Phosphorus nutrition and mycorrhiza effects on grass leaf growth. P status- and size-mediated effects on growth zone kinematics

MONIKA KAVANOVÁ, AGUSTÍN A. GRIMOLDI, FERNANDO A. LATTANZI & HANS SCHNYDER

Lehrstuhl für Grünlandlehre, Technische Universität München, Am Hochanger 1, D-85350 Freising-Weihenstephan, Germany

ABSTRACT
This study tested whether leaf elongation rate (LER, mm h⁻¹) and its components – average relative elemental growth rate (REGRavg, mm mm⁻¹ h⁻¹) and leaf growth zone length (LLEGZ, mm) – are related to phosphorus (P) concentration in the growth zone (PLEGZ, mg P g⁻¹ tissue water) of Lolium perenne L. cv. Condesa and whether such relationships are modified by the arbuscular mycorrhizal fungus (AMF) Glomus hoi. Mycorrhizal and non-mycorrhizal plants were grown at a range of P supply rates and analysed at either the same plant age or the same tiller size (defined by the length of the sheath of the youngest fully expanded leaf). Both improved P supply (up to 95%) and AMF (up to 21%) strongly increased LER. In tillers of even-aged plants, this was due to increased REGRavg and LLEGZ. In even-sized tillers, it was exclusively due to increased REGRavg. REGRavg was strictly related to PLEGZ (r² = 0.95) and independent of tiller size. Conversely, LLEGZ strictly depended on tiller size (r² = 0.88) and not on PLEGZ. Hence, P status affected leaf growth directly only through effects on relative tissue expansion rates. Symbiosis with AMF did not modify these relationships. Thus, no evidence for P status-independent effects of AMF on LER was found.

Key-words: arbuscular mycorrhizal fungi; Glomus hoi; leaf elongation rate; leaf growth zone; Lolium perenne L.; relative elemental growth rate

Abbreviations: AMF, arbuscular mycorrhizal fungus/fungi; LER, leaf elongation rate; LLEGZ, length of the leaf growth zone; PLEGZ, concentration of P in leaf growth zone; REGRavg, average relative elemental growth rate; WSC, total water soluble carbohydrates.

INTRODUCTION
Phosphorus (P) is an essential macronutrient required for plant growth and development, but plants have to cope with limiting soil P availability in many terrestrial ecosys-
tiller size might subsequently affect plant growth independently of the nutrient status (Niklas 1994). It is presently unknown whether the decrease in \( L \) under P deficiency is related exclusively to a decreased plant P status or it is also partly related to effects mediated by the reduced tiller size. Interestingly, an allometric relationship between the length of the sheath enclosing the growing leaf and one of the \( L \) components, \( L_{LGZ} \), has been observed in a study of successive leaves in wheat (Kemp 1980b) and in eight grasses compared at different sheath lengths (Arredondo et al. 1999) showed that artificial shortening of sheaths reduced \( L \) by decreasing \( L_{LGZ} \). Usually, studies of the effects of environmental or nutritional factors on leaf growth have not distinguished direct from size-dependent treatment effects. However, such a distinction is essential for understanding the mechanisms controlling leaf growth.

The aims of this study were: (1) to assess the effects of P supply on \( L \) and its components, \( REGR_{avg} \) and \( L_{LGZ} \); (2) to verify if these parameters are related to growth zone P status; and (3) to investigate if these relationships are modified by AMF. \( L \) and its components were determined in perennial ryegrass (Lolium perenne L. cv. Condessa) grown over a wide range of P supply, with and without AMF (Glomus hoi). In order to account for both P status- and tiller size-mediated effects, comparisons were made between the responses of even-aged plants with different tiller sizes and plants with even-sized tillers.

**MATERIALS AND METHODS**

**Plant culture, AMF inoculation and growth conditions**

Surface-sterilized seeds of perennial ryegrass (L. perenne) were sown in plastic pots (diameter 5 cm, height 35 cm) containing a mixture of quartz sand supplemented with fine powdered Hyperphos (63 mg P per pot), providing a source of P with low availability for all plants. Each pot contained one plant. Half of the pots were inoculated with AM fungus G. hoi (15 mL inoculum per pot). The inoculum consisted of a mixture of sand and roots originating from a single-spore pot culture of G. hoi BEG104 propagated on Plantago lanceolata L. Pots with and without inoculation were placed in separate containers (76 × 56 × 37 cm) in growth chambers. Two independent experiments were conducted.

