

# Winter damage to perennial forage crops in eastern Canada: Causes, mitigation, and prediction

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Bélanger, G., Castonguay, Y., Bertrand, A., Dhont, C., Rochette, P., Couture, L., Drapeau, R., Mongrain, D., Chalifour, F.-P. and Michaud, R. 2006. **Winter damage to perennial forage crops in eastern Canada: Causes, mitigation, and prediction.** *Can. J. Plant Sci.* **86**: 33–47. Harsh winter climate results in frequent losses of stands and yield reduction in many forage-growing areas of Canada and other parts of the world. Climatic conditions and crop management both affect the winter survival of perennial forage crops. In this review, we present the main causes of winter damage in eastern Canada and we discuss crop management practices that help mitigate the risks of losses. Predictive tools available to assess the risks of winter damage both spatially and temporally are also presented. Our understanding of the causes of winter damage and of the plant adaptation mechanisms to winter stresses, particularly the role of N and C organic reserves, has improved. Forage species commonly grown in eastern Canada differ in their tolerance to subfreezing temperatures and to anoxia caused by the presence of ice on fields. Some improvement in winter hardiness of forage legume species has been achieved through breeding in eastern Canada but new technologies based on laboratory freezing tests and the identification of molecular markers may facilitate the future development of winter-hardy cultivars. Crop management practices required for good winter survival are now better defined, particularly those involving cutting management and the interval between harvests. Simulation models and climatic indices derived from our current knowledge of the causes of winter damage provide general indications on the risk of winter damage but their degree of precision and accuracy is still not satisfactory. Further improvements in winter survival require a more thorough understanding of the different causes of winter damage and, primarily, of their complex interactions with genetic, climatic, and management factors.

**Key words:** Alfalfa, organic reserves, cultivars, species, management, climate

Bélanger, G., Castonguay, Y., Bertrand, A., Dhont, C., Rochette, P., Couture, L., Drapeau, R., Mongrain, D., Chalifour, F.-P. et Michaud, R. 2006. **Les dommages hivernaux aux cultures fourragères pérennes dans l'Est du Canada: causes, atténuation et prédiction.** *Can. J. Plant Sci.* **86**: 33–47. Le climat rigoureux de l'hiver entraîne des pertes fréquentes de peuplement et des diminutions de rendement dans plusieurs régions fourragères du Canada et ailleurs dans le monde. Les conditions climatiques et le mode d'exploitation des cultures affectent tous les deux la survie à l'hiver des cultures fourragères pérennes. Dans cette synthèse, nous présentons les principales causes des dommages hivernaux dans l'Est du Canada et nous discutons des modes d'exploitation des cultures qui permettent d'atténuer les risques de pertes. Les outils de prédiction disponibles pour évaluer les risques de dommages hivernaux dans l'espace et dans le temps sont aussi présentés. Notre compréhension des causes des dommages hivernaux et des mécanismes d'adaptation des plantes aux stress hivernaux, particulièrement le rôle des réserves organiques en C et en N, s'est améliorée. Les espèces fourragères couramment cultivées dans l'Est du Canada diffèrent pour leur tolérance aux températures froides de l'hiver et pour l'anoxie causée par la présence de glace dans les champs. Des améliorations de la résistance à l'hiver des espèces de légumineuses fourragères ont été obtenues par sélection génétique dans l'Est du Canada mais les nouvelles technologies, basées sur des tests de congélation en laboratoire et l'identification de marqueurs moléculaires, devraient faciliter le développement futur de cultivars résistants à l'hiver. Les modes d'exploitation des cultures pour assurer une meilleure survie hivernale sont maintenant mieux définis, particulièrement ceux impliquant la gestion de la fauche et l'intervalle entre les récoltes. Les modèles de simulation et les indices climatiques, dérivés de nos connaissances des causes de dommages hivernaux, donnent des indications générales des risques de dommages hivernaux mais leur degré de précision et d'exactitude n'est toujours pas satisfaisant. Les améliorations futures de cultivars, de modes d'exploitation et de prédiction des risques viendront d'une meilleure compréhension des différentes causes des dommages hivernaux et, principalement, de leurs interactions complexes avec les facteurs génétiques, climatiques et de mode d'exploitation.

**Mots clés:** Luzerne, réserves organiques, cultivars, espèces, mode d'exploitation, climate

Perennial forage crops are grown on more than 2.1 million ha in eastern Canada (Ontario, Québec, and the Atlantic provinces), which represent about 40% of the cultivated land (Statistics Canada 2001) and an annual estimated farm value of Can\$1.3 billion. They are a key element of the current transition towards more sustainable agricultural sys-

tems and, consequently, there is a need to improve our understanding of how environmental stresses limit their pro-

**Abbreviations:** BSA, bulk segregant analysis; DM, dry matter; GDD, growing degree-days; TNC, total non-structural carbohydrates; VSP, vegetative storage proteins

ductivity and long-term persistence (Sanderson et al. 1997). Harsh winter climatic conditions are a major constraint for perennial crops and have historically been the cause of frequent losses of stands and yield reduction in many forage-growing areas of Canada (Suzuki 1972; Ouellet 1976) and other parts of the world. For example, a small indemnity program of the Québec Crops Insurance Board covering winter damage in pure stands of alfalfa paid average yearly compensations of Can\$1.2 million during the period 1985 to 1999 (S. Pion, Québec Crops Insurance Board, personal communication, 2001). In addition, it is estimated that each year from 1990 to 1999, approximately 30% of insured forage crop areas in Québec incurred significant losses due to winter damage.

Climatic conditions influence winter survival of perennial crops directly by setting the intensity of the environmental stress and indirectly by modulating plant hardiness. Several studies conducted under both natural and controlled conditions have provided information on the effects of environmental factors on survival of winter-sensitive forage species (McKenzie and McLean 1980a,b; Paquin and Pelletier 1980; Paquin and Mehuys 1980; Paquin 1985). Correlation studies have also been used to determine on a larger geographical scale the links between climate variables and winter damage (Ouellet 1976, 1977; Ouellet and Desjardins 1981). These studies of plant-climate interactions provided key information for the determination of optimal climatic conditions for winter survival. This knowledge was also used in the development of agroclimatic indices calculated from readily available climate variables such as air temperature and precipitation (Rochette and Dubé 1993a,b; Bélanger et al. 2002a) and in modelling winter hardiness (Kanneganti et al. 1998a,b).

In this review, we present the main causes of winter damage, and we discuss crop management practices that help mitigate the risks of losses. We also present some of the predictive tools available to assess the risks of winter damage both spatially and temporally. This review focuses primarily on commercially important winter-sensitive species like alfalfa (*Medicago sativa* L.) and relies largely on research conducted in eastern Canada.

### CAUSES OF WINTER DAMAGE

The winter hardiness of perennial herbaceous species is determined by their ability to tolerate a wide range of environmental stresses such as subfreezing and fluctuating temperatures, excess soil moisture, ice encasement, heaving, and low temperature pathogens (Andrews 1987).

