

ECOLOGY, BEHAVIOR AND BIONOMICS

Effect of Nitrogen on *Passiflora suberosa* L. (Passifloraceae) and Consequences for Larval Performance and Oviposition in *Heliconius erato phyllis* (Fabricius) (Lepidoptera: Nymphalidae)

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Efeito do Nitrogênio sobre *Passiflora suberosa* L. (Passifloraceae) e Conseqüências para o Desempenho Larval e Oviposição de *Heliconius erato phyllis* (Fabricius) (Lepidoptera: Nymphalidae)

RESUMO - Avaliou-se a influência do nitrogênio no crescimento e nas características morfológicas e nutricionais de *Passiflora suberosa* L. e as conseqüências no desempenho larval e oviposição de *Heliconius erato phyllis* (Fabricius). Foram utilizados três níveis de nitrogênio no solo (tratamentos) para o cultivo de *P. suberosa*: 0, 150 e 300 mg L⁻¹. Larvas recém-eclodidas foram criadas em laboratório (25 ± 1°C), individualmente, em ramos das plantas cultivadas em cada tratamento e fêmeas capturadas em campo foram submetidas a testes de escolha para oviposição. A taxa de crescimento, a área foliar e o comprimento dos internódios de *P. suberosa* aumentaram significativamente com a adição de nitrogênio. A dureza das folhas jovens nas plantas cultivadas sem adição de nitrogênio foi maior, as quais também apresentaram menor conteúdo de água. Houve maior concentração de nitrogênio e potássio nas plantas cultivadas com a adição de nitrogênio no solo. A qualidade nutricional do alimento expressa pela maior concentração de nitrogênio e água, diminuiu o tempo de desenvolvimento larval e aumentou o tamanho dos adultos, embora não tenha afetado a sobrevivência de forma significativa. As fêmeas escolheram os ramos das plantas fertilizadas para oviposição, o que foi positivamente relacionado com desempenho das larvas. Isto poderia explicar a manutenção desse comportamento, bem como a escolha por ramos maiores, registrado em estudos anteriores.

PALAVRAS-CHAVE: Dureza foliar, interação inseto-plantas, qualidade nutricional, tamanho, tempo de desenvolvimento

ABSTRACT - The present study examines the influence of nitrogen on growth rates, morphological and nutritional characteristics of *Passiflora suberosa* L., and the corresponding consequences for *Heliconius erato phyllis* (Fabricius) growth and oviposition. The treatments consisted of different nitrogen doses (0, 150 and 300 mg L⁻¹) applied to the soil. Newly emerged larvae were individually reared in the laboratory (25 ± 1°C) on branches of *P. suberosa* obtained from plants grown under the three nitrogen levels, and field collected females were submitted to oviposition choice tests. *P. suberosa* growth rates, leaf area and internode length increased significantly when grown with nitrogen enriched soil. *P. suberosa* presented significantly greater leaf toughness when grown without nitrogen addition. Leaves of this species also presented lower water content and toughness. Nitrogen addition increased the concentration of nitrogen and potassium on plant leaf tissues. The greater nitrogen concentration on plants did not affect *H. erato phyllis* larval survival, but led to a reduction in larval development time and increased adult size. Females significantly preferred to lay eggs in plants cultivated in nitrogen enriched soil. This choice was positively correlated with larval performance, which could be a possible explanation for the maintenance of that behaviour, as well as the choice of larger branches as reported in previous studies.

KEY WORDS: Leaf toughness, insect-plant interaction, insect development rate, insect body size, and plant nutritional value

The use of different species of host plants with varied physical characteristics and nutritional contents can influence survival, growth, development time, reproduction and choice behaviour of herbivorous insects (Scriber 1982, 1984; Mattson & Scriber 1987; Awmack & Leather 2002). Intraspecific differences among host plants can result from their own development or from environmental factors, as soil nutrient availability (Bernays & Chapman 1994, Schoonhoven *et al.* 1998). As an answer to such factors, plants can present morphological or chemical variation. Among the morphological characteristics, internode length, leaf area, toughness, hairiness and leaf thickness can influence nutrition or even acceptance or rejection of a certain plant by an insect (Fernandes 1994, Coley & Barone 1996, Kerpel & Moreira 2005). Among the chemical characteristics, nutritional content, water content and the presence of secondary metabolite compounds can have such an influence (Scriber & Feeny 1979, Slansky 1992, Bernays & Chapman 1994, Schoonhoven *et al.* 1998).

