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Chemical composition of 24 wild species differing in relative growth rate

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ABSTRACT

The chemical composition of 24 plant species which showed a three-fold range in potential growth rate was investigated. The carbon content of whole plants was lower for fast-growing species than for slow-growing ones. Fast-growing species accumulated more organic N-compounds, organic acids and minerals, whereas slow-growing species accumulated more (hemi)cellulose, insoluble sugars and lignin. No correlations with relative growth rate were found for soluble phenolics, soluble sugars and lipids. The costs to construct 1 g of plant biomass were rather similar for fast- and slow-growing species, both when expressed as C needed for C-skeletons, as glucose to provide ATP and NAD(P)H, and as total glucose costs. Therefore, we conclude that, despite the differences in chemical composition between fast- and slow-growing species, variation in the costs of synthesis of whole plant biomass cannot explain interspecific variation in relative growth rate of herbaceous species.

Key-words: chemical composition; construction costs; interspecific variation; relative growth rate.

INTRODUCTION

The process of CO_2 fixation is a prerequisite for plant growth and has received considerable attention from (eco)physiologists, who have analysed inherent variation in relative growth rate (RGR; Duncan & Hesketh 1968; Dijkstra & Lambers 1989; Poorter, Remkes & Lambers 1990). However, it is only the first step in a series of biochemical reactions that leads to the construction of biomass. Plants are composed of a wide array of compounds. The biosynthetic pathways to construct these compounds differ, and consequently, so does the amount of fixed carbon required for their synthesis. This implies that interspecific variation in relative growth rate may be affected by inherent variation in the chemical composition.

The chemical constituents within a plant can roughly be divided into six quantitatively important categories: lipids, lignin, organic N-compounds, carbohydrates, organic acids and minerals (Penning de Vries, Brunsting & Van Laar 1974). The carbon costs for the synthesis of these different (organic) compounds can be separated into two components. The first is associated with C needed to provide carbon skeletons. Some compounds, like organic acids, have a low C-content, whereas the C-content of others (e.g. lipids and lignin) is high. Differences in the chemical composition will thus affect the total amount of C needed for C-skeletons of 1 g of plant material. In this way, the C-content of the plant biomass is the parameter that bridges the gap between the growth rate of a plant on one hand, and the rates of photosynthesis and respiration per unit plant weight on the other.

The second part of the synthesis costs is associated with the amount of reduced carbon needed to provide for the amount of ATP and NAD(P)H, necessary to drive the biosynthetic pathways. Penning de Vries *et al.* (1974) have calculated these costs for the different groups of organic compounds mentioned above. The synthesis of organic N-compounds from glucose and NO_3^- requires substantial amounts of ATP and NAD(P)H, whereas during the formation of organic acids from glucose ATP and NAD(P)H are produced. The CO_2 released during the production of ATP and NAD(P)H forms part of the respiration as measured in gas exchange.

The aim of this paper is threefold. Firstly, it investigates whether there are differences in carbon content between inherently fast- and slow-growing species. Secondly, it analyses how variation in C-content can be explained in terms of the chemical composition, according to the functional groups discerned by Penning de Vries *et al.* (1974). Finally, it evaluates to what extent possible differences in chemical composition between fast- and slow-growing species affect the total costs for construction of biomass.

MATERIALS AND METHODS

Growth of the plants

Plants of 24 wild species with a C_3-type of photosynthesis, common in Western Europe, were grown from seed. These species were the monocotyledons

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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., and the dicotyledons *Anthriscus sylvestris* (L.) Hoffm., *Galinsoga parviflora* 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. The seedlings were placed in a growth room with the following conditions: (day) 14h, photosynthetic photon flux density 315±30 μmol m^{-2} s^{-1}, temperature 20±0.5°C, relative humidity circa 70%; (night) l0h, temperature 20±0.5°C. Light was provided by fluorescent lamps (Philips TL-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 2mol m^{-3} (Poorter & Remkes 1990).

Experimental design

The growth experiment started when the plants had reached a fresh weight of approximately l00mg (day 0). Eight plants were harvested on days 0, 3, 7, 10, 14 and 17, 4-8 h after the start of the light period (Poorter & Remkes 1990). All this material was oven-dried for 24h at 80°C and mixed for chemical analyses. A separate group of eight plants was used for the measurements of photosynthesis and shoot and root respiration (Poorter *et al.* 1990). Between day 11 and day 17, depending on the size of the plants, 4h after the start of the light period, a third group of plants was collected and freeze-dried.

In all cases, plant material was separated into leaves, roots and a remaining fraction, termed stem throughout this paper. For each of these fractions, individual plants were combined to form two independent groups to be used in the subsequent chemical analyses.

Chemical analyses

Organic N-content was determined on oven-dried material of plants for which photosynthesis and shoot and root respiration were measured. Ground plant material was digested with a modified micro-Kjeldahl method using concentrated sulphuric acid and a mixture of sodium sulphate, copper sulphate and selenium in a ratio of 1.55:97:1.55 (w/w) as a catalyst (Bradstreet 1965). The N-content was determined colorimetrically using the indophenolblue method (Novozamsky *et al.* 1974).