#### Exposure to Subfreezing Temperatures

The capacity to tolerate subfreezing temperatures is probably the single most important factor that influences plant winter hardiness across a wide range of environments. The impact of numerous environmental stresses that cause winter damage is often indirectly the result of their effect on the capacity of the plants to tolerate subfreezing temperatures. For example, unacclimated alfalfa will not tolerate a mild freeze of around  $-5^{\circ}\text{C}$  during the summer but it will withstand winter temperatures of  $-15$  to  $-20^{\circ}\text{C}$  after a fall accli-

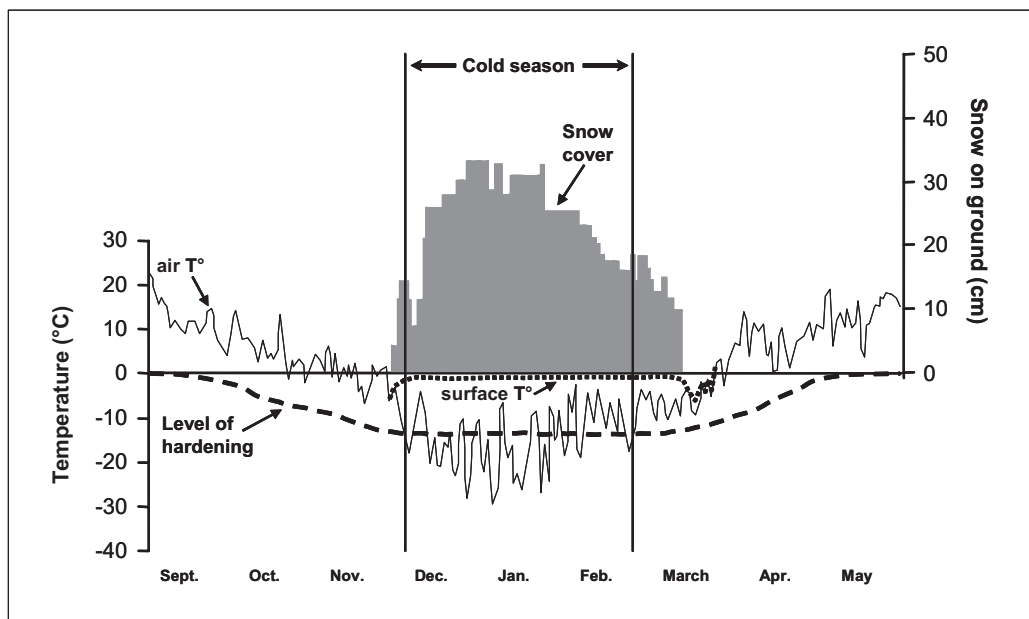
mation period (McKenzie et al. 1988). This increase in freezing tolerance occurs progressively during fall in response to environmental changes (Fig. 1). Growth reduction in late summer or early fall triggered by a shorter photoperiod and lower temperatures is generally recognized as the initial stage of winter hardening in alfalfa (Hodgson 1964; McKenzie et al. 1988; Stout and Hall 1989). The acquisition of freezing tolerance accelerates upon exposure to non-freezing temperatures below  $5^{\circ}\text{C}$  (Paquin and Pelletier 1980). Field observations (Paquin 1984), later confirmed by experiments conducted under controlled conditions (Castonguay et al. 1993), revealed that hardening of alfalfa continues under snow cover and that maximum freezing tolerance is achieved in early winter after the soil has frozen.

A high level of cold hardiness is maintained in fully acclimated alfalfa plants if crown temperature remains below freezing and if adequate levels of organic reserves are maintained (McKenzie and McLean 1980b). Conversely, exposure to temperatures above  $0^{\circ}\text{C}$  in winter can cause a loss of cold hardiness (Sakai and Larcher 1987) and thus increase the susceptibility to injury by subsequent exposure to low subfreezing temperatures (Ouellet and Desjardins 1981; Suzuki 1981). Such dehardening occurs at a much faster rate than hardening, and winter survival can be affected by exposure to even short periods of warm temperatures during winter (Eagles et al. 1997). There is evidence that a photoperiodic control of dehardening occurs in some species. Eagles et al. (1997) reported that a cold-hardened cultivar of timothy (*Phleum pratense* L.) adapted to northern Norway maintained a high level of hardiness when exposed to  $10^{\circ}\text{C}$  under a short photoperiod (8 h), whereas it dehardened significantly at temperatures above  $4^{\circ}\text{C}$  under a long photoperiod (16 h). Such photoperiodic regulation of dehardening could explain anecdotal and non-documented observations that temperatures above  $0^{\circ}\text{C}$  have less impact on the loss of winter hardiness when they occur early in winter. Photoperiodic control of dehardening has not yet been tested on alfalfa or on other winter-sensitive species grown in Canada and it deserves a closer scrutiny in the perspective of warmer winters under predicted climate change (Bélanger et al. 2002a).

In eastern Canada, air temperature frequently drops below  $-15^{\circ}\text{C}$ , the potential  $\text{LT}_{50}$  (temperature that causes 50% mortality of a population) of field-grown alfalfa exposed to optimal hardening conditions (Paquin and Mehuys 1980), with minimum temperatures ranging from  $-20$  to  $-44^{\circ}\text{C}$ . Therefore, winter survival of perennial forage crops depends on insulation of roots and crown buds by snow cover (Fig. 1). Snow provides excellent insulation against variations in air temperature. With little snow cover, temperature at the crown level remains close to air temperature (Baadshaug 1973). A snow cover of 0.1 m is considered sufficient to maintain the temperature at the crown level around the freezing point (Leep et al. 2001).

#### Soil Moisture and Ice

Wet soils will prevent plants from reaching their full hardening potential in the fall (Calder et al. 1965; Suzuki et al.



**Fig. 1.** Graphical representation of the ideal scenario for the winter survival of perennial forage crops under the conditions of eastern Canada. This scenario mainly takes into account air temperature and snow insulation throughout the overwintering period in relation to cold hardening and dehardening.

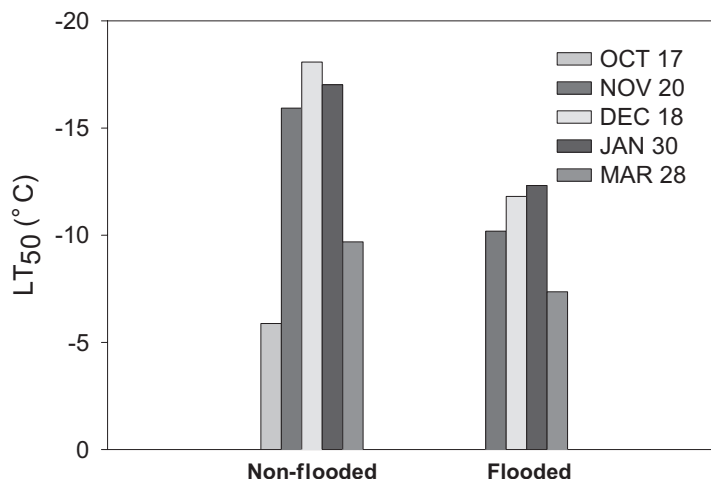
1975; Suzuki 1977; Paquin and Mehuys 1980; McKenzie and McLean 1980b). Olién (1984) presented evidence that reversible changes in the concentrations of intercellular solutes during freezing could help prevent adhesion of cells to extracellular ice and the lethal distortions of tissues. Increased tissue water content in plants exposed to excess soil humidity could reduce the concentration of intercellular solutes and lessen their protective effect. The amount of rain in fall, which directly affects soil moisture (low evapotranspiration), is negatively correlated with winter survival (Ouellet and Desjardins 1981). Rainfall is therefore a useful index reflecting soil moisture during acquisition of cold hardiness (Bélangier et al. 2002a). The acquisition of cold hardiness can also be affected by low soil water content (Ouellet and Desjardins 1981). Dry conditions in early fall may decrease the accumulation of organic reserves important for cold tolerance. Later in the fall, however, dry conditions may promote fall hardening. Work by Cloutier and Siminovitch (1982) showed that limited desiccation can induce freezing tolerance in winter cereal seedlings at non-acclimating temperatures. Fowler et al. (1981) also concluded that crown moisture content was inversely related to the field survival of winter wheat. This is likely due to the fact that tolerance to both freezing and dehydration is based, in part, on common adaptive traits allowing cells to withstand extensive desiccation (Xin and Browse 2000).

