Effects of Nitrogen and Potassium Fertilization on Perennial Ryegrass Cold Tolerance During Deacclimation in Late Winter and Early Spring

D.E. Webster and J.S. Ebdon¹

Department of Plant, Soil, and Insect Sciences, Stockbridge Hall, University of Massachusetts, Amherst, MA 01003

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Abstract. Turf loss from freezing injury results in costly re-establishment, especially with turfgrasses such as perennial ryegrass (*Lolium perenne* L.) having poor low-temperature hardiness. Studies are limited as to the influence of N and K on cold tolerance during dehardening periods in late winter when grasses are most susceptible to freezing injury. The objective of this study was to evaluate perennial ryegrass low temperature hardiness during deacclimation in response to N and K and associated effects on crown hydration, median killing temperature (LT₅₀), shoot growth rate, tissue K concentration, soil exchangeable K, and low temperature disease. Treatments included five rate levels of N (49, 147, 245, 343, and 441 kg·ha⁻¹·yr⁻¹) in all factorial combinations with 3 rate levels of K (49, 245, and 441 kg·ha⁻¹·yr⁻¹). Low temperature tolerance was assessed using whole plant survival and electrolyte leakage (EL). Interactions between N and K were detected for all field measurements. The effects of N and K on survival LT₅₀ were detected only during late winter periods in February 2004, N and K differences were lost by March. Late winter cold survival was negatively correlated with crown moisture, growth rate, and tissue K. Tissue K concentrations ranged from 28.6 to 35.9 g·kg⁻¹ DM while soil K ranged from 121 to 261 mg·kg⁻¹. Soil extractable K was not correlated with tissue K. Survival and EL LT₅₀ were uncorrelated due to N and K interaction. Survival LT₅₀ ranged from -9.0 to -13.6 °C. Maximum cold hardiness occurred when low to moderate N (49 to 147 kg·ha⁻¹·yr⁻¹) was applied with medium-high to high levels of K (245 to 441 kg·ha⁻¹·yr⁻¹), which corresponded to soil exchangeable K levels ranging from 200 to 260 mg·kg⁻¹. Alternatively, similar K fertilization and soil K levels combined with high rates of N (343 and 441 kg·ha⁻¹·yr⁻¹) increased freeze stress and low temperature fungi (*Typhula incarnata*). At N rates routinely applied to perennial ryegrass, higher soil extractable K beyond those levels currently recommended for optimum shoot growth could provide some benefit in enhancing cold hardiness. Late fall applied N did not appear to increase the potential for winter injury.

Turf loss due to freezing injury results in costly reestablishment, increased weed pressure and soil loss from erosion, and a reduction in the aesthetic value and function of turf areas (DiPaola and Beard, 1992). The extent of freeze stress injury from low temperature is a function of the species, physiological state of the plant, environmental conditions, and the mechanism of injury (Beard, 1973). Research has shown that creeping bentgrass (*Agrostis stolonifera* L.) is one of the most tolerant cool-season grasses to low temperature while perennial ryegrass (*Lolium perenne* L.) is one of the most susceptible (Beard, 1966; Carroll, 1943; Gusta et al., 1980; Rajashekar et al., 1983). Ebdon et al. (2002) reported a direct relationship between perennial ryegrass susceptibility to freezing stress and turf (quality) performance. Perennial ryegrass is planted extensively throughout the U.S. and its use has

increased greatly in recent decades (Meyer and Funk, 1989). Therefore, developing strategies to decrease the susceptibility of this species to freeze stress especially in northern latitudes would assist turfgrass managers immensely.

Balanced nutrition is essential to quality turf. Nitrogen is a critical nutrient in cold hardiness and heavy applications of N in the fall period may predispose turfgrass to greater winter injury from low temperature (Carroll, 1943). The biological explanation underlying low temperature injury is crown hydration, which increases with tissue N (Carroll and Welton, 1939). Turfgrasses depend mainly upon the survival of the regenerative parts of the plant such as the crown, stolons, and rhizomes for recovery from severe winter stresses including abiotic and biotic stresses such as low temperature fungi. Increased tissue N and crown hydration levels decrease winter hardiness thus increasing the likelihood of low temperature kill. Welterlen and Watschke (1985) found a direct relationship with relative conductivity of crown tissue extract (membrane leakage and injury) and N fertilization rate.

There are numerous reports that provide different results as to the relationship between N and K and winter hardiness through their

effects on carbohydrate reserves and crown hydration. Potassium, like N, is reported to effect crown hydration and, as a result, winter hardiness. Markland and Roberts (1967) found that tissue hydration levels decreased with increasing amounts of K. Hurto and Troll (1980) evaluated low temperature hardiness of 'Manhattan' perennial ryegrass using sand culture. They reported an inverse relationship between winter kill and water-soluble carbohydrate content and a positive correlation between winter kill and tissue K levels with maximum tiller survival provided by a 2 to 1 (N to K) ratio. Although Cook and Duff (1974) reported tissue K contents ranging from 0.7 to over 3% during the cold hardening period in tall fescue (*Festuca arundinacea* Schreb.), no connection was detected between K nutrition and freezing tolerance. Similarly, no relationship was observed with K and freezing tolerance of tall fescue and carbohydrate content (Cook and Duff, 1976).

As reported above, tissue N and K can alter crown hydration and winter hardiness. An overall state of maximum low temperature hardiness corresponds closely with maximum tissue dehydration. This period of maximum cold hardiness in cool temperate regions occur following acclimation to low temperature in late December and early January (Beard, 1966; Watschke, 1981). Tompkins et al. (2000) reported a highly significant (negative) correlation between cold hardiness and crown moisture. Furthermore, by early spring creeping bentgrass had lost its maximum cold hardiness advantage over annual bluegrass (*Poa annua* L.). A small increase in crown moisture of 6% for creeping bentgrass was sufficient to accelerate deacclimation during this period. Accordingly, maximum low temperature injury occurs during deacclimation of late winter and early spring (Beard, 1966; White and Smithberg, 1980).