Freeze-dried plants were utilized for the determination of the C-content and the concentration of soluble and insoluble sugars. Total C-content of the samples was determined with an automatic C-H-N analyser (Carlo Erba, model 1106, Milano, Italy) using combustion gas chromatography (Pella & Colombo 1973). For the determination of soluble and insoluble sugars the samples were extracted with chloroform, 80% ethanol and water [2:4:2.5 (v/v)]. The residue, obtained after centrifugation, was boiled for 3h in 3% (v/v) HCl to hydrolyse the insoluble sugars (starch and fructans). The total soluble and insoluble sugar content was determined colorimetrically using the anthrone reagent (Fales 1951).

Oven-dried plant material was used to determine the ash content, ash alkalinity and nitrate content. Ash content was measured after combustion of the sample in a muffle furnace at 550°C for l0h. Thereafter, ash alkalinity was determined acidimetrically (Jungk 1968). Nitrate was determined in water extracts of the same samples (Cataldo *et al.* 1975). Lipid content, soluble phenol concentration and crude cell wall content were also determined on oven-dried plant material. Firstly, ground plant material was extracted with chloroform, methanol and water in a ratio of 2:2:1 (v/v) (Bligh & Dyer 1959). Lipids were determined gravimetrically on the residue left after drying off the chloroform phase over N_2. Soluble phenol content was determined colorimetrically in the methanol-water phase as indicated in Singleton (1988), using Folin-Ciocalteu's phenol reagent (SIGMA F-9252) and p-coumaric acid as a standard. After the extraction with chloroform, methanol and water, the pellet was boiled for 3h in 3% (v/v) HC1 to remove all insoluble sugars. The residue was considered to be crude cell wall material and was measured gravimetrically after drying at 70°C for 24h. This fraction was analysed for organic nitrogen as described above. The lignin content in the residue was determined colorimetrically at 280nm after treatment with acetyl bromid in acetic acid (Morrison 1972). p-Coumaric acid was used as a standard. Negligible amounts of ash were found after combustion of the residue (<0.5%).

Calculations

The weight of total organic N-compounds was calculated by multiplying the Kjeldahl-N-content by 6.25. Organic acid content was determined by correcting ash alkalinity (meq g^{-1}) for the NO_3^--concentration (meq g^{-1}), and multiplying by 62.1 g meq^{-1}, assuming that the organic acid composition of the 24 species equalled the mean composition of *Deschampsia flexuosa* and *Holcus lanatus,* as determined by GC. Total mineral content was calculated as the sum of ash and NO_3^--content, subtracting 30 * meq g^{-1} ash alkalinity to correct for oxides, which turn into CO_3^{2-} after cooling. The fraction of plant material that remained after removing lipids, soluble and insoluble sugars, and after subtraction of its concentration organic N-compounds was considered to be composed of (hemi)cellulose and lignin. For the conversion of p-coumaric acid units into total lignin

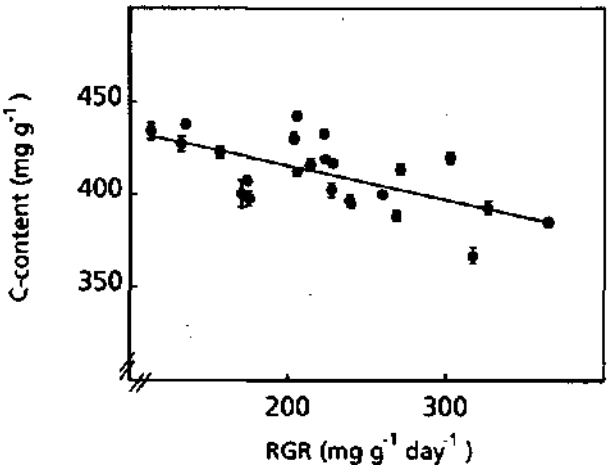


Figure 1. Carbon content of whole plants of 24 species differing in relative growth rate: mean values±SE (determinations of two independent bulk samples per species). Regression lines with a slope significantly different from zero ($P < 0.05$) are drawn continuously, others as broken lines.

weight, we assumed lignin to consist of equal amounts of p-hydroxyphenol, guaiacyl and syringyl units, connected by ß-O-4' linkages (cf. Monties 1989).

To arrive at an estimate of the total amount of glucose required for synthesis of plant material and the amount of CO_2 produced during this process, we used the approach of Penning de Vries *et al.* (1974). However, we split the group of carbohydrates, as used by Penning de Vries *et al.* (1974) into three: (hemi)cellulose, which we assumed to comprise 50% cellulose and 50% hemicellulose; insoluble sugars, which we assumed to be starch in all cases; and soluble sugars, which we assumed to consist of glucose, fructose and sucrose in equal amounts. Moreover, we treated soluble phenolics as a separate group, assuming it to be all p-coumaric acid produced at a cost of 3 mol NADH and 1 mol ATP per mol formed. All concentrations are expressed per unit dry weight.