A remote sensing survey of alfalfa winterkill by Paquin et al. (1977) confirmed that the incidence of damage was much higher in field depressions and in poorly drained soils. Reduction in cold tolerance in plants acclimated under excess soil moisture (Fig. 2; Paquin and Mehuys 1980;

Paquin et al. 1987) increases the risks of winterkill, especially when the insulation provided by the snow cover is insufficient.

Rainfall or snow melt during winter may induce ice-sheet formation at the soil surface and ice encasement of plants (Gudleifsson 1993). Ice restricts gas exchange between soil and the atmosphere, and oxygen consumption by soil microbes and overwintering plants can result in anoxic conditions. The switch to anaerobic respiration accelerates the use of plant carbon reserves, reduces substrates for respiration and regrowth, and results in the accumulation of potentially phytotoxic end products such as ethanol and lactic acid (Andrews 1996; Bertrand et al. 2001). Damage to ice-encased plants could be attributable to exposure to a high  $\text{CO}_2$  concentration or a combination of a high  $\text{CO}_2$  concentration with ethanol and lactic acid (Andrews and Pomeroy 1989). Early work by Freyman and Brink (1967) indicated that the accumulation of  $\text{CO}_2$  was more damaging to ice-encased alfalfa than the reduction of  $\text{O}_2$ .

Ice encasement may physically damage plants. Physical damage to the roots can also be caused by soil heaving, primarily in imperfectly drained soils exposed to frequent freeze-thaw cycles (Holmes and Robertson 1960; Russell et al. 1978). Significant losses of alfalfa stands due to frost heaving frequently occur in the Atlantic provinces of Canada (Grant and Saini 1973) and in the southern areas of Québec (Pesant et al. 1978). In addition, ice sheets without snow cover can also result in the exposure of roots and crowns to potentially damaging temperatures (Paquin 1984). The presence of snow, however, can prevent this effect from occurring.



**Fig. 2.** The effect of excess soil moisture on freezing tolerance expressed as the lethal temperature for 50% of the plants ( $LT_{50}$ ). Pot-grown plants of the cultivar AC Caribou were flooded during a 2-wk period in early fall and were subsequently drained and kept saturated throughout fall and winter. Control plants (non-flooded) were kept under well-watered conditions during the same period. Both groups of plants were acclimated to natural fall and winter conditions in an unheated and ventilated greenhouse located at a site near Québec City. Data from Y. Castonguay and P. Nadeau (unpublished).

## Diseases

Perennial forage legumes and grasses are prone to a number of diseases. Winter survival is likely affected by the severity of diseases, especially at the crown and root level, because of a reduction in organic reserves. For instance, *Fusarium*-infected alfalfa roots accumulated less soluble sugars than non-infected plants (Richard and Martin 1993). In short-lived red clover (*Trifolium pratense* L.), the first winter produced conditions conducive to root infection during the ensuing growing season that resulted in significant plant losses in the second winter (Gagnon 1979).

An analysis of disease resistance ratings obtained from cultivar performance trials in the United States revealed that levels of disease incidence having a significant impact on persistence are less widespread than generally believed, and that factors other than diseases must contribute to genetic differences in alfalfa stand decline (Volenc et al. 2002). However, the six diseases in their study did not include *Fusarium* crown and root rot and *Sclerotinia* crown and stem rot, two common diseases in eastern Canada. For example, in a study in eastern Canada, Richard et al. (1982) reported that *Fusarium*-infected plants did not withstand freezing as well as healthy plants. It is also well established that infection reduces the longevity of individual plants, so that the stand becomes progressively less productive (Gossen 2003).

Management practices that affect plant reserves, such as intensive harvesting, are likely to influence the level of infection by root pathogens. Assessment of regrowth after cutting, performed in both sterile and non-sterile media, suggests that frequently clipped alfalfa plants die from both reserve depletion and infection (Lukezic et al. 1969). In field-grown alfalfa, Couture et al. (2002) reported that a fall harvest increased the severity of *Fusarium* root rot, especially when the regrowth interval between the last summer harvest and the fall harvest was short.

Snow moulds are the predominant disease organisms infecting forages during winter. The various snow moulds occurring in Canada have been recently reviewed by Gaudet and Gossen (2003). These pathogens are adapted to grow at low temperatures and are promoted by snow cover. In stands severely infected by snow moulds, plants are killed when there is insufficient living tissue remaining in the crown. When the infection is less severe, spring regrowth is delayed. The incidence of snow moulds is higher in south-western Ontario than in other areas of eastern Canada. While snow moulds are better known in winter cereals and turf, all perennial forage crops are thought to be susceptible to at least some species of snow mould, although limited data are available on their incidence. In a survey of the snow moulds of winter cereals in Québec, four species of snow moulds were detected across the province (Pouleur and Couture 1988). The single sample of orchardgrass (*Dactylis glomerata* L.) collected in this survey was found to be affected by two species. The prevalence of snow mould diseases in forage grasses is likely similar to the one occurring in cereals as the pathogens are the same (Couture et al. 2003). In forage legumes, we suspect *Sclerotinia* crown and stem rot (*Sclerotinia trifoliorum* Eriks.) to be on the increase in eastern Canada.

## MITIGATING THE RISKS OF WINTER DAMAGE

The choice of species, cultivars, and crop management practices during the entire growing season, but primarily in the fall, affect the risk of winter damage to perennial crops.

### Species and Cultivars

Forage species differ in their ability to withstand winter stresses. Some alfalfa cultivars can tolerate temperatures at the crown level of  $-20$  to  $-26^{\circ}\text{C}$  for a few hours but will be damaged when exposed to temperatures of  $-8$  to  $-10^{\circ}\text{C}$  for

**Table 1. Relative shoot regrowth, relative total non-structural carbohydrates (TNC), and ethanol concentration in shoot bases and crowns of oxygen deficiency-treated plants of four forage species following 100 d of treatment. Relative shoot regrowth and relative total non-structural carbohydrates (TNC) were calculated as values from oxygen deficiency-treated plants divided by values from plants maintained under normal atmospheric conditions. Adapted from Bertrand et al. (2001, 2003)**

	Timothy	Orchardgrass	Alfalfa	Red Clover
Relative shoot regrowth	0.69	0.49	0.30	0.04
Relative TNC	1.24	0.95	0.46	0.62
Ethanol (mg g <sup>-1</sup> DM)	3.07	11.04	10.41	14.28

a few days (Paquin 1984). Timothy, however, can tolerate temperatures as low as  $-30^{\circ}\text{C}$  (Paquin 1984). In one of the most severe plant winterkill events in the history of Prince Edward Island attributed to a prolonged exposure to sub-freezing soil temperatures, red clover sustained the greatest losses, followed by alfalfa and orchardgrass (Suzuki 1972). Birdsfoot trefoil (*Lotus corniculatus* L.) and smooth brome grass (*Bromus inermis* Leyss.) suffered less damage, while timothy remained relatively unaffected. Species differences in the rates of dehardening were also reported with alfalfa losing its cold tolerance faster than timothy but more slowly than smooth brome grass (Paquin 1984).

