Birch (1984) correlated the presence of NPAAs in *Vicia* to aphid (*Aphis fabae*, *Acyrtosiphon pisum* (Harr.), *Megoura viciae* (Buckt.)) resistance and found that the most domesticated species were also the least resistant. β-Cyanoalanine has been shown to be active against *Locusta migratoria* where it exhibited diuretic effects and led to an inhibition of moulting (Schlesinger *et al.*, 1976); it has also been documented as an effective feeding inhibitor for three species locust (Navon and Bernays, 1978) and one bruchid species (Janzen *et al.*, 1977). Canavanine is toxic to almost every organism examined so far (Rosenthal, 1991). The insecticidal properties of these compounds alone, without considering their edaphic, allelopathic and possible drought tolerance functions, suggest that it may be prudent to aim for seed specific deletion[2] of these factors whilst preserving and enhancing their beneficial role in other parts and phenological growth stages of the plant. The most attractive feature of *Vicia* and *Lathyrus* spp. as a group of grain and forage legumes for dry areas is the tolerance of individual species or genotypes to stress factors such as drought, cold, temporary waterlogging, pests, diseases and infertile soils. It is clearly undesirable to convert a crop with a minimum input requirement into one that would require higher inputs for its production.

The economics of producing low toxin varieties (with their added-value through improved palatability, reduced toxicity and hence marketability), versus the benefits derived from the protective and adaptive functions of these compounds are going to determine whether genetic deletion of toxins or post-harvest detoxification (itself a costly process) could become viable options for the further development and utilisation of these crops. Their sustainable development, especially for resource-poor farmers in underdeveloped countries may favour post-harvest processing, which has a long tradition in many parts of the world and is of especial importance in traditional cultures that still subsist on a variety of otherwise toxic or unpalatable food stuffs.

A wide variety of fermented foods are produced and eaten around the world (Yokutsuka, 1991; Campbell-Platt, 1980; Campbell-Platt, 1987; Reddy and Salunkhe, 1989). The potential for the further development of fermented foods has been advocated amongst nutritionists because of the intrinsic nutritional benefits associated with such products (Hesseltine, 1983). Fermentation is also an effective means for food preservation (Nout and Rombouts, 1992). Fermented foods can be prepared at both, an industrial and the household scale. Indeed, many fermented foods are prepared by very simple techniques and represent grass roots technology which is already widespread; a fact which facilitates their further refinement, transfer and adoption in underdeveloped countries for the detoxification of alternative food sources.

Ayyagari *et al.* (1989) compared various Indian household food preparation techniques for their effectiveness in detoxifying *L. sativus*, and their data show that those methods which included a fermentation step were the most effective in reducing ODAP levels, eliminating 95% of this toxin. Further improvements in detoxification are likely to be made with selection for better ODAP degradation. Such methods can, in principle, also be used for the post-harvest detoxification of *Vicia* seeds, thus providing an alternative approach to the wider utilisation of these grains without the need for genetic removal of their low molecular weight antinutritive and unpalatability factors.

The incorporation of fermentation processes into other simple food technologies also offers good prospects for a detoxification of food sources while simultaneously giving flexibility in the manipulation of flavour, texture and colour of the raw material.

---

[2] Seed predators may necessitate the introduction of, or selction for, other defence mechnisms e.g. proteinase inhibitors

The minor *Vicia* grain legumes, viz. *V. ervilia*, *V. sativa* and *V. narbonensis* are used as supplemental feeds for ruminant production, which is another form of post-harvest detoxification. The maximum dietary inclusion levels for individual varieties in ruminant diets, detoxification in the rumen and the effects of such diets on end-product quality require further research attention. Because these grains have been used for millennia as a ruminant feed in the Mediterranean, Middle-East and West-Asia, this ancient knowledge should be documented, if not already recorded, and verified by experimentation.

Conclusion

Canavanine and γ-glutamyl-S-ethenyl-cysteine have been identified as unpalatability factors from the seeds of *V. villosa* and *V. narbonensis*, respectively. The selection of genotypes with low or nil levels of anti-nutritive factors provides an opportunity to assess the ecological function of these toxins and to manipulate the temporal and spatial expression of their biosynthesis within in the plant. The available evidence concerning the biology of non-protein amino acids indicates that these compounds are important in plant defence and adaptation, but more detailed evidence and economic assessment of their roles are required.

Traditional utilisation practices including ruminant feeding practices and preparative methods for *Vicia* grain should be assessed in detail for their effectiveness in minimising the ingestion of toxic factors whilst preserving their nutritive properties. Innovative application of this knowledge can reasonably be expected to provide the basis for the wider utilisation of *Vicia* spp. as grain legumes.

Acknowledgments:

The help, co-operation and support of Dr. Max E. Tate and Lynne Giles (Waite Agricultural Research Institute), the late Dr. Richard Davies (Department of Agriculture, South Australia), Brendan Greirson (Chemistry Centre Western Australia), the Australian Grains Research Development Corporation (GRDC) and CLIMA are gratefully acknowledged

Bibliography

Ayyagari R., Narasinga Rao B. S., Roy D. N. (1989) Lectins, trypsin inhibitors, BOAA and tannins in legumes and cereals and the effects of processing. *Food Chemistry* 34:229-238.

Bell E. A. (1971) Comparative biochemistry of non-protein amino acids. In: Chemotaxonomy of the *Leguminosae*. (Harborne J. B., Boulter D., Turner B. L., Eds)pp. 179-204, Academic Press, London & New York.

