Plant, Cell and Environment (1994) **17,** 963–970

The chemical composition and anatomical structure of leaves of grass species differing in relative growth rate

J. J. C. M. VAN ARENDONK & H. POORTER

Department of Plant Ecology and Evolutionary Biology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands

ABSTRACT

To arrive at a better understanding of variation in specific leaf mass (SLM, leaf weight per unit leaf area), we investigated the chemical composition and anatomical structure of the leaves of 14 grass species varying in potential relative growth rate. Expressed on a dry weight basis, the fast-growing grass species with low SLM contained relatively more minerals and organic N-compounds, whereas slow-growing species with high SLM contained more (hemi)cellulose and lignin. However, when expressed per unit leaf area, organic N-compounds, (hemi)cellulose, total structural carbohydrates and organic acids increased with increasing SLM.

For the 14 grasses, no trend with SLM was found for the leaf volume per unit leaf area. Leaf density was positively correlated with SLM. Variation in density was not caused by variation in the proportion of intercellular spaces. The proportion of the total volume occupied by mesophyll and veins did not differ either. A high SLM was caused, at least partly, by a high proportion of non-veinal sclerenchymatic cells per cross-section. The epidermal cell area was negatively correlated with SLM.

We conclude that the differences in SLM and in the relative growth rate (RGR) between fast- and slow-growing grass species are based partly on variation in anatomical differentiation and partly on chemical differences within cell types.

Key-words: chemical composition; epidermal cells; Gramineae; leaf anatomy; leaf density; relative growth rate; sclerenchymatic cells.

INTRODUCTION

When grown under close to optimum conditions, species from ruderal or competitive environments generally have inherently high relative growth rates (RGRs, rates of biomass increase per unit biomass), while species from environments where the nutrient supply is restricted predominantly have low RGRs (Grime & Hunt 1975, Poorter & Remkes 1990). In an analysis of the growth and carbon economy of 24 herbaceous species from Western Europe, the rate of photosynthesis per unit leaf area was found not to correlate with the potential RGR for these species (Poorter, Remkes & Lambers 1990). Rather, the up to 3-fold difference in RGR was correlated with an almost 3-fold difference in the leaf area:total plant weight ratio (Poorter & Remkes 1990). The main reason for the higher leaf area:plant weight ratio of the fast-growing species was their higher specific leaf area (SLA, leaf area: leaf weight). A similar conclusion was reached in a quantitative analysis of 46 comparative growth experiments (Lambers & Poorter 1992).

Since the SLA is so important in explaining differences in growth rate between species, it is imperative to obtain more insight into the interspecific variation in this parameter. Two avenues are open (cf. Dijkstra & Lambers 1989). First, differences in SLA, or rather its inverse, the specific leaf mass (SLM, leaf weight: leaf area), might be explained by different chemical compositions. For example, if species A and B have similar concentrations of chemical compounds per unit leaf area, but species A has an additional amount of lignin per unit leaf area, this extra lignin might explain the difference in specific leaf mass between species A and B. An alternative approach to reducing the variation in SLA is to analyse the anatomical structure of the leaves. For example, if species A has considerably more sclerenchymatic tissue than species B, this might be the main factor determining the interspecific variation in SLM. Clearly, such an anatomical difference will have consequences for the chemical composition of the leaves.

The aim of this paper is to investigate the differences in the chemical composition and the anatomy of leaves that relate to the observed variation in SLM. To this end, we determined both the chemical composition and the anatomy of the leaves of 14 grass species, which differ 2-fold in SLM.

MATERIAL AND METHODS

Growth of plants

Plants of 14 grass species that are common in Western Europe were grown from seeds. Table 1 lists these species

Correspondence: Jeroen J.C.M. van Arendonk, Department of Plant Ecology and Evolutionary Biology, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands.

Table 1. Values of the relative growth rate (RGR; mg g^{-1} d^{-1}) and the specific leaf area (SLA; m^2 kg^{-1}) for 14 grass species. Plants were grown in nutrient solution with a nitrate concentration of 2 mol m^{-3}. Life form and habitat are as described in Hubbard (1968)

Species	Habitat	RGR	SLA
Corynephorus canescens (L.) Beauv.	coastal sand-dunes; perennial	113	33·1
Festuca ovina L.	acid, dry, nutrient-poor sand; perennial	132	25·3
Deschampsia flexuosa (L.) Trin.	sandy and peaty soils; perennial	135	27·6
Briza media L.	hill calcarous grasslands; perennial	157	35·1
Brachypodium pinnatum (L.) Beauv.	neglected open calcarous grasslands; perennial	174	40·7
Cynosorus cristatus L.	old grasslands; perennial	176	32·0
Festuca arundinacea L.	by side of streams, shorter types drier calcarous and sandy soils; perennial	184	40·5
Poa pratensis (L.) Schreb.	well-drained sandy, gravelly, loamy soils; perennial	185	40·7
Lolium perenne L.	nutrient-rich grasslands; perennial	214	38·8
Phleum pratense L.	field margins, roadsides; perennial	227	47·8
Dactylis glomerata L.	nutrient-rich haymeadows, pastures, roadsides; perennial	229	50·2
Holcus mollis L.	various soils from sands to heavy loams; perennial	243	43·5
Holcus lanatus L.	nutrient-rich grasslands; perennial	268	43·7
Poa annua L.	disturbed and trampled places; annual	272	46·7

[nomenclature according to Hubbard (1968) and Van der Meijden *et al.* (1987)], together with their main habitats. The seeds of *Festuca arundinacea* and *Poa pratensis* were obtained commercially (Kieft BV, Blokker, The Netherlands); seeds of *Holcus mollis* were kindly provided by Dr J.P. Grime (Sheffield, UK), and seeds of other species were collected from a natural population in the Netherlands.

