PROSPECTS OF BREEDING NON-BLOATING CLOVERS FOR RUMINANTS

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Although bloat may occur in sheep or cattle grazing on many types of forage it is particularly associated with cattle grazing lush legumes (Todd, 1969). Bloat is a result of the foaming of the rumen contents when gases which are part of the end-products of digestion, normally voided by eructation, are partially, and in extreme cases totally, retained in what has been described as a stable foam. This results in the build-up of intra-ruminal pressures often causing death of the animal.

According to Johns (1961) the annual loss from animal deaths in New Zealand alone may be valued at more than $2 million. To this must be added the cost of control measures, principally pasture spraying, trough water treatment and animal drenching. Pasture management to reduce the balance of clover in the sward has also been recommended as a means of controlling bloat. This, however, will reduce N fixation and consequent pasture productivity (Sears et.al 1965), and will limit the contribution of an otherwise highly nutritive forage (Butler et. al. 1968). These problems could be overcome by breeding non-bloating legumes. The realization of this objective requires examination of the inherent variability of forage legumes to cause bloat. Thus while it is very common in cattle grazing red clover (Trifolium pratense), white clover (T. repens) and alfalfa (Medicago sativa), it occurs only occasionally in cattle grazing subterranean clover (T. subterraneum), and there is no record of bloat from lotus major (Lotus pedunculatus). There is no doubt at all, therefore, that the genetic make-up of the forage legume is an extremely important factor in the production of bloat.

Before plants can be tailored to desired requirements, these requirements have to be specified in terms of plant constituents which can be identified and selected for or against. In this context certain recent findings are very significant. Cooper and associates, in 1966, using the method of Kendall (1964), demonstrated that there was a close relationship between the foam formation in plant leaf extracts of 27 legume species and their known bloat potentials. Mangan in 1959 and subsequently McArthur, Stifel and others have demonstrated that most of the foam is caused by a protein factor in the leaves (McArthur et.al. 1964); Stifel et.al. 1968).

Kendall's work in 1966 on a number of legumes, including red clover, suggested that all legumes contain proteins which under appropriate conditions in vitro may produce equal volume of foam. He inferred that foam production in nature is limited in the non-bloating legumes by the presence of tannin in leaves which combine with and precipitate the foam-producing proteins. Kendall further showed that the foaming ability could be restored in the leaf extracts of these plants by the addition of a reagent, soluble polyvinyl pyrrolidone (PVP), known for its ability to combine with phenolics and tannins. He demonstrated that addition of PVP increased the volume of foam which could be produced from the non-bloating forages, for example lotus major, lespedeza (Lespedeza stipulacea), crown vetch (Coronilla varia), and birdsfoot trefoil (Lotus corniculatus). From these results Kendall concluded that tannins, or tannin-like substances, were involved in the non- bloating legumes. This conclusion has been further confirmed by Jones, Lyttelton and Clarke (1970) using the gel electrophoretic technique, and shows that non-bloating legume forages may fail to produce bloat because the soluble leaf proteins are precipitated by tannins and are therefore not available for foam production.

Although tannin is a rather vague, collective term, based on the ability to tan leather, its biochemical properties in the present context of precipitating the soluble proteins are specific enough. The discovery that tannins, more precisely in the form known as condensed tannins, may be involved as leaf constituents in characterising non-bloating legumes could, therefore, be highly important in tackling the problem of bloat. It has been shown that both of the main forage legumes in New Zealand, white and red clover, lack tannin (Kendall, 1966). Of the three other species of clover already examined, T. arvense, T. hybridum and T. subterranean (Jones and Lyttelton, unpub.), T. arvense has tannin but this is an annual of no agronomic potential.

Since early 1970, the Genetics Unit of the Grasslands Division has been engaged in examining wild clovers for the presence or absence of tannin. Seeds of white clover, obtained from 17 sources including parts of the Mediterranean, Near East, Europe, Africa and North America were sown, and leaves of individual plants were tested for tannin by the method of gel electrophoresis. Owing to the expensive, laborious technique involved, usually only 10-15 plants from each accession were examined. Of a total of over 250 white clover plants tested none had an appreciable amount of tannin. Four plants showed only traces of tannin. Thus it was possible that in white clover the occurrence of tannin, if present at all, is extremely rare. An examination of ten local stray plants of red clover showed no tannin in them.

Recently, a further examination of over 300 plants of white clover in local pastures, by a quick spot-test (Ferric Chloride Test - a modification of the Ferric Ammonium Citrate Test of Burns (1963)) again showed no plant with an appreciable amount of tannin.

T. uniflorum is a species of good agronomic potential closely related genetically to, and capable of successful hybridization with _T. repens_ (Pandey, 1957). We examined the species _T. uniflorum_ grown from seeds obtained from four sources in the Mediterranean. Of a total of 110 plants studied, again none showed any appreciable amount of tannin.