Forage species also differ in their tolerance to anoxia caused by the presence of ice on fields. In a study conducted under simulated winter conditions, Bertrand et al. (2001) observed a differential sensitivity of forage species to long-term exposure to anoxic conditions with the following ranking in spring regrowth reduction: red clover > alfalfa > orchardgrass > timothy (Table 1). A greater tolerance of timothy to anoxia relative to other grass species was also reported by Gudleifsson et al. (1986). Bertrand et al. (2003) discovered that timothy delays the development of anoxic conditions by maintaining a relatively slow metabolic rate during winter. This attribute allows timothy to both avoid high  $\text{CO}_2$  concentration and the accumulation of ethanol in plant tissues and to maintain high carbohydrate reserves (Table 1).

Few studies have been conducted on the winter survival of legume species when grown in a mixture with one or several grass species. Belzile (1987) reported that the presence of timothy can improve red clover persistence. When the winter was unfavourable to legumes, the red clover population the following spring was higher in populations mixed with timothy than in a pure clover population. On the other hand, when alfalfa was grown with an aggressive grass species such as smooth brome grass, only the hardy cultivars with substantial fall dormancy and lower growth potential persisted well under Saskatchewan conditions (Bittman et al. 1991). The presence of one or more grass species along with alfalfa in the sward is therefore likely to affect the persistence of the legume species either through an effect on the winter survival of the legume species or through interspecies competition modulated by the relative winter hardiness of the legume and grass species or cultivar.

Extensive variability for tolerance to winter stresses has been reported within some forage species. Winter survival of ecotypes of white clover (*Trifolium repens* L.) originating from eastern Canada and tested in the same region ranged

from 0 to 90% (Caradus and Christie 1998). Differences in cold tolerance among 23 alfalfa entries were observed in laboratory freezing tests (Schwab et al. 1996). Genetic variability for tolerance to three types of winter stresses (freezing, low temperature flooding, and ice encasement) was observed among 16 cultivars of alfalfa (Bowley and McKersie 1990). Differential ranking obtained with each stress indicated that tolerance to one winter stress is not necessarily associated to tolerance to other stresses. Gudleifsson et al. (1986) also reported a limited association between cold tolerance and the tolerance to anoxic conditions among several grass species. This led them to conclude that, in areas where both low temperature and ice encasement stresses are prevalent, the tolerance to both cold and anoxia should be tested when comparing species, cultivars, or breeding lines.

Some improvements in winter hardiness of legume species have been achieved through plant breeding in eastern Canada. The alfalfa cultivar Apica selected for local adaptation had 10–20% better persistence than the cultivars Iroquois and Saranac while having similar DM yields (Michaud et al. 1983). The red clover cultivar AC Charlie performed better than the check cultivars in seasons following severe winterkilling (Choo et al. 1994). Those are only two examples of cultivars successfully developed to withstand eastern Canadian winters. In contrast, Volenec et al. (2002) recently concluded that efforts in the United States to improve alfalfa persistence by breeding for greater winter hardiness have not been effective. The introgression of superior winter stress tolerance in high-yielding cultivars is difficult because of the complexity of the trait. From those observations, Volenec et al. (2002) concluded that variability for winter stress tolerance remains largely untapped by alfalfa breeding programs.

The dormancy response during cold acclimation is a survival strategy in which plant growth in fall and spring is sacrificed in favour of persistence. This, however, represents an undesirable trait from a forage yield standpoint, and breeders have historically tried to reach a compromise between an extended growing season and long-term persistence. The extent to which winter hardiness can be dissociated from the short-day-induced decline in forage growth is still unclear. There are indications that winter hardiness can be improved within populations having intermediate or low levels of fall dormancy without concurrent reduction in seasonal growth (Bowley and McKersie 1990; McCaslin et al. 1990; Brummer et al. 2000; Weishaar et al. 2005). Progress, however, will require a better understanding of the mechanisms

involved in the relationship between dormancy, winter survival, and plant growth at different levels of plant organization, and of the genetic control of those mechanisms (Cunningham et al. 2001).

Progress is also impeded by the lack of a rapid and accurate means of assessing winter hardiness potential and the reliance on the unpredictable occurrence of test winters that selectively discriminate between sensitive and tolerant genotypes (McKenzie and McLean 1984). In addition, the uniqueness of each winter in terms of the nature of the abiotic stress makes it difficult both for the evaluation of progress and the genetic selection.

New technologies based on laboratory freezing tests (Nadeau et al. 2002) and the identification of molecular markers (Castonguay et al. 2005) may provide useful alternatives to field tests. In the past 10 to 15 yr, molecular and genetic studies have contributed important information on the nature of key adaptations for survival at low temperatures [see reviews by Castonguay et al. (1997) and Volenec et al. (2002)]. For instance, a close relationship between the capacity of alfalfa cultivars to accumulate the cryoprotective oligosaccharides stachyose and raffinose and freezing tolerance has been shown (Castonguay and Nadeau 1998; Cunningham et al. 2003). These soluble sugars are thought to play a role in helping stabilize membranes and proteins during freeze-induced desiccation. It has also been shown that low temperatures induce extensive changes in gene expression. Some of the cold-induced genes were more highly or specifically expressed in cold-tolerant than in cold-sensitive cultivars (Castonguay et al. 1993; Monroy et al. 1993) and their expression has been positively associated with winter survival under field conditions (Cunningham et al. 2001). The quantification of gene transcripts or metabolites closely associated with the winter hardiness potential has been proposed as an approach for the identification of genotypes better adapted to cold (Castonguay et al. 1998; Cunningham et al. 2001). However, large variations due to environmental interactions and difficulties in scaling up analytical procedures constitute practical limitations to the development of these applications.

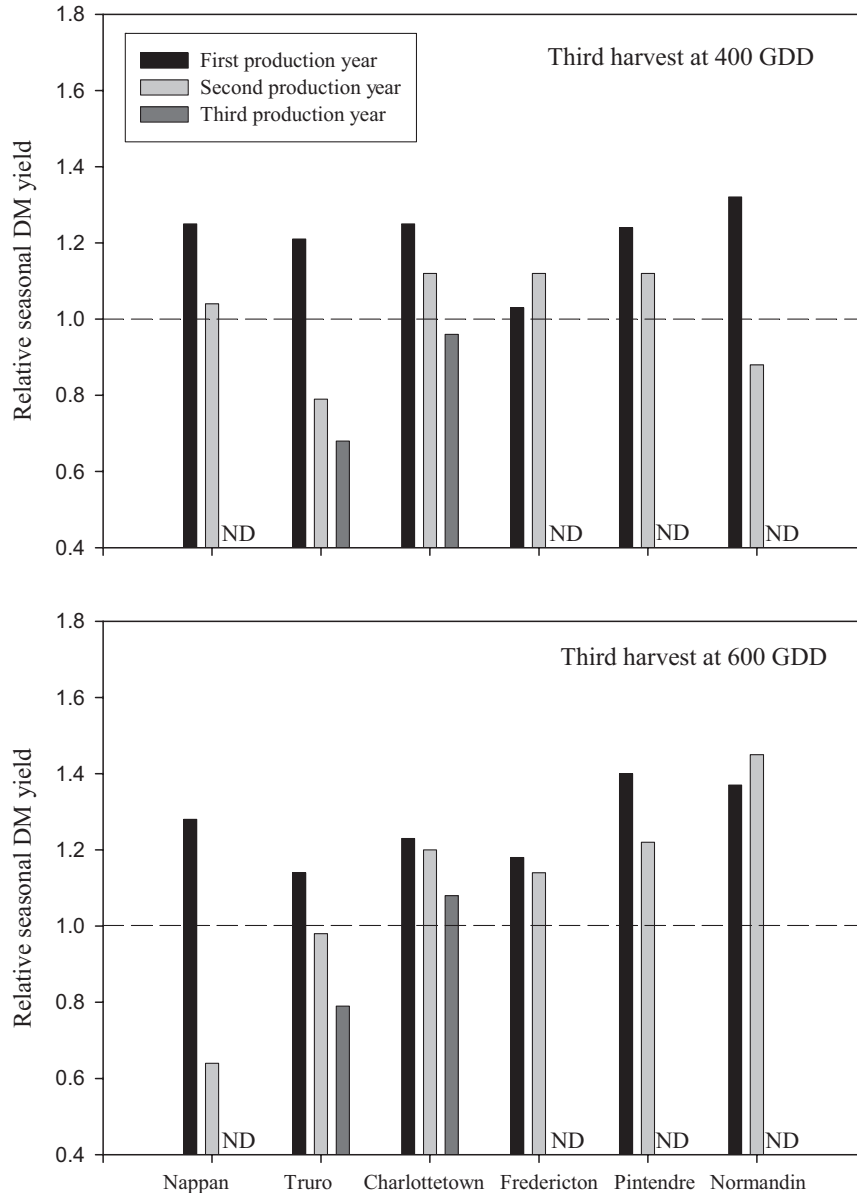
The identification of neutral genetic markers associated with superior adaptation to cold would provide a more robust approach for selection. The search for such genetic markers has been mostly based on the linkage association between genetic polymorphisms and the inheritance of traits of interest in segregating populations, a process known as quantitative trait locus (QTL) analysis. However, genotyping of large mapping populations is a tedious and costly process, and the QTL analysis is not easily applicable to outbred species with complex genomes. An effective alternative, the bulk segregant analysis (BSA), has recently been developed for the identification of genes with major effects on QTL (Michelmore et al. 1991). The BSA approach allows DNA pools from populations selectively improved for a trait of interest to be tested for differences in frequency for candidate genes with a high probability of being associated with that trait (Quarrie et al. 1999). The BSA has recently been used to identify genes associated with superior adaptation to cold in alfalfa populations selectively improved for their freezing tol-