Growth occurred in 33 dm^3 containers in a growth room under the following conditions: day: 14 h, photosynthetic photon flux density 315±30 μmol $m^{-2}s^{-1}$, temperature 20±1°C, relative humidity 70%; night: 10 h, temperature 20±1°C, relative humidity 70%. The plants received a modified Hoagland solution with a nitrate concentration of 2 mol m^{-3}. Further details on experimental conditions are given in Poorter & Remkes (1990).

The growth experiment started when the plants had reached a fresh weight of approximately 100 mg (day 0). Eight plants were harvested on days 3, 7, 10 and 14, and 16 plants on days 0 and 17. The relative growth rate (RGR) was calculated according to Poorter (1989). Both RGR and SLA were calculated at the time when plants had attained total dry weights of 30 to 100 mg.

For the chemical investigations, plants of the last three harvests were pooled into two independent bulk samples; for the anatomical investigations, plants were harvested on day 17.

Chemical analyses

Oven-dried leaf material was ground and extracted with water at room temperature, followed by methanol and water in a ratio of 1:1 (v/v) at 70 °C, chloroform and methanol in a ratio of 1:1 (v/v) at room temperature (3 times) and acetone at room temperature (3 times). The residue was dried at 45 °C for 24 h. The water and methanol extracts were combined, and the chloroform/methanol extract was discarded. The soluble phenol content in the methanol–water phase was determined colorimetrically (Singleton 1988), using Folin-Ciocalteu's phenol reagent (SIGMA F-9252) and p-coumaric acid as a standard. The lignin content in the residue was also determined colorimetrically, after treatment with acetyl bromide in acetic acid (Morrison 1972), again using p-coumaric acid as a standard.

For analysis of the content of lipid, organic nitrogen, (hemi)cellulose, insoluble and soluble sugars, organic acids, minerals, nitrate and total C and N, we refer to Poorter & Bergkotte (1992). For the calculation of the concentration of organic N-compounds we multiplied the total organic nitrogen concentration by 6·25.

Leaf anatomy

Leaf sections were taken either from fresh material (*Poa annua*, *P. pratensis*, *Festuca arundinacea*, *H. mollis*) at a point 1/3 of the distance from the leaf base to the leaf apex, or from the middle parts of the youngest fully grown leaves of material (*Brachypodium pinnatum, Briza media, Corynephorus canescens, Cynosurus cristatus, Dactylis glomerata, Deschampsia flexuosa, Festuca ovina, Holcus lanatus, Lolium perenne* and *Phleum pratense*) that had been stored in FPA fixative [formalin, propionic acid and 70% ethanol in a ratio of 1:1:18 (v/v/v)].

The leaf sections were embedded in historesin (LKB Bromma, Sweden), and 5 μm slices were cut by microtome and coloured with safranine, which stains phenolics red, and astro-blue, which stains cellulose blue. We assume that the distortion and collapse of the epidermal cells of the 14 grass species were equal for all species. Light microscopy and image analyses with an image-processing system (Interactiv Bild Analyse Sytem, I.B.A.S.-2000, Germany) were used to quantify the anatomical differ-

ences amongst the leaf cross-sections. The total area of each cross-section was determined, as well as the area occupied by epidermis, veins (including the sclerenchyma around them), non-veinal sclerenchyma and the intercellular spaces. The area of the mesophyll was then determined by substraction of the other areas from the total area. The width of the section was measured through the middle of the section and the middle of all the veins. All measurements were carried out in triplicate.

Statistics

The relation between the various parameters and the SLM (the inverse of the SLA) was tested with a linear regression analysis. SLM rather than SLA was used as it scales linearly with extra investment of biomass per unit leaf area. Terms were considered significant at the 5% level.

RESULTS

Growth analyses

The values of SLA and RGR for the 14 grass species are given in Table 1, together with the main habitats of these species. RGR, corrected for size-dependent ontogenetic drift, varied 2·4-fold between the slowest (*Corynephorus canescens*) and the fastest (*Poa annua*) growing species. SLA varied 2-fold, and there was a strong positive correlation ($p<0·001$, $r^2=0·69$) between the two variables. There is a problem with analysing variation in SLA in terms of variation in chemical composition per unit leaf area, in that they scale inversely. We have therefore used the inverse of SLA, i.e. SLM, throughout the rest of this paper. SLM ranged from 20 g m^{-2} for *Dactylis glomerata* to 40 g m^{-2} for *Festuca ovina*.

