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THE DEVELOPMENT OF CATERPILLARS OF GYPSY MOTH (*Lymantria dispar* L.) FEEDING ON FOOD AFFECTED BY NITROGEN

RAZVOJ GUSENICA GUBARA (*Lymantria dispar* L.) NA HRANLJIVOM SUBSTRATU OBOGAĆENOM DUŠIKOM

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Summary

In laboratory rearing, affects were monitored of the differentiated content of nitrogen (17.17–38.89 mg.g^{-1}) in birch (*Betula pendula* Roth) leaves on the development, mortality and consumption of food of caterpillars of *Lymantria dispar* L. The low content of nitrogen in food was the cause of the higher mortality of caterpillars, smaller weight of pupae and the prolongation of development. Caterpillars of future males fed on food with the insufficient content of nitrogen needed the higher amount of food to complete their development. This experiment supported a hypothesis that spring phytophages preferred tissues with the higher content of nitrogen. Caterpillars can respond to the above-standard content of nitrogen similarly as to the lack of nitrogen.

KEY WORDS: nitrogen, stress, *Betula pendula*, *Lymantria dispar*, caterpillars, laboratory rearing, development

Introduction
Uvod

Nitrogen shows an irreplaceable role in the metabolism of many biopolymers: – proteins, amines, amides, pigments, growth substances etc. It affects the creation and quality of biomass. Nitrogen is the basic component of protoplasm and enzymes (Larcher 1988). Stress in a plant caused e.g. by air pollutants or changes of weather produces the growth of the content of nitrogen in some tissues and the increased mobility of available nitrogen. Thus, the plant can become the source of food of higher quality for herbivores (White 1984). The growth and production of phytophagous insect is affected by the quantity and quality of proteins and amino acids in food (McNeil, Southwood 1978). During the growing season, the assimilatory apparatus of trees is exposed to the attack of the broad spectrum of insects (Feeny 1970, Lindquist, Miller 1976). Herbivores are adapted to obtain necessary energy and food, to overcome defensive responses of plants and other factors of the environment (Mattson 1980). The level of nitrogen in the assimilatory organs of broadleaved species (10–40 mg.g^{-1}) (Larcher

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1988) generally decreases in the course of the growing season (Hrdlička, Kula 2001, Šrámek et al. 2009, Rodin, Bazilevich 1967).

A hypothesis: The increased content of nitrogen in leaves can affect positively the development of caterpillars of species feeding during the growing season and negatively of species occurring as late as the second half of the growing season. The aim of the paper is to verify responses of caterpillars of gypsy moth reared on leaves of birch (*Betula pendula* Roth) with the differentiated content of nitrogen.

Gypsy moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) – an Eurasian species spread from Europe to North America where it became the most important pest (McManus, Csóka 2007). It is a widely polyphagous species attacking more than 300 species of trees and shrubs from at least 14 plant families (Doane, McManus 1981, Lechowicz, Mauffette 1986). In Europe, it prefers oak, hornbeam, beech, chestnut, but also birch, linden, willow, poplar, maple, alder and larch. At the same time, it does harms to fruit trees (Schwenke 1978). In the USA, even defoliation of conifers (particularly of spruce) occurs at gradations (McManus, Csóka 2007).

The growth and mortality of caterpillars of *L. dispar*, duration of their development, the weight of pupae and fertility of females are affected by a host plant (Barbosa, Greenblatt 1979, Roden, Mattson 2008, Kinney et al. 1997).

In Central Europe, gypsy moth hatches usually in August and September. Females create clutches containing 500–800 eggs on the bark of broadleaved species. In an egg, the embryogenesis of which was finished during the growing season, a diapausing caterpillar overwinters. Caterpillars hatch in April or at the beginning of May, climb to crowns of trees or spread on web fibres to the surroundings (Schwenke 1978). Male caterpillars come through 5 (exceptionally 6) instars, female 6 (exceptionally 7) instars (Leonard 1966). Under Central-European conditions, caterpillars develop 6–12 weeks depending on weather and food quality. At the turn of June and July, they pupate on trees. Pupae are fixed to the stem bark by means of thin web. After 2–3 weeks, moths hatch (Schwenke 1978).

Birch (*B. pendula*) grows as an admixture in cultivated forests, on derelict land often disturbed by anthropogenic effects (mine dumps, spoil banks). In air-polluted areas of the ČR, it is a dominant substitute tree species (Slodičák et al. 2008) creating also spontaneous monocultures. The species occurs on poor dry soils as well as on extremely acid sites (pH 3.5–5.0); it is nearly missing on mesotrophic sites (particularly limestone) (Hejný, Slavík 1990). It responds negatively by decreased increment to reclamation liming (Kula 2009). Increased inputs of nitrogen at simultaneous drought stress cause intensive summer defoliation (Kaňová, Kula 2004) and the high level of nitrogen limits height and diameter increment (Kula, Pešlová, Martinek 2012).

Methods of research
Metode istraživanja

Annual future nutrient plants of birch *B. pendula* were planted out into containers (volume 10 l) with soil substrate taken in the Cambic mineral horizon of a forest soil and placed into plastic greenhouses in a forest nursery (Brno – Řečkovice, altitude 220 m) (Kula, Pešlová, Martinek 2012). Changes in the content of nitrogen in the substrate were caused by the application of ammonium nitrate (NH_4NO_3) to a plant in three treatments: 0.5 g (T1), 1 g (T2) and 1.5 g (T3). The fertilization was carried out in the year of outplanting (2006) in regular five-week intervals (four-times), in further years (2007–2009) five times in the growing season. The amount of applied ammonium nitrate was derived from nitrogen depositions in the Ore Mountains according to the ČHMÚ (Czech Hydrometeorological Institute) data.

