

Effects of nitrogen on growth properties and phenology of silver birch (*Betula pendula* Roth)

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ABSTRACT: Effects of stress caused by differentiated inputs of nitrogen after the application of ammonium nitrate (NH_4NO_3) on silver birch (*Betula pendula* Roth) were monitored in a greenhouse. The applied ammonium nitrate did not affect pH values but increased differently the content of nitrogen in soil and leaves. With increased inputs of nitrogen the height and diameter increment of birch also decreased, budbreak was delayed and the autumn leaf-fall slowed down. Frost heaving of shoots occurred particularly in the lower half of the birch stem. With the increasing content of nitrogen in leaves the content of phosphorus decreased and the level of potassium increased.

Keywords: nitrogen; silver birch; stress; phenology of budbreak; leaf-fall; frost heaving

The emissions of nitrogen pollutants (NH_3 , NO_x) in Central Europe stopped in the second half of the 1990s and an increase in dry and wet depositions of nitrogen was found out at some locations (FADRHOŇSOVÁ et al. 2002; OULEHLE et al. 2006; PRIETZEL et al. 2006). A critical nitrogen load with a short-term ($10 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) and long-term ($5 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) effect was reported by CHADWICK and HUTTON (1990) and 7–20 $\text{kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ by GRENNFELT and NILSSON (1988). On 96% of the area of the Krušné hory Mountains, the critical amount ($15 \text{ kg}\cdot\text{N}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) was exceeded and so eutrophication and worsening of the health condition of forest stands occurred (ŠRÁMEK et al. 2008).

Acidification caused by nitrogen oxides is a long-term, cumulative and dynamic process and its consequences are manifested with a delay (ZAPLETAL et al. 2003). Synergic effects of SO_2 and NO_2 cause the reduction of biomass and of the root system as well as the precocious leaf fall in *Betula pendula* Roth and *Betula pubescens* Ehrh. (WRIGHT 1987).

At the lack of nitrogen, chlorosis occurs, small leaves with limited activities of stomata are developed (LARCHER 1976), dwarf growth occurs, precocious yellowing of leaves (LARCHER 1976;

PODRÁZSKÝ 2001), the growing season is shortened (late leaf unfolding, precocious termination of growth and leaf fall) (PODRÁZSKÝ 2001).

The increased content of nitrogen accelerates the growth of aboveground organs at the expense of roots reducing the stability of trees (KREUTZER 1994), extends the growing season of trees increasing their sensitivity to frost (MARSCHNER 1995; THOMAS, BLANK 1996; KAŇOVÁ, KULA 2004). With the input of nitrogen, its content in leaves increases, a disproportion in the growth of roots and aboveground biomass increases, the effectiveness of photosynthesis and vitality decrease, the content of sulphur, potassium and magnesium in leaves declines (MARSCHNER 1995; HEILMEIER et al. 2000). A negative correlation between nitrogen depositions and concentrations of phosphorus in leaves has been found for example for beech, Norway spruce (BRAUN et al. 2010) and Scots pine (PALÁTOVÁ, MAUER 1998; BOROWIEC et al. 2005). At the > 2% nitrogen concentration in leaves the growth of beech seedlings stagnates and its resistance to frost decreases (SPINNER et al. 1996). In birch, frost damage occurs at nitrogen concentrations > 2.5% (KAŇOVÁ, KULA 2004).

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Nitrogen represents 2–4% in the biomass of herbs, 1–4% in leaves of deciduous species and 1–2% in needles (LARCHER 1976). BERGMANN (1988) determined the optimum level of nitrogen in leaves of birch (2.5–4%), oak (2–3%), lime (2.3–2.8%), spruce (1.35–1.7%), larch, maple and ash (1.6–2.3%), beech (1.9–2.5%). Nitrogen accumulates particularly in young shoots, leaves, buds, seeds and storage organs (LARCHER 1976). The response to nitrogen fertilization in forest woody species (*B. pendula*, *Fagus sylvatica* L., *Picea abies* [L.] Karst., *Abies alba* Mill.) is not always the same (POKORNÝ, ŠALANSKÁ 1999; HEILMEIER et al. 2000; PALÁTOVÁ et al. 2008). In leaves, the nitrogen concentration decreases during the growing season (HRDLIČKA, KULA 2001) and as a consequence of ageing in the plant tissues (FENG et al. 2008; ŠRÁMEK et al. 2009).

