Effects of nitrogen on the selection of food by *Phyllobius arborator* (Herbst)

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ABSTRACT: The selection of a nutritive plant and the consumption of food (*Betula pendula* Roth) affected by differentiated inputs of nitrogen after the repeated application of ammonium nitrate into soil was monitored at *Phyllobius arborator* (Herbst) under field (polythene greenhouse) and laboratory (Climacell) conditions. In birch leaves, the content of nitrogen increased. The diameter and height increment was stimulated by the application of 0.5–1 g, higher doses induced stress and the fall of increment. According to the frequency of feeding marks on leaves and food consumption by weevils of the genus *Phyllobius* in a polythene greenhouse, birch with the higher content of nitrogen was preferred. In laboratory rearing, females showed higher food requirements. In short-term rearing, differentiation did not occur in the amount of consumed food in males and females depending on the nitrogen content.

Keywords: nitrogen; *Betula pendula* (Roth); *Phyllobius arborator* (Herbst)

Nitrogen is an important macroelement, the uptake of which by trees is carried out not only by roots from the soil environment but also in the form of ammonium or nitrate ions from liquids on the surface of leaves. It significantly affects growth properties and the creation of below-ground and above-ground biomass. High nitrogen inputs induce asymmetry in the growth of roots and assimilatory organs and the lack of nutrients due to the inhibition of K^+, Mg^{2+}, Ca^{2+} and water. They also lower the effectiveness of photosynthesis, increment and vitality and extend the growing season. Thus, the danger of frost damage to annual shoots occurs (Marschner 1995; Heilmeier et al. 2000; Kulhavý, Formánek 2002; Kaňová, Kula 2004a). Ammonia in the plants changes to amino acids and thus the concentration of proteins increases (Srivastava, Ormrod 1984).

In the second half of the 90s of the 20th century, the fall of emissions of nitrogen pollutants (NH_3, NO_x) stopped, but at some localities, it was possible to note an increase of dry and wet nitrogen depositions (Fadrhonsová et al. 2002) exceeding the critical amount (15 kg N/ha/year) on 4% area of the Krušné hory Mts. (Hadaš 2002, 2004).

High nitrogen inputs (200 kg/ha) reduce insect fauna by ⅓ in soil and by ¾ in vegetation (Anonymous 1991). As for pine, population density decreased, but species diversity was preserved (Jones, Paine 2006). The response of *Psylloidea* to fertilization was, however, opposite. Their species diversity decreased and numerical proportion increased (Prestidge 1982). The quality of food for phytophages is positively affected by the increased concentration of organic nitrogen in leaves and phloem of plants (White 1984; Haddad et al. 2000). Mining insect *Stigmella* sp. and *Lithocolletis* sp. and imagoes of weevils of the genus *Phyllobius* preferred leaves of birch affected by ammonium sulphate (Jansson, Smilowitz 1985; Heliövaara, Väisänen 1993; Kaňová, Kula 2004b). Pronos et al. (1999) mention responses of

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZe 1G46002, Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6215648902.

phytophages to air pollution and increased inputs of nitrogen into plants.

METHODS

The experiment was established in April 2006 by planting one-year birch plants (*Betula pendula* Roth) into containers (10-litre volume) with a soil substrate from the Cambic mineral horizon of forest soil. Soil properties were modified adding 1 l peat to a plant. After an interval of three weeks, 128 rooted birch plants (32 plants in each variant) were placed into a polythene greenhouse devised to monitor the ethology of weevils (selection of food). In other two polythene greenhouses, 480 birch plants were placed as the source of food for laboratory rearing. The microclimate of polythene greenhouses in July at the introduction of weevils of the genus *Phyllobius* (monitored by a temperature-humidity sensor AMET) is characterized by mean daily temperatures 18–24°C, daily maximum 21–37°C, daily minimum 11–16°C, daily mean relative air humidity 56–84%, watering 2–3 times per week, altitude 220 m. Simultaneously with a control (T0), changes in the content of nitrogen were induced in three variants with applications of 0.5 g (T1), 1 g (T2) and 1.5 g (T3) ammonium nitrate $(NH_4)NO_3$ to a plant into the soil in four repetitions. The amount of applied ammonium nitrate was derived from nitrogen depositions in the Krušné hory Mts. according to data of the Czech Hydrometeorological Institute in Prague (ČHMÚ).

