

Propositions

1. Increasing biomass production during the critical period of grain number determination can help to increase the yield potential both in wheat and oilseed rape (this thesis).
2. Yield formation in oilseed rape is more source-limited during grain filling than in wheat (this thesis).
3. The photosynthetic efficiency at low light depends on the leaf N content in photosynthetic tissues (this thesis).
4. The light-associated vertical leaf N distribution changes dynamically during crop growth and is regulated by N availability (this thesis).
5. N availability, through its effects on tiller survival and growth, limits the effects of high CO₂ on biomass and leaf area production in wheat (this thesis).
6. Oscar Wilde thought that among the arts, literature was superior to painting because of its temporal component, which allows involving a psychic response to one's own story. Physiological modelling will remain more next to painting than to literature until acclimation to environmental factors is dynamically simulated.
7. In international relations between countries, the recognition of human rights abuse is generally overshadowed by the promise of a profitable trade balance.
8. The first difficult task of young democracies is to reform their corrupt and inefficient judiciary systems.
9. International co-operation for the development of low-cost vaccines against infectious diseases can have an enormous positive impact on the welfare of people in need in developing countries.
10. Matching long term objectives to short term cash flow is one of the major problems of universities today.

F. Dreccer

Radiation and nitrogen use in wheat and oilseed rape crops
Wageningen, 8 December 1999

Acknowledgements

The research done for this thesis was financed by the Laboratory of Theoretical Production Ecology of Wageningen University (The Netherlands), the Research Institute for Agrobiolgy and Soil Fertility (The Netherlands), Cátedra de Cerealicultura de la Facultad de Agronomía, Universidad de Buenos Aires (Argentina) and Consejo Nacional de Investigaciones Científicas y Técnicas (Argentina).

The publication of this thesis was possible thanks to Wageningen University and the foundation 'Fonds Landbouw Export-Bureau 1916/1918'.

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**Radiation and nitrogen use
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ANOS 201, 2780.

Radiation and nitrogen use in wheat and oilseed rape crops

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
dr. C.M. Karssen
in het openbaar te verdedigen
op woensdag 8 december 1999
des namiddags half twee in de Aula

15N 972186

DRECCER, María Fernanda

Radiation and nitrogen use in wheat and oilseed rape crops

María Fernanda Dreccer .- [S.l.:s.n.]

Thesis Wageningen Universiteit. - With ref. - With summary in Dutch and Spanish

ISBN 90-5808-162-1

Subject headings: biomass, radiation use efficiency, nitrogen, vertical leaf nitrogen distribution, leaf photosynthesis, CO₂, wheat, oilseed rape

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BIBLIOTHEEK
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Abstract

Dreccer, M. F. **Radiation and nitrogen use in wheat and oilseed rape crops.** PhD thesis, Laboratory of Theoretical Production Ecology, Wageningen Agricultural University, The Netherlands. 136 pp. with English, Dutch and Spanish summaries.

This thesis focuses on understanding the physiological bases of biomass production per unit intercepted irradiance (RUE) in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.), combining traditional experimentation with modelling. RUE has been indicated as a physiological attribute that limits yield potential in different crops (Chapter 2) and is affected by N availability. The response of wheat and oilseed rape to N availability was compared in terms of yield, intercepted radiation, RUE, photosynthesis and N use efficiency during the critical period for grain number definition and grain filling (Chapters 3 and 4). Oilseed rape had lower harvest index for biomass, and yield was more limited by the capacity for assimilate production than that of wheat (Chapter 3). During the critical period for grain number definition, RUE in oilseed rape reached a higher value than wheat at high N and was more sensitive to N availability (Chapter 3). The higher leaf photosynthesis per unit leaf N in oilseed rape than wheat and the fact that oilseed rape leaves were increasingly less-saturated by light due to the inflorescence growth (more at high N supply) contributed to explain this response (Chapter 4). The vertical pattern of leaf N distribution in a canopy determines canopy photosynthesis, and manipulating it has been suggested as a route to maximise RUE (Chapter 2). In wheat and oilseed rape, leaf N was partitioned in relation to the gradient of absorbed irradiance (Chapters 4,5,6). The light-associated leaf N distribution changed dynamically during crop growth and was regulated by N availability but not by atmospheric CO₂ concentration (Chapter 4,5,6). The vertical leaf N distribution was such that kept the capacity for photosynthesis at high and low light in balance (Chapter 5), close to the theoretical optimum maximising canopy photosynthesis (Chapter 4 and 5) and did not differ remarkably between species (Chapter 4). Finally, the response of canopy photosynthesis to N availability could change under high atmospheric CO₂ if acclimation occurs, i.e. photosynthesis and the synthesis of photosynthetic enzymes decrease and N is re-allocated within the photosynthetic machinery and within the plant. The interaction between CO₂ level and N availability was studied in wheat during the critical period for grain number definition. At high N availability, high CO₂ resulted in increased biomass production due to enhanced tiller growth. Supplying N in proportion to growth could not prevent photosynthetic acclimation in response to CO₂ with leaf age (Chapter 6). The contribution of the results to the existing knowledge and its applicability are discussed in the context of genetic crop improvement and N fertilisation and future research needs are indicated (Chapter 7).

