# A review of the development of a bloat-reduced alfalfa cultivar

B. Coulman[1], B. Goplen[1], W. Majak[2], T. McAllister[3], K.-J. Cheng[3], B. Berg[4], J. Hall[5], D. McCartney[6] and S. Acharya[3]

*[1]Agriculture and Agri-Food Canada Research Centre, 107 Science Place, Saskatoon, Saskatchewan, Canada S7N 0X2; [2]Agriculture and Agri-Food Canada Range Research Unit, Kamloops, British Columbia, Canada V0H 1Z0; [3]Agriculture and Agri-Food Canada Research Centre, Lethrbridge, Alberta, Canada T1J 4B1; [4]Alberta Department of Agriculture and Agri-Food; [5]Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia Canada V0H 1Z0; [6]Agriculture and Agri-Food, Lacombe Research Centre, Lacombe, Alberta, Canada T4L 1W1.* *Received 28 June 1999, accepted 27 September 1999.*

Coulman, B., Goplen, B., Majak, W., McAllister, T., Cheng, K.-J., Berg, B., Hall, J., McCartney, D. and Acharya, S. 2000. **A review of the development of a bloat-reduced alfalfa cultivar**. Can. J. Plant Sci. **80**: 487–491. The studies that led to the development of a bloat-reduced alfalfa began in 1970. Initial work focused on the causes of bloat, the characteristics of bloat-safe and bloat-causing legumes and the development of screening methodologies. The characteristics that were examined as causal factors of bloat in legume species included soluble proteins, saponins, absence of condensed tannins, and rapid breakdown of cells in the rumen. The factor or combination of factors responsible for causing bloat in animals grazing legumes have not been clearly identified; however, of the above, rapid cell breakdown was considered to be the most promising line of research to pursue in selecting a bloat-safe legume. A 4-h nylon-bag rumen digestion technique was developed, which was used to assess the initial rate of digestion (IRD) of fresh leaf material in large numbers of alfalfa genotypes. Using this technique on a population of four alfalfa cultivars, four cycles of recurrent selection for low initial rate of digestion (LIRD) were carried out over an 11-yr period. The resulting selected population, LIRD-4, had a vegetative stage initial rate of digestion that was approximately 85% of that of unselected alfalfa. To determine whether this reduction in IRD would reduce or eliminate bloat, grazing experiments comparing LIRD-4 and unselected cv. Beaver were conducted at three locations over 3 yr. Over all grazings, LIRD-4 caused significantly fewer incidences of bloat than Beaver; thus it is a bloat-reduced, rather than a bloat-safe cultivar. LIRD-4 was released in 1997 and named AC Grazeland Br.

**Key words**: Bloat, cattle, alfalfa, low initial rate of digestion, grazing, bloat-reduced alfalfa

Coulman, B., Goplen, B., Majak, W., McAllister, T., Cheng, K.-J., Berg, B., Hall, J., McCartney, D. et Acharya, S. 2000. **Historique de la création d'un cultivar de luzerne à effet météorisant réduit**. Can. J. Plant Sci. **80**: 487–491. Les travaux qui ont conduit à la création d'une lignée de cultivar possédant un effet météorisant moins violent remontent à 1970. Au début, l'accent était porté sur les causes du météorisme, sur les caractères des légumineuses météorisantes et non météorisantes et sur la mise au point de méthodes de tri génétique. Parmi les facteurs causaux éventuels du météorisme, il y avait les protéines solubles, les saponines, l'absence de tanin condensé et la décomposition rapide des cellules végétales dans le rumen. Le facteur ou la combinaison de facteurs responsables du météorisme chez les animaux pâturant des légumineuses n'a pas encore été clairement identifié. Mais de tous ceux énumérés plus haut, c'est la dégradation rapide des cellules dans le rumen qui apparaît comme l'avenue la plus intéressante dans la recherche d'une légumineuse non météorisante. Une technique de digestion ruminale en sachets de nylon pendant 4 heures a été mise au point pour évaluer le taux initial de digestion (TID) des feuilles fraîches provenant d'un grand nombre de génotypes de luzerne. En appliquant la technique à une population de 4 cultivars de luzerne, 4 cycles de sélection récurrente sur le caractère faible taux initial de digestion (FTID) étaient réalisés dans une période de 11 ans. La population sélectionnée résultante, LIRD-4, démontrait un taux initial de digestion au stade végétatif correspondant à environ 85 % de celui de la luzerne non sélectionnée. Pour déterminer si cette diminution est suffisante pour réduire ou pour éliminer les cas de météorisme, des expériences de pâturage comparant la population LIRD-4 au cultivar ordinaire Beaver étaient réalisées à trois emplacements pendant 3 ans. Tous épisodes de pâture confondus, l'emploi de LIRD-4 donnait lieu à moins de cas de météorisme que Beaver, c'est pourquoi on le considère comme un cultivar à effet météorisant réduit plutôt que comme un cultivar non météorisant. En 1997, LIRD-4 était mis au commerce sous la désignation AC Grazeland Br.