According to the ICP Forests methodology, mature leaves were sampled from the upper half of a birch crown (except four terminal) at the turn of August and September. In the leaves, the content of nitrogen was determined after desiccation at 70 °C according to the method of Kjeldahl using a tecator Kjeltec analyzer UNIT 2300 (Kula, Pešlová, Martinek 2012).

On 22 March 2009, eggs of gypsy moth were transported from oak stands of the Lednice-Valtice area (48°44'45.085"N, 16°49'7.276"E). The rearing of caterpillars was carried out in Petri dishes. In total, 600 caterpillars of the 1st instar were divided into 60 Petri dishes at 10 pieces. Each of the treatments (T1, T2, T3) including control (T0) showed 15 repetitions. The rearing of caterpillars was carried out under controll. Day light 10 hours, temperature 17.8 °C (14 June increased to 20.3 °C), RH (relative humidity) 60 %.

Without exposure 6 hours, temperature 13 °C (14 June increased to 15.5 °C), RH 85 %.

Transitional light conditions with 20 % intensity twice at four hours with the continuous change of temperature and humidity.

Twigs with leaves from nutrient plants (*B. pendula*) according to treatments (T0–T3) were sampled at an interval of 48-hours in plastic greenhouses and transported to a laboratory in a tempered box with a laboratory temperature (circa 20 °C) in the course of 1 hour. Leaves with the determined area were used for the rearing.

The instar of caterpillars was noted continuously [according to the cranium width 0.6–1.2–2.2–3.2–4.4–6 mm (Schwenke 1978)] and mortality of caterpillars, pupation and pupa weight. Due to the increased consumption of food caterpillars were reared individually from the 4th instar. The area of feeding was determined as a difference between the input area of a leave and the leaf remainder after the caterpillar feeding using an ADC BioScientific Leaf Area Meter AM300.

The fine feeding of caterpillars of the 1st instar, which could not be detected by this apparatus, was determined in a biometric laboratory by means of the system of analysis and image processing NIS – Elements AR (digital camera 5 Mpix Nikon DS – Fi 1 with Navitar macroobjective, exposure KAISER RB 5000 DL, exciter lamp KAISER prolite basic, computer with NIS program – Elements AR, version 2.30, processor X86, 2533 MHz, HD 230 GB, RAM 2 MB). The system operated in the MS Windows XP Professional environment (Kula et al., in print).

After the experiment termination, the content of nitrogen was determined in remainders of leaves of *B. pendula* from the period of the whole rearing in a 14-day interval. To evaluate data obtained the Statistica.cz program was used (StatSoft 2007).

Results
Rezultati

The content of nitrogen in birch leaves – Sadržaj dušika u lišću breze

The level of nitrogen in leaves of control birch trees (T0) was markedly lower in comparison with leaves of fertilized treatments (T1–T3). Maximum values were achieved in the first 14 days of rearing (28.21 $mg.g^{-1}$), then a continuous decline occurred to a minimum value in the final stage of rearing (17.17 $mg.g^{-1}$). The content of nitrogen in leaves of treatment T1 decreased from a maximum at the beginning of the rearing (33.91 $mg.g^{-1}$) to a value of 27.38 $mg.g^{-1}$ (26 May – 9 June) with a subsequent slight increase to 29.82 $mg.g^{-1}$. The content of nitrogen in leaves of birch treatment T2 ranged within the limits 30.38–36.25 $mg.g^{-1}$

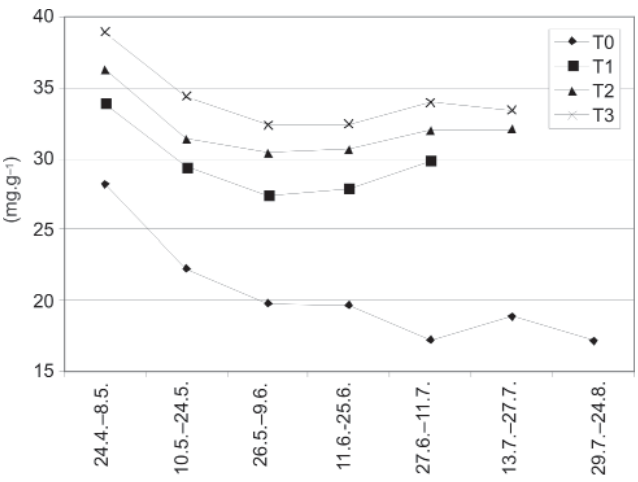

Figure 1. The average content of nitrogen in leaves of nutritive plants of *Betula pendula* during the rearing of *Lymantria dispar*

Slika 1. Prosječna količina dušika u biljkama breze *Betula pendula* za vrijeme hranjenja gubara

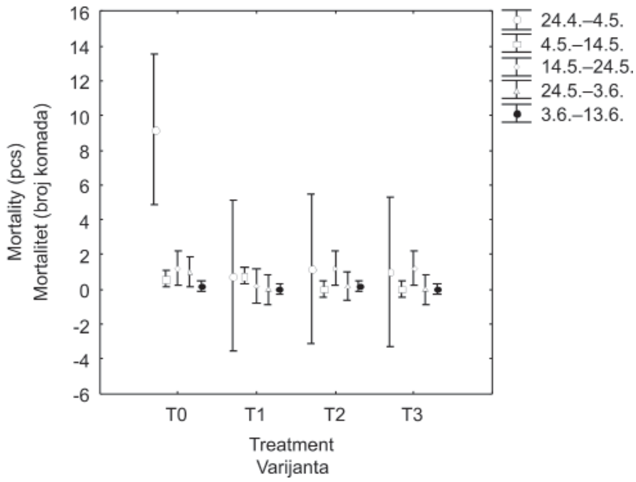

Figure 2 The number of died caterpillars of *Lymantria dispar* within an 48-hour interval in particular stages of rearing depending on the content of nitrogen in food (treatments T0, T1, T2, T3) (0.95 confidence intervals)

Slika 2. Broj uginulih gusjenica gubara u intervalu od 48 sat za svaki stadij ovisno o sadržaju dušika u hrani (tretmani TO, T1, T2. T3) (intervali pouzdanosti 0.95)

and at treatment T3 within the limits 32.35–38.89 $mg.g^{-1}$, maximum values being determined at the beginning of the rearing (Fig. 1).