Growth properties were evaluated according to the plant height and root collar diameter. The phenology of leaf fall was quantified on the level of a plant in categories 0.1–5, 6–25, 26–50, 51–75, 76–100% at the simultaneous registration of colour changes.

Imagoes of the genus *Phyllobius* were released into the area of the polythene greenhouse (2. 7. 2006). After 14 days, leaves damaged by feeding were registered in all plants of the experiment (128). Under laboratory conditions, individual rearings of *Phyllobius arborator* (Herbst) (34 ♂♂, 46 ♀♀) were established in Petri dishes of a diameter of 13.5 cm. At an interval of 48 hours, branches with birch leaves were offered to the weevil in variants T0–T3 (24 repetitions). The rearings were placed into a Climacell 707 with a set up 10-hour day-time (temperature 24°C, relative air humidity 40%, insolation 100%) and 6-hour night-time regime (temperature 14.4°C, relative air humidity 60%, insolation 0%) (with a 4-hour gradual transition between them) for the period of monitoring (6 days).

Using a Leave Area Meter (AM 300), the area of leaves with feeding marks was scanned right on the

Table 1. Characteristics of a soil substrate for growing nutritive plants

		pH KCl	pH H_2O	Nkjehl	C_{ox}	C:N	N-NH_4^+	N-NO_3^-	Σ N-min
				(%)			(mg/kg)		
Starting position	Ø	3.47 ± 0.01	4.43 ± 0.03	0.08 ± 0.00	2.08 ± 0.22	25.4 ± 2.95	2.81 ± 1.47	8.23 ± 1.2	11.03 ± 2.28
Variant	T0	3.48	4.16	0.10	1.89	18.9	6.12	5.68	11.80
	T1	3.38	3.99	0.08	1.64	20.5	31.20	28.30	59.50
	T2	3.40	3.93	0.09	2.12	23.6	67.10	44.10	111.20
	T3	3.37	3.91	0.10	1.48	14.8	75.70	59.60	135.30

plant. The extent of damage was calculated using the Photoshop program with the resolution of 64 × 240 pixels.

The chemistry of a mixed soil sample was analyzed before the experiment establishment and then at the end of the growing season from variants T0-T3 by an accredited laboratory Ekola Ltd. Bruzovice. Following analyses were carried out: oxidizable carbon (C_{ox}), C:N (C_{ox}/Nkjehl) ratio, the content of available nutrients N-NO_3^-, N-NH_4^+ (determined in the leach in 1% potassium sulphate solution), pH value (H_2O, KCl) (Table 1). The C:N fluctuation could be caused by the uneven proportion of the originally applied peat substrate in the mixed soil sample.

After the completion of weevil feeding, leaves were sampled from branches in the tree profile (except four terminal). The leaves were then analyzed after drying at 70°C. Nitrogen was determined according to Kjeldahl using a tecator Kjeltec analyzer UNIT 2300 device. ANOVA LSD test (Statistica Cz) was used for statistical evaluation.

RESULTS

Effects on a nutritive plant

A basic condition of the rapid increase in the nitrogen content in birch leaves was demonstrated by the analysis of its level in the dry matter of leaves during the spring season. The amount of nitrogen increased nonlinearly with the increasing rate of ammonium nitrate into the substrate, differences being statistically significant ($P < 0.001$ at the significance level $\alpha = 0.05$); only between variants T2 and T3, statistically significant differences were not found (T0 24.8; T1 29.8; T2 32.0; T3 32.9 mg/g) (Fig. 1).

