

Winter versus Summer Dormancy: Alfalfa Fall Dormancy

Jeff Volenec

Department of Agronomy, Purdue University, West Lafayette, IN U.S.A.

INTRODUCTION

Dormancy is a phenomenon that impacts reproduction, productivity, and survival of most perennial plants. The ability to alter dormancy has numerous potential benefits in agriculture; however, manipulation of this key plant developmental feature is impaired by our fragmented and incomplete knowledge of mechanisms controlling dormancy. Several forms of dormancy have been described. Endodormancy, also known as innate dormancy, is caused by factors found in dormant tissues that prevent growth even under conditions conducive for development. Seeds, corms, tubers, rhizomes, as well as axillary buds are all capable of exhibiting endodormancy. Buds routinely survive environmental injury of winter or summer via endodormancy, entering this physiological state during fall in temperate regions or just prior to the dry summer season in Mediterranean environments. Paradormancy, sometimes referred to as apical dominance or correlative inhibition, is arrested bud development due to the presence of growth-inhibiting signals produced by developing shoots. Removal of existing shoots (or apical meristems) often releases axillary and adventitious buds from paradormancy. Ecodormancy, sometimes referred to as imposed or forced dormancy, occurs when plants are placed in environmental conditions that are not conducive to growth such as cold or drought stress. Although very little is known about the mechanisms by which growth is inhibited by these conditions, it is speculated that abscisic acid metabolism is involved.

Like most plants alfalfa exhibits paradormancy. Crown buds remain dormant until shoots are removed, then some, but not all of the previously dormant buds on crowns elongate. Mechanisms controlling bud development after alfalfa defoliation are poorly understood despite important agricultural implications. For example, the well-known shoot growth rate advantage of tetraploid alfalfa over near-identical diploid plants is due to crown bud mass (size, number) at defoliation and is not associated with the relative growth rates of individual buds/shoots once dormancy breaks and shoot growth resumes (Volenec, 1988). More scientific attention has focused on fall dormancy of alfalfa. A form of ecodormancy, it is a measure of natural shoot height in fall, and ranges from 1 to 11 (<http://www.naaic.org/stdtests/Dormancy2.html>). More than any other characteristic fall dormancy influences adaptation of alfalfa to specific agro-ecosystems (Castonguay et al., 2006). Dormant alfalfa plants are generally more tolerant of abiotic stress, and especially winter-related stresses, than are non-dormant alfalfa cultivars. The goal of this paper is to integrate our fragmented understanding of morphological, physiological and biochemical mechanisms associated with alfalfa fall dormancy with its impact on environmental stress tolerance.

Fall Dormant Alfalfa Cultivars Have Reduced, Decumbent Shoot Growth in Autumn.

The “hallmark” morphological attribute that identifies fall dormant alfalfa plants is the drastic reduction in shoot growth in autumn. Smith (1961) characterized the variation in fall dormancy both between and within alfalfa cultivars and reported a seven-fold range in shoot height in autumn (4 to 27 cm). With recent cultivar development focusing on non-dormant cultivars, a wider range in alfalfa fall dormancy and autumn height exists today (<8 cm up to >45 cm) corresponding to fall dormancy ratings that range from <1 to >11, respectively (Cunningham et al., 2001). The shorter shoots and slower shoot growth rates of fall dormant germplasm and

plant introductions resulted from fewer nodes and shorter internodes than non-dormant plants (Volenec, 1985). In addition, dormant plants had more, but smaller shoots than non-dormant alfalfa germplasms. Using reciprocal grafting Heichel and Henjum (1990) concluded that relatively greater reductions in internode length occurred in fall dormant cultivars exposed to autumn-like conditions, and that this reduction was primarily conditioned by shoot, not root genotype. However, rootstock could influence herbage production per plant and suggested that graft-transmissible substances were involved in fall dormancy responses of alfalfa.

Root and crown morphology also is altered by fall dormancy. Greenhouse studies revealed that both root and crown mass of dormant plants was less than that of non-dormant plants (Volenec, 1985). This was confirmed in field studies where solid-seeded fall dormant plants had smaller roots and crowns than did plants of non-dormant cultivars (Juan et al., 1994). However, Johnson et al. (1998) screened a diverse array of germplasms in the field and reported that taproots of dormant alfalfa were intermediate in diameter, but in agreement with others, these taproots had numerous, large branches, and greater fibrous root mass when compared to semi- and non-dormant alfalfa germplasms.

