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THE CONTROL OF BIOMASS PARTITIONING IN PLANTS FROM “FAVOURABLE” AND “STRESSFUL” ENVIRONMENTS: A ROLE FOR GIBBERELLINS AND CYTOKININS

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Summary. Plants which naturally occur on nutrient-poor sites have inherently lower growth rates when compared to plants from more favourable habitats. This difference in growth rate is maintained when the species are compared under conditions which are optimal for their growth. Genetic variation in plant growth rate is predominantly due to variation in the pattern of biomass allocation in leaves, stems and roots: fast-growing genotypes have more leaf area per unit plant weight, but the leaves have a low biomass density. Gibberellins are involved in some of these inherent differences in biomass allocation.

Under nitrogen-stress, i.e. when exposed to nitrogen-poor conditions, a plant’s growth rate is reduced, when compared to that at an optimum supply of nutrients. This is partly accounted for by a smaller investment of resources in leaves and a greater investment in roots. Rates of photosynthesis per unit leaf area may also be less at a growth-limiting supply of nitrogen, whereas carbon requirements for respiration, expressed as a proportion of carbon fixed in photosynthesis, are increased. This is largely due to increased carbon requirements for root respiration, due to the relative increase in size of the root system. Under nitrogen-stress, the leaves of inherently fast-growing as well as those of slow-growing *Poa* species accumulate less protein and more cell-wall components. This is associated with an increase in leaf mass per unit leaf area and a relatively greater investment in sclerenchymatic cells. The volume occupied by epidermal cells is reduced, despite their relative increase in number, due to the decrease in epidermal cell size. Cytokinins play a major role in the shift in biomass allocation from leaves to roots, as dependent on nitrogen supply. There is no information to support a role of cytokinins in the changes in leaf anatomy.

This paper reviews recent information on the mechanisms accounting for the pattern of biomass allocation between leaves and roots. The role of gibberellins and cytokinins in the control of biomass allocation is also discussed. We conclude that gibberellins account for part of the genotypic variation in biomass allocation, whereas cytokinins are responsible for part of the phenotypic changes upon nitrogen-stress.

***Key words*:** biomass partitioning, cytokinins, gibberellins, nitrogen stress, relative growth rate

Introduction

Plants vary widely in their growth rate achieved under near-optimum conditions (RGR$_{max}$) and this variation is closely correlated with the species' ecological distribution. Fast-growing species tend to occur in temperate, productive environments, whereas slow-growing ones are from nutrient-poor, cold, or otherwise unfavourable habitats (Grime and Hunt, 1975; Chapin, 1980; Lambers and Poorter, 1992; Atkin et al., 1996). In a comparison of fast- and slow-growing grass species, the variation in RGR$_{max}$ is largely accounted for by variation in specific leaf area (leaf dry mass per unit leaf area, SLA; Poorter and Remkes, 1990; Garnier, 1992; Atkin et al., 1996). The low SLA of slow-growing species is associated with more non-veinal sclerenchymatic cells, relatively small epidermal cells (Van Arendonk and Poorter, 1994) and a high leaf-mass density (Garnier and Laurent, 1994; Ryser and Lambers, 1995). Gibberellins are likely to be involved in the control of some of these ecologically important genetic differences, because the level of this hormone is under genetic control (*Zea mays,* Rood et al., 1990a; *Brassica rapa,* Rood et al., 1990b; *Lycopersicon esculentum,* Koornneef et al., 1990; *Plantago major,* Dijkstra et al., 1990). Recent information on the role of gibberellins in the genetic variation in biomass allocation, partly from our own group, will be discussed in this paper.

When plants are growing at a limiting supply of nitrogen, their growth rate decreases, largely due to a decrease in their leaf area production leading to a lower leaf area per unit plant dry mass (LAR) (Gastal and Belanger, 1993; Van der Werf, 1993b; Belanger et al., 1994). The rate of photosynthesis may also decline, whereas the fraction of photoassimilates required in respiration increases (Van der Werf et al., 1993b, 1994). The decrease in LAR is invariably associated with a lower leaf weight ratio (leaf mass per unit plant mass, LWR; e.g. Hirose et al., 1988), and sometimes, but not invariably (Van der Werf et al., 1993b), with a decrease in specific leaf area as well. The fraction of biomass invested in roots (root weight ration, RWR) is invariably increased (e.g. Poorter et al., 1995; Ryser and Lambers, 1995). It is likely that cytokinins play a role in the control of biomass partitioning as affected by nitrogen supply (Van der Werf, 1995), as will be discussed as the second major topic in this paper. Table 1 gives a list of the parameters describing biomass partitioning and of other terms used throughout the text.