The above-limit content of nitrogen in leaves can inhibit the uptake and assimilation of some ions, especially K^+ , Mg^{2+} , Ca^{2+} (INGESTAD 1990; MARSCHNER 1995), reduce photosynthesis, increase the content of water in leaves, vitality of trees (THOMAS et al. 2006). Nitrogen is an important stress factor of forest ecosystems (KULHAVÝ, FORMÁNEK 2002).

The aim of the present paper is to determine the effects of differentiated inputs of nitrogen into soil on the chemistry of leaves, growth properties and phenology of birch *B. pendula*.

METHODS

In April, one-year birch plants (*B. pendula*) were planted into containers (volume 10 l) with soil substrate from the Cambic mineral horizon of a forest soil poor in nutrients (Table 1). One litre of peat was poured by a single application on the soil surface around all plants with an aim to decrease their mortality. The planting stock originated from the fourth forest vegetation zone (VIEWEGH et al. 2003) of the Bohemian-Moravian Upland forest region. After three weeks, 128 rooted plants were placed into a plastic greenhouse (Řečkovice forest nursery, altitude 220 m a.s.l.).

The microclimate of ventilated plastic greenhouses monitored by a HOBO temperature-humidity sensor (produced by AMET Velké Bílovice Ltd., Czech Republic) deviated from temperatures outside the greenhouse by 0.5–1°C (Czech Hydrometeorological Institute, Brno-Tuřany, Czech Republic). Minimum temperatures reached –12.9°C in January 2007, –14.1°C in February 2008, –16.7°C

in January 2009 and –18.1°C in December 2009. The occurrence of late frosts (May 2007, –2.4°C) and the relatively early terminated growing season (October 2007, –3.4°C) with related winter frosts are characteristic of the site. High summer temperatures cause precocious defoliation of birch stressed by an increased content of nitrogen. The birch trees were watered using the same intensity in all treatments as necessary in the growing season and in the winter season, soil moisture was provided by watering or snow.

The amount of applied ammonium nitrate to a plant was determined in such a way to achieve the differentiated content of nitrogen in birch leaves for the following study of the response of selected phytophages (KULA et al. 2012a, b, c). Changes in the content of nitrogen were induced by an application of 0.5 g (T1), 1 g (T2) and 1.5 g (T3) of ammonium nitrate NH_4NO_3 to a plant on the moist soil surface with the following gradual dissolution by watering. In each of the treatments, there were 32 plants. The fertilization was realized repeatedly in monthly intervals in the year of planting (2006) four times and in 2007 and 2008 five times. In 2009, no fertilization was carried out. Determined applications in treatments T1–T3 corresponded to nitrogen doses at the amount of 178–357–535 $kg \cdot ha^{-1} \cdot year^{-1}$. Thereby, a differentiated stress was purposefully caused.

Growth properties of birch were evaluated through its height and root collar diameter. The phenology of budbreak was monitored at buds of sample tree branches of each of the trees (in total 1,280 buds) in categories 0 to 5 (0 – bud with an intact bud scale, 1 – bud with an intact bud scale and showing through green colour, 2 – apical part of a bud with a green tip, 3 – a disturbed bud top exceeding 1 mm, 4 – unfolding leaves, 5 – developed leaves). The phenology of leaf fall was quantified for the whole plant by the degree of defoliation (0, 1–5, 6–25, 26–50, 51–75, 76–100%) and colour changes, namely visually in two categories (yellowing leaves and yellow leaves).

The soil used in this experiment was analysed before its establishment. At the end of the growing season, a composite sample was analysed by an accredited laboratory (Laboratoře Morava Ltd., Studénka, Czech Republic) that was taken from eight plants for each of the treatments (T0–T3). The C/N ($C_{ox} = C_{tot}/N_{tot}$) ratio was determined, the content of available nutrients [$N-NO_3^-$, $N-NH_4^+$ in the extract of a 1% solution of potassium sulphate (K_2SO_4)] and the pH value (KCl) (Table 1).

