

## Acclimation capability of *Calamagrostis epigejos* and *C. arundinacea* to changes in radiation environment

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### Abstract

Three different approaches were used in quantification of acclimation capability of perennial grasses colonizing deforested areas: (a) nondestructive estimation of their relative growth rate (RGR), (b) analysis of photosynthetic and respiratory characteristics of individual organs, (c) measurement and calculation of the whole plant carbon balance over the 24 h cycle. The experiments were made in controlled conditions with plants growing continuously in extremely low photosynthetic photon flux (PPF),  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and at moderately high PPF ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Responses of plants to reciprocal transfer between low and high PPF environment were also studied. Fully acclimated plants of both species grew successfully at low PPF (LI) with RGR of about 20 to  $40 \text{ g kg}^{-1} \text{ d}^{-1}$ , and increased their RGR three to five times immediately after transfer to high PPF (HI). Lowered respiration rate and increased specific leaf area (SLA) were the most powerful mechanisms in acclimation to LI. Variation in the net photosynthetic rates per unit leaf area ( $P_N$ ) did not contribute significantly to acclimation. *C. epigejos* had considerably higher RGR under both irradiances, although its photosynthetic characteristics ( $P_N$  and apparent quantum yield) were not better than those of *C. arundinacea*. Higher values of SLA were of key importance for better performance of *C. epigejos* under both LI and HI.

*Key words:* carbon balance; deforestation; grasses; growth rate; photosynthesis; quantum yield; respiration.

### Introduction

Progressive forest disturbance is one of the most significant ecological problems in central Europe. Rapid expansion of perennial grasses has been observed in a majority of damaged forests and in open deforested areas (Fiala 1989, Pyšek 1991). The new grass stands are surprisingly stable for long period of time and prevent spontaneous reforestation by tree seedlings. This contradicts to ecological theories explaining

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vegetation dynamic in disturbed ecosystems. In a typical case, secondary succession after heavy disturbance should be pioneered by annuals or biennial herbs with a ruderal strategy of growth, followed by perennial herbs and trees (Grime 1981).

The most likely explanation for the success of some fast growing perennials after deforestation could be in their ability to survive for long periods of time in shade of the original undisturbed forest canopy, where their populations are formed by scattered and sterile individuals. In improved radiation conditions they could expand more rapidly by vegetative spreading than could the potentially fast growing annuals or biennials from newly dispersed seeds.

To the most abundant plants colonizing deforested sites belong two perennial grasses, *C. epigejos* and *C. arundinacea*. Both species can be characterized as fast growing sun plants, but their ability to persist in shaded habitats is remarkable. In extreme cases they can be found beneath the canopy of dense coniferous forests, where maximum values of photosynthetic photon flux are seldom above  $20 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Such adverse irradiances are already close to the lowest limit for survival of vascular plants, including obligate shade species.

As has been found in our previous field observations, there are some differences between the two species. The more robust *C. arundinacea* (up to 1.5 m in height) is usually less successful in occupation of new spaces than the smaller *C. epigejos* (for its photosynthetic characteristics see Gloser and Barták 1994). The stands of *C. epigejos* are much denser and more widely distributed under contrasting irradiances than those of *C. arundinacea*.

The aim of our work was to evaluate the ability of the two *Calamagrostis* species to acclimate to different irradiances, and to specify the underlying mechanisms. The acclimation to radiation is usually based both on morphological and physiological plasticity of a plant, and can be studied on different levels of complexity (organelles, organs, or whole plant, for reviews see Boardman 1977, Björkman 1981). In our first approach to the comparative study of responses of *C. epigejos* and *C. arundinacea* to changes in radiation environment, the growth rate and carbon economy on whole plant and on organ level were examined.

## Materials and methods

**Plant cultivation and measurement of growth rate:** Plants of *C. epigejos* (L.) Roth and *C. arundinacea* (L.) Roth were grown from seeds collected in a forest clearing near Brno. Immediately after germination on wet sand the seedlings were transplanted into small pots containing moist *Perlite*, and placed in a glasshouse. After four weeks, selected seedlings were washed free of *Perlite* and grown hydroponically in containers with aerated and circulated Hoagland nutrient solution (pH 4.8 - 5.2). The nutrient solution was renewed once a week. One week after transfer of plants to the hydroponic culture the main experiment was started (*i.e.*, day zero). Containers with plants were placed in cultivation room with temperature  $22 \pm 2 \text{ }^\circ\text{C}$  and relative air humidity  $60 \pm 10 \%$ . Irradiation was supplied by *Gro-Lux* fluorescent tubes. One half of containers with plants of both species was placed close to the radiation source.