**Experiment 1: comparison at even age**

The aim of this experiment was to analyse the response of \( L \) and its components to P supply and AMF in even-aged tillers. Thus, plants were grown on different levels of soluble P supply for the same time period: 61–63 d after sowing (DAS). Growth room (VKZPH 005-120-S, Heraeus Vötch, Balingen, Germany) conditions were 20/15°C (day/night), 70% relative air humidity and 425 µmol m⁻² s⁻¹ photosynthetically active photon flux density (PPFD) at plant height for 16 h day⁻¹. In order to promote AMF colonization, all plants were initially (first 34 DAS) irrigated four times a day with 25 mL of modified P-free half-strength Hoagland’s solution [2.5 mM KNO₃, 2.5 mM Ca(NO₃)₂, 1 mM MgSO₄, 0.5 mM KCl, 0.5 mM NaCl, 0.125 mM Fe-EDTA, 23 µM H₂BO₃, 4.5 µM MnSO₄, 0.38 µM ZnSO₄, 0.16 µM CuSO₄ and 0.05 µM Na₂MoO₄]. Thereafter and until the end of the experiment, four concentrations of soluble P (0, 0.02, 0.1 and 0.5 mM) in the form of KH₂PO₄ were supplied to both mycorrhizal and non-mycorrhizal plants.

**Experiment 2: comparison at even size**

The aim of this experiment was to analyse the response of \( L \) and its components to P supply and AMF of plants with even-sized tillers, that is, similar sheath length. To this end, seeds for low-P plants were germinated 14 d in advance of seeds for high-P plants. Measurements were performed at 60–61 DAS in low-P plants and at 46–47 DAS in high-P plants when plants in the different treatments had mature tillers of similar size (sheath length of the youngest expanded leaf: 100 ± 11 mm). The plants were grown in four growth chambers (E15, Conviron, Winnipeg, Canada), with 20/15 °C (day/night), 70% relative air humidity and 525 µmol m⁻² s⁻¹ PPFD at plant height for 16 h day⁻¹. All plants were first irrigated for 21 DAS with the nutrient solution as described above, except that 0.02 mM KH₂PO₄ was included. Thereafter, two levels of soluble P supply were applied in both the mycorrhizal and non-mycorrhizal plants: 0.02 mM (low P) and 1 mM (high P).
**AMF colonization**

Root colonization by AMF was determined at 68 DAS in experiment 1 and at 63 DAS (low-P treatments) and 49 DAS (high-P treatments) in experiment 2. Colonization was estimated on roots stained with Trypan Blue by using the gridline intersect method (McGonigle *et al.* 1990). The root length colonized (%) is represented by the percentage of total intercepts where hyphae were present.

**LER**

Representative mature tillers (i.e. tillers having at least two fully expanded leaves) were selected for measurement of **LER** and leaf growth zone properties. Leaf elongation rate (**LER**, mm h⁻¹) was measured on the most rapidly growing leaf on a tiller. The measured leaf was the youngest visible leaf during the phase of its maximal expansion when **LER** was near constant (Schnyder *et al.* 1990). **LER** was determined as the ratio of change of distance between the tip of the elongating blade and the ligule of the youngest fully expanded leaf, which was measured daily with a ruler. The length of the sheath of the youngest expanded leaf was recorded at every measurement. In experiment 1, **LER** was measured on four plants per treatment at 61, 62 and 63 DAS. In experiment 2, five plants per treatment were measured at 60 and 61 DAS in the low-P treatments, and at 46 and 47 DAS in the high-P treatments. No difference in **LER** was observed between sampling dates (P > 0.1) or growth chambers (P > 0.1). Therefore, data from different sampling times and chambers were pooled.

**Components of **LER**: L_{LGZ} and REGR**

**LER** components were estimated by determining the growth spatial distribution within the leaf growth zone of mature tillers immediately after **LER** measurements on the same measured leaves using a pin-pricking method (Schnyder, Nelson & Coutts 1987). Briefly, 2 h after the start of the light period, a series of holes 3 mm apart along the basal 40–60 mm of a tiller was made with a fine needle. The plants were returned to the growth chamber for 4–6 h. Thereafter, distances between the holes both along the base of the growing leaf and along the non-growing surrounding sheath were measured with 0.1 mm accuracy. Leaves with the ligule located farther than 2 mm from the point of attachment were discarded, which assured that only the blade elongation was assessed.