Fully developed birch leaves were taken annually at the turn of August and September from the

Table 1. Characteristics of the soil substrate

Treatment	N-NH ₄ ⁺	N-NO ₃ ⁻	Σ N-min	pH (KCl)	N _{tot}	C _{ox}	C:N
	(mg·kg ⁻¹)				(%)		
Pretreatment							
T0							
T1							
T2	2.81	8.23	11.03	3.47	0.08	2.08	25.4
T3							
2006							
T0	6.12	5.68	11.8	3.48	0.10	1.89	18.9
T1	31.2	28.3	59.5	3.38	0.08	1.64	20.5
T2	67.1	44.1	111.2	3.40	0.09	2.12	23.6
T3	75.7	59.6	135.3	3.37	0.10	1.48	14.8
2007							
T0	7.51	3.41	10.9	3.59	0.07	2.26	32.3
T1	15.7	10.2	25.9	3.49	0.08	2.19	27.4
T2	68.6	28.2	96.8	3.48	0.08	1.99	24.9
T3	93.4	48.4	141.8	3.51	0.09	1.99	22.1
2008							
T0	9.21	4.85	14.1	3.74	0.08	1.83	22.9
T1	11.8	8.94	20.7	3.72	0.08	1.66	20.8
T2	31.1	27.0	58.1	3.65	0.09	1.36	15.1
T3	44.1	46.3	90.4	3.64	0.09	1.78	19.8
2009							
T0	4.15	4.15	8.30	3.52	0.13	2.70	21
T1	3.93	5.13	9.06	3.54	0.11	2.92	26
T2	12.09	10.08	22.2	3.45	0.08	2.87	34
T3	20.63	17.73	38.4	3.58	0.10	2.72	27

T0 – application of 0 g NH₄NO₃, T1 – 0.5 g NH₄NO₃, T2 – 1 g NH₄NO₃, T3 – 1.5 g NH₄NO₃

whole tree profile, dried at a temperature of 70°C and subsequently, nitrogen was determined by Kjeldahl (Kjeltec analyser UNIT 2300).

For statistical evaluations, the Statistica Cz (StatSoft 2007) program was used. The difference among treatments T0–T3 was evaluated using ANOVA and LSD tests; in case that the condition of the normal distribution of data was not satisfied, non-parametric statistics (Kruskal-Wallis test) was used.

RESULTS

Site and growth conditions

Growth conditions and stress reactions of birch were affected by fertilization (ammonium nitrate) in 2006–2008. Statistically significant differences in the content of nitrogen in soil were found between

the control (T0) and fertilized treatments (T1–T3) (Table 1). The amount of N_{tot} in treatments T0–T3 was identical in monitored years. The content of oxidizable carbon was increased. The fluctuation of C/N ratio was caused by the decomposition of peat due to high doses of fertilization and the leaching of carbonaceous substances from peat to lower layers of soil (organomineral horizon), which served for sampling. This process did not affect the achieved specific differentiated levels of nitrogen in leaves of a nutrient plant. The values of pH_{KCl} in soil did not markedly increase after the three-year application of ammonium nitrate (from 3.47 to 3.64–3.74) and subsequently declined to the initial value after fertilization was interrupted.

With the increasing amount of nitrogen in birch leaves the content of phosphorus decreased and the level of potassium increased. In other elements (Mg, Ca, S), no linear change in the content of ele-

Table 2. The content of elements in dry matter of leaves in particular treatments in 2008

Treatment	N (%)	P	Mg	Ca	K	S
T0	2.01	1.06	3.38	10.55	5.65	1.37
T1	3.15	0.41	3.13	8.45	5.41	1.86
T2	4.11	0.45	3.05	9.37	6.50	1.60
T3	4.13	0.32	3.06	9.68	7.90	1.43

T0 – application of 0 g NH₄NO₃, T1 – 0.5 g NH₄NO₃, T2 – 1 g NH₄NO₃, T3 – 1.5 g NH₄NO₃

ments due to the increased content of nitrogen was determined (Table 2). The basic hypothesis of a fast increase in the content of nitrogen in birch leaves during the growing season was proved by the analysis of its level in the leaf dry matter (August 2006). The amount of nitrogen increased non-linearly with the increasing rates of ammonium nitrate applied to the substrate, differences among treatments being statistically significant ($P < 0.001$, chosen significance level $\alpha = 0.05$). Statistically significant differences in the content of nitrogen in leaves were not observed only between T2 and T3 treatments (T0/24.71; T1/29.92; T2/32.14; T3/32.88 mg·g⁻¹) in the year 2006. In 2007–2009, there were statistically significant differences among all treatments (Fig. 1).