Key words: biomass, radiation use efficiency, nitrogen, vertical leaf nitrogen distribution, leaf photosynthesis, CO₂, wheat, oilseed rape

PREFACE

Many people contributed to the completion of this work from inside and outside the 'research field', but none of this would have been possible without the support, love and good sense of humour of my husband Daniel and the long-distance back-up of our families. A mi familia quiero agradecerle el cariño, el apoyo incondicional y la paciencia que tuvieron en estos últimos años. Saber que cuento siempre con ustedes es lo mejor que me puede pasar!

The start of this project was marked by its approval by Rudy Rabbinge, to whom I also have to thank for letting me participate in the various activities of the graduate school and facilitating my attendance to international conferences. I'm most indebted to my co-promotor, Ad Schapendonk, for his interest and determination to make things possible, for letting me learn something new after each discussion and last but not least for being a sensitive person. I hope we can continue working together in the future. To Gustavo Slafer, I have to thank his commitment to my higher education and his critical and stimulating spirit. I'm also most thankful to Marcel van Oijen for being always open for discussion and for helping me with the modelling.

The experimental work was possible thanks to the collaboration of WAU and AB, and several people helped me during the process. My special thanks go to Ad van Ast for offering his unconditional support and experience during the struggle to co-ordinate the experimental plan. Sander Pot made the photosynthesis measurements possible, thanks to his skills, and endurable, thanks to his good humour. I also have to thank Els Mathijssen and Wim den Dunnen from UNIFARM for their help in logistics and for giving me the opportunity of having professional help from R.Siep and J.H. Scholten. I'm most thankful to Jan van Kleef, Adrie Kooijman and the AB-technical support team (Peter de Man, Herman Peters, Cees Jansen and Johan Derksen), Peter van Leeuwen and Jaap Uttien for support and advice during the experiments in AB. I'm also grateful to Ans Hoffman, Wim Valkenburg, Wim Pape, Johan Uitenbroek and Geert Stooopen for help in different stages of the chemical analyses. Thanks also to Jan van Walsem for welcoming me in the TON-lab and bearing my endless number of N-analysis. The experiment in CPRO was possible thanks to the collaboration with Robert van Loo and the help of Ab de Vos. Advice and equipment loan from Peter van der Putten are greatly appreciated. To Jacques Withagen thanks for understanding what was it that I really wanted with the statistical analysis and to Els Geurts, the gentle guardian of the AB-library, thanks for all your help and affection.

Jan Goudriaan and Peter Leffelar were always available for giving valuable advice. To the TPE and AB-'youngsters' Annemarie, Bjorn, Cor, Corine, Eddy, Jacaranda, Jacco, Jan-Willem, Joost, Lammert, Leo, María Rosa, Reinoud, Sergio and Shana thanks for being good company. Thanks to the PE-group members for the discussions. Ria Boleij is acknowledged for keeping the communication going on, and Gon van Laar for her nice dinners and help with the manuscript. I particularly thank Ries de Visser for his contagious enthusiasm and for sharing with me his experience with isotope labelling. To Tom Dueck thanks for his friendliness and not fearing sharing experimental

chambers with me. I also thank Barbara Habekotté for advice on oilseed rape growth, Ko Groenwold and Bert Smit for enlightening me on the root sampling, and Peter de Man and Geert Versteeg for making it possible. The help of the Dean's Office for sorting daily life problems is greatly appreciated. Last but not least, thanks Anita van Polen for having always a nice word in the long and lonely AB-evenings and to Eva for being a cheerful office-mate.