**Mots clés**: météorisme, bovin, luzerne

The actual selection of a bloat-reduced cultivar began in 1979; however, the background research to produce a bloat-safe or bloat-reduced alfalfa was initiated in 1970. The breeding program was carried out at the Saskatoon Research Centre of Agriculture and Agri-Food Canada (AAFC) under the supervision of B. P. Goplen and R. E. Howarth. Other key scientists involved in the work that led to the development of this cultivar were K.-J. Cheng of the Lethbridge Research Centre and W. Majak of the Kamloops Range Research Unit. The development of a bloat-reduced alfalfa

**Abbreviations**: **AAFC**, Agriculture and Agri-Food Canada; **IRD**, initial rate of digestion; **LIRD**, low initial rate of digestion

**Table 1. Characteristics of bloat-safe and bloat-causing legumes**

| Species | Bloat-safe | Condensed tannins | Reticulate veins | Thick mesophyll cell walls | Thick epidermal cell walls | Low initial rate of digestion |
|---|---|---|---|---|---|---|
| Alfalfa | No | No | No | No | No | No |
| Birdsfoot trefoil | Yes | Yes | No | Yes | No | Yes |
| Cicer milkvetch | Yes | No | Yes | Yes | Yes | Yes |
| Sainfoin | Yes | Yes | No | ? | ? | Yes |

can be divided into three phases: 1) pre-breeding (1970–1979); 2) selection (1979–1991); and 3) evaluation (1990–1996).

## PRE-BREEDING

Much of the work in the 1970s concentrated on elucidating the causes of bloat, studying the characteristics of bloat-causing and bloat-free legume species (Table 1), and developing screening methodologies. Bloat occurs when the mechanism for the eructation of rumen gas is inhibited or impaired and gas production exceeds the animal's ability to expel it (Howarth et al. 1991). In legume pasture bloat, the eructation mechanism is commonly inhibited by frothy or foamy rumen contents. Consequently, much of the early work focused on the constituents of legumes that could cause foam to form in the rumen.

Legume forages contain high concentrations of soluble proteins that are surface-active foaming agents. Initially, a protein known as Fraction I or 18s (ribulose bisphosphate carboxylase) was examined, but it was soon realized that Fraction II proteins also had foaming properties (Howarth et al. 1973); consequently the program began to assess total soluble protein concentrations. To investigate the possibility of selection for lower concentrations of these protein fractions in alfalfa, Miltimore et al. (1974) found a low broad-sense heritability estimate of 0.28 for Fraction I protein. Gutek et al. (1976) also reported low narrow-sense heritability estimates for Fraction I (19–20%), Fraction II (19–27%) and total soluble (23–31%) proteins. It was concluded that it would be possible to select for lower soluble protein concentration in alfalfa using recurrent selection with progeny testing. It was later estimated that a 50% reduction in soluble protein concentration would be required to produce a bloat-free alfalfa (Howarth et al. 1977). Given the importance of the Fraction I chloroplast proteins in photosynthesis, this may have been difficult to achieve. In addition, it was found that rumen soluble protein concentrations were not strongly correlated with the occurrence of bloat in cattle fed fresh alfalfa.

Saponins are other surface active foaming agents in alfalfa that were considered to be a potential cause of bloat (Lindahl et al. 1957). No significant differences in the occurrence of bloat and frothy rumen contents were found in animals fed high versus low saponin alfalfas (Majak et al. 1980). Consequently, the AAFC program did not consider saponin concentration as a suitable selection criterion to produce a bloat-free alfalfa.