Nutrients interact with each other in such ways that are essential to plant survival. In Kentucky bluegrass (*Poa pratensis* L.) under greenhouse conditions using soilless media, Monroe et al. (1969) found that clipping weights increased more with K from 0 to 400 ppm under high nitrogen (130 ppm) compared to low N (65 ppm). Nitrogen to potassium ratios approximating 1 to 1.5 appear to provide maximum shoot growth response. In a field study conducted by Christians et al. (1981), they found that N and K were observed to interact in their effects on Kentucky bluegrass shoot growth. At lower levels of applied N, tissue production increased with increasing levels of applied K with maximum growth favored by a low N to K ratio less than 1. At high levels of applied N, shoot growth decreased slightly with increasing levels of K with maximum growth favored by a N to K ratio greater than 1. Ebdon et al. (1999) reported similar interactive effects of N and K on Kentucky bluegrass shoot growth. These interactive effects of N and K indicate that the ratio of N to K for optimum growth is not constant but varies with N. Thus, K through its interaction with N can effect shoot growth and in turn alter freeze stress tolerance by influencing carbohydrate

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¹To whom reprint requests should be addressed; e-mail sebdon@pssci.umass.edu.

Table 1. Monthly N (urea) and K (potassium sulfate) schedule.

Yearly rate (kg·ha ⁻¹)	Monthly schedule						
	April	May	June	August	September	October	November
N							
49					X ^z		
147		X			X		X
245		X	X		X	X	X
343	X	X	X	X	X	X	X
441	X	X	X	X	2X ^y	X	2X
K							
49					X		
245	X	X	X		X		X
441	X	X	X	X	2X	X	2X

^zX = 49 kg·ha⁻¹.^y2X = 98 kg·ha⁻¹.

reserves and hydration level. However, Beard and Rieke (1966) reported that a N to K ratio of 2 to 1 provided maximum low temperature survival in common kentucky bluegrass and 'Toronto' creeping bentgrass at all levels of N applied from 0 to 784 kg·ha⁻¹·yr⁻¹. Their results suggest that a balance of potassium that is one-half that of nitrogen is important for maximum cold hardiness rather than low N or high K.

Evaluation of survival or recovery after exposure to low temperature is a reliable method for assessment of low temperature tolerance within diverse plant genera (Steponkus, 1978). This method unfortunately does not provide adequate information as to the mechanisms that cause death of the freeze-stressed plant. To assess the level of cell injury due to low-temperature stress, the electrolyte leakage (EL) technique is commonly used as an alternative to regrowth-survival. Leakage methods are more rapid and have been shown to be correlated with survival (Cardona et al., 1997; Ebdon et al., 2002; Fry et al., 1991; Gusta et al., 1980; Maier et al., 1994). Studies have shown, however, that EL may underestimate actual survival (Cardona et al., 1997; Fry et al., 1993) because a high leakage level may not always equate to greater membrane injury when plants are allowed to adjust to low temperature through acclimation (Uemura and Stepokus, 1994). Therefore, survival evaluations should be included with EL assays to provide a more reliable assessment of actual low-temperature survival. Furthermore, slope estimates derived from EL curves when plotted against temperature are good indicators of mortality rate during freezing stress (Zhu and Liu, 1987).

In recent years there has been a practitioner trend towards applying relatively high rates of K equal to or exceeding N. Although the effects of N or K applied alone and in combination have been studied, there is no agreement among turfgrass managers as to the optimum K fertilization program. This in large part is due to the complexity of soil-plant-environment interaction and varied effects on nutrients (and fertilizer) programs. Limited information is available as to the mechanism of cold hardiness by which N and K interact especially during periods of deacclimation when grasses are most susceptible. Perennial ryegrass is one of the most widely used and least tolerant turfgrass species to low temperature, so additional study is needed in this area and species. The

objective of this study was to evaluate perennial ryegrass low temperature hardiness in response to N and K during deacclimation in late winter and its effects on crown hydration, lethal freezing temperature, shoot growth rate, tissue K concentration, soil exchangeable K, and low temperature disease.

Materials and Methods

Fertilizer treatments. Fertilizer studies were initiated at the University of Massachusetts Joseph Troll Turf Research Center (South Deerfield, Mass.) beginning in April 2000. Fifteen N-K combinations were applied to established 'Palmer II' perennial ryegrass turf growing on a Hadley silt loam (coarse, silty, mixed, nonacid, mesic Typic Udifluent). Morgan extractable K before treatment was 121 mg·kg⁻¹ (medium-high) with a soil pH of 6.3. Morgan extractable P before treatment was 23 mg·kg⁻¹ (high), so no supplemental P was applied. Treatments included five rate levels of N (49, 147, 245, 343, and 441 kg·ha⁻¹·yr⁻¹) in all factorial combinations with 3 rate levels of K (49, 245, and 441 kg·ha⁻¹·yr⁻¹). Treatment plots were 1.5 × 3.0 m and were mown twice per week at 3.175 cm using reel mowers with clippings returned except when collections were made for growth determination and tissue analysis. The 15 N-K treatment combinations were arranged as a randomized complete block with four replicates. Urea (45-0-0) was used as the sole N source while potassium sulfate (0-0-41.5) was used as the sole source of K, which were applied by hand. Monthly fertilizer N and K were applied at the same time at a rate of 49 kg·ha⁻¹ from 2000 through the 2003 growing seasons according to the schedule shown in Table 1. A 98 kg·ha⁻¹ rate (N and K) was used during the months of September and November for the 441 kg·ha⁻¹·yr⁻¹ treatment. Monthly fertilizer applications were generally applied during the last week of each month and irrigated after fertilization with 1 cm of water. Plots were irrigated during the growing season to prevent visual moisture stress. About 60% to 70% of the total annual N was applied during the fall period from late August through late November (Table 1). Late fall N in November was applied following the last mowing after shoot growth ceased.

