Growth temperature effects on nitrogen concentration in shoots and roots of seven temperate grass species

GUDNI THORVALDSSON[1]

AND

H. TAPANI KUNELIUS[2]

[1]*Agricultural University of Iceland, Keldnaholti, 112 Reykjavík, Iceland*
E-mail: gudni@lbhi.is
[2]*Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Avenue, Charlottetown, Prince Edward Island, CIA 4N6 Canada*
E-mail: kuneliust@agr.gc.ca

ABSTRACT

The effect of temperature on nitrogen concentration of seven grass species (*Poa pratensis* L., *Phleum pratense* L., *Alopecurus pratensis* L., *Lolium perenne* L., *Festuca rubra* L., *Deschampsia caespitosa* L. Beauv. and *Festuca pratensis* Huds.) was investigated in a growth chamber experiment. The grass species were sown in pots in a greenhouse and placed in three growth chambers with day/night temperatures at 9/5, 13/9 and 17/13 °C when the seedlings were either two weeks old (5-8 cm high) or five weeks old (14-37 cm high). Three pots of each species and temperature were harvested at weekly intervals during the subsequent three weeks, as well as three pots of each species from the greenhouse. Nitrogen concentration was on average, 53% higher in shoots than in roots. The difference in nitrogen concentration between shoots and roots increased with increasing temperature. On average 79% of the nitrogen found in the plants were in the shoots. The highest amount of nitrogen was found in *P. pratense* but the lowest in *F. rubra.*

Key words: growth chamber, nitrogen uptake, temperature, shoots, roots, grasses

YFIRLIT

Áhrif hita á innihald niturs í rótum og ofanjarðarhlutum sjö norðlægra grasstegunda

Áhrif hita á nitur í blöðum og rótum hjá sjö grastegundum (vallarsveifgrasi, vallarfoxgrasi, háliðagrasi, fjölæru rýgresi, túnvingli, snarrótarpunti og hávingli) voru athuguð í ræktunarklefatilraun. Þessum tegundum var sáð í potta í gróðurhúsi og þær fluttar á tveimur mismunandi tímum í þrjá ræktunarklefa, þar sem hitinn (dagur/nótt) var 9/5, 13/9 og 17/13 °C. Fyrri hópurinn var settur í ræktunarklefa þegar plönturnar voru tveggja vikna gamlar (5-8 sm á hæð) en sá síðari þegar þær voru fimm vikna gamlar (14-37 sm á hæð). Þrír pottar af hverri tegund og úr hverjum ræktunarklefa voru uppskornir í hverri viku næstu þrjár vikurnar. Samtímis voru þrír pottar úr gróðurhúsinu uppskornir til samanburðar. Niturprósenta var að meðaltali 53 % hærri í ofanjarðarhluta plantnanna en rótum. Munurinn í niturprósentu milli ofanjarðarhluta og róta jókst með auknum hita. Að meðaltali voru 79% af nitri plantnanna í ofanjarðarhlutanum. Heildarniturupptaka var mest í vallarfoxgrasi en minnst hjá túnvingli.

INTRODUCTION

Hydrogen, carbon and oxygen are the most abundant nutrients in plants and nitrogen (N) is the fourth most abundant. As N is the main nutrient in chemical fertilizers, it is of great importance to minimize N losses and get as much out of the N fertilizers as possible. To this end it is important to gain a good understanding of the N accumulation and distribution in plants.

The accumulation and distribution of N in crops depends on N supply, plant genotype, and environmental factors, and N is diluted by DM growth (Gillet 1982). Concentration of total N varies between plant organs and changes within the same organ according to stage of development. Young grass shoots can contain more than 4 % total N in stem and leaves, but at maturity less than 1 %; Clearly, N uptake does not correlate directly with the growth rate (Langille & Calder 1968, Thorvaldsson & Andersson 1986, Lindberg & Lindgren 1988, Thorvaldsson et al. 2000).