Mortality of caterpillars – Mortalitet gusjenica

Hatched caterpillars of *L. dispar* in the laboratory rearing in control treatment showed 30.7 % mortality. However, at caterpillars fed on food with the higher content of nitrogen (treatments T1–T3), we determined 2.7–4 % mortality. Differences in the mortality of caterpillars within a 48-hour interval between treatment T0 and treatments T1–T3 were statistically significant in the first 10 days of rearing (p = 0.010–0.013) (Fig. 2).

In control treatment, the mortality of caterpillars continuously increased up to a level of 40.0 % until the 40th day of rearing where it remained until the end of the rearing (40.7 %) (Fig. 3). Caterpillars of the 1st instar were most sensitive in the control treatment (31.3 %). In the consequential 2nd – 6th instars, the decline gradually stopped (6–3.3–0–0–0 %).

The mortality of caterpillars in treatment T1 after 20 days of rearing increases to 5.3 %. After 30 days of rearing, it reaches the final value of 6 % while 3.3 % caterpillars died in the 1st instar and 2.7 % in the 2nd instar.

Mortality in treatments T2 and T3 remained at the initial level (3.3–4 %) in the first 20 days of rearing and differed significantly ($p = 0.022$) from the increasing mortality in treatment T1 (Fig. 2). In further 10 days, it doubled (7.3–8 %). In treatment T3, the value of 7.3 % is final while mortality in T2 increases up to 9.3 % (Fig. 3). In treatment T3, caterpillars died only in the 1st (3.3 %) and 2nd (4 %) instars while in treatment T2, dead caterpillars occur also in the 3rd and 4th instars.

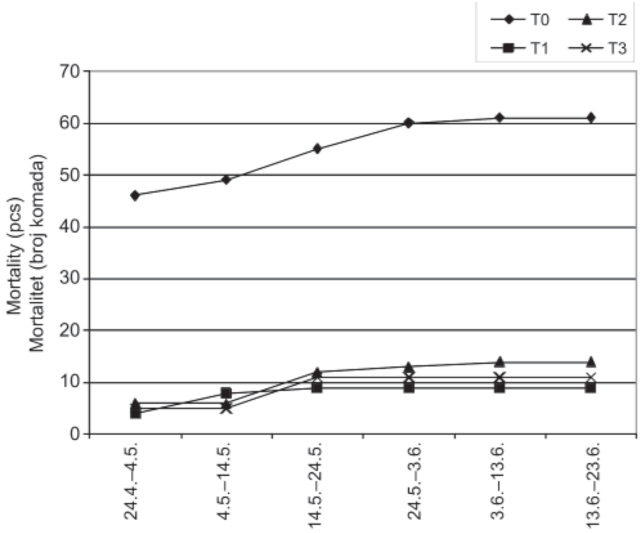

Figure 3. Mortality of caterpillars of *Lymantria dispar* in laboratory rearing with food affected by nitrogen (*Betula pendula*)

Slika 3. Mortalitet gusjenica gubara u laboratorijskom uzgoju sa lišćem breze i dodanim dušikom (*Betula pendula*)

The development of caterpillars – Razvoj gusjenica

The development of caterpillars of *L. dispar* came through six instars according to the sex of a future imago, differences being noted in the duration of particular instars depending on the quality of food.

The shortest total development was determined in the 1st–3rd instars of caterpillars in treatments T1 (32 days) and T2 (34 days). Slower development occurred in treatments T3 (40 days) and T0 (47 days). Statistically significant differences were proved among all treatments with the exception of T1 and T2, T0 and T3 at the 1st instar, T2 and T3 at the 2nd instar, T1 and T2 at the 3rd instar (Fig. 4).

In treatments T1–T3, a trend was kept of the prolongation of the development of caterpillars in the 1st instar (11–12–15), the 2nd instar (12–13–14) and the 3rd instar (9–9–11).

Because caterpillars were reared individually from the 4th instar, a difference was determined in the duration of particular instars between caterpillars of future males and females regardless of the quality of ingested food. Duration of the 4th and 5th instars (males 16, 21 days, females 14, 13 days) and the period of pupae (males 21 days, females 17 days) were statistically significantly longer at males than at females ($p < 0.001$ at the level of significance $\alpha = 0.05$). However, the total duration of the development of males is shorter as compared to females with respect to the absence of the 6th instar, which took at females on average 22 days.

The length of the development of the 4th and 5th instars of caterpillars of future males showed the same dependence on the food quality as the 1st–3rd instars. However, differences at the 4th instar were not significant (T0/18, T1/15, T2/16, T3/17 days). At the 5th instar, a difference was statistically significant between control treatment (T0/26 days) and all other treatments (T1 and T2/20 days, T3/21 days) ($p = 0.001–0.022$) (Fig. 5).