Clippings were collected monthly following mowing events for a total of five collections per growing season from April through August

in 2002 and 2003. Clippings were oven dried at 70 °C for 48 h, weighed and expressed as g DM·m⁻²·d⁻¹. Total tissue K was determined in 2002 and 2003 from chemical analysis of dried clippings collected in April before fertilizer treatments. Also, soil samples for exchangeable soil K (Morgan extractable) were taken in April during 2002 and 2003 from each N-K treatment plot. Chemical analysis for tissue K was performed by the University of Massachusetts Soil and Tissue Testing Laboratory using inductively coupled argon plasma technology (model ICAP-61; Thermo Jarrell Ash, Franklin, Mass.). Plots were rated for gray snow mold (*Typhula incarnata*) as percent of plot in early Spring 2002 (6 Apr.) and 2003 (23 Mar.). Identity of snow mold disease was confirmed by the University of Massachusetts Turfgrass Disease Diagnostic Laboratory.

Freeze stress field sampling. Samples were taken from each N-K treatment field plot during late winter and early spring of 2004. Four plugs (366 cm²) were removed from each plot for freeze stress evaluations (EL and survival). Samples were taken during the deacclimation phase when grasses are most susceptible to direct low temperature kill (Tompkins et al., 2000; White and Smithberg, 1980). The samples were placed in a freezer at 0 °C to ensure no growth would occur. Samples were kept at this temperature for two days in preparation for EL and survival determination. Temperatures of the treatment plots were monitored in the field using five copper-constantan thermocouples, attached to a datalogger (CR10X; Campbell Scientific, Inc., Logan, Utah). Electrolyte leakage and whole plant survival were determined on all N-K treatment combinations within a replicate (block), which required 1 week to complete. Therefore, 4 weeks were required to complete one run (experiment) on all four replications (blocks) and two experiments were conducted representing two deacclimation environments (late winter and early spring). Samples were pulled in 2004 for the late winter phase beginning 2 Feb. (block 1) and ending 23 Feb. (block 4) while the early spring samples were removed beginning 8 Mar. (block 1) and ending 29 Mar. (block 4).

Freeze stress whole plant survival. Freeze shock and subsequent recovery of plant material was evaluated by submitting individual plants to a range of 11 decreasing treatment temperatures consisting of a nonfrozen control (5 °C), and 10 freeze-stress temperatures: -1, -3, -5, -7, -9, -11, -13, -15, -17, and -19 °C. Treatment temperatures were applied using a ScienTemp freezer (ScienTemp Corp. Adrian, MI). The temperature of the plant material was cooled to the desired treatment level at a rate of 2 °C·h⁻¹ maximum. The material was then soaked at that temperature for a minimum of 1 h. Treatment temperatures were adjusted manually and monitored using six copper-constantan thermocouples, attached to a datalogger (CR10X; Campbell Scientific, Inc., Logan, Utah).

Ten plants were exposed to each of 11 treatment temperatures and 15 N-K treatment combinations for a total of 1650 perennial ryegrass plants that were required to evaluate

plant survivability [10 (individual plants) $\times$ 11 (temperature treatments) $\times$ 15 (N–K treatments)]. The 10 plants from each temperature by fertilizer (N–K) combination were used to calculate (percent) whole plant survival. Four replications of this procedure was conducted. Plant material was washed free of soil, using cold water, and separated into individual plants. Shoots and roots were trimmed to 2 cm each. To ensure ice nucleation, samples were wrapped in paper toweling moistened with deionized water and placed into poly freezer bags for freeze stress exposure. During sample preparation, all plant material was temporarily stored at 5 °C, nonfrozen control temperature. The temperature of the plant material was cooled to the desired treatment level.

After temperature exposure, the plant material was removed from the freezer and assessed for survival as a percentage of viable-green shoots. The temperature at which 50% of the plant (crown) tissue that survive based on regrowth recovery was expressed as the LT_{50} survivability (lethal temperature at which 50% of the plant material survive). Regrowth recovery was determined by the following procedure. After each treatment temperature exposure, freezer bags containing samples were removed from the freezer and allowed to thaw slowly, for a minimum of 12 h, at 5 °C. Samples were then be replanted into cell trays and placed in the greenhouse (about 20 °C average air temperature) for a four week recovery period. Plants with any green surviving tissues or any new growth were counted as survivors. All others were considered as having been killed by the treatment temperature. Percent survival was calculated as: survival (%) = (no. of plants survived $\div$ total no. of plants) $\times$ 100.

Freeze stress electrolyte leakage. Leaves and roots were removed from plant samples, leaving only crowns. Five crown samples were placed into each of three test tubes and stoppered for a total of 15 crowns for each N–K treatment combination. To ensure

ice nucleation, test tubes were filled with 4 ml deionized water, frozen, and held at -2 °C before plant material was added. Crowns were then placed in the test tubes in contact with the ice and an additional 1 ml deionized water was added. During sample preparation plant material was temporarily stored at 5 °C. A total of 9900 perennial ryegrass crowns [15 (individual crowns per N–K treatment) $\times$ 11 (temperature treatments) $\times$ 15 (N–K treatments) $\times$ 4 replications] were used for electrolyte leakage evaluations.

After sample preparation, the treatment-temperature schedule and protocol used for freeze-shock survival was followed. After each treatment-temperature exposure, three test tubes per N–K combination were removed from the freezer and allowed to thaw for a minimum of 12 h at 5 °C. Samples were then subjected to infiltration (test tubes uncovered) under partial vacuum (0.033 MPa) for 20 min, then incubation (test tubes covered) at 5 °C for 12 h, and then placed on a centrifugal (rotary) shaker table (about 150 rpm) at about 21 °C for 8 h. Initial conductivity (IC) of the extract of each test tube was measured using a conductivity bridge (model 1054; VWR Scientific, Boston, Mass.). Crowns were then killed by overnight exposure to -40 °C. Samples were thawed at room temperature for at least 4 h and placed on a shaker table for 2 h at room temperature, after which the final conductivity (FC) of the extract was measured. Electrolyte leakage was expressed as a relative percentage: EL (%) = (IC $\div$ FC) $\times$ 100. The three test tube samples were averaged, and the procedure was repeated for a total of four replications.

At the time of field sampling, 165 crowns from each N–K treatment plot were used to determine percent crown moisture content. Crown fresh mass (FM) was measured, followed by oven drying for 48 h and weighed to obtain dry mass (DM). Percent crown moisture was calculated as [1 – (DM $\div$ FM)] $\times$ 100.