The objective of the present study was to quantify the effects of temperature on nitrogen uptake and nitrogen concentration of different grass species and test whether the species react similarly to increasing temperature. The experiment was also used to quantify the temperature effect on growth of these grass species (Thorvaldsson & Martin 2004) and effects of temperature on digestibility and fibre content (Thorvaldsson, Tremblay & Kunelius unpublished).

MATERIAL AND METHODS

Species and soil

Seven cool-season grass species were sown in 80 pots each, in a greenhouse at the Nova Scotia Agricultural College, Truro, NS, Canada (45° 22' N - 63° 16' W). The species, cultivars, their origin, seeding dates and emergence dates are given in Table 1. Each pot was 127 mm in diameter at the top and 90 mm at the bottom and 118 mm deep. A peat-based growing medium designed for the cultivation of horticultural plants (Pro-Mix BX from Premier Horticulture LTD, QC) was used. The medium is a blend of Canadian sphagnum peat moss (80 %), perlite, vermiculite, and dolomitic and calcitic limestone. Three or four seeds were placed in each of 9 equally distributed 2 mm deep holes in each pot and thinned to 9 plants per pot after emergence of seedlings.

The species were seeded at different times (Table 1) as some species germinate and grow faster than others. Seeds of *F. pratensis* Huds. and *L. perenne* L. were seeded six days later than originally planned due to late receipt of seed. Seedling establishment was good for all species except for *D. caespitosa* L. Beauv. which had a too low seed germination in some pots.

On 22 May, 2 June, and 14 June, each pot received 0.064 g N, 0.028 g P and 0.051 g K with trace amounts of boron (B), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn).

Growth chambers and greenhouse

Two of the growth chambers were of the same size, 185 cm x 77 cm. The growth chamber with the highest temperature was 248 cm x 125 cm and of a different type. Growth chambers were provided with cool-white fluorescent tubes supplemented with incandescent bulbs. The light level in the growth chambers was measured weekly with an LI COR quantum sensor, which measures photosynthetically active radiation (PAR) in the 400-700 nm wave band. The light level was kept as steady as possible and adjusted by moving the lights up or down. The lights were 90 - 100 cm above the floor of the chambers where the pots were standing. The measurements at leaf height were for the experimental period, on average, 152 $\mu mol\ m^{-2}\ s^{-1}$. Relative humidity in the growth chambers was about 80 % at 9 °C, 70 % at 13 °C and 60 % at 17 °C. Plants were watered daily, as required. The average temperature in the greenhouse was 21 °C. The light was not measured.

Experimental design

At the beginning of the treatment period (29

Table 1. Cultivar, origin, seeding date and beginning of emergence for each species.

Species	Cultivar	Origin	Seeding date	Beginning of emergence
Alopecurus pratensis L.	Seida	Norway	8 May	14 May
Deschampsia caespitosa L. Beauv.	Unnur	Iceland	3 May	10 May
Festuca pratensis Huds.	Norild	Norway	16 May	22 May
Festuca rubra L.	Sámur	Iceland	7 May	13 May
Lolium perenne L.	Svea	Sweden	16 May	21 May
Phleum pratense L.	Adda	Iceland	9 May	13 May
Poa pratensis L.	Fylking	Sweden	2 May	9 May

May) three pots of all grass species were harvested from the greenhouse. On the same dates, nine pots (3 harvest dates x 3 replicates) of each species (Group 1) were moved into each of three growth chambers at different temperatures. The day/night temperature regimes were 9/5, 13/9 and 17/13°C with a day length of 19 hours as most of the cultivars are from regions with long days during the summer. The pots were rotated two to four times weekly during the experimental period. Three pots of each grass species and growth chamber were harvested each week for the subsequent three weeks, as well as three pots of each species from the greenhouse. However, analyse of N were only performed on samples from the third and the sixth week. The plants from the greenhouse provided a reference for growth with light and temperature more similar to outdoor conditions. The rate of germination in the *Deschampsia* pots was low and some pots had to be discarded. Therefore, this species could only be harvested from the greenhouse in the last week, whereas in the growth chambers it was consistently harvested.