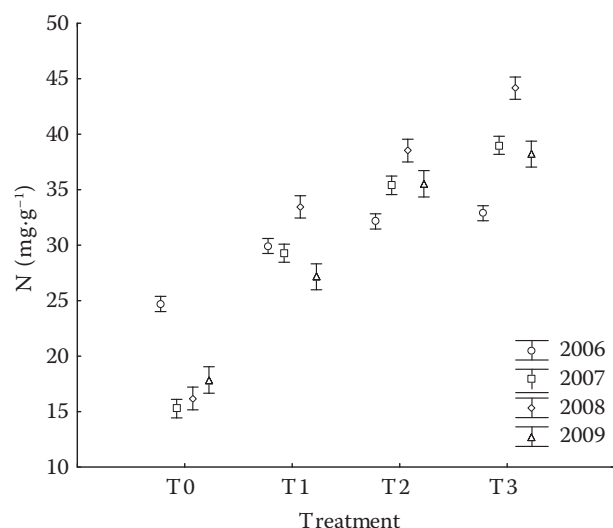


Fig. 1. The average content of nitrogen in the dry matter of birch (*B. pendula*) leaves (2006–2009) depending on the application of ammonium nitrate – treatments T0–T3 (T0 – application of 0 g NH₄NO₃, T1 – 0.5 g NH₄NO₃, T2 – 1 g NH₄NO₃, T3 – 1.5 g NH₄NO₃) (error bars depict 0.95 confidence intervals)

Effects of nitrogen on growth parameters of birch

At the end of the first growing season (September 2006), a statistically significant difference ($P < 0.001$, $\alpha = 0.05$) in the height of plants was found only between the control (84.6 cm) and treatments with nitrogen application (92.8–96.4 cm), but not between doses of nitrogen (T1–T3). The root collar diameter corresponds with findings mentioned above because a statistical difference ($P < 0.001$, $\alpha = 0.05$) was found only between the control (7.7 mm) and treatments with nitrogen (8.4–8.6 mm) after the first effect of nitrogen. At increased rates of nitrogen (1.5 g), a statistically insignificant decline in the height and diameter of plants was evident (Figs. 2 and 3).

At the end of the second growing season, statistically significant differences were found between the control and treatments with nitrogen application, as regards both height (control treatment 101.6 cm, other treatments 118.7–124.6 cm, $P < 0.001$, $\alpha = 0.05$) and diameter (control treatment 10.8 mm, other treatments 11.6–12.4 mm, $P = 0.000–0.022$, $\alpha = 0.05$).

As for height, a statistically significant difference ($P = 0.027$, $\alpha = 0.05$) was moreover noted between treatments T2 (124.6 cm) and T3 (118.7 cm) and for diameter between treatments T1 (12.4 mm) and T3 (11.6 mm) ($P = 0.017$, $\alpha = 0.05$) (Figs. 2 and 3).

The height increment determined in autumn 2008 proved the same trend as in the previous year as it was apparent from average heights (T0/122.6; T1/146.4; T2/150.3; T3/142.6 cm). The diameter increment in treatment T3 was inhibited by the high input of nitrogen so much that the values did not statistically differ from the control treatment (T0/12.2; T1/13.7; T2/13.2; T3/12.7 mm) (Figs. 2 and 3).

At a final measurement in autumn 2009, a statistically significant difference ($P = 0.000–0.008$, $\alpha = 0.05$) was noted in height among all treatments with the exception of T1 and T2 (T0/133.4; T1/160.6; T2/164.8; T3/149.8 cm). In the root collar diameter, a significant difference occurred only between treatments T0 × T1 ($P = 0.003$, $\alpha = 0.05$) and T1 × T3 ($P = 0.011$, $\alpha = 0.05$) (T0/13.2; T1/14.4; T2/13.9; T3/13.4 mm) (Figs. 2 and 3).