erance (Castonguay et al. 2004). The identification of candidate genes associated with improved freezing tolerance in alfalfa could be an important step towards the future utilization of DNA markers as selection tools in breeding programs. Marker-assisted selection could advantageously complement conventional breeding approaches for the improvement of complex traits like cold hardiness.

### Cutting Management

Fall cutting management of alfalfa in Canada and adjacent areas of the United States has long been centred around a critical fall rest period; 4–6 wk of non-harvested growth preceding the expected date of the first killing frost (Langille et al. 1965; Calder and McLeod 1966; MacLeod et al. 1972; Gervais and Bilodeau 1985). However, this concept of critical fall rest period based on calendar dates has been questioned by several authors (Tesar and Yager 1985; Sheaffer et al. 1986; Bélanger et al. 1992). It was suggested that the regrowth interval between the fall harvest and the preceding one is a better determinant of winter survival and spring regrowth than calendar dates (Sheaffer et al. 1986; Bélanger et al. 1992). Although the impact of a fall harvest on seasonal DM yield is advantageous as long as an interval of 500 or 600 growing degree-days (GDD, 5°C basis) is kept between the last summer harvest and the fall harvest, the yield benefits from a fall harvest can be relatively short-term, particularly if plants are subsequently exposed to harsh winter conditions (Bélanger et al. 1999; Dhont et al. 2004). In studies conducted in eastern Canada, major winter damage to alfalfa occurred at three out of six sites in either the second or third production year when a fall harvest had been taken (Bélanger et al. 1999; Dhont et al. 2004). Even in winters with no major plant mortality, the spring regrowth may be reduced if a fall harvest was taken the previous fall. At most of the six sites in the above studies, the yield benefits from a third harvest taken in the fall, relative to a two-harvest system with no fall harvest, declined with time (Fig. 3). The authors concluded that, in eastern Canada, harvesting alfalfa in September and October increases the risk of winter damage and decreases the regrowth during the following spring. Bélanger et al. (1999) proposed that the fall cutting management of alfalfa should be based on the following scale from low to high risk: 1, No fall harvest; 2, A fall harvest taken 600 GDD after the last summer harvest; 3, A fall harvest taken 500 GDD after the last summer harvest; 4, A fall harvest taken 400 GDD after the last summer harvest. More recent research conducted in Québec confirmed the appropriateness of this risk scale based on the regrowth interval between the last two harvests (Dhont et al. 2004).

The response to fall cutting management may also depend on cultivars. In the United States, a fall harvest resulted in greater winter injury in semi-dormant and non-dormant alfalfa cultivars than in fall dormant cultivars (Volenec et al. 2002). In a study on the effects of a fall harvest conducted in Atlantic Canada, two alfalfa cultivars with the same dormancy rating (Oneida VR and Apica) responded differently to a severe winter (Bélanger et al. 1999). In a recent study in Québec, however, two cultivars (WL 225 and AC Caribou) in the same dormancy group responded similarly to fall harvest treatments (Dhont et al. 2004).



**Fig. 3.** Relative seasonal DM yield of alfalfa (DM yield with three harvests per year with the third harvest in the fall divided by the DM yield with only two harvests) in the first, second, or third production year of alfalfa harvested a third time in the fall at either 400 or 600 GDD after the second harvest, at six sites in eastern Canada. ND, not determined in the third production year. Adapted from Bélanger et al. (1999) and Dhont et al. (2004).

The effect of fall cutting management on persistence and the spring regrowth potential has not been extensively assessed in other perennial forage species. Most studies conducted on other forage species have focussed on the regrowth interval and the number of harvests during spring and summer. In the northern agricultural areas of eastern Canada (< 1400 growing degree-days, 5°C basis), three harvests before September did not affect the persistence of orchardgrass, a winter-sensitive forage grass species, if the second harvest was taken before July 20 (Drapeau 1999). In the same region, intensive harvest management (three and

five harvests per year) reduced tall fescue (*Festuca arundinacea* Schreb.) seasonal DM yield in the second and third production years compared with a less-intensive harvest management (two harvests per year); this observation suggests a potential negative impact on persistence (Drapeau et al. 2005). McKenzie et al. (2004) concluded that harvesting birdsfoot trefoil more than once per year in Atlantic Canada significantly reduced stand density. Most studies have shown a limited impact of harvest management on timothy persistence (Kunelius et al. 1974, 1976; Kunelius and McRae 1986).

A fall harvest and the height at which it is taken may affect the depth of snow and consequently, the temperatures to which the plants will be exposed during the winter and the likelihood of plant survival (McKenzie et al. 1988). Along with affecting snow cover, fall cutting management also changes the biochemical composition of the plants with a potential impact on winter survival and spring regrowth.

### Organic Reserves

The capacity of perennial forages to withstand winter and to resume growth in spring relies on the availability of endogenous carbon (C) and nitrogen (N) reserves stored in vegetative organs during the cold acclimation period in the preceding fall. The negative effect of fall harvests on alfalfa persistence and yield has historically been attributed to a decrease in total non-structural carbohydrates (TNC) concentrations in taproots (Graber et al. 1927; Reynolds 1971). In a 3-yr study conducted in Québec, a fall harvest reduced the TNC concentrations of alfalfa taproots and alfalfa productivity (Gervais and Girard 1987). Several other field observations, however, indicated that root TNC concentrations in fall and winter were not reduced by fall harvests, and were poorly related with yields in the following spring (Edminsten and Wolf 1988; Sheaffer et al. 1988; Brink and Marten 1989). More recent research even showed an increase in TNC concentrations ( $\text{g kg}^{-1}$  DM) of taproots of alfalfa harvested in the fall (Dhont et al. 2002; Haagensohn et al. 2003). Dhont et al. (2002, 2003), however, observed that the increase in total amounts ( $\text{g plant}^{-1}$ ) of TNC and N reserves in alfalfa taproots during fall was strongly limited by a fall harvest, especially with shorter regrowth intervals between the last two harvests (Fig. 4). They concluded that total amounts of TNC and N reserves in roots are better determinant factors of the shoot regrowth of alfalfa than their concentrations.