From the other side of the ocean I counted always with the support of my colleagues at the Cátedra de Cerealicultura (FAUBA). In particular I want to thank Daniel Calderini, Daniel Miralles and Gabriela Abeledo for letting me work with them despite the distance. I was lucky to count with the help of Gabriela, Roxana, Mariana, Valeria and Santiago during experimental work. Furthermore, I greatly appreciate the generosity of Atilio Barneix for letting me use his lab and the advice provided by Carla Caputo.

My last words are for my friends. Thanks to Ariane for being always there. To Paola, Vicky and Herman, Maritza and Hans, Marta and Wim, thanks for the relax-hours, for being always supportive and moreover for letting me into your families. I will miss you a lot!!

Thanks again to all,

FER>

A los míos

List of abbreviations

$A_{\max,n}$	Leaf net photosynthesis at light saturation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
$A_{\max,g}$	Leaf gross photosynthesis at light saturation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
EFF_I	Quantum efficiency based on incident light	$\text{mol CO}_2 \text{ mol}^{-1} \text{ quanta}$
EFF_A	Quantum efficiency based on absorbed light	$\text{mol CO}_2 \text{ mol}^{-1} \text{ quanta}$
DAS	Days after sowing	d
DAG	Days after germination	d
DTNA	Daytime total net CO_2 assimilation by the canopy	$\text{mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$
canopy-PNUE	Canopy photosynthetic nitrogen use efficiency	$\text{mol CO}_2 \text{ mol N d}^{-1}$
I_{AL}	PAR absorbed per unit leaf area	$\mu\text{mol quanta m}^{-2} \text{ leaf}$
IPARc	Cumulative intercepted PAR	MJ m^{-2}
k	Coefficient of light extinction	-
LAI	Leaf area index	$\text{m}^2 \text{ m}^{-2}$
leaf N	Leaf nitrogen content	$\text{mmol N m}^{-2} \text{ leaf}$ or $\text{g N m}^{-2} \text{ leaf}$
leaf-PNUE	Leaf photosynthetic nitrogen use efficiency	$\mu\text{mol CO}_2 \text{ mmol N s}^{-1}$
R_d	Respiration in the dark	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
RUE	Canopy radiation use efficiency	$\text{mol CO}_2 \text{ mol quanta}^{-1}$
PAR	photosynthetically active radiation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$; $\text{MJ m}^{-2} \text{ d}^{-1}$
P_n	Leaf net photosynthesis rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
t	Light transmission coefficient	-
WSC	Water soluble carbohydrates	mg g^{-1}

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Chapter 1

General introduction

Background

Improvement of crop yields and increases in global population have been linked for more than 10,000 yrs, when domestication of today's cultivated plants started (Evans, 1993b). What makes our century particular is the striking increase in yield potential of the major crops, driven by the widespread utilisation of nitrogen (N) fertilisers and agro-chemicals. The achievement has been such that, in recent decades, the inequality in nutrition of the world-population was caused by restricted access to food rather than by low production at the world scale (IFPRI, 1995). However, for several reasons, there is an urging need to continue increasing yield potential. Firstly, demographers agree that human population will approach 8 billion by 2020, i.e. 2 billion more people than today (FAO, 1997). Secondly, increasing production through high-input agriculture and expansion of agricultural areas are no longer viable alternatives in many regions of the world. In industrialised countries, over-use of fertilisers has led to serious environmental problems, and conservation of natural and recreational areas are increasingly valued. In developing countries, agricultural land has been over-exploited, and major works of land improvement are not affordable. Raising the yield potential of the major agricultural crops through genetic improvement seems the more appropriate way to ensure sustainable production in very different agricultural systems. High-input systems, by fine-tuning of the use of fertilisers, will benefit from high yielding resource-use efficient varieties. In addition, most high yielding cultivars are also recommendable for stressed or minimum-input environments, where they may not achieve their potential but have superior performance (Reynolds et al., 1996a), at least for stresses ranging from low to moderate in severity (Slafer and Araus, 1998). In this context, the present thesis investigated options to raise yield potential in two crops, wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.).