A number of bloat-safe legumes, such as birdsfoot trefoil (*Lotus corniculatus* L.) and sainfoin (*Onobrychis viciaefolia* Scop.), contain condensed tannins in their foliage (Sarkar et al. 1976). It was suggested that these tannins could prevent the occurrence of bloat by acting as protein precipitants (Goplen et al. 1980). Cicer milkvetch (*Astragalus cicer* L.) is a non-bloating legume that does not contain condensed tannins. This indicated that, although condensed tannins are effective bloat-preventing compounds, there are other mechanisms, which play a role in bloat-safe legumes. A survey indicated that condensed tannins were not found in the foliage of a large number of annual and perennial *Medicago* spp., common alfalfa cultivars, and mutagenized populations of diploid and tetraploid *M. sativa* and *M. falcata* (Goplen et al. 1981). Consequently, it was not possible to produce a leaf-tannin containing alfalfa by conventional selection techniques. Condensed tannins do occur in the coats of alfalfa seeds (Goplen et al. 1980), indicating that the pathway for the biosynthesis of these compounds exists in alfalfa. Production of a bloat-safe alfalfa with foliar condensed tannins requires the transfer of appropriate biochemical and/or regulatory genes from other species (McMahon et al. 2000). A program to produce such alfalfa germplasm is continuing at the Saskatoon Research Centre of AAFC under the supervision of M. Gruber.

In the late 1970s, the AAFC bloat program began to focus on the breakdown of cells of forage legumes and the release of their cellular constituents into the rumen. As well as soluble proteins, it was suggested that small particles such as chloroplast fragments released in the rumen upon cellular breakdown contribute to bloat (Howarth et al. 1991). Leaf cells of various legumes were ruptured by crushing them with a mortar and pestle. It was found that the mesophyll cells of bloat-safe legumes were more resistant to mechanical rupture than those of bloat-causing legumes (Howarth et al. 1978). In addition, Lees (1984) found that epidermal and mesophyll cell walls were thicker in bloat-safe than bloat-causing legumes and whole leaves of bloat-safe legumes were consistently less digested than bloat-causing legumes in short term rumen fermentations (Howarth et al. 1979). Thus, initial rates of digestion were slower in bloat-safe legumes resulting in a lower concentration of intracellular leaf constituents (including soluble proteins and chloroplast fragments) in rumen fluid. These constituents likely remain below a critical threshold that would cause bloat.

The challenge was to develop routine screening methods to estimate initial rate of digestion in breeding populations. Howarth and Goplen (1983) and Goplen et al. (1981) described four methods that were evaluated: 1) nylon bag digestion; 2) in vitro enzyme (pectin and cellulose) digestion; 3) mechanical strength of mesophyll cell walls; and 4) dry matter loss by leaching. Nylon bag digestion was the technique chosen because it takes all causal factors into account, including the effect of plant intracellular constituents and rumen salivary proteins. Using this technique, the variation in leaf tissue disruption, as measured by dry matter disappearance after various short digestion periods in the rumen, was determined among various forage legumes and among and within alfalfa cultivars (Howarth et al. 1982). Among species, red clover (*Trifolium pratense* L.), white clover (*T. repens* L.) and alfalfa (bloat-causing legumes) had the highest relative rates of leaf disruption. The bloat-safe legumes sainfoin, cicer milkvetch and birdsfoot trefoil had lower rates of leaf disruption. There were no differences among alfalfa cultivars; however, among the 12 individual clones of cv Beaver, there were differences ($P < 0.05$) in dry matter disappearance (range 47–59%). The rank order of these differences was consistent over spring and summer field growths and greenhouse-grown plants.

These results validated the use of the nylon bag technique to select for a bloat-reduced/safe alfalfa and indicated that there was variability for leaf disruption rate among genotypes within alfalfa populations. An hypothesis describing the time course of dry matter disappearance was proposed (Fig. 1) which estimated that a 25–30% reduction in initial rate of digestion (6–8 h) would be required to produce a bloat-safe alfalfa. This would make alfalfa similar in initial digestion rate to cicer milkvetch and birdsfoot trefoil, both bloat-safe legumes. The nylon bag technique described by Howarth et al. (1982) was impractical for screening large populations for reduced IRD, thus, a modified technique was developed (Goplen et al. 1993).