Segmental elongation rate (**SER**; mm h⁻¹) was then calculated as:

\[
\text{SER}_i = \frac{L_{i}-L_{i-1}}{\Delta t},
\]

where \(L_{i-1}\) is the length of a segment delimited by two neighbouring holes in the growing blade (measured \(\Delta t\); h, after pinning) and \(L_{i-1}\) is the length of the corresponding segment measured in the surrounding non-growing leaf sheath. **SER** was corrected by the ratio between the **LER** of a non-pierced leaf (**LER_{control}**) measured on the same leaf before pinning and the **LER** of the pierced leaf (**LER_{pierced}** determined as the sum of all **SER** along the leaf). This was done to account for the effects of pinning on **LER**. It has been repeatedly shown that growth reductions caused by pinning do not modify the relative distribution of growth rates and \(L_{LGZ}\) (Schnyder *et al.*, 1987, 1990; Hu & Schmidhalter 2000), validating its use for the assessment of the spatial distribution of growth rates (e.g. Ben-Haj-Salah & Tardieu 1995; Fricke & Peters 2002; Assuero *et al.* 2004).

The velocity of displacement (\(V_i\); mm h⁻¹) of a given segment \(i\) was calculated as the sum of elongation rates of all segments located more basally. The Richards function was fitted to each \(V_i\) profile (all fittings \(r^2 \geq 0.99\); TableCurve 2D v.5.01, Systat, Richmond, CA, USA):

\[
V_i = a \frac{1}{(1 + \exp(b - ax))^c}, \quad (2)
\]

where \(x\) is the distance from the leaf base, \(a\) is the asymptotic maximal \(V_i\), and \(b, c\) and \(d\) are constants.

Relative elemental growth rate (**REGR**, mm mm⁻¹ h⁻¹) was estimated as the first derivative of the fitted Richards function at the midpoint of each 3-mm-long segment:

\[
\text{REGR} = \frac{ac \exp\left(\frac{b}{2}x\right) + (\exp(b) + \exp(2b))}{d}, \quad (3)
\]

\(L_{LGZ}\) (mm) was defined as the distance from the leaf base to the midpoint of the last segment where **SER** was positive. The average relative elemental growth rate (**REGR_{avg}**) was then determined as:

\[
\text{REGR}_{avg} = \frac{\text{LER}_{control}}{L_{LGZ}}. \quad (4)
\]

\(L_{LGZ}\) was alternatively calculated as the position where 95% of the \(a\) value (the asymptotic \(V_i\), predicted by the fitted Richards function) was reached:

\[
L_{LGZ} = -\ln(20^a - 19^b) + \frac{\ln 19}{c} + \frac{b}{c}. \quad (5)
\]

The different estimations of \(L_{LGZ}\) gave near-identical results because of the high values of \(r^2\) fittings of the Richards function (data not shown).

**Sampling and chemical analyses**

In experiment 1, five plants were sampled at the end of the dark period at 68, 74 and 83 DAS. A piece of tissue 1.7 times the length of the \(L_{LGZ}\) was dissected out from the base of elongating leaves of mature tillers similar to those used in **LER** measurements. In experiment 2, 12 plants were sampled at the end of the light period at 63 DAS in low-P and 49 DAS in high-P treatments, and leaf growth zones (\(L_{LGZ}\) determined by the pin pricking) were dissected out. The rest of the shoot tissue was pooled. In all cases, fresh weight was recorded and the samples were immediately frozen in liquid N\(_2\), freeze-dried for 72 h at \(-80^\circ\text{C}\), weighed, ground and stored at \(-25 ^\circ\text{C}\) before analyses.

P concentration was determined on 10–20 mg of pooled samples by a modified phosphovanado-molybdate colorimetric method following acid digestion (Hanson 1950).
Experimental design and statistical analysis

Both experiments were complete two-way factorials. The first experiment consisted of two levels of AMF treatment and four levels of P supply arranged in a completely randomized design. The second experiment consisted of two levels of AMF treatment and two levels of P supply arranged in a randomized complete block (growth chambers) design. Analysis of variance (ANOVA) revealed no effect of growth chamber on LER (P > 0.1). In both experiments, the effects of P supply and AMF on the nutritional status and parameters of the kinematic analysis were then tested by two-way ANOVA, with the main factors P supply and AMF treatment. The relationships between $P_{LGZ}$ and LER and its components were tested by linear regression analyses of treatment averages, and slopes and intercepts for AMF treatments were compared with F-test (Statistica 6.0, Statsoft, Tulsa, OK, USA). Results are shown as means ± 1 SE.