This failure to find tannin among plants of the species _T. repens_, _T. pratense_ and _T. uniflorum_ led us to investigate the possibility of looking for tannin in other cultivated and wild species of _Trifolium_. Twenty-seven additional species have now been examined. In most cases plants from two or three different sources and in two species, _T. hybridum_ (2x and 4x) and _T. ambiguum_ (2x, 4x and 6x), plant materials of different ploidy levels were studied.

It can be seen from Table 1 that none of the nine agriculturally important species of clover has tannin. And 16 other wild or locally cultivated species studied showed no tannin. The study, however, revealed five species of _Trifolium_ which had considerable amount of tannin. One of which, _T. arvense_, an annual weed, has already been referred to before. The four new species, _T. alpestre_, _T. trichocephalum_, _T. rubens_ and _T. hohenackeri_, are all perennials and are potentially acceptable agronomically. Not all plants of these species have tannin. In _T. alpestre_ and _T.rubens_ it occurs only in a very small proportion of plants, but in _T. trichocephalum_, in which we have now examined 122 plants from two sources, about 30-40% of the plants show tannin. In _T. hohenackeri_, on the other hand, more than 90% of the plants may have tannin.

Our work has shown that there is polymorphism not only with regard to the presence or absence of tannin but also with regard to the physiology of tannin production. In _T. alpestre_, _T. rubens_ and _T. trichocephalum_, all tannin-producing plants examined so far produced tannin under a condition of water-stress; the same plants when grown under a normal, fairly moist condition, often showed no trace of tannin. In _T. hohenackeri_, on the other hand, tannin production was free of moisture-stress requirement, and occurred under both dry and wet conditions.

The discovery of tannin-producing agronomically acceptable species of _Trifolium_ raises two interesting possibilities: (1) inclusion of promising strains of a tannin containing _Trifolium_ species into the normal pasture seed mixture; (2) introduction of the tannin character into our two most important legume forages, red and white clover, through hybridization. The first method is of a temporary, experimental nature but can be tried almost immediately. If successful, it can bring quick relief to the most susceptible areas. The second offers a permanent solution but requires extensive research investigations. For, before the long-term, interspecific-hybridization project is attempted there is one serious question to be answered and there is also one serious technical problem to be overcome.

The serious question relates to the apparent relationship of the presence of tannin with unpalatability and lower nutritive value of the forage. Although no data are available on Trifolium species, observations in two forage legumes, sericea and Desmodium, which have very high amounts of tannin, can be a helpful guide in our own evaluation of this problem. Much of the information, however, has been obtained from sericea.

Sericea lespedeza (Lespedeza cuneata) is a perennial legume which is coming into usage in South-Eastern United States as a forage and soil conserving crop, although it is less palatable than most other forage legumes. Feeding trials by Wilkins and associates (1953) have indicated that tannin is a major cause of this unpalatability. Stitt and Hyland (1946) found an inverse relationship between the levels of total tannin and crude protein. However, a great variability exists in the amount of tannin between plants of the same species as well as between different species of Lespedeza. Significantly, a comparative study by Donnelly and Anthony (1970) of DDM (Dry Matter Digestibility) by the in vivo nylon bag technique on the low - and high-tannin sericea plants suggested a tannin threshold which is critical in relation to DDM.

Environmental factors such as moisture, temperature, light intensity, day length and season of growth greatly influence the amount of tannin present. These are much more important than soil types, although where a nutritional deficiency limits growth of sericea, the tannin content may be significantly increased. In most cases tannin content was lower in plants that were stunted and growing very slowly. However, plants infected with disease have generally higher tannin than the corresponding healthy plants. In general, young leaves were a little higher in tannin than were older leaves of the same plant (Wilson, 1955: Bates, 1955; Donnelly, 1959).

The other forage which has attracted some attention with regard to its tannin content and palatability is the tropical legume Desmodium. Bryan (1966) found that D. uncinatum having 3.6% tannin and D. intortum having 7% tannin, are readily eaten, particularly the tannin-rich leaves, and that yearling steers and heifers grazing them gave good weight gains.

TABLE 1: *Trifolium* Species Examined for the Presence or Absence of Tannin

SPECIES	TOTAL NO. OF SPECIES	TANNIN (+ -)
Agriculturally more important species.		
T. repens (White clover (> 250 plants)		
T. pratense (Red clover)		
T. hybridum (2x,4x) (Alsike clover)		
T. incarnatum (Crimson clover)		
T. alexandrinum Egyptian clover)	9	-
T. fragiferum (Strawberry clover)		
T. subterraneum (Subterranean clover)		
T. resupinatum (Persian clover)		
T. semi-pilosum (Kenya White clover)		
Wild or locally cultivated species		
T. hirtum, T. pannonicum,		
T. badium, T. occidentale,		
T. nigrescens, T. ambiguum (2x, 4x, 6x)		
T.sarosiense, T. medium,	16	-
T. squarrosum, T. burchellianum,		
T. rueppellianum, T. usambarense,		
T. baccarinii, T. parviflorum,		
T. neglectum, T. uniflorum (109 plants)		

... Continued

Cont.