Three weeks later, on June 19 and 20, a new group of pots (Group 2) was placed in the growth chambers from the greenhouse and the same harvest procedure was followed. The remaining five pots of each species were kept in reserve.

Root mass was harvested before the start of each group and at the last harvest date of each group. Tillers were cut as close to the soil surface as possible, and the small stubble remaining was weighed with roots. Roots were separated from the soil by washing with water.

The three pots of the same treatment were treated as independent replicates in the statistical analysis, although they grew in one chamber. Any differences between chambers other than those due to temperature were confounded with temperature. The error component due to chambers was expected to be small, but in the case of small temperature effects, spurious results due to lack of replication of chambers can not be excluded.

Statistical analyses were carried out by GenStat® (Genstat 5 Committee 1993). Analyses of variance were performed for each harvest date separately and differences between growth chambers and between species tested for statistical significance. Greenhouse treatment was analysed separately.

Total nitrogen (N) was determined by combustion method with the LECO-CNS-1000 analyzer (LECO Instruments Ltd., Mississauga, ON).

RESULTS AND DISCUSSION

Plant development

When the plants in Group 1 were put into the growth chambers they were on average 5-8 cm long (measured to the top of the leaves) with 1-2 leaves. The plants in Group 2 had 2-3 leaves when they were put into the chambers and the height was 14-37 cm, *F. rubra* L. being the lowest and *A. pratensis* L. the highest. Plant height increased significantly with increasing temperature and the difference between species

Table 2. Nitrogen concentration (% of DM) in shoots at three harvest dates and after three weeks at different temperatures. Species and temperature differed significantly (P<0.001) at both harvest dates. Species x temperature interaction was significant for 19 June.

Species	Harvested 29 May	Harvested 19 June				Harvested 10 July			
		Growth chambers			Green-	Growth chambers			Green-
		9/5°	13/9°	17/13°	house	9/5°	13/9°	17/13°	house
Alopecurus pratensis	8.88	5.06	5.42	5.20	5.33	2.23	1.75	1.83	1.88
Deschampsia caespitosa	7.31	5.42	5.74	5.54	5.93	2.87	2.66	2.60	1.99
Festuca pratensis	too small sample	5.58	6.16	6.34	6.33	2.64	2.52	2.14	1.85
Festuca rubra	7.26	5.45	6.04	5.73	6.25	3.70	3.64	3.50	2.98
Lolium perenne	5.67	4.72	5.60	5.87	5.80	2.26	2.36	2.16	1.83
Phleum pratense	7.01	5.55	5.90	5.49	5.58	2.33	2.52	2.76	1.83
Poa pratensis	6.72	5.54	6.03	5.85	5.95	2.80	3.04	2.94	2.06
Average	7.14	5.33	5.84	5.72	5.88	2.69	2.64	2.56	2.06
Standard deviation (all temperatures)		0.14			0.32				

was also significant (data not presented).

The plants remained in a vegetative state for most of the experiment. The first nodes appeared in a few plants at the last harvest date for *P. pratensis L., A. pratensis, D. caespitosa* and *F. pratensis. In P. pratense L*. a few nodes were also present on 3 July in plants grown in the greenhouse and on 10 July in plants grown in chambers at 13/9 °C and 17/13 °C. Two timothy tillers had formed heads in the greenhouse by the last harvest date. All cultivars tested came from Scandinavia and Iceland, where the days during summer are longer than in Nova Scotia. This, together with the fact that the plants grew from seeds without exposure to cool temperatures and short days, decreased the probability of reproductive growth.

Nitrogen

Tables 2 and 3 show the concentration of N in shoots and roots at different temperatures and harvest dates. As expected, the content was

Table 3. Nitrogen concentration (% DM) in roots at three harvest dates and after three weeks at different temperatures. Species and temperature differed significantly (P<0.001) at both harvest dates. Species x temperature interaction was significant for 19 June.