RESULTS

Plant growth at different levels of P supply and AMF treatment

When sampled at the same age (experiment 1), plants grown at low P supply and/or in the absence of AMF had substantially lower plant biomass (Grimoldi et al. 2005) and had mature tillers with shorter sheaths than plants grown at high P and/or in the presence of AMF (Fig. 2a). In contrast, and as aimed for, tiller sheath length did not differ among treatments when plants were sampled at different dates in experiment 2 (60 and 46 DAS) (Fig. 2b). Thus, the responses of LER and its components to P supply and AMF were analysed in plants where any possible tiller size-mediated effects could (experiment 1) or could not (experiment 2) interfere with P status-mediated effects.

Spatial distribution of growth along the leaf base

The profile of velocity of displacement along the leaf growth zones had the expected form in all treatments. Velocity increased with distance from the leaf base until the end of the leaf growth zone, where it became constant and equal to LER. P deficiency consistently reduced the maximal displacement velocity, i.e. LER, by up to 60% in mature tillers of even-aged plants and by 45% in even-sized mature tillers (P < 0.05; Fig. 3a–c).

On the other hand, P deficiency had contrasting effects on $L_{LGZ}$ in the two experiments. It shortened $L_{LGZ}$ by up to 46% when mature tillers of even-aged plants were compared (P < 0.05). However, neither P supply nor AMF affected $L_{LGZ}$ when even-sized tillers were compared (P > 0.05; Fig. 3c), even though they clearly differed in PLGZ. Likewise, mycorrhizal plants had higher LER and longer...
Table 1. Nutrient status of perennial ryegrass plants. The effect of soluble P supply and presence (AMF+) or absence (AMF−) of the arbuscular mycorrhizal fungus *Glomus hoi* on the concentrations of P, N and C in the form of WSC, and total C in the leaf growth zone biomass (\(P_{\text{LGZ}}, N_{\text{LGZ}}, C_{\text{WSC-LGZ}}, C_{\text{LGZ}}\), respectively). Values in brackets are SE (\(n = 2–10\)).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>AMF treatment</th>
<th>P supply (mM)</th>
<th>(P_{\text{LGZ}})</th>
<th>(N_{\text{LGZ}})</th>
<th>(C_{\text{WSC-LGZ}})</th>
<th>(C_{\text{LGZ}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Even age</td>
<td>AMF−</td>
<td>0</td>
<td>0.42 (0.02)</td>
<td>4.77 (0.22)</td>
<td>23.4 (0.7)</td>
<td>73.0 (4.3)</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.67 (0.04)</td>
<td>6.23 (0.29)</td>
<td>24.9 (2.3)</td>
<td>59.6 (2.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.90 (0.05)</td>
<td>6.85 (0.18)</td>
<td>18.9 (1.7)</td>
<td>57.3 (2.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.89 (0.08)</td>
<td>6.35 (0.40)</td>
<td>17.0 (1.9)</td>
<td>50.4 (4.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMF+</td>
<td>0</td>
<td>0.47 (0.08)</td>
<td>5.07 (0.07)</td>
<td>22.0 (1.7)</td>
<td>63.5 (3.6)</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>0.66 (0.05)</td>
<td>5.53 (0.26)</td>
<td>21.9 (2.3)</td>
<td>56.4 (4.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.83 (0.07)</td>
<td>5.97 (0.17)</td>
<td>20.7 (2.4)</td>
<td>55.8 (3.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.10 (0.05)</td>
<td>6.06 (0.37)</td>
<td>15.1 (1.2)</td>
<td>50.4 (3.1)</td>
<td></td>
</tr>
<tr>
<td>Even size</td>
<td>AMF−</td>
<td>0.02</td>
<td>0.71 (0.03)</td>
<td>6.97 (0.21)</td>
<td>46.0 (2.8)</td>
<td>71.8 (4.3)</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.21 (0.11)</td>
<td>8.63 (0.24)</td>
<td>20.8 (4.3)</td>
<td>55.0 (1.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AMF+</td>
<td>0.02</td>
<td>0.77 (0.10)</td>
<td>7.25 (0.28)</td>
<td>56.2 (2.6)</td>
<td>76.9 (3.1)</td>
</tr>
<tr>
<td></td>
<td>1.00</td>
<td>1.47 (0.14)</td>
<td>8.82 (0.30)</td>
<td>23.8 (1.8)</td>
<td>59.1 (2.1)</td>
<td></td>
</tr>
</tbody>
</table>