Fall Dormancy in Alfalfa is Driven by Short Photoperiods and Cold temperatures.

Cold, non-freezing temperatures result in reduced herbage growth characteristic of alfalfa fall dormancy; a response that is intensified by short (< 8hr) photoperiods (Tysdal, 1933; Shih et al., 1967). Short photoperiods in autumn are confounded with changes in light quality; however, shifting spectral composition of incident white light to be red- or blue-enriched light reduced winter survival of all cultivars irrespective of fall dormancy (Tysdal, 1933). This suggests that alfalfa fall dormancy reaction may be more than a simple phytochrome or cryptochrome response. Alternating temperatures (25/2°C) intensify fall dormancy and freezing tolerance, especially for fall dormant cultivars (Tysdal, 1933). In addition, Castonguay et al. (1995) showed that exposure to sub-freezing (-2°C) temperatures preferentially enhanced dormancy attributes including freezing tolerance of fall dormant alfalfa cultivars.

Fall Dormancy is Associated with Enhanced Winter Hardiness.

Fall dormancy has been used as a surrogate for predicting alfalfa winter survival because of the expense and time (multiple years) needed to accurately determine this important agronomic attribute. Early research (Janssen, 1929) clearly identified winter dormancy was an essential prerequisite for winter survival and that varieties that did not resume growth during brief warm periods in winter were highly desirable. Smith (1961) extended this finding by characterizing the association of fall dormancy with winter hardiness both between and within cultivars, and showed that these traits were closely correlated ($r = 0.94$). Subsequent work using 251 cultivars confirmed that fall dormancy could be used to predict winter injury with a high degree of reliability ($R^2=0.85$) (Schwab et al., 1996). Because only 7 of 251 cultivars deviated significantly from this relationship, these authors concluded that, in the absence of data on winter hardiness *per se*, that fall dormancy remained a useful predictor of this key trait. However, fall dormant plants tend to have relatively conservative growth patterns, and this is manifested as lower forage yield when compared to non-dormant plants.

Others have observed variation in winter survival that is not closely associated with fall dormancy. Busbice and Wilsie (1965) reported positive correlations between fall growth and spring recovery across and within F2 progenies of dormant by semi-dormant crosses. They speculated, but did not prove, that simultaneous selection for winter hardiness and fall non-

dormancy would be effective. More recently, Brummer et al. (2000) reported a weak genetic correlation between fall height and winter injury (-0.16) which implies that within this population, less fall non-dormant plants incurred less winter injury. These results provide compelling evidence that simultaneous improvement in both fall growth and winter hardiness may be possible. However, bi-directional selection for contrasting fall dormancy from within cultivars that themselves differed in fall dormancy resulted in the predicted response: plants selected for greater fall dormancy exhibited improved winterhardiness, whereas populations selected for less fall dormancy incurred greater winter injury (Cunningham et al., 1998; 2001).

Winter hardiness and drought stress tolerance have several biochemical and biophysical processes in common, including tolerance of cellular dehydration, that have led to hypotheses regarding the impact of fall dormancy on alfalfa drought tolerance. For example, imposing severe water deficit stress by withholding water for two weeks improved freezing tolerance of several alfalfa cultivars (Tysdal, 1933). We examined the association between taproot water relations and freezing tolerance in alfalfa cultivars differing in fall dormancy (Haagenson and Volenec, unpublished). To our surprise, relative water content (RWC) of roots of winter hardy, dormant cultivars (Vernal, 5454) was greater than that of CUF 101 (non-dormant) that did not survive winter. However, consistent with our expectations, osmolality of taproots of dormant plants increased during cold acclimation more than that of non-dormant plants and was associated with improved winter hardiness. Transcript abundance for dehydrins increased with cold acclimation, especially in the dormant cultivars Vernal and 5454. Thus, many of the physiological and biochemical adjustments that occur in response to dehydration stress also occur as alfalfa taproots cold acclimate and are associated with fall dormancy.