Chemical analyses

All of the eight groups of compounds measured increased with increasing SLM, when expressed as quantity per unit leaf area (Table 2). However, in a number of cases (lipids, minerals, soluble phenolics), the slopes did not significantly differ from zero. In the case of lignin the slope was significant ($p<0·01$, $r^2=0·61$). Approximately 1·0 g m^{-2} of the total variation in SLM could be explained by lignin. Clearly significant differences were found for organic N-compounds ($p<0·01$, $r^2=0·70$), (hemi)cellulose ($p<0·01$, $r^2=0·74$), total non-structural carbohydrates ($p<0·001$, $r^2=0·82$) and organic acids ($p<0·001$, $r^2=0·84$). These compounds explained 2·0, 6·5, 3·5 and 2·5 g m^{-2}, respectively, of the total variation in SLM of 20 g m^{-2}. Recovery was not 100 % and was lower for species with high SLMs (75%) than for those with low SLMs (95%, $p<0·05$, $r^2=0·43$).

Anatomy

Examples of cross-sections are given in Fig. 1, which shows cross-sections of the fast-growing *Poa annua* and the slow-growing *Poa pratensis*.

Although there was variation between species, the total volume of tissue per unit leaf area (Fig. 2a) did not show any relationship with SLM. The density of the total tissue, however, was positively correlated with the SLM and varied 2-fold between fast- and slow-growing species (Fig. 2b; $p<0·01$, $r^2=0·58$).

Fig. 3 shows the proportions of the area of the leaf cross-section taken up by the various tissues and intercellular spaces. There was no correlation between the SLM and the fractional areas occupied by the mesophyll cells (Fig. 3a), the veins (Fig. 3b) and the intercellular spaces (Fig. 3c) in the 14 grass species. The total area occupied by non-veinal

Table 2. The specific leaf mass (SLM; g m^{-2}), the chemical composition (g m^{-2}) and the recovery (%) of the 14 grass species differing in relative growth rate. TNC = total non-structural carbohydrates. Chemical concentrations and recovery were correlated with SLM. The significances of these linear regressions are given at the bottom of the table (ns, not significant; * $p<0·05$; ** $p<0·01$; *** $p<0·001$)

	SLM	Lipid	Lignin	Organic N-compounds	(Hemi) cellulose	TNC	Organic acids	Minerals	Soluble phenolics	recovery
1. *D. glomerata*	19·9	1·12	0·37	7·46	2·50	2·14	1·18	2·67	0·06	88
2. *P. pratense*	20·9	0·95	0·59	7·79	3·80	2·29	0·78	3·34	0·10	94
3. *P. annua*	21·4	1·10	0·51	8·91	3·04	2·40	1·05	2·83	0·13	93
4. *H. lanatus*	22·9	0·92	0·41	8·61	2·76	3·27	0·73	3·52	0·09	89
5. *H. mollis*	23·0	1·68	0·10	6·87	1·83	2·35	0·58	3·70	0·68	77
6. *B. pinnatum*	24·6	1·19	0·67	7·13	5·37	3·32	1·03	2·48	0·09	87
7. *P. pratensis*	24·6	1·47	0·22	5·97	4·45	1·32	1·02	3·09	0·34	73
8. *F. arundinacea*	24·7	2·15	0·33	6·40	3·95	3·83	1·28	3·42	0·36	88
9. *L. perenne*	25·8	0·72	0·72	9·64	5·19	3·62	1·48	3·45	0·08	97
10. *B. media*	28·5	1·15	0·66	8·86	4·14	3·21	1·31	3·06	0·64	81
11. *C. canescens*	30·2	1·04	1·29	8·80	7·15	4·26	1·16	2·14	0·15	86
12. *C. cristatus*	31·3	1·58	0·62	10·05	5·36	3·36	1·83	4·36	0·08	87
13. *D. flexuosa*	36·2	1·18	0·68	9·24	4·85	4·99	2·09	3·07	0·14	72
14. *F. ovina*	39·5	1·20	0·99	11·57	6·90	5·01	1·95	3·43	0·08	79
p		ns	**	**	**	***	***	ns	ns	*