Phenology of budbreak

Already in the initial stage of budbreak (22. 3. 2007), statistically significant deviations occurred

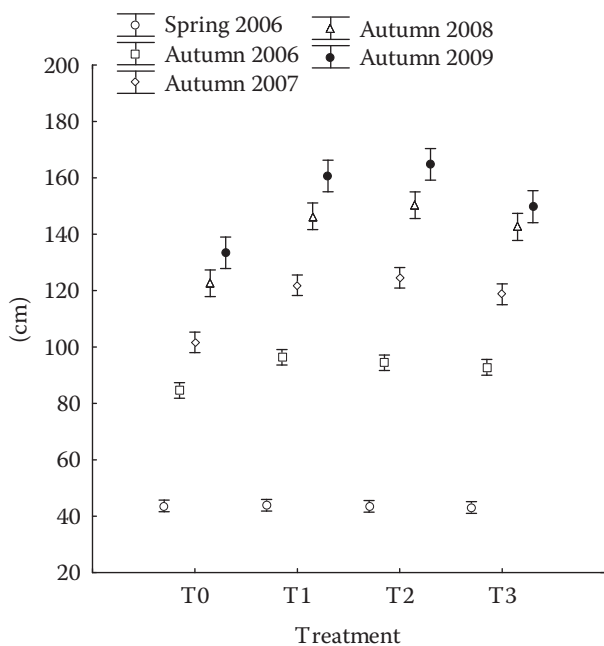


Fig. 2. The height of birch plants (*B. pendula*) in particular treatments (see Fig. 1) (error bars represent 0.95 confidence intervals) (2006–2009)

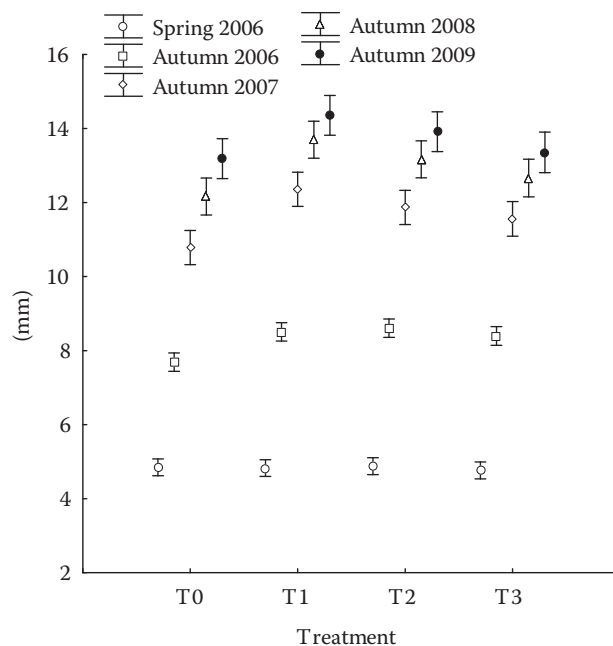


Fig. 3. The root collar diameter of birch plants (*B. pendula*) in particular treatments (see Fig. 1) (error bars represent 0.95 confidence intervals) (2006–2009)

within the pairs of treatments T0 and T1 ($P = 0.008$, $\alpha = 0.05$), T0 and T3 ($P = 0.004$, $\alpha = 0.05$), T1 and T2 ($P = 0.015$, $\alpha = 0.05$), T2 and T3 ($P = 0.007$, $\alpha = 0.05$). In the course of budbreak, a statistically significant deviation remained between the control and all treatments with the nitrogen input. The final stage of budbreak (12. 4. 2007) was characterized by a statistically significant deviation between the control and all treatments, which were stressed by nitrogen ($P < 0.001$, $\alpha = 0.05$) and between T1 \times T2 ($P = 0.021$, $\alpha = 0.05$) and T2 \times T3 ($P = 0.015$, $\alpha = 0.05$) (Fig. 4).

In 2008 at the end of March, a statistically significant deviation was noted among treatments T0 and T1, T2, T3 ($P < 0.001$, $\alpha = 0.05$). In the course of the first half of April, partial deviations were determined between T1 \times T2 and T1 \times T3 ($P = 0.000$ – 0.047 , $\alpha = 0.05$) (Fig. 4).

The course of budbreak in 2009 proves relationships mentioned above in such a way that in the first decade of April, statistical deviations were noted not only between the control treatment (T0) and all nitrogen treatments ($P < 0.001$, $\alpha = 0.05$) but also between treatments T1 \times T2 and T1 \times T3 ($P = 0.001$ – 0.020 , $\alpha = 0.05$) (Fig. 4).

The course of birch budbreak was slowed down in treatments affected by nitrogen in comparison with the control and between nitrogen-affected treatments (T1 \times T2 and T1 \times T3).