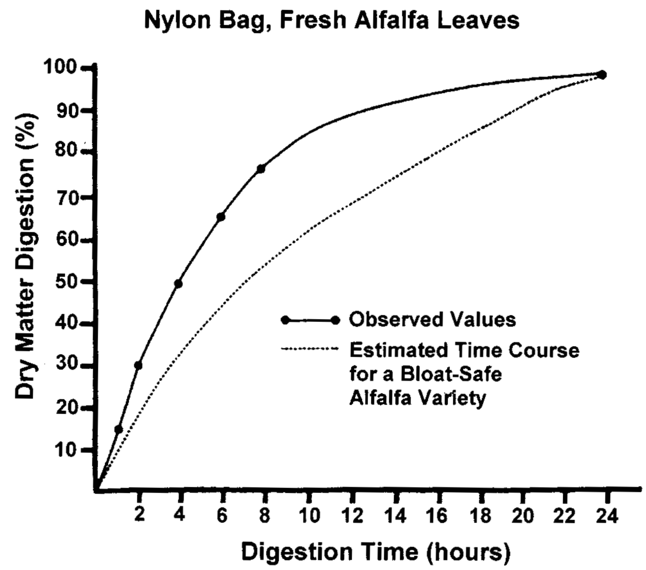


**Fig. 1**. The proposed time-course of dry matter loss from alfalfa forage in a bloat safe cultivar (from Howarth et al. 1982).

## SELECTION

The selection of a bloat-reduced alfalfa cultivar was carried out at the research farm of the Agriculture and Agri-Food Canada Research Farm at Saskatoon, SK. Canada (52°38 lat, 106°38 long), with a Sutherland clay loam soil type (Typic Haploboroll). The modified nylon bag (in sacco) technique used to screen alfalfa plants for IRD has been previously described (Goplen et al. 1993) and only the highlights are presented here.

The original population that was evaluated consisted of 1200 plants of the cultivars Beaver, Vernal, Anchor and Kane. All four cultivars were in use in western Canada at the initiation of the selection. Kane is a creeping-rooted type of alfalfa whereas the other three are tap-rooted types. These cultivars are resistant to bacterial wilt (*Clavibacter michiganese)* and are winterhardy under western Canadian conditions.

Plants were established in spaced nurseries on 1-m centers. They were sampled for IRD determinations at a vegetative (pre-bud to mid-bud) stage of growth, with two or three collections per year. No samples were collected in the year of establishment. Fresh samples were collected from the upper 15 cm of alfalfa plants, chopped into 1-cm segments, and 5 g subsamples were placed in duplicate nylon bags. The nylon bags were attached to a weighted polyethylene bottle (10–12 bags per bottle) and placed in the rumen of a fistulated cow for 4 h. The bags were retrieved from the rumen and washed, and the undigested residue was dried and weighed to determine dry matter disappearance. It was possible to screen 40–60 samples per day; however, given the large number of plants to analyze, it was necessary to cut portions of the nursery to maintain growth at the pre- to mid-bud stage for sampling.

Four cycles of selection for LIRD were carried out (Table 2) with 1050 to 1600 plants screened in each cycle and the selection intensity varying from 2 to 5%. When the desired plants were identified, they were transferred from the field to a growth cabinet where they were polycrossed to produce seed for the next generation of selection. The seed produced after each polycross in each cycle was designated LIRD-1, -2, -3, and -4. To produce LIRD-3 and LIRD-4, progeny testing was carried out, respectively, on the 20 and 12% least digested plants, thus cycles 3 and 4 required 4 yr to complete, rather than the 2 yr for cycles 1 and 2. The progenies were planted in year 3 and analyzed in year 4 of the selection cycles 3 and 4. The selection of the 2% lowest IRD clones in cycles 3 and 4 (Table 2) was based on the results of the progeny screening. The selections made in each cycle of selection included plants originating from each of the four cultivars in the original source nursery. Seed production nurseries (0.45 ha) were established for the LIRD-3 and LIRD-4 populations to provide adequate seed for the establishment of grazing trials to evaluate bloat incidence.