Statistical analysis and LT_{50} estimates. Lethal temperatures at which 50% survival

and EL occurred (LT_{50}) was determined mathematically by curve fitting using a four-parameter sigmoid model (Sigma Plot, SPSS Inc., Chicago Ill.). Parameter estimates were substituted back into the nonlinear equation and the temperature where Y (survival or EL) is 50% was determined for each replicate and N–K treatment combination. A four parameter nonlinear regression model as described by Cardona et al. (1997) was used to estimate the slope of the EL curve by fitting EL to temperature. Slope estimates were determined using the user defined sub-routine of nonlinear regression (Sigma Plot, SPSS Inc., Chicago, Ill.).

Analysis of variance (ANOVA) of LT_{50} estimates (EL and survival), slope estimates, crown hydration, shoot growth rate, tissue K, soil exchangeable K, and snow mold severity were analyzed by MINITAB (State College, Pa.). The effects of N were evaluated by partitioning the total N–K treatment sum of squares (SS) and the 5 rate levels of N into single degree of freedom (df) orthogonal contrasts to determine N linear (NL), N quadratic (NQ), N cubic (NC), and N quartic (NQR) components. Similarly for K, the 3 rate levels of K were partitioned to test for K linear (KL) and K quadratic (KQ) components. Nitrogen and potassium main effects and associated contrasts were crossed to partition N $\times$ K interaction SS to test for interaction components. No interaction with year (2002 and 2003) was detected for shoot growth rate (pooled over monthly collections), soil exchangeable K, tissue K, and snow mold, therefore the data were pooled and reported here as average over year. Surface response curves for cold survival, crown hydration, shoot growth rate, soil exchangeable K, tissue K concentrations, and snow mold were generated using significant ($P \leq 0.10$) polynomial N and K main effects and interaction components from ANOVA using the user defined sub-routine of nonlinear regression (Sigma Plot; SPSS Inc., Chicago Ill.). Nonsignificant ($P > 0.10$) lower ordered terms were included in the final model if higher

Table 2. Mean squares (MS) from ANOVA of LT_{50} estimates (survival and EL), slope estimates from EL curves, and crown moisture during late winter and early spring periods of cold deacclimation as influenced by N and K fertilization.

ANOVA		P values							
		Late winter			Early spring				
		LT_{50}			Crown	LT_{50}		EL slope	Crown
F test source	df	Survival	EL	EL slope	moisture	Survival	EL	EL slope	moisture
Block	3	****	****	***	*	****	***	****	NS
N	4	**	****	****	**	NS	****	****	NS
N _{Linear} (NL)	1	**	****	****	****	NS	****	NS	*
N _{Quadratic} (NQ)	1	**	****	*	NS	NS	NS	****	NS
N _{Cubic} (NC)	1	NS	NS	***	NS	NS	**	*	NS
N _{Quartic} (NQR)	1	NS	NS	NS	NS	*	NS	****	NS
K	2	NS	****	NS	NS	NS	**	*	NS
K _{Linear} (KL)	1	NS	***	NS	NS	NS	**	*	NS
K _{Quadratic} (KQ)	1	NS	***	NS	NS	NS	NS	NS	NS
N $\times$ K	8	*	*	NS	NS	NS	NS	*	NS
NL $\times$ KL	1	***	***	NS	**	NS	NS	NS	NS
NL $\times$ KQ	1	NS	NS	NS	NS	NS	NS	NS	NS
NQ $\times$ KL	1	NS	NS	NS	NS	NS	NS	NS	NS
NQ $\times$ KQ	1	NS	NS	*	NS	NS	NS	NS	NS
NC $\times$ KL	1	NS	*	NS	*	NS	NS	NS	NS
NC $\times$ KQ	1	NS	NS	NS	NS	NS	NS	NS	NS
NQR $\times$ KL	1	NS	*	NS	NS	NS	NS	***	NS
NQR $\times$ KQ	1	NS	NS	NS	NS	NS	NS	NS	NS

NS, ****, ***, **, * Nonsignificant or significant at $P \leq 0.001$, 0.01, 0.05, and 0.10, respectively.

Table 3. Means for LT₅₀ estimates (survival and EL), slope estimates from EL curves, and crown moisture during late winter and early spring periods of cold deacclimation as influenced by N and K fertilization.

Rate (kg·ha ⁻¹ ·yr ⁻¹)	Late winter				Early spring			
	LT ₅₀ (°C)		EL	Crown	LT ₅₀ (°C)		EL	Crown
	Survival	EL	slope	moisture (%)	Survival	EL	slope	moisture (%)
N								
49	-11.8	-6.9	0.65	84.1	-9.9	-3.0	0.71	83.5
147	-12.3	-7.0	0.30	84.7	-10.1	-1.7	0.61	84.6
245	-11.9	-6.5	0.40	85.3	-9.3	-1.7	1.00	84.9
343	-12.1	-5.3	0.38	85.5	-10.3	-1.6	0.71	85.8
441	-10.3	-4.4	0.28	86.9	-9.8	-0.5	0.53	84.8
LSD (0.05)	-1.3	-0.6	0.16	1.9	NS	-0.8	0.18	NS
K								
49	-11.5	-5.5	0.47	84.9	-10.3	-2.2	0.81	84.7
245	-11.9	-6.4	0.36	85.0	-9.6	-1.6	0.67	84.4
441	-11.5	-6.2	0.37	86.0	-9.7	-1.3	0.69	85.0
LSD (0.05)	NS	-0.4	NS	NS	NS	-0.7	0.12	NS
N-K								
49-49	-10.5	-7.1	0.77	84.1	-9.9	-3.5	0.75	83.4
49-245	-11.5	-7.3	0.67	83.9	-9.5	-2.9	0.67	82.7
49-441	-13.3	-6.4	0.51	84.3	-10.2	-2.6	0.70	84.4
147-49	-11.5	-6.2	0.32	86.7	-10.8	-2.8	0.51	84.0
147-245	-13.6	-7.1	0.29	82.8	-9.8	-1.4	0.60	83.6
147-441	-11.8	-7.5	0.29	84.7	-9.6	-0.9	0.73	86.2
245-49	-11.7	-6.2	0.50	85.1	-10.1	-1.8	1.33	85.3
245-245	-12.2	-7.0	0.28	85.1	-9.0	-1.8	1.00	84.9
245-441	-11.7	-6.4	0.41	85.8	-8.8	-1.6	0.77	84.6
343-49	-12.1	-4.6	0.52	83.4	-10.9	-1.9	0.78	86.1
343-245	-12.3	-5.6	0.25	85.9	-9.7	-1.8	0.58	86.4
343-441	-11.8	-5.7	0.36	87.2	-10.3	-1.2	0.78	85.1
441-49	-11.8	-3.3	0.23	85.3	-9.7	-0.8	0.68	84.8
441-245	-10.0	-5.0	0.31	87.3	-10.1	-0.2	0.48	84.7
441-441	-9.0	-4.8	0.29	88.2	-9.7	-0.5	0.45	84.8
LSD (0.05)	-1.9	-0.8	0.23	2.7	NS	-1.5	0.26	NS
CV (%)	13.4	11.5	49.2	2.7	13.6	59.6	30.5	2.9