Statistical analysis

Data were analysed with the SAS statistical package (Joyner 1985). Relations between the various chemical compounds and RGR are described with linear regression analysis. To summarize the data, a principal component analysis was carried out using procedure factor of the SAS statistical package.

RESULTS

The mean carbon content was high in leaves, intermediate in stem and lowest in roots (Table 1). In all plant parts, the C-content correlated negatively with the RGR of a plant species. The C-content of whole plants varied between 370 and 440 mg g^{-1} (Fig. 1) and decreased significantly with RGR ($P < 0.001$). H-content also decreased with increasing RGR ($P < 0.001$), whereas N-content was positively correlated ($P < 0.001$).

Ten different plant compounds were analysed. Compared to other organs, leaves contained relatively high amounts of lipids and organic N-compounds, and low concentrations of lignin. Stems had high concentrations of NO_3^- and lignin. Roots showed relatively high concentrations of (hemi)cellulose and insoluble sugars, and low concentrations of lipids.

The correlations between the various components and elements are depicted in a correlation diagram (Fig. 2). Three different groups could be discerned. Positive correlations were found between organic N-compounds, organic acids, minerals, NO_3^- and total N-content (Fig.

Table 1. Chemical composition (in mg g^{-1}) of a typical slow-growing plant species (RGR = 110 mg g^{-1} d^{-1}) and a typical fast-growing one (RGR = 370mg g^{-1} d^{-1}), as calculated from linear regressions of the concentration of each compound in a species with the RGR of that species. Values are given for leaves, stem and roots separately; r^2, fraction of variance explained by the regression line

	Leaves			Stem			Roots		
Compound	Slow	Fast	r^2	Slow	Fast	r^2	Slow	Fast	r^2
Organic N-compounds	285	371	0.18*	235	289	0.08	174	296	0.38**
Organic acids	30	135	0.34**	42	84	0.10	17	44	0.10
Minerals	94	138	0.14$^+$	74	242	0.53***	138	194	0.19*
Lignin	26	14	0.l0	45	23	0.26*	40	26	0.09
(Hemi)cellulose	183	110	0.29**	234	176	0.19*	272	232	0.06
Insoluble sugars	86	100	0.02	179	156	0.05	279	181	0.23*
Lipids	42	40	0.00	21	28	0.04	16	26	0.19*
Soluble sugars	46	33	0.05	76	76	0.00	39	59	0.02
Soluble phenolics	9	2	0.10	6	2	0.08	3	3	0.00
Total recovery	801	943	0.35**	911	1076	0.39**	977	1060	0.16$^+$
NO_3^-	18	36	0.08	0	94	0.58***	31	69	0.48***
Carbon	445	397	0.27**	435	359	0.52***	414	362	0.46***
Hydrogen	61	55	0.30**	60	47	0.66***	57	49	0.46***
Nitrogen	46	59	0.16$^+$	34	50	0.27**	32	47	0.32***