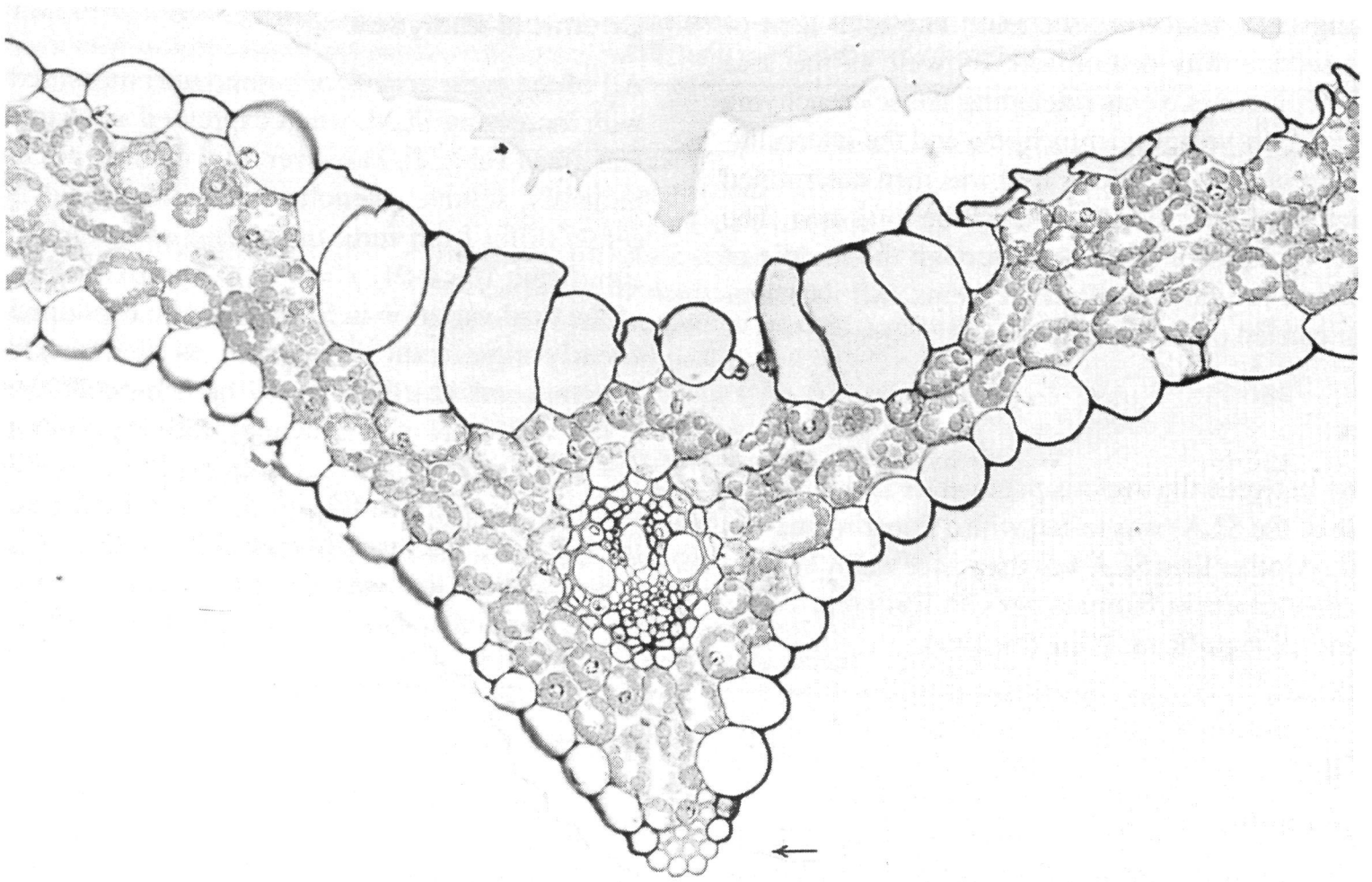

Figure 1. Cross-sections of leaves of (a) the fast-growing *Poa annua* (RGR = 272 mg g^{-1} d^{-1}) and (b) the slow-growing *Poa pratensis* (RGR = 185 mg g^{-1} d^{-1}). The slices are 5 μm thick, and the stains are safranine and astro-blue. The arrows indicate the non-veinal sclerenchymatic cells.