Differentiated inputs of nitrogen caused different non-linear diameter and height increments in birch plants. Differences in the height increment were statistically significant ($P < 0.001$ at the significance level $\alpha = 0.05$) only between the control (40.5 cm) and variants with nitrogen applications (49.5 to 53.9 cm), however, not between particular doses of

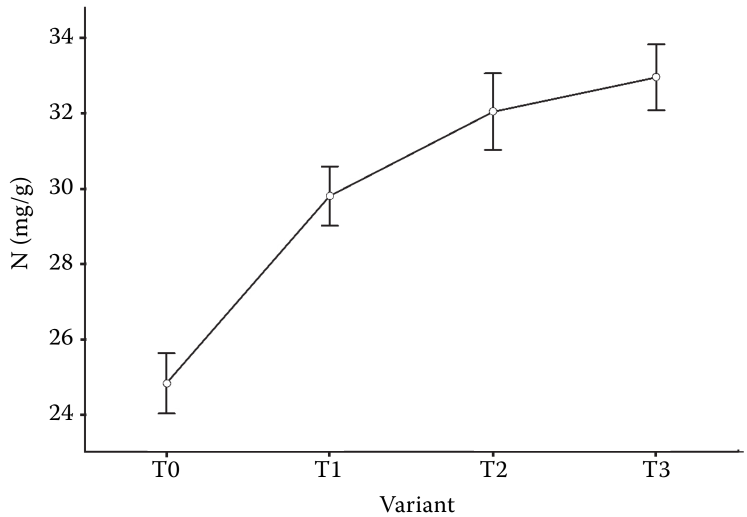

Fig. 1. Mean content of nitrogen in the dry matter of birch leaves depending on the application of ammonium nitrate (0.95 reliability intervals)

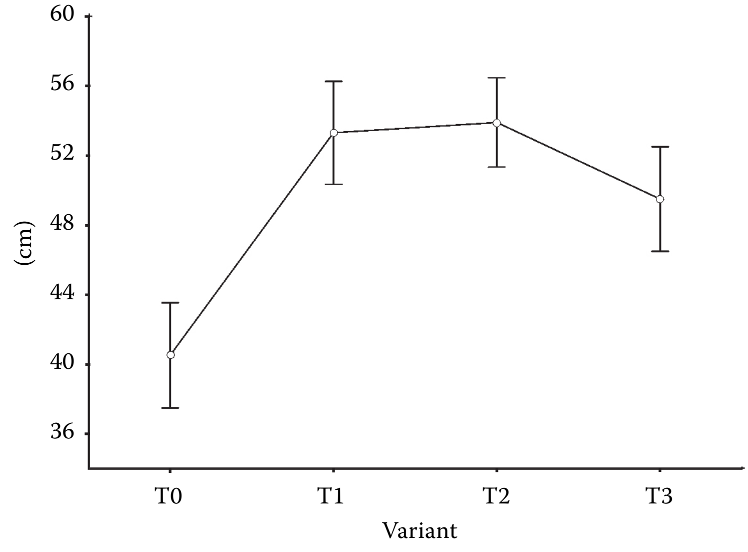

Fig. 2. Mean height increment of birch (0.95 reliability intervals)