$+ 0.05 < P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

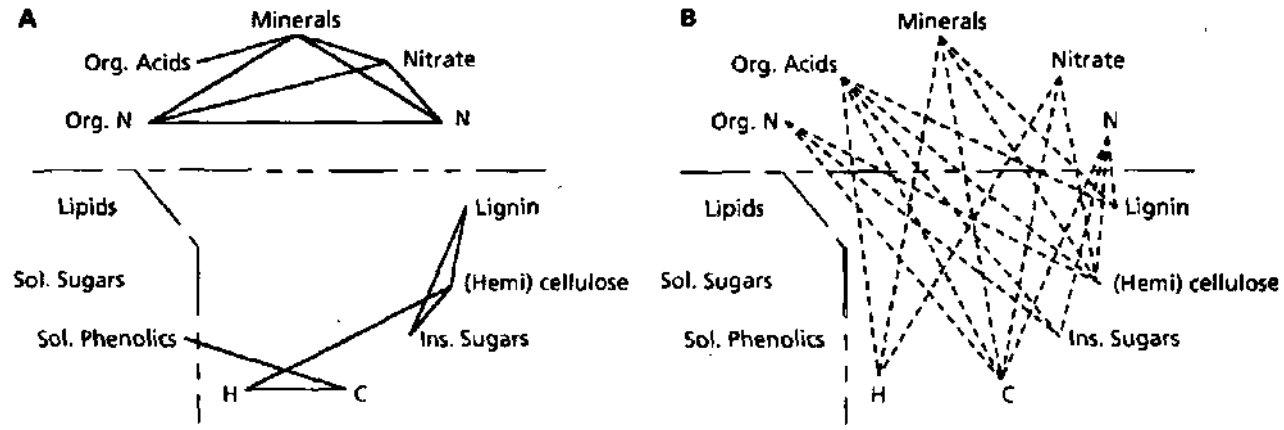
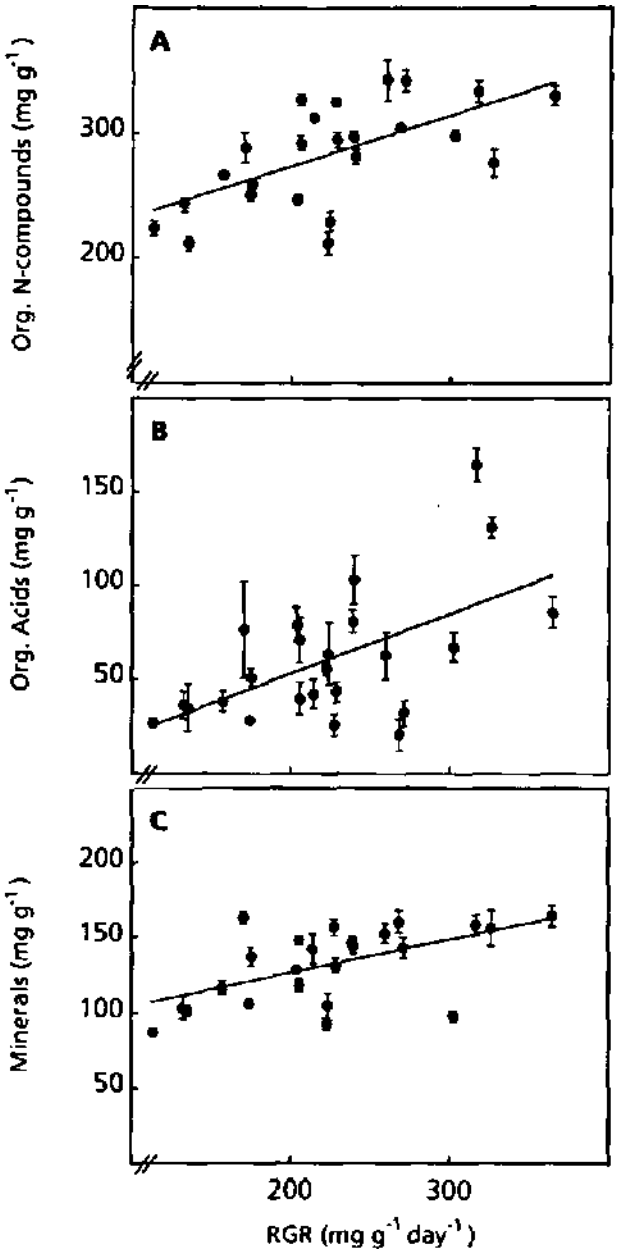


Figure 2. Correlation diagram of the concentrations of the various compounds and elements of 24 species differing in growth rate: (A) positive correlations; (B) negative correlations. All correlations were significant at $P < 0.05$ or lower.



2A). A second group which showed positive interrelations were lignin, (hemi)cellulose, insoluble sugars, C-content and H-content. These two groups were correlated negatively with each other (Fig. 2B). A third group was formed by soluble phenolics, soluble sugars and lipids, which showed hardly any negative or positive correlation with other compounds. This separation was confirmed by a principal component analysis, where all variables of the first group loaded highly positively on the first axis, the variables from the second group loaded highly negatively, and the elements of the third group did not load substantially. Although RGR correlated significantly with this first factor ($P < 0.001$), growth rate explained only 56% of its variation.

Similar results were found when individual components were considered. Organic N-compounds (Fig. 3A; $P < 0.01$), organic acid (Fig. 3B; $P < 0.01$) and mineral content (Fig. 3C; $P < 0.01$), including NO_3^- ($P < 0.01$) all correlated positively with RGR. Lignin (Fig. 4A; $P < 0.05$), (hemi)cellulose (Fig. 4B; $P < 0.01$) and insoluble sugars (Fig. 4C; $P < 0.05$) correlated negatively with RGR. In general, RGR explained 20-45% of the variation in these compounds. No correlation was found with lipids (Fig. 5A), soluble sugars (Fig. 5B) or soluble phenolics (Fig. 5C). All these regressions refer to whole plants. Table 1 gives values for leaves, stem and roots separately.

Total recovery differed for the three organs. Mean recovery was 87% for leaves, 100% in stems and 104% in roots. A significant positive correlation was found between recovery and RGR in each of these organs (Table 1).

The dry weight:fresh weight ratio decreased with increasing RGR for both leaves, stem and roots (all $P <$

Figure 3. The concentration of (A) organic N-compounds, (B) organic acids and (C) minerals of whole plants of 24 species differing in relative growth rate: mean values±SE (determinations of two independent bulk samples).