LIRD-3 and LIRD-4 had initial rates of digestion approximately 85% of that of cv. Beaver when sampled at a vegetative stage (Goplen et al. 1993). At more mature stages, however, these populations were not different from Beaver in IRD. Hall et al. (1994) found IRDs of LIRD-3 to be

**Table 2. Selection program for a bloat-reduced alfalfa, 1979–1991**

| Cycle | Year | No. of plants | Percent of plants selected | Progeny test |
|---|---|---|---|---|
| 1 | 1979 | 1200 | 3 | No |
| 2 | 1981 | 1560 | 5 | No |
| 3 | 1983 | 1050 | 2 | Yes |
| 4 | 1987 | 1600 | 2 | Yes |

89–96% of those of Beaver at the vegetative to mid-bud stage of growth. These results indicated that: 1) there was no change in IRD from cycle 3 to cycle 4; 2) the reduction in IRD fell short of the 20–30% reduction that was predicted to be required for bloat prevention (Howarth et al. 1982); and 3) the reduction in IRD occurred only at the vegetative stage, which was the stage of growth used in the screening and selection. The question that had to be answered at this point was whether such a reduction in IRD would prevent or reduce bloat incidence in grazing cattle.

## EVALUATION

The evaluation of LIRD-3 for bloat incidence was carried out at Kamloops, BC, and Lethbridge, AB, from 1990 to 1992 (Hall et al. 1994). LIRD-3 was compared with Beaver in feedlot and grazing trials with alfalfa being fed or grazed for only 6 h $d^{-1}$. This interrupted feeding system provoked a very high incidence of bloat and in only one of six trials was a reduced incidence of bloat observed with LIRD-3.

LIRD-4 was evaluated at Kamloops, BC, Lethbridge, AB, and Melfort, SK, from 1994 to 1996 in grazing trials where animals were on pastures 24 h $d^{-1}$. In a number of the grazing periods there were significantly fewer incidences of bloat with LIRD-4 than Beaver. A more comprehensive presentation of these experiments can be found elsewhere in this symposium proceedings (Berg et al. 2000).

## RELEASE

Based on the reduced incidence of bloat for LIRD-4, this population was released in 1997 and named AC Grazeland Br. Certified seed was available in Canada in 2000. Compared to Beaver, AC Grazeland Br has thicker epidermal and mesophyll cell walls (Goplen et al. 1993), longer stems and internodes, and slightly higher concentrations of neutral detergent fiber and acid detergent fiber (Coulman, unpublished data). Similarly, Shenk and Elliott (1971) found that synthetics of alfalfa selected for low 6 h in vitro dry matter disappearance were higher in neutral detergent fiber concentration than synthetics selected for high in vitro dry matter disappearance.

It is expected that the availability of AC Grazeland Br will increase the hectarage of alfalfa grazed in western Canada. Producers who want to reduce the risk of bloat occurring in their grazing animals will be interested in this cultivar, although it will reduce the incidence of bloat, but not completely eliminate it. Research is underway to test AC Grazeland Br in combination with other bloat-reducing practices such as: mixtures with meadow bromegrass (*Bromus riparius* Rehm.) or sainfoin; Rumensin boluses; and the use of detergents. The goal is to provide a management package that will reduce the probability of bloat close to 0.


**Berg, B. P., Majak, W., McAllister, T. A., Hall, J. W., McCartney, D., Coulman, B. E., Goplen, B. P., Acharya, S. N., Tait, R. M. and Cheng, K.-J. 2000**. Bloat in cattle grazing alfalfa cultivars selected for a low initial rate of digestion: A review. Can. J. Plant Sci. **80**: 493–502.

**Goplen, B. P., Howarth, R. E. and Lees, G. L. 1993**. Selection of alfalfa for a lower initial rate of digestion and corresponding changes in epidermal and mesophyll cell wall thickness. Can. J. Plant Sci. **73**: 111–122.

**Goplen, B. P., Howarth, R. E., Lees, G. L., Majak, W., Fay, J. P. and Cheng, K.-J. 1981**. Evolution of selection techniques in breeding for bloat-safe alfalfa. Proc. XIV International Grassland Congress. Lexington, KY. pp. 221–223.

