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Differential chemical allocation and plant adaptation: A Py-MS Study of 24 species differing in relative growth rate

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Abstract

The chemical composition of leaves of 24 wild species differing in potential relative growth rate (RGR) was analysed by pyrolysis-mass spectrometry. The variation in RGR significantly correlated with differences in chemical composition: slow-growing species were richer in glucan-based polysaccharides and in C16:0 fatty acid, whereas fast growing ones contained more protein (other than those incorporated in cell walls) and chlorophyll, sterols and diglycerides. Other, apparently significant correlations, e.g. for pentose-based hemicellulose and for guaiacyl lignin appeared solely based on a group separation between mono- and dicotyledonous species.

Considering the eleven monocotyledonous and thirteen dicotyledonous species separately, correlations were found in addition to the previously mentioned general ones. Within the group of the monocotyledons the low-RGR species were significantly enriched in pentose-based hemicellulose, ferulic acid and (hydroxy)proline-rich cell wall protein and nearly significant in guaiacyl and syringyl lignin, fast-growing species contained more potassium. Within the group of the dicotyledons slow-growing species were enriched in triterpenes and aliphatic wax esters.

In general, the monocotyledons contained more cell wall material such as pentose-based hemicellulose, ferulic acid, glucans (including cellulose) and guaiacyl-lignin, and also more aliphatic wax esters, than the dicotyledons. The dicotyledons, on the other hand, contained somewhat more protein than the grasses.

Per unit weight of cell wall, the amount of (hydroxy)proline- rich protein in low-RGR species was comparatively low. A higher investment of cell wall proteins to explain the low rate of photosynthesis per unit of leaf nitrogen of slow-growing species as suggested by Lambers and Poorter (1992), therefore, seems unlikely.

Abbreviations: HPRP – (hydroxy)proline-rich protein(s), LAR – leaf area ratio, LWR – leaf weight ratio, MVA – multivariate analysis, NAR – net assimilation rate, PC – principal component, PNUE – photosynthetic nitrogen use efficiency, PyGCMS – pyrolysis - gas chromatography-mass spectrometry, PyMS – pyrolysis mass spectrometry, RGR – relative growth rate, SLA – specific leaf area, SLM – specific leaf mass

Introduction

Species normally found in nutrient-poor habitats tend to have an inherently low maximum relative growth rate (RGR). That is, even at near-optimum conditions they have a much lower dry weight increase per unit of biomass and time than species from habitats with a high nutrient availability (Grime and Hunt, 1975). A low potential growth rate per se does not seem to confer ecological advantage. Therefore, it has been postulated that the target of selection for the occurrence of slow-growing species in nutrient-poor habitats has been one or more of the components linked with RGR, rather than the RGR itself (Lambers and Poorter, 1992). Various characteristics vary with potential RGR. Inherently slow-growing species have a relatively large investment of leaf biomass per unit of leaf area (high specific

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leaf mass or SLM) (Poorter and Remkes, 1990) and a relatively low rate of photosynthesis per unit of leaf nitrogen (low PNUE) and leaf weight (Poorter et al., 1990). Dicots, furthermore, also have a relatively low investment of total biomass in the leaves (low leaf weight ratio or LWR) (Poorter and Remkes, 1990). One of the causes of the variation in PNUE might be a difference in investment of protein in cell walls (Lambers and Poorter, 1992). Primary cell walls are claimed to contain up to 10 (Fry, 1988) or even up to 20% (Jones and Robinson, 1989) of structural proteins. These proteins are rich in (hydroxy)proline and appear among others to be associated with defense against microbial attack (Benhamou et al., 1991; Esquerré-Tugayé et al., 1979; Kratka, 1989). Enrichment in hydroxyproline-rich proteins (HPRP), therefore, could also add to leaf survival. Variation in SLM could, among others, be the consequence of variation in the number and/or shape of leaf cells or of variation in cell wall thickness. Evidence has been obtained (Niemann et al., 1992a; Poorter and Bergkotte, 1992) that the inherent variation in RGR is accompanied by differences in chemical composition. For a group of monocotyledons, a relative enrichment of cell wall elements was found for the slow-growing species (Niemann et al., 1992a). This enrichment is probably to a large extent based on a comparatively high proportion of sclerenchymatic cells (Van Arendonk and Poorter, 1994). For these species, therefore, the variation in SLM can at least partly be explained by variation in anatomical differentiation. Changes in relative amounts of cell wall material affect the palatability (Bastide et al., 1988) and digestibility (Kephart et al., 1990) of the leaves to foraging herbivores and therefore could lead to comparatively high leaf longevity, a possible target of selection in adaptation.

During our investigations on the interspecific variation of growth rate a number of mono- and dicotyledons had already been subjected to a wet chemical analysis (Poorter and Bergkotte, 1992). The limited number of chemical data collected and a still unexplained variation in recovery also led to a further investigation by analytical pyrolysis of, in the first place, the monocotyledons of the group (Niemann et al., 1992a). Clear correlations between RGR, SLM and chemical (and anatomical) differentiation were found for the grasses; the question whether such correlations were present in the dicotyledons as well, however, still had to be answered. Therefore, this group also was investigated further for a possible variation in chemical allocation between cell wall and cytoplasm material. In view of the large differentiation found for the grass leaves, attention was focussed on leaves.

The combined technique of pyrolysis mass spectrometry (PyMS) is, among others, especially suited to the analysis of lignocellulosic material (Boon, 1989, 1992; Pouwels et al., 1987, 1990). It further provides information on a large array of other constituents as, for instance, fatty acids, diglycerides and sterols; it also enables to distinguish between (hydroxy)proline-rich- and other proteins (Niemann et al., 1992b). Py(GC)MS has meanwhile proven its value in the analysis of bacteria as well as plant and animal cells (for relevant literature see Boon, 1992). Pyrolysis data have often been compared to those obtained with other analysis techniques (Moers et al., 1990; Saiz-Jimenez et al., 1987; Scheijen, 1991; Schulten et al., 1982), including a worldwide comparison of biomass data (Round-Robin report, 1991, cp. Van der Hage et al., 1993), and have not only been found in good agreement, but, furthermore, for tobacco leaves PyMS data have been demonstrated to give a more accurate description of the chemical variability than a dataset of non-mass spectrometric origin (Scheijen, 1991).

With the use of PyMS analysis it was tried to answer (part of) the following questions: 1) is relative enrichment in cell wall elements a general aspect of slow-growing species or is it restricted to certain taxa such as the Gramineae; 2) is there other general chemical differentiation among fast- and slow-growing species which could explain the differences in SLA, and 3) can interspecific variation in PNUE indeed be explained by a change in relative amounts of wall protein as suggested (Lambers and Poorter, 1992)? In addition, attention was paid to differences in chemical composition between mono- and dicotyledons.

Material and methods

Growth of the plants

Plants of 24 wild species, all non-woody plants with a C_3 type of photosynthesis but obtained from a large range of habitats in western Europe and varying in potential relative growth rate, were used. Plants of 13 dicotyledons: *Anthriscus sylvestris* (L.) Hoffm., *Galinsoga parvflora* Cav., *Geum urbanum* L., *Hypericum perforatum* L., *Lysimachia vulgaris* L., *Origanum vulgare* L., *Pimpinella saxifraga* L., *Plantago major* ssp *major* L., *Rumex crispus* L., *Scrophularia nodosa* L., *Taraxacum officinale* Weber, *Trifolium repens* L., and

Urtica dioica L.; and 11 monocotyledons: *Brachypodium pinnatum* (L.) Beauv., *Briza media* L., *Corynephorus canescens* (L.) Beauv., *Cynosurus cristatus* L., *Dactylis 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 with the following conditions: Day: 14 h, photosynthetic photon flux density approximately 315 μmol m^{-2} s^{-1}, temperature 20°C, relative humidity approximately 70%. Night: 10 h, temperature 20°C. Light was provided by fluorescent lamps (Philips TI-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 m*M* (Poorter and Remkes, 1990). Part of the plants was used for extensive growth analyses as described by Poorter et al. (1990). Full details are given in Poorter et al. (1990) and in Poorter and Remkes (1990); these authors also measured the following relative growth rates (in mg g^{-1} day^{-1}): *Brachypodium pinnatum*, 174; *Briza media*, 157; *Corynephorus canescens*, 113, *Cynosurus cristatus*, 176; *Dactylis glomerata*, 229; *Deschampsia fluxosa*, 135; *Festuca ovina*, 132; *Holcus lanatus*, 268; *Lolium perenne*, 214; *Phleum pratense*, 227; *Poa annua*, 272; *Anthriscus sylvestris*, 239; *Galinsoga parviflora*, 365; *Geum urbanum*, 224; *Hypericum perforatum*, 205; *Lysimachia vulgaris*, 223; *Origanum vulgare*, 203; *Pimpinella saxifraga*, 171; *Plantago major*, 240; *Rumex crispus*, 327; *Scrophularia nodosa*, 302; *Taraxacum officinale*, 260; *Trifolium repens*, 206; and *Urtica dioica*, 317. The selection thus includes species from all three groups distinguished by Grime and Hunt (1975): high-RGR (here, RGR > about 260, indicative for nutrient-rich and/or disturbed habitats); ubiquitous moderate-RGR species (200 - 260) and low-RGR (< about 210, indicative for nutrient-poor habitats). Leaf blades were harvested during a period of 17 days after the plants had reached a fresh weight of approximately 100 mg. At this growth stage, RGRs for the leaves were virtually equal to those of the roots and those of whole plants. The plant material was oven-dried for 24 h at 80 °C, ground and homogenised in a freeze mill. Freeze-dried leaves were utilized for the determination of the concentration of insoluble sugars, assumed to be mainly starch and fructans (Poorter and Bergkotte, 1992).

For two species, *Poa annua* and *Anthriscus sylvestris*, freeze-dried leaf material was soxhlet-extracted for 48 h with 80% ethanol to compare ethanol-insoluble residues with the original rough leaf material.

Pyrolysis-mass spectrometry

Analysis by pyrolysis-mass spectrometry has some major advantages over other analysis techniques. Preseparation or purification of the samples is not required, the technique is very fast, can be used for very small amounts of material, and it gives in one analysis information on a very large array of both low- and high molecular components. Especially where the nature of possible chemical changes which might be related to RGR variation was unknown, the choice for such a universal technique was an obvious one. A disadvantage of the use of PyMS is the fact that the interpretation of the data requires more practice than with several other techniques.

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. 2.5 μL samples 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. To avoid day-to-day variation the 13 dicotyledons were analysed, in a triplicate series, on the same day. For the same reason a separate sample set of all 24 species was analysed on one day as well. The difference between replicate analyses for the summed abundancies of polymers such as protein or lignin, was less than 3%. The scan cycle time was 1 s and the source temp 180 °C, with a heating rate of 16 °C s^{-1} up to 800 °C. To avoid further fragmentation during ionization low voltage EI at about 16 eV was applied. An extensive description is given by Boon (1989, 1992).

Interpretation of PyMS spectra

Pyrolysis - mass spectra of plant materials contain the summed masses of ionized fragments and molecules originating from desorption of lower-molecular-weight components and from pyrolysis of the different bio-polymers. Single masses may represent several structurally different ions which also may differ in origin. Phenol (*m/z* 94), for instance, may be derived either from lignin or from protein; *m/z* 180 represents at least six different fragments, all identified by GC-MS, from several (fractions of) plant species (Boon, 1989; Hempfling and Schulten, 1990; Niemann et al., 1990; Pouwels et al., 1987; Van der

Table 1. List of pyrolysis low voltage EI mass peaks found for certain chemical constituents in plant material, compiled from data by Boon (1989), Scheijen et al. (1989) and Pouwels and Boon (1990); with added unpublished data

Compound	Mass peaks
Cellulose, amylose	57, 60, 73, 85, 86, 96, 98, 100, 102, 110, 112, 126, 144
Hemicellulose (pentosan)	58, 85, 86, 114
Pectin (rhamnose)	128
Pectin (methylgalacturonan)	140, 172
Residual pectin	31, 32, 85
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, 216 252, 270
Ribonucleic acids	111, 126, 135, 151
Fatty acids[c]	129, 228, 236, 256, 264, 284
Sterols[c]	368–386, 380–398, 382–400, 394–412, 396–414
Triterpenoids[c]	424, 426, 428, 456, 470
Diglycerides[c]	550, 574, 592
Chlorophyll (phytadienes)	278, 280
Aliphatic wax esters[c]	592, 620, 648, 660, 676, 704, 732, 760, 788
Alkanes[c]	436, 464
Tannin	94, 110, 126
Phtalate	149, 279

[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.

Hage et al., 1993; Van Smeerdijk and Boon, 1987), of which the major ones, however, are derived from guaiacyl lignin, which still renders *m/z* 180 a marker for this polymer. Biomacromolecular systems such as bacterial cells and plant cells have been the subject of many Py(GC)MS studies in which the fragments pyrolytically released have been identified and classified. These studies showed that, as summarized in Table 1 and in spite of overlap, many fragments are, as such or in combination, specific for certain polymers or molecules. References to this article, especially the reviews by Boon (1989, 1992) give access to the large pile of relevant literature.

For the plant polysaccharides, the occurrence of the characteristic masses like *m/z* 114 or 126 has to be interpreted with care as in PyMS only mono- and/or oligomeric units are visible. *M/z* 114 is an indicator of xylose and/or arabinose and therefore indicates the xylan and xyloglucan fraction of hemicellulose together with the (comparatively low) arabinan and arabinogalactan fraction of pectin, but not of the $\beta(1-3)$, $(1-4)$-glucans which may be abundant in grasses (Fry, 1988). *M/z* 126 indicates hexoses and does not distinguish between different types of glucans, thus, this mass represents both cellulose, amylose and the glucan fraction of hemicellulose. The amylose

content, therefore, was determined separately using anthrone reagent (Poorter and Bergkotte, 1992). Pectin is characterized via rhamnose and methylgalacturonan. Rhamnose, which is also present in homogalacturonan (Fry, 1988), has been found to give a series 4 dianhydrodeoxysugars with *m/z* 128 as the molecular ion peak (Boon et al., 1988)

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 could be used for a temperature-resolved separation of those compounds from the biopolymer fragments.

Multivariate data analysis of PyMS data and other statistics

Principal component (PC) analyses were performed on the PyMS data files, using a modified Arthur package, adapted to PyMS data (Boon et al., 1984). In this method, relative abundances of the 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. Appropriate PCs were found by simple and stepwise regression with the RGR. For the multivariate data analysis the recorded mass spectra were averaged over the pyrolysis time (35 seconds for scans 15 – 50).

Statistic validity of the calculated coefficients was determined using Student's t-test.

Results

Typical PyMS spectra of leaves of a fast-growing dicotyledon, *Gallinsoga parviflora*, and of those of a slow-growing monocotyledon, *Deschampsia flexuosa*, are given in Figure 1. These spectra, and those of the other 22 species, show characteristic fragments from polysaccharides such as glucans, pentose-based hemicellulose (inclusive some arabinan and arabinogalactan from pectic side-chains) and pectin, from phenolic acids, proteins, fatty acids, guaiacyl (G) and syringyl (S) lignin, steroids, diglycerides and aliphatic wax esters as summarized in Table 1. At first sight the spectra of the eleven monocotyledons investigated were more or less similar (Niemann et al., 1992a), whereas the dicotyledon leaf spectra differed somewhat more, especially in the relative contribution of the mass peaks *m/z* 110 (dihydroxybenzene) and *m/z* 126 (derived from glucans such as cellulose and amylose). For all species the contribution of polysaccharide, protein and fatty acid fragments in the total ion current was relatively high, that of lignin fragments on the contrary very low when compared to spectra of other (often older) plant material (Boon, 1989; Niemann et al., 1990).

Evaluation of the results of the two data sets (24 species together and 13 dicotyledons, respectively) was based on comparison of the relative intensities in the total ion current of certain specific fragments and on multivariate analysis of the data of the two sets for three separate mass ranges, *m/z* 30–220 which is mainly determined by pyrolysis fragments of the biopolymers, *m/z* 300–720 which is mainly determined by thermal desorption, and *m/z* 100–650 to cover the overlap.

The PyMS spectra of the ethanol-insoluble part of the leaves of *P. annua* and *A. sylvestris* in the lower mass ranges (*m/z* 30–220) were essentially the same as those of total leaf material. Most of the more prominent fragments or molecular ions in the higher mass ranges, however, like for instance *m/z* 278 and 280 (from chlorophyll-derived phytadienes), *m/z* 336 and 364 (dehydrated alcohols) and *m/z* 313, 396 and 414 (from sterols), were absent in the ethanol-insoluble fraction. These masses were found in the spectra of the ethanol extracts, which further contained among others several protein fragments and *m/z* 136 (cytoplasm terpenoid). The extracted sugars were represented by fragments *m/z* 57, 60 and 73 (from levoglucosan, a pyrolysis product of polyhexosan) and not, or only to a low extent, by *m/z* 126 or 114.

Comparison of relative intensities

In comparison with the dicotyledon spectra, those of the monocotyledons showed a relatively high abundance of fragment *m/z* 150 derived from ferulic acid (not shown) and *m/z* 114 from pentose-based hemi-

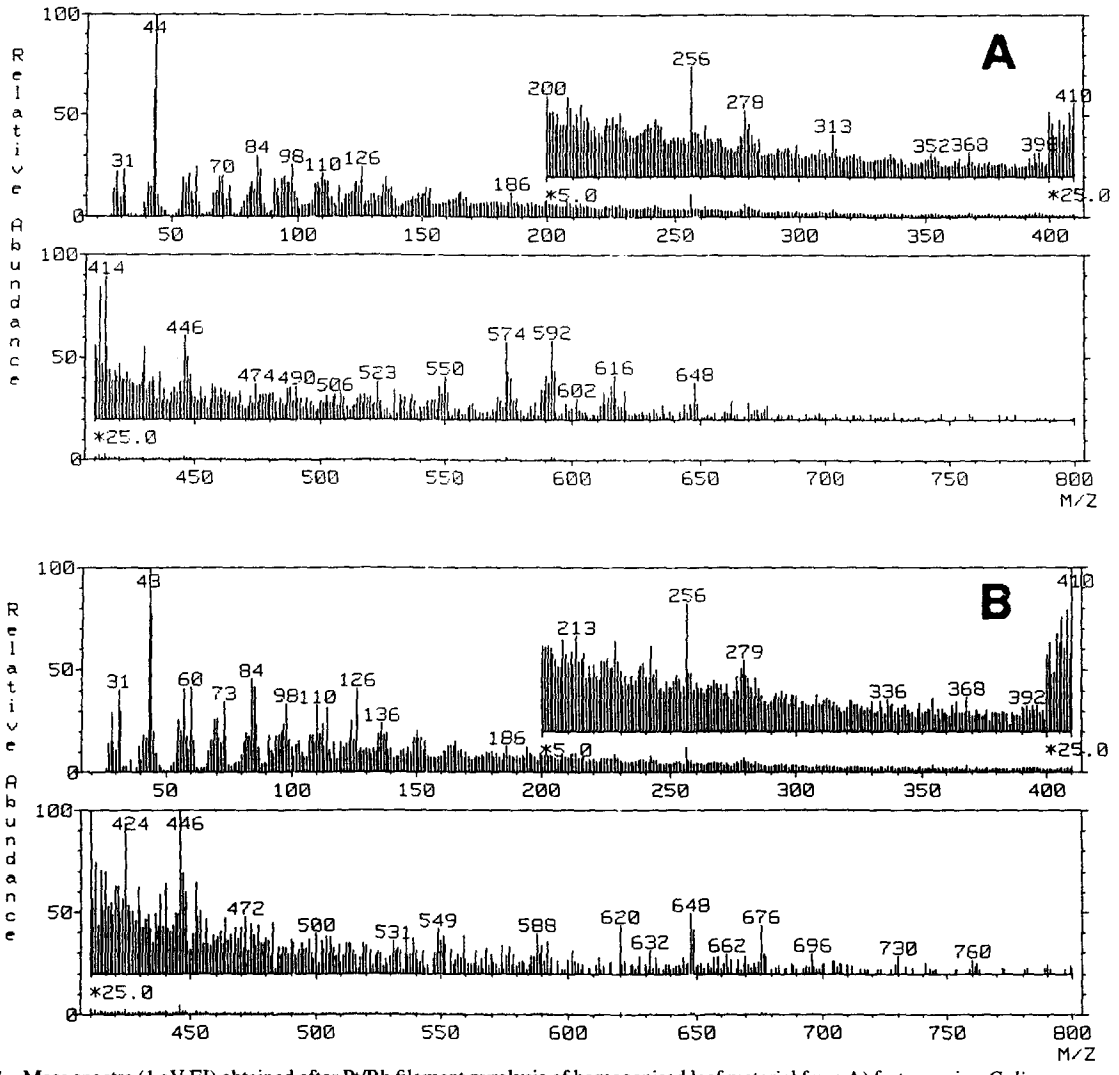


Fig. 1. Mass spectra (1 eV EI) obtained after Pt/Rh filament pyrolysis of homogenised leaf material from **A**) fast-growing *Galinsoga parviflora* and **B**) slow-growing *Deschampsia flexuosa*. *Inserts*: enlarged scales, y axis × 5 for *m/z* 200–400, × 25 for *m/z* 400–800.

cellulose (inclusive some pectic arabinan and arabinogalactan; Fig. 2), and were somewhat richer in glucan (Fig. 3A) and lignin fragments and in the molecular ions *m/z* 592, 620, 648, 660, 676, 704, 732 and 760 of the aliphatic wax esters (not shown). The dicotyledons on the other hand were somewhat enriched in protein (Fig. 3B) and in diglycerides (*m/z* 550, 574 and 592; not shown).

Considering all investigated species together, the relative abundances of typical polysaccharide (*m/z* 114, $p < 0.001$, not shown; *m/z* 126, Fig. 3A) or guaiacyl lignin (*m/z* 124 – 180, $p < 0.05$, not shown) fragments, but also that of the C16:0 fatty acid (*m/z* 256, $p < 0.01$; not shown), correlated inversely with the RGR, whereas those of protein fragments like *m/z* 34, 48 or 117, or the combined relative abundances of *m/z* 34, 48, 67, 81, 91, 92, 100, 117, 131, 174, 176, 186, 188, 190, 202 and 216 (Fig. 3B), and those of chlorophyll (*m/z* 278 and 280, $p < 0.01$, not shown) correlated positively with RGR. Potassium (*m/z* 39, $p < 0.1$, not shown) showed a positive tendency. Within the group of dicotyledons alone, however, these correlations were only (nearly) significant for the protein fragments ($p < 0.1$), for chlorophyll ($p < 0.1$) and for *m/z* 256 ($p < 0.01$), but not for the polysaccharide or lignin fragments or the potassium ion, although the curves showed similar tendencies. The abundance of the hydroxyproline-derived cell-wall fragment *m/z* 81

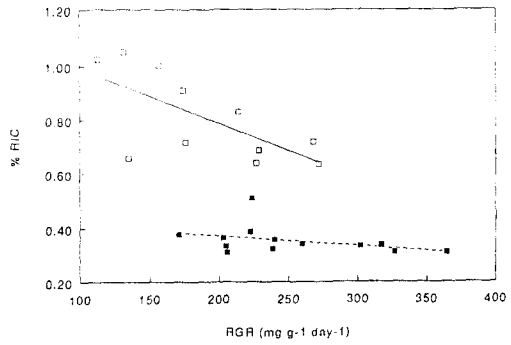


Fig. 2. Relative abundances of fragment *m/z* 114 from pentose-based hemicellulose in percent of the total ion current (% RIC) in pyrolysis low voltage EI spectra of 11 grasses (□) and 13 dicotyledons (■). The straight lines indicate a linear regression, which is significant for the 11 grasses ($p < 0.05$), but not for the dicotyledons.

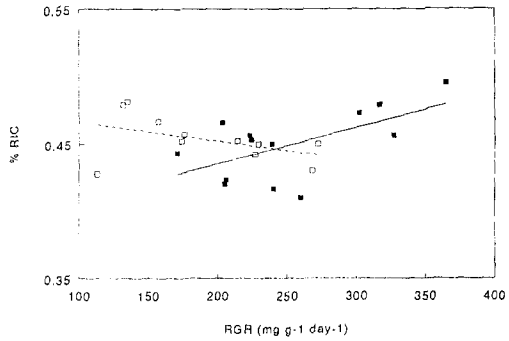
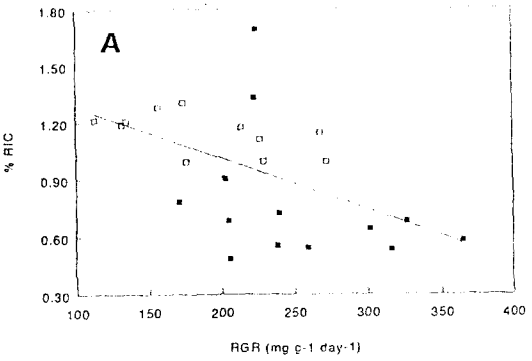


Fig. 4. The relative abundance of fragment *m/z* = 81 (from hydroxyproline-rich protein) in percent of the total ion current (% RIC) in pyrolysis low voltage EI mass spectra of homogenised leaf material of 13 dicotyledons (■) and 11 grasses (□). The straight line indicates a linear regression, which is significant for the dicotyledons ($p < 0.05$), but not for the grasses.



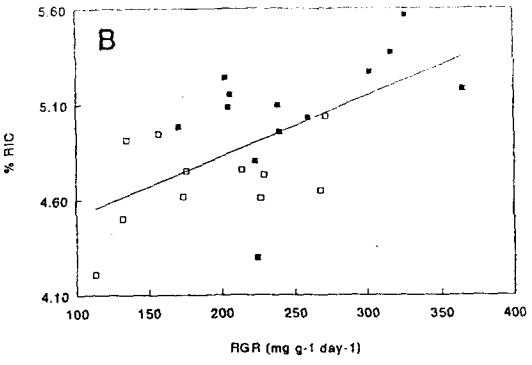


Fig. 3. The relative abundance of **A**) fragment *m/z* 126 from glucans and **B**) the combined fragments *m/z* 34 – 216 from protein in percent of the total ion current (% RIC) in pyrolysis low voltage EI mass spectra of leaf material of 24 species differing in relative growth rate (RGR); □ = monocotyleclons, ■ = dicotyleclons. The straight lines indicate a significant linear regressions, $p < 0.01$.

correlated positively with RGR as well (Fig. 4). Within the group of monocotyledons, the abundancies of the polysaccharides (*m/z* 114 – $p < 0.05$, *m/z* 126 – $p < 0.1$), of guaiacyl – ($p < 0.1$) and syringyl – ($p < 0.1$) lignin and of ferulic acid ($p < 0.01$) correlated inversely, that of potassium *m/z* 39 ($p < 0.05$) correlated positively with the RGR. Protein values were only significantly (inversely) correlated for the relative abundance of hydroxyproline fragment *m/z* 81 (vs. the combined fragments *m/z* 34 – 216; $p < 0.01$), the other protein fragment abundancies showed a positive tendency.

Multivariate analysis of the PyMS data

For both data sets and in all mass ranges group correlations were found which appeared to depend on the potential RGR. For the mass range *m/z* 30–220 for 24 species, the correlation between RGR and the separation based on principal component 1 (PC 1), describing 35% of the total variation, is shown in Figure 5A together with the reconstructed mass spectra of the PCs (Fig. 5B). It is mainly a group separation; carbohydrate, phenolic acid and some G-lignin characteristics are plotted in the negative principal component spectrum, determined by monocotyledons (PCl^-, Fig. 5B), and those of CO_2, protein and a fragment *m/z* 136 (cytoplasm-specific terpenoid (Scheijen, 1991)) in PCl^+, which is mainly determined by dicotyledons (Fig. 5B). Considering the dicotyledons separately, however, a more or less similar correlation was described by PC2 (selected by stepwise regression with the RGR and showing 13% of the total variation;

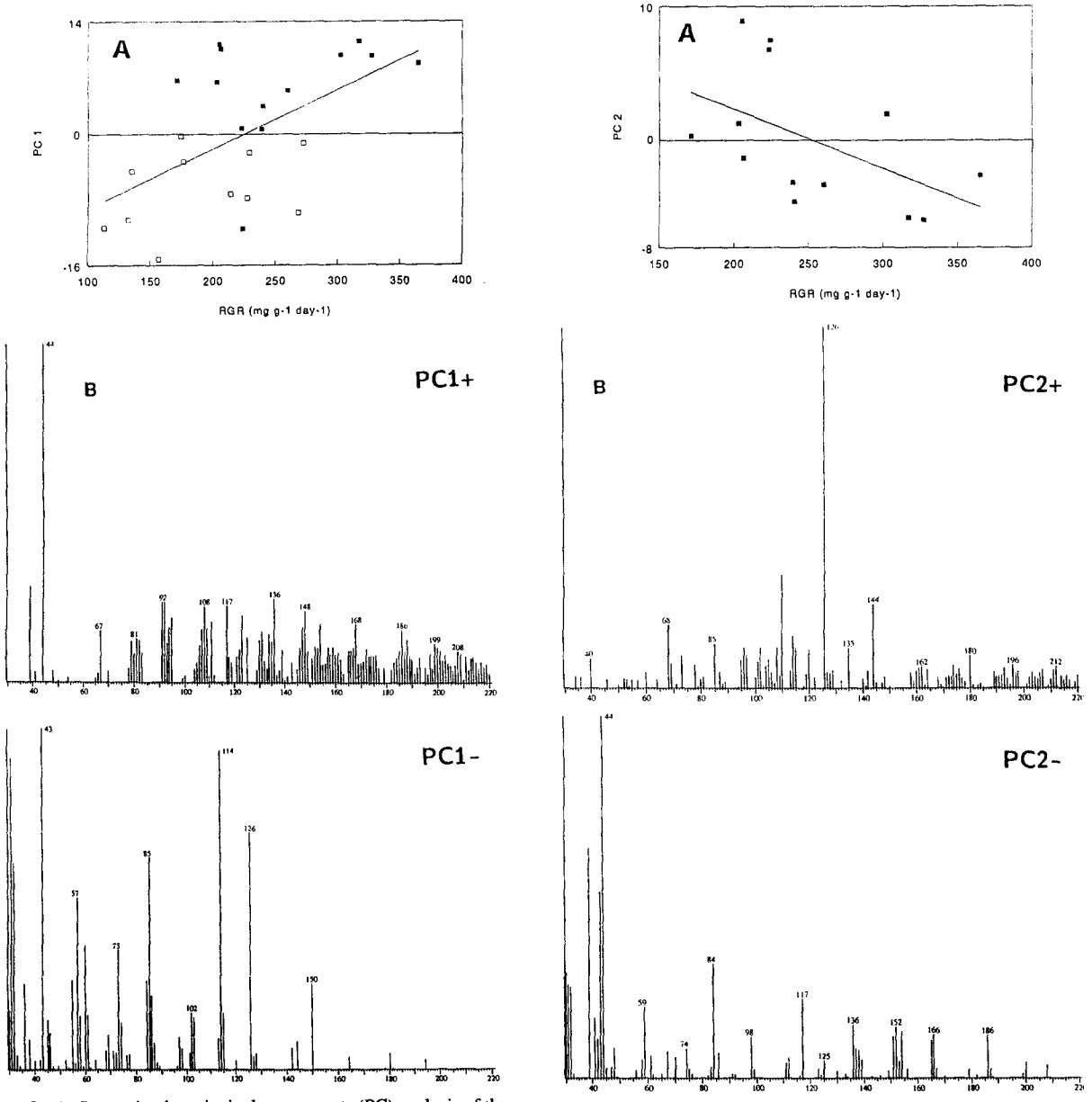


Fig. 5. **A.** Separation by principal components (PC) analysis of the pyrolysis low voltage EI mass spectra (mass range *m/z* 30–220) of leaf material of 11 grasses (□) and 13 dicotyledons (■) differing in relative growth rate (RGR); the straight line indicates a significant regression, $p < 0.01$, and **B.** the principal components spectra of PC1 on which this separation is based, describing 35% of total variation. Principal components are reconstructed mass spectra (see text).

Fig. 6. **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 13 dicotyledons differing in relative growth rate (RGR); the straight line indicates a linear regression, $p < 0.1$, and **B.** the principal components spectra of PC2 (describing 13% of total variation). Principal components are reconstructed mass spectra (see text).

Fig. 6A, B). In addition to the intense glucan (*m/z* 126–144) and some other, low intensity polysaccharide (*m/z* 114 – mainly pentose-based hemicellulose *m/z* 85-113-140-172 – methylated pectin) and G- and S-lignin characteristics, $PC2^+$ also shows the hydroxyproline fragment *m/z* 81, but lacks fragment *m/z* 150 from ferulic acid. $PC2^-$ plots *m/z* 39 of potassium, *m/z* 44 of CO_2, *m/z* 136, and a number of protein fragments other than *m/z* 81.

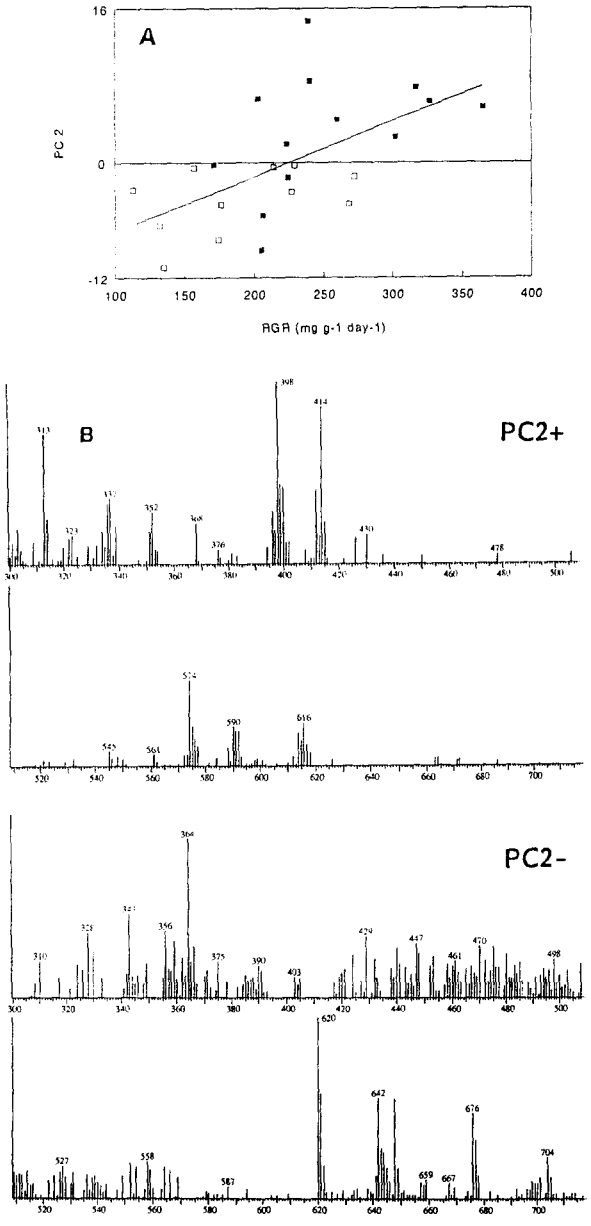


Fig. 7. **A.** Separation by principal components (PC) analysis of the pyrolysis low voltage EI mass spectra (mass range *m/z* 300–720) af leaf material of 11 grasses (□) and 13 dicotyledons (■) differing in relative growth rate (RGR); the straight line indicates a significant regression, $p < 0.01$, and **B.** the principal components spectra of PC2 on which this separation is based describing 13% of total variation. Principal components are reconstructed mass spectra (see text).

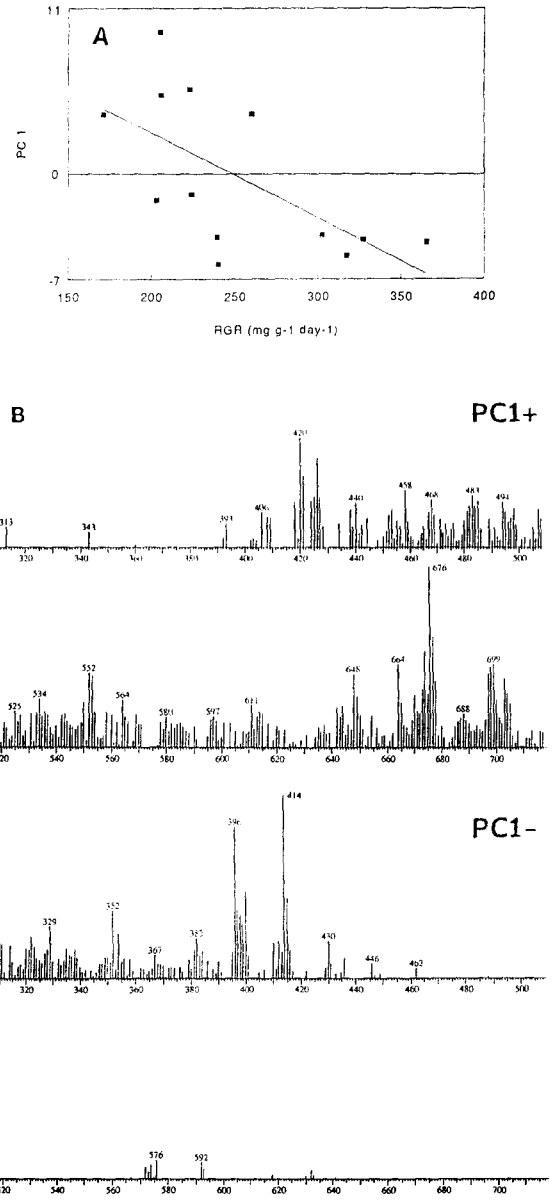


Fig. 8. **A.** Separation by principal components (PC) analysis of the pyrolysis low voltage EI mass spectra (mass range *m/z* 300–720) of leaf material of 13 dicotyedons differing in relative growth rate (RGR); the straight line indicates a significant linear regression, $p < 0.05$, and **B.** the principal components spectra of PC 1 (describing 9% of total variation). Principal components are reconstructed mass spectra (see text).

For the mass range *m/z* 300/720 the correlation between RGR and the separation based on PC2 (24 species, describing 13% of the total variation) or PC1 (13 dicotyledons, 9% of total variation) is shown in Figure 7 and 8 together with the reconstructed mass spectra of the PCs. Although again a group separation is evident (Fig. 7), still in both cases high RGR correlated with a comparatively high contribution of sterols and a somewhat lower one of diglycerides (cf. Table 1) and of tocopherol (*m/z* 430). Low RGR is mainly deter-

mined by aliphatic wax esters ($PC2^-$ Fig. 7B, PCl^+ Fig. 8B) and, for dicotyledons alone, by some triterpenes (*m/z* 424, 426, 428, 456; PCl^+ Fig. 8B). Multivariate data analysis in the mass range *m/z* 100/650 in addition to the sterols also showed a clear contribution of the chlorophyll-derived phytadienes (*m/z* 278, 280) for the high-RGR dicotyledons (not shown).

Insoluble sugars

The amounts of insoluble sugar of the leaves were nearly significant negatively correlated with the RGR for the monocotyledons ($p < 0.1$), but showed a reverse, not significant, tendency for the dicotyledons or the whole group of 24 species (not shown).

Comparison of PyMS and wet-chemical data

Only part of the wet chemical data obtained by Poorter and Bergkotte (1992) for the leaves of the 24 species (published in detail for 2 of the 24 only) can be compared with the PyMS data as a fraction like e.g. 'soluble phenolics' has no direct counterpart in PyMS fragments. For the polysaccharides the % RIC of the glucan fragment *m/z* 126 is positively correlated both to the residual (hemi)cellulose fraction ($p < 0.01$, not shown) and to the combined fractions insoluble sugars and (hemi)cellulose (Fig. 9A) or those inclusive soluble sugars ($p < 0.001$, not shown); there is no significant correlation to the separate fraction of soluble sugars, however. *M/z* 114, the indicator of pentose-based hemicellulose (inclusive arabinan and arabinogalactan sidechains from pectin) only correlates significantly to those fractions for the grasses and not for the dicotyledons (Fig. 9B). The % RIC of the summed protein fragments *m/z* 34 – 216 is, significantly, positively correlated to the nitrogen content (measured by CHN-analysis) for the dicots ($p < 0.05$), and nearly significant for the grasses ($0.05 < p < 0.1$) or the total group ($0.05 < p < 0.1$). For the Kjeldahl-N-content similar, but not, or only nearly significant, tendencies are apparent. For a very good protein marker fragment like *m/z* 117 (Ralph and Hatfield, 1991; Tsuge and Matsubara, 1985) of indole (from tryptophan) and benzylcyanide (from phenylalanine), *p*-values < 0.001 (N) and < 0.01 (Kjeldahl-N) were found for the 24 species. *M/z* 39 of potassium is positively correlated to the mineral content ($p < 0.01$ for the 24 species as well as for the two subgroups) and *m/z* 44 of CO_2 to the organic acid fraction ($p < 0.001$ for 24 species). For the colorimetrically determined lignin values the correlation with the summed fragments of guaiacyl- and/or syringyl lignin only was nearly significant ($p < 0.1$) for the 24 species. Specific fragments like *m/z* 180 (guaiacyl lignin, $p < 0.01$) or *m/z* 154 and 167 (syringyl lignin, $p < 0.01$), however, correlated significantly. Table 2 summarizes the correlations between the wet chemical data and various pyrolysis fragment markers.

Discussion

PyMS analysis of the non-extracted leaf material showed both cell wall (lower mass range) and cytoplasm (higher masses) markers. Ethanol extraction, intended for a crude separation of the cell wall, did not improve the analysis as it did not remove the major part of) the intracellular proteins, starch and RNA; also the major part of *m/z* 256 (n-hexadecanoic acid, possibly derived from membrane glycerolipids) was retained in the ethanol insoluble fraction. Direct analysis of the dried and ground leaves, therefore, was preferred.

For all mass ranges principal component analysis revealed group separations which correlated with the potential RGR. For the 24 species together this is partly based on a separation of monocotyledons from dicotyledons (Figs. 5A, 7A). Obviously, trends in these groups are different from the overal trend. This aspect was not considered by Poorter and Bergkotte (1992) for the wet chemical analysis data of the same group. Part of the correlations found in their work actually were determined by grass data only. Basis of the RGR-correlated variation for the dicotyledons is a change in comparative amounts of glucan polysaccharide (Fig. 6) on one hand and protein (Fig. 6), chlorophyll (not shown) and sterols and diglycerides (Fig. 8) on the other. A large starch content might obscure an interpretation of the hexose masses as cell wall indicators. For the dicots, the insoluble sugar fraction, however, showed a positive, though not significant, correlation to the RGR indicating that the inverse relation found for *m/z* 126 (Figs. 3A, 6A) is to a large extent based on cellulose, as also indicated by Fig 9A. RGR-correlations for the separate mass fragments, based on % RIC, for the dicotyledons are not (for *m/z* 126) or only slightly ($0.05 < p < 0.1$ for protein fragments) significant (for comparison, in the wet chemical analysis of the leaves of the dicotyledons alone: not significant for the (hemi)cellulose fraction and the organic nitrogen fraction). Wet-chemical and PyMS data of comparable fractions are not quite comparable because, among others, different components of polymers like protein or

Table 2. Comparison of wet chemical data of 24 wild plants (Poorter and Bergkotte, 1992) with pyrolysis mass spectral marker fragments

Compound	(Sum) *M/z*	(marker for)	r^2
Lipids	129	(fatty acids)	0.15[a]
	278–280	(chlorophyll)	0.12[a]
	256	(C16:0 fatty acid)	0.05
	396	(dehydrositosterol)	0.01
	136	(cytoplasmic terpenoid)	0.01
	44	(CO_2)	0.21[b]
Lignin	(124–180[e])	(G-Lignin)	0.15[a]
	150	(")	0.30[c]
	178	(")	0.22[b]
	180	(")	0.24[c]
	(154–210[f])	(S-lignin)	0.16[a]
	154	(")	0.25[c]
	167	(")	0.28[c]
	208	(")	0.19[b]
	210	(")	0.08
	44	(CO_2)	0.14[a]
Org N-comp.	(34–216[g])	(protein)	0.06
	48	(methionine)	0.28[c]
	81	(proline)	0.06
	100	(dialkylamine)	0.57[d]
	117	(tryptophan/phenylalanine)	0.32
	186	(tyrosine)	0.54[d]
	135	(nucleic acid)	0.30[c]
	44	(CO_2)	0.02
(Hemi)cellulose	85	(pectin)	0.33[c]
	114	(pentosans)	0.38[d]
	126	(glucans)	0.24[c]
	128	(pectin)	0.00
	44	(CO_2)	0.23[c]
Insol. sugars	126	(glucans)	0.12
	44	(CO_2)	0.10
Sol. sugars	114	(pentosans)	0.00
	126	(glucans)	0.05
Org. acids	44	(CO_2)	0.48[d]
Minerals	39	(potassium)	0.34[c]
	39[h]	(" [h])	0.45[d]
NO_3	39	(potassium)	0.03
	39[g]	(" [h])	0.37[d]
Nitrogen[i]	(34–216[g])	(protein)	0.14[a]
	117	(tryptophan)	0.36[d]
	135	(nucleic acid)	0.23[c]
Sol. phenolics	120	(*p*-coumaric acid)	0.08
	150	(ferulic acid)	0.00

[a] $0.05 < p < 0.1$.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$.
[e] Sum *m/z* 124, 137, 138, 150, 152, 164, 166, 178 and 180.
[f] Sum *m/z* 154, 167, 168, 182, 194, 196, 208 and 210.
[g] Sum *m/z* 34, 48, 67, 81, 91, 92, 100, 117, 131, 174, 176, 186, 188, 190, 202 and 216.
[h] Series of 24 species minus the values of *Rumex crispus* (with an exceptional high potassium value).
[i] Total nitrogen does not differ much from Kjeldahl-N in its correlation with protein pyrolysis fragments.

lignin have a different response factor in the total relative intensity (Van der Hage et al., 1993) and, therefore, % RIC to a certain extent also reflects the compositon in addition to the amount. However considering the large variability (20 – 30%) found for e.g. various polysaccharides in the same 'classic' analyses in a comparison of biomass analyses of the same samples by 19 laboratories (Milne et al., 1992), the correspondance between the PyMS and wet-chemical data seems quite reasonable. The discrepancy between the wet-chemical and PyMS data for lignin is probably mainly based on the inaccuracy of the colorimetric lignin determination (J J C M Van Arendonk, pers. commun. also illustrated by the variation in the data of Poorter and Bergkotte, 1992) combined with a comparatively low variation in lignin content in the leaves of the different species.

The major advantage of the PyMS analysis over a wet chemical one, is the determination of a very wide range of components and a more detailed analysis of the biopolymers. Thus, among others a more detailed interpretation was obtained for the polysaccharides (pentose-/hexose-based/pectin vs. residual (hemi)cellulose), for proteins (hydroxyproline rich/others vs. N-analysis) and for lignin (separation into guaiacyl and syringyl, determination of ferulic acid). For the lipids, for example, PyMS analysis showed significant RGR-correlations for lipophylic compounds such as chlorophyll and C 16:0 fatty acid, and, by multivariate analysis also correlated the RGR with sterols and diglycerides, and (for the dicotyledons) with triterpenes and aliphatic wax esters; for the wet chemical total lipid fraction, however, no correlation with RGR had been found at all (Poorter and Bergkotte, 1992).

For comparable fractions wet-chemical data and comparison based on the % RIC of certain fragments agree quite well as far as the (lack of) correlation between RGR and composition is concerned. At the growth stage of the plants investigated leaf RGRs are virtually equal to those of whole plants; correlations between leaf composition and leaf RGR obviously are quite similar to such correlations for the whole plant.

The taxonomic distance among the dicots (from different families) may partly mask correlations apparent within a family like the grasses. As seen from principal component analysis, also for the dicotyledons a more general, RGR-dependent chemical variation seems to exist which supersedes the large interspecific diversification. Though much less clear than for the grasses (Niemann et al., 1992a), this correlation appears also based on a division between cell-wall-derived and cytoplasm-derived fragments or molecules.

In two inbred lines of *Plantago major*, the fast-growing line was also found to contain less cell-wall components, although no differentiation was found for organic N-compounds (Dijkstra and Lambers, 1989). A shift in chemical allocation between cytoplasm and cell-wall material which correlates with the RGR, appears to be a more general phenomenon.

The di- and monocotyledons differed in RGR correlations with respect to ferulic acid and (hydroxy)proline-rich protein (HPRP), both considered to be cell-wall components (Cassab and Varner, 1988; Harris and Hartley, 1980; Marcus et al., 1991). A comparatively high accumulation of polysaccharide-bound phenolic acids is typical for monocotyledons (Harris and Hartley, 1980), however, and therefore it is not surprising that especially for the monocotyledons a very significant negative (Niemann et al., 1992a) correlation with the RGR was found for ferulic acid. For HPRP, represented by pyrolysis fragment *m/z* 81, for the dicotyledons a positive correlation with the RGR was found (Fig. 4). More cell-wall material would suggest more wall protein and therefore a negative correlation with RGR as indicated for the grasses (Fig. 4, not significant in absolute amounts, significant comparative to other protein fragments). However, also in the grasses the correlation of the abundance of *m/z* 81 with RGR shifts to a positive one when it is considered relative to other cell-wall components rather than as an absolute amount. Obviously, although they have less cell-wall material, fast-growing species contain more wall protein per unit of wall material. For the correlation of *m/z* 81 with RGR for the dicotyledons the differences in wall protein concentration probably supersede the differences in amount of wall material. The wall-bound character of the greater part of the *m/z* 81-related protein was demonstrated by its comparatively difficult accessibility by pronase in enzyme-treated leaf material (Niemann et al., 1992b; J J C M Van Arendonk, pers. commun.).

Lambers and Poorter (1992) suggested that one of the causes of the comparatively low photosynthetic nitrogen use efficiency (PNUE) of slow-growing species (Poorter et al., 1990) may be their relatively greater investment in cell-wall proteins. The amount of HPRP per unit weight of cell walls of slow- and fast-growing species is not the same, however, but is lower in low-RGR species. Also, when for the 24 species the ratio *m/z* 81 / *m/z* protein (sum of protein fragments) was plotted against the PNUE (data from Poorter et al.,

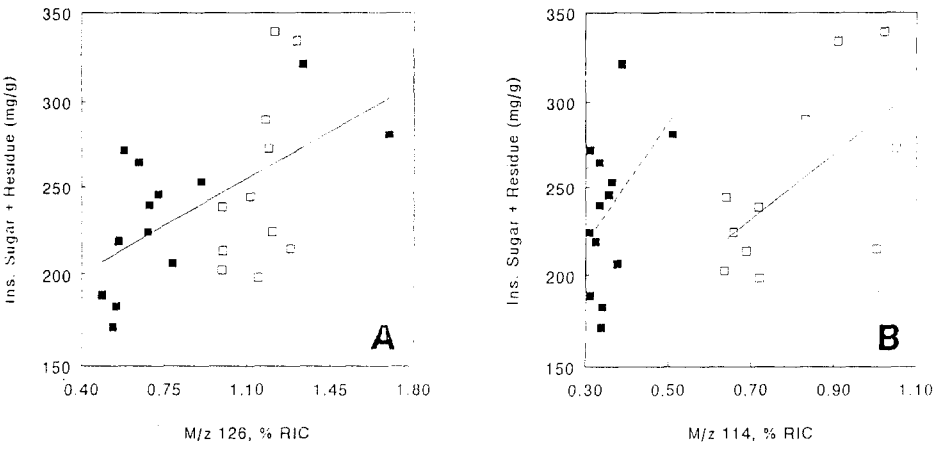


Fig. 9. Comparison of the relative amounts of the combined fractions of insoluble sugars and residual (hemi)cellulose as determined by Poorter and Bergkotte (1992) for the leaves of 11 grasses (□) and 13 dicotyledons (■) differing in RGR, with the percentages of the total ion current (RIC) of A) fragment *m/z* 126 and B) fragment *m/z* 114. The straight solid lines indicate a significant linear regression, **A.** $p < 0.01$, **B.** $p < 0.05$.

1990), no correlation was found (data not shown). If HPRP is representative for all cell-wall protein, these results lead to the rejection of this hypothesis put forward by Lambers and Poorter (1992). For any definite conclusion, however, a further distinction of both cytoplasm and cell wall proteins and their relation to RGR is necessary.

In addition to differences with respect to RGR correlations for ferulic acid and HPRP, the di- and monocotyledons also differ for polysaccharides and wax esters. The monocotyledons are much richer in both pentose- and hexose-based polysaccharides, a difference which can only partly be explained by a comparatively high xylan and low xyloglucan content of grass cell walls (Fry, 1988). The monocotyledons were further not only found richer in aliphatic wax esters than dicots, but also the RGR-correlation appeared reversed, being positive for the grasses and negative for the dicotyledons. Similar to the correlation for wax esters, low-RGR dicots also appeared somewhat richer in triterpenes than the high-RGR species.

Some of the differences found between the slow- and fast-growing species might be explained by differences in size of the plant cells. Inherent differences in tissue differentiation for instance leading to smaller cells in low-RGR species, might form an explanation for the present results. For two subspecies of *Plantago major*, Dijkstra and Lambers (1989) found a relative decrease in epidermal cell size associated with low RGR. For 14 grasses Van Arendonk and Poorter (1994) also found an increase in the small-type epidermal cells and an increased number of sclerenchyma cells, associated with lower RGR. Garnier and Laurent (1994) in fast-growing annual grass species found a higher proportion of mesophyll tissue and lower proportions of sclerenchyma and vascular bundles than in con-generic, slow-growing perennial ones.

The benefit of the plant of these inherent differences in chemical allocation still have to be guessed at. Phenolic acids have been associated with resistance against fungi (Glazener, 1980; Ismail et al., 1987; Kuc et al., 1956; Niemann and Baayen, 1988). Increases in cell-wall material may lead to an increase in protection against mechanical perturbation, e.g. trampling (Kokubu et al., 1990) and a decrease in palatability (Bastide et al., 1988) and digestibility (Classen et al.,1990; Hartley and Jones, 1978; Kephart et al., 1990). Increased leaf toughness (Kokubu et al., 1990) and leaf longevity (Reich et al., 1991) as a consequence of structural changes and/or changes in amounts of cell wall material may have formed part of the process of natural selection.

Conclusions

Fast-growing dicotyledonous species contain more protein, chlorophyll, sterol and diglycerides, whereas slow-growing ones are richer in glucan-based polysaccharides, C16:0 fatty acid, triterpenes and aliphatic wax esters. Fast-growing monocotyledons differ more clearly from slow-growing ones, having more cytoplasmic elements and less cell-wall components (Niemann et al., 1992a). For the monocotyledons this is

probably to a large extent based on anatomical differences (Van Arendonk and Poorter, 1994). Because of the taxonomic distance among the dicotyledons, which belong to different families, correlations present within one family like e.g. the Gramineae of the monocotyledons, may partly be masked. Relative enrichment in cell-wall elements may be a more general aspect of slow-growing species.

A relatively greater investment in cell wall protein in slow-growing species, which could explain the low rate of photosynthesis per unit of leaf nitrogen as suggested by Lambers and Poorter (1992) was not found.

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References

Bastide P, Massonie G and Macheix J-J 1988 Influence in vitro des composés phénoliques des jeunes filles du pêcher, *Prunus persixa* (L.) Batsch, sur le puceron vert du pêcher, *Myzus persicace* Sulzer. Agronomie 8, 787–792.

Benhamou N, Mazau D, Grenier J and Esquerré-Tugayé M-T 1991 Time-course study of the accumulation of hydroxyproline-rich glycoproteins in root cells of susceptible and resistant tomato plant infected by *Fuxarium oxysporum* f.sp *Radicis-lycopersici*. Planta 184, 196–208.

Boon J J 1989 An introduction to pyrolysis mass spectrometry of lignocellulosic material: case studies on barley straw, corn stem and *Agropyron*. *In* Physicochemical Characterization of Plant Residues for Industrial and Feed Use. Eds. A Chesson and E R Ørskov. pp 25–45. Elsevier Applied Science, Amsterdam.

Boon J J 1992 Analytical pyrolysis mass spectrometry: new vistas opened by temperature-resolved in-source PyMS. Int. J. Mass Spectrom. Ion. Process. 118/ 119, 755–787.

Boon J J, Nip M, Eijkel G B, Brandt-de Boer B and Van Amsterdam M 1988 Characterization of citrus and apple pectins by pyrolysis mass spectrometry in Py-EIMS, DCI-MS and PYGCMS modes. *In* Advances in Mass Spectrometry, Vol 11. Ed. P Longevialle. pp. 1100–1102. Heyden and Sons, London.

Boon J J, Tom A, Brandt B, Eijkel G B, Kistemaker P G, Notten F J W and Mikx F H M 1984 Mass spectrometric and factor discriminant analysis of complex organic matter from the bacterial culture environment of *Bacteroides gingivalis*. Anal. Chim. Acta 163, 193–205.

Cassab G I and Varner J E 1988 Cell wall proteins. Ann. Rev. Plant Physiol. 39, 321–353.

Classen D, Arnason J T, Serratos J A, Lambert J D H, Nozzolillo C and Philogéne B J R 1990 Correlation of phenolic acid content of maize to resistance to *Sitophilus zeamais*, the maize weevil, in cimmyt's collections. J. Chem. Ecol. 16, 301–315.

Dijkstra P and Lambers H 1989 A physiological analysis of genetic variaton in relative growth rate within *Plantago major* L. Funct. Ecol. 3, 577–587.

Esquerré-Tugayé M T, Lafitte C, Mazau D, Toppan A and Touzé A 1979 Cell surfaces in plant microorganism interactions. II. Evidence for the accumulation of hydroxyproline-rich glycoproteins in the cell wal of diseased plants as a defense mechanism. Plant Physiol. 64, 320–326.

Fry S C 1988 The growing plant cell wall: chemical and metabolic analysis. Longman Scientific and Technical, Harlow. 333p.

Garnier E and Laurent G 1994 Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. New Phytol. 725–736.

Glazener J A 1980 Defense mechanisms in tomato fruit after infection with *Botrytis cinerea*.Thesis, Utrecht University, 81p.

Grime J P and Hunt R 1975 Relative growth-rate: its range and adaptive significance in a local flora. J. Ecol. 63, 393–422,

Harris P J and Hartley R D 1980 Phenolic constituents of the cell wall of monocotyledons. Biochem. Syst. Ecol. 8, 153–160.

Hartley R D and Jones E C 1978 Phenolic components and degradability of the cell walls of the brown midrib mutant, bm_3, of *Zea mays*. J. Sci. Food Agric. 29, 777–789.

Hempfling R and Schulten H-R 1990 Chemical characterization of the organic matter in forest soils by Curie point pyrolysis-GC/MS and pyrolysis-field ionozation mass spectrometry. Org. Geochem. 15, 131–145.

Ismail M K, Salama A-A M, Ali M I A and Ouf S A-E 1987 Effect of some phenolic compounds on spore germination and germ-tube length of *Aspergillus fumigatus* and *Fusarium oxysporum* f.sp. *lycopersici*. Cryptogamie - Mycologie 8, 51–60.

Jones R L and Robinson D G 1989 Protein secretion in plants. New Phytol. 111, 567–597.

Kephart K D, Buxton D R and Hill R R Jr 1990 Digestibility and cell wall components of alfalfa selected for divergent herbage lignin concentration. Crop Sci. 30, 207–212.

Kokubu A, Kuraishi S and Sakurai N 1990 Culm strength of barley. Correlations among maximum bending stress, cell wall dimensions and cellulose content. Plant Physiol. 91, 876–882.

Kratka J 1989 Changes of hydroxyproline content in the cell wall of carnation after inoculation with *Fusarium oxysporum* f.sp *dianthi*. Z. Mikrobiol. 144, 485–488.

Kuc J, Henze R E, Ullstrup A J and Quackenbush F W 1956 Chlorogenic and caffeic acids as fungistatic agents produced by potatoes in response to inoculation with *Helminthospozium carbonum*. J. Am. Chem. Soc. 78, 3128–3125.

Lambers H and Poorter H 1992 Inherent variation in growth rate between higher plants: A search for physiological causes and ecological consequences. Adv. Ecol. Res. 23, 187–261.

Marcus A, Greenberg J and Averyhurt-Fullard V 1991 Repetitive proline-rich proteins in the extracellular matrix of the plant cell. Physiol. Plant. 81, 273–279.

Milne T A, Chum H L, Agblevor F and Johnson D K 1992 Standardized analytical methods. Biomass Energy 2, 341–366.

Moers M E C, Baas M, Boon, J J and De Leeuw J W 1990 Molecular characterization of total organic matter and carbohydrates in peat samples from a cypress swamp by pyrolysis-mass spectrometry and wet-chemical methods. Biogeochem. 11, 251–277.

Niemann G J and Baayen R P 1988 Involvement of phenol metabolism in resistance of *Dianthus caryophyllus* to *Fusarium oxysporum* f.sp. *dianthi*. Neth. J. Plant Pathol. 94, 289–301.

Niemann G J, Baayen R P and Boon J J 1990 Differentiation between tissues from carnation (*Dianthus caryophyllus* L.) stems by pyrolysis-mass spectrometry. Ann. Bot. 65, 461–472.

Niemann G J, Pureveen J B M, Eijkel G B, Poorter H and Boon J J 1992a Differences in relative growth rate in 11 grasses correlate with differences in chemical composition as determined by pyrolysis mass spectrometry. Oecologia 89, 567–573.

Niemann G J, Van Arendonk J J C M and Boon J J 1992b Tryptophan-rich protein in plant cell walls? Abstr Sixth Cell Wall Meet, Nijmegen, August 25–28. p 104.

Poorter H and Bergkotte M 1992 Chemical composition of 24 wild species differing in relative growth rate. Plant Cell. Environ. 15, 221–229.

Poorter H and Remkes C 1990 Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. Oecologia 83, 553–559.

Poorter H, Remkes C and Lambers H 1990 Carbon and nitrogen economy of 24 wild species differing in relative growth rate. Plant Physiol. 94, 621–627.

Pouwels A D, Tom A, Eijkel G B and Boon J J 1987 Characterisation of beech wood and its holocellulose and xylan fractions by pyrolysis-gas chromatography-mass spectrometry. J. Anal. Appl. Pyrolysis 11, 417–436.

Pouwels A D and Boon J J 1990 Analysis of beech wood samples, its milled wood lignin and polysaccharide fractions by Curie-point and platinum filament pyrolysis-mass spectrometry. J. Anal. Appl. Pyrolysis 17, 97–126.

Ralph J and Hatfield R D 1991 Pyrolysis-GC-MS characterization of forage materials. J. Agric. Food Chem. 39, 1426–1437.

Reich P B, Uhl C, Walters M B and Ellsworth D S 1991 Leaf lifespan as a determinant of leaf structure and function among 23 Amazonian tree species. Oecologia 86, 16–24.

Saiz-Jimenez C, Boon J J, Hedges J J, Hessels J K C and De Leeuw J W 1987 Chemical characterization of recent and buried woods by analytical pyrolysis: Comparison of pyrolysis data with ^{13}C NMR and wet chemical data. J. Anal. Appl. Pyrolysis 11, 437–450.

Scheijen M A 1991 Analytical pyrolysis studies on Tobacco. PhD Thesis, University of Amsterdam, 139p.

Scheijen M A, Brandt-de Boer B, Boon J J, Hass W and Heemann V 1989 Evaluation of a tobacco fractionation procedure using pyrolysis mass spectrometry combined with multivariate analysis. Beitr. Tabakforsch. 14, 261–282.

Schulten H R, Bahr U, Wagner H and Hermann H 1982 Pyrolysis field ionization mass spectrometry of pharmaceutial plant mucilages. Biomed. Mass Spectrom. 9 115–118.

Tsuge S and Matsubara H 1985 High-resolution pyrolysis-gas chromatograpy of proteins and related materials. J. Anal. Appl. Pyrolysis 8, 49–64.

Van Arendonk J J C M and Poorter H 1994 The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate. Plant Cell. Environ. 17, 963–970.

Van der Hage E R E, Mulder M M and Boon J J 1993 Structural characterization of lignin polymers by temperature-resolved in-source pyrolysis mass spectrometry and Curie-point pyrolysis gas chromatography/mass spectrometry. J. Anal. Appl. Pyrolysis 25, 149–183.

Van Smeerdijk D G and Boon J J 1987 Characterisation of sub-fossil *Sphagnum* leaves, rootlets of Ericaceae and their peat by pyrolysis-high-resolution gas chromatography-mass spectrometry. J. Anal. Appl. Pyrolysis 11, 377–402.

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