Is Fall Dormancy Associated with Enhanced Drought Tolerance and Water Use Efficiency?

Carbon isotope discrimination has been used to compare water use efficiency (WUE) of contrasting alfalfa cultivars. Using diverse plant introductions (PIs), Johnson and Tieszen (1994) reported a significant range in carbon isotope discrimination (18.9 to 20.3%) in the field. They did not report fall dormancy of these PIs, but fall growth values (surrogate for fall dormancy) reported in the Germplasm Resources Information Network (GRIN, <http://www.ars-grin.gov/>) for these PIs were not generally associated with carbon isotope discrimination values. However, additional greenhouse study of PI 434600 (fall dormant) revealed a relatively low carbon isotope discrimination value and higher WUE when compared to PI 420400 (non-dormant). The greater WUE of PI 434600 was associated with greater herbage growth and not less water use *per se*. These results were confirmed by Ray et al. (1998) who observed higher carbon isotope discrimination values (poorer WUE) in non-dormant alfalfa germplasms when compared to dormant germplasms. Plants with higher WUE efficiency tended to have low forage yield and were relatively slow to develop and flower; both characteristics of dormant alfalfa. Other studies have found no association between fall dormancy and alfalfa WUE. For example, Collino et al. (1997) reported that herbage growth, evapotranspiration, relative water content, stomatal conductance, and soil water extraction were similar for dormant and non-dormant alfalfa cultivars. By comparison Hattendorf et al. (1990) reported that CUF 101 (non-dormant) had lower transpiration than Vernal (dormant), and offered an opportunity to improve WUE of alfalfa. Selection for improved WUE using carbon isotope discrimination has been successful in tall fescue (*Festuca arundinacea* Schreb.) (Johnson et al., 2008), however, there are no similar published reports for alfalfa. Thus, the relationship between genetic variation in fall dormancy and WUE measured using isotope discrimination in alfalfa remains to be fully defined.

The WUE of alfalfa also can be manipulated with management. For example, withholding water for one or two regrowth cycles (30 to 60 d) reduced water use more than forage yield and resulted in 25% higher water use efficiency (Metochis and Orphanos, 1981). Part of this effect was due to compensatory growth that occurred with rewatering when growth of previously unwatered plants was greater than that of plants continuously irrigated. However, such advantages to withholding water have not been reported in all studies. Guitjens and Goodrich (1994) imposed dormancy on alfalfa by withholding water and measured WUE and evapotranspiration (ET). They observed that up to one-half of the water lost during dormancy was ET associated with herbage growth, while the remaining one-half to two-thirds of the water lost during imposed dormancy was unaccounted for in their analysis. They ascribed the high losses during dormancy to greater than normal evaporative losses, unmeasured losses in herbage yield, or to root growth that was not measured in their study.

Dormancy-based Physiological and Biochemical Adjustments that Occur During Abiotic Stress Acclimation of Alfalfa.

Early efforts to discriminate dormant from non-dormant alfalfa varieties on the basis of taproot composition were largely unsuccessful due in large part to the rudimentary techniques used in these studies (Mark, 1936). Recently we have shown that selection for greater fall dormancy reduced the height of the non-dormant cultivar CUF 101 and markedly improved its winter survival (Cunningham et al., 1998). Concentrations of sugars and proteins in roots of the dormant CUF 101 population increased to values similar to those observed for the fall-dormant, winter-hardy Norseman. Greater sugar accumulation during cold acclimation was also observed for suspension cells derived from dormant alfalfa suggesting that the fundamental mechanisms stimulating sugar accumulation in dormant cultivars transcends morphological attributes (Kalengamaliro et al., 2000). Later work showed that concentrations of the raffinose family oligosaccharides (RFO) raffinose and stachyose, and activity of galactinol synthase, the first committed step in RFO synthesis, were higher in dormant when compared to non-dormant alfalfa during cold acclimation (Cunningham et al., 2003). This increase in RFO synthesis parallels that of plants (Urano et al., 2008) and seeds (Corbineau et al., 2000) exposed to drought/dehydration, and is thought to involve membrane protection. This same membrane protective function may enhance winter survival of dormant alfalfa cultivars where RFO accumulation is high (Cunningham et al., 2003).