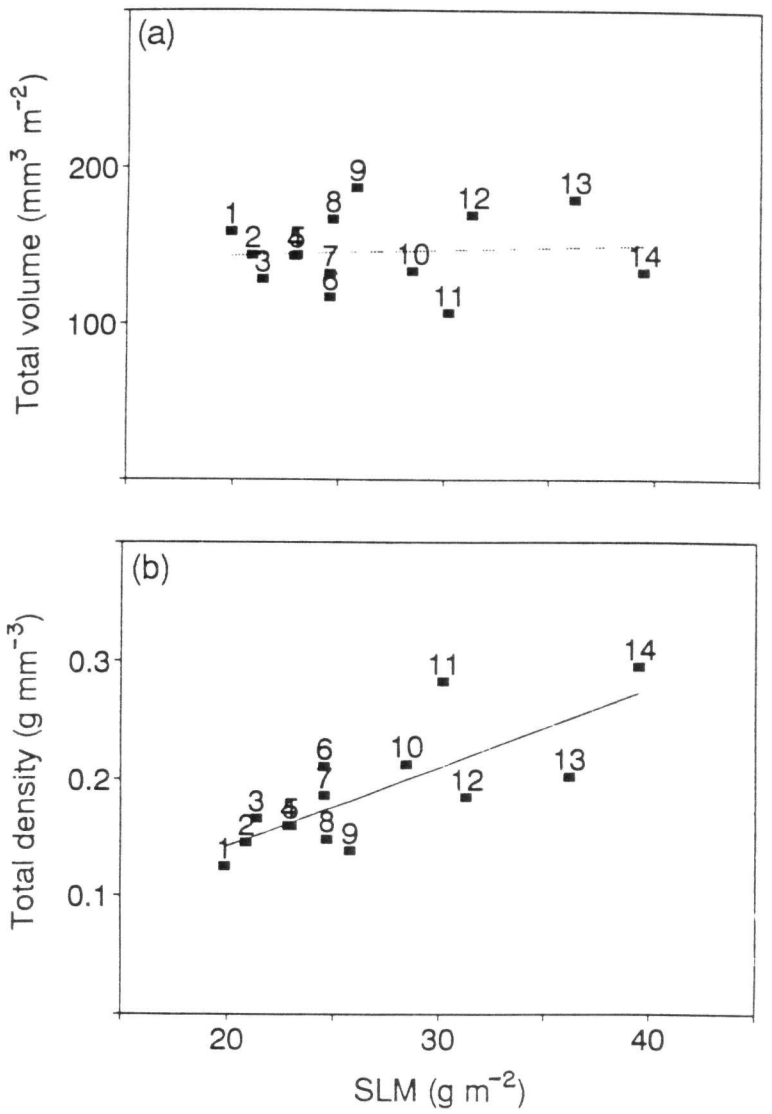

Figure 2. (a) The total volume of the tissue per unit leaf area ($y = 0{\cdot}368x + 136$, ns) and (b) the total density of the tissue ($y = 7{\cdot}43 \times 10^{-3}x + 0{\cdot}009$, $p < 0{\cdot}001$, $r^2 = 0{\cdot}58$) for the 14 grass species that differ in SLM. The numbers refer to the numbers in Table 2. The continuous straight line indicates a significant linear regression, the broken line a non-significant regression.

sclerenchymatic cells showed a positive correlation with SLM (Fig. 3d; $p < 0{\cdot}05$, $r^2 = 0{\cdot}46$), and the epidermal cell area a negative one (Fig. 3e; $p < 0{\cdot}05$, $r^2 = 0{\cdot}35$). The positive correlation of the sclerenchymatic cell area and SLM was mainly due to variation in the number of sclerenchymatic cells per unit area (Fig. 4a; $p < 0{\cdot}01$, $r^2 = 0{\cdot}53$). The average size of the sclerenchymatic cells (Fig. 4b) did not vary systematically. This is at variance with the data on the epidermis. The number of epidermal cells per section area (Fig. 4c) did not show a correlation with the SLM, but the average area of a single epidermal cell did (Fig. 4d; $p < 0{\cdot}01$, $r^2 = 0{\cdot}50$).

DISCUSSION

The chemical analyses confirm and extend previous data by Poorter & Bergkotte (1992) and Niemann *et al.* (1992), which indicate that the interspecific variation in RGR and SLA (Table 1) is related to a change in organic nitrogen, minerals, total non-structural carbohydrates, organic acids and cell wall constituents such as lignin and (hemi)cellulose. This means that slow-growing grass species appear to be relatively rich in cell wall material and relatively poor in cytoplasmic and vacuolar compounds.

Which of the chemical constituents of the leaves can explain the 20 g m^{-2} variation in SLM? Two extreme alternatives can be discerned. In the first, all chemical constituents have equal concentrations for all species, and thus variation in SLM scales with variation in all compounds. In the second, there are differences in chemical composition per unit dry weight between species with low and high SLM, such that all but one constituent are present in equal amounts per unit leaf area, whilst one constituent is higher in the high-SLM leaves than in the low-SLM leaves. Variation in SLM would thus be due to variation in just one constituent. Given the higher concentration of cell wall components in the slow-growing, high-SLM plants we might expect that (hemi)cellulose and lignin together might cause the variation in SLM. Although this is indeed the case, most of the other constituents also correlate positively with the SLM, with the noticeable exception of lipids and minerals. Dijkstra (1989) arrived at a similar conclusion when analysing the difference in SLM and RGR between two *Plantago major* subspecies.

How can this be explained? The chemical difference per unit leaf area is a reflection of variation in anatomy. Variation in SLM can be the result of differences in volume per square metre of leaf area or in leaf density, i.e. leaf dry weight per unit leaf volume (Witkowski & Lamont 1991). The average leaf volume per unit leaf area did not show any correlation with the SLM (Fig. 4a). Consequently, systematic variation in SLM is solely related to leaf density (Fig. 2b). A rather similar conclusion was reached in an analysis of the anatomy of perennial and annual Mediterranean grass species, where variation in average leaf thickness was far less important in explaining variation in SLM than was leaf density (E. Garnier, personal communication). What causes the differences in leaf density between species? It may be that a large proportion of the low-SLM leaves is occupied by intercellular spaces. However, there was no correlation between SLM and the fractional area occupied by intercellular spaces (Fig. 2c). Alternatively, a high SLM may be caused by a high proportion of dense material. Indeed, a positive correlation was found between SLM and the fraction of non-veinal sclerenchymatic tissue (Fig. 2d). On the other hand, the proportion of the leaf occupied by epidermal cells was negatively correlated with the SLM (Fig. 2e). The proportions of mesophyll (Fig. 2a) and vein (Fig. 2b) area per section did not vary systematically.

How can the higher proportion of sclerenchymatic tissue in higher SLM leaves be explained? This does not depend on variation in sclerenchymatic cell size between the species, but rather on the number of cells per unit leaf area (Figs 4a,b). In contrast, differences in the proportion of the cross-section occupied by the epidermis were reflected in differences in size rather than in the number of these epidermal cells (Figs 4c,d).