\(P_{\text{LGZ}}\) than non-mycorrhizal plants in tillers of even-aged plants (\(P < 0.05\)). Such an effect was not found when even-sized tillers were compared (\(P > 0.05\)).

The spatial distribution of \(REGR\) within the growth zone had a common bell shape in all treatments, with a maximum near the centre of the growth zone (Fig. 3d–f). P deficiency lowered maximum \(REGR\), an effect particularly evident in even-sized tillers.

**The effect of P supply and AMF on P, N and WSC concentrations**

\(P_{\text{LGZ}}\) increased strongly with increasing P supply in both experiments (\(P < 0.01\); Table 1). AMF increased \(P_{\text{LGZ}}\) at the highest P supply level in the experiment comparing even-aged plants (\(P < 0.05\); Table 1), although AMF colonization was low compared with the other treatments (Table 2). In the other treatments, AMF had no significant effect on \(P_{\text{LGZ}}\) (\(P > 0.05\), Table 1).

Other components of leaf growth zone biomass were also affected by P supply. In both experiments, increasing P supply led to 20–33% higher nitrogen (N) and 27–58% lower WSC concentrations in the leaf growth zone (Table 1). Because increasing P supply had a greater effect on P concentrations (by 70–134%), growth zone N:P and WSC:P ratios (w w\(^{-1}\)) were highest at the lowest P supply. AMF colonization increased N and decreased WSC concentrations in the growth zone only when \(P_{\text{LGZ}}\) was also improved.

**Relationship between growth zone P status and \(LER\) and its components**

In both even-aged and even-sized tillers, \(LER\) was linearly related to \(P_{\text{LGZ}}\) (\(P < 0.05\); Fig. 4a & b, Table 3). The slope and intercept of the regression tended to be higher in even-aged mycorrhizal plants, but this effect was not statistically significant (\(P > 0.1\); Table 3).

Regression analyses also demonstrated a positive linear relationship between \(REGR_{\text{avg}}\) and \(P_{\text{LGZ}}\) in both even-aged and even-sized tillers (\(P < 0.05\); Fig. 4c & d, Table 3). The slope of this relationship was somewhat higher in even-sized tillers, possibly because of a slightly different definition of the growth zone tissue sampled for P analysis (see Materials and methods).

The relationship between \(L_{\text{LGZ}}\) and \(P_{\text{LGZ}}\) was more complex. When treatments differed in tiller sheath length (even-aged plants), \(L_{\text{LGZ}}\) was related linearly to \(P_{\text{LGZ}}\) (\(P < 0.05\); Fig. 4e, Table 3). Furthermore, AMF colonization increased \(L_{\text{LGZ}}\) over the whole range of \(P_{\text{LGZ}}\) in even-aged plants (\(P > 0.1\); Table 3). Notably, in this experiment, AMF also increased the tiller size over the whole range of P supplies (Fig. 2a). Conversely, none of these effects were observed when size-mediated effects were avoided: \(P_{\text{LGZ}}\) and \(L_{\text{LGZ}}\) were unrelated, and AMF had no effect on \(L_{\text{LGZ}}\) when tillers of the same size were compared (\(P > 0.1\); Fig. 4f, Table 3), even though their \(P_{\text{LGZ}}\) were very different.

Table 2. Mycorrhizal colonization of perennial ryegrass roots. The effect of soluble P supply on the percentage of root length colonized by the AMF *Glomus hoi* was determined in even-aged plants at 68 DAS, and in even-sized plants at 63 DAS (0.02 mM P) and 49 DAS (1 mM P) on Trypan Blue stained roots by the gridline intersect method. Mycorrhizal colonization of the even-aged plants was reported before (Grimoldi et al. 2005). Non-mycorrhizal plants had null root colonization in both experiments. Values in brackets are SE (\(n = 4–9\)).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>P supply (mM)</th>
<th>Root length colonized (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Even age</td>
<td>0</td>
<td>49.3 (3.8)</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>48.1 (1.7)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>15.2 (1.6)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>8.3 (0.3)</td>
</tr>
<tr>
<td>Even size</td>
<td>0.02</td>
<td>33.7 (3.5)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.4 (0.4)</td>
</tr>
</tbody>
</table>
Accounting for sheath length effects on LER and its components

The data already presented revealed a strict allometric relationship between $L_{LGZ}$ and the sheath length of the youngest expanded leaf through which the growing leaves emerged (Fig. 5). This relationship was not modified by AMF and did not differ between the two experiments.

