

'The functional equilibrium', nibbling on the edges of a paradigm¹

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Summary

This paper discusses the mechanisms underlying the 'functional equilibrium' between above-ground and below-ground organs of higher plants. Evidence is presented that the alternative path contributes to a significant extent in root respiration. Since this path is only engaged when the supply of carbohydrates to a sink exceeds its demand, it is suggested that root growth is not limited by the supply of carbohydrates. Accordingly, it is concluded that the close correlation between carbohydrate supply to the roots and root growth cannot be explained by a limitation of root growth by carbohydrates. A satisfactory alternative explanation cannot be provided yet.

Information is included on the translocation of N from the leaves to the roots at both an optimal and a limiting supply of NO₃ to wheat plants. The proportion of N, first translocated to the leaves and then retranslocated to the roots was highest at a limiting N supply. It is suggested that leaves are unable to incorporate all imported N under N-limiting conditions and that the excess N is then translocated back to the roots. This finding indicates that the close correlation between N supply to the plants and growth of the shoot cannot simply be explained by the roots having first priority with respect to recently absorbed N.

Introduction

Brouwer (1963) proposed a theory to explain the 'functional equilibrium' between above-ground and below-ground organs of higher plants. It is clear that Brouwer's theory has provided an excellent framework to understand the plant's response to its environment (nutrient and water supply, light intensity), and continues to do so. Now, 20 years after Brouwer proposed his theory, there is reason to believe that the mechanisms underlying the functional equilibrium are more complicated than was originally thought. It is the aim of this paper to indicate discrepancies between Brouwer's theory on the 'functional equilibrium' and recent results on a) respira-

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tion via a non-phosphorylating electron transport pathway; b) circulation of N between shoots and roots.

Brouwer's theory on the 'functional equilibrium' has definitely provided many scientists with a framework for the last 20 years. And the work presented in this paper, which culminated in the nibbling on the edges of the 'functional equilibrium', has clearly been stimulated by Brouwer's paradigm.

The functional equilibrium and a description of the problem to be discussed

This theory is based on two major observations. The first one is that upon removal of parts of the shoot, root growth is reduced. Similarly, under conditions of low light intensity, root growth is more reduced than shoot growth. Root growth is supposed to be limited by the supply of carbohydrates from the leaves (Brouwer, 1963, 1967). This explanation diametrically opposes recent results on the contribution of the alternative pathway in root respiration. As will be discussed in the next section this pathway is only engaged when there is an excess of carbohydrates. Such an *excess* must clearly be unreconcilable with a *limitation*!

The second observation is that under nitrogen deficiency roots continue to develop whereas shoot growth is reduced. Based on this observation it was postulated that 'at impending nitrogen deficiency the greater part of the nitrogen uptake is used by the root system for growth, leaving only a small part to be translocated to the shoot' (Brouwer, 1963). Simpson et al. (1982a) recently showed that under N-deficiency vegetative wheat roots export large quantities of N to the leaves. As will be further discussed in a following section, a large proportion of the translocated N was subsequently translocated back to the roots. This suggests that although N was preferentially used for growth of roots, this was not due to a low rate of export of N to the shoots, as postulated by Brouwer.

This paper deals particularly with the mechanism underlying the functional equilibrium, whereas the functional equilibrium per se is accepted as a well-established fact.

Root respiration and the energy overflow hypothesis

Until about a decade ago plant respiration was considered as a process to generate metabolic energy by the oxidation of substrates such as carbohydrates and fats. Then, Bahr & Bonner (1973a) discovered that mitochondria from the spadix of skunk cabbage contain an electron transport path, which, unlike the classical cytochrome path, is not coupled to the conservation of metabolic energy. Since then it has been demonstrated that mitochondria from many higher plant tissues contain this non-phosphorylating, CN-resistant electron transport path, the 'alternative path' (Laties, 1982). Bahr & Bonner (1973b) showed that in isolated mitochondria the alternative path does not compete for electrons with the cytochrome path and that it is only engaged upon saturation of the cytochrome path with electrons. Theologis & Laties (1978) showed that also in slices of sweet potato the alternative path is only engaged upon saturation of the phosphorylating chain. This observation was

confirmed with roots of *Pisum sativum* (De Visser & Blacqui re, 1984).

Root respiration often occurs to a large extent via the alternative path, e.g. 40 % in *Zea mays* roots (Lambers et al., 1982). In maize roots saturation of the cytochrome path was shown to be due to a constraint of the flux through the cytochromes by oxidative phosphorylation (Day & Lambers, 1983). The alternative path in maize roots is not only operative in the mature parts but also in the tips (Lambers & Posthumus, 1980). It is therefore concluded that the engagement of the alternative path in maize roots is due to a restriction of the cytochrome path by the shortage of ADP, due to a limitation by the energy-requiring processes. If the demand for metabolic energy were higher, the cytochrome path would be less restricted.

The alternative path is only engaged when the cytochrome path becomes saturated with electrons. It is therefore not unexpected that the activity of the alternative path in roots is high when the availability of respiratory substrates (carbohydrates) is high (Table 1). The activity declines when the availability of carbohydrates in the roots decrease, e.g. in cucumber roots at the end of a long night, when growth occurs at low light intensity (Lambers, 1982). Addition of glucose to slices of wheat leaves, harvested at the end of the night (when the respiratory activity is considerably lower than in slices harvested at the end of the day), increases the activity of the alternative path, but not that of the cytochrome path (Azc n-Bieto et al., 1983). In a recent review (Lambers, 1982) more examples are included which show that the alternative path is only operative when the input of respiratory substrates is high. It is concluded that carbohydrates are only oxidized via the alternative path when all other carbohydrate-requiring processes in the tissue are saturated: the alternative path acts as an 'energy overflow' (Lambers, 1982). This implies that whenever the alternative path is shown to be significantly contributing to root respiration, then these roots are receiving more carbohydrates than is required for growth and other C-requiring processes. So it is concluded that when the alternative path contributes to root respiration, root growth is *not* limited by carbohydrates.

It is too early to state with any degree of certainty what an 'energy overflow me-

Table 1. Soluble carbohydrate content and respiration via the cytochrome and alternative pathways.

Species	Soluble carbohydrate content	Respiration via			Reference
		cytochrome path	alternative path	expressed in unit	
<i>Cucumis sativus</i> (roots)	2.5 g kg ⁻¹ in dry wt	0.20	0.01	mg O ₂ s ⁻¹ g ⁻¹	Challa, 1976; Lambers, 1980
	10 g kg ⁻¹ in dry wt	0.20	0.20	mg O ₂ s ⁻¹ kg ⁻¹	
<i>Solanum tuberosum</i> (ten-day-old callus-forming tubers)	5 g kg ⁻¹ in fresh wt	0.50	0.17	mol O ₂ s ⁻¹ kg ⁻¹	Van der Plas et al., 1983
	25 g kg ⁻¹ in fresh wt	0.50	0.33	mmol O ₂ s ⁻¹ kg ⁻¹	
<i>Triticum aestivum</i> (leaves)	7 mmol C m ⁻²	0.42	0	mol O ₂ m ⁻² s ⁻¹	Azc�n-Bieto et al., 1983
	74 mmol C m ⁻²	0.46	0.38	mol O ₂ m ⁻² s ⁻¹	

tabolism' really means for the functioning of an intact plant. *Plantago coronopus* responded upon a change in the salinity level in the root environment (NaCl 0-50 mmol/l) with a decreased activity of the alternative path in the roots and the accumulation of sorbitol (Lambers et al., 1981). Neither growth nor photosynthesis were affected by the change in salinity. It was found that the amount of C 'saved' by reducing the activity of the alternative path was the same as that utilized in the synthesis of sorbitol in the roots. This, and other experiments in the same vein, included in a recent review (Lambers, 1982), has led to the suggestion that the alternative path may be significant in dampening the effect of environmental fluctuations.

In an agricultural context, it is important to elucidate if the alternative path is indeed significant in 'dampening' the effect of fluctuations in the environment. The alternative path consumes a significant portion of the daily produced photosynthates, and selection against such a wasteful trait might thus lead to improved productivity, provided no penalties are involved in such a selection.

Towards a mechanism explaining 'limitation' simultaneously with 'excess'

Brouwer (1963, 1967) demonstrated that root growth is, in the long run, correlated with the carbohydrate supply to the roots. In the preceding section it has been shown that carbohydrates do not limit root metabolism. To explain this discrepancy it has to be assumed that the carbohydrate supply is a regulating factor in root growth, without being limiting. How can this be envisaged? According to the Münch hypothesis (Canny, 1973) transport of all compounds in the phloem depends on the loading and unloading of sucrose. If it is assumed that the factor(s) regulating root growth travel in a fixed ratio with sucrose, a close correlation of carbohydrate supply to the roots and root growth is expected. As to the nature of these growth-regulating factors one can merely speculate. They could be of hormonal nature, they might be vitamins or perhaps even protons or potassium ions.

It may appear that the subtle shift from the *limitation*, as proposed by Brouwer, to the *regulation* by carbohydrates, as proposed here, is of academic significance only. However, there are also important applied aspects which can best be illustrated by some recent results. At a decreasing rate of photosynthesis and of the carbohydrate supply to the roots of *Plantago lanceolata*, root growth (in mg g⁻¹ day⁻¹) was constant (Lambers et al., 1981). This was possible since the decreased supply of carbohydrates caused a decreased activity of the alternative path, whilst utilization of C by the cytochrome path and in growth was not affected. Similarly, in a comparison of two genotypes of *P. lanceolata* (D. Kuiper, unpublished) it was found that the fast growing genotype respired only to a small extent via the alternative path in the roots, whereas the slow growing genotype respired to a large extent via this non-phosphorylating path. Total root respiration (i.e. the sum of the fluxes via both the cytochrome and alternative paths) was about the same for the two genotypes (Table 2). Similar results have been obtained with genotypes of *Plantago major*, (Kuiper, 1983).

Table 2. Root respiration ($\text{mg O}_2 \text{ s}^{-1} \text{ kg}^{-1}$ in dry weight) and root growth ($\text{g day}^{-1} \text{ kg}^{-1}$ in dry weight) of two genotypes of 47-day-old *Plantago lanceolata* plants (unpublished data from D. Kuiper).

Genotype	Growth rate	Respiration	
		cytochrome path	alternative path
Male sterile	'high'	1.39	0.42
Hermaphrodite	'low'	0.97	0.83

Uptake, transport and utilization of nitrogen

I now would like to make some remarks on the second aspect of the functional equilibrium. Brouwer (1963) postulates that the roots enjoy first priority for N at impending N deficiency. Under such conditions the roots are supposed to export only a small portion of the recently absorbed N.

In recent years Pate and co-workers (e.g. Pate 1980, Pate et al., 1979a, 1979b) have provided a wealth of information on translocation of N in white lupin. Pate et al. (1979a) demonstrated that a large proportion of all N exported from the roots to the shoots is translocated back to the roots. Under conditions of impending N deficiency this proportion was 58 % in ca. 60-day-old lupin plants. Lambers et al. (1982) used a 'split root technique' to quantify translocation of N from the leaves to the roots. Half of the root system was supplied with NO_3 , whilst the other half was deprived of nitrate. The N supply to the NO_3 -deprived roots was calculated from the N increment in these roots. C translocation to both the NO_3 -supplied and the NO_3 -deprived roots was derived from data on growth and respiration of the roots. This allowed calculation of the C/N ratio of the phloem stream feeding the NO_3 -deprived roots. Assuming that the C/N ratio is similar for the phloem sap feeding the NO_3 -fed roots, the amount of N exported from the shoot to the NO_3 -fed roots can be calculated. In vegetative wheat plants the proportion of N which is first translocated to the shoot and then translocated back to the roots was higher under N-deficient conditions than at an optimal supply of NO_3 (Table 3). This indicates that at a limiting N supply to wheat plants the roots continue to export N to the shoot, but that the shoot returns a high proportion of the imported N. Consequently, the roots enjoy first priority with respect to N incorporation, in agreement with the functional equilibrium. Only the mechanism is different from that proposed by Brouwer (1963). Whereas Brouwer suggested that the roots incorporate relatively much N and *consequently* export less N under N-deficient conditions, the present results indicate that the roots' priority is largely determined by the fact that the shoots return a greater proportion of the imported N. It has been suggested (Simpson et al., 1982b) that this phenomenon can be explained by the effect of N in the environment on the production of cytokinin in the root tips. At a limiting N supply the production of cytokinin is reduced (Torrey, 1976). Consequently, the export of cytokinins to the leaves is reduced and this leads to reduced incorporation of N in the leaves. This then would cause increased export of N from the leaves to the roots. Although

Table 3. Transport and utilization of N in wheat grown under N-optimal or N-deficient conditions, in $\mu\text{g day}^{-1}$. Data from Lambers et al., 1982.

	N-optimal conditions	N-deficient conditions
N increment in shoots	515	281
N translocation to roots (from the shoots)	60	60
N translocation to roots/N translocation to shoots	0.11	0.18
N increments in roots	43	34

Simpson et al. (1982b) obtained evidence in favour of their hypothesis, further testing is warranted. It should also be added that despite their pronounced effect on N incorporation in the leaves, kinetin did not have any effect on the dry-matter production of the shoot.

Concluding remarks

The data discussed in the present paper have not at all changed our view on the *existence* of a functional equilibrium. However, I believe that the present data provide evidence that carbohydrates, although significant in *regulating* root growth, are not really *limiting* this process. Further research will have to be done to explain the clear correlation between carbohydrate supply to the roots and root growth. The mechanism of regulating shoot growth by N also appears to be more subtle than simply by *limitation*. Cytokinins may be the link between N-supply and the shoot's N-metabolism, but there is no evidence to suggest that they are of major importance for shoot dry-matter production.

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References

- Azcón-Bieto, J., H. Lambers & D. A. Day, 1983. The effect of photosynthesis and carbohydrate status on respiratory rates and the involvement of the alternative path in leaf respiration. *Pl. Physiol.* (in press).
- Bahr, J. T. & W. D. Bonner Jr., 1973a. Cyanide-insensitive respiration. I. The steady states of skunk cabbage spadix and bean hypocotyl mitochondria. *J. biol. Chem.* 248: 3441-3445.
- Bahr, J. T. & W. D. Bonner Jr., 1973b. Cyanide-insensitive respiration. II. Control of the alternate pathway. *J. biol. Chem.* 248: 3446-3450.
- Canny, M. J., 1973. Phloem translocation. Cambridge University press. ISBN 0-521-20047-4.
- Brouwer, R., 1963. Some aspects of the equilibrium between overground and underground plant parts. *Meded. Inst. biol. scheikd. Onderz. Landb. Gewass.* 213: 31-39.

- Brouwer, R., 1967. Beziehungen zwischen Spross- und Wurzelwachstum. *Angew. Bot.* 41: 244-254.
- Challa, H., 1976. An analysis of the diurnal course of growth, carbon dioxide exchange and carbohydrate reserve content of cucumber. Ph. D. Thesis, Wageningen. Pudoc, Wageningen, ISBN 90-220-0623-9.
- Day, D. A., & H. Lambers, 1983. The regulation of glycolysis and electron transport in roots. *Physiologia Plant.* 58: 155-160.
- De Visser, R. & T. Blacqui re, 1983. Inhibition and stimulation of root respiration in *Pisum* and *Plantago* by hydroxamate. Its consequences for the assessment of the activity of the alternative path activity. *Pl. Physiol.* (submitted for publication).
- Kuiper, D., 1983. Genetic differentiation in *Plantago major*: Growth and root respiration and their role in phenotypic adaptation. *Physiologia Plant.* 57: 222-230.
- Lambers, H., 1980. The physiological significance of cyanide-resistant respiration. *Plant, Cell, and Environment* 3: 293-302.
- Lambers, H., 1982. Cyanide-resistant respiration: A non-phosphorylating electron transport pathway acting as an energy overflow. *Physiologia Plant.* 55: 478-485.
- Lambers, H. & F. Posthumus, 1980. The effect of light intensity and relative humidity on growth and root respiration of *Plantago lanceolata* and *Zea mays*. *J. exp. Bot.* 31: 1621-1630.
- Lambers, H., T. Blacqui re & C. E. E. Stuiwer, 1981. Interactions between osmoregulation and the alternative respiratory pathway in *Plantago coronopus* as affected by salinity. *Physiologia Plant.* 51: 63-68.
- Lambers, H., D. A. Day & J. Azc n-Bieto, 1983. Cyanide-resistant respiration in roots and leaves. Measurements with intact tissues and isolated mitochondria. *Physiologia Plant.* 58: 148-154.
- Lambers, H., F. Posthumus, I. Stulen, L. Lanting, S. J. Dijk van de & R. Hofstra, 1981. Energy metabolism of *Plantago lanceolata* L. as dependent on the supply of mineral nutrients. *Physiologia Plant.* 51: 93-98.
- Lambers, H., R. J. Simpson, V. C. Beilharz & M. J. Dalling, 1982. Growth and translocation of C and N in wheat (*Triticum aestivum*) grown with a split root system. *Physiologia Plant.* 56: 421-429.
- Laties, G. G., 1982. The cyanide-resistant, alternative path in higher plant respiration. *A. Rev. Pl. Physiol.* 33: 519-555.
- Pate, J. S., 1980. Transport and partitioning of nitrogenous solutes. *A. Rev. Pl. Physiol.* 31: 313-340.
- Pate, J. S., D. B. Layzell & C. A. Atkins, 1979a. Economy of carbon and nitrogen in a nodulated and non-nodulated (NO₃-grown) legume. *Pl. Physiol.* 64: 1083-1088.
- Pate, J. S., D. B. Layzell & D. L. McNeil, 1979b. Modeling the transport and utilization of carbon and nitrogen in a nodulated legume. *Pl. Physiol.* 63: 730-737.
- Simpson, R. J., H. Lambers & M. J. Dalling, 1982a. Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Physiologia Plant.* 56: 11-17.
- Simpson, R. J., H. Lambers & M. J. Dalling, 1982b. Kinetin application to roots and its effects on uptake translocation and distribution of nitrogen in wheat (*Triticum aestivum*) grown with a split root system. *Physiologia Plant.* 56: 430-435.
- Theologis, A. & G. G. Laties, 1978. Relative contribution of cytochrome-mediated and cyanide-resistant electron transport in fresh and aged potato slices. *Pl. Physiol.* 62: 232-237.
- Torrey, J. G., 1976. Root hormones and plant growth. *A. Rev. Pl. Physiol.* 27: 435-460.
- Van der Plas, L. H. W. & M. J. Wagner, 1983. Regulation of the activity of the alternative oxidase in callus-forming potato tuber tissue discs. *Physiologia Plant.* 58: 311-317.