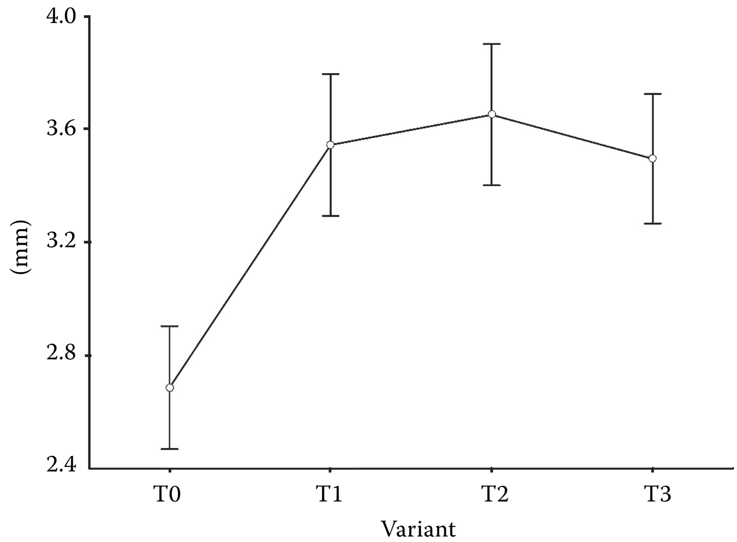

Fig. 3. Mean diameter increment of birch in a root collar depending on the application of ammonium nitrate (0.95 reliability intervals)

nitrogen (Fig. 2). Changes in the diameter of a root collar correspond to findings mentioned above because statistical differences occurred only between the control (2.7 mm) and variants with nitrogen (3.5–3.7 mm) (Fig. 3). With an increased rate of nitrogen (1.5 g), statistically insignificant decrease occurred in the height and diameter increment.

As for the fall of leaves at the end of the growing season, no differences were noted between particular variants. On the basis of the evaluation of colour changes at the end of the growing season using non-parametric statistics (Kruskal-Wallis test) a statistically significant difference occurred ($P < 0.001$ at the significance level $\alpha = 0.05$) between the control (T0) when leaves of plants got yellow more intensively and variants affected by nitrogen.

Selection and consumption of food

In a polythene greenhouse, *P. arborator* damaged birch leaves in all variants in a differentiated range from 336 (T0) to 430 leaves (T2) (Fig. 4). Differences in the frequency of the feeding mark occurrence were not accidental ($\chi^2 = 12.67$, df = 3, $P = 0.005$). Insect attack increased with the increasing mean content of nitrogen in the dry matter of leaves from variant T0 (24.8 mg/g) to variant T2 (32 mg/g) (Fig. 5) while at the nitrogen level of 32.9 mg/g (T3) the attack moderately decreased. This conclusion also corresponds to the amount of food in the control (T0 – 51 cm^2) and in variant T2 (69 cm^2) (Fig. 6) and converted dry matter (194–263 mg) (Fig. 7).

In the laboratory rearing of *P. arborator* imagoes, statistically significant differences were found in the consumption of leaf biomass by males and females not distinguishing variants ($P < 0.001$, Fig. 8). Females consumed on average 60.6 ± 15.4 mm^2 per day leaf area (2.3 mg dry matter per day) while males consumed on average 45.7 ± 10.5 mm^2/day (1.7 mg dry matter per day). Differences between particular variants in

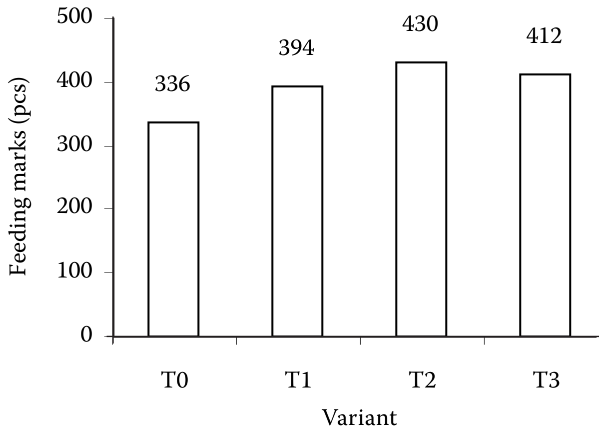

Fig. 4. The number of feeding marks created by weevils of the genus *Phyllobius* on birch leaves affected by differentiated inputs of nitrogen