The correlation between $L_{LGZ}$ and sheath length in Fig. 5 includes data from different P supply treatments. We verified that the same relationship also existed in data subsets with uniform P status. Figure 6 shows one such subset including even-aged tillers with 0.83–0.90 mg P g$^{-1}$ tissue water in the leaf growth zone. In these tillers, LER was positively correlated with the sheath length of the youngest expanded leaf ($r^2 = 0.51$, $P < 0.05$; Fig. 6a). The correlation was entirely due to the relationship between $L_{LGZ}$ and sheath length ($r^2 = 0.63$, $P < 0.05$; Fig. 6c) because $REGR_{avg}$ was independent of sheath length ($r^2 = 0.00$, $P > 0.1$; Fig. 6b). These results were confirmed by regression analyses within each treatment in both experiments, and $REGR_{avg}$ never correlated with sheath length (data not shown).

Table 3. Linear regression analysis of the relationship between leaf growth variables and P status of the growth zone in non-mycorrhizal (AMF–) and mycorrhizal (AMF+) plants. Parameters of the linear regression of leaf elongation rate (LER) and its two components, average relative elemental growth rate ($REGR_{avg}$) and leaf growth zone length ($L_{LGZ}$) against $P_{th}$ are presented. In the experiment with even-sized tillers, AMF– and AMF+ data were combined. Values in brackets are SE ($n = 4$) for parameters different from zero ($P < 0.05$).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Variable</th>
<th>AMF treatment</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Even age</td>
<td>LER</td>
<td>AMF–</td>
<td>1.78 (0.39)</td>
<td>0.08 (n.s.)</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMF+</td>
<td>2.01 (0.29)</td>
<td>0.13 (n.s.)</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>REGR$_{avg}$</td>
<td>AMF–</td>
<td>0.022 (0.005)</td>
<td>0.033 (0.004)</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMF+</td>
<td>0.023 (0.003)</td>
<td>0.035 (0.002)</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>$L_{LGZ}$</td>
<td>AMF–</td>
<td>23.6 (4.8)</td>
<td>10.3 (n.s.)</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMF+</td>
<td>26.0 (5.6)</td>
<td>12.3 (n.s.)</td>
<td>0.92</td>
</tr>
<tr>
<td>Even size</td>
<td>LER</td>
<td>AMF–/AMF+</td>
<td>1.47 (0.25)</td>
<td>0.33 (n.s.)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>REGR$_{avg}$</td>
<td>AMF–/AMF+</td>
<td>0.035 (0.006)</td>
<td>0.015 (n.s.)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>$L_{LGZ}$</td>
<td>AMF–/AMF+</td>
<td>3.65 (n.s.)</td>
<td>32.6 (2.2)</td>
<td>0.62</td>
</tr>
</tbody>
</table>

n.s., not significant at $P = 0.05$.
Phosphorus effects on grass leaf elongation

Figure 6. Relationships between the sheath length of the youngest expanded leaf enclosing the growing leaf and (a) leaf elongation rate (LER) (b) average relative elemental growth rate (REGRavg) and (c) leaf growth zone length (L_{LGZ}) in the experiment with even-aged tillers. Data correspond to a subset of plants with similar growth zone P concentration (0.83–0.90 mg g⁻¹ tissue water), with symbols as for Fig. 3. Linear regression equations are: \( y(LER) = 0.017x + 0.088; r^2 = 0.52 P < 0.001 \); \( y(REGR_{avg}) = 0.000x + 0.046; r^2 = 0.03 P = 0.41 \); \( y(L_{LGZ}) = 0.27x + 6.84; r^2 = 0.65 P < 0.001 \). Intercepts different from zero are marked with an asterisk (\( P < 0.05 \)).