Species	Harvested 29 May	Harvested 19 June				Harvested 10 July			
		Growth chambers			Green-	Growth chambers			Green-
		9/5°	13/9°	17/13°	house	9/5°	13/9°	17/13°	house
Alopecurus pratensis	4.82	4.42	4.28	3.53	3.34	1.56	1.10	1.02	1.02
Deschampsia caespitosa	4.28	4.15	4.17	3.67	3.33	1.92	1.63	1.54	1.00
Festuca pratensis	3.62	4.86	4.43	3.28	3.56	1.89	1.52	1.17	1.02
Festuca rubra	4.31	4.72	4.29	3.78	3.58	2.53	2.20	2.22	1.30
Lolium perenne	3.93	4.82	4.45	3.41	2.91	1.47	1.33	1.17	1.00
Phleum pratense	4.74	4.51	3.97	3.27	2.73	1.54	1.54	1.44	0.93
Poa pratensis	3.59	4.11	4.06	3.54	2.75	1.69	1.69	1.55	1.03
Average	4.18	4.51	4.24	3.50	3.17	1.80	1.57	1.45	1.04
Standard deviation (all temperatures)		0.26			0.21				

Table 4. Total nitrogen in the grasses (g in shoots and roots pot^{-1}) after three weeks at different temperatures and at two harvest dates. Species and temperature, and their interaction differed significantly (P<0.001) at harvest on 19 June. Species differed significantly (P<0.001) on 10 July.

	Harvested 19 June				Harvested 10 July			
	Growth chambers			Green-	Growth chambers			Green-
Species	9/5°	13/9°	17/13°	house	9/5°	13/9°	17/13°	house
Alopecurus pratensis	0.053	0.090	0.133	0.188	0.243	0.233	0.227	0.273
Deschampsia caespitosa	0.049	0.087	0.114	0.142	0.190	0.197	0.221	0.202
Festuca pratensis	0.023	0.062	0.129	0.171	0.233	0.249	0.244	0.270
Festuca rubra	0.038	0.053	0.069	0.097	0.158	0.168	0.127	0.155
Lolium perenne	0.049	0.094	0.179	0.175	0.243	0.273	0.223	0.291
Phleum pratense	0.077	0.114	0.182	0.234	0.292	0.267	0.272	0.307
Poa pratensis	0.063	0.109	0.136	0.180	0.212	0.226	0.229	0.235
Average	0.050	0.087	0.135	0.170	0.224	0.2300.220		0.248
Standard deviation (all temperatures)	0.013			0.030				

very high when the plants were small but as the biomass increased the nitrogen was diluted in an increasing amount of dry matter (Deinum 1966, Gillet 1982). The average yield of shoots increased from 0.08 g pot^{-1} to 0.83 (9°C), 1.39 (13°C), 1.90 (17°C) and 2.62 (greenhouse) g pot^{-1} during the three weeks in Group 1. In Group 2 the DM yield of shoots increased from 2.62 g pot^{-1} to 6.11 (9°C), 6.04 (13°C), 6.36 (17°C) and 8.57 (greenhouse) during the three weeks (Thorvaldsson & Martin 2004).

The concentration of nitrogen was always higher in the shoots than in the roots, in average 53 % higher, which was very similar to results from a growth chamber experiment with timothy (Thorvaldsson, 1992). However, the difference in nitrogen concentration between shoots and roots increased with temperature, being lowest at 9°C and highest in the green house. The difference in nitrogen concentration between shoots and roots increased with time, was greater on 10 July than 19 June. The difference in nitrogen concentration among the species was to a large extent dependent on the difference in DM yield. Whitehead (1970) found that N concentration in the roots of *L. Perenne, P. pratense and D. glomerata* averaged 1.6% and the differences among species were small even though *P. pratense* was lowest. Larsson and Steen (1984) found on average a nitrogen content of 1.87 % in roots for four grass species at different growth stages and three nitrogen levels, but the differences between species were non-significant.