PPF in this HI treatment was  $200 \pm 20 \mu\text{mol m}^{-2} \text{s}^{-1}$  (measured in the middle height of plant shoots). The second half of containers was placed in such a distance from the radiation source, that the PPF near the plants was only  $25 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$  (LI treatment). Photoperiod was kept at 16 h in both variants.

The growth rate of plants was estimated by a nondestructive method. Twelve plants in each treatment were selected and their mass was periodically determined (at 3 to 5 d intervals) for 34 d. Only the fresh mass increments of each plant were thus recorded. The surface water was removed from roots before mass determination using filter paper. The remaining plants were used to determine biomass partitioning, leaf area, and for measurements of  $P_N$  and dark respiration rate ( $R_D$ ).

After 15 d of growth in controlled conditions, half of the plants in each treatment were transferred to the other treatment (*i.e.*, from LI to HI, and from HI to LI). The non-destructive estimation of growth rate was done in the same time intervals as in plants which were not transferred. Most of the morphological and physiological parameters were measured in fully acclimated plants, 33-35 d from the beginning of experimental treatment.

RGR was calculated for each plant using a classical approach (difference in  $\ln$ -transformed plant fresh mass divided by the time interval of the two subsequent determinations). For estimation of time course of RGR, the calculated values for individual plants were fitted by a polynomial, as suggested by Poorter (1989).

The other calculated growth-analysis characteristics were: leaf area ratio (LAR, projected leaf area per unit of whole plant dry mass), leaf mass ratio (LMR, dry mass of leaves per unit of whole plant dry mass), and SLA (projected leaf area per unit of leaf dry mass).

**Gas exchange measurements:**  $P_N$  and  $R_D$  were calculated from measurements of  $\text{CO}_2$  exchange. An open type system with differential infra-red gas analyser (*Uras 2*, Hartmann and Braun, Germany) was used in all cases. The relationship between  $P_N$  and PPF was measured in the youngest fully developed leaves placed in an assimilation chamber (Gloser 1970). The chamber was ventilated, temperature controlled, and contained quantum flux sensor (*Li-190*, Licor, USA). The air entering assimilation chamber was prepared from decarbonized ambient air and pure  $\text{CO}_2$  using a cascade of mixing pumps (*Wösthof*, Germany).  $\text{CO}_2$  concentration in the prepared air was  $400 \text{ cm}^3 \text{ m}^{-3}$ , and its relative humidity was 80 %. PPF was supplied by a quartz iodine 1 kW lamp filtered by a 0.15 m thick layer of water, and, if necessary, by a set of neutral grey filters. The temperature of leaves during measurements of  $P_N$  was  $20.0 \pm 0.3 \text{ }^\circ\text{C}$ .

$R_D$  was measured both in intact leaves exposed in assimilation chamber, and in detached plant parts (roots, stem, leaves) placed in thermostated glass cuvettes (at  $20 \text{ }^\circ\text{C}$ ), and connected to the same gasometric system, as described above. To prevent any desiccation of plant material during measurements, the dew point of air entering the cuvettes was set at the temperature of measured sample ( $20 \text{ }^\circ\text{C}$ ). No significant effect of excision on respiration rate was found in preliminary experiments.

The diurnal carbon gain of whole plants was calculated from separate gasometric measurements of shoots and roots.  $\text{CO}_2$  exchange of shoots was continually recorded *in situ*, *i.e.*, in the same place and conditions in which the plants grew during the

whole experiment, among the other plants used for estimation of growth rate. During measurements of  $\text{CO}_2$  exchange the shoots were covered by an enclosure made from a thin polypropylene foil. Root respiration was measured subsequently (on the second day) after detachment, as described above.

**Chemical analyses and statistical evaluation:** Total nitrogen and carbon content in plant biomass was determined by combustion gas chromatography (*Elemental Analyser 1106, Carlo Erba, Italy*). Data were analyzed using a *Statgraphics* program.

## Results

**Growth, photosynthesis and respiration in plants continuously grown at two irradiances:** Time courses of RGR of *C. epigejos* and *C. arundinacea*, calculated from fresh-mass increments of permanent sample plants (Fig. 1) showed that *C. epigejos* had considerably higher RGR under both irradiances. The difference was about 50 % at HI treatment, and even more (about twice as much) in LI.