Combining the chemical and anatomical results, we conclude, first, that a low SLM is mainly caused by less dense material. Niemann *et al.* (1992) showed that species with high RGRs were richer in cytoplasm. This is at least

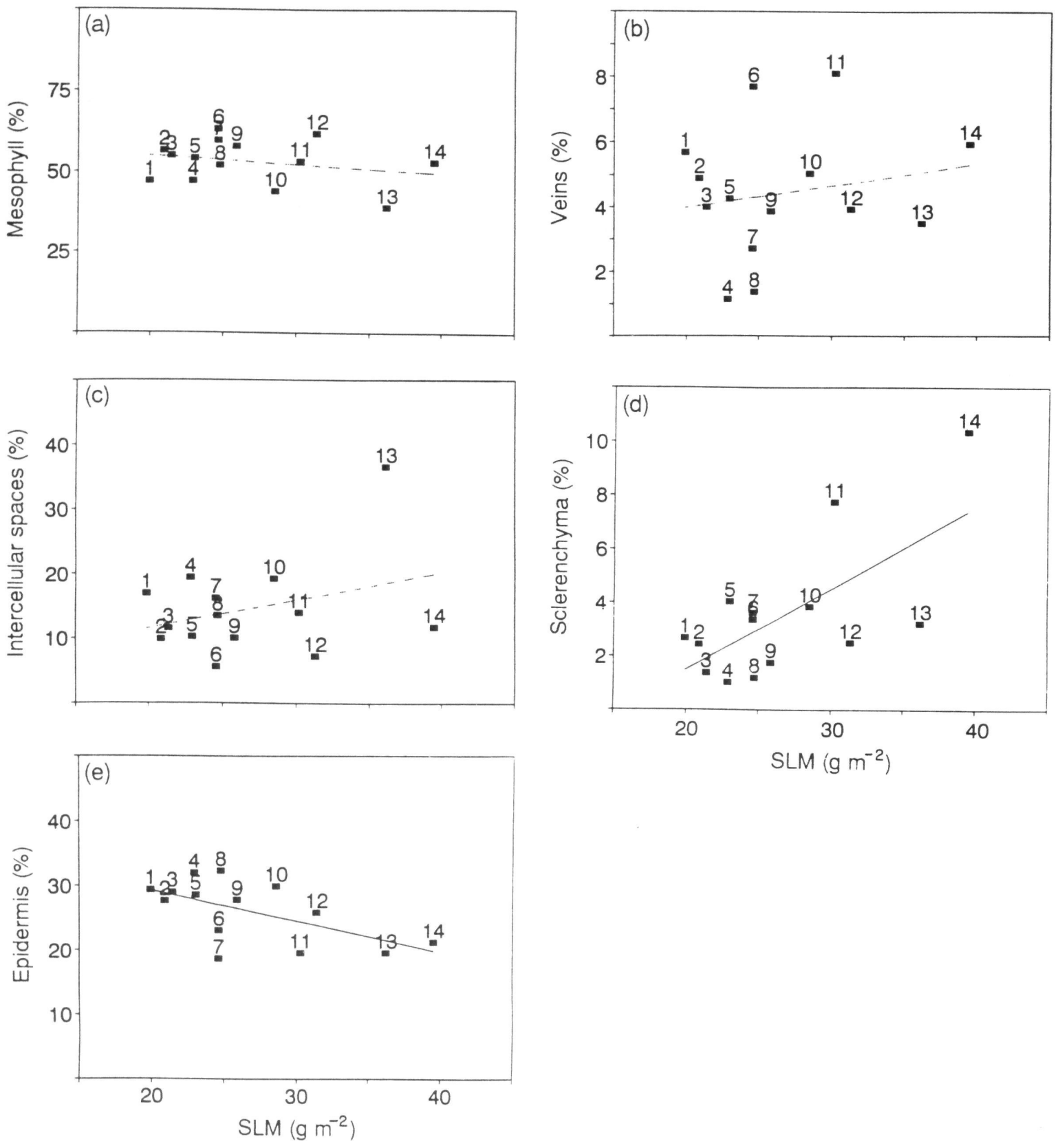

Figure 3. (a) Proportion of the total cross-sectional area occupied by mesophyll cells ($y = -0{\cdot}279x + 61{\cdot}4$, ns), (b) veins ($y = 0{\cdot}067x + 3{\cdot}24$, ns), (c) intercellular spaces ($y = 0{\cdot}441x + 2{\cdot}67$, ns), (d) non-veinal sclerenchymatic cells ($y = 0{\cdot}301x - 5{\cdot}33$, $p < 0{\cdot}01$, $r^2 = 0{\cdot}46$) and (e) epidermal cells ($y = -0{\cdot}482x + 39{\cdot}1$, $p < 0{\cdot}01$, $r^2 = 0{\cdot}35$) for the 14 grass species differing in SLM.

partly due to their relatively low content of cell wall material. Whether the larger volume of the epidermal cells also contributes to this higher cytoplasm content remains to be investigated. Secondly, we conclude that the grass species with high SLMs contain more of the comparatively thick sclerenchymatic cell walls. This might explain why relatively large amounts of cell wall compounds were found in species with high SLMs. In addition to changes in the amount of cell wall material per unit leaf area, changes in their chemical compositions might occur.

