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Differences in relative growth rate in 11 grasses correlate with differences in chemical composition as determined by pyrolysis mass spectrometry

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Summary. Eleven grass species varying in potential relative growth rate (RGR) were investigated for differences in chemical composition by pyrolysis mass spectrometry. The spectral data revealed correlations between RGR and the relative composition of several biopolymers. Species with a low potential RGR contained relatively more cell wall material such as lignin, hemicellulose, cellulose, polysaccharide-bound ferulic acid and hydroxyproline-rich protein, whereas species with a high potential RGR showed relatively more cytoplasmic elements such as protein (other than those incorporated in cell walls) and sterols.

Key words: Cell components – Grasses – Interspecific variation – Pyrolysis mass spectrometry – Relative growth rate

Even when grown under optimum conditions plant species may differ considerably in growth rate. Variation in the maximum relative growth rate (RGR), the dry weight increase per unit of biomass and per unit of time for species grown under more or less optimal conditions, may be as large as 350 mg g^{-1} day^{-1} (Grime and Hunt 1975). Species adapted to environments with growth-limiting conditions like nutrient-poor soils tend to have an inherently low RGR (Grime and Hunt 1975). It is not known if a relatively low RGR is essential for such an adaptation, nor what the advantage of such a low RGR could be (Lambers and Dijkstra 1987; Poorter 1989). A relationship with increased tolerance of stress is however very probable (Lambers and Poorter 1992). Poorter and Remkes (1990) investigated the factors causing the differences in RGR in 24 wild species, including the 11 grasses studied here. They found a positive correlation between the RGR and the leaf area ratio (LAR), the ratio between the total leaf area and total plant weight. This was mainly due to the specific leaf area (SLA), the ratio between leaf area and leaf weight. Selection in nutrient-poor habitats has possibly led to species that have a relatively large investment of leaf biomass per unit of leaf area (low SLA) and a relatively low investment of total biomass in the leaves (low LWR). A comparatively low SLA may be advantageous in such habitats when this is caused by extra investment in secondary metabolites, for instance phenolic compounds which may give protection under unfavorable circumstances (Waterman and McKey 1989). Chemical analysis indicated that low-RGR species have a comparatively high carbon level, which is partly caused by a difference in mineral level and partly by a comparatively high investment in carbon-rich components (Poorter and Bergkotte 1992). In extreme cases differences in the carbon content alone can explain a difference of 40% in RGR between two species which are similar with respect to their amount of leaf area, stem and root tissue, and even have the same rate of photosynthesis and respiration (Poorter 1989). A high C content of organic material might be explained by a relatively high content of carbon-rich components such as lipids or lignin (Table 1). Indeed, slow-growing species accumulated more lignin, and also more (hemi)cellulose and insoluble sugars, but no correlation was found between RGR and lipids (Poorter and Bergkotte 1992). Fast-growing species accumulated more organic N com-

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Table 1. Carbon content of different plant compounds in g g^{-1}, and chemical compositions of a young vegetative maize plant and the aboveground portion of sorghum (adapted from Poorter 1989)

Fraction	Carbon content	Chemical composition (%)	
		Maize	Sorghum
Lipids	0.776	2.5	3.0
Lignin	0.689	8.0	2.7
Organic N compounds	0.530	23.0	9.5
(Hemi)cellulose	0.461	48.0	57.8
Fructan, starch, sugars	0.412	8.5	21.0
Organic acids	0.375	5.0	1.3
Minerals	0.000	5.0	4.8

pounds and organic acids. Changes in relative amounts of cell wall components and protein may also effect the digestibility of the plant to foraging herbivores (Kephart et al. 1990) and, therefore, possibly also the plant's survival.

More data were wanted on the possible relationship between RGR and investment in cell wall substances and, therefore, more detailed information on the polymeric cell wall material of the different plant types was needed. Such high-molecular-weight material cannot be directly analysed. Instead of some form of chemical degradation, analytical pyrolysis was chosen as an alternative method. The combined technique of pyrolysis mass spectrometry (PyMS) is especially suited to the analysis of lignocellulosic material (Boon 1989). It provides information on biopolymers such as lignin, carbohydrates, lipids and protein and on ratios of these constituents. The resulting PyMS spectra are also accessible for principal component and discriminant analyses (Boon 1989).