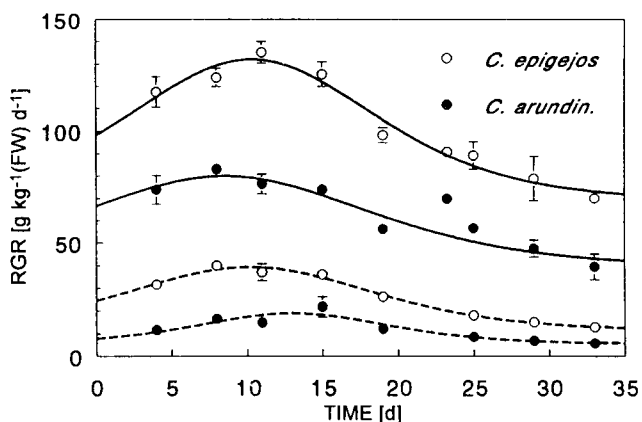


Fig. 1. Changes in relative growth rate (RGR) of *Calamagrostis epigejos* and *C. arundinacea* cultivated at high irradiance ( $\text{PPF} = 200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , solid lines) and in extremely low irradiance ( $\text{PPF} = 25 \mu\text{mol m}^{-2} \text{s}^{-1}$ , dashed lines). Mean values from six plants ( $\pm \text{SE}$ ).

More detailed analysis of these differences was problematic. Calculation of net assimilation rate (NAR) and LAR as components of RGR was not possible for the whole period of cultivation, because the data on leaf area development were lacking. Nevertheless, the most important structural characteristics were assessed in fully acclimated plants during the final week of experiment (Table 1). *C. epigejos* had evidently better morphological parameters for fast growth in both PPF regimes - higher values of LAR and SLA were the most important of all. There were no significant differences in root/shoot dry mass ratio in both treatments, and in LMR and tillering rate at the LI treatment.

$P_N$  per unit leaf area at saturating PPF was highest in *C. arundinacea* from the HI treatment (Table 2). The overall shape of dependences of  $P_N$  (measured in leaf blades

only!) was different for plants cultivated at different irradiances - maximum values of  $P_N$  (at saturation irradiance) and saturating values of PPF were always much smaller in plants from the LI treatment (Fig. 2). The interspecific differences were less pronounced. There were even smaller differences between the two species in their  $P_N$  (per unit leaf area), when compared at pertinent growth irradiances (25 or 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF). Also very similar were the values of apparent quantum yield of  $P_N$ , which were derived from the initial slope of curves  $P_N \times \text{PPF}$  up to 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF. Leaves grown under LI and HI were not much different in this parameter (Table 2).

Table 1. Morphological characteristics of plants of *Calamagrostis epigejos* (CE) and *C. arundinacea* (CA) grown at two levels of photosynthetic photon flux (PPF) [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]: leaf area ratio (LAR,  $\text{m}^2 \text{kg}^{-1}$  of whole plant dry mass), specific leaf area (SLA,  $\text{m}^2 \text{kg}^{-1}$  of leaf dry mass), leaf mass ratio (LMR, leaf dry mass/whole plant dry mass), root/shoot dry mass ratio (R/S), and number of tillers per plant. Day 35 of the experiment, mean values  $\pm$  SE,  $n = 6$ . Significant interspecific differences ( $p < 0.05$ ) are marked as +.

	Growth PPF		Sign.	200		Sign.
	25	CA		CE	CA	
LAR	25.60	19.37	+	15.70	10.70	+
SLA	52.63	39.84	+	37.43	21.57	+
LMR	0.53	0.49	-	0.42	0.50	+
R/S	0.34	0.42	-	0.41	0.39	-
Tillers	5.00	4.83	-	18.67	10.83	+

Table 2. Net photosynthetic rate of leaves at saturating photon flux ( $P_{N,\text{max}}$ ) and at the growth radiation level ( $P_{N,\text{growth}}$ ) [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ], dark respiration rate of whole plants ( $R_D$ ) [ $\mu\text{mol}(\text{CO}_2) \text{kg}^{-1} \text{s}^{-1}$ ], apparent quantum yield of photosynthesis ( $\Phi_{\text{app}}$ ) [ $\text{mol mol}^{-1}$ ], carbon content in the whole-plant biomass (CC) [ $\text{kg kg}^{-1}$ ], specific diurnal carbon gain (CG) [ $\text{mol}(\text{C}) \text{kg}^{-1} \text{d}^{-1}$ ], and relative growth rate (RGR) [ $\text{g kg}^{-1} \text{d}^{-1}$ ] calculated from CG. Significant interspecific differences ( $p < 0.05$ ) are marked as +. PPF during growth [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ].