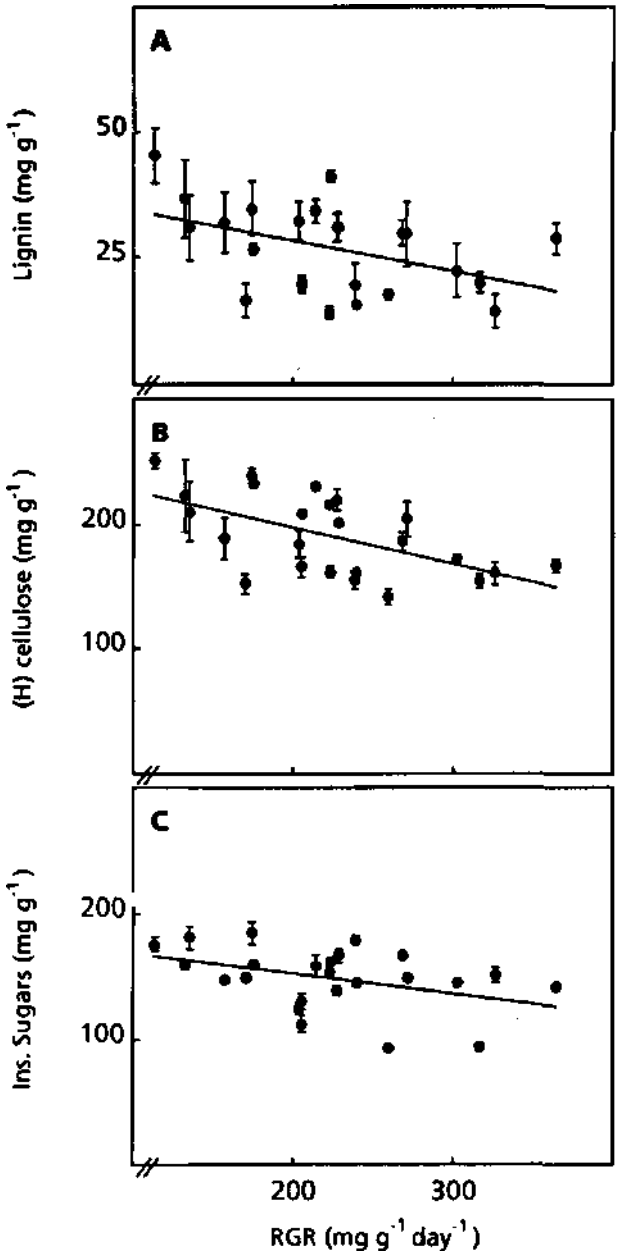


Figure 4. The concentration of (A) lignin, (B) (hemi)cellulose and (C) insoluble sugars of whole plants of 24 species differing in relative growth rate: mean values±SE (determinations of two independent bulk samples).

0.001), and also for total plant biomass (Fig. 6; $P < 0.001$).

DISCUSSION

Carbon content

Leaves, stem and roots differ with respect to their C-content as well as the concentration of chemical compounds. If one seeks to understand the variation in growth of whole plants, it is imperative to first express these concentrations on a whole plant basis. Thereafter, concentrations in leaves, stem and roots, and biomass allocation to these organs can be considered to explain variation on a whole plant level.

Carbon content of whole plants decreased with increasing relative growth rate (Fig. 1). The difference is not due to a systematic trend in biomass allocation, although there was a small, but significant increase in allocation to the leaves with increasing RGR (Poorter & Remkes 1990). As leaves have a higher carbon content than stems or roots, we would expect this increase in allocation to leaves to be accompanied by an increased