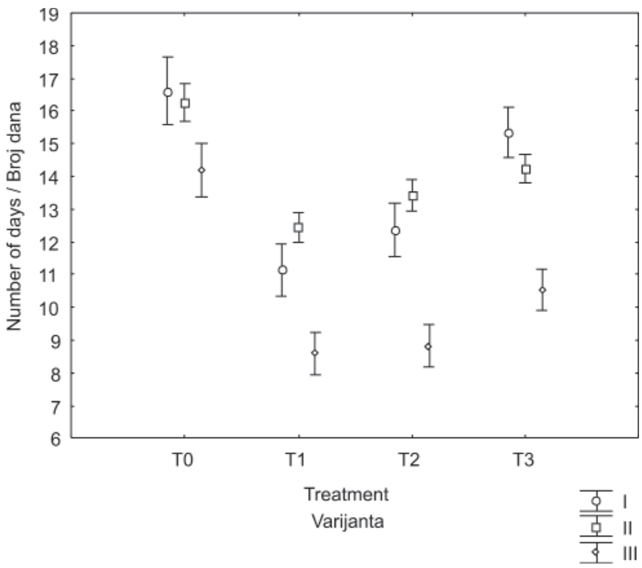

Figure 4. Duration of the development of caterpillars of the 1st, 2nd and 3rd instars of *Lymantria dispar* in laboratory rearing with food affected by nitrogen (*Betula pendula*) (0.95 confidence intervals)

Slika 4. Trajanje razvoja gusjenica gubara 1., 2., i 3. stadija u laboratorijskom uzgoju brezovim lišćem s dodatkom dušika (*Betula pendula*) (intervali pouzdanosti 0.95)

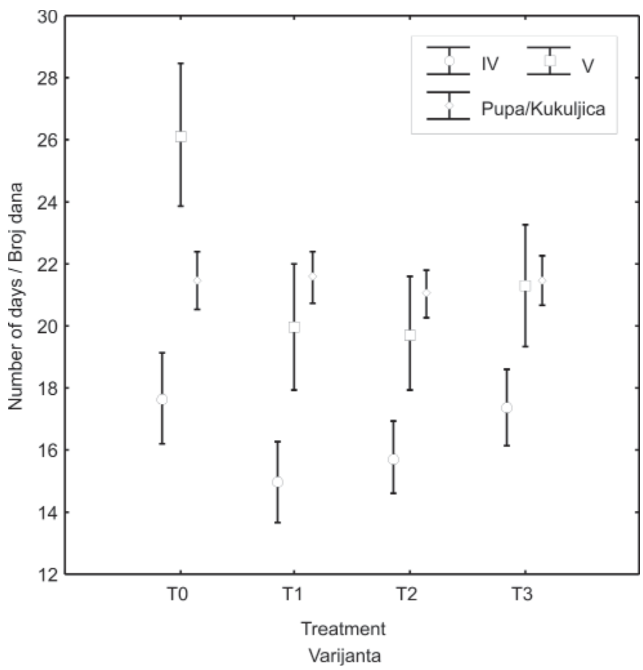

Figure 5. Duration of the development of caterpillars of the 4th and 5th instars and duration of the pupa stage at future males of *Lymantria dispar* in laboratory rearing with food affected by nitrogen (*Betula pendula*) (0.95 confidence intervals)

Slika 5. Trajanje razvoja gusjenica 4. I 5. Stadija i trajanje stadija kukuljica budućih mužjaka gubara u laboratorijskom uzgoju s dodatkom dušika breze (*Betula pendula*) (intervali pouzdanosti 0.95)

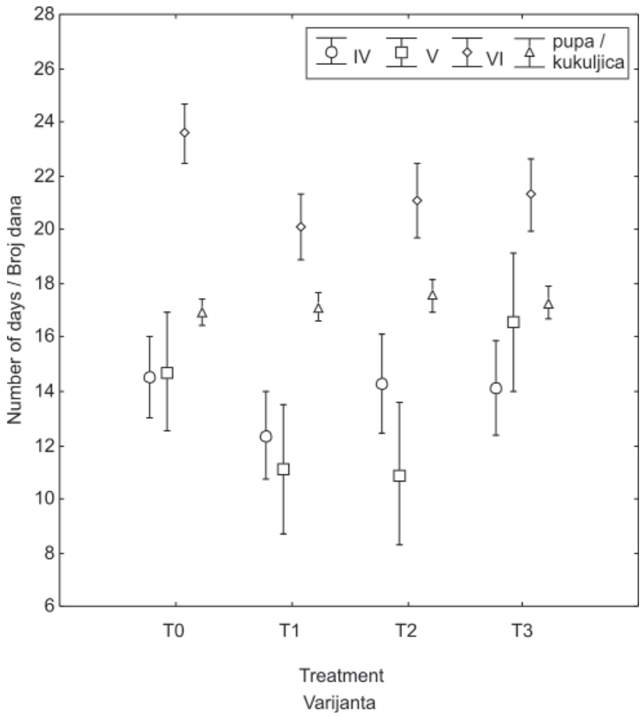

Figure 6. Duration of the development of caterpillars of the 4th, 5th and 6th instars and duration of a female pupa of *Lymantria dispar* in laboratory rearing with food affected by nitrogen (*Betula pendula*) (0.95 confidence intervals)

Slika 6. Trajanje razvoja gusjenica 4. I 5. stadija i trajanje stadija kukuljica budućih ženki gubara u laboratorijskom uzgoju hranom lišća breze s dodatkom dušika (*Betula pendula*) (intervali pouzdanosti 0.95)

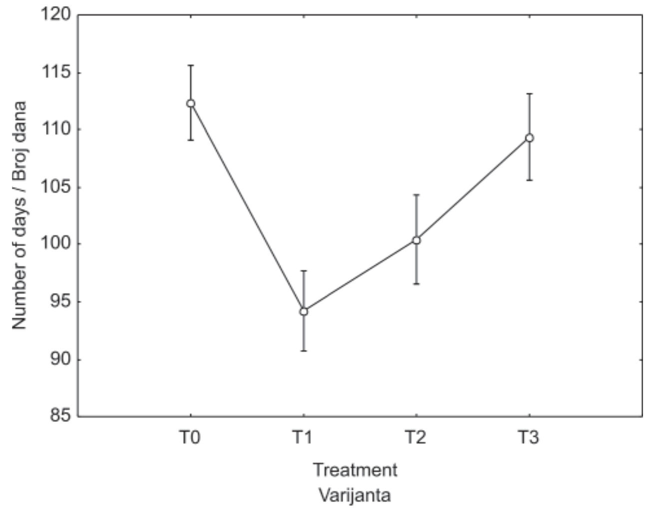

Figure 8. Total duration of the development of females of *Lymantria dispar* depending on the content of nitrogen in food (0.95 confidence intervals)

Slika 8. Ukupno trajanje razvoja ženki gubara ovisno o količini dušika u hrani (intervali pouzdanosti 0.95)

The development of the 4th instar of caterpillars of future females was fastest in treatment T1, but differences were statistically not significant (T0/15, T1/12, T2 and T3/14 days). The 5th instar in treatments T1 and T2 responded in the same way (11 days). The development was prolonged in treatments T0 (15 days) and T3 (17 days). Statistically significant differences occurred between treatments T1 and T3 as well as T2 and T3 ($p = 0.018$). The 6th instar was shortest in treatment T1 (20 days), which differed significantly ($p = 0.001$) from control treatment (24 days) (Fig. 6). The duration of a pupa was independent on the food quality ingested by caterpillars (Figs. 5, 6) both in males and females.

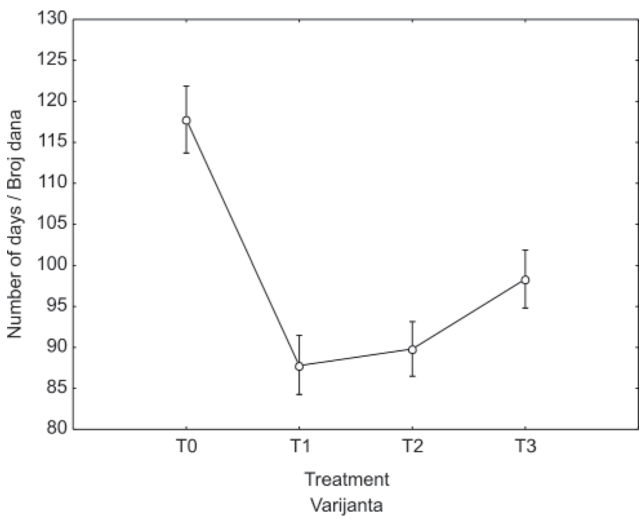

Figure 7. Total duration of the development of males of *Lymantria dispar* depending on the content of nitrogen in food (0.95 confidence intervals)

Slika 7. Ukupno trajanje razvoja mužjaka gubara ovisno o sadržaju dušika u hrani (intervali pouzdanosti 0.95)

The total duration of development from hatching a caterpillar to a new moth was at males and females shortest in treatment T1 (males 88, females 94 days) followed by treatment T2 (males 90, females 100 days), T3 (males 98, females 109 days) and T0 (males 118, females 112 days). Differences between particular treatments were statistically significant except T1 and T2 at males; T1 and T2, T0 and T3 at females (Figs. 7, 8).

The weight of pupae – Težina kukuljice

The weight of female pupae (0.61 g) regardless of the food quality of caterpillars was significantly higher ($p < 0.001$) than the weight of male pupae (0.25 g) (Fig. 9). At males, effects of the food quality on the weight of pupae were not proved (T0/0.20 g, T1/0.29 g, T2/0.26 g, T3/0.21 g) because of the small number of males in treatment T0, which caused the large dispersion of values (Fig. 9). At females, treatment T0 (0.45 g) significantly ($p < 0.001$) differed from other treatments T1–T3 (0.72–0.74–0.67 g) (Fig. 9).

Food consumption – Potrošnja hrane

The total consumption of food of caterpillars of the 1st–3rd instars was highest in treatment T0 (0.06 g), which significantly ($p < 0.001$) differed from treatments T1–T3 (0.05–0.04–0.04 g) (Fig. 10).

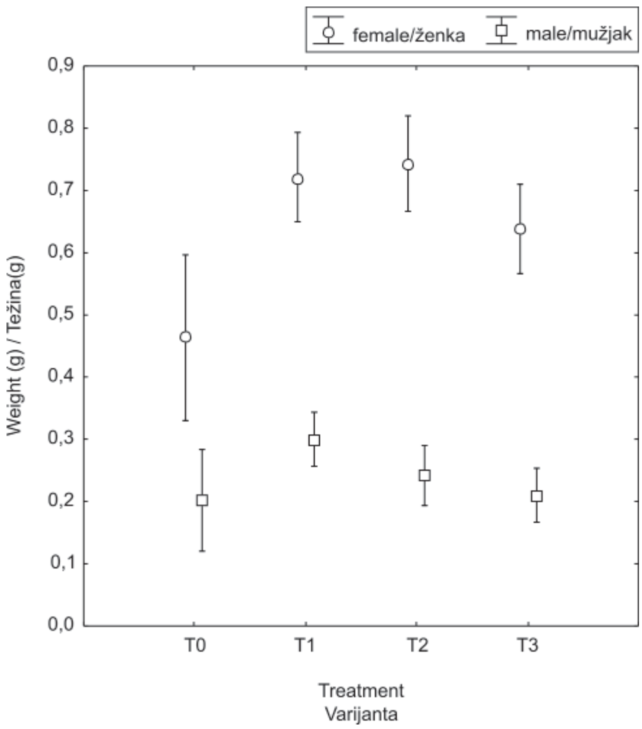

Figure 9. The weight of male and female pupae of *Lymantria dispar* depending on the content of nitrogen in food (*Betula pendula*) (0.95 confidence intervals)

Slika 9. Težina muških i ženskih kukuljica gubara ovisno o sadržaju dušika u hrani brezova lišća (*Betula pendula*) (intervali pouzdanosti 0.95)

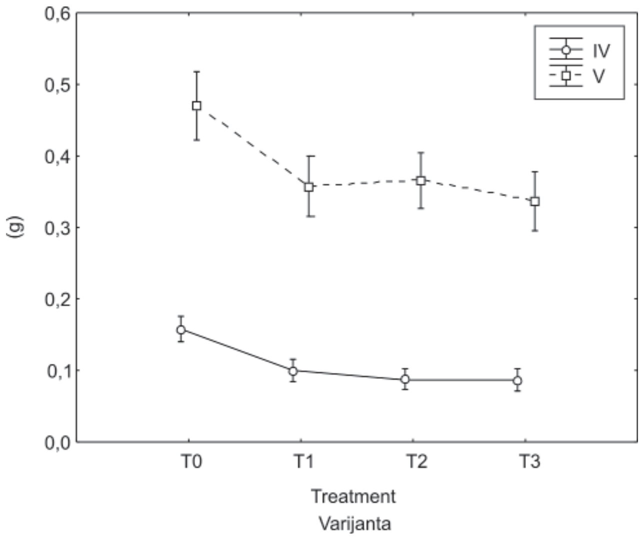

Figure 11. The total food consumption (g DM of leaves) of male caterpillars of *Lymantria dispar* in the course of the 4th and 5th instars (0.95 confidence intervals)

Slika 11. Ukupna konzumacija hrane (g DM suhe tvari lišća) gusjenica budućih ženki gubara tijekom 4. i 5. stadija (intervali pouzdanosti 0.95)

The same trend showed the total consumption of food of caterpillars of future males of the 4th instar (T0/0.16 g, T1/0.10 g, T2/0.09 g, T3/0.09 g; $p < 0.001$) and the 5th instar (T0/0.47 g, T1/0.36 g, T2/0.37 g, T3/0.34 g; p=0.001–0.015) (Fig. 11), which became evident in the whole consumption of food of caterpillars of future males (T0/0.74 g, T1/0.50 g, T2/0.49 g, T3/0.47 g; $p < 0.001$) (Fig. 12).

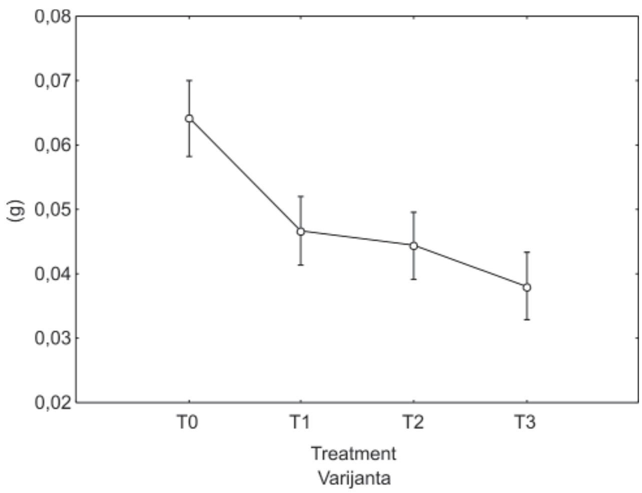

Figure 10. The total consumption of food (g DM of leaves) of caterpillars of *Lymantria dispar*, the 1st–the 3rd instars (0.95 confidence intervals)

Slika 10. Ukupna konzumacija hrane (g DM suhe tvari lišća) gusjenica gubara 1. do 3. stadija (intervali pouzdanosti 0.95)

At caterpillars of future females, statistically significant differences in the food consumption were not noted except the 4th instar where treatment T1 (0.12 g) differed from treatment T3 (0.08 g) ($p = 0.025$). The total consumption of food of caterpillars of the 5th instar reached 0.24–0.30 g and of the 6th instar 1.01–1.21 g (Fig. 13). In the total consumption of food of females, significant differences were not determined among particular treatments (T0/1.51 g, T1/1.57 g, T2/1.61 g, T3/1.40 g) (Fig. 14). Caterpillars of future females showed (as expected) significantly higher total consumption of food than caterpillars of future males (1.52 and 0.54 g; $p < 0.001$).

Discussion
Rasprava

Gypsy moth is a polyphagous species occurring in the majority of broadleaved species and in some conifers (Doane, McManus 1981). Although birch does not rank among the most attractive species, it was selected as a nutritive species to compare a possible response with other phytophages trophically related particularly to birch – *Cabera pusaria* L., *Lochmaea caprea* L., *Phyllobius* sp., *Euceraphis betulae* Koch (Kula et al. 2012). The content of nitrogen in leaves of deciduous species is given within the limits 10–40 $mg.g^{-1}$ (Larcher 1988). Through the fertilization of birch by ammonium nitrate significantly differentiated values were achieved of the concentration of nitrogen in leaves (17.17–38.89 $mg.g^{-1}$).

The low content of nitrogen in food causes its increased consumption and feeding, digestion and development are generally prolonged. Other mechanisms to manage the inadequate content of nitrogen consist in the presence of symbiotic organisms in the digestive system or casual carnivory (Mattson 1980). With respect to the natural decline of nitrogen in the assimilatory organs of trees during the growing season it is possible to suppose that spring species of leaf-eating or sucking species profit from the higher level of nitrogen unlike species with summer feeding where the natural level of nitrogen is lower.

Comparing the mortality and duration of the development of caterpillars and the weight of pupae of *L. dispar* on birch, beech, maple and oak the highest mortality, the longest development of caterpillars and the lowest weight of pupae were noted in maple while the caterpillars developed best in birch (Barbosa, Greenblatt 1979). It could be related to the different level of nitrogen in leaves of trees – birch 25–40 $mg.g^{-1}$, maple 16–23 $mg.g^{-1}$ (Bergmann 1988).

Decreasing the level of nitrogen in artificial food from 24.4 to 12.6 $mg.g^{-1}$ did not show any affect on the mortality of caterpillars of the 4th instar of *L. dispar* (Lindroth et al. 1991) due to the higher tolerance of older caterpillars to changes of the food quality. Subsequently, it was proved that the mortality of *L. dispar* caterpillars fed on food with the low content of nitrogen (15 $mg.g^{-1}$) was 2.4-times higher than in caterpillars fed on food with the high content of nitrogen (37 $mg.g^{-1}$) (Lindroth et al. 1997).

In rearing on birch, we determined that this difference was even six fold. Karowe, Martin (1989) came to similar conclusions at caterpillars of a moth *Spodoptera eridania* Stoll, which showed 4 generations per year in Florida (Mitchell, Tumlinson 1994). On the other hand, at a sawfly *Neodiprion swainei* Midd., the feeding of which culminates in August and at the beginning of September (McLeod 1970), the mortality of caterpillars with the content of nitrogen in needles of *Pinus banksiana* Lamb. increased (Smirnoff, Bernier 1973). Also caterpillars of *C. pusaria* (summer and late summer occurrence) responded to the higher level of nitrogen in leaves of birch by increased mortality (Kula et al., in print).

Slightly increased content of nitrogen (27.38–33.91 $mg.g^{-1}$) shortened the duration of development of males of L. *dispar* by 30 days and that of females by 18 days as compared

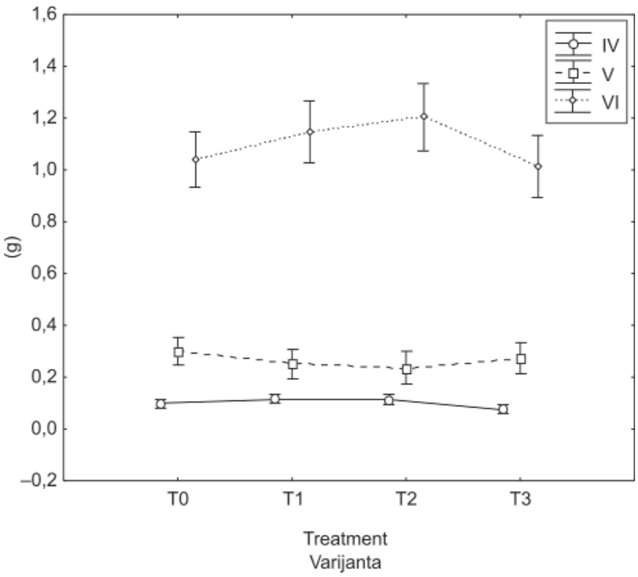

Figure 13. The total food consumption (g DM of leaves) of female caterpillars of *Lymantria dispar* during the 4th, 5th and 6th instars (0.95 confidence intervals)

Slika 13. Ukupna konzumacija hrane (g DM suhe tvari lišća) budućih ženki gubara za vrijeme 4., 5., 6. stadija (intervali pouzdanosti 0.95)

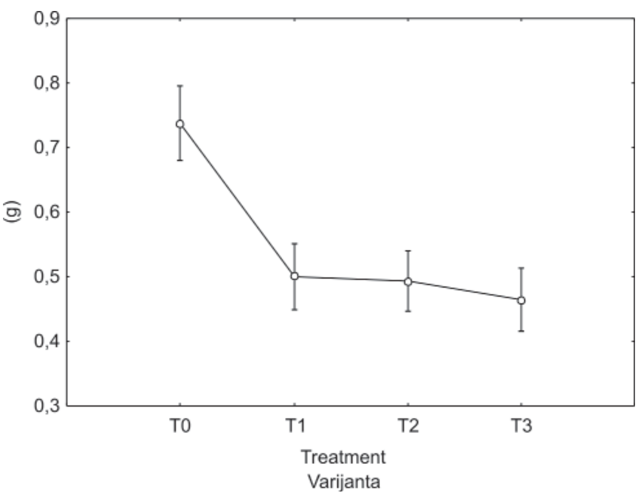

Figure 12. The total food consumption of male caterpillars of *Lymantria dispar* depending on the content of nitrogen in food (0.95 confidence intervals)

Slika 12. Ukupna konzumacija hrane budućih mužjaka gubara ovisno o sadržaju dušika u hrani (intervali pouzdanosti 0.95)

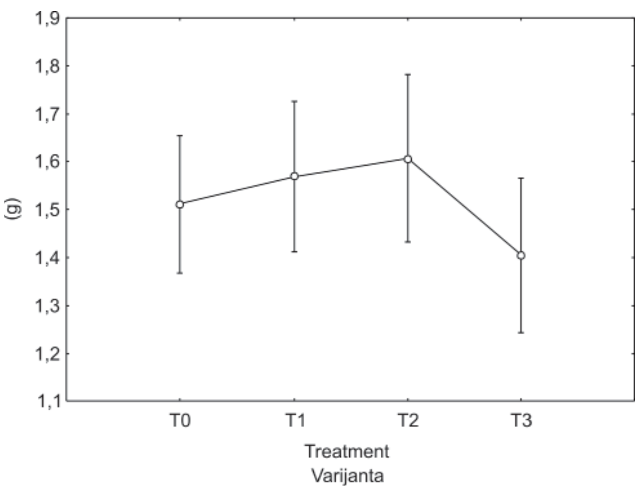

Figure 14. The total food consumption of female caterpillars of *Lymantria dispar* depending on the content of nitrogen in food (0.95 confidence intervals)

Slika 14. Ukupna konzumacija hrane gusjenica budućih ženki gubara ovisno o dušiku u hrani (intervali pouzdanosti 0.95) with development affected by the low level of nitrogen in leaves (17.17–28.21 $mg.g^{-1}$). Lindroth et al. (1991, 1997) noted the prolonged development of males and females of *L. dispar* fed on food with the low content of nitrogen. In pine looper moth (*Bupalus piniarius* L.), the caterpillars of which hatch usually in the 2^{nd} half of July (Křístek, Urban 2004, Schwenke 1978), increased levels of nitrogen showed negative effects causing the prolongation of development (Katzel, Loffler 1995). At caterpillars of *Choristoneura fumiferana* Clem. feeding early in spring (Crummey, Otvos 1980), the content of nitrogen in needles of *Abies balsamea* (L.) Mill. did not affect the length of development (Shaw, Little 1972). Reasons of the development prolongation in treatment T3 can consist in the slightly stressing response to the high concentration of nitrogen as compared with natural conditions of oak forests.

The weight of female pupae of *L. dispar* fed on leaves of unfertilized birch trees was markedly lower as compared with treatments enriched by nitrogen. The fall of weight of pupae at the reduced content of nitrogen in the food of caterpillars of *L. dispar* was noted by a number of authors (Joseph et al. 1993, Lindroth et al. 1991, 1997, Giertych et al. 2005), further Shaw, Little (1972) at pupae of *Ch. fumiferana* and Bryant et al. (1987) at pupae of *Choristoneura conflictana* Walk, which are early spring species (Prentice 1955).

Male caterpillars fed on food with the low content of nitrogen consumed higher amounts of food to complete their development, which was also proved by Lindroth et al. (1991, 1997).

Although *Danaus plexippus* L. is a regularly migrating moth with four generations per year (Ackery, Vane-Wright 1984), it responded similarly as *L. dispar* (Schroeder 1976). However, Hättenschwiler, Schafellner (1999) noted the growth of the consumption of food with the increasing content of nitrogen in needles of spruce at caterpillars *Lymantria monacha* L., which is, however, surprising at an early spring species (Křístek, Urban 2004, Schwenke 1978).

Different responses of males and females to changes in the quality of food of *L. dispar* were also confirmed by Lindroth et al. (1991, 1997) and Giertych et al. (2005). More than one half of the amount of nitrogen assimilated by the caterpillar of a future female is used for the production of eggs (Montgomery 1982).

Conclusions
Zaključci

In the laboratory rearing of caterpillars of gypsy moth (*L. dispar*) the lack of nitrogen in food became evident as a stress factor. This stress manifested itself in the increased mortality and prolonged development of caterpillars of *L. dispar* as well as in the decreased weight of pupae in control rearing. Under conditions of the lack of nitrogen, male caterpillars needed to ingest the higher amount of food to complete their development. Through this experiment a hypothesis has been supported that spring phytophagous species prefer tissues with the higher content of nitrogen. Nevertheless, caterpillars can respond to very high inputs of nitrogen in food also negatively.

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Sažetak:

Rast i mortalitet, trajanje razvoja gusjenica, težina kukuljica i plodnost ženki gubara ovisna je o biljci domaćinu. U laboratorijskom uzgoju o utjecaju različitog sadržaja dušika (17.17–38.89 mg.g^{-1}) na razvoj, mortalitet i konzumaciju gusjenica gubara istraživano je s lišćem breze (*Betula pendula* Roth).

Jednogodišnje biljke breze uzgajane su izvan kontejnera na zemljanom supstratu, gdje su razlike u sadržaju dušika dobivene primjenom amonijevog nitrata (NH_4NO_3) u tri tretmana 0.5g (T1); 1g (T2) i 1.5g (T3). Sadržaj dušika u lišću određeno je po Kjeldahl metodi u uređaju Kjeltec analizatoru UNIT 2300. Uzgoj gusjenica proveden je u kontroliranim uvjetima u Climacell 707 termostatu, gdje je konzumacija hrane praćena primjenom uređaja ADC Bio Scientific Leaf Meter AM 300 ili pomoću amalizatora NIS – Elements AR.

Niski sadržaj dušika u hrani bio je uzrok većeg mortaliteta u kontrolnom tretmanu (40.7 %) u usporedbi s gusjenicama tretiranima s dušikom (6–9.3 %) (sl. 2, 3). Lindoth et. (1997) al je utvrdio da smanjeni sadržaj dušika uzrokuje porast mortaliteta gusjenica. Barbose, Greenblatt (1979) su smatrali da je breza u spektru hranidbenih vrsta preferirana za gusjenice gubara. Postoji korelacija s količinom dušika u lišću (Bergman 1988). Najkraća dužina razvoja kod malog povećanja sadržaja dušika (27.38–33.91 mg.g^{-1}) traje 88–94 dana, a kod povećanja razine dušika (30.38–36.25 mg.g^{-1}) razvoj traje 90–100 dana, a kod velikog povećanja dušika (32.35–38.39 mg.g^{-1}) 98–109 dana, a kod kontrolnog tretmana s dušikom 17.17–28.21 mg.g^{-1} ekstremno je produžen razvoj (118–112 dana) (sl. 7, 8). Ukupna konzumacija hrane 1. do 3. stadija bila je veća u kontrolnom tretmanu signifikantno od tretmana s povećanim dušikom (sl. 10). Kod mužjaka utjecaj kvalitete hrane na težinu kukuljica nije utvrzen, ali se kod ženki, statističko signifikantne razlike javljaju između kontrole i tretmana s dušikom (sl. 9).

KLJUČNE RIJEČI: dušik, stres, *Betula pendula*, gubar, gusjenica, laboratorijski uzgoj, razvoj