However, we do not know the specific gravity of these sclerenchymatic cell walls, so we cannot calculate how much of the variation in the total amount of cell wall compounds is due to variation in sclerenchyma. Part of the variation is probably due to extra investment of cell walls in other parts of the leaf. Such an analysis is beyond the scope of the present experiment. It thus appears that the high SLM (low SLA) of the slow-growing grasses is caused by anatomical differentiation. The observed chemical variation is likely to be a consequence of the anatomical differentiation.

Ecological implications

Interspecific variation in SLA, as observed in this growth chamber experiment, is correlated with variation in the natural habitats of these plants. That is, the species with high SLAs occur in productive habitats, whereas low-SLA

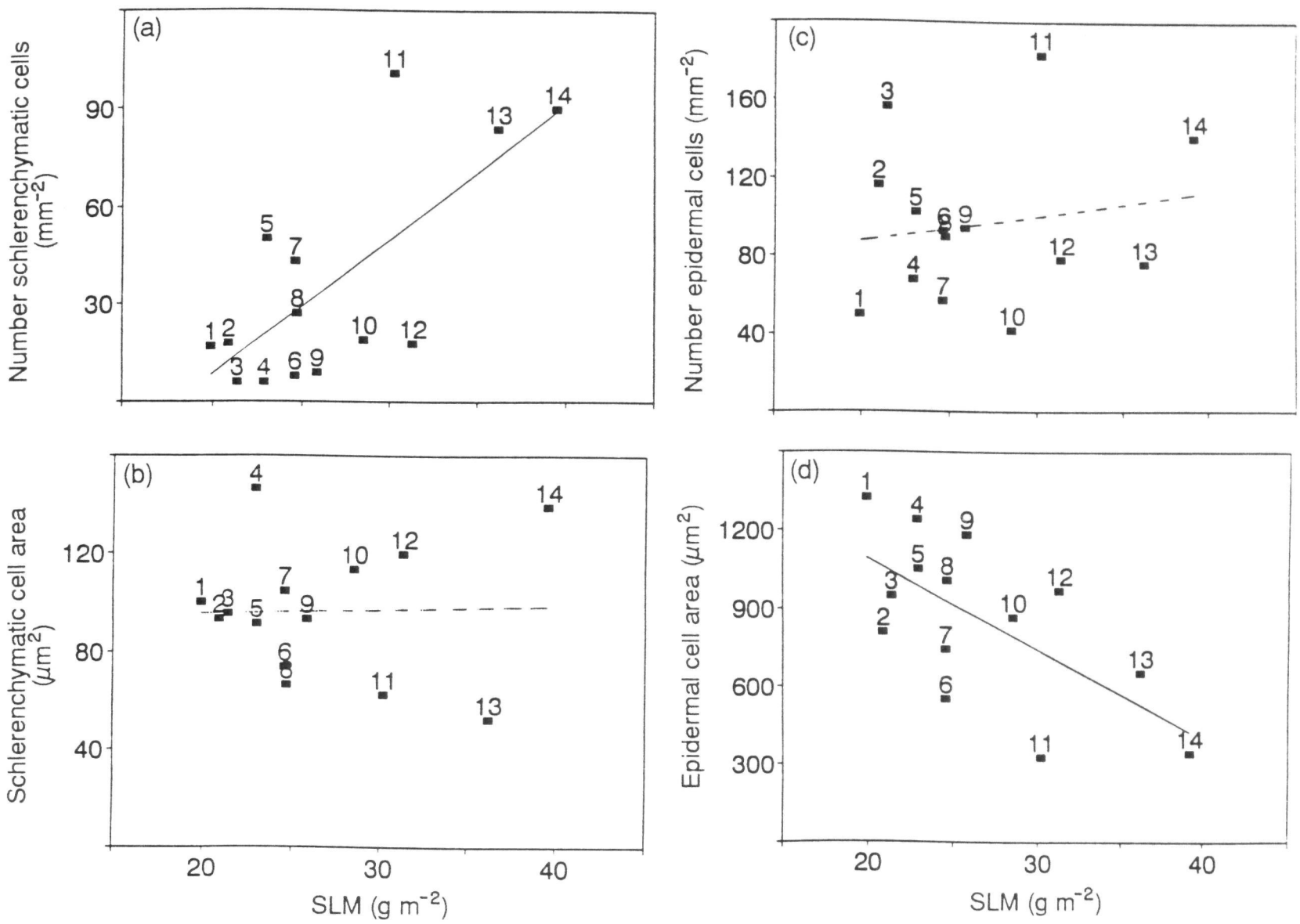

Figure 4. (a) The number of sclerenchymatic cells per cross-sectional area ($y = 1{\cdot}33x - 11{\cdot}3$, $p < 0{\cdot}01$, $r^2 = 0{\cdot}53$), (b) the average size of a single sclerenchymatic cell ($y = 0{\cdot}153x + 92$, ns), (c) the number of epidermal cells per unit area ($y = 1{\cdot}28x + 62{\cdot}2$, ns) and (d) the average size of a single epidermal cell ($y = -0{\cdot}532x + 1480$, $p < 0{\cdot}01$, $r^2 = 0{\cdot}41$) for the 14 grass species differing in SLM.

species occur in nutrient-poor environments. The SLA, or more precisely the factors causing a high or low SLA, partly determine the success of a species in a specific habitat. A high investment in (hemi)cellulose and secondary compounds increases the strength of the leaf. Moreover, secondary compounds such as lignin reduce the digestibility of the leaves. Secondary compounds also protect the leaves against herbivores or allow growth under less favourable conditions (Bell 1981; Bazzaz *et al.* 1987; Baas 1989). As a result, leaf longevity (Coley 1983) as well as the nutrient use efficiency (Aerts 1990) are increased.