**Goplen, B. P., Howarth, R. E., Sarkar, S. K. and Lesins, K. 1980**. A search for condensed tannins in annual and perennial species of *Medicago*, *Trigonella*, and *Onobrychis*. Crop Sci. **20**: 801–804.

**Gutek, L. H., Goplen, B. P. and Howarth, R. E. 1976**. Heritability of soluble proteins in alfalfa. Crop Sci **16**: 199–201.

**Hall, J. W., Majak, W., Stout, D. G., Cheng, K.-J. Goplen, B. P. and Howarth, R. E. 1994**. Bloat in cattle fed alfalfa selected for a low initial rate of digestion. Can. J. Anim. Sci. **74**: 451–456.

**Howarth, R. E. and Goplen, B. P. 1983**. Improvement of forage quality through production management and plant breeding. Can. J. Plant Sci. **63**: 895–902.

**Howarth, R. E., Chaplin, R. K., Cheng, K.-J., Goplen, B. P., Hall, J. W., Hironaka, R., Majak, W. and Radostits, O. M. 1991**. Bloat in cattle. Agriculture Canada, Ottawa, ON. Publ. 1858/E. 34 pp.

**Howarth, R. E., Goplen, B. P., Brandt, S. A. and Cheng, K.-J. 1982**. Disruption of leaf tissues by rumen microorganisms: An approach to breeding bloat-safe forage legumes. Crop Sci. **22**: 564–568.

**Howarth, R. E., Goplen, B. P., Fay, J. P. and Cheng, K.-J. 1979**. Digestion of bloat-causing and bloat-safe legumes. Ann. Rech. Vet. **10**: 332–334.

**Howarth, R. E., Goplen, B. P., Fesser, A. C. and Brandt, S. A. 1978**. A possible role for leaf cell rupture in legume pasture bloat. Crop Sci. **18**: 129–133.

**Howarth, R. E., Majak, W., Waldern, D. E., Brandt, S. A., Fesser, A. C., Goplen, B. P. and Spurr, D. T. 1977**. Relationships between ruminant bloat and the chemical composition of alfalfa herbage. I. Nitrogen and protein fractions. Can. J. Anim. Sci. **57**: 345–357.

**Howarth, R. E., McArthur, J. M., Hikichi, M. and Sarkar, S. K. 1973**. Bloat investigations: Denaturation of alfalfa fraction II proteins by foaming. Can. J. Anim. Sci. **53**: 439–443.

**Lees, G. L. 1984**. Cuticle and cell wall thickness: Relation to mechanical strength of whole leaves and isolated cells from some forage legumes. Crop Sci. **24**: 1077–1081.

**Lindahl, I. L., Davis, R. E., Tertell, R. T., Whitmore, G. E., Shalkop, W. T., Dougherty, R. W., Thompson, C. R., Van Atta, G. R., Bickoff, E. M., Walter, E. D., Livingston, A. G., Gugolz, J., Wilson, R. H., Sideman, M. B. and DeEds, F. 1957**. Alfalfa saponins, studies on their chemical, pharmacological and physiological properties in relation to ruminant bloat. US Dep. Agric. Tech. Bull. 1161, Agr. Res. Service, Washington, DC. 83 pp.

**Majak, W., Howarth, R. E., Fesser, A. C., Goplen, B. P. and Pederson, M. W. 1980**. Relationships between ruminant bloat and composition of alfalfa herbage. II. Saponins. Can. J. Anim. Sci. **60**: 699–708.

**McMahon, L. R., McAllister, T. A., Berg, B., Majak, W., Acharya, S. N., Popp, J. D., Coulman, B. and Cheng, K.-J. 2000**. A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. Can. J. Plant Sci. **80**: 469–485.

**Miltimore, J. E., McArthur, J. M., Goplen, B. P., Majak, W. and Howarth, R. E. 1974**. Variability of fraction 1 protein and heritability estimates for fraction 1 protein and total phenolic constituents in alfalfa. Agron. J. **66**: 384–386.

**Sarkar, S. K., Howarth, R. E. and Goplen, B. P. 1976**. Condensed tannins in herbaceous legumes. Crop Sci **16**: 543–546.

**Shenk, J. S. and Elliott, F. C. 1971**. Plant compositional changes resulting from two cycles of directional selection for nutritive value in alfalfa. Crop Sci. **11**: 521–524.