Eleven grass species of which the RGR had been determined in earlier experiments (Poorter and Remkes 1990) were investigated to evaluate a possible relation between the chemical composition of their leaves and the interspecific variation in RGR.

Material and methods

Growth of the plants

Plants of 11 monocotyledons: *Brachypodium pinnatum* (L.) Beauv., *Briza media* L., *Corynephorus canescens* (L.) Beauv., *Cynosurus cristatus* L., *Dactylus glomerata* L., *Deschampsia flexuosa* (L.) Trin., *Festuca ovina* L., *Holcus lanatus* L., *Lolium perenne* L., *Phleum pratense* L. and *Poa annua* L., were grown from seed in a growth room. The following conditions were used: day: 14 h, photosynthetic photon flux density ca. 315 $\mu mol\ m^{-2}\ s^{-1}$, temperature 20° C, relative humidity ca. 70%; night: 10 h, temperature 20° C. Light was provided by fluorescent lamps (Philips T1–33–RS, 215 W) and incandescent bulbs (Philips, 40 W) in a ratio of 4:1. Plants were grown in a frequently replenished modified Hoagland solution with a nitrate concentration of 2 mM. Full details are given in Poorter and Remkes (1990). Leaf blades were harvested during a period of 17 days after the plants had reached a fresh weight of approximately 100 mg. The plant material was oven-dried for 24 h at 80° C.

Relative growth rate

RGRs ($mg\ g^{-1}\ day^{-1}$) measured by Poorter and Remkes (1990) were: *Brachypodium pinnatum*, 174; *Briza media*, 157; *Corynephorus canescens*, 113; *Cynosurus cristatus*, 176; *Dactylis glomerata*, 229; *Deschampsia flexuosa*, 135; *Festuca ovina*, 132; *Holcus lanatus*, 268; *Lolium perenne*, 214; *Phleum pratense*, 227; and *Poa annua*, 272.

Pyrolysis mass spectrometry

Pt, Rh filament pyrolysis mass spectrometry was performed on a JEOL DX–303 double focussing mass spectrometer equipped with a platinum-rhodium 90/10 filament in-source pyrolysis probe. Samples (2.5 µl) of a suspension of 2 $mg\ ml^{-1}$ dry material in ethanol were used for the PyMS analysis with a mass range of 20–750 or 50–2000 amu. The scan cycle time was 1 s and the source temperature 180° C, with a heating rate of 20–800° C s^{-1}. To avoid further fragmentation during ionization low voltage EI at about 16 eV was applied. An extensive description is given by Boon (1989).

Thermal extraction

The combination of comparatively high volatility and a comparatively low degree of degradation of the wax esters during the pyrolysis of the samples allowed temperature-resolved separation of those compounds from the biopolymer fragments.

Multivariate data analysis of PyMS data

Principal component (PC) and discriminant analyses were performed on the PyMS data files, using a modified Arthur package, adapted to PyMS data (Boon et al. 1984). In this method, spectra are considered to be points in a multidimensional space with the mass numbers as coordinate axes. The relative distribution of mass intensities in each spectrum determines the position in the multidimensional space. Similar spectra will cluster as one group. From the file of selected spectra an overall average spectrum ("zero point") is calculated which serves as reference point for the individual spectra. Mathematically, the differences between the individual spectra are determined by comparison with the zero point spectrum. PC analysis is performed on these data yielding sets of correlated mass peaks (PCs), which can be represented by reconstructed mass spectra. When multiple analyses are available discriminant analysis can be performed. The PCs are linearly combined into new independent variables, discriminant functions (DFs), which are represented graphically by reconstructed mass spectra. Dissimilarity is quantitatively expressed in discriminant function scores. PCs and DFs are numbered in order of the amount of variance accounted for. For the multivariate data analysis the recorded mass spectra were averaged over the pyrolysis time (60 s = 60 scans). Multiple analyses were carried out, giving three locations for each individual plant in the score plot.

Results

Figure 1 shows the PyMS spectra of two grasses with an inherently high (*Holcus lanatus*, Fig. 1A) and an inherently low RGR (*Festuca ovina*, Fig. 1B), respectively. In general, for both grasses these spectra show similar characteristics with fragments from the polysaccharides cellulose, hemicellulose and pectin, from phenolic acids, proteins, fatty acids, guaiacyl (G) lignin and syringyl (S) lignin, aliphatic wax esters and steroids, summarized in Table 2. In the total ion profile the contribution of polysaccharide and protein fragments is relatively high, that of lignin fragments on the contrary very low. Similar spectra were obtained for the other nine grasses.