DISCUSSION

AMF and P supply control leaf growth via the same P status- and size-dependent mechanisms

This is the first quantitative assessment of the relationship between the P status of leaf growth zones and the components of LER. The results revealed that AMF and P supply enhanced leaf growth via identical mechanisms. These included both P status-dependent and tiller size-mediated effects on LER (Fig. 7). Remarkably, REGRavg was strictly related to the P status of the leaf growth zone (i.e., \( P_{LGZ} \)) and was independent of tiller size and AMF. Conversely, \( L_{LGZ} \) was a function of the sheath length of the youngest expanded leaf through which the growing leaf emerged (i.e., tiller size) and was independent of P status and AMF. Accordingly, in plants of similar tiller size, P supply and AMF affected LER only via the effect of P status on REGRavg. Conversely, in plants of the same age, differences in tiller size contributed to treatment differences in LER via the effect of tiller size on \( L_{LGZ} \). These results illustrate the need for a distinction between P status-dependent and size-mediated effects on LER.

Our results for the even-aged plants agree with those of the only other study on effects of P supply on leaf growth kinematics in grasses reported by Assuero et al. (2004). In their study with even-aged (but uneven-sized) plants, P deficiency caused a 63% reduction in LER, which resulted from a 56% shorter \( L_{LGZ} \), and a 7% reduction in REGRavg. Their work also demonstrated a higher cell production rate in the P sufficient tillers. The present data suggest that the responses observed by Assuero et al. (2004) resulted mainly from the size-mediated changes of LER and only marginally from the direct effect of growth zone P status on LER. Thus, it raises the question whether cell production rate is also related to tiller size. Clearly, the cellular mechanisms underlying the effects of P supply and AMF on leaf growth merit further study.

Our data also directly demonstrate that P deficiency did not limit leaf growth through a reduced C availability. In fact, the growth zones of P deficient plants had a higher WSC concentration than the growth zones of P sufficient plants, thus corroborating previous findings that P deficiency has a stronger effect on C utilization than on assimilation (Rao & Terry 1989; Rodríguez, Andrade & Goudriaan 2000). AMF have been reported to consume up to 20% of C fixed by the plant (Jakobsen & Rosendahl 1990). However, this cost was not reflected in WSC concentrations in the growth zone, suggesting that the presence of AMF did not negatively affect the amount of C available for leaf growth.

\[
\text{LER} = L_{LGZ} \times \text{REGR}_{avg}
\]

Figure 7. Graphical summary of the effects of P supply and mycorrhizal colonization on LER and its components. P supply and AMF treatment affect the leaf growth zone P status, which then directly affects LER through a change in REGRavg. In turn, changes in the sheath length of the youngest expanded leaf (related to either the direct effect of P status on LER or to any other P-independent influence) will affect LER through an effect on \( L_{LGZ} \).
The uptake and subsequent metabolism of N are also known to be reduced under P deficiency (Rufty et al. 1993). In the present study, N concentration in leaf growth zone biomass decreased with P deficiency, but the N:P ratio of the growth zone biomass was highest in P-deficient plants. This typical response of P-limited plants (but not of N-limited plants) (Agren 2004) indicates that the reduction of $\text{REGR}_{\text{avg}}$ under P deficiency was related exclusively to the low P status of the growth zone.

**Tissue expansion rate is directly related to P status of the growth zone**

This study demonstrated a direct and strong relationship between the $P_{\text{LGZ}}$ and $\text{REGR}_{\text{avg}}$. Because $\text{REGR}$ is a relative rate, it does not depend on the number of cells or cell length, and for a given position is equal to the relative rate of cell expansion (Schneider et al. 1990; Ivanov, Dobrochoave & Baskin 2002). The actual mechanism by which expansion rate is affected by P status is not known. However, relative cell expansion rate is a function of cell wall extensibility and turgor pressure in excess of the yield threshold of the cell wall (Van Volkenburgh 1999). Arguably, P deficiency can affect both parameters because it leads to low leaf ATP concentrations (Jacob & Lawlor 1993) and causes a variety of transcriptional and hormonal changes (Franco-Zorrilla et al. 2004). It also remains to be elucidated whether the growth reduction is mediated by unavailability of P as a substrate or by P as a signal (Ticconi & Abel 2004). Certainly, observed lower $P_{\text{LGZ}}$ under P deficiency may result from decreased availability of P in xylem/phloem and may directly limit the synthesis of P-containing cell components, although this can be partially compensated, e.g. by galactoolipids and sulfolipids partly replacing phospholipids in membranes (Dörmann & Benning 2002). Yet, decreased $P_{\text{LGZ}}$ may result from lower P deposition due to cytokinin-mediated decreases in cell division/expansion as well (Werner et al. 2003).

**What controls the length of the leaf growth zone?**

The mechanism how the length of the leaf growth zone is actually regulated is so far unknown. Possible determinants include both positional and temporal controls of tissue expansion. We evaluated the possibility that cells require a fixed time interval to complete expansion. However, time-position trajectories (cf. Gandar & Hall 1988) generated from the present data suggested that such a ‘time-control’ mechanism was unlikely. This is because P deficiency led to significantly longer residence times of tissue in the growth zone when size effects were taken into account (data not shown). The present – and also previous (Kemp 1980b; Casey et al. 1999; Arredondo & Schnyder 2003) – studies showed that $L_{\text{LGZ}}$ is proportional to the sheath length of the youngest expanded leaf, suggesting a positional rather than temporal control. It is known that termination of cell expansion is associated with increased apoplastic peroxidase activity (Bacon, Thompson & Davies 1997; de Souza & MacAdam 1998). In turn, peroxidase activity in maize coleoptiles responded to changes in light quality in a phytochrome-mediated response (Kim, Shinkle & Roux 1989). Thus, coordination between $L_{\text{LGZ}}$ and enclosing sheath mediated by morphogenic effects of light quality seems a reasonable hypothesis (cf. Skinner & Simmons 1993; Gautier & Varlet-Grancher 1996), albeit direct experimental evidence is still missing.

A review of kinematic studies evaluating the control of leaf elongation under different abiotic stresses indicates that when experimental designs produced no substantial difference between sheath length of control and treated plants, changes in $\text{LER}$ were chiefly due to changes in $\text{REGR}_{\text{avg}}$ and not $L_{\text{LGZ}}$ (e.g. light-dark cycles: Schnyder & Nelson 1988; salinity: Fricke & Peters 2002; N deficiency: Fricke, McDonald & Mattson-Djos 1997; general nutrient deficiency: Snir & Neumann 1997; source limitation: Fricke 2002; temperature: Ben-Haj-Salah & Tardieu 1995; ABA accumulation: Dodd & Davies 1996). Conversely, in studies where treatments altered sheath length substantially, both $L_{\text{LGZ}}$ and $\text{REGR}_{\text{avg}}$ contributed to differences in $\text{LER}$ (e.g. irradiance: Schnyder & Nelson 1989; salinity: Bernstein, Läucli & Silk 1993; N deficiency: Gastal & Nelson 1994, Töth et al. 2002; P deficiency: Assuero et al. 2004). Therefore, it seems these abiotic stresses initially affected $\text{LER}$ solely by reducing $\text{REGR}$. Eventually, however, the treatment-related changes in tiller sheath length brought about an additional (indirect) effect: a change of $L_{\text{LGZ}}$.

In conclusion, this study showed that the effects of P supply and AMF on $\text{REGR}_{\text{avg}}$ and thus on $\text{LER}$, were closely and linearly related to their effects on $P_{\text{LGZ}}$. The other component of $\text{LER}$, $L_{\text{LGZ}}$, was strictly related to the sheath length of the youngest expanded leaf independently of P supply or AMF treatment. Thus, tissue expansion rate was directly associated with P status, but the position at which expansion stopped was unrelated. AMF and P supply affected leaf growth through identical mechanisms.

**ACKNOWLEDGMENTS**

This research was supported by Deutsche Forschungsgemeinschaft (SFB 607) and Deutscher Akademischer Austausch Dienst (Procope D/033624). The G. hoi inoculum was provided by Andreas Heinemeyer (University of York, UK). We thank Marie Prud’homme and Annette Morvan-Bertrand (UMR INRA-UCBN Ecophysiologie Végétale, Agronomie et Nutrition NCS, Université de Caen, France) for their hospitality, access to their laboratory and help with WSC analyses. All the technical staff at Lehrstuhl für Grünlandlehre provided invaluable assistance, particularly Wolfgang Feneis, Anja Schmidt and Angela Ernst-Schwärzli.

**REFERENCES**


Received 18 April 2005; received in revised form 8 July 2005; accepted for publication 6 August 2005