	Growth PPF		Sign.	200		Sign.
	25	CA		CE	CA	
$P_{N,\text{max}}$	8.20	9.83	+	12.60	17.30	+
$P_{N,\text{growth}}$	1.15	1.28	-	8.56	9.70	-
$\Phi_{\text{app}}$	0.06	0.06	-	0.06	0.07	-
$R_D$	8.10	7.50	-	16.20	15.40	-
CC	0.39	0.39	-	0.41	0.40	-
CG	0.83	0.31	+	3.50	2.16	+
RGR	25.70	9.61	+	103.40	64.80	+

Expression of  $P_N$  values per leaf dry mass unit (Fig. 3) resulted in PPF curves of different shape from those presented in Fig. 2. The leaves of HI-grown plants of *C. epigejos* showed much better photosynthetic performance than those of *C. arundinacea* at all PPF applied during measurements. The differences in LI-grown plants were much smaller.

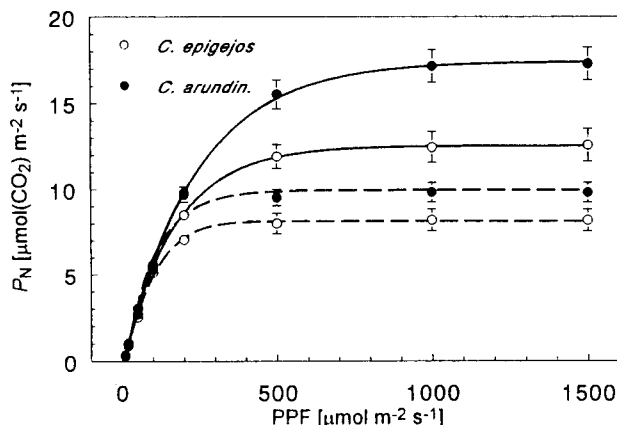


Fig. 2. Response of net photosynthetic rate,  $P_N$  per leaf area unit to photosynthetic photon flux (PPF) in leaves of *Calamagrostis epigejos* and *C. arundinacea* grown at high irradiance (solid lines) and at low irradiance (dashed lines). Vertical bars denote  $\pm$  SE.

There were no significant differences between the two species in specific respiration rate when comparing plants grown in the same treatment. Plants of both species grown at HI always had much higher  $R_D$  than plants grown at LI (Table 2, Fig. 5).

Diurnal carbon gain of whole plants (CG, calculated from diurnal integral  $\text{CO}_2$  exchange) was much higher in *C. epigejos* than in *C. arundinacea* grown in both PPF treatments. The lowest CG was found always in *C. arundinacea* grown at LI. The values of CG given in Table 2 were expressed in a standardized form (per one gram of whole-plant biomass) for the sake of better comparability. Instantaneous values of RGR can be calculated from the carbon gain data if we know carbon content (CC, relative values) in newly formed plant biomass:

$$\text{RGR [g kg}^{-1} \text{ d}^{-1}] = 12 \text{ CG (CC)}^{-1}$$

There were no differences between the species in mean values of CC (Table 1). Carbon gain was thus directly proportional to biomass increments and to the RGR.

**Responses of acclimated plants to reversed PPF regime:** Plants of both species initially grown at  $25 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPF responded to improved PPF conditions ( $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) by rapid increase of their RGR up to values exhibited by plants grown permanently at HI (Fig. 4). In plants transferred from HI to LI their RGR dropped below the values found in plants continuously grown at LI and no acclimatory changes leading to increments of RGR were observed till the end of experiment.