A high m/z 150, a fragment derived from ferulic acid, is typical for grasses with a comparatively high percentage of cell-wall-bound phenolic acids (Harris and Hartley 1980). The relative abundance of m/z 150 correlated inversely with the RGR; low-RGR species showed a relatively high abundance of m/z 150 (Fig. 2). A similar tendency, but no significant correlation, was found for m/z 120, a fragment derived from *p*-coumaric acid (not shown). Other special grass components are the aliphatic wax esters with molecular ions m/z 592, 620, 648, 660, 676, 704, 732 and 760 with a carbon chain length ranging from C_{40} to C_{54}. Both spectra also show a relatively high

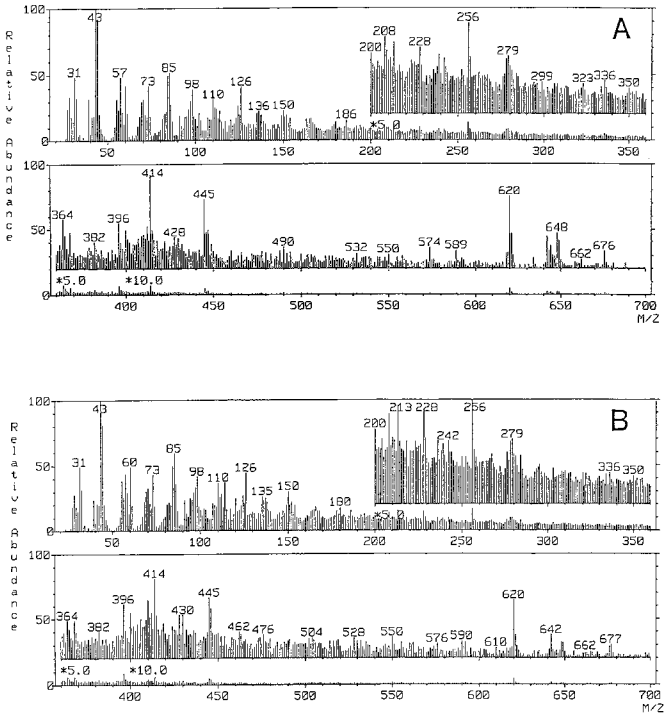


Fig. 1A, B. Mass spectra (16 eV EI) obtained after Pt, Rh filament pyrolysis of homogenised leaf material from **A** *Holcus lanatus* and **B** *Festuca ovina. Inserts:* enlarged scales, *y* axis × 5 for *m/z* 200–400, × 10 for *m/z* 400–700

contribution of the sterols *m/z* 386 and 414 and their dehydration products *m/z* 368 and 396 (cf. van der Heiden et al. 1990). The identity of compounds such as e.g. sitosterol (*m/z* 414) was confirmed by cap-GC–MS of dichloromethane extracts of the dried leaf material. The identification of the various compounds is subject to further studies with GCMS and PyGCMS.

Multivariate data analysis of the PyMS data from the different grasses was performed separately for the mass ranges *m/z* 30–220 which is mainly determined by pyrolysis fragments of the biopolymers, and *m/z* 300–740 which is mainly determined by thermal desorbtion. In both mass ranges species-specific variation was found. In the mass range *m/z* 30–220, for example, PC 4, describing 7% of the variation, separates *Dactylis glomerata* from the other ten species (Fig. 3), due to a comparatively high contribution of hemicellulose (*m/z* 58, 85, 86, 114), and the phenolic fragments *m/z* 107, 108, 122, 136 (Fig. 3B, $PC4^+$). *Deschampsia flexuosa,* on the other hand, plots close to $PC4^-$ (Fig. 3A) and apparently is relatively poor in those components and enriched in *m/z* 110 (dihydroxybenzene), cellulose and possibly in tryptophan (*m/z* 117). Similarly, two other grasses, *Briza media* and *Cynosurus cristatus,* appeared enriched in syringyl lignin compared with the other species (not shown).

D. flexuosa was also separated from the other ten species by PC1 and DF1 in the mass range *m/z* 300–740 (not shown), apparently due to a comparatively low contribution of *m/z* 336 and 364 (C24 and C26 alkenes), *m/z* 396–414 (sitosterol), *m/z* 574 (diglyceride), *m/z* 620 (aliphatic wax ester) and *m/z* 642 and 644 (unknown).

Table 2. List of pyrolysis low voltage EI mass peaks found for certain chemical constituents in plant material, compiled from data from Boon (1989), Scheijen et al. (1989) and Pouwels and Boon (1990); with added unpublished data

Compound	Mass peaks
Non-reducing polysaccharides	31, 32, 43, 55, 58, 60, 72, 74, 82
Cellulose, amylose	57, 60, 73, 85, 86, 96, 98, 100, 102, 110, 112, 126, 144
Hemicellulose (pentosan)	58, 85, 86, 114
Rhamnose	128
Methylgalacturonan	140, 172
Phenolic acids (esters)	120, (164), 136 (180), 150 (194), 180 (224), 196[a]
Guaiacyl lignin (monomers)	124, 137, 138, 150, 152, 164, 166, 178, 180
Syringyl lignin (monomers)	154, 167, 168, 180, 182, 194, 196, 208, 210
Mixed G–S lignin dimers	272 (G–G), 302 (G–S), 332 (S–S), 358 (G–G), 388 (G–S), 418 (S–S)
Protein[b]	17, 34, 41, 48, 55, 67, 68, 69, 70, 81, 83, 91, 92, 94, 100, 108, 117, 131, 138, 152, 154, 166, 174, 176, 178, 186, 188, 190, 192, 202, 204, 216
Ribonucleic acids	135
Fatty acids[c]	129, 228, 236, 256, 264, 284
Sterols[c]	368–386, 382–400, 396–414
Triterpenoids[c]	424, 426, 428, 456, 470
Aliphatic was esters[c]	592, 620, 648, 660, 676, 704, 732, 760, 788
Diglycerides[c]	550, 574, 592
Chlorophyll (phytadienes)	278, 280

[a] *m/z* 196 from dihydroferulic acid (Niemann et al. 1990)
[b] Part of EI values, courtesy of Martin Scheijen, FOM, Amsterdam
[c] Only molecular ions of some major compounds are given

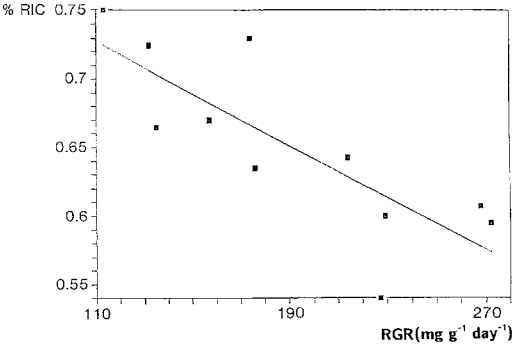


Fig. 2. The relative abundance of fragment *m/z* 150 in percent of the total ion current (% *RIC*) in pyrolysis low voltage EI mass spectra of leaf material of 11 grass species differing in relative growth rate (RGR, in mg g^{-1} day^{-1}). The *straight line* indicates a significant linear regression, $P<0.01$

Other PCs and/or DFs revealed group correlations for the eleven grasses which appeared to depend on the potential RGR. For example for the mass range *m/z* 30–220 the correlation between RGR and the separation based on PC3, describing 11% of the total variation, is shown in Fig. 4 together with the reconstructed mass

spectra of the PCs. Lignin and carbohydrate characteristics are plotted in the negative principal components spectrum ($PC3^-$, Fig. 4B) and those of protein and a fragment *m/z* 136 correlated with a cytoplasm-specific terpenoid (Scheijen 1991; Boon unpublished) in $PC3^+$, Fig. 4B).

As for *m/z* 150 (Fig. 2) the relative abundance of typical polysaccharide (*m/z* 114, 126) or lignin (*m/z* 180, 210) fragments correlated inversely with the RGR (not shown), whereas the relative abundance of the terpenoid fragment *m/z* 136, or that of protein fragments like *m/z* 34, 48 or 117, or the combined relative abundances of protein fragments *m/z* 34, 48, 67, 81, 91, 92, 100, 117, 131, 174, 176, 186, 188, 190, 202 and 216, correlated positively with RGR (not shown). The relative abundance of protein fragment *m/z* 81, derived from hydroxyproline, on the other hand, correlated inversely with RGR; Fig. 5 shows the relation between RGR and the ratio of the relative abundance of *m/z* 81 to *m/z* 34–216. The ratio of *m/z* 81 to the fragments of the other cell wall constituents (ratio *m/z* 81 to sum *m/z* 114, 126, 150, 180 and 210), however, was positively correlated with RGR (not shown).

In the mass range 300–739 multivariate analysis also revealed a group correlation dependent on the RGR, described by PC2 (9% of total variance, Fig. 6). High RGR ($PC2^-$ in Fig. 6B) correlates with a comparatively high contribution of sterols (*m/z* 382 and 400 from campesterol, *m/z* 313, 396 and 414 from sitosterol), C24 and C26 alkenes (*m/z* 336 and 364, dehydrated alcohols) and aliphatic wax esters (*m/z* 620, 648). Low RGR is possibly determined by a high contribution of *m/z* 368 (probably a C24 fatty acid) and a diffuse mass peak pattern of unidentified compounds ($PC2^+$, Fig. 6B). Some aspects of correlation with RGR are also included in other PCs: discriminant analysis further differentiates low RGR species from high RGR species in a scoreplot based on discriminant functions 1 and 2 (not shown) and describing 17.7% of total variance. Basically the pattern is the same as that shown in Fig. 6.

Discussion

In general, the grasses showed a comparatively similar chemical composition which resembles that of maize and sorghum (Table 1). Variation in chemical composition among the eleven grasses was low and appeared partly species-specific. Nevertheless, another part of the variation was significantly correlated with the potential RGR. Slow-growing species accumulated more lignin, hemicellulose, cellulose, polysaccharide-bound ferulic- and *p*-coumaric acid and hydroxyproline-rich protein (another wall constituent, Marcus et al. 1991), and appeared enriched in a group of as yet unidentified higher molecular

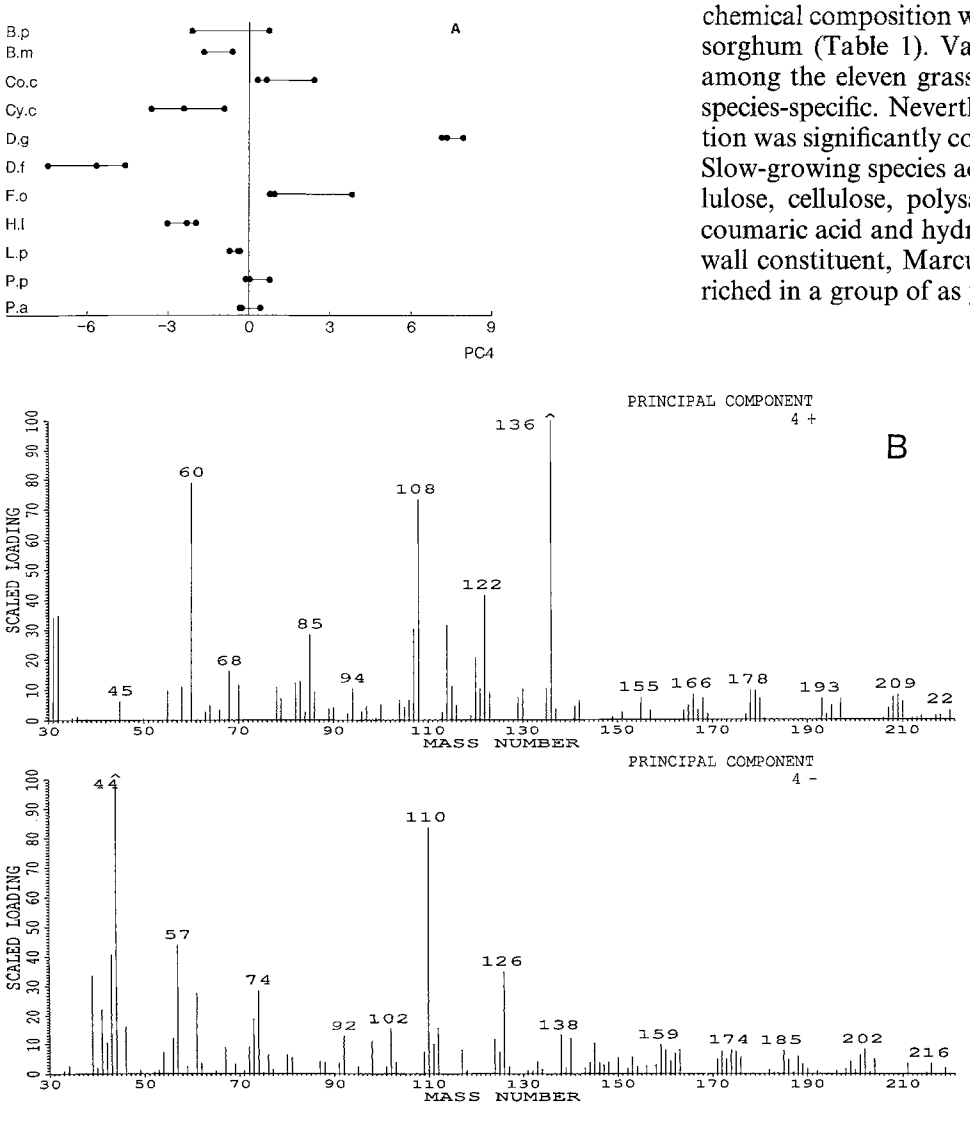


Fig. 3. A Scoreplot and **B** principal components spectra (PC4) for a series of pyrolysis low voltage EI mass spectra, mass range *m/z* 30–220, of leaf material of 11 grasses differing in relative growth rate, describing 7% of the total variation. Principal components spectra are the reconstructed mass spectra on which the scoreplot is based (see text). Each plant is represented by two or three sample analyses; replicate sample from the same plant are connected. *B.p. Brachypodium pinnatum; B.m. Briza media; Co.c. Conynephorus canescens; Cy.c. Cynosorus cristatus; D.g. Dactylis glomerata; D.f. Deschampsia flexuosa; F.o. Festuca ovina; H.l. Holcus lanatus; L.p. Lolium perenne; P.p. Phleum pratense; P.a. Poa annua*

weight compounds. Fast-growing species were comparatively enriched in protein (other than those incorporated in cell walls), sterols and aliphatic wax esters. Thus, in general, high investment in cell wall material is a clear marker of the slow-growing species, whereas the fast-growing species are apparently richer in cytoplasm compounds.

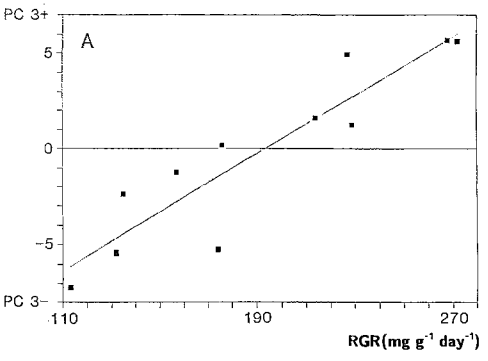
Enrichment in m/z 136, a cytoplasm-specific terpenoid (Scheijen 1991, and unpublished), in the fast-growing species further supports this idea. A comparative accumulation of aliphatic wax esters in the fast-growing species could be explained by their leaf morphology. Those species have thinner leaves with a comparatively large leaf surface (a relatively high SLA) and, therefore, a higher contribution of cuticular wax components is expected.

Relative to their cellulose and lignin content, cell walls of high-RGR species were enriched in hydroxyproline-rich protein, in spite of the fact that the leaves of these species accumulated less of this protein than low-RGR species. This suggests a change in cell wall composition as well as in relative amount. No significant correlations were found, however, for the lignin/polysaccharide or the syringyl-/guaiacyl-lignin ratio (data not shown).

The PyMS data substantiate and extend the observations with wet chemical methods recently obtained by one of us on lignin, polysaccharides and protein (Poorter and Bergkotte 1992). Contrary to the present results, in these analyses no correlation was found between RGR and total lipid concentration. The present data indicate that lipids also show a correlation with the RGR which is apparently based on qualitative rather than quantitative differences.

Preliminary results indicate that the RGR-correlated differences in composition are also found in a larger group of plants including many dicotyledons. This may indicate the existence of a more general underlying mechanism. A relative enrichment of cell wall elements in slow-growing species could explain the low SLA (Poorter and Remkes 1990) found in such species. A low SLA correlated with low RGR and associated with a comparative increase in epidermal cell number, has also been found for some subspecies of *Plantago major* (Dijkstra and Lambers 1989). The slow-growing subspecies was, among other things, characterized by a higher amount of cell wall material per unit leaf dry weight. Apparently, large differences in allocation pattern accompany the genetic variation in relative growth rate.

The benefit to the plant of such inherent differences is still a subject of speculation. Considering the different functions of the plant leaves, such as photosynthesis, support, defense against herbivory, and storage, a shift towards more cell wall material seems to point to a comparative increase in the direction of support and/or defense, possibly at the expense of photosynthetic capacity. Relative increases of cell wall material have been associated with, among other things, an increase in



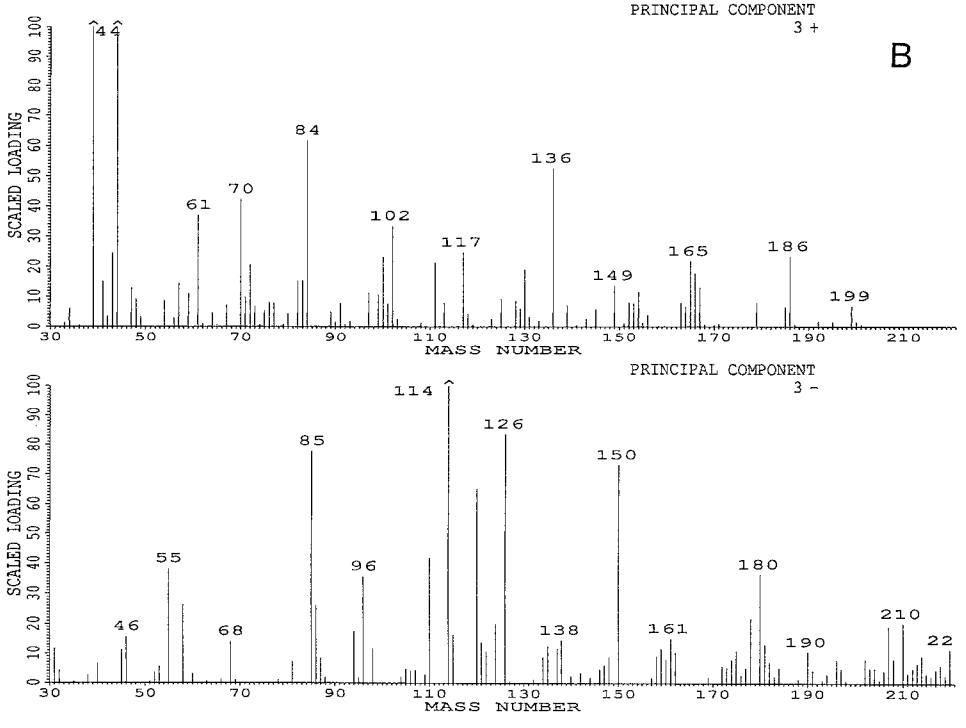


Fig. 4. A Separation by principal components (PC) analysis of the pyrolysis low voltage EI mass spectra (mass range m/z 30–220) of leaf material of 11 grass species differing in relative growth rate (RGR in mg g^{-1} day^{-1}; the *straight line* indicates a significant linear regression, $P < 0.001$), and **B** the principal components spectra of PC3 on which this separation is based, describing 11% of the total variation. Principal components spectra are reconstructed mass spectra (see text)

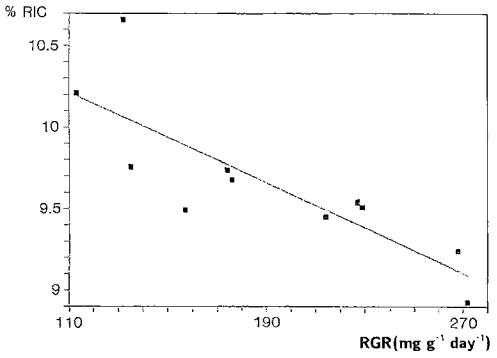
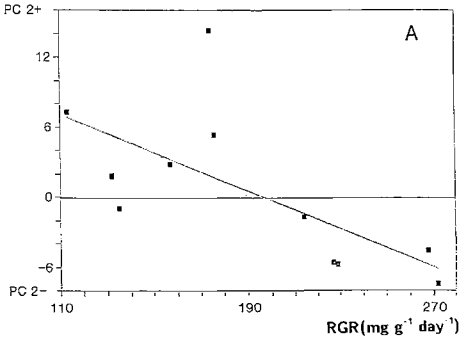


Fig. 5. The ratio of the relative abundance (RA) of hydroxyproline fragment *m/z* 81 vs the combined RAs of protein fragments *m/z* 34, 48, 67, 81, 91, 92, 100, 117, 131, 174, 176, 186, 188, 190, 202 and 216, in the total ion current in pyrolysis low voltage EI mass spectra of leaf material of 11 grass species differing in relative growth rate (RGR in mg g^{-1} day^{-1}). The *straight line* indicates a significant linear regression, $P<0.01$. *% RIC*, percentage of the total ion current

protection against trampling (Kokubu et al. 1990) and with decreased digestibility to herbivores (Kephart et al. 1990). An increase in *p*-coumaric- and ferulic acid, as found in this study for the low-RGR species, also results in decreased palatability (Bastide et al. 1988) and digestibility (Hartley and Jones 1978; Classen et al. 1990). Both phenolic acids (Glazener 1980; Ismail et al. 1987; Kuc et al. 1956; Niemann and Baayen 1988) and hydroxyproline-rich protein (Kratka 1989) have been associated with resistance against fungi.

In a recent treatise on the physiological causes and ecological consequences of inherent variation in growth rate, Lambers and Poorter (1992) conclude that a low potential growth rate *per se* does not confer ecological advantage. The target of selection for the occurrence of slow-growing species in unfavourable habitats is supposed to have been one of the components linked with RGR, rather than the RGR itself. High leaf longevity, realized by a high degree of protection against detrimental factors as a consequence of extra investment in phenolics and other cell wall components, could well be such a component.



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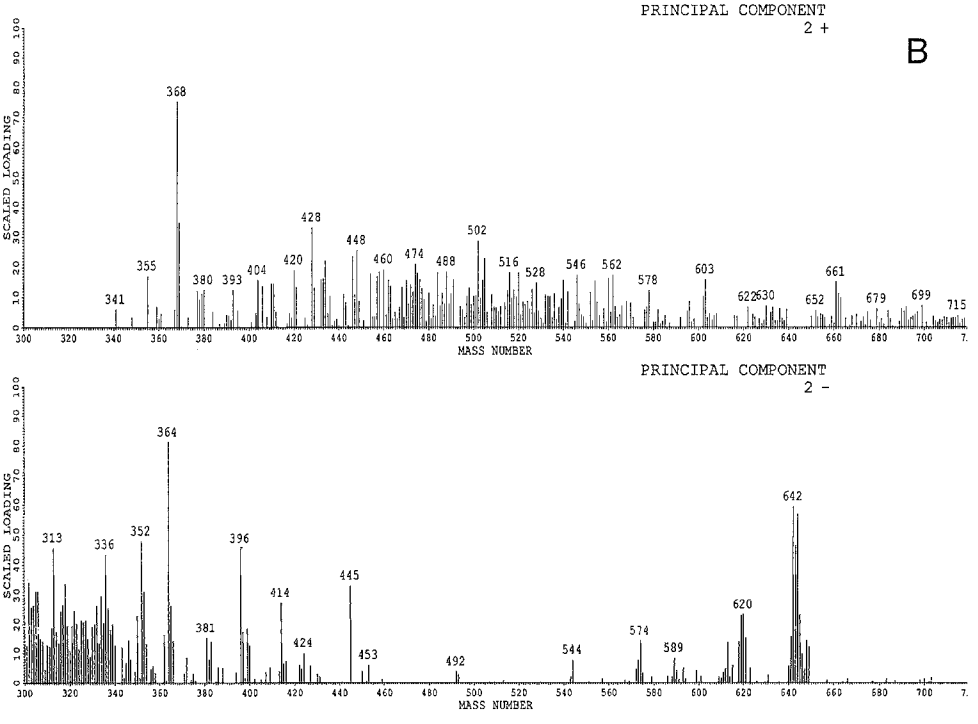

Fig. 6. A Separation of the pyrolysis low voltage EI mass spectra (mass range *m/z* 300–739) of 11 grass species differing in relative growth rate (RGR in mg g^{-1} day^{-1}; the *straight line* indicates a significant linear regression $p<0.05$) and **B** the principal components spectra of PC2 on which this separation is based, describing 9% of the total variation. Principal components spectra are reconstructed mass spectra (see text)

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