SPECIES	TOTAL NO. OF SPECIES	TANNIN (+ -)
T. arvense (20: +) T. rubens (9: 1+, 8-) T. alpestre (21: 1+, 20-) T. trichocephalum (122: 30+, 19 trace, 73-) T. hohenackeri (8: 7+, 1-)	5	+
	30	

TABLE 2: Taxonomic Affinity and Chromosome Number of Certain *Trifolium* species

Tribe or Section	Species	Chromosome Number (2n)
Euamoria	T. repens	32
	T. nigrescens	16
	T. hybridum	16
	T. ambiguum	32, 48
Cryptosciadium	T. uniflorum	32
	T. pratense	14
	T. pallidum	16
	T. diffusum	16
	T. arvense	14
Eulagopus	T. alpestre	16
	T. rubens	16
	T. trichocephalum	48
	T. hohenackeri	16 + 2B

Similarly, the work of Wilkins and associates (1953) with sericea lespedeza indicated that its consumption by sheep was reduced markedly only when the tannin content was around 12%.

Considering these and other pertinent observations together, there is no doubt that a very high tannin content is definitely detrimental as regards palatability and nutritive value. However, there are two promising features which may help in the solution of this problem. Firstly, there is often a considerable store of genetic variability available for selection of low - or moderate-tannin genotypes. Secondly, there appears to be a tannin threshold, which may be specific to plant species and foraging animal, and at which the tannin level is completely acceptable to the animal and yet may be sufficient to resist bloat and produce herbage of a high quality and quantity. The observations on lespedeza and Desmodium are highly suggestive of this. Unfortunately, owing to differences in the techniques of extraction of tannin the given percentages of tannin by different workers in different species and strains are not comparable. It is, therefore, absolutely necessary that qualitative and quantitative work with regard to tannin content, palatability and nutritive value should be done on the Trifolium species and the desired parameters of selection be properly defined, before the feasibility of this research project can be truly evaluated.

The other obstacle to the production of white and red clovers with sufficient tannin content lies in the difficulties involved in the hybridization of Trifolium species. Among the flowering plants, Leguminosae as a family, to which Trifolium belongs, is notorious for having many genera characterised by the paucity of interspecific hybridization. T. repens has so far been successfully hybridized with only two species, T. nigrescens and T. uniflorum (Trimble, 1951; Brewbaker and Keim, 1953; Pandey, 1957). T. pratense has also been successfully hybridized with only two species, T. diffusum and T. pallidum (Taylor et. al. 1963); Armstrong and Cleveland, 1970).

Table 2 gives the taxonomic tribe and chromosome number of certain Trifolium species. It can be seen from the Table that T. repens and T. pratense have so far been successfully hybridized generally with members of the same tribe.

In the tribe Euamoria to which T. repens belongs no species has yet been found containing tannin. All the five species so far found to have tannin belong to the tribe Eulogopus, to which the forage species T. pratense belongs. This is according to Ascherson and Graebner's classification (1906-10) of the genus Trifolium. But even if we consider different classific-ations, we find that of the 10 species related to T. repens which have been studied here, none show tannin. On a similar basis out of 11 studied species which are related to *T. pratense*, five showed tannin. The tannin data thus demonstrate the chemotaxonomic affinity between the species of the tribe *Eulagopus*. At present it is not known whether *T. pallidum* and *T. diffusum*, with which *T.pratense* has been shown to hybridize, have tannin or not. Nevertheless, at this stage, judging from the taxonomic affinity, the chances of introducing tannin character through interspecific hybridization is better for *T. pratense* than for *T. repens*. However, genetically, it is possible that a character which has been lost in two parents may reappear in certain progeny of the hybrids as a result of genetic complementation. The hybrids of *T.repens* and *T. uniflorum* which are known to be vigorous and fertile may offer that possibility.

The modern techniques of hybridization involving heat treatment (Newton et. al. 1970), embryo culture, hormone application (Pandey, 1967) and careful choice of parents based on inherent polymorphism (Pandey, 1969) provide a reasonable chance of success even in such a difficult genus as *Trifolium*, as is evidenced by the few successes already achieved.

At the Genetics Unit of the Grasslands Division we have also started mutation experiments in *T. repens* and *T. pratense* through seed treatment with the mutagenic chemical ethyl methane sulfonate (EMS). The treated seedlings and their progenies will be examined for mutants which might produce tannin. An incorporation into the established varieties of the tannin character from such mutants, if they could be obtained, would be far simpler than that to be achieved through interspecific hybridization.