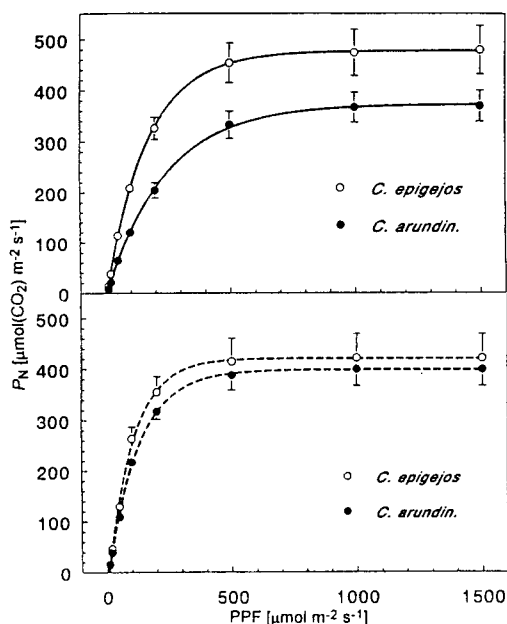


Fig. 3. Dependence of net photosynthetic rate,  $P_N$  per leaf dry mass unit on photosynthetic photon flux (PPF) in leaves of *Calamagrostis epigejos* and *C. arundinacea* grown at high irradiance (top, solid lines) and at low irradiance (bottom, dashed lines). Vertical bars denotes  $\pm$  S.E.

$R_D$  was measured during the final week of experiment both in plants permanently grown at LI and HI, and in plants after reciprocal transfer (Fig. 5). In most cases,  $R_D$  of the transferred plants was nearly the same as in plants permanently grown in the pertinent radiation regime. Respiration rate of roots was always more sensitive to changes in radiation during growth than shoot respiration.

## Discussion

Long-term survival of plants in forest understory would be not possible without efficient morphological and physiological adaptative traits, enabling efficient capture and use of limited radiant energy. These features are at best expressed in obligate shade tolerant plant species (Boardman 1977, Björkman 1981). In most plants normally growing in fully insolated habitats (sun plants), some acclimatory changes increasing their tolerance to shade can be induced. Such changes are based on similar mechanisms as in obligate shade tolerant species, but seldom are as efficient. The potential for acclimation to shade varies widely among plant species and a clear delimitation between sun and shade species is thus difficult. Grasses are usually considered as plants with rather limited capacity for acclimation to shade environments (Pierson and Mack 1990).

There are difficulties with quantification of the acclimatory changes in plants growing in different radiation environments. Simultaneous use of several experimental approaches may be very helpful. Measurements of RGR of shaded

plants provide an overall estimate of whole-plant responses but do not allow determination of the responsible particular traits, which are involved in acclimation. Measurements of the carbon metabolism of individual organs ( $\text{CO}_2$  exchange rates) are helpful in deeper analysis. We therefore utilized both approaches.

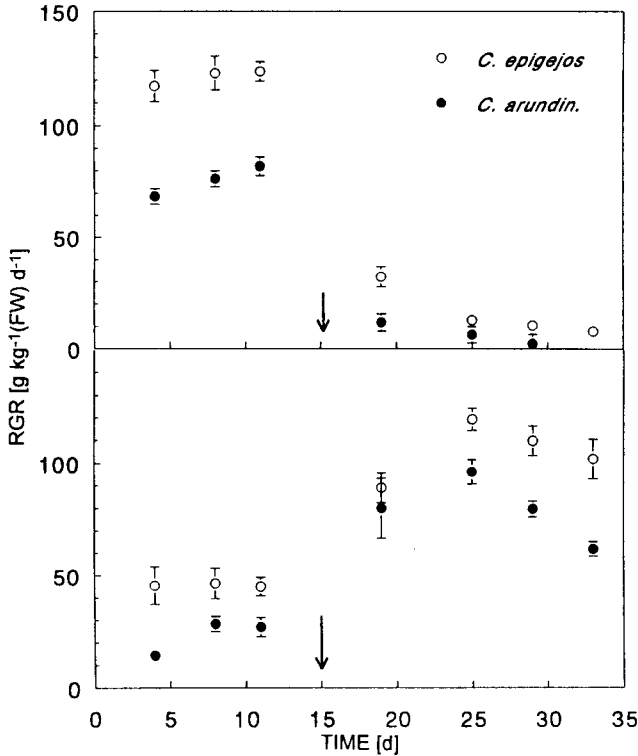


Fig. 4. Changes in relative growth rate (RGR) in plants of *Calamagrostis epigejos* and *C. arundinacea* transferred from high irradiance to low irradiance (top), and from low irradiance to high irradiance (bottom). Date of the transfer is denoted by an arrow.

Both species of *Calamagrostis* were able to continue in growth and in formation of new tillers even in extremely low irradiances ( $25 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Such a low PPF was set in our experiments intentionally in order to simulate critical radiation environment in the understory of closed forests. The values of PPF in the HI treatment were in fact not very high (about one tenth of maximum PPF in fully insulated areas), and resembled the regime in open forests, in which the two *Calamagrostis* species become highly invasive.