Nitrogen is a mineral nutrient required by all organisms, being the main component of the body, the genetic code and the metabolism (Mattson 1980, Scriber 1984, Simpson & Raubenheimer 1993, Simpson *et al.* 1995). Scriber (1984) compiled 115 studies on herbivorous insects response to plant fertilization with nitrogen, showing that many of these responses are contradictory. For example, there was an increment on some performance components of species fed with fertilised plants and in 44 cases the latter were less attacked by herbivores. On the other hand, in some cases there were decreases in the populations of the herbivores in sites with low nitrogen content. Besides the physiological changes, insect responses could have been caused by morphological change on the part of the plants, as in leaf area and corresponding microclimate. The latter factor interferes in the relationship with other plants and with pathogens (Scriber 1984).

Some insects are capable of recognising the physiological state of their host plants, including their nutritional value. Adults of *Pieris rapae* L. (Lepidoptera: Pieridae), for example, choose plants treated with fertiliser (Southwood 1972, Myers 1985). Other herbivorous species depend on young tissues in their early stages of development (Thomas 1987). Larvae of *Heliconius erato phyllis* Fabricius (Lepidoptera: Nymphalidae), for example, present a high mortality rate when fed with mature leaves during the early instars (Rodrigues & Moreira 1999). Apart from physical characters, as lower toughness and thickness, young tissues are known to have higher percentages of nitrogen and water than mature tissues (Slansky & Weller 1992, Bernays & Chapman 1994).

The larvae of the heliconians can use many species of passionflowers as food sources (Benson *et al.* 1976, Brown 1981, Menna-Barreto & Araújo 1985). *Passiflora suberosa* L. is one of the most frequently used species by *H. erato phyllis* in Rio Grande do Sul. Females select larger shoots and apical regions free from eggs of conspecifics for oviposition, among other parameters (Mugrabi-Oliveira & Moreira 1996a,b; Kerpel & Moreira 2005). Natural populations of *P. suberosa* have diverse morphological and

nutritional characteristics, probably because of edaphic and environmental factors (Barp *et al.* in press). The aim of this study is to verify 1) the effect of nitrogen on the morphological and nutritional characteristics of *P. suberosa*, as well as on its growth, and 2) the corresponding influences on larval development time, adult size and *H. erato phyllis* oviposition behaviour.

Materials and Methods

Soil preparation. Approximately 0.7 m³ of a lithosoil (Bastos *et al.* 1998) was taken from the sub superficial layer (20-40 cm), in an area of the Campus do Vale, Universidade Federal do Rio Grande do Sul (UFRGS). After screening in a 4-mm mesh and homogenising, a sample of approximately 1 L was taken to chemical analysis, in October 2000. A basic correction of the soil fertility was performed after the analysis results and recommendations of the Comissão of Fertilidade do Solo - RS/SC (1994) and the Comissão of Fertilidade do Solo of Minas Gerais (1999). The fertility correction consisted on applying 200 mg L⁻¹ of P, 150 mg L⁻¹ of K and a solution of micronutrients (Bo, Cu, Fe, Mn, Mo, Zn). The sources were triple superphosphate (42 % P₂O₅ = 21 % P) and potassium chloride (60 % KCl = 50% K), for P and K, respectively. A basic fertilisation was applied in order to avoid possible limitations in other nutrients essential for plant growth. Treatments consisted on applying three doses of N: 1) zero (treatment N0); 2) 150 mg L⁻¹ (treatment N150); 3) 300 mg L⁻¹ of soil (treatment N300). Urea was used as a source of N (45% N). Given the relatively high pH (6,2) and because passionflowers are supposedly tolerant to acidic soils (Mânica 1981) no limestone correction was used.

P. suberosa seedlings that were obtained from the Horto Florestal Barba Negra (Barra do Ribeiro, RS) were transplanted to 3-L pots, between November 24th 2000 and December 5th 2000. The 84 pots were divided in four blocks (21 pots/block); that is, each treatment had seven replicates per block. After planting, the pots were distributed equidistantly in a shelter with 7 x 3.5 x 2.3 m, with 50%-sombrite screen for protection, in the Departamento de Ecologia of UFRGS. Pots placement was randomised according to Zar (1999), with restriction (trios of the same treatment). Plants were watered daily.

A micronutrients solution made of a mixture of salts containing Bo, Cu, Mn, Mb, Zn, Fe was used. Overall, 30 ml of a "half-strength" solution was applied per pot; with 10 ml one week after planting, 10 ml one day after pruning (10/01/2001), and other 10 ml after two months (10/03/2001). Two N topdressings were applied on the treatments N150 and N300: one on 07/02/2001 and another on 07/04/2001, at the doses 84 mg and 168 mg of nitrogen per kg of soil, respectively.

Leaf morphometry. To obtain morphometric parameters of the leaves we followed the procedure used by Barp *et al.* (in press). Three pots of each block were randomly chosen, for each treatment (n = 12 pots/treatment). From each plant one shoot was chosen and cut off 10 cm from the soil level,

and then marked with tape. The shoots were evaluated fortnightly for length, number of leaves and leaf area. The number of leaves was recorded so that internode length could later be calculated. The evaluations were done until 98 days after cutting. This period included the beginning of shoot development through to its stabilisation.

On each sampling, the leaves were counted and the shoot length measured. The leaf area was measured by placing the leaves, one by one, on a leaf paper, and drawing the contour. Leaf drawings were digitised and images measured with Image Tool 2.0® software for Windows. Internode length was obtained by dividing shoot length by number of leaves. Shoot length, internode length and leaf area (seven samples throughout time for each of these three parameters) were compared by means of analysis of variance (repeated measures ANOVA), followed by Tukey's multiple comparison tests.

At the end of the 98-day period (April 16th 2001), the leaves were removed from the plants and thickness, toughness and hairiness of the first and fifth apical leaves of the marked shoots were evaluated in the lab. The first and fifth apical leaves were used because *H. erato phyllis* larvae consume first the apical region of the shoot and continue toward the plant base, where tissues are mature (Rodrigues & Moreira 1999). To allow comparisons between leaves on the same position among the three treatments, we used the first leaves as young ones and the fifth leaves as mature ones, given that most shoots in treatment N0 had the maximum of five leaves.

Thickness was obtained by a transversal cut on the median region of the leaf blade. Cuts were done with shaving metal razors and mounted on excavated glass slides containing distilled water. Then, thickness was measured under microscope equipped with a micrometric scale ocular. Hairiness was quantified from a 1-mm² area, on the central region of the leaf blade, beside the midrib on the adaxial leaf face. A microscope equipped with a micrometric scale ocular was used to count the trichomes. Toughness was determined indirectly by the force necessary to perforate a 1-mm² area of the first and fifth leaves, using a perfurometer (Barp et al. in press, Sanson et al. 2001).

Toughness, thickness and hairiness were compared with Kruskal-Wallis's tests, followed by Dunn's multiple comparison tests, because data did not follow a normal distribution.

Leaf nutritional analyses. Shoots representing five samples from each treatment were taken in minimal amounts, just enough to employ the kind of analyses used. Freshly collected material was weighted on an analytical scale, to obtain fresh weight. Afterwards, the material was placed in packets of brown paper and kept on a kiln under $50 \pm 5^\circ\text{C}$, during a week, and again weighted to obtain dry weight. Water content was obtained by subtracting dry weight from fresh weight. Water percentages were arcsine transformed (Zar 1999) and then submitted to analysis of variance (ANOVA), followed by Tukey's multiple comparison tests.

Dried material was pounded on a knife mill in the Laboratório de Solos of the Faculdade de Agronomia, UFRGS,

and then taken to the analyses, following procedures described in Tedesco et al. (1985). Results from each sample were obtained from the average of two measurements. Macronutrient content was obtained in percentages, and transformed to g kg⁻¹ for statistical analyses. Where ANOVA were significant, treatments were compared with Tukey's multiple comparison tests. In cases where data did not follow a normal distribution (under a Kolmogorov-Smirnov's test) Kruskal-Wallis' tests were used, followed by Dunn's multiple comparison tests.

Larval rearing. Freshly hatched larvae of *H. erato phyllis* were individualised on shoots of *P. suberosa* obtained from the pots on different nitrogen levels. Larvae came from butterfly captured in the Vale Campus (UFRGS) and kept in an insectary in the Departamento de Ecologia. Shoots containing the larvae were kept in bottles with water, covered with fine cloth and maintained in the lab ($25 \pm 1^\circ\text{C}$; 14h of light/day) (described in detail by Mugarab-Oliveira & Moreira 1996a). Shoots were examined daily and changed when necessary, so that adequate food was always available. Also, larval death or ecdysis was registered daily, with instar duration and lifetime recorded from the first instar to adult emergence. Also, right anterior wing length of the adults was measured with a calliper.

Larval survival was compared with a "logrank" test. Larval development time and adult size were analysed with a Kruskal-Wallis's test, followed by Dunn's multiple comparisons, since they did not follow normal distributions. Wing length was analysed with an ANOVA, followed by Tukey's multiple comparisons test.

Oviposition test. To test the capacity to recognise shoot quality for oviposition, females were captured in forest edges in the Vale Campus of UFRGS. After capture, butterflies were kept in insectary for one day and then tested individually (n = 20). The tests took place inside a wire frame insectary 10 x 5 x 2.7 m, covered with black, 50%-sombrite, divided in ten compartments. Five shoots obtained from plants cultivated on soil with the three levels of nitrogen were offered to each butterfly, totalling 15 shoots (multiple choice tests). The shoots had five leaves open and intact apical regions and were kept in bottles containing water and distributed in equidistant compartments, alternating the different treatments. Each butterfly was tested during a day (from 9 a.m. to 5 p.m.). At the end of each period, shoots were checked and the number of eggs in each condition was recorded. The females were freed afterwards. At the beginning of the tests, food was offered to each female. Food consisted of a semi-natural diet with distilled water (50 ml), saccharose (7.5 g), and commercial pollen (1.5 g) (Mugarab-Oliveira & Moreira 1996a). Tests were suspended during rainy days, given the low oviposition activity.

Results of the oviposition tests were compared with a Kruskal-Wallis test, followed by Dunn's multiple comparison tests, for not following a normal distribution (Kolmogorov-Smirnov's test).

Results

Morphometric measurements. Shoot growth, leaf area and

internode length of *P. suberosa* were significantly different among plants cultivated under the three levels of nitrogen, increasing with the amount of mineral added (Fig. 1).

There were no significant differences in thickness,

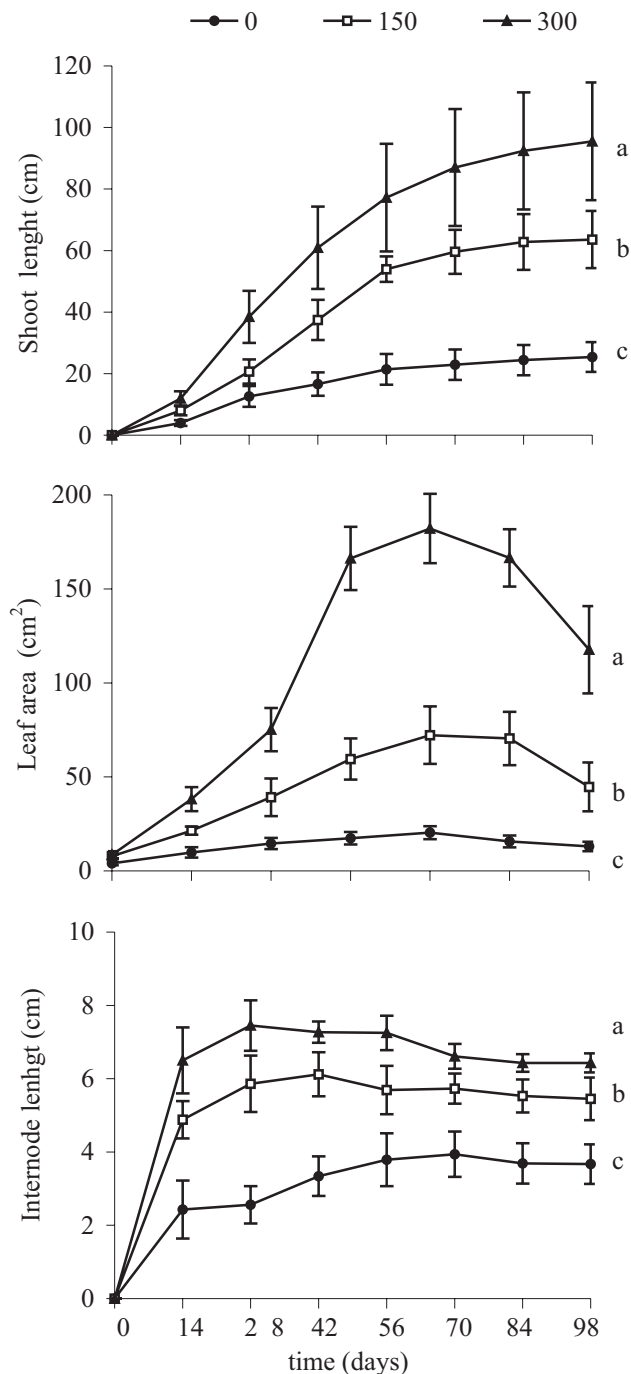


Fig. 1. Temporal variation (average \pm standard error) in shoot growth, leaf area and internode length of *P. suberosa* shoots cultivated under different nitrogen levels in the soil (mg L^{-1}). Curves followed by different letters differ significantly (repeated measures ANOVA, followed by Tukey's multiple comparison tests, $\alpha < 0.05$; $n = 9$ per treatment).

neither for the first or the fifth leaf of *P. suberosa* plants cultivated with different levels of nitrogen (Fig. 2). There was a higher toughness only for the first leaves of plants without nitrogen addition compared with those cultivated with N300. There were no differences in hairiness among treatments, neither for the first nor for the fifth leaf.

Water content in flowers cultivated without nitrogen supplementation was significantly lower than those cultivated with addition of N150 and N300. There were no significant differences between the latter two treatments (Fig. 3).

Nutritional leaf analyses. Plants cultivated with addition of 300 and 150 mg L^{-1} of nitrogen had higher concentrations of this mineral in their tissues than plants cultivated in N0. There were no differences between the concentration of nitrogen in plants cultivated under N150 and N300 (Table 1). There was a significant difference between the concentration of potassium in plants cultivated under N300 and without nitrogen addition. Plants cultivated with N300 had significantly less calcium than plants without nitrogen addition. There were no significant differences in the concentration of magnesium, phosphorous and sulphur among the three treatments. For the micronutrients, there were higher concentrations of iron, copper and sodium for plants without nitrogen addition, relative to plants with N300 addition, and no differences among the other treatments. Zinc concentration was higher on plants cultivated under N0 than on plants cultivated under N150. *P. suberosa* cultivated with N300 had higher concentrations of manganese compared to plants in treatment N0.

There were no significant differences in larval survival for larvae fed shoots of *P. suberosa* cultivated under different soil nitrogen levels and those without addition of the mineral (Fig. 4). Development time was significantly longer for larvae fed shoots from soils without nitrogen addition, compared to those fed shoots of *P. suberosa* with addition of 150 mg L^{-1} and 300 mg L^{-1} of nitrogen (Fig. 5). Adults obtained from larvae that fed on nitrogen-supplemented shoots of *P. suberosa* were larger than those from larvae reared with plants without nitrogen addition. Females laid significantly more eggs on shoots of *P. suberosa* with addition of 300 mg L^{-1} of nitrogen. There were no significant differences among number of eggs oviposited on shoots with addition of 150 mg L^{-1} of nitrogen and without nitrogen addition.

Discussion

A greater nutritional quality expressed mainly by the greater concentration of nitrogen and water in food used by larvae of *H. erato phyllis* decreased development time, as well as resulted in a larger adult size, although it did not affect survival significantly. Females were highly selective, choosing shoots with higher levels of nitrogen and water content. Thus, female choice for fertilised plants was positively related to larval performance. Smiley & Wisdom (1985) also obtained a positive significant relationship between larval growth rate of *Heliconius ismenius* Latreille and *H. melpomene* (L.) (Lepidoptera: Nymphalidae) and

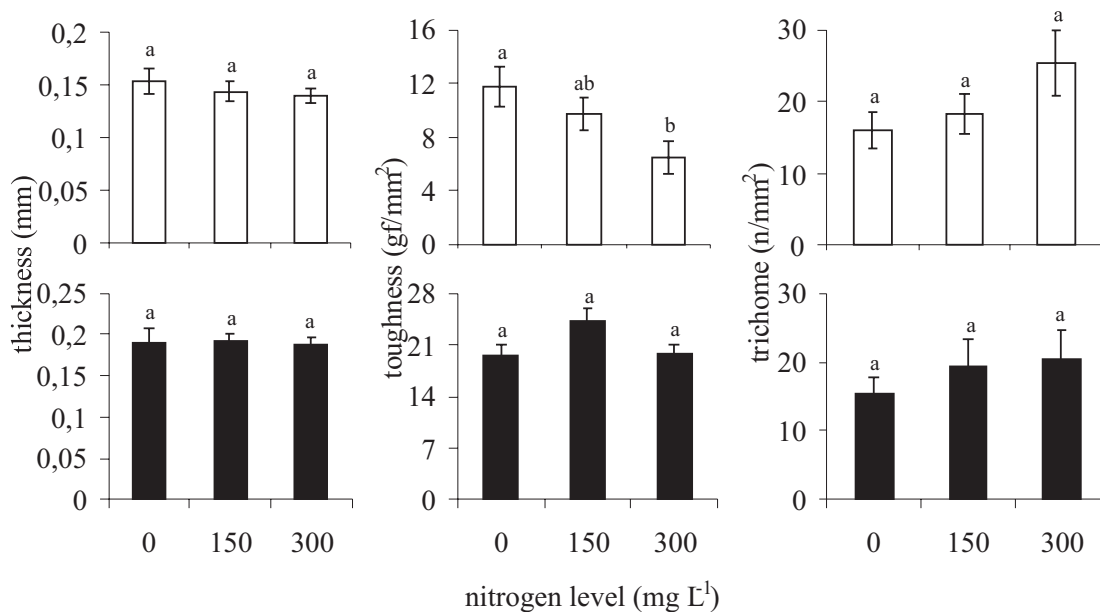


Fig. 2. Variation (average \pm standard error) on characteristics of the first (open bars) and fifth leaf (closed bars) of *P. suberosa* cultivated under different nitrogen levels in the soil (mg L^{-1}). Within leaf ages, bars followed by different letters differ significantly (Kruskal-Wallis' tests followed by Dunn's multiple comparison tests; $\alpha < 0.05$; $n = 20$ per treatment).

higher nitrogen content of different passion vine species.

This study adds the nutritional host plant quality, at an intraspecific level, as another attribute evaluated by *H. erato phyllis* female for oviposition. The ability of *H. erato phyllis* females to discriminate plants that will improve larval performance was already known at both interspecific and intraspecific levels for other qualitative aspects of host plant shoots. According to Kerpel & Moreira (2005), the females choose preferentially *Passiflora misera* Humbold, Bonpland

& Kunth compared to *P. suberosa*. Larvae fed shoots of *P. misera* result in larger adults, and body size is directly correlated to higher fecundity in this species (Rodrigues & Moreira 2002). According to Mugarab-Oliveira & Moreira (1996a), other qualitative aspects of the shoot and host plant inspected by the female is the presence of an intact apical region, shoot size, leaf area and internode size. Fertilised plants possess advantages in such attributes, since shoot growth rate, leaf area and internode length of *P. suberosa* were higher in those cultivated with nitrogen addition.

A delay in development time of the larvae fed shoots of *P. suberosa* without nitrogen addition can be considered as a compensatory response to lower nutrient availability. Probably, in similar situations in nature, *H. erato phyllis* increases development time to accumulate important resources. Besides, herbivorous insects need to ingest a large quantity of food to reach their nutritional needs, either for the low nutrient availability or inefficiency of the digestive process (Schoonhoven *et al.* 1998, Jaenike & Marcow 2003). It is known that in restricted environments, faced with low quality diets, insects can use mechanisms to compensate for nutritional deficiencies (Simpson & White 1990, Slansky & Wheeler 1992, Browne 1995). If in one hand, a longer development time can be a strategy used by organisms to accumulate proteins, carbohydrates and essential fats for the adult phase (Simpson & White 1990, Slansky & Wheeler 1992), on the other hand, ecologically a longer development time might mean a higher vulnerability to predation, to desiccation and to the action of pathogens, decreasing the chances for reaching maturity and reproducing (Häggestrom & Larsson 1995). Thus, plants with low nutritional quality can affect herbivore survival, even if indirectly (Clancy &

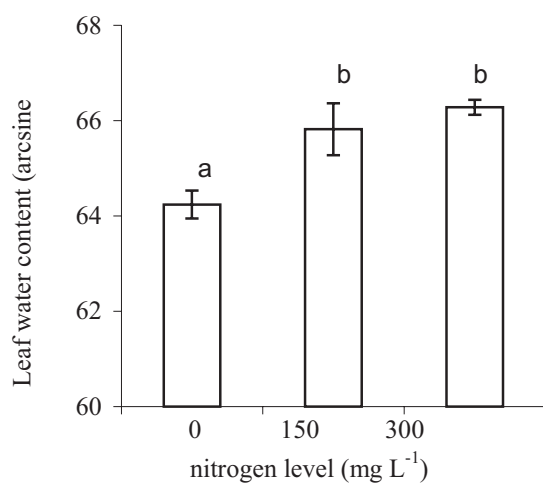


Fig. 3. Water content (average \pm standard error) of *P. suberosa* cultivated under different nitrogen levels in the soil (mg L^{-1}). Treatments followed by the same letters do not differ significantly (ANOVA, followed by Tukey's multiple comparison tests, $\alpha < 0.05$; $n = 5$ per treatment).

Table 1. Concentration (average \pm standard error) of macro (g kg⁻¹) and micronutrients (mg kg⁻¹) in *P. suberosa* branches cultivated under three levels of nitrogen. Within nutrients, treatments followed by same letters do not significantly statistically differ (tests of Kruskal-Wallis, followed by Dunn's multiple comparison tests; $\alpha < 0.05$, n = 5 per treatment).

Nutrients	Nitrogen level (mg L ⁻¹)		
	0	150	300
N	23.6 \pm 1.20 ^a	34.8 \pm 0.58 ^b	38.6 \pm 0.67 ^b
Ca	15.4 \pm 0.92 ^a	12.0 \pm 0.70 ^b	8.3 \pm 0.47 ^b
Mg	7.2 \pm 0.36 ^a	6.0 \pm 0.17 ^a	5.8 \pm 0.20 ^a
P	2.4 \pm 0.034 ^a	2.1 \pm 0.01 ^a	2.6 \pm 0.01 ^a
K	26.4 \pm 0.74 ^a	33.4 \pm 1.28 ^b	36.8 \pm 0.73 ^b
S	2.3 \pm 0.17 ^a	2.1 \pm 0.05 ^a	2.2 \pm 0.05 ^a
Cu	12.7 \pm 0.79 ^a	11.5 \pm 1.13 ^{ab}	6.8 \pm 0.95 ^b
Zn	62.0 \pm 1.09 ^a	43.0 \pm 1.94 ^b	47.2 \pm 3.26 ^{ab}
Fe	2046.4 \pm 332.12 ^a	901.4 \pm 89.65 ^b	533.8 \pm 46.82 ^b
Mn	56.0 \pm 3.72 ^a	66.8 \pm 7.15 ^{ab}	149.2 \pm 28.15 ^b
Na	609.4 \pm 171.12 ^a	234.2 \pm 27.89 ^b	167.4 \pm 17.97 ^b

Price 1987).

Plant growth rate, leaf area and internode length increased with nitrogen addition. Such aspects can be limited by the low photosynthetic rate and reduced leaf expansion, faced with nitrogen supplies lower than those required by the plant (Marschner 2002). At the cell level, a decrease in these parameters results from a reduction in cell number and volume (Chapin 1980, Fosket 1994). To herbivores, from an ecological point of view, shoots with slow growth rates and small leaf areas can result in lower quantities of food resources available, given the short time during which development of an immature insect occurs.

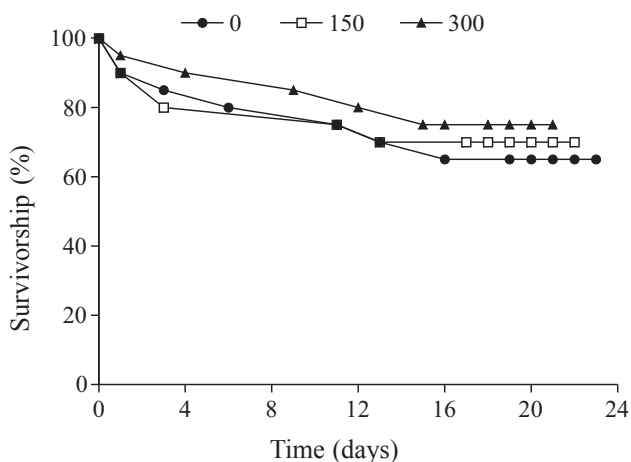


Fig. 4. Survivorship (average \pm standard error) from eclosion to adult emergence, for larvae (n = 20/treatment) of *H. erato phyllis* reared on *P. suberosa* shoots cultivated under different nitrogen levels in the soil (mg L⁻¹). There were no significant differences among treatments (Logrank tests; $\alpha = 0.05$).

Higher or lower nitrogen levels in the soil can also lead to changes in plant anatomy and morphology (Schoonhoven *et al.* 1998). In this study, higher toughness of the first leaves of *P. suberosa* was detected for plants cultivated without nitrogen addition. Therefore, in addition to the nutritional aspect, the physical aspect must also be considered, since larvae fed shoots of *P. suberosa* without nitrogen addition also faced higher tissue toughness on the first leaves, during the first instars, compared to the larvae receiving shoots cultivated with more nitrogen. Silveira (2002) found higher toughness on leaves of *P. suberosa* compared with leaves of *P. misera*. The author detected more wear and tear in the mandibles of larvae consuming tougher leaves. The wear and tear of these structures results in a larger cost in acquiring food.

According to Scriber (1984), higher leaf toughness results from increases in lignin content, tannins and also from decreases in water content of the tissues. This author also states that, normally, these characteristics are attributed to mature leaves. Also, for herbivorous insects, plant tissue is normally the main source of water (Bernays & Chapman 1994). Thus, lower water content in the leaves can impair the development of these organisms, what seems to happen in this study. Effectively, the first leaves of *P. suberosa* with lower water content had greater toughness. Plants cultivated without nitrogen addition in the soil had slower growth rates, which was practically stabilised when sampling took place. This means the cessation of young leaves production. Thus, it is possible that, although they were the first leaves on a shoot, they were actually mature when analysed morphologically. According to Chapin (1980), maturation is a common plant response to insuitable nutrient supplies.

Changes in the concentration of macro and micronutrients on *P. suberosa* tissues were detected for the nitrogen addition treatments. In analyses of passionflower

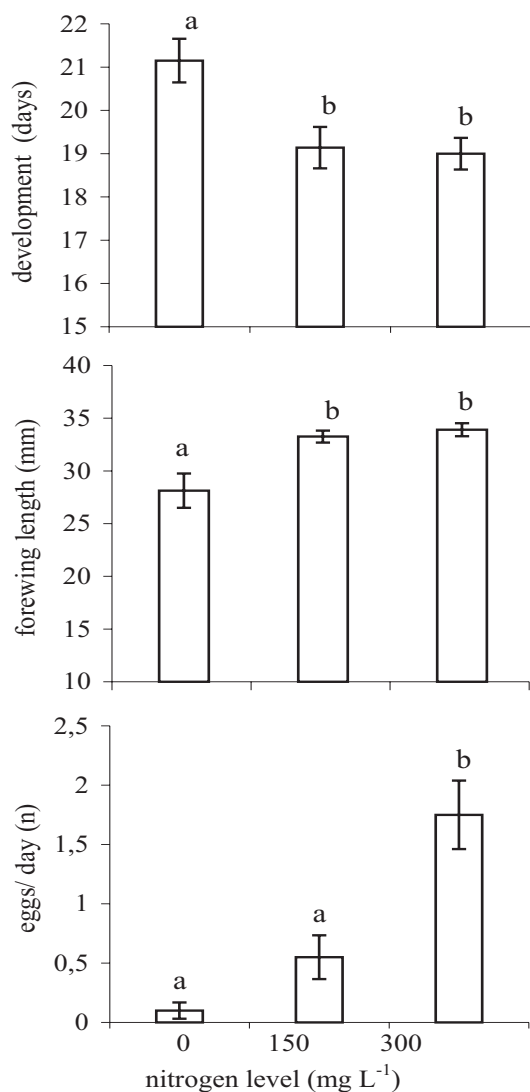


Fig. 5. Influence of *P. suberosa* shoots cultivated under different nitrogen levels in the soil (mg L^{-1}) on larval performance and oviposition of *H. erato phyllis* (average \pm standard error; $n = 20/\text{treatment}$). Treatments followed by the same letters do not differ significantly (ANOVA followed by Tukey's multiple comparison tests, $\alpha < 0.05$; $n = 5$ per treatment).

tissues from natural populations, these concentrations varied between 28 g kg^{-1} and 31 g kg^{-1} (Kerpel 2004). Thus, it is confirmed that the passionflower responds to higher availability of nitrogen in the soil, and in such conditions, can vary the concentration of this mineral in their tissues bringing consequences to their consumers. The dependence of larvae of *H. erato phyllis* on young tissues in their initial instars is already known (Rodrigues & Moreira 1999). Since we obtained a positive relationship between larval performance and nitrogen levels, it can be inferred that this dependence is also due to the high concentration of this element in the leaf tissues.

Among the microelements, except for manganese, all

others were found in higher concentrations in plants cultivated without nitrogen addition to the soil. Iron and sodium concentration, for example, was nearly four times higher in plant tissues without nitrogen addition. According to Marschner (2002), micronutrients can influence plant resistance indirectly. This happens because some of these nutrients have a fundamental role in the metabolism of some substances. For example, manganese and copper function in the metabolism of phenolic compounds and lignin, which can be considered plant defence substances against pathogens and herbivores, and calcium is essential for membrane and cell wall stability (Marschner 2002). According to this author, plants with high calcium contents are less susceptible to fungi and bacteria. Possibly, this lower susceptibility can extend to insects ingesting their tissues, since these are subject to more resistant cell walls.

In summary, our data showed clearly that fertilization leads to nutritional changes on *P. suberosa* plants, which affect *H. erato phyllis* larval performance and oviposition choices.

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