Increasing radiation use efficiency to raise yield potential

Raising yield potential in crops that have already been subjected to a high selection pressure for yield is not an easy task. In wheat, genetic gains in yield potential declined in the last ten years (Reynolds et al., 1996b), compared to 0.5 to 1% per year in the two previous decades (Slafer et al., 1994). In oilseed rape, empirical selection has also increased yields, but yields are comparatively lower than in wheat (even after accounting for seed composition) (Habekotté, 1997a). Understanding the physiology of yield formation and targeting for specific physiological attributes could help to accelerate genetic gains in traditional breeding schemes (Shorter et al., 1991), and profit more rapidly from advances in molecular biology and biotechnology.

A crop's yield is the proportion of total biomass partitioned to the harvestable organs. Past genetic improvement in yield potential of cereal crops can be mostly attributed to the increase in harvest index, and particularly in the number of grains per m^2 (for review see Slafer et al., 1994). The next avenue to raise yield potential is then to increase biomass, particularly during the period of definition of the potential grain number. This stage of the crop cycle in wheat and oilseed rape coincides with the period of rapid inflorescence growth (Fischer, 1985; Mendham et al., 1981). By then, determinate crops have attained the maximum radiation interception, thus any increase in total biomass production will come from a higher net biomass production per unit absorbed radiation, so-called radiation use efficiency (RUE).

The radiation use efficiency depends on total net canopy photosynthesis, which is the integration of the photosynthesis and respiration of all the aboveground organs in the plant. Since the response of photosynthesis to light depends on the amount of enzymes, pigments and other N-rich photosynthesis-related compounds present in the leaf (Evans, 1989a), the partitioning of leaf N between canopy layers with respect to the irradiance gradient is a critical characteristic determining the canopy photosynthetic capacity (Field, 1983). For instance, for a canopy with a fixed leaf area and a certain limited amount of N in green organs, it has been hypothesised that canopy photosynthesis per unit absorbed irradiance could be maximised by distributing leaf N preferentially to the more-illuminated parts of the canopy (Field and Mooney, 1986).

The strong relation between photosynthesis and leaf N, in dependence of environmental factors, is the basis for analysing the possibilities of biomass improvement through N redistribution among leaf layers. Variability exists between species in their capacity for photosynthesis per unit leaf N (Evans, 1989a; Poorter and Evans, 1998). Inter-specific variation in photosynthetic N use efficiency can originate in different attributes, such as the capacity for CO_2 uptake (Henson et al., 1990), enzyme functioning (Makino et al., 1988) or the partitioning of leaf N between the main photosynthetic functions, i.e. light capture, carboxylation capacity and energy conversion (Evans, 1989a). Knowledge on the reasons for species-related differences in the use of N at the leaf and canopy level will help to pinpoint desirable characteristics for the design of higher yielding, N-use efficient crop genotypes.

Finally, it is known that the observed rise in atmospheric CO_2 level will continue during the next decade (Keeling et al., 1995). Leaf photosynthesis in C_3 plants and biomass production respond positively to it (Lawlor, 1995). However, several lines of evidences indicate that the initial stimulation of leaf photosynthesis by CO_2 declines in time (Sage, 1994) and seems to be strongly regulated by N supply (Rogers et al., 1996). Considering the economic and social importance of wheat, and the fact that higher wheat yields at high CO_2 have been related to increases in radiation use efficiency (Monje and Bugbee, 1998), factors that could affect it deserve particular attention.