There is one other significant, genetic feature of the published data which is worth considering. Alfalfa (*Medicago sativa*) is a bloating legume. However, using Kendall's method of foam production, Rumbaugh (1969) has found large differences between individual plants of alfalfa in the amount of stable foam produced. His study to evaluate the genetic potential of a number of experimental populations of alfalfa as sources of clones which would have low stable foam values are very significant. Results from some of the plant populations clearly demonstrated that low foam potential, and therefore the resistance to bloat, is quite compatible with desirable agronomic attributes in alfalfa. Since these plants presumably did not contain significant amount of tannin, as is suggested from the gel electrophoretic tests (Jones and Lyttelton, 1971), the high genetic variability in the ability to produce foam suggests an independent approach to the bloat question that would appear to be uncomplicated by the knotty problem of the association of tannin with unpalatability and deficient nutritive value.

A similar lack of association between tannin and bloat resistance is suggested in Trifolium subterraneum which do not show the presence of tannin and yet animals feeding on this forage rarely produce bloat. It is, therefore, possible that chemical compound(s) other than tannin may also be involved in bloat resistance. The unknown factor(s) may be another phenolic compound or it may not be phenolic at all.

In our effort to achieve an inexpensive and more permanent solution of the problem of bloat we have, thus, at present, certain exciting new possibilities open to us. The co-operation of geneticists, biochemists, animal and plant physiologists, and agronomists is essential in defining and charting out the new parameters of genetic and biochemical enquiry which must precede a full-fledged plant breeding programme. The prospects are, nevertheless, promising.

REFERENCES

Armstrong, K.C.: Cleveland, R.W., 1970: Crop Sci. 10: 354-357.

Ascherson, P.: Graebner, P., 1906-1910: Synopsis der Mitteleuropaischen Flora. Leguminosae. Band 6, Teil 2, Wilhelm Englemann, Leipzig.

Bates, R.P., 1955 Agron. J. 47: 564-567.

Brewbaker, J.L.: Keim, W.F., 1953: American Naturalist 87: 323-326.

Bryan, W.W., 1966: Proc. 10th Inter. Grassld Congr., Helsinki, 311-315.

Burns, R.E., 1963: Univ. Georgia Coll. Agric. Tech. Bull. N.S. 32, 5-14.

Butler, G.W.: Rae, A.L.: Bailey, R.W., 1968: N.Z. agric. Sci. 3: 8-12.

Cooper, C.S.: Eslick, R.F.: McDonald, P.W., 1966 Crop Sci. 6: 215-216.

Donnelly, E.D., 1959: Agron. J. 51: 71-73.

Donnelly, E.D.: Anthony, W.B., 1970: Crop Sci. 10: 200-202.

Johns, A.T., 1961: Sheepfarming Ann., 178-186.

Jones, W.T.: Lyttelton, J.W., 1971: N.Z. J. Agric. Res. 14: 101-107.

Jones, W.T., Lyttelton, J.W.: Clarke, R.T.J., 1970: N.Z. J. Agric. Res. 13: 149-56.

Kendall, W.A., 1964: Crop Sci. 4: 391-393.

__________ 1966: Crop Sci. 6: 487-488.

McArthur, J.M.: Miltimore, J.E.: Pratt, M.J., 1964: Can.J. An. Sci. 44: 200-206.

Mangan, J.L., 1959: N.Z.J. Agric. Res. 2: 47-61.

Newton, D.L.: Kendall, W.A.: Taylor, N.L., 1970: Theoret. Appl. Gen. 40: 59-62.

Pandey, K.K., 1957: J. Hered. 48: 278-281.

__________ 1967: Heredity 22: 255-284.

__________ 1969: Genetica 40: 477-474.

Rumbaugh, M.D., 1969: Crop Sci. 9: 438-440.

Sears, P.D.: Goodall, V.C.: Jackman, R.H.: Robinson,G.S. 1965: N.Z. J. Agric. Res. 8: 270-83.

Stifel, R.B.: Vetter, R.L.: Allen, R.S., 1968: J. Agric. Fd Chem. 16: 500-504.

Stitt, R.E.: Hyland, H.L., 1946: J. Amer. Soc. Agron. 38: 454-455.

Taylor, N.I.: Stroube, W.H.: Collins, G.B.: Kendall, W.A., 1963: Crop Sci. 3: 549-552.

Todd ,J.R., 1969: Brit. Grassld Soc. Occ. Symp. No. 6: 297-307.

Trimble, J.P., 1951: M.S. Thesis, Pennsylvania State College (unpubl.).

Wilkins, H.L.: Bates, R.P.: Henson, P.R.: Lindahl,I.L.: Davis, R.E., 1953: Agron. J. 45: 335-336.

Wilson, C.M., 1955: Agron. J. 47: 83-86.