In forage legumes, N reserves stored as amino acids or vegetative storage proteins (VSPs) are extensively mobilized for the initial growth of new shoots in the spring and were shown to be closely related to shoot biomass production (Ourry et al. 1994; Volenec et al. 1996; Avice et al. 1997; Dhont et al. 2003). The much larger pool of C reserves is used mainly to sustain respiration of perennial tissues at the beginning of the regrowth period (Avice et al. 1996). Circumstantial evidence suggests that VSPs may also help confer protection against environmental stresses during fall and winter (Volenec et al. 2002). For instance, a 32-kDa alfalfa VSP possesses high homology with class III chitinases involved in plant defence against pathogens while a 17.3-kDa VSP from white clover has a high content of neutral and hydrophilic amino acids characteristic of numerous stress-induced proteins (Avice et al. 2003). Chitinases also possess antifreeze properties and could play a role in plant adaptation to cold (Yeh et al. 2000). Combination of daylength reduction and lowering temperatures resulted in a preferential allocation of N to roots in alfalfa (Noquet et al. 2001). However, the accumulation of a specific VSP of 32 kDa during the fall was found to be due mainly to the reduction of photoperiod, with cold temperatures having a negative impact on its deposition in storage organs (Avice et al. 2003). It is well established that the deposition of C and N reserves in the fall occurs in response to

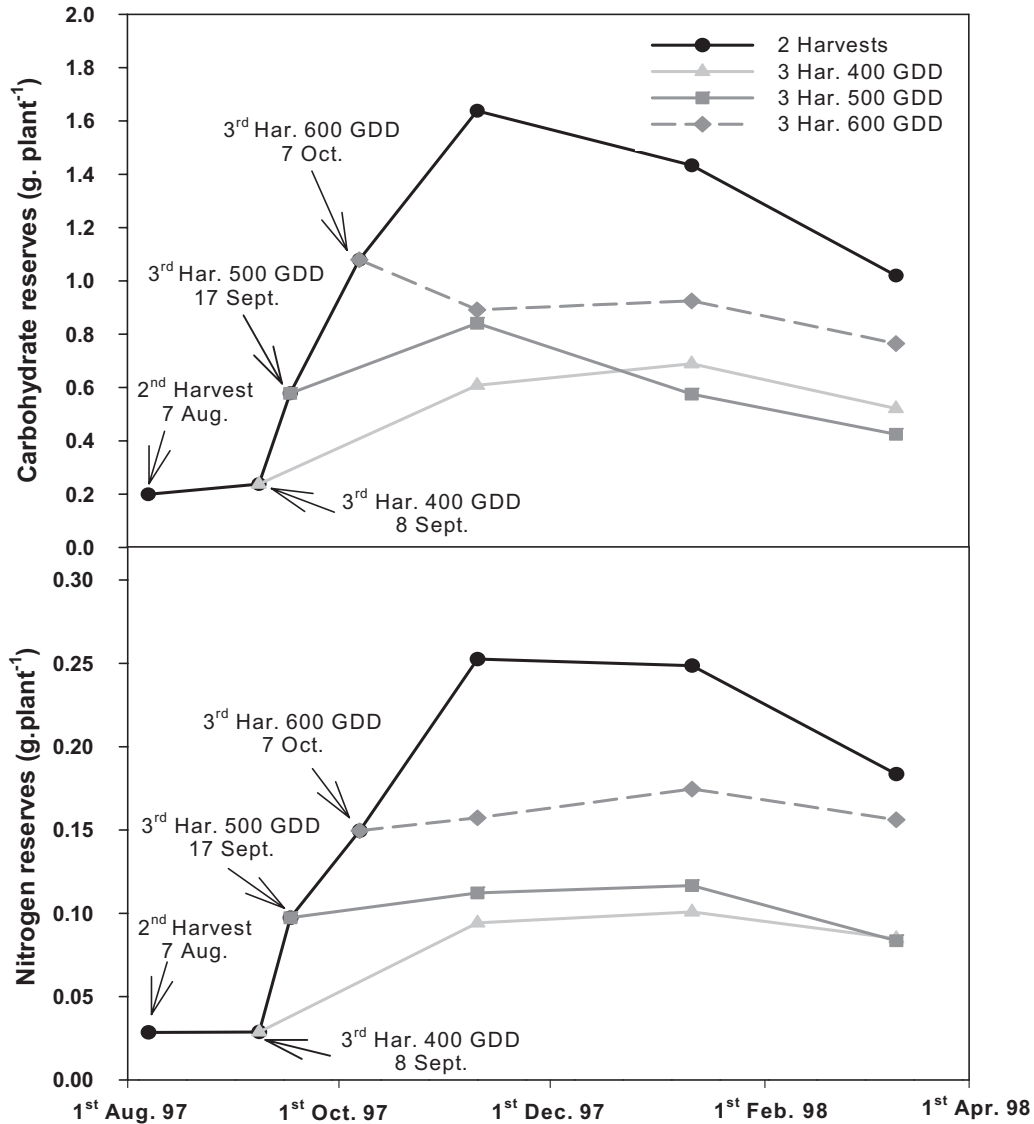
changes in photoperiod and temperature, but the impact of the interaction between these environmental conditions and fall cutting management on the accumulation of C and N reserves is still poorly understood. Therefore, predicting the levels of C and N reserves available for winter survival and regrowth remains difficult.

Alfalfa persistence is not only affected by the levels of root organic reserves required to sustain metabolism in overwintering plants and mobilized for spring regrowth, but also depends on the capacity of the plants to alter their biochemical composition and accumulate known cryoprotectants that include soluble sugars (Castonguay and Nadeau 1998), proline (Paquin 1985), and cold-regulated proteins (Castonguay et al. 1997). Extensive conversion of starch reserves into soluble sugars during the cold acclimation period is well documented in alfalfa (McKenzie et al. 1988). Differences in field survival among alfalfa cultivars of contrasting winterhardiness were closely related to the accumulation of the low-abundance galactose-containing oligosaccharides, raffinose and stachyose (Castonguay and Nadeau 1998; Cunningham et al. 2003). Recent observations from a study of fall cutting management suggest that fall harvests do not reduce the accumulation of cryoprotective sugars (Dhont et al. 2002, 2004). However, fall harvests have a major impact on the accumulation of the stress-related VSP of 32kDa and amino acids such as proline, arginine, and histidine during cold acclimation (Dhont et al. 2003). It has been suggested that these free amino acids play an important osmoregulatory role in response to abiotic stresses (Rai 2002) while contributing N for the regrowth in the spring (Hendershot and Volenec 1993).

### Soil Moisture and Fertility

Adequate surface and subsurface drainage is required when growing winter-sensitive species like alfalfa and red clover. Excessive water in alfalfa fields decreases the level of cold tolerance, increases the extent of soil freezing, and promotes the formation of ice in the soil (McKenzie et al. 1988). The influence of soil nutrients on cold tolerance and winter survival is not well understood (McKenzie et al. 1988). Very little research on the role of K and P fertilization on winter survival of alfalfa has been conducted over the past 20 yr. The importance of both K and P fertilization for the cold resistance of alfalfa was first recognized by Jung and Smith (1959); they concluded that plant survival following exposure to freezing temperatures was good if K and P were present at a ratio of 5 to 2. High levels of K in the soil can also reduce the stress induced by harvesting alfalfa in the fall (Sheaffer et al. 1988). However, in a soil with a high level of exchangeable K, increasing annual K fertilization from 200 to 400  $\text{kg K ha}^{-1} \text{ yr}^{-1}$  did not affect DM yield or persistence of alfalfa under any fall cutting management in Atlantic Canada (Bélanger et al. 1992).