Target crops

Wheat and oilseed rape are the target crops of this study. Wheat is the most important food crop in terms of world annual production (FAO, 1997), and a major source of energy, protein and fibre in human nutrition (Gooding and Davies, 1997). Annual and perennial oilseed crops and their by-products are the second most valuable commodity in agricultural trading (Scarisbrick and Ferguson, 1995). Among them, oilseed rape production and consumption has expanded rapidly in the past two decades (Verheijen and Jimmink, 1995; Scarisbrick and Ferguson, 1995). This crop is the single most important winter-spring oilcrop. The primary application of its oil is the manufacture of edible products, but industrial applications are increasing (Verheijen and Jimmink, 1995; Sandars, 1995; Walker, 1995). After oil extraction, the high protein seed residue is used as animal feedstuff, which makes it an interesting option considering the steady increase in demand of meat and dairy products world-wide (IFPRI, 1995). At the cropping system level, wheat and oilseed rape are alternative crops in winter-spring rotations. The oilseed can add profitability to a traditionally cereal-based rotation (Zentner et al., 1996), used as a break crop helps to disrupt the cycle of soil borne-pathogens (Kirkegaard et al., 1997) and makes possible a more rational and effective control of weeds and insects. In addition, spring types of both crops are suitable for double-cropping, thus creating opportunities to increase productivity on an area basis at the farm level.

Objectives and approach

The objective of the present thesis is to investigate options for increasing biomass production to raise yield potential, while optimising the use of N at the canopy level in wheat and oilseed rape. In this context, emphasis is given to study (a) the dynamics of the vertical leaf N distribution along crop ontogeny in response to plant characteristics and environmental conditions, and (b) their impact on canopy radiation use efficiency. The study of N partitioning within the leaves is used as a basis to understand differences between species in photosynthetic N use efficiency. The research is focused on wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.), the most important winter-spring components of cereal-oilseed rotations of temperate regions.

To address the objectives, physiological insight gained from different levels of observation in the experiments, mainly leaf, plant and canopy, is integrated by means of modelling. This combined approach allows studying the sensitivity of canopy photosynthesis to different crop characteristics and helps in the identification of morpho-physiological traits that might be manipulated in order to enhance crop productivity.

Outline

In Chapter 2, the need to increase biomass production in crops, with particular reference to winter-spring cereals, is reviewed. The relation between vertical leaf N distribution and canopy photosynthesis is explored as a particular option to improve

radiation use efficiency and yield potential. Main assumptions underlying the prediction of the optimum vertical leaf N allocation, i.e. the role of light climate within the canopy and total leaf nitrogen content, are revised.

Chapters 3 and 4 are devoted to the comparative ecophysiology of wheat and oilseed rape in relation to N availability during the critical period for grain number definition and grain filling. In Chapter 3, traditional growth analysis is used to understand the differences between species in the response of yield to N availability, by studying the limitations imposed by N shortage on radiation interception, radiation use efficiency and biomass partitioning to different organs. The capacity for N absorption and use is also compared between species. Possibilities to increase yield potential in both crops are discussed in terms of the photosynthetic efficiency of the different organs and the source-sink ratio during grain filling. In Chapter 4, the reasons for inter-specific differences in radiation use efficiency and its sensitivity to N availability were investigated with a bottom-up approach. Experimental and modelling work were combined to distinguish between process dynamics at leaf and canopy levels. Canopy photosynthesis and daytime radiation use efficiency were calculated with a model based on N-dependent leaf photosynthesis and intra-canopy light and leaf N distribution. The model was also used for exploring the sensitivity of canopy photosynthesis and radiation use efficiency to different crop characteristics.

In Chapter 5, the dynamics of leaf N distribution was determined during wheat vegetative growth. The actual vertical leaf N distribution was compared with the optimal one and the sensitivity of the optimal distribution to changes in the photosynthetic capacity at low and high light is discussed. Chapter 6 deals with the response of growth to CO₂ in combination with N availability with focus on the period of grain number definition. The question was addressed whether the phenomenon of photosynthetic acclimation to high CO₂ is a simple response to changes in the plant N status and its influence on the source-sink balance. In the experiments reported in Chapters 5 and 6, plants were grown hydroponically and N was provided in proportion to the expected growth, to keep the plant N concentration steady and avoid complex time-dependent effects of N availability and dilution of N in plant biomass.

Finally, in Chapter 7, the contribution of the results to the existing knowledge is highlighted, and the applicability is discussed in the context of genetic crop improvement and N fertilisation.

Chapter 2

Light-associated canopy N distribution. Revision of assumptions and possibilities of manipulation to improve the yield potential in winter cereals

Abstract

This chapter reviews the literature on the relation between vertical leaf nitrogen distribution and canopy photosynthesis, as a possible route to maximise radiation use efficiency, biomass production and yield potential in winter cereals. Main assumptions underlying the prediction of the optimum vertical leaf nitrogen allocation, i.e. the role of light climate within the canopy and total leaf nitrogen content, are revised. Dynamic aspects and possible impact of manipulation of vertical leaf N distribution on yield and quality of winter cereals are discussed and areas for future research highlighted.

Keywords: leaf nitrogen distribution, optimum leaf nitrogen profile, radiation use efficiency, yield potential, winter cereals.

Abbreviations: A_{\max} , light saturated photosynthesis rate; EFF_1 , quantum efficiency based on incident light; k , light extinction coefficient; k_N , nitrogen extinction coefficient; LAI, leaf area index; RUE, radiation use efficiency.

Introduction

General

Breeding efforts, in interaction with improvement in agronomic techniques, have been the routes to increase crop yields since the beginning of agriculture. The distinction between past contribution of each of these factors to increases in grain yield of agricultural crops, though difficult to separate completely, has been attempted by several authors (Jensen, 1978; Slafer and Andrade, 1991). For the most important winter cereal, bread wheat (*Triticum aestivum* L.), yield increases in different countries since the beginning of this century were due equally to genetic gain in yield potential vs. improvement in management practices and gains in genetic resistance (see Slafer et al., 1994). Among the management practices, the increase in nitrogen (N) fertiliser use has been responsible for a great deal of the gain in biomass, yield and quality of cereals (Bell et al., 1995). The fast adoption of this technique was linked to the widespread introgression of major dwarfing genes (Rht) or direct selection for short stature in most winter cereals (Richards, 1992), which reduced the risk of lodging. However, the danger of environmental pollution in conjunction with the trend towards less subsidised agriculture in many cereal growing countries, call for reconsideration of the level of N use. In this context, plant breeding may play a leading role in the search for future increases in grain yields and nitrogen use efficiency.

Crop yields in winter cereals: past breeding achievements and future prospects

For most winter cereals, actual prospects for yield improvement contrast widely with those in the beginning of the century. The imposition of organised selection pressure has resulted in a sharp increase in harvest index, the proportion of total biomass allocated into grains (e.g. oats: Lawes, 1977; Peltonen-Sainio, 1990, 1994; barley: Riggs et al., 1981; Bulman et al., 1993; bread-wheat: Austin et al., 1980; Austin et al., 1989; Siddique et al., 1989; Slafer and Andrade, 1989; Calderini et al., 1995; durum wheat: Waddington et al., 1987) (some examples in Fig. 2.1). As harvest index cannot be increased beyond certain limits (i.e. 62% as calculated for wheat by Austin et al., 1980), and many crops are already close to this theoretical threshold, the present scope for future increases in potential yield through this characteristic is very narrow. Therefore, increasing biomass production must be considered, sooner or later, as the main avenue towards further raising potential yields.

Substantial success has been achieved in increasing yield potential through the empirical selection approach of trial and error directed to yield per se (Loss and Siddique, 1994), however, using physiological attributes as selection criteria could accelerate future genetic gains (Shorter et al., 1991). To achieve this target, it will be necessary to identify traits to help in the detection of potential parents and/or the selection of the progeny. In the following sections of the introduction we will analyse crop level physiological components of final biomass and try to recognise which could

photosynthesis, would result in increased biomass has often been considered (Austin et al., 1989; Carver and Nevo, 1990; Austin, 1992). However, a striking lack of association between leaf photosynthesis and biomass production has been frequently found, particularly when comparing old vs. new varieties or genotypes with different levels of ploidy (Austin et al., 1982; Johnson et al., 1987; Carver et al., 1989). One of the reasons might be that the rate of photosynthesis and leaf size are so often negatively correlated that pleiotropic effects of genes for high photosynthesis rate on reduced leaf area have been suggested (Bhagsari and Brown, 1986; Austin, 1989). Another limitation of the approach could be that the impact of a trait from a low level of organisation (organ) was expected to be additive on scaling to a higher level of organisation (crop). However, the basic idea of improving leaf photosynthesis might still be useful, provided the potential for photosynthesis at different leaf layers in the canopy is considered.

Radiation use efficiency: routes for maximisation

Because leaf photosynthesis responds to incident radiation and leaf N content, different possