NITRATE REDUCTASE ACTIVITY IN FIELD-GROWN *PUERARIA LOBATA* (KUDZU) IN SOUTHEASTERN BRAZIL[1]

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ABSTRACT - Although *Pueraria lobata* (Willd.) Ohwi (*P. thunbergiana*) (Sieb. & Zucc.) Benth.) is thus ecologically and economically important in much of the world, its physiological ecology and other factors contributing to its invasive growth are not well understood. Nitrate reductase activity (NRA) measurements have long been used to indicate the effect of changes in the environment on a plant's capacity to assimilate nitrate nitrogen. The objective of this paper was to describe how naturally occurring changes in air temperature (T_a) and solar radiation flux density (S_i), affect the *in vivo* nitrate reductase activity in field-grown *P. lobata* and consequently the ability of this species to assimilate nitrate nitrogen in non-native areas. A meteorological thermometer and a thermocouple pyranometer were used to measure changes in the air temperature and the solar radiation flux density, respectively. The highest nitrate reductase activity found in this study [5.8 μmol NO_2 h^{-1} (g FW)$^{-1}$] was observed when T_a was 27.4°C. A significant reduction in nitrate reductase activity was observed with T_a below 21.8°C or above 33.6°C. Nitrate reductase activity was linear and positively related to S_i. A 12% increase in nitrate reductase activity was observed when S_i rose from 531 to 852 W m^{-2}. These results suggest that *P. lobata* can be a successful competitor in regions located at the transition between subtropical and tropical biomes.

Index terms: cover crop, ecophysiology, forage, Leguminosae, nitrate assimilation.

ATIVIDADE DA REDUTASE DO NITRATO EM PLANTAS DE *PUERARIA LOBATA* CRESCIDAS NO CAMPO, NO SUDESTE DO BRASIL

RESUMO - Embora *Pueraria lobata* (Willd.) Ohwi (*P. thunbergiana*) (Sieb. & Zucc.) Benth.) seja ecológica e economicamente importante em vários países, sua fisioecologia e outros fatores que contribuem para seu hábito invasivo não são bem conhecidos. Estimativas da atividade da redutase do nitrato (NRA) têm sido empregadas como indicativo do efeito de alterações no ambiente sobre a capacidade das plantas de assimilar nitrato. O objetivo deste trabalho foi o de descrever como alterações naturais na temperatura do ar (T_a) e na densidade de fluxo de radiação solar (S_i) afetam NRA *in vivo* em *P. lobata* crescida no campo, e, conseqüentemente, sua capacidade de assimilar nitrogênio na forma de nitrato em áreas não-nativas. Utilizou-se um termômetro meteorológico para medir as alterações da temperatura do ar, e um piranômetro à base de termopares, para medir a densidade do fluxo da radiação solar. O mais alto valor para NRA [5,8 μmol NO_2 h^{-1} (g FW)$^{-1}$] foi observado quando a T_a era 27,4°C. Redução significativa em NRA foi observada quando a T_a se encontrava abaixo de 21,8°C ou acima de 33,6°C. Elevação em S_i foi acompanhada por elevação proporcional em NRA. Aumento de 12% em NRA foi observado quando S_i aumentou de 531 para 852 W m^{-2}. Estes resultados sugerem que *P. lobata* pode ser um competidor efetivo em regiões localizadas na transição entre os biomas tropical e subtropical.

Termos para indexação: cultura de cobertura, ecofisiologia, forragem, Leguminosae, assimilação de nitrato.

INTRODUCTION

Pueraria lobata Ohwi (kudzu) is a perennial twining vine used as a cover crop and as forage throughout the subtropics and in some warm temperate regions (Bogdan, 1977). However, this species is also

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the most important weed associated with forestry in the United States (Carter et al., 1989). In subtropical and in temperate climates *P. lobata* is very invasive, and concerns about its spread are documented (Sasek & Strain, 1990). Although *P. lobata* is thus ecologically and economically important in most of the world, its physiological ecology and other factors contributing to its invasive growth are not well understood (Carter & Teramura, 1988). Information on the ecophysiological aspects of nitrogen assimilation in *P. lobata* is limited, especially for plants growing in regions that represent the transition from subtropical to tropical biomes. Nitrate is considered to be the major source of inorganic nitrogen for most angiosperms. The first step on the nitrate reduction process is catalyzed by the enzyme nitrate reductase (NR) (Solomonson & Barber, 1990). Activity of this enzyme is considered to be a limiting factor for growth, development, and protein production in plants (Solomonson & Barber, 1990). NR activity (NRA) measurements have long been used to indicate the effect of changes in the environment on a plant's capacity to assimilate nitrate nitrogen (Nicholas et al., 1976; Fredeen et al.,1991).

The objective of this paper was to describe how naturally occurring changes in air temperature (T_a) and solar radiation flux density (S_i) affect the *in vivo* nitrate reductase activity in field-grown *P. lobata*, and consequently the ability of this species to assimilate nitrate nitrogen in non-native areas.

MATERIAL AND METHODS

A *Pueraria lobata* (Leguminosae-Faboideae) stand measuring 4,000 m², established at the Instituto Agronômico Experimental Station (Campinas, SP, Brazil: latitude 22°49'S and longitude 47°06'W, altitude 669 m), was used in this study. Plants were propagated from cuttings and field-grown in a typical haplorthox soil without irrigation or fertilization. Measurements of the nitrate reductase activity, air temperature and solar radiation flux density were performed in a weekly basis between Sep. 2nd and Nov. 24th, 1987.

Measurements of the nitrate reductase activity (NRA) were performed separately on five completely expanded uppermost terminal leaflets randomly chosen in the area of the whole stand.

Air temperature (T_a) was measured at 13h (solar time) with a shaded meteorological thermometer (R. Fuess) set over the top of the plants.

Immediately after T_a measurements and just before leaflet harvesting, solar radiation flux density (S_i) perpendicularly incident on the leaflet surface was measured with a thermocouple pyranometer calibrated according to a standard pyranometer (Kipp & Zonen, Delft, Netherlands).

In vivo nitrate reductase activity assay

Terminal leaflets measuring around 60 cm² were used for the NRA assay. Leaflets exposed to direct sunlight were randomly harvested and placed in black polyethylene bags on ice until NRA assays could be done (within 30 minutes of harvesting). Nitrate reductase activity *in vivo* was assayed according to Harper & Hageman (1972) with modifications. Leaflets were cleaned with distilled water and 200 mg leaflet strips (50 mm²/strip) were vacuum infiltrated for three minutes with phosphate buffer (K_2HPO_4 + KH_2PO_4; 100 mM), pH 6.5, containing 50 mM KNO_3 and 1% (v/v) n-propanol (Pereira-Netto et al., 1989). The leaflet strips were then incubated in the phosphate buffer solution at 32°C for 60 minutes in a shaking water bath in the dark.

RESULTS AND DISCUSSION

Temperature dependence curve for NRA

Fig. 1 shows a typical bell-shaped temperature dependence curve for nitrate reductase activity (NRA). The range of naturally occurring changes in air temperature observed during this study limited the NRA measurements to air temperatures ranging between 21.0°C and 36.0°C. A 45% enhancement in NRA was related to increase in air temperature from 21.0°C to 27.4°C. Further increase in temperature (up to 36.0°C) resulted in a 27% reduction in NRA.

NRA data showed in this study indicates the enzyme's potential to reduce nitrate. This potential is dependent on the amount of enzyme present in the leaflet and reflects limitations on the reducing power and also difficults for the nitrate ion to reach the enzyme's active site. However, NRA is modulated by microclimate conditions existing at the time of the harvesting. Reductions of NRA observed at higher temperatures could be due to enzyme inactivation or degradation, while reductions of NRA at lower temperatures could be attributed to the limita-

tion of NADH and nitrate supply (Beevers & Hageman, 1980).

The lowest NRA levels observed in this study are higher than NRA levels found for other temperate and tropical indoor-grown legume species (Andrews et al., 1984; Pereira-Netto, 1992). Nevertheless, exceptionally high activities, 50-250 μmol NO_2 h^{-1} (g FW)$^{-1}$, have been recorded for members of the genus *Erythrina* (Smirnoff et al., 1984).

The optimum temperature for NRA found in this study is close to the temperature optimum found for other metabolic processes, such as photosynthesis (26.0°C), in field-grown *P. lobata* (Forseth & Teramura, 1987). In *Glycine max* plants grown at different temperatures, the highest NRA observed was in plants growing at 32.5°C (Magalhães et al., 1976).

The high NRA activity found at air temperatures around 30.0°C helps to understand, in part, the successful establishment of *P. lobata* in an area that represents the transition from subtropical to tropical biomes, and also indicates that *P. lobata* can be a successful competitor in this region, where annual average air temperature is typically closer to the optimum temperature for NRA found in this study, when compared to subtropical-temperate biomes.