NS Nonsignificant at $P > 0.05$ level.

ordered terms were statistically significant. Estimates for LT₅₀ (EL and survival), EL slope, and crown moisture that were measured during periods of deacclimation from late winter to early spring are reported separately by period because of late winter-to-early spring interaction. Least significant difference (LSD) values at the 0.05 level are reported for comparisons between N-K treatments and main effects. No departures from the assumptions of ANOVA were detected in homogeneity of variance or normality. Correlation coefficients (r) were computed to assess the relationship among various response variables.

Results and Discussion

Effects of N on cold hardiness. Nitrogen was an important factor affecting low temperature hardiness based on survival and EL evaluations (Table 2). We view whole plant survival LT₅₀ as the absolute indicator of cold hardiness and EL LT₅₀ as a predictor of low temperature hardiness. Nitrogen significantly reduced low temperature hardiness (survival) in late winter when 441 kg·ha⁻¹·yr⁻¹ was applied. The 441 kg·ha⁻¹·yr⁻¹ rate corresponded to three applications applied from September through November totaling 245 kg·ha⁻¹ (Table 1), which is the critical period for N and associated winter injuries (Carroll, 1943). The late fall applied N in November did not appear to increase the potential for low temperature kill. Alternatively, it was excessive N in September (98 kg·ha⁻¹) followed by 49 kg·ha⁻¹ in October that was associated with significantly higher

killing temperatures (less negative LT₅₀) (Table 3). The 49 kg·ha⁻¹·yr⁻¹ N fertilization applied in September was no different in cold hardiness from other fall applied N treatments such as 98 and 147 kg·ha⁻¹, all of which incorporate late fall applied N (Tables 1 and 3). In this study, late fall N was always applied after shoot growth ceased and this limited the potential for surge growth (excess shoot growth) and winter kill from low temperature and snow mold fungi.

The potential for low temperature kill (survival LT₅₀) among the 15 N-K treatment combinations was closely associated with

crown hydration (moisture) in late winter ($r = 0.68$, $P \leq 0.01$). So, as crown moisture increased (Fig. 1), lethal killing temperature increased (Fig. 2). The 441 kg·ha⁻¹·yr⁻¹ N rate during the late winter period, which was associated with the poorest cold hardiness, significantly increased crown moisture content compared to the 49 kg·ha⁻¹·yr⁻¹ rate (Table 3). During the early spring period, no difference between N-K treatments was detected in crown hydration and survival LT₅₀ (Tables 2 and 3). The low temperature hardiness and low crown hydration advantage of N at 49 kg·ha⁻¹·yr⁻¹ during the late winter sampling period (2 to 23 Feb.) was lost by early spring (8 to 29 Mar.).

A major contributor to dehardening during

Fig. 1. Predicted crown moisture content of perennial ryegrass during late winter in 2004 fertilized with varying rates of N and K: Predicted crown moisture = $85.3 + 0.64\text{NL} + 0.09\text{NQ} + 0.13\text{NC} + 0.54\text{KL} + 0.56\text{NL} \times \text{KL} + 0.10\text{NQ} \times \text{KL} - 0.44\text{NC} \times \text{KL}$. $R^2 = 0.806$ ($P \leq 0.05$).

Fig. 2. Predicted survival LT₅₀ of perennial ryegrass during late winter in 2004 fertilized with varying rates of N and K: Predicted survival LT₅₀ = $-9.44 - 0.91\text{NL} + 0.07\text{NQ} - 0.36\text{KL} + 0.07\text{NL} \times \text{KL}$. $R^2 = 0.724$ ($P \leq 0.01$).

Fig. 3. Mean daily air and soil temperatures during deacclimation.

Table 4. Means for shoot growth rate, tissue K concentration, soil exchangeable K, and snow mold (typhula blight) severity as influenced by N and K fertilization (2002–03 averages).

Rate (kg·ha ⁻¹ ·yr ⁻¹)	Shoot growth (g·m ⁻² ·d ⁻¹ dry wt)	Tissue K (g·kg ⁻¹ dry wt)	Soil K (mg K/kg)	Snow mold (%)
N				
49	0.9	29.6	202	49.8
147	3.0	31.9	192	55.1
245	3.6	32.6	182	50.8
343	6.1	33.2	179	56.2
441	7.0	34.6	162	62.2
LSD (0.05)	0.6	0.9	9	6.5
K				
49	3.8	31.3	134	50.1
245	4.3	32.7	184	54.9
441	4.3	33.1	232	59.4
LSD (0.05)	0.4	0.7	7	5.0
N–K				
49–49	0.8	28.6	146	45.8
49–245	0.9	29.7	200	55.4
49–441	0.9	30.7	261	48.2
147–49	3.0	31.6	141	56.5
147–245	2.8	31.9	197	50.1
147–441	3.1	32.2	238	58.7
245–49	3.6	32.0	135	47.4
245–245	3.4	32.1	179	47.6
245–441	3.7	33.6	232	57.3
343–49	5.3	31.5	127	48.9
343–245	6.8	33.9	179	57.5
343–441	6.3	34.1	230	62.0
441–49	6.0	32.9	121	51.6
441–245	7.5	35.9	164	64.1
441–441	7.6	34.9	200	70.0
LSD (0.05)	0.9	1.4	15	11.2
CV (%)	25.4	5.1	5.9	24.5

late winter and early spring is increasing soil temperature. Plants deacclimated quickly during the late winter period as soil temperatures increased dramatically (Fig. 3). During the late winter sampling period, survival LT_{50} averages of -13.7 , -12.6 , -11.8 , and -8.6 °C were observed for sampling dates corresponding to the 2, 9, 16, and 23 Feb., respectively, which correlated with rising soil temperatures (Fig. 3). Crown moisture also increased significantly during this late winter sampling period from 84.1% on 2 Feb. to 86.4% by 23 Feb. No differences were observed, however, in survival LT_{50} and crown moisture between sampling periods during the early spring after soil temperatures peaked. Tompkins et al. (2000) reported similar relationships between crown hydration and LT_{50} with soil temperature. A highly significant block effect was generally observed during deacclimation (Table 2), suggesting that blocking over time was effective

in controlling variation in LT_{50} and crown moisture introduced by soil temperature.

The slope of the EL curve is an important indicator of mortality resulting from freeze stress (Gudleifsson et al., 1986; Zhu and Liu, 1987). Differences in slope between hardened and nonhardened perennial turfgrasses (Rajasekar et al., 1983) and forage grasses (Gudleifsson et al., 1986) suggest that most hardy species have smaller slopes (flatter curves), while nonhardy species are associated with EL curves having steep slopes. Slope estimates derived from EL curves indicated differences occurred due to deacclimation. A highly significant block effect ($P \leq 0.001$, Table 2) was detected during the late winter and early spring sampling periods. Slope of the EL curve increased significantly with time during the 4-week freeze-stress evaluation period in late winter and early spring. Specifically, plant material from the 2 Feb. sampling date had flatter slopes (0.35)

Fig. 4. Predicted EL LT_{50} of perennial ryegrass during late winter in 2004 fertilized with varying rates of N and K: Predicted EL $LT_{50} = -1.71 + 0.50NL - 0.02NQ + 0.24NC + 0.01NQR + 0.41KL - 0.13NL \times KL - 0.03NQ \times KL + 0.08NC \times KL - 0.06NQR \times KL$. $R^2 = 0.974$ ($P \leq 0.01$).

compared to the last sampling date on 23 Feb. in late winter (EL slope of 0.57), suggesting that plants were dehardening. The increase in mortality during deacclimation based on slope estimates continued into the early spring period as slope estimate increased from 0.57 on 9 Mar. to 1.31 by 29 Mar.

A difference in EL slope also implies that deacclimation was affecting the rate of membrane leakage, and in turn, EL LT_{50} estimates in N and K treated perennial ryegrass. For example, EL LT_{50} estimates during late winter were on average lower (more winter hardy, EL LT_{50} of -6.0 °C) with smaller slope estimates (0.40) compared to early spring EL LT_{50} (-1.7 °C) and slope estimates (0.72) (Table 3). The slope of the EL curve has been shown to be correlated (positively) with the lethal killing temperature of the tissue in cultivars of *Paspalum vaginatum* Swartz (Cardona et al., 1997) and perennial ryegrass (Ebdon et al., 2002). In our N–K study, the slope of the EL curve among the 15 N–K combinations was uncorrelated with EL and survival LT_{50} . Also, we found no correlation among the 15 N–K treatments in EL slope between late winter and early spring dehardening periods. Previous studies have emphasized species and cultivar differences. However, EL slope may not necessarily be relevant in cold hardness studies involving N and K possibly due to their interactive effects on predicted (EL) and actual cold tolerance.

Effects of K and interaction with N on cold hardness. Potassium through its interaction with N significantly altered LT_{50} survival and EL (Table 2). Electrolyte leakage prediction of cold tolerance was affected by K interaction with N in ways different from actual whole plant survival. For example, with N at 49 kg·ha⁻¹·yr⁻¹ increasing levels of K significantly decreased predicted (EL) cold hardness (Fig. 4 and Table 3) while actual cold survival increased with K during the late winter sampling period (Fig. 2 and Table 3). Conversely, with N at 441 kg·ha⁻¹·yr⁻¹ increasing levels of K increased EL cold hardness while actual (survival) cold tolerance decreased. Accordingly, EL LT_{50} and Survival LT_{50} were uncorrelated. These results are not consistent with species and cultivar evaluations, which have suggested EL as a potential screening method for improved low temperature tolerance because of its correlation with actual cold survival (Cardona et al., 1997; Ebdon et al., 2002; Fry et al., 1991; Gusta et al., 1980; Maier et al., 1994; Murdoch et al., 1990). Furthermore, LT_{50} for survival during late winter was lower on average by about

Fig. 5. Predicted shoot growth rate (2002–03 average) of perennial ryegrass fertilized with varying rates of N and K: Predicted shoot growth rate = $4.12 + 1.55\text{NL} - 0.03\text{NQ} - 0.02\text{NC} - 0.10\text{NQR} + 0.29\text{KL} - 0.07\text{KQ} + 0.20\text{NL} \times \text{KL} - 0.09\text{NL} \times \text{KQ}$. $R^2 = 0.992$ ($P \leq 0.001$).

Fig. 6. Predicted *Typhula incarnata* (2002–03 average) of perennial ryegrass during early spring fertilized with varying rates of N and K: Predicted *Typhula incarnata* = $54.8 + 2.6\text{NL} + 0.8\text{NQ} + 4.7\text{KL} - 0.1\text{KQ} + 0.1\text{NL} \times \text{KQ} - 0.9\text{NQ} \times \text{KQ}$. $R^2 = 0.738$ ($P \leq 0.05$).

Table 5. Mean squares (MS) from ANOVA of shoot growth rate, tissue K concentration, soil exchangeable K, and snow mold (typhula blight) severity as influenced by N and K fertilization (2002–03 averages).

F test source	df	Shoot growth	Tissue K	Soil K	Snow mold
Block	3	***	NS	****	NS
N	4	****	****	****	**
N _{Linear} (NL)	1	****	****	****	***
N _{Quadratic} (NQ)	1	NS	NS	NS	NS
N _{Cubic} (NC)	1	NS	**	NS	NS
N _{Quartic} (NQR)	1	****	NS	NS	NS
K	2	*	****	****	***
K _{Linear} (KL)	1	**	****	****	***
K _{Quadratic} (KQ)	1	NS	NS	NS	NS
N × K	8	NS	NS	NS	NS
NL × KL	1	**	**	***	**
NL × KQ	1	*	NS	NS	NS
NQ × KL	1	NS	*	NS	NS
NQ × KQ	1	NS	NS	NS	*
NC × KL	1	NS	NS	**	NS
NC × KQ	1	NS	NS	NS	NS
NQR × KL	1	NS	NS	NS	NS
NQR × KQ	1	NS	NS	NS	NS

NS, ****, ***, **, * Nonsignificant or significant at $P \leq 0.001$, 0.01, 0.05, and 0.10, respectively.

5.7 °C compared to those derived from fitted EL curves (Table 3), therefore EL underestimated actual survival. Similarly, in paspalum (Cardona et al., 1997), centipedegrass [*Eremochloa ophioides* (Munro) Hack] (Fry et al., 1993), and perennial ryegrass (Ebdon et al., 2002) EL assays underestimated survival by about 3.5 °C.

One plausible explanation for the lack of agreement between predicted (EL) and actual cold survival is the effect differential concentrations of K in tissues may have on the electrical conductivity of the bathing solution used in EL evaluations. For example, we observed luxury consumption of K with N at 49 kg·ha⁻¹·yr⁻¹ in response to increasing levels of K from 49 to 441 kg with no commensurate increase in shoot growth rate (Table 4) or crown hydration (Table 3). Leakage of cell contents from crowns during EL evaluations is based on the assumption that greater ion leakage equates to greater injury and higher killing temperatures. Healthy (nonfrozen) tissues leak ions (Palta et al., 1977) and K is readily exchangeable (highly mobile cation). The significantly higher tissue K concentrations associated with the 49N–441K treatment may have increased the

conductivity of crown tissue extract during EL evaluations, thus increasing EL LT₅₀ when compared to the other K combinations with 49 kg N. Furthermore, the luxury consumption of K that was observed is the most likely explanation for the superior cold hardiness afforded by the 49N–441K kg·ha⁻¹·yr⁻¹ treatment combination (survival LT₅₀ of –13.3 °C) when compared with 49N–49K kg·ha⁻¹·yr⁻¹ (survival LT₅₀ of –10.5 °C) (Table 3). Potassium's ability to bind water during the formation of ice crystals has been suggested as a possible mechanism in cold tolerance, however, the role of K in plants is complex because of its many physiological functions (Wagner, 1967).

Nitrogen main effect means for survival LT₅₀ and EL LT₅₀ were in agreement for cold hardiness (Table 3), which were linear and curvilinear (quadratic) in response to N (Table 2). However, results based on predicted (EL) cold tolerance were distinctly different and opposite to that observed with actual cold survival due in large part to N × K interaction (Tables 2 and 3, Figs. 2 and 4). Unlike predicted (EL) cold tolerance, its important to recognize that actual low temperature survival can be interpreted through N and K main effects and

interaction in biologically meaningful ways by crown hydration, the rate of shoot growth, and tissue K concentrations.

Biological interpretation of N and K effects on cold tolerance. We detected significant linear effects with N (NL) on cold survival and crown moisture (Table 2). The NL component accounted for 92.1% of crown hydration and 85.6% of cold survival total nitrogen SS. Therefore, the NL component was the single most important trend explaining cold tolerance and crown moisture in response to N. As previously reported in this study, the potential for low temperature kill among the 15 N–K treatment combinations was highly correlated with crown moisture in late winter (Figs. 1 and 2), which is consistent with the findings reported by Beard (1966), Gusta et al. (1980), and Tompkins et al. (2000). Crown moisture (Fig. 1) was positively correlated with the rate of shoot growth (Fig. 5) ($r = 0.68$, $P \leq 0.01$) and the rate of shoot growth increased with N (Table 4).

The effects of K were N dependent and K through its interaction with N (i.e., NL × KL interaction) was important (Table 2). Potassium interaction with N had a greater influence on cold tolerance and crown moisture than the K main effects. For example, the NL × KL interaction SS was about 12 times larger than the K main effect SS for cold survival and 1.5 times larger than the K main effect SS for crown hydration. No effect on shoot growth or crown hydration was observed with K when fertilized with N at 245 kg·ha⁻¹·yr⁻¹ and lower. Conversely, a 25% increase in the rate of shoot growth was detected with increasing K when fertilized with N at 343 kg·ha⁻¹·yr⁻¹ and higher (Table 4), which was sufficient to cause a significant increase in crown moisture (Table 3). However, only the 441 kg·ha⁻¹·yr⁻¹ N treatment was associated with significantly greater low temperature kill in response to K (Table 4). The higher leaf growth rate with 441 kg N compared to 343 kg N rate mostly likely accelerated the depletion of nonstructural carbohydrate reserves (Hull and Smith, 1974), and along with crown hydration increased the potential for low temperature kill. Percent tiller kill in perennial ryegrass has been reported to be negatively correlated with water-soluble carbohydrate content (Hurto and Troll, 1980).