Other authors have reported a relation between SLA, leaf anatomy and habitat similar to that found in the present experiment. For example, Baruch, Ludlow & Davis (1985) investigated the anatomy and physiology of African grasses introduced to Venezuela. These grasses, which have high SLA and a low proportion of rigid tissue, replaced a native grass on fertile sites. However, on less fertile sites, the native grass, which has a much lower SLA and a higher proportion of rigid tissue, resisted replacement. Similarly, Pammenter, Drennan & Smith (1986) reported on the introduction of *Agrostis* species on sub-antarctic islands. On these islands a native *Agrostis* species occurs which has a much lower SLA, due partly to a greater leaf thickness and partly to a higher proportion of support tissue. The introduced species was able to survive in wind-sheltered places, but on exposed sites only the native species could thrive.

We conclude that SLA, or more precisely the factors underlying SLA, are important in determining the success of species in stressful environments.

CONCLUSIONS

At the chemical level, differences in SLA are due to variation in lignin, organic N-compounds, (hemi)cellulose, total non-structural carbohydrates and organic acids. At the anatomical level these differences are due to leaf density, not to leaf thickness. The higher density of low-SLA leaves is partly due to the increased number of sclerenchymatic cells.

ACKNOWLEDGMENTS

We thank Dr Maarten Terlou for his help with the I.B.A.S. Image Processing System, Jopie Elings and Jan van Roessel for making the cross-sections and Yvonne van Berkel for her help with the chemical analyses. Furthermore, we would like to thank Gerard Niemann, Hans Lambers, John Evans and Eric Garnier for their valuable comments on earlier versions of this manuscript.

REFERENCES

Aerts R. (1990) Nutrient use efficiency in evergreen and deciduous species from heathlands. *Oecologia* **84,** 391–397.

Baas W.J. (1989) Secondary compounds, their ecological significance and consequences for the carbon budget. Introduction of the carbon/nutrient cycle theory. In *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants* (eds H. Lambers, M. L. Cambridge, H. Konings & T. L. Pons), pp. 313–340. SPB Academic Publishing, The Hague.

Baruch Z., Ludlow M.M. & Davis R. (1985) Photosynthetic response of native and introduced C_4 grasses from Venezuelan savannas. *Oecologia* **67,** 388–393.

Bazzaz F.A., Chiariello N.R., Coley P.D. & Pitelka L.F. (1987) Allocating resources to reproduction and defense. *Bioscience* **37,** 58–67.

Bell J.J. (1981) The physiological role(s) of secondary (natural) products. In The Biochemistry of Plants, Vol. 7 (ed. E. E. Conn), pp. 1–19. Academic Press, New York.

Coley P.D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* **53,** 209–233.

Dijkstra P. (1989) Cause and effect of differences in specific leaf area. In *Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants* (eds H. Lambers, M. L. Cambridge, H. Konings & T. L. Pons), pp. 125–140. SPB Academic Publishing, The Hague.

Dijkstra P. & Lambers H. (1989) A physiological analysis of genetic variation in relative growth rate within *Plantago major* L. *Functional Ecology* **3,** 577–587.

Grime J.P. & Hunt R. (1975) Relative growth rate: Its range and adaptive significance in a local flora. *Journal of Ecology* **63,** 393–422.

Hubbard C.E. (1968) *Grasses*. Penguin Books Ltd, Harmondsworth.

Lambers H. & Poorter H. (1992) Inherent variation in growth rate between higher plants: A search for physiological causes and ecological consequences. *Advances in Ecological Research* **23,** 187– 261.

Morrison I.M. (1972) Improvements in the acetyl bromide technique for the determination of lignin and the digestibility and its applications to legumes. *Journal of the Science of Food and Agriculture* **23,** 1463–1469.

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

Pammenter N.W., Drennan P.M. & Smith V.R. (1986) Physiological and anatomical aspects of photosynthesis of two *Agrostis* species at a sub-antarctic island. *The New Phytologist* **102,** 143– 160.

Poorter H. (1989) Growth analysis: towards a synthesis of the classical and functional approach. *Physiologia Plantarum* **75,** 237–244.

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

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

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

Singleton V.L. (1988) Wine analyses. In *Modern Methods of Plant Analysis*, New Series, Vol. 6 (eds H. F. Linskens & J. F. Jackson), pp. 200–207. Springer-Verlag, Berlin.

Van der Meijden R., Weeda E.J., Adema F.A.C.B. & De Joncheere G. J. (1987) *Flora van Nederland*. Wolters-Noordhoff, Groningen.

Witkowski E.T.F. & Lamont B.B. (1991) Leaf specific mass confounds leaf density and thickness. *Oecologia* **88,** 486–493.

Received 25 October 1993; received in revised form 23 February 1993; accepted for publication 14 March 1994