Phenology of leaf fall

No differences among particular treatments were noted in defoliation at the end of the first growing season (2006). The differences occurred only in subsequent growing seasons (2007–2009). In 2007, the defoliation occurred in the summer season. In the autumn aspect, when the leaf fall evaluation started, there was a statistically significant difference among individuals affected by higher inputs of nitrogen compared with the control. This trend was maintained until 25 October with partial deviations between treatments T1, T2, T3 (Fig. 5). In 2008, the leaf fall was affected by the degree of defoliation in the summer aspect and, therefore, it was significantly higher in treatments affected by the input of nitrogen as compared with the control ($P = 0.000$ – 0.035 , $\alpha = 0.05$) (Fig. 5) for the entire monitored period (25. 9.–31. 10.).

A trend of the higher level of defoliation in birch affected by nitrogen was proved in 2009 already in the third decade of September. Simultaneously, partial deviations appeared between treatments affected by nitrogen (T1 \times T2 and T1 \times T3) (Fig. 5).

With respect to the fall of leaves in the summer season, it is not possible to evaluate total deviation in the development of defoliation in the autumn season. Based on the colour change of senescent leaves, a statistically significant difference was

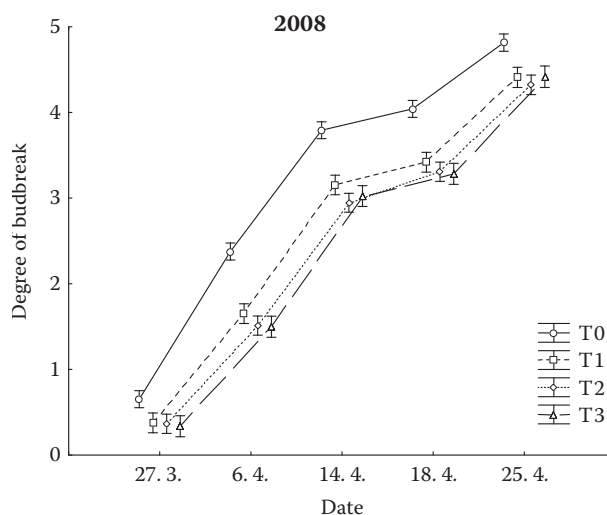
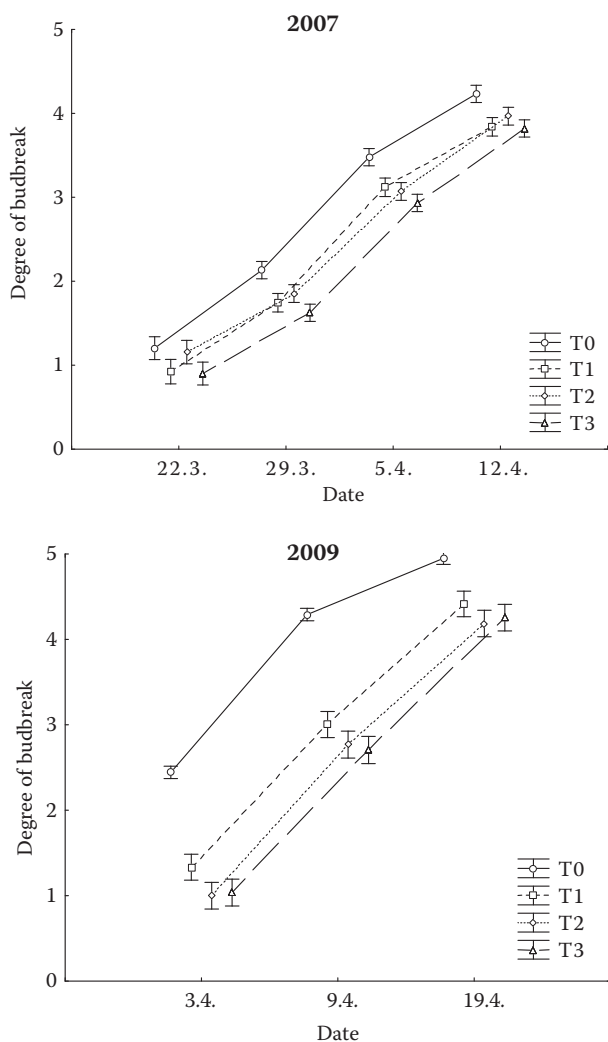


Fig. 4. The course of budbreak in categories 0–5 (0 – bud with an intact bud scale, 1 – bud with an intact bud scale and showing through green colour, 2 – apical part of a bud with a green tip, 3 – disturbed bud top exceeding 1 mm, 4 – unfolding leaves, 5 – developed leaves) of birch *B. pendula* in particular treatments T0–T3 (T0 – application of 0 g NH_4NO_3 , T1 – 0.5 g NH_4NO_3 , T2 – 1 g NH_4NO_3 , T3 – 1.5 g NH_4NO_3) (error bars represent 0.95 confidence intervals) (2007–2009)

proved ($P < 0.001$, $\alpha = 0.05$) between the control (T0) and fertilized treatments when leaves of control plants yellowed more intensively than treatments affected by nitrogen.

DISCUSSION

Under conditions of the pollution-disturbed forest area of the Krušné hory Mountains, birch has become an important component of stands of substitute tree species because it is resistant to high soil acidity. Reclamation liming, growing pH and increasing content of nitrogen in soil affect the growth properties of birch (KULA 2009). We have proved by this experiment that birch shows decreased increments at high intake of nitrogen.

In the forest region of the Krušné hory Mountains, the level of nitrogen in *B. pendula* leaves decreased insignificantly from the average value $28.60 \text{ mg}\cdot\text{g}^{-1}$ (year 1995) to $26.08 \text{ mg}\cdot\text{g}^{-1}$ (1998) (HRDLIČKA, KULA 2004) and $25 \text{ mg}\cdot\text{g}^{-1}$ (2011) (KULA, unpublished). In our experiment in the Řečkovice forest

nursery, the content of nitrogen in birch leaves in the control treatment ($15.26\text{--}24.71 \text{ mg}\cdot\text{g}^{-1}$) was below this level while in treatments T1–T3 it reached above-standard values ($29.27\text{--}44.15 \text{ mg}\cdot\text{g}^{-1}$) in 2006–2008. Through fertilization, we reached the differentiated content of nitrogen in birch *B. pendula* leaves to conduct the subsequent experiment aimed at the determination of the response of phytophages (*Lymantria dispar* L., *Cabera pusaria* L., *Lochmaea caprea* L.) to increased inputs of nitrogen (KULA et al. 2012a, b, c).

Frost heaving of unripe annual shoots at early frosts became evident in birch trees with the high content of nitrogen, which was proved by findings of PALÁTOVÁ and MAUER (1998). Although repeated late frosts occur at the locality, the experiment could continue because birch was not sensitive to them (KULA 2000a, b). At the concentration of nitrogen in leaves $> 2\%$, the resistance of beech plants to winter frosts decreases (SPINNER et al. 1996). This negative phenomenon was not even proved in birch at 3–4.4% of nitrogen in leaves.

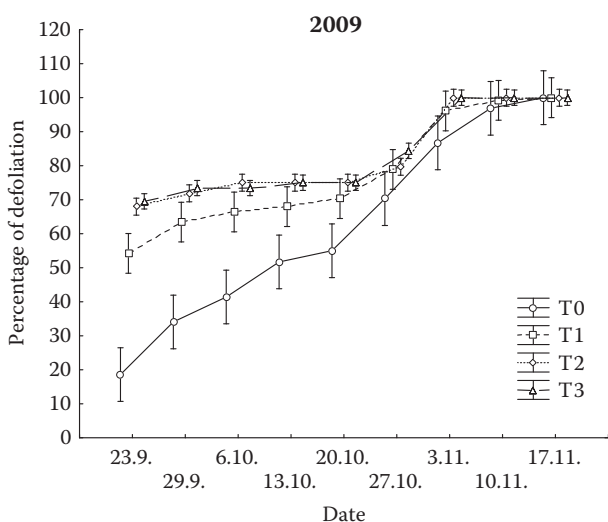
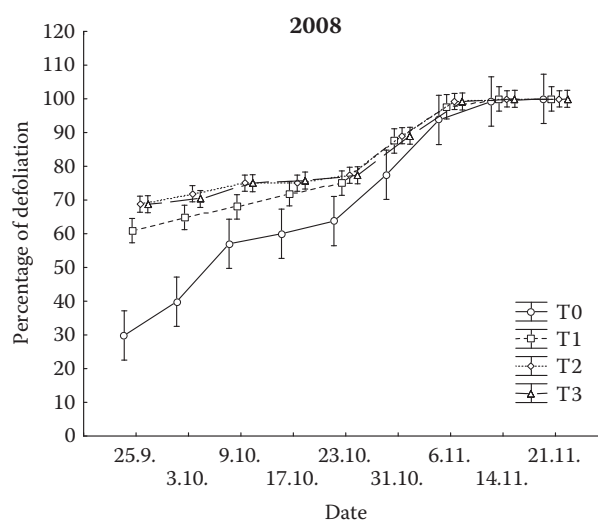
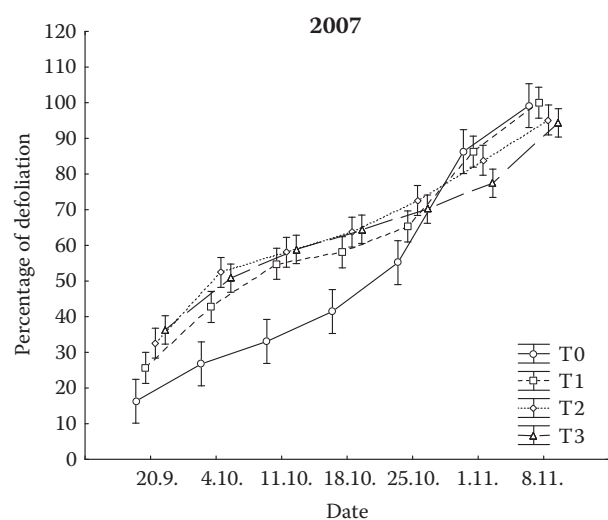