Accumulation of amino acids and proteins also occurs in taproots during cold acclimation and differences, both quantitative and qualitative, occur between dormant and non-dormant alfalfa cultivars. From September to November, buffer-soluble low molecular weight N increased two-fold in non-dormant cultivars whereas it increased four-fold in dormant cultivars (Cunningham and Volenec, 1998). Subsequent work revealed that taproot amino-N was generally higher in taproots of winter hardy, dormant alfalfa cultivars when compared to non-dormant cultivars (Haagenson et al., 2003a, b). The physiological roles played by amino N accumulation in stressed alfalfa remain unclear, but could be several including: colligative effects such as freezing point depression or osmotic adjustment during drought; serving as a readily available source of N when stress is alleviated; or may have specific effects on protein or membrane stability.

Dormant alfalfa cultivars also accumulate higher taproot protein concentrations than do non-dormant alfalfa populations (Cunningham and Volenec, 1998; Haagenson et al., 2003a). Protein concentrations were 15 to 20% greater in December in taproots of dormant when compared to

non-dormant cultivars; a difference that was closely related to genetic differences in winter hardiness ($R^2=0.67^{**}$) (Cunningham et al., 2001). Other work confirmed this relationship using selection for contrasting fall dormancy within cultivars that themselves differed in fall dormancy (Cunningham et al., 1998). Selection for greater fall dormancy within CUF 101 (non-dormant) increased both fall dormancy and winter hardiness of the fall dormant version of CUF 101. In addition to changes in protein concentration, changes in polypeptide composition also were observed. A substantial portion of this protein accumulation results from preferential synthesis of four polypeptides that serve as vegetative storage proteins (VSP) in alfalfa taproots (Cunningham and Volenec, 1996). These proteins accumulate in taproots in autumn as plants cold-acclimate, and preferentially disappear when alfalfa shoot growth begins in spring (Hendershot and Volenec, 1993).

Water relations impact alfalfa taproot VSP accumulation. For example, irrigation during establishment increased taproot VSP accumulation (Justes et al., 2002) probably through its effect on increasing seedling development rate (Kalengamaliro et al., 1997). However, Erice et al. (2007) observed that both elevated CO_2 and moderate drought stress stimulated taproot VSP accumulation in alfalfa. Recently, Pembleton et al. (2009) also reported that mild to moderate water deficit stress increased taproot VSP accumulation, especially in fall dormant alfalfa. However, severe water deficit stress greatly reduced taproot VSP accumulation in both cultivars. The accumulation of VSPs during moderate drought may serve two purposes: first, VSPs may be an N salvage mechanism and this N is used for shoot regrowth when water again becomes available; and second, these VSPs may have a direct impact on plant drought tolerance through some uncharacterized protective mechanism. This latter concept has been proposed for proteins that, like the taproot VSPs, are heavily glycosylated and protect plants from drought and freezing stresses (Yu and Griffith, 2001). Preferential accumulation of VSPs when exposed to stress may be one of several mechanisms conferring greater abiotic stress tolerance to dormant alfalfa. VSP gene knock-out studies are underway that will provide better insight into the multiple physiological and biochemical roles these proteins have in alfalfa stress tolerance.

Dormancy and Forage Improvement.

Despite the importance of fall dormancy to successful deployment of alfalfa cultivars worldwide, our understanding of internal mechanisms controlling this response remains meager. Clearly, fall dormancy in alfalfa is positively associated with abiotic stress tolerance, but also results in lower forage yield when compared to non-dormant alfalfa (Brummer et al., 2002). Understanding the key physiological and biochemical basis of the dormancy-stress tolerance-yield relationship might enable negative aspects of this relationship to be altered (low yield-high dormancy), while retaining other positive features like stress tolerance. Recent genetic studies suggest that this may be possible (Brouwer et al., 2000; Brummer et al., 2000). Nevertheless, proof-of-concept awaits commercial release of a non-dormant cultivar with exceptional winter survival and superior drought tolerance.