Bell E. A. (1977) The possible significance of uncommon amino acids in plant-vertebrate, plant-insect and plant-plant relationships. In: Natural products & the protection of plants. Proc. of a study week at the Pontificial Academy of Sciences, Oct 18-23, 1976. (Marini-Bettòlo G. B., Ed)pp. 571-595, Elsevier, Amsterdam.

Bell E. A., Tirimanna S. L. (1965) Associations of amino acids and related compounds in the seeds of forty-seven species of *Vicia*: their taxonomic and nutritional significance. *Biochemical Journal* 97:104-111.

Cacho J., Garcia M. A., Ferrando I. (1989) Selective spectrophotometric determination of canavanine. *Analyst* 114:965- 968.

Campbell-Platt G. (1980) African locust bean (*Parkia* species) and its West African fermented product, dawadawa . *Ecology of Food and Nutrition 9:123-132*.

Campbell-Platt G. (1987) Fermented foods of the world. A dictionary and guide. Butterworth

Enneking D. (1994) The biological chemistry of *Vicia* toxins. Ph. D. thesis, University of Adelaide, South Australia

Enneking D., Giles L. C., Tate M. E., Davies R. L. (1993) L Canavanine: A natural feed intake inhibitor for pigs (isolation, identification and significance). *Journal of the Science of Food and Agriculture* 61: 315-325

Griffiths D. W., Ramsay G. (1992) The concentration of vicine and convicine in *Vicia* faba and some related species and their distribution within mature seeds. *Journal of the Science of Food and Agriculture* 59: 463-468

Hesseltine C. W. (1983) The future of fermented foods. *Nutrition Reviews* 41:293-301.

Holt J., Birch N. (1984) Taxonomy, evolution and domestication of *Vicia* in relation to aphid resistance. *Annals of Applied Biology* 105:547-556.

Janzen D. H., Juster H. B., Bell A. E. (1977) Toxicity of secondary compounds to the seed-eating larvae of the bruchid beetle *Callosobruchus maculatus*. *Phytochemistry* 16:223-227.

Khattab A. M. A. (1988) Taxonomic studies on the close wild relatives of the faba bean (*Vicia* faba L.) in section Faba. Doctoral Dissertation, University of Southampton, UK. Dissertation Abstracts International B (The Sciences and Engineering) 49:617B.

Mattikala E. J., Virtanen A. I. (1962) A new γ- glutamylpeptide, γ-L-glutamyl-S-(propenyl)-L-cysteine, in the seeds of chives (*Allium schoenoprasum*). *Acta Chemica Scandinavica* 16:2461-2462.

Navon A., Bernays E. A. (1978) Inhibition of feeding in acridids by non-protein amino acids. *Comparative Biochemistry and Physiology A Comparative Physiology* 59A:161-164.

Nout M. J. R., Rombouts F. M. (1992) Fermentative preservation of plant foods. *Journal of Applied Bacteriology Symposium Supplement* 73:136S-147S.

Pitz W. J., Sosulski F. W., Hogge L. R. (1980) Occurrence of vicine and convicine in seeds of some *Vicia* species and other pulses. *Journal of the Canandian Institute for Food Science and Technology* 13:35-39.

Reddy N. R., Salunkhe D. K. (1989) Fermentation. In: CRC handbook of world food legumes: Nutritional chemistry, processing technology, and utilisation Vol. 3. (Salunkhe D. K., & Kadam S. S., Eds)pp. 177-217, CRC Press, Boca Raton, Florida.

Rosenthal G. A. (1972A) Deaminocanavanine formation from canavanine. *Phytochemistry* 11: 2827-2832

Rosenthal G. A. (1991) Nonprotein amino acids as protective chemicals. Chapter 1 In:: Herbivores: Their interaction with secondary plant metabolites Vol I: The chemical participants. pp. 1-34, Academic Press, San Diego, California.

Saxena M. C., Abd El Moneim A. M., Ratinam M. (1993) Vetches (*Vicia* spp.) and chicklings (*Lathyrus* spp.) in the farming systems in West Asia and North Africa and improvement of these crops at ICARDA. Proceedings of the workshop on the breeding and selection of *Vicia* and *Lathyrus* for grain production. University of Western Australia, September 22.-23. 1992. (Garlinge J., Perry M. W., Eds.) Occasional Publication No.1 pp. 1-7, Co-operative Research Center for Legumes in Mediterranean Agriculture (CLIMA), Perth, Western Australia.

Schlesinger H. M., Applebaum S. W., Yehudith Birk (1976) Effect of ß-cyano-L-alanine on the water balance of *Locusta migratoria*. *Journal Insect Physiology* 22:1421-1425.

Tschiersch B., Hanelt P. (1967) Die freien Aminosäuren der Samen von *Vicia* L. und die systematische Gliederung der Gattung. *Flora, Abteilung A* 157:389-406.

Yasui T., Endo Y., Ohashi H. (1987) Infrageneric variation of the low molecular weight carbohydrate composition of the seeds of the genus *Vicia* (*Leguminosae*). *Botanical Magazine*, *Tokyo* 100:255-272.

Yokutsuka T. (1991) Proteinaceous fermented foods and condiments with koji molds. In: Handbook of Applied Mycology Vol. 3: Foods and Feeds. (Arora D. K., Mukerji K. G., & Marth E. H., Eds), Marcel Dekker, New York.
Zohary D., Hopf M. (1988) Domestication of Plants in the Old World. Clarendon, Oxford.