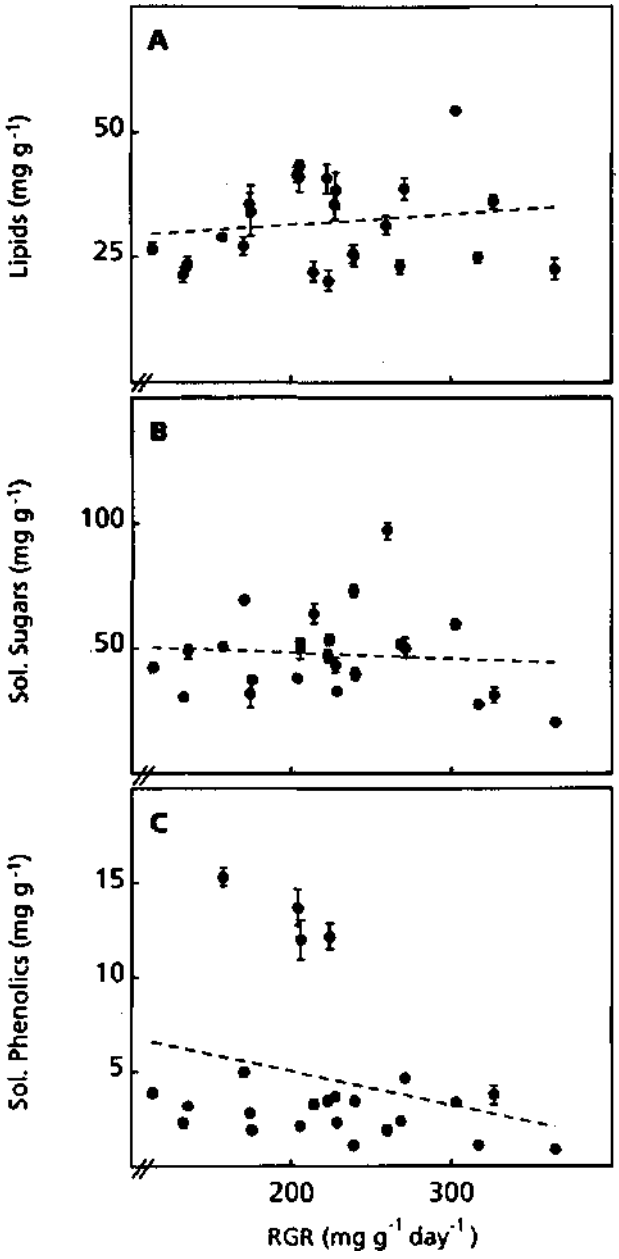


Figure 5. The concentration of (A) lipids, (B) soluble sugars and (C) soluble phenolics of whole plants of 24 species differing in relative growth rate: mean values±SE (determinations of two independent bulk samples).

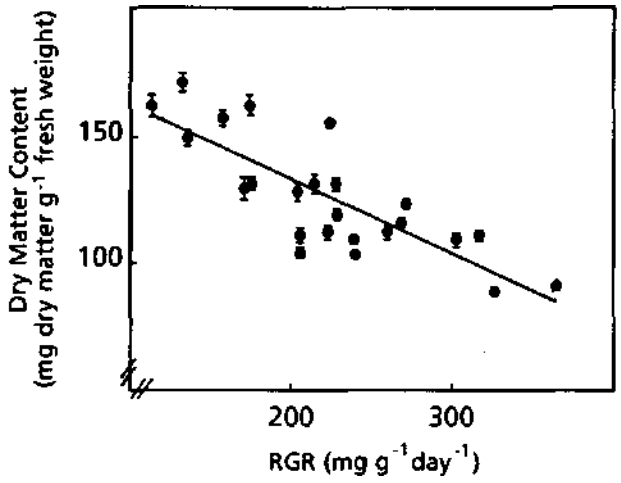


Figure 6. Dry weight:fresh weight ratio of whole plants of 24 species differing in relative growth rate. Mean values calculated over the total dry weight range of 30-100mg (cf. Poorter & Remkes 1990).

carbon content. This not being the case, we conclude that the decrease in C-content of total plant biomass is due to the decrease in each of the separate organs (Table 1).

Few data are available on the whole plant carbon content of different species. From data on the chemical composition of a number of plant species, varying from greenhouse-grown cucumbers (Challa 1976) to the above-ground biomass of coniferous forest (Lieth 1975), Poorter (1989) calculated that C-content ranges at least from 390 to 550mg g^{-1}. All other things being equal, this would cause RGR to vary by 40%. Using the same formula as Poorter (1989; Eqn 3), it is calculated that in the present experiment, differences in carbon content explain only 5% of the difference in RGR. Thus, we conclude that C-content does not play an important role in explaining interspecific variation in RGR, unless species of different growth forms are compared.

Chemical composition

Chemical composition varied with RGR, both when expressed per unit total plant weight and per unit weight of the various organs. Fast-growing species accumulated more organic N-compounds as well as organic acids and minerals (Fig. 3), whereas slow-growing species accumulated more lignin, (hemi)cellulose and insoluble sugars (Fig. 4). Dijkstra & Lambers (1989) found partly deviating results for two inbred lines of *Plantago major,* differing in growth rate. These lines contained equal amounts of organic N-compounds and the fast-growing line had a higher soluble and insoluble sugar concentration. In accordance with the present results, the fast-growing line contained less cell wall components [(hemi)-)cellulose plus lignin] and more minerals (30% higher concentration in the shoot of the rapid-growing line), in part due to a higher NO_3^- content. Similar results were reported for a fast-growing lettuce cultivar, which had a higher NO_3^--content than a slower-growing one (Blom-Zandstra, Lampe & Ammerlaan 1988). The increased NO_3^--content was accompanied by a decrease in organic acids. Blom-Zandstra *et al.* (1988) concluded that nitrate replaces sugars as an osmoticum and that this contributes to the difference in growth rate. A negative correlation between nitrate and organic acids was not observed for the present species (Fig. 2). However, as fast-growing species contain more water per unit dry weight (Fig. 6), it is expected that the extra amount of NO_3^- (and other minerals) in fast-growing species is needed to maintain the osmotic potential at a comparable high level as that of the slow-growing species.

Which changes in chemical composition are responsible for the decrease in C-content with increasing RGR (Fig. 1)? The decrease in lignin and (hemi)cellulose causes a small decrease in C-content. Taken together, these decreases are as important as the increase due to a higher concentration of organic N-compounds. However, the major factor causing changes in C-content is the difference in accumulation of minerals (Fig. 3C). The mineral fraction is also important in explaining variation in C-content in data compiled from the literature (Poorter 1989), and a similar result is expected on the basis of an almost complete analysis of the chemical composition of two *Plantago major* lines (Dijkstra & Lambers 1989).