Table 1. Abbreviations used and the units in which they are expressed

LAR	=	leaf area ratio	(m^2 leaf kg^{-1} plant)
LWR	=	leaf weight ratio	(g leaf g^{-1} plant)
NAR	=	net assimilation rate	(g m^{-2} day^{-1})
RGR	=	relative growth rate	(mg g^{-1} day^{-1})
RWR	=	root weight ratio	(g root g^{-1} plant)
SLA	=	specific leaf area	(m^2 leaf kg^{-1} leaf)

The control of biomass allocation to roots

The mechanism of phloem unloading in roots has only been studied in detail for a very limited number of species, e.g. *Pisum sativum* (Dick and ap Rees, 1975) and *Zea mays* (Giaquinta et al*.,* 1983). Based on these studies it is widely believed that in roots sucrose moves from the phloem to other root cells via symplasmic diffusion and that this process does not require respiratory energy. If carbohydrates enter the sink cells in the roots via symplasmic diffusion, it appears that roots can exert little control over the rate of carbon import into the roots. The only way roots might determine the rate of phloem import is by their rate of structural growth, storage, respiration and exudation. However, current evidence indicates that the rate of sucrose import controls rates of growth and respiration, rather than being controlled by these processes (Lambers and Atkin, 1995). It is concluded that roots are a poor sink, to which only those assimilates are exported which are left over by sinks which have a capacity to exert control over the rate of phloem unloading. This model has been advocated for several decades (e.g. Brouwer, 1963, 1983) and we will briefly review evidence in support of this model.

In an attempt to test Brouwer's (1963) model, Nagel et al*.* (1994) compared the growth, photosynthesis, water relations and pattern of biomass partitioning of an ABA-deficient mutant *(sitiens)* with a wildtype tomato *(Lycopersicon esculentum)* . As found before for another ABA-deficient tomato mutant *(flacca;* Bradford et al*.,* 1983), *sitiens* has a greater stomatal conductance, a reduced hydraulic conductance of the roots and a decreased water potential in the leaves. It also incorporates less biomass into leaves and shows a relatively greater allocation of biomass to the roots. Nagel et al*.* (1994) concluded that carbon allocation to roots is enhanced as a result of less biomass accumulation in leaves and that their results support Brouwer's model. Similar results have been obtained with a number of gibberellin-deficient tomato mutants (Table 2). The gibberellin (GA) concentration in the mutants is too low to support normal leaf and stem growth. Consequently, more carbon is left for translocation to the roots. Thus, when leaf sink strength is reduced as a consequence of a low rate of GA production, more carbon is allocated to the roots. If leaf sink growth is reduced as a result of a low water potential in the root environment, again relatively more carbon is invested in roots (Creelman et al., 1990). The low water potential does not sustain leaf growth at the rate of control plants, because of the accumulation of ABA, which strongly inhibits leaf growth, whereas root growth is affected considerably less (Saab et al., 1990). As long as photosynthesis is inhibited less at a low water potential than leaf growth is, relatively more carbon is available for translocation to the roots. The control in plants grown at a low nitrogen supply, which also allocate relatively more carbon to their roots, is likely to be more complicated (Van der Werf, 1995), as will be discussed in a separate section.

Table 2. Relative growth rate, root weight ratio, specific leaf area, leaf and stem biomass density, and photosynthesis per unit leaf area of wild type and two GA-mutants of tomato

	WT	Mutant 1	Mutant 2
Growth rate mg g^{-1} day^{-1}	228.0	193.0	150.0
Root weight ratio g g^{-1}	0.08	0.12	0.18
Specific leaf area m^2 g^{-1}	22.0	17.0	13.0
Leaf mass density g DW g^{-1} FW	0.147	0.134	0.117
Stem mass density g DW g^{-1} FW	0.054	0.063	0.081
Leaf thickness mm	0.32	0.35	0.39
Photosynthesis μmol m^{-2} s^{-1}	7.9	7.8	7.7

We conclude that roots have very little *direct* control over the rate of carbon export from the leaves. If they do exert control, it is in an *indirect* manner, via their control of leaf growth. This lack of control over carbon import might well require a respiratory system which not only functions to generate ATP, but also allows the oxidation of sucrose to proceed with little ATP production (Lambers and Atkin, 1995; Wagner and Krab, 1995).