More recently, research has shown the importance of the balance of P and K fertilization on the carbohydrate and protein metabolism of alfalfa during fall acclimation. In a field trial performed in Indiana, Berg et al. (2005) noted that a fall application of K increased root starch concentrations and decreased soluble sugar concentrations in taproots of alfalfa.



**Fig. 4.** Carbohydrate and nitrogen reserves in taproots of alfalfa harvested either only twice during the summer, or three times with the third harvest taken in the fall 400, 500, or 600 growing degree-days (GDD, 5°C) after the second harvest. Plants were first grown under controlled conditions; following acclimation under natural hardening conditions, they overwintered in an unheated greenhouse. Carbohydrate reserves consist of soluble sugars and starch; nitrogen reserves consist of total amino acids and total soluble proteins. Adapted from Dhont et al. (2002, 2003).

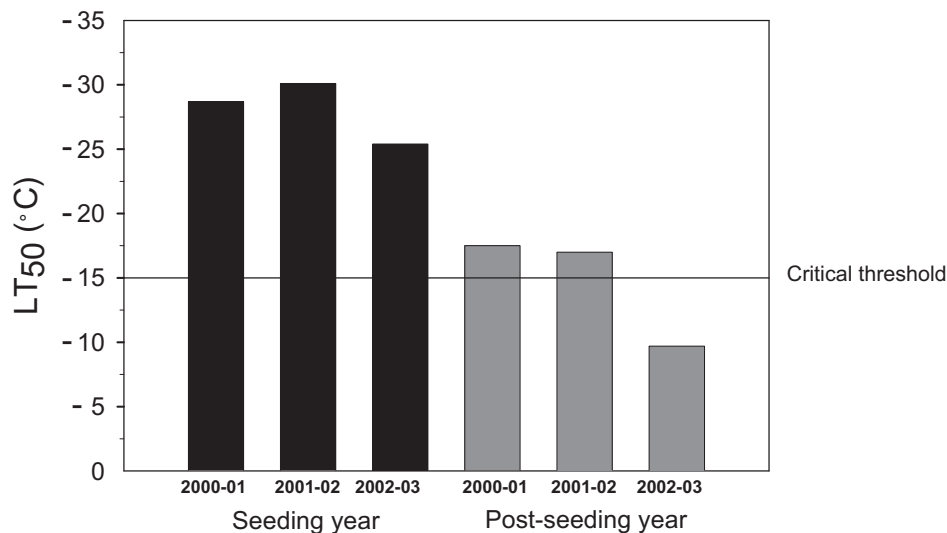
The application of P without K reduced root starch concentration, whereas a combined application of P and K resulted in the greatest starch concentration. Even though we can assume that adequate fertilization will improve winter hardiness and spring regrowth of alfalfa grown in P and K deficient soils, the relationship between P and K concentration in plant tissues and persistence as it relates to C and N reserves in overwintering alfalfa remains to be established.

Although N fertilisation is generally thought to interfere with the cold acclimation process by promoting active growth of plants in the fall, its impact on winter survival of overwintering plants is still unclear. Alfalfa fertilized with nitrates was slightly more freezing tolerant than N-deprived plants (Vézina and

Nadeau 1991). Winter wheat seedlings receiving high levels of N in a hydroponic system were found to be less hardy than unfertilized plants in the initial stages of the cold acclimation process (Tyler et al. 1981). Gusta et al. (1999), however, reported no effect of N fertilization on the freezing tolerance of winter wheat seedlings. The effect of N fertilization on cold tolerance is probably affected by the amount of N and the timing of its application in relation to the cold acclimation process.

#### Age of Stand

The effect of the age of the stand on cold tolerance and winter survival of alfalfa is the subject of conflicting reports. Using field observations, Paquin and Pelletier (1987) and



**Fig. 5.** Freezing tolerance, expressed as the lethal temperature for 50% of the plants ( $LT_{50}$ ), for alfalfa established and maintained for 3 consecutive years in a field plot (post-seeding years) at a site near Québec City as compared with the  $LT_{50}$  of seedlings that were sown under environmentally controlled conditions in late summer each year (seeding years). Both groups of plants were acclimated under natural hardening conditions and overwintered in an unheated greenhouse. Freezing tolerance was assessed in mid-January of each year. Critical threshold of  $-15^{\circ}\text{C}$  for field survival is indicated. Data from Y. Castonguay, P. Nadeau, and R. Michaud (unpublished).

Suzuki (1991) reported that alfalfa plants in the post-seeding year had greater fall hardening and were more cold tolerant than seeding-year plants. In contrast, other studies indicated that seeding-year plants of alfalfa showed less winter injury (Smith 1964; Heinrichs 1973) and yield reduction when exposed to a freezing stress than plants in their first post-seeding year (Bowley and McKersie 1990). More recent observations tend to confirm that plants in the seeding year can develop greater cold tolerance than those in post-seeding years (Fig. 5). Reasons for this are unclear, but preliminary data indicate that the greater cold tolerance of alfalfa seedlings is associated with more pronounced molecular changes typically related to freezing tolerance in alfalfa (Y. Castonguay, P. Nadeau, and R. Michaud, unpublished data). The potentially lesser cold tolerance and the increased incidence of diseases in older plants represent a greater risk of winter damage in aging stands. The age of the stand should therefore be considered when estimating the risk of winter damage to alfalfa (Undersander et al. 1994). As well, a balanced age distribution of alfalfa stands on the farm would help reduce the risks of losses due to winters.