Solar radiation flux density dependence curve for NRA

In order to avoid the interference of changes in air temperature on the light dependence curve for NRA, all data presented on Fig. 2 refer to leaflets harvested at air temperature ranging from 23.4°C to 33.6°C, when temperature had very little effect on NRA. NRA was linear and positively ($p \leq 0.05$) related to enhancement in solar radiation flux density (S_r) from 531 to 852 W m^{-2} (Fig. 2). Enhancement in NRA in response to increases in solar radiation flux density was found for *Glycine max* (Nicholas et al., 1976) and *Sorghum sudanese* leaves (Hallmark & Huffaker, 1978). In rainforest species of *Piper* leaf NRA was strongly correlated with average daily photosynthetically active photon flux (Fredeen et al.,

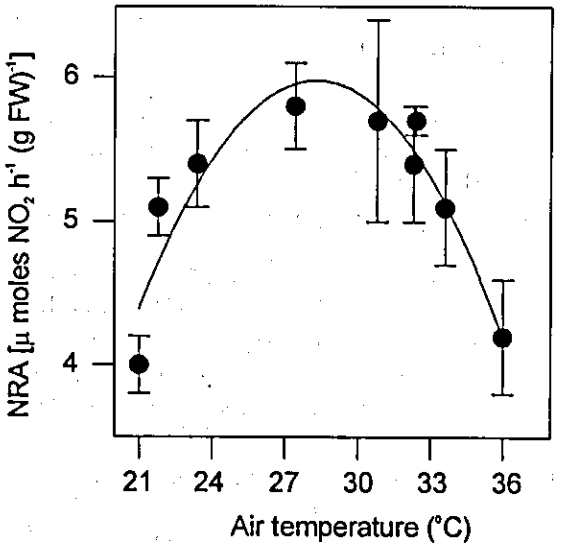

FIG. 1. Temperature dependence curve for the nitrate reductase activity (NRA); S_r ranging between 531 and 908 W m^{-2}; each point in the graph represents average NRA for 5 leaflets; vertical bars indicate standard error; $Y = -29.3497 + 2.9254X - 0.0736X^3$; $r^2 = 0.8926$.

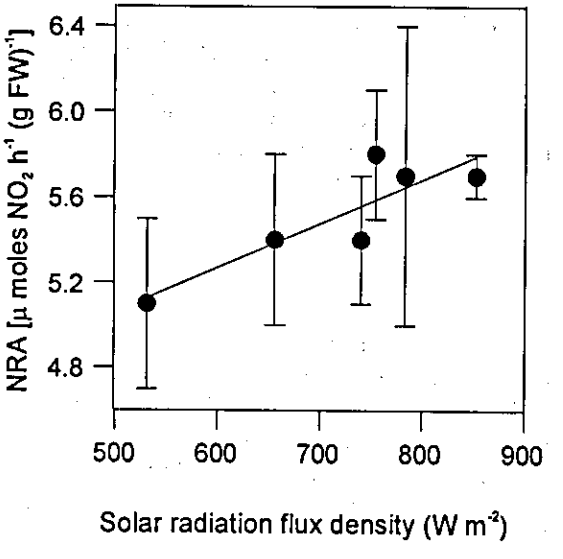

FIG. 2. Light dependence curve for the nitrate reductase activity (NRA); air temperature ranging between 23.4°C and 33.6°C; vertical bars indicate standard error; each point in the graph represents average NRA for 5 leaflets; $Y = 4.0351 + 0.0021X$; $r^2 = 0.7643$.

1991). Light acts by promoting synthesis of NR as well as causing activation or modulation of the preformed enzyme (Pilgrim et al., 1993). In dark-adapted green barley seedlings, it was shown that white light stimulated NR mRNA accumulation in an intensity-dependent manner (Melzer et al., 1989). Analysis of wild type and chimeric NR gene expression in plants of *Nicotiana plumbaginifolia* revealed that light regulates NR mRNA translation and/or the stability of NR protein (Vincentz & Caboche, 1991).

Increased membrane permeability (Kannangara & Woolhouse, 1967) and short-term accumulation of end products of photosynthesis at high S_t levels are considered to be partly responsible for the increase in maximum NRA observed in various species (Cheng et al., 1992). Furthermore, enhanced nitrate uptake and nitrate efflux from the metabolically inert storage pool into the active metabolic pool in response to light has also been reported to increase NR activity (Melzer et al., 1989).

The decrease in *P. lobata* NRA following reductions in S_t found in this study is consistent with its previously reported poor adaptability to the forest understory (Carter & Teramura, 1988). In addition, the NRA responses to changes in solar radiation flux density is in agreement with the idea that physiological performance of *P. lobata* may be related to vine climbing mechanics, which permits maximization of light interception and utilization (Carter & Teramura, 1988).

CONCLUSIONS

1. Variations in air temperature induce significant changes on the nitrate reductase activity in kudzu.

2. Nitrate reductase activity in kudzu is directly related to the solar radiation flux density.

3. Data found in this study help to understand the sucessful establishment of kudzu at the border between the subtropical and tropical regions.

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REFERENCES

ANDREWS, M.; SUTHERLAND, J.M.; THOMAS, R.J.; SPRENT, J.I. Distribution of nitrate reductase activity in six legumes: the importance of the stem. **New Phytologist**, v.98, p.301-310, 1984.