The overall capture and utilization of incident radiation, integrally manifested in growth rates, was much better in *C. epigejos* than in *C. arundinacea*. This finding could be helpful in explanation of invasive character of growth of *C. epigejos* in natural habitats, although some other characteristics favourable for better vegetative spreading of this species should be taken into account (e.g., formation of longer rhizomes).



In analyzing the contribution of different traits to fast growth of *C. epigejos*, the rather low rates of  $P_N$  per unit leaf area were not significant. Of course, the standard measurements of  $P_N$  were done in selected leaves with the best performance, and the variability of  $P_N$  in the other leaves was not tested. Measurement of gas exchange of the whole plants and the subsequent calculation of diurnal carbon balance was a more appropriate approach to explain differences in growth rate between the two species.

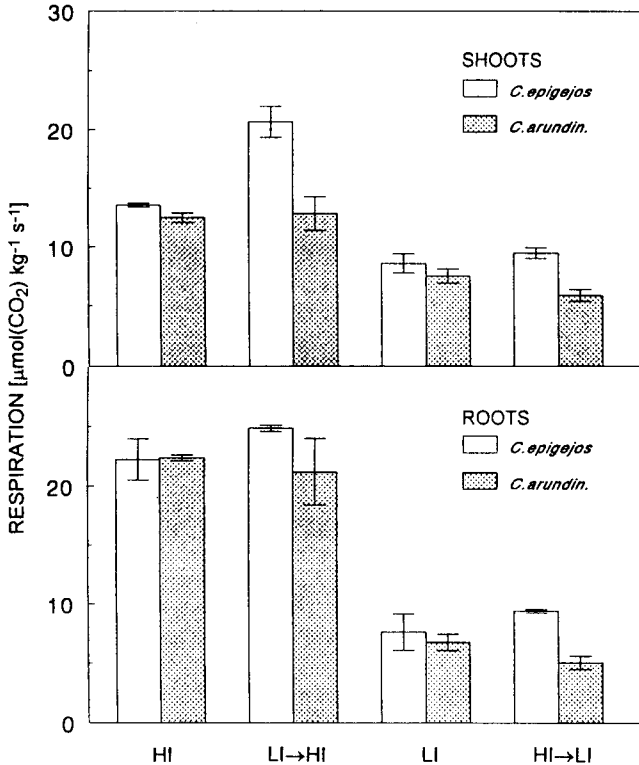


Fig. 5. Shoot and root respiration rates of *Calamagrostis epigejos* and *C. arundinacea* cultivated permanently in high irradiance (HI) or in low irradiance (LI), and respiration rates of plants transferred from HI to LI and from LI to HI. Mean values from six plants  $\pm$  SE.

Comparisons based on instantaneous  $P_N$  calculated per unit leaf area did not include all operational costs of the leaves (night respiration) and costs of leaf construction. Night respiration can be incorporated by calculating  $P_N$  on a 24 h cycle. Leaf construction costs can be indirectly included expressing  $P_N$  per leaf mass unit, as suggested by Givnish (1988). In this case, the better performance of *C. epigejos* becomes especially apparent. The leaves of *C. epigejos* were much thinner and, consequently, less costly in terms of energy investment into growth of unit leaf area, than the leaves of *C. arundinacea*.

In spite of striking differences in shape of the curves  $P_N \times \text{PPF}$  between the plants from HI and LI, the differences in apparent quantum yield of photosynthesis were much smaller. It is now generally accepted that acclimation to shade is not connected with changes in quantum yield (Evans 1987, and some other authors, as reviewed by Boardman 1977 and Björkman 1981). We have measured only apparent quantum yield ( $\Phi_{\text{app}}$ ) from incident quantum flux, and not the true quantum yield from absorbed quanta. Slight (but significant) decrease of  $\Phi_{\text{app}}$  in LI grown plants could be caused not only by lower efficiency of conversion of absorbed radiation, but also by lower absorptance of leaves. This parameter was, unfortunately, not measured in our experiments.

We conclude that both species tested in our experiments exhibited extraordinary high potential to acclimate to changes in radiation environment, as compared with some other grasses (e.g., Patterson 1979, Pierson and Mack 1990). Both species made very similar adjustments in their photosynthetic and respiratory activity, and in biomass partitioning. Morphological attributes of leaves, best expressed in extended range of SLA, were the most important traits responsible for better performance of *C. epigejos* in both LI and HI environments.

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