The higher leaf growth rate and crown moisture content reported here in response to K with N at 343 and 441 kg·ha⁻¹ also increased the severity of low temperature disease (*Typhula incarnata*) by as much as 25% to 35% over low K (49 kg K) combinations (Table 4, Fig. 6). So, gray snow mold severity increased with K in direct proportion with the rate of shoot growth (about 25%). Shoot growth and damage from gray snow mold were correlated ($r = 0.50$, $P \leq 0.10$). Although the potential for K to influence winter injuries is complicated by its interaction with N, N–K combinations were similar in their effect on cold tolerance and disease. Low temperature survival (LT₅₀) and disease severity among the 15 N–K treatment combinations were positively correlated ($r = 0.54$, $P \leq 0.05$). Tissue (crown) moisture is a predisposing condition with both winter inju-

Fig. 7. Predicted tissue K concentration (2002-03 average) of perennial ryegrass fertilized with varying rates of N and K: Predicted tissue K = $3.23 - 0.11\text{NL} - 0.01\text{NQ} + 0.02\text{NC} + 0.09\text{KL} - 0.02\text{KQ} + 0.01\text{NL} \times \text{KL} - 0.02\text{NL} \times \text{KQ} + 0.01\text{NQ} \times \text{KL} - 0.01\text{NQ} \times \text{KQ}$. $R^2 = 0.975$ ($P \leq 0.01$).

ries, and like low temperature kill, gray snow mold injury was highly correlated with tissue moisture content ($r = 0.78$, $P \leq 0.001$).

N and K for maximum cold survival. Lethal killing temperature for survival in perennial ryegrass ranged from -9.0 to -13.6 °C in response to N and K (Table 3). Maximum low temperature tolerance was associated with N applied at low ($49 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) to moderate ($147 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) levels in combination with annual K rates ranging from moderately high ($245 \text{ kg} \cdot \text{ha}^{-1}$) to high ($441 \text{ kg} \cdot \text{ha}^{-1}$). The lowest median lethal temperature of -13.6 °C in our study (Table 3) was achieved by applying 147 kg N with K at $245 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$. These N-K combinations associated with maximum cold survival are in contrast to the 2 to 1 (N to K) ratio suggested by Beard and Rieke (1966) and Hurto and Troll (1980). Nitrogen rates ranging from 49 to $245 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ are routinely applied to perennial ryegrass. These yearly N rates when applied in combination with the highest levels of K used in our study caused no detrimental effects on either winter disease or low temperature hardiness. Alternatively, N when applied on an annual basis ranging from 343 to $441 \text{ kg} \cdot \text{ha}^{-1}$, the potential for winter kill (freeze stress and disease) increased with K (Figs. 2 and 6). As reported earlier, winter injuries in response to N and K were closely associated with shoot growth rate and crown moisture, and growth rate (Fig. 5) was highly correlated with tissue K concentrations (Fig. 7) ($r = 0.89$, $P \leq 0.001$).

Monroe et al. (1969) reported that added K and high N increased the K content of Kentucky bluegrass leaf tissues in sand cultures. Similarly, we found that tissue K concentrations increased significantly with N and K (Tables 4 and 5, Fig. 7). Potassium in tissues ranged from $28.6 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$ ($49\text{N}-49\text{K} \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) to $35.9 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$ ($441\text{N}-245\text{K} \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) (Table 4). These K concentrations are about three times the minimum concentration of $10 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$ suggested for K deficiency (Turgeon, 2002). Potassium response to N and K was strongly linear ($P \leq 0.001$, Table 5). However, a significant N by K interaction ($\text{NL} \times \text{KL}$) was

Fig. 8. Predicted exchangeable soil K (2002-03 average) of perennial ryegrass fertilized with varying rates of N and K: Predicted soil K = $183 - 9.5 \text{NL} - 0.6 \text{NQ} - 1.5 \text{NC} + 49.3 \text{KL} - 3.3 \text{NL} \times \text{KL} - 0.3 \text{NQ} \times \text{KL} - 2.4 \text{NC} \times \text{KL}$. $R^2 = 0.996$ ($P \leq 0.001$).

detected, suggesting that K concentrations in tissues in response to K fertilization was N dependent. Generally, significant increases in tissue K in response to K fertilization were only observed when fertilized with N at 343 to $441 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$. At these N rates, K at $49 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ was growth limiting, so shoot growth and winter kill increased with K. Maximum shoot growth, tissue K, and winter disease generally occurred at 343 and 441 kg N when fertilized with K at $245 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$. These yearly N rates are typically not recommended for cool-season turfgrass. However, the data suggests that over fertilization with water soluble N in the fall period (i.e., 98 and $49 \text{ kg} \cdot \text{ha}^{-1}$ in September and October, respectively) in combination with K at $245 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ and higher may increase freeze stress injury or low temperature disease.

Tissue K concentrations and soil exchangeable K levels were not correlated ($r = 0.11$). Exchangeable soil K increased linearly with K fertilization (Table 5, Fig. 8) and the Morgan extractable K levels (2-year average across N) ranged from $134 \text{ mg} \cdot \text{kg}^{-1}$ ($49 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) to $232 \text{ mg} \cdot \text{kg}^{-1}$ ($441 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) (Table 4). Exchangeable soil K decreased linearly with N fertilization as soil pH decreased (data not shown). Potassium fertilization is not recommended according to the University of Massachusetts Soil and Tissue Testing Laboratory when soil extractable K levels exceed about $125 \text{ mg} \cdot \text{kg}^{-1}$. No shoot growth response in perennial ryegrass was observed in this study with increasing soil exchangeable K at N rates of $245 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ and lower. At these N rates, soil exchangeable K of $135 \text{ mg} \cdot \text{kg}^{-1}$ (and possibly lower) were more than adequate for maintaining optimum shoot growth (Table 4). However, maximum cold hardiness was achieved at low to moderate N rates (49 and $147 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$) fertilized with K at 245 to $441 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$, which corresponded to soil extractable K levels ranging from 200 to $260 \text{ mg} \cdot \text{kg}^{-1}$. Conversely, at N rates of $343 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$ and higher, the rate of shoot growth peaked with K at $245 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{yr}^{-1}$, and soil exchangeable K levels ranged from 164 to $179 \text{ mg} \cdot \text{kg}^{-1}$ (Table 4). At these high

N rates, winter injuries from freeze stress and low temperature fungi increased with K fertilization at soil exchangeable K levels of about $125 \text{ mg} \cdot \text{kg}^{-1}$ and higher (Table 4). These results indicate at low to moderate N rates, higher soil extractable K beyond those levels typically recommended for optimum shoot growth may provide some benefit in enhancing perennial ryegrass cold hardiness.

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