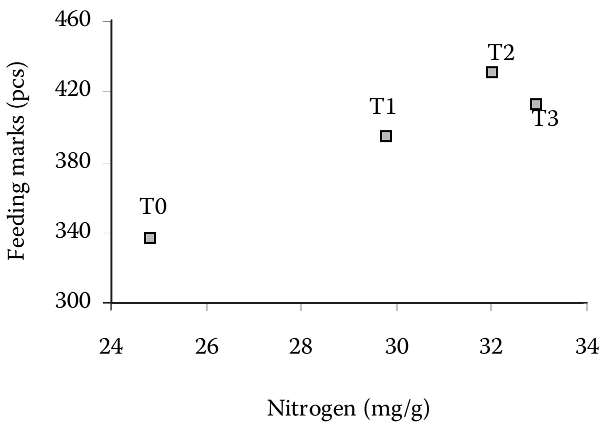

Fig. 5. Relationship between the occurrence of feeding marks of weevils of the genus *Phyllobius* and the content of nitrogen in birch leaves

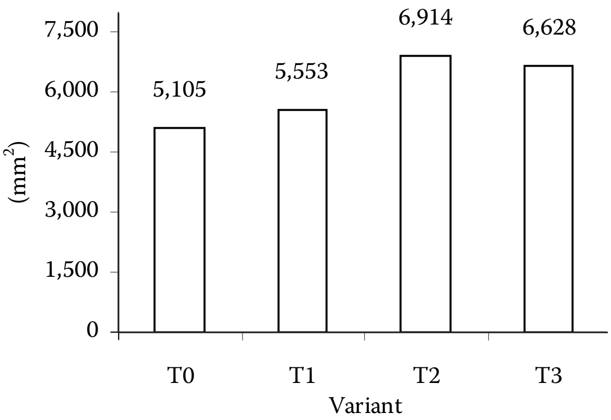

Fig. 6. Weevils of the genus *Phyllobius* – consumption of food on birch affected by differentiated inputs of nitrogen

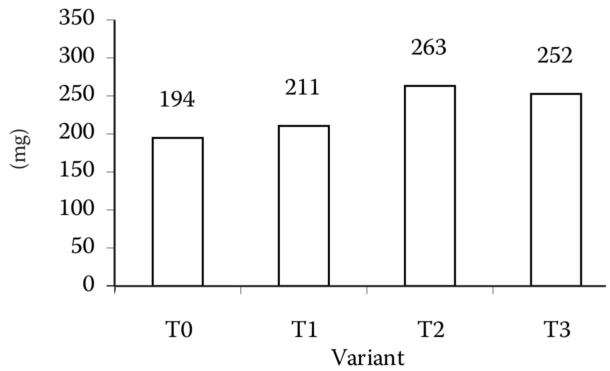

Fig. 7. The weight of the dry matter of birch leaves affected by the application of ammonium nitrate consumed by weevils of the genus *Phyllobius*

the consumption of food by males and females were not statistically significant (Figs. 9 and 10).

DISCUSSION

Data given by Marschner (1995) showing that nitrate ions or ammonium cations, which were added in the form of ammonium nitrate to the soil substrate, were taken by a plant very well were demonstrated in an experiment with *B. pendula*. Their uptake began to be limited only after the nitrogen content in leaves exceeding a value of 32 mg/g dry matter. Higher levels can be connected with a negative response induced by the nitrogen surplus when the content of nitrogen ions in the substrate probably acted as a stress factor causing the disturbance of the uptake of other nutrients (Kulhavý, Formánek 2002).

P. arborator attacked more frequently plants with the increased nutrition of nitrogen according to conclusions on stress impacts (Kaňová, Kula 2004b, 2005). Changed nutrition or stress could affect the quality aspects of food, e.g. the production of secondary metabolites and thus, the acceptability of plant biomass for phytophages (Mattson 1980; White 1984; Bryant et al. 1993). However, it is necessary to take into account the different response of particular plant species to stress and subsequently the differentiated response of insect induced by changes in the quality of food (Larsson 1989).