Fig. 5. The course of the defoliation of birch *B. pendula* in particular treatments T0–T3 (see Fig. 1) (error bars represent 0.95 confidence intervals) (2007–2009)

Birch defoliation in the summer season was caused by stress which was induced by drought in plants with the increased content of nitrogen. KAŇOVÁ and KULA (2004) came to similar conclusions. The loss of chlorophyll at the end of the growing season in treatments T1–T3 was more gradual than in the control. The increased content of nitrogen prolonged the growth stage.

The course of birch budbreak was slowed down in treatments affected by nitrogen as compared to the control and among treatments affected by nitrogen (T1–T2 and T1–T3). Delayed birch budbreak occurred in trees heavily affected by inputs of nitrogen. According to OMAROV et al. (1999), this phenomenon is associated with the surplus of ammonium nitrogen, which induces the fall of cytokinins, increase of abscisic acid and thus, the start of budbreak is inhibited.

The higher level of nitrogen in substrate probably functions as a stressing factor, which disturbs the uptake of other nutrients (KULHAVÝ, FORMÁNEK 2002). We confirmed findings of PALÁTOVÁ and

MAUER (1998), BOROWIEC et al. (2005), HRDLIČKA and KULA (2007) and BRAUN et al. (2010) that the increased content of nitrogen even in leaves of birch causes the decline of phosphorus. Unlike HEILMEIER et al. (2000), potassium increased at the increasing nitrogen, however, it did not affect other elements.

Thus, the response of particular tree species may not be identical and nitrogen fertilization does not always show positive effects. In Scots pine (*Pinus sylvestris* L.), nitrogen is the cause of the height increment decline at the expense of the higher quality of assimilatory apparatus (PALÁTOVÁ, MAUER 1998).

Birch responded to the increased input of nitrogen also negatively by reduced increment. KULA (2009) reported the decline of increment in birch trees with the high application dose of dolomitic limestone when the content of calcium and magnesium increased and the proportion of potassium decreased in assimilatory organs. HEJNÝ and SLAVÍK (1990) stated that birch grows well at poorer as well as extremely acid sites (pH 3.5–5.0) being absent at nutritive substrates and particularly limestone.

CONCLUSIONS

The birch (*B. pendula*) responds to ammonium nitrate fertilization by the increased content of nitrogen in leaves depending on its amount in soil. The decreased content of nitrogen in birch shows stimulating effects whereas higher doses of nitrogen cause stress and differentiated decline of height and diameter increment.

The increased level of nitrogen in birch inhibits budbreak, reduces the degree of chlorophyll degradation and the progress of senescence and autumn defoliation prolonging the growth stage and increasing the risk of early frosts. The high content of nitrogen and drought stress are the causes of drying and marked loss of assimilatory organs of birch in the summer season.

The increased content of nitrogen in birch leaves is related to the decline of phosphorus and increase of potassium. Mg, Ca and S and soil pH do not change.

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