REFERENCES

- Brouwer, D.J., S.H. Duke, and T.C. Osborn. 2000. Mapping genetic factors associated with winterhardiness, fall growth, and freezing injury in autotetraploid alfalfa. *Crop Sci.* 40:1387-1396.
- Brummer, E.C., M.M. Shah, and D. Luth. 2000. Reexamining the relationship between fall dormancy and winter hardiness in alfalfa. *Crop Sci.* 40:971-977.
- Brummer, E.C., K.J. Moore, and N.C. Bjork. 2002. Agronomic consequences of dormant-nondormant alfalfa mixtures. *Agron. J.* 94:782-785.
- Busbice, T.H., and C.P. Wilsie. 1965. Fall growth, winterhardiness, recovery after cutting and wilt resistance in F2 progenies of Vernal x Dupuits alfalfa crosses. *Crop Sci.* 5:429-432.
- Castonguay, Y., S. Laberge, E.C. Brummer, and J.J. Volenec. 2006. Alfalfa winter hardiness: A research retrospective and integrated perspective. *Adv. Agron.* 90:203-265.
- Chen, T.H.H., and F.S.C. Chen. 1988. Relations between photoperiod, temperature, abscisic acid, and fall dormancy in alfalfa (*Medicago sativa*). *Can. J. Bot.* 66:2491-2498.
- Collino, D.J., A.E. López, J. Dardanelli, R. Sereno, and R.W. Racca. 1997. Effect of soil water availability on water use strategies and dry matter production by two alfalfa cultivars differing in winter dormancy. *Phyton* 61:45-53.
- Corbineau, F., M. A. Picard, J.-A. Fougereux, F. Ladonne, and D. Côme. 2000. Effects of dehydration conditions on desiccation tolerance of developing pea seeds as related to oligosaccharide content and cell membrane properties. *Seed Sci. Res.* 10:329-339.
- Cunningham, S.M., and J.J. Volenec. 1996. Purification and characterization of vegetative storage proteins from alfalfa (*Medicago sativa* L.) taproots. *J. Plant Physiol.* 147:625-632.
- Cunningham, S.M., J.J. Volenec, and L.R. Teuber. 1998. Plant survival and root and bud composition of alfalfa populations selected for contrasting fall dormancy. *Crop Sci.* 38:962-969.
- Cunningham, S.M., J.A. Gana, J.J. Volenec, and L.R. Teuber. 2001. Winterhardiness, root physiology, and gene expression in successive fall dormancy selections from 'Mesilla' and 'CUF 101' alfalfa. *Crop Sci.* 41:1091-1098.
- Cunningham, S.M., P. Nadeau, Y. Castonguay, S. Laberge, and J.J. Volenec. 2003. Raffinose and stachyose accumulation, galactinol synthase expression, and winter injury of contrasting alfalfa germplasms. *Crop Sci* 43:562-570.
- Erice, G., J.J. Irigoyen, M. Sanchez-Diaz, J.C. Avice, and A. Ourry. 2007. Effect of drought, elevated CO₂ and temperature on accumulation of N and vegetative storage proteins (VSP) in taproot of nodulated alfalfa before and after cutting. *Plant Sci.* 172:903-912.
- Guitjens, J.C., and M.T. Goodrich. 1994. Dormancy and nondormancy alfalfa yield and evapotranspiration. *J. Irr. Drain. Eng.* 120:1140-1146.
- Haagenson, D.M., S.M. Cunningham, and J.J. Volenec. 2003a. Root physiology of less fall dormant, winter hardy alfalfa selections. *Crop Sci* 43:1441-1447.
- Haagenson, D.M., S.M. Cunningham, B.C. Joern, and J.J. Volenec. 2003b. Autumn defoliation effects on alfalfa winter survival, root physiology, and gene expression. *Crop Sci* 43:1340-1348.
- Hattendorf, M.J., D.W. Evans, and R.N. Peaden. 1990. Canopy temperature and stomatal conductance of water-stressed dormant and nondormant alfalfa types. *Agron. J.* 82:873-877.
- Hendershot, K.L., and J.J. Volenec. 1993. Taproot nitrogen accumulation and use in overwintering alfalfa (*Medicago sativa* L.) *J. Plant Physiol.* 141:68-74.
- Johnson, R.C., and L.L. Tiesen. 1994. Variation in water-use efficiency in alfalfa germplasm. *Crop Sci.* 34:452-458.