The role of gibberellins to account for inherent variation in biomass partitioning

The role of gibberellins (GAs) in the control of stem growth is well documented (Métraux, 1987). However, leaf growth is also controlled by GA, as evidenced by the leaf growth and leaf size of tomato mutants with decreased levels of GA (O.W.

Nagel, unpubl.). Gibberellins also control leaf size in *Brassica rapa* (Zanewich et al., 1990), *Thlaspi arvense* (Metzger and Hassebrock, 1990) and *Triticum aestivum* (McCaig and Morgan, 1993). It is likely that variation in leaf size between fast- and slow-growing species is at least partly associated with differences in the concentration of endogenous gibberellins (reviewed in Lambers and Poorter, 1992).

The exact mechanism by which GA controls leaf elongation has not been studied in such detail as the control by GA of stem growth. However, based on detailed work with stem and hypocotyl tissue, GA may promote cell elongation by enhancing cell wall extensibility due to removal of calcium from the apoplast (Métraux, 1987). Consequently, in genotypes with a low rate of GA production the rate of leaf cell expansion is less. In addition, cell division is likely to be less also. The lower rate of cell elongation and cell division is associated with smaller leaves, with less and smaller epidermal cells (H. Konings, unpublished). Possibly leaf cells can undergo cell division during a restricted period of their life-time only. However, they may continue to optimum conditions (Lambers and Poorter, 1992), Van Arendonk (unpubl.) made a comparison of four congeneric grass species *(Poa annua, P. trivialis, P. compressa* and *P. pratensis,* grown at both an optimum and a limiting nitrogen supply. At an optimum nutrient supply, the species differ in their maximum RGR, ranging from 184 mg g^{-1} day^{-1} for the slowest-growing *P. pratensis* to 248 mg g^{-1} day^{-1} for the fastest-growing *P. annua.* RGR does not correlate with NAR, but is significantly correlated with LAR, which is entirely accounted for by variation in SLA.

As expected, RGR and nitrogen concentrations decrease upon nitrogen stress (low nitrogen supply). The carbon concentration in leaf tissue decreases in *P. annua,* but increases in *P. pratensis.* In the other two species an increase in carbon concentration is found from optimal N-supply to a moderate N-limitation, followed by a decrease upon severe nitrogen stress. The decrease in RGR is accounted for by the decrease in LAR (both SLA and LWR). A pyrolysis-mass spectrometry analysis (cf. Niemann et al. 1995) showed that nitrogen stress decreases the concentration of protein and enhances that of (hemi)cellulose and lignin. The changes are largest in the fastest-growing *P. annua.*

We subsequently analyzed the anatomical basis of the decrease in SLA and changes in chemical composition, using transverse leaf sections of all four *Poa* species. Nitrogen stress invariably enhances the proportion of leaf tissue which is occupied by of sclerenchymatic cells from about 0.5 to 6%, predominantly due to an increase in number of these sclerencymatic cells. The area occupied by veinal tissue doubles, from approx. 4.5 to 9%, whereas that occupied by epidermal cells is more or less constant (25%), despite a substantial decrease in size of the epidermal cells, especially in *P. annua.* Mesophyll + intercellular spaces occupy a variable area of approx. 60% in all species and treatments.

A reduced nitrogen supply reduces the cytokinin export to the leaves (Kuiper et al., 1989; Fetene and Beck, 1993). Due to the lower cytokinin import into leaves, protein synthesis and growth of the leaves is less (Simpson et al., 1982; Kuiper and Staal, 1987). As long as photosynthesis declines less than leaf growth, more carbon is available for root growth. The roots may either grow at the same rate as those of control plants, or their growth may be increased (Van der Werf, 1995). Based on this information, it appears that the relative increase in biomass allocation to roots upon nitrogen stress is largely accounted for by the decrease in production and export of cytokinins from the roots. We have no information if cytokinins might also be involved in the anatomical changes in leaves upon nitrogen-stress. These anatomical changes are probably ecologically functional, in that the increase in sclerenchymatic and veinal tissue is likely to give better protection of the leaves (Lambers and Poorter, 1992). It is therefore of interest to reveal the physiological basis of these anatomical and chemical changes.

Conclusions

It is concluded that current evidence indicates that roots have very little direct control over the rate of carbon import from the leaves. However, they do exert indirect control, via their control of leaf growth which depends on the supply of cytokinins and water from the roots. This lack of control over carbon import might well require a respiratory system which not only functions to generate ATP, but also allows the oxidation of sucrose to proceed with little ATP production.

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References

Atkin, O.K., B. Botman, H. Lambers, 1996. The causes of inherently slow growth in alpine plants: an analysis based on the underlying carbon economies of alpine and lowland *Poa* species. Funct. Ecol., submitted

Belanger, G., F. Gastal, F. R. Warembourg, 1994. Carbon balance of tall fescue *(Festuca arundinacea* Schreb.): Effects of nitrogen fertuilization and the growing season. Ann. Bot., 74, 653-659.

Bradford, K. J., 1983. Water relations and growth of the *flacca* tomato mutant in relation to abscisic acid. Plant Physiol., 72, 251-255.

Brouwer, R., 1963. Some aspects of the equilibrium between overground and underground plant parts. Meded. Inst. Biol. Scheikd. Onderzoek Landbouwgewassen, 213, 31-39.

Brouwer, R., 1983. Functional equilibrium: sense or nonsense? Neth. J. Agric. Sci., 31, 335-348.

Chapin, F. S. III, 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst., 11, 233-260.

Creelman, R. A., H. S. Mason, R. J. Bensen, J. S. Boyer, J. E. Mullet, 1990. Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings. Analysis of growth, sugar accumulation, and gene expression. Plant Physiol., 92, 205-214.

Dick, P. S., T. ap Rees, 1975. The pathway of sugar transport in roots of *Pisum sativum.* J. Exp. Bot., 26, 305-314.

Dijkstra, P., 1989. Cause and effect of differences in SLA. 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, SPB Academic Publishing, The Hague.

Dijkstra, P., Ter Reegen, H. Kuiper, P. J. C. 1990. Relation between relative growth rate, endogenous gibberellins, and the response to applied gibberellic acid for *Plantago major.* Physiol. Plant., 79, 629-634.

Fetene, M., E. Beck, 1993. Reversal of direction of photosynthete allocation in *Urtica dioica* L. plants by increasing cytokinin import into the shoot. Bot. Acta, 106, 235-240.

Garnier, E., 1992. Growth analysis of congeneric annual and perennial grass species. J. Ecol., 80, 665-675.

Garnier, E., G. Laurent, 1994. Leaf anatomy, specific leaf mass and water content in congeneric annual and perennial grass species. New Phytol., 128, 725-736.

Gastal, F., G. Belanger, 1993. The effects of nitrogen fertilization and the growing season on photosynthesis of field-grown tall fescue *(Festuca arundinacea* Schreb.) canopies. Ann. Bot., 72, 401-408.

Giaquinta, R. T., W. Lin, N. L. Sadler, V.R. Franceschi, 1983. Pathway of phloem unloading of sucrose in corn roots. Plant Physiol., 72, 362-367.

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

Hirose, T., A. H. J. Freijsen, H. Lambers, 1988. Modelling of the response to nitrogen availability of two *Plantago* species grown at a range of exponential nutrient addition rates. Plant Cell Environ., 11, 827-834.

Koornneef, M., T. D. G. Bosma, C. J. Hanhart, J. H. van der Veen, J. A. D. Zeevaart, 1990. Isolation and characterization of gibberellin-deficient mutants in tomato. Theor. Appl. Gen., 80, 852-857.

Kuiper, D., M. Staal, 1987. The effect of exogenously supplied plant growth substances on the physiological plasticity in *Plantago major* ssp *major*: Responses of growth, shoot to root ratio and respiration. Physiol. Plant., 69, 651-658.

Kuiper, D., P. J. C. Kuiper, H. Lambers, J. T. Schuit, M. Staal, 1989. Cytokinin contents in relation to mineral nutrition and benzyladenine addition in *Plantago major* ssp. *pleiosperma.* Physiol. Plant., 75, 511-517.

Lambers, H., H. Poorter, 1992. Inherent variation in growth rate between higher plant: A search for physiological causes and ecological consequences. Adv. Ecol. Res., 22, 187-261.

McCaig, T. N., J. A. Morgan, 1993. Root and shoot dry matter partitioning in near-isogenic wheat lines differing in height. Can. J. Plant Sci., 73, 679-689.

Métraux, J. -P., 1987. Gibberellins and plant cell elongation. In: Plant Hormones and Their Role in Plant Growth and Development. Ed. P. J. Davies, Martinus Nijhoff Publishers, Dordrecht., 296-317.

Metzger, J. D., A. T. Hassebrock, 1990. Selection and characterization of a gibberellin-deficient mutant of *Thlaspi arvense* L. Plant Physiol., 94, 1655-1662.

Nagel, O. W., H. Konings, H. Lambers, 1994. Growth rate, plant development and water relations of the ABA-deficient tomato mutant *sitiens.* Physiol. Plant., 92, 102-108.

Niemann, G. J., J. B. M. Pureveen, G.B. Eijkel, H. Poorter, J.J. Boon, 1995. Differential chemical allocation in plant adaptation: a PY-MS study of 24 species differing in relative growth rate. Plant Soil, in press.

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

Rood, S. B., R. I. Buzzell, D. J. Major, R. P. Pharis, 1990a. Gibberellins and heterosis in maize, Quantitative relationships. Crop Sci., 30, 281-286.

Rood, S. B., K. P. Zanewich, D. F. Bray, 1990b. Growth and development of *Brassica* genotypes differing in endogenous gibberellin content. II. Gibberellin content, growth analyses and cell size. Physiol. Plant., 79, 679-685.

Ryser, P., H. Lambers, 1995. Root and leaf attributes accounting for the performance of fast- and slow-growing grasses at different nutrient supply. Plant Soil, 170, 251-265.

Saab, I. N., R. R. Sharp, J. Pritchard, G. S. Voetberg, 1990. Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth of maize seedlings at low water potentials. Plant Physiol., 93, 1329-1336.

Simpson, R. J., H. Lambers. M. J. Dalling, 1982. Kinetin application to roots and its effects on uptake, translocation and distribution of nitrogen in wheat *(Triticum aestivum)* grown with a split root system. Physiol. Plant., 56, 430-435.

Terry, N., 1970. Developmental physiology of sugar-beet. II. Effect of temperature and nitrogen supply on the growth, soluble carbohydrate content and nitrogen content of leaves and roots. J. Exp. Bot., 21, 477-496.

Van Arendonk, J. J. C. M., H. Poorter, 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 Werf, A., 1995. Growth analysis and photoassimilate partitioning. In: Photoassimilate Distribution in Plants and Crops: Source-sink Relationships. Eds. E. Zamski, A.A. Schaffer, Marcel Dekker, in press.

Van der Werf, A., M. Van Nuenen, A. J. Visser, H. Lambers, 1993a. Contribution of physiological and morphological plant traits to a species' competitive ability: a hypothesis for inherently fast- and slow-growing monocotyledonous species. Oecologia, 94, 434-440.

Van der Werf, A., F. Schieving, H. Lambers, 1993b. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast- and slow-growing species. Funct. Ecol., 7, 63-74.

Van der Werf, A., H. Poorter, H. Lambers, 1994. Respiration as dependent on a species' inherent growth rate and on the nitrogen supply to the plant. In: A Whole-Plant Perspective of Carbon-Nitrogen Interactions, Eds. J. Roy, E. Garnier, SPB Academic Publishing, The Hague, 61-77.

Wagner, A. M., K. Krab, 1995. The alternative respiration pathway in plants: role and regulation. Physiol. Plant., in press.

Zanewich, K. P., S. B. Rood, P. H. Williams, 1990. Growth and development of *Brassica* genotypes differing in endogenous gibberellin content. II. Leaf and reproductive development. Physiol. Plant., 79, 673-678.