The total amount of nitrogen in shoots and roots at different temperatures and two harvest dates are shown in Table 4. The differences between species were significant at both harvest dates. *P. pratense* was highest and *F. rubra* lowest in total nitrogen at both harvest dates, and on average *P. pratense* absorbed twice as much N as *F. rubra. L. perenne* absorbed on average 13% less N than *P. pratense* and *A. pratensis*, and *P. pratensis* and *F. pratensis* around 20% less than *P. pratense. D. caespitosa* absorbed 30% less N than *P. pratense. F. pratensis* absorbed relatively little N in Group 1, probably because the plants were small in the beginning of the experiment. Genotypic variation in mineral accumulation has been found within many crop plants (Perby & Jensen 1983). Even though the species absorbing most N were also among the highest yielding species, the yield pattern was not exactly the same as the one for N uptake. The yield of *A. pratensis* was almost the same as *P. pratense* even though the N uptake was 18 % less and *P. pratensis* gave a relatively lower DM yield than

Table 5. Proportion of total nitrogen in the grasses found (%) in the shoots after three weeks at different temperatures and at two harvest dates. Species and temperature differed significantly (P<0.001) at harvest on 19 June. Temperature differed significantly (P<0.001) on 10 July.

	Harvested 19 June				Harvested 10 July			
	Growth chambers			Green-	Growth chambers			Green-
Species	9/5°	13/9°	17/13°	house	9/5°	13/9°	17/13°	house
Alopecurus pratensis	70.2	72.3	78.2	83.9	79.7	76.7	78.4	79.2
Deschampsia caespitosa	71.7	75.3	81.3	83.3	80.8	80.2	83.1	76.5
Festuca pratensis	73.1	76.7	85.9	84.5	79.2	80.9	81.3	78.8
Festuca rubra	68.5	74.8	80.1	80.6	80.5	83.6	83.6	75.8
Lolium perenne	72.6	76.1	83.1	85.0	81.1	80.9	80.7	75.4
Phleum pratense	68.1	74.2	78.4	85.7	79.8	79.0	81.9	80.2
Poa pratensis	73.1	76.4	82.2	86.6	80.2	79.8	81.9	75.1
Average	71.0	75.1	81.3	84.2	80.2	80.2	81.6	77.3
Standard deviation (all temperatures)	2.16			1.96				

N yield. Some N was mineralized from the soil as more N was absorbed than was provided with fertilization.

It is interesting to note that the difference between temperature treatments in N uptake was highly significant on 19 June but not on 10 July (Table 4). The same results were found for DM yield in this experiment (Thorvaldsson & Martin 2004), which was significantly affected by temperature in the younger plants (Group 1) but not in the older plants (Group 2). The explanation could be that young and small roots are more dependent on temperature for N uptake than the large and older ones. It is also interesting to see that at 17/9 °C, N uptake of *L. perenne, F. pratensis* and *P. pratense* increased faster than the uptake of the other species.

Table 5 shows the proportion of total nitrogen of grass species found in the shoots at the different temperatures and harvest dates. The proportion of nitrogen found in shoots varied between 68.1 and 86.6 % (average 78.9%). In a growth chamber study of timothy in Sweden, the percentage of nitrogen found in shoots was on average 71 % (Thorvaldsson 1992). In a study on wheat, 70 % of absorbed nitrogen was detected in shoots 18 days after sowing but 81% 26 days after sowing (Simpson et al. 1982). Hansson et al. (1987) found 21 and 28% of plant nitrogen in the roots of barley fertilized with N and in unfertilized barley, respectively. On 19 June there was a clear difference between temperatures in the proportion of nitrogen found in shoots, which increased with increasing temperature, which was not the case on 10 July. The difference between species in proportion of N in shoots was significant on 19 June. The average proportion was highest for *F. pratensis* (80.1) and lowest for *F. rubra* (76.0). On 10 July all the species were close to 80 % and the difference not significant.

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