- Johnson, R.C., A.A. Hopkins, and M.A. Evans. 2008. Carbon isotope discrimination, selection response, and forage production of tall fescue in contrasting environments. *Crop Sci* 48:1048-1054.
- Janssen, G. 1929. The relationship of organic root reserves and other factors to the permanency of alfalfa stands. *Agron. J.* 21:895-911.
- Johnson, L.D., J.J. Marquez-Ortiz, J.F.S. Lamb, and D.K. Barnes. 1998. Root morphology of alfalfa plant introductions and cultivars. *Crop Sci.* 38:497-502.
- Juan, N.A., C.C. Sheaffer, and D.K. Barnes. 1994. Root and crown characteristics of alfalfas varying in fall dormancy. *Can. J. Plant Sci.* 74:125-127.
- Justes, E., P. Thiebeau, J-C. Avice, G. Lemaire, J.J. Volenec, and A. Ourry. 2002. Influence of summer sowing dates, N fertilization and irrigation on autumn VSP accumulation and dynamics of spring regrowth in alfalfa (*Medicago sativa* L.). *J. Exp. Bot.* 53:111-121.
- Kalengamaliro, N.E., J.J. Volenec, S.M. Cunningham, and B.C. Joern. 1997. Seedling development and deposition of starch and storage proteins in alfalfa roots. *Crop Sci.* 37:1194-1200.
- Kalengamaliro, N.E., J.A. Gana, S.M. Cunningham, and J.J. Volenec. 2000. Mechanisms regulating differential freezing tolerance of suspension cell cultures derived from contrasting alfalfa genotypes *Plant Cell Tiss. Organ Cult.* 61:143-151.
- Mark, J.J. 1936. The relation of reserves to cold resistance in alfalfa. Iowa State Univ., Agric. Exp. Stn., Botany Plant Path. Section, Farm Crops Subsection, Research Bull. 208. pp. 304-335.
- Metochis, C., and P.I. Orphanos. 1981. Alfalfa yield and water use when forced into dormancy by withholding water during summer. *Agron. J.* 73:1048-1050.
- Pembleton, K.G., J.J. Volenec, R.P. Rawnsley and D.J. Donaghy. 2009. Partitioning of taproot assimilates and crown bud development is affected by water deficit in regrowing alfalfa (*Medicago sativa* L.). *Crop Sci.* 49:(in press).
- Ray, .I.M., M.S. Townsend, and J.A. Henning. 1998. Variation for yield, water-use efficiency, and canopy morphology among nine alfalfa germplasms. *Crop Sci.* 38:1386-1390
- Schwab, P.M., D.K. Barnes, and C.C. Sheaffer. (1996). The relationship between field winter injury and fall growth score for 251 alfalfa cultivars. *Crop Sci.* 36:418-426.
- Shih, S.C., G.A. Jung, and D.C. Shelton. 1967. Effects of temperature and photoperiod on metabolic changes in alfalfa in relation to cold hardiness. *Crop Sci.* 7:385-389.
- Smith, D. 1961. Association of fall growth habit and winter survival in alfalfa. *Can. J. Plant Sci.* 41:244-251.
- Tysdal, H.M. 1933. Influence of light, temperature, and soil moisture on the hardening process in alfalfa. *J. Agric. Res.* 46:483-515.
- Urano, K, K. Maruyama, Y. Ogata, Y. Morishita, M. Takeda, N. Sakurai, H. Suzuki, K. Saito, D. Shibata, M. Kobayashi, K. Yamaguchi-Shinozaki, and K. Shinozaki. 2008. Characterization of the ABA-regulated global responses to dehydration in *Arabidopsis* by metabolomics *Plant J.* 57:1065-1078.
- Volenec, J.J. 1985. Leaf area expansion and shoot elongation of diverse alfalfa germplasms. *Crop Sci.* 25:822-827.
- Volenec, J.J. 1988. Herbage growth and carbohydrate metabolism of diploid and tetraploid alfalfa. *Crop Sci.* 28:128-132.
- Yu, X.M., and M. Griffith. 2001. Winter rye antifreeze activity increases in response to cold and drought, but not abscisic acid. *Physiol. Plant.* 112:78-86.