Therefore, the response of *P. arborator* cannot be generalized. Other species or the same insect species on another plant species can behave differently (Heliövaara, Väisänen 1993).

The amount of biomass consumed by weevils depending on a variant did not differ. Significant differences between males and females are not surprising because, in general, females show higher food requirements for the creation of a fat body and eggs. Kula (1988) mentions similar results in the increased consumption of food in the creation of a fat body in females of *Lochmaea caprae* (L.) Differences of imagoes in the consumption of food in particular variants cannot be confronted with findings obtained under conditions of the polythene greenhouse where

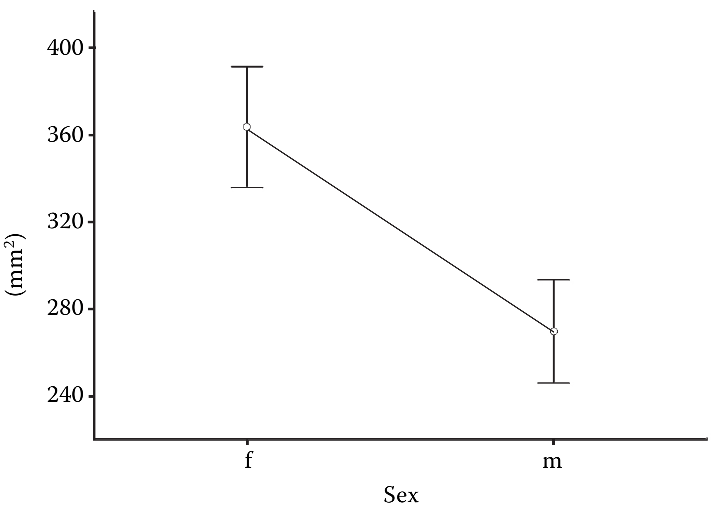

Fig. 8. Differences in the consumption of food by *Phyllobius arborator* according to sex (laboratory rearing)

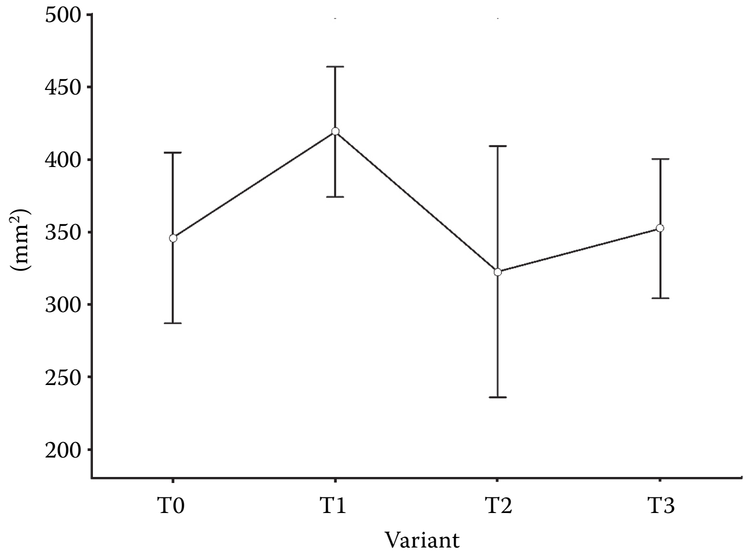

Fig. 9. Mean consumption of food by females of *Phyllobius arborator* after 6 days of feeding in a laboratory rearing (0.95 reliability intervals)