BEEVERS, L.; HAGEMAN, R.H. Nitrate and nitrite reduction. In: MIFLIN, B.J. (Ed.). **The biochemistry of plants**. New York: Academic Press, 1980. v.5, p.115-168.

BOGDAN, A.V. **Tropical pasture and fodder plants**: grasses and legumes. London: Longman, 1977. 475p.

CARTER, G.A.; TERAMURA, A.H. Vine photosynthesis and relationships to climbing mechanics in a forest understory. **American Journal of Botany**, v.75, p.1011-1018, 1988.

CARTER, G.A.; TERAMURA, A.H.; FORSETH, I.N. Photosynthesis in an open field for exotic versus native vines of the southeastern United States. **Canadian Journal of Botany**, v.67, p.443-446, 1989.

CHENG, C.-L.; ACEDO, G.N.; CRISTINSIN, M.; CONKLING, M.A. Sucrose mimics the light induction of *Arabidopsis* nitrate reductase gene transcription. **Proceedings of the National Academy of Sciences of the United States of America**, v.89, p.1861-1864, 1992.

FORSETH, I.N.; TERAMURA, A.H. Field photosynthesis, microclimate and water relations of an exotic temperate liana, *Pueraria lobata*, kudzu. **Oecologia**, v.71, p.262-267, 1987.

FREDEEN, A.L.; GRIFFIN, K.; FIELD, C.B. Effects of light quantity and quality and soil nitrogen status on nitrate reductase activity in rainforest species of the genus *Piper*. **Oecologia**, v.86, p.441-446, 1991.

HALLMARK, W.B.; HUFFAKER, R.C. The influence of ambient nitrate, temperature and light on nitrate assimilation in sudan grass seedlings. **Physiologia Plantarum**, v.44, p.147-152, 1978.

HARPER, J.E.; HAGEMAN, R.H. Profiles and seasonal profiles of nitrate reductase in soybeans (*Glycine max* (L.) Merr.). **Plant Physiology**, v.49, p.146-154, 1972.

KANNANGARA, C.G.; WOOLHOUSE, H.M. The role of carbon dioxide, light and nitrate in the synthesis and degradation of nitrate reductase in leaves of *Perilla frutescens*. **New Phytologist**, v.66, p.553-561, 1967.

MAGALHÃES, A.C.; PETERS, D.B.; HAGEMAN, R.H. Influence of temperature on nitrate metabolism and leaf expansion in soybean (*Glycine max* (L.) Merr.) seedlings. **Plant Physiology**, v.58, p.12-16, 1976.

MELZER, J.M.; KLEINHOFS, A.; WARNER, R.L. Nitrate reductase regulation: Effects of nitrate and light on nitrate reductase mRNA accumulation. **Molecular and General Genetics**, v.217, p.341-346, 1989.

NICHOLAS, J.C.; HARPER, J.E.; HAGEMAN, R.H. Nitrate reductase activity in soybeans (*Glycine max* (L.) Merr.). I. Effects of light and temperature. **Plant Physiology**, v.58, p.731-735, 1976.

PEREIRA-NETTO, A.B. Atividade da redutase de nitrato em *Cajanus cajan* e *Desmodium barbatum*: padronização das condições do ensaio. **Pesquisa Agropecuária Brasileira**, Brasília, v.27, n.7, p.1011--1015, 1992.

PEREIRA-NETTO, A.B.; MAGALHÃES, A.C.N.; PINTO, H.S. Atividade da redutase de nitrato *in vivo* em *Pueraria phaseoloides*: padronização das condições do ensaio. **Revista Brasileira de Fisiologia Vegetal**, v.1, p.133-137, 1989.

PILGRIM, M.L.; CASPAR, T.; QUAIL, P.H.; MCCLUNG, C.R. Circadian and light-regulated expression of nitrate reductase in *Arabidopsis*. **Plant Molecular Biology**, v.23, p.349-364, 1993.

SASEK, T.W.; STRAIN, B.R. Implications of atmospheric CO_2 enrichment and climatic change for the geographical distribution of two introduced vines in the USA. **Climatic Change**, v.16, p.31-51, 1990.

SMIRNOFF, N.; TODD, P.; STEWART, G.R. The occurrence of nitrate reduction in the leaves of woody plants. **Annals of Botany**, v.54, p.363-374, 1984.

SOLOMONSON, L.P.; BARBER, M.J. Assimilatory nitrate reductase: functional properties and regulation. **Annual Review of Plant Physiology**, v.41, p.225--253, 1990.

VINCENTZ, M.; CABOCHE, M. Constitutive expression of nitrate reductase allows normal growth and development of *Nicotiana plumbaginifolia* plants. **European Molecular Biology Organization Journal**, v.10, p.1027-1035, 1991.