## PREDICTING THE RISKS OF WINTER DAMAGE

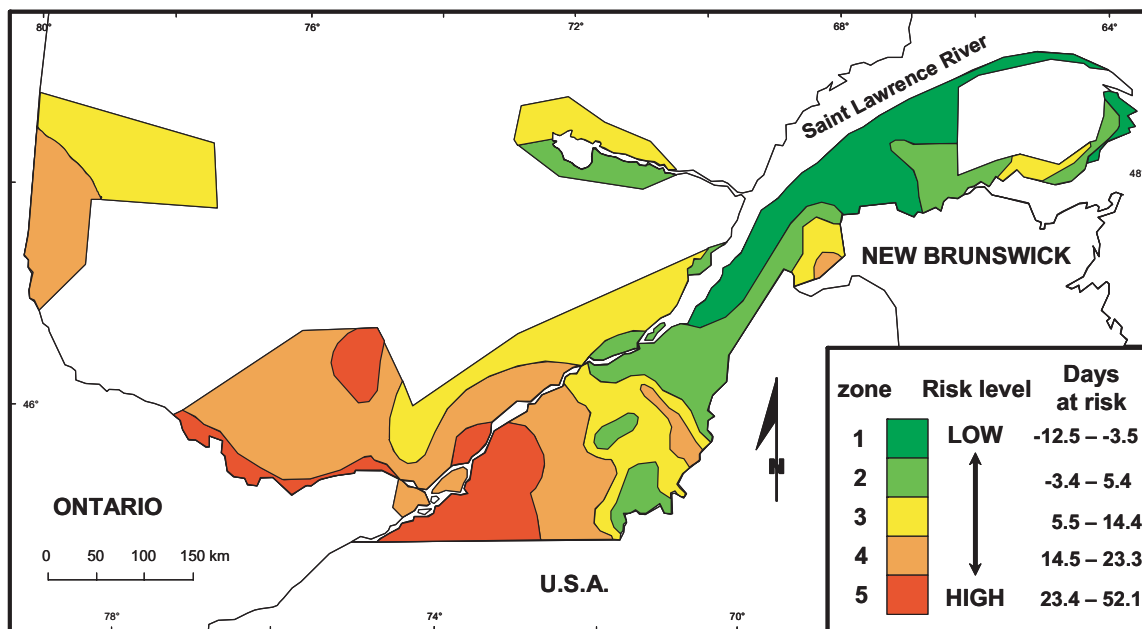
### Spatial and Temporal Variability

Indices to estimate the relative risks associated with the most probable climatic causes of damage to herbaceous perennials during fall and winter were developed by Rochette and Dubé (1993a,b) and modified by Bélanger et al. (2002a). Fall indices express the influence of air temperature and soil moisture on the acquisition of cold hardiness. Winter indices assess the impact of (i) subfreezing air temperatures, (ii) the loss of cold hardiness due to temperatures

above  $0^{\circ}\text{C}$ , and (iii) the potential damage to the root system by soil heaving and ice encasement. One of those indices was used for map zonation of the number of days at risk of winterkill for herbaceous perennials in agricultural areas of Québec (Fig. 6; Rochette and Dubé 1993a). The number of days at risk was calculated as the difference between the length of the cold period (days between the first and last occurrence of air temperature  $\leq -15^{\circ}\text{C}$ ) and the number of days with a snow cover  $\geq 0.1$  m. Based on this risk assessment, exposure to killing temperatures does not normally represent a significant threat to perennial forages in agricultural regions of northern Québec as a result of abundant snow cover. Conversely, thinner and intermittent snow cover in southern Québec increases markedly the likelihood of winterkill damage despite milder winter temperatures.

Although a direct validation of these indices cannot be done due to the lack of quantitative field data on winter damage to perennial forage crops, they reflect well-established regional differences in observed winter damage. Predictions that conditions in southern areas of eastern Canada are bound to be less favourable for overwintering are in close agreement with the observed occurrence of winter damage to forage crops (Bolduc et al. 1977). For example, from 1985 to 1999, an annual average of 63% of the alfalfa areas insured by the Québec Crops Insurance Board incurred losses due to winterkill in southern Québec compared with only 19% in the Lower St. Lawrence-Gaspé peninsula (S. Pion, personal communication, Québec Crops Insurance Board).

These indices were also used to predict how changes in climate over the next 50 yr are likely to affect the risks of winter injury. Bélanger et al. (2002a) predicted that the risks



**Fig. 6.** Spatial variation in the number of days at risk of winter damage in agricultural areas of Québec (1961–1990). The number of days at risk is calculated as the difference between the length of the cold period (days between the first and last occurrence of air temperature  $\leq -15^{\circ}\text{C}$ ) and the number of days with a snow cover  $\geq 0.1$  m. Adapted from Rochette and Dubé (1993a).

of winter damage to perennial forage crops will increase in eastern Canada because of (i) reduction in fall hardening due to warmer temperatures; (ii) less cold hardiness during winter due to warmer temperatures; and (iii) significant loss of snow cover protection, which will likely increase risks of damage due to subfreezing temperatures and ice (Table 2).

### Forecasting the Risk

Several factors must be considered when attempting to predict the risk of winter damage at a given site and year. Simulation models can be used to integrate several factors and make predictions of winter injury. A process-based simulation model of cold hardiness and winter injury was developed to predict alfalfa yield as a function of weather, cultivar, and management (Kanneganti et al. 1998a,b). This model, however, focusses only on cold hardiness and does not take into account other factors involved in winter survival.

A scoring sheet for assessing risks of alfalfa winter injury was developed in the United States by Undersander et al. (1994) and adapted for the conditions in Québec by Bélanger et al. (2002b). The scoring sheet accounts for stand age, alfalfa cultivar, soil pH, level of soil K, soil drainage, soil moisture during fall/winter, harvest frequency, and presence or absence of a stubble in the fall.

For predictive purposes, several conditions including minimum soil temperatures at different depths, the occurrence and duration of subfreezing soil temperatures, and the frequency of alternate freezing and thawing during the winter must be considered (Suzuki 1973). Soil temperatures in winter were shown to be negatively correlated to winter survival

**Table 2.** Values of indices for current conditions and two future periods in eastern Canada. Adapted from Bélanger et al. (2002a)

	1961–1990	2010–2039	2040–2069
Fall – Cold (CDD $< 5^{\circ}\text{C}$ ) <sup>z</sup>	86.4	77.3	68.8
Fall – Rain (mm $\text{d}^{-1}$ ) <sup>y</sup>	2.97	2.83	2.75
Winter – Days at risk (d) <sup>x</sup>	12.1	23.7	27.8
Winter – Rain (mm $\text{d}^{-1}$ ) <sup>w</sup>	0.86	0.92	0.98
Winter – Thaw (DD $> 0^{\circ}\text{C} \text{d}^{-1}$ ) <sup>v</sup>	0.27	0.47	0.59

<sup>z</sup>Net accumulation of cold degree-days (CDD) during the fall hardening period.

<sup>y</sup>Mean daily rainfall during the hardening period.

<sup>x</sup>Difference between the length of the cold period (days between the first and last occurrence of air temperature  $\leq -15^{\circ}\text{C}$ ) and the number of days with snow cover  $\geq 0.1$  m.

<sup>w</sup>Mean daily rainfall accumulation during the cold period.

<sup>v</sup>Mean daily accumulation of degree-days above  $0^{\circ}\text{C}$  during the cold period.

of alfalfa (Ouellet and Desjardins 1981); this was also confirmed in a study where prolonged periods with soil temperatures between  $-8$  and  $-12^{\circ}\text{C}$  were associated with significant winter damage (Dhont et al. 2004). Although it is well established that low soil temperatures can cause damage, it is unlikely that it could be used as a single predictor of winterkill (Ouellet and Desjardins 1981). The concept proposed by Suzuki (1973), which includes predictors such as soil temperature and level of cold hardiness (indirectly assessed by freeze-thaw cycles during the winter), is still not operational and progress is impeded by insufficient in situ temperature data. This, however, could change with the availability of low-cost data logging equipment and the possibility of using remote sensing for estimating soil temperature.

Simulation models, scoring sheets, and climatic indices are useful tools to assess the risks of winter injury due to the climatic conditions and crop management. However, these tools need to be refined if they are to be used with confidence in the future. They can provide general indications on the risk of winter injury at a given site but their degree of precision and accuracy is still not satisfactory nor properly validated. Validation would require numerous multi-site and multi-year studies.

### CONCLUSION

Winter stresses to perennial forage crops in eastern Canada include subfreezing temperatures without a sufficient snow cover, excess soil moisture during the cold-hardening period and throughout winter, the presence of ice, and diseases. Our understanding of those causes of winter damage and of the plant adaptation mechanisms to winter stresses, particularly the role of N and C organic reserves, has improved. However, the development of new cultivars and the prediction of risks of winter losses remain difficult because the causes of winter damage often act in combination and in interaction with species, cultivars, and crop management. Future improvements in winter survival will come from a better understanding of the different causes of winter damage and of their complex interactions with genetic, climatic, and management factors. Progress towards the development of a more comprehensive understanding of winter damage to perennial forage crops will be fostered through multidisciplinary studies involving agronomy, physiology, biochemistry, pathology, and molecular biology.

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