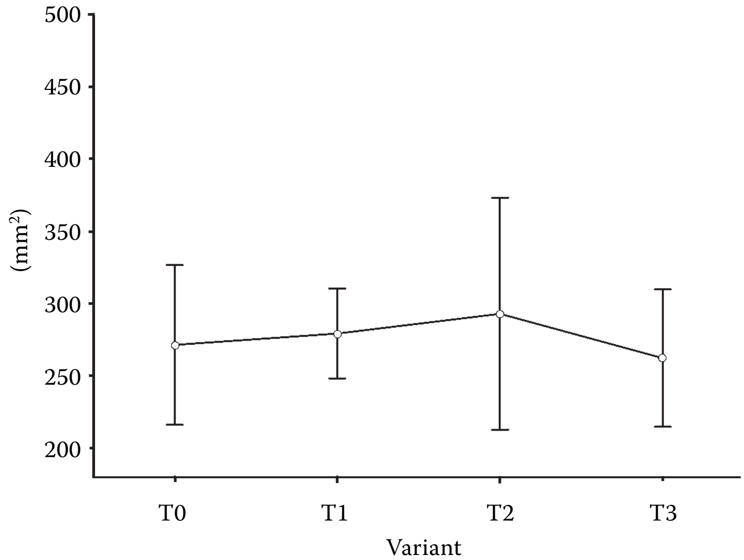

Fig. 10. Mean consumption of food by males of *Phyllobius arborator* after 6 days of feeding in a laboratory rearing (0.95 reliability intervals)

the proportion in feeding according to sex could not be evaluated.

CONCLUSION

The input of nitrogen into a plant through soil became evident by the increased content of nitrogen in birch leaves and changes in height and diameter increment. Applications of ammonium nitrate up to 1 g to a plant (32 mg/g) showed stimulation effects, higher rates induced stress and fall in increment.

The frequency of feeding marks and the consumption of food by weevils of the genus *Phyllobius* proved their preference to plants affected by nitrogen.

In general in laboratory rearings (Climacell), increased uptake of food occurred in females. Effects of the various level of nitrogen in food did not differentiate the height of uptake within a sex.

References

ANONYMOUS, 1991. IUCN East European Programme, the lowland grasslands of Central and Eastern Europe. Environmental Research Ser., *4*: 1.

BRYANT J.P., REICHARDT P.B., CLAUSEN T.P., WERNER R.A., 1993. Effect of mineral nutrition on delayed inducible resistance in Alaska paper birch. Ecology, *74*: 2072–2084.

FADRHONSOVÁ V., ŠRÁMEK V., KROUPOVÁ M., 2002. Vývoj depozic v oblasti Krušných hor. In: Sborník referátů Výsledky lesnického výzkumu v Krušných horách v roce 2001, Teplice 14. 3. 2002. Jíloviště-Strnady, VÚLHM: 49–54.

HADAŠ P., 2002. Celková potenciální depozice síry, dusíku a vodíkových iontů na území PLO Krušné hory v roce 1999. In: Sborník referátů Výsledky lesnického výzkumu v Krušných horách v roce 2001, Teplice 14. 3. 2002. Jíloviště-Strnady, VÚLHM: 55–66.

HADAŠ P., 2004. Potenciální podkorunové depozice síry, dusíku a iontů vodíku na území PLO Krušné hory v roce

2001. In: Sborník referátů Výsledky lesnického výzkumu v Krušných horách v roce 2003, Teplice 22. 4. 2004. Jíloviště-Strnady, VÚLHM: 13–28.

HADDAD N.M., HAARSTAD J., TILMAN D., 2000. The effects of long-term nitrogen loading on grassland insect communities. Oecologia, *124*: 73–84.

HEILMEIER H., BARONIUS K., KUHN A.J., NEBE W., 2000. Biomass and nutrient relationships of *Betula pendula, Fagus sylvatica, Picea abies* and *Abies alba* with varying nitrogen and sulphur additions in a pot experiment. Forstwissenschaftliches Centralblatt, *119*: 161–176.

HELIÖVAARA K., VÄISÄNEN R., 1993. Insects and Pollution. Boca Raton, CRC Press Inc.: 393**.**

INGESTAD T., 1990. Nitrogen and plant grow; maximum efficiency of nitrogen fertilizers. In: PERALA D.A., ALM A.A., Reproductive ecology of birch: A Review. Forest Ecology and Management, *32*: 1–38.

JANSSON R.K., SMILOWITZ Z., 1985. Influence of nitrogen on population parameters of potato insects: abundance, population growth and within-plant distribution of the green peach aphid, *Myzus persicae* (Homoptera: Aphididae). Environmental Entomology, *15*: 49–55.

JONES M.E., PAINE T.D., 2006. Detecting changes in insect herbivore communities along a pollution gradient. Environmental Pollution, *143*: 377–387.

KAŇOVÁ D., KULA E., 2004a. The effect of stress factors on birch *Betula pendula* Roth. Journal of Forest Science, *50*: 399–404.

KAŇOVÁ D., KULA E., 2004b. Vliv stresu na břízu *Betula pendula* Roth a její atraktivitu pro fytofágy. [Projekt NAZV QC 1144/2001.] Brno, MZLU: 82.

KAŇOVÁ D., KULA E., 2005. Effect of experimental acidification on birch *Betula pendula* Roth and several feed-linked insects. In: International Conference Acid Rain 2005, June 12–17 2005. Prague, Czech Hydrometeorological Institute: 626.

KULA E., 1988. The willow leaf beetle (*Lochmaea capreae* L.) in birch stands. Acta Universitatis Agriculturae, *57*: 261–307.

KULHAVÝ J., FORMÁNEK P., 2002. Dusík v lesních ekosystémech Krušných hor. In: Sborník referátů Výsledky lesnického výzkumu v Krušných horách v roce 2001, Teplice 14. 3. 2002. Jíloviště-Strnady, VÚLHM: 37–46.

LARSSON S., 1989. Stressful times for the plant stress – insect performance hypothesis. Oikos, *56*: 277.

MARSCHNER H., 1995. Mineral Nutrition of Higher Plants. 2nd Ed. London, Academic Press: 889.

MATTSON W.J., 1980. Herbivory in relation to plant nitrogen content. Annual Review of Ecology and Systematics, *11*: 119.

PRESTIDGE R.A., 1982. The influence of nitrogenous fertilizer on the grassland Auchenorrhyncha (Homoptera). Journal of Applied Ecology, *19*: 735–749.

PRONOS J., MERRILL L., DAHLSTEN D., 1999. Insects and pathogens in a pollution-stressed forest. Oxidant air pollution impacts in the montane forests of Southern California: A case study of the San Bernardino Mountains. Ecological Studies, *134*: 317–337.

SRIVASTAVA H.S., ORMROD D.P., 1984. Effects of nitrogen dioxide and nitrate on growth and nitrate assimilation in bean leaves. Plant Physiology, *76*: 418.

WHITE T.C.R., 1984. The abundace of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia (Berlin), *6*: 90–105.

Received for publication June 23, 2007
Accepted after corrections October 16, 2007

Vliv dusíku na výběr potravy listohlodem stromovým (*Phyllobius arborator* Herbst)

ABSTRAKT: Výběr živné rostliny a spotřeba potravy (*Betula pendula* Roth) ovlivněné diferencovanými vstupy dusíku po opakované aplikaci dusičnanu amonného do půdy byl sledován u *Phyllobius arborator* (Herbst) v podmínkách terénních (fóliovník) a laboratorních (Climacell). V listech břízy se zvýšil obsah dusíku. Tloušťkový a výškový přírůst byl stimulován aplikací 0,5–1 g $(NH_4)NO_3$, vyšší dávky vyvolaly stres a pokles přírůstu. Podle frekvence požerků na listech a spotřeby potravy nosatci rodu *Phyllobius* ve fóliovníku byla preferována bříza s vyšším obsahem dusíku. V laboratorním chovu měly vyšší potravní nároky samice, přičemž se v krátkodobém chovu neprojevila diference u samců a samic v množství přijaté potravy v závislosti na obsahu dusíku.

Klíčová slova: dusík; *Betula pendula* (Roth); *Phyllobius arborator* (Herbst)

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