The present experiment focuses on young, vegetative plants and does not include ontogenetic aspects. Poorter & Pothmann (1992) studied ontogenetic drift for a range of parameters in two of the 24 species, the fast-growing grass species *Holcus lanatus* and the slow-growing *Deschampsia flexuosa.* These plants were grown under the same conditions as in the present experiment, but during a much longer period of time (up to 50d). Although slight changes in parameters like C-content, organic N concentration and the dry weight:fresh weight ratio of the plants were found, differences between the two species essentially remained constant for the duration of the experiment. From this observation, we infer that the differences between species, as observed here, are likely to hold over longer periods of growth.

Recovery

To arrive at an estimate of the total costs of construction of biomass and the associated CO_2-production, one has to determine all of the quantitative important compounds. In the present experiment, this goal was not fully achieved. Mean recovery was around 100% in roots and stems, but 86% in leaves. Moreover, in all organs a positive correlation was found between recovery and RGR of the species. Miller & Stoner (1979) also found lower recoveries in leaves (58%) than in stem (85%) and roots (87%). However, they did not analyse organic

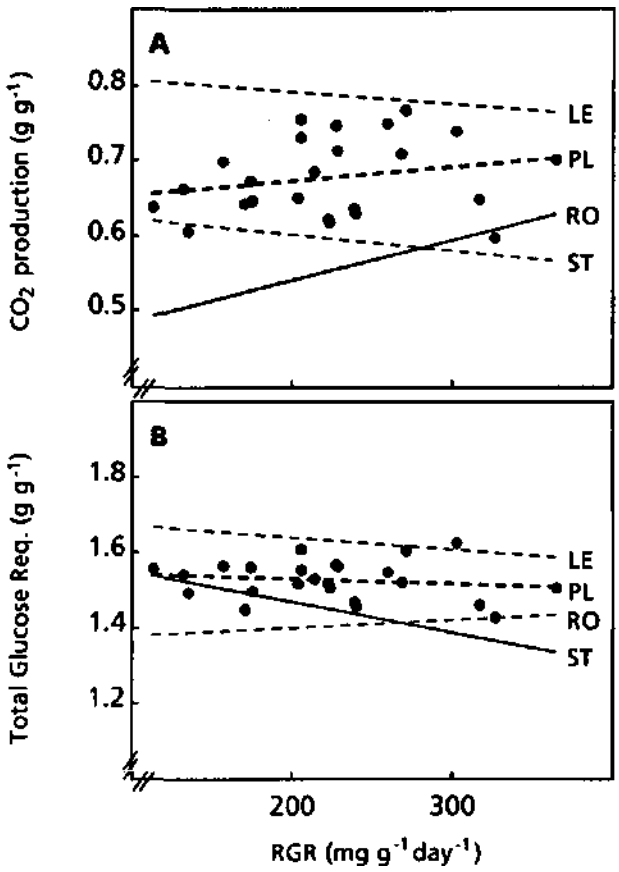


Figure 7. (A) CO_2 evolution associated with the production of 1 g of biomass, (B) total glucose requirement to produce 1 g of biomass for 24 species differing in growth rate. Data points and thick lines refer to calculated values and regression lines for whole plants (PL). Thin lines pertain to calculated regression lines for leaves (LE), stem (ST) and roots (RO), respectively.

acids and minerals. Lafitte & Loomis (1988) found recoveries of 87-91% for above-ground parts of *Sorghum bicolor*. In a study on leaves of one evergreen and two deciduous shrubs, Merino, Field & Mooney (1984) reported recoveries ranging from 64 to 98%. In this case, the evergreen shrub had much lower recoveries than the deciduous ones, comparable to the slow-growing and fast-growing species in this study. Chapin (1989) found recoveries of ca. 95%, but did not check for systematic differences between species with different growth forms. An explanation for the systematic trend with RGR may be that the chemical composition within each group of compounds varies systematically. So, by assuming the same multiplication factor (as in the case of organic N-compounds and organic acids) or the same reaction to a coloured substance (as in the case of soluble and insoluble sugars), we may have overestimated the weight of these fractions in fast-growing species and underestimated it in the slower-growing ones. Alternatively, some of the secondary plant compounds which have not been determined were present in higher quantities in the slower-growing species and/or might have hindered a proper colorimetric determination. For subsequent calculations the total composition of each organ was normalized to 100%.

The costs of biomass production

To arrive at the total costs for biomass production and the consequent CO_2 evolution the approach of Penning de Vries *et al.* (1974) was adopted. The assumptions, implicit in this analysis, are discussed by Chiariello, Mooney & Williams (1989). Calculated growth respiration (CO_2 released per gram of biomass produced) was higher for leaves than for stems or roots (Fig. 7A), in part associated with the leaves' higher concentration of (expensive) organic N-compounds (Table 1), which are costly in terms of NAD(P)H and ATP. Growth respiration of roots increased significantly with increasing RGR (cf. Poorter *et al.* 1991). No such increase was found for leaves or stems, where the increase in costly organic N-compounds with RGR was smaller, and accompanied by a larger increase in cheap organic acids or minerals. For the growth respiration of whole plants, no significant correlation with RGR was found.

The amount of glucose needed to construct 1g of whole-plant biomass (for both C-skeletons and energy production) did not differ between fast- and slow-growing species (Fig. 7B). The glucose costs for the synthesis of leaves and roots were not significantly different for fast- and slow-growing species, but those for stems decreased with increasing RGR, due to a higher concentration of minerals. What is the reason for the absence of a correlation between total glucose requirements and RGR? Expensive compounds, like lignin and lipid, change so little with RGR, that they do not contribute significantly to variation in costs. Carbohydrates have an average cost, and therefore, do not add much to the variation in total cost. The main reason for the observed constancy is that a large increase in costly organic N-compounds with RGR is associated with an increase in cheap compounds (organic acids, minerals; cf. Fig. 2).

To our knowledge, costs of growth have not been determined for whole plants before. Miller & Stoner (1979) found the glucose costs for leaves to be higher than those for stems or roots, comparable to the present results on 24 species. However, as they do not give data on biomass allocation, costs for the synthesis of total biomass cannot be calculated. Chapin (1989) found similar carbon costs for leaves of tundra species with different growth forms (ranging from mosses to evergreen shrubs), although their chemical composition varied considerably. He concluded that the rather constant costs are due to a negative correlation between two groups of expensive compounds, proteins on one hand and compounds like lignin and tannins on the other. As he did not consider the concentration of organic acids and minerals, which showed a positive correlation with organic N in the present 24 species, it cannot be excluded that such a positive correlation with

protein also holds for tundra species. On average, reported glucose requirements for the construction of leaf biomass are slightly higher for species from unproductive habitats (1.59 g glucose per gram biomass; data of Chung & Barnes 1977; Miller & Stoner 1979; Merino *et al.* 1984; Chapin 1989), than for crop and weed species (1.41 g glucose per gram biomass; data of Penning de Vries *et al.* 1974; Lafitte & Loomis 1988; Mooney, cited in Chapin 1989). This suggests that the glucose costs for the construction of one unit of whole plant weight might be somewhat lower for fast-growing species than for slow-growing ones. However, since different authors used different analytical methods, and since their plant material was grown under vastly different conditions, generalizations based on these data are virtually impossible. Our own data show that differences in construction costs between fast- and slow-growing herbaceous species are negligible. Therefore, the conclusion of Chapin (1989), that there is no difference in the construction costs of leaves of deciduous and evergreen tundra species, can be generalized to a wider range of plant species, and is valid for whole plant biomass as well.

Ecological implications

Beside the above-mentioned differences (e.g. in organic N-compounds and lignin), the fast-growing species in this experiment also differed from the slow-growing ones with respect to their specific leaf area (leaf area:leaf weight ratio; Poorter & Remkes 1990) and the rates of photosynthesis and respiration per unit leaf weight (Poorter *et al.* 1990). These differences generally coincide with differences in leaf toughness and leaf longevity under field conditions (Coley, Bryant & Chapin 1985; Reich *et al.* 1991) and also correlate with the nutrient availability in their natural habitat (Grime & Hunt 1975; Poorter & Remkes 1990). In simplified terms, plants from nutrient-rich habitats could be characterized by having short-lived leaves with a high specific leaf area, high concentrations of organic N and a high physiological activity per unit leaf weight. Plants from nutrient-poor sites, on the other hand, have longer-living leaves with a low specific leaf area and relatively more protective structures and components, like lignin and other secondary compounds, even when grown at near-optimum conditions. Co-occurrent with variation in leaf anatomy, these differences in chemical composition seem to determine at least partly the success of a species in a specific habitat. From the present results, we conclude that, within the herbaceous species, the inherent adaptations to a nutrient-poor environment do not come at a cost as far as the total amount of glucose needed for the construction of leaves (per unit weight) is concerned. However, partly as a result of these differences in chemical composition, the daily carbon gain per unit leaf weight is lower. Consequently, the time period before a leaf of a slow-growing species has 'payed back' its own construction costs is much longer. A similar conclusion was reached by Williams, Field & Mooney (1989) for a range of tropical tree species.

CONCLUSIONS

Fast-growing species have a lower carbon content and accumulate more organic N-compounds, organic acids and minerals, whereas slow-growing species contain relatively more lignin, (hemi)cellulose and insoluble sugars. However, the costs to construct 1 g of total plant biomass are remarkably similar. Therefore, variation in construction costs of biomass of whole plants is not an important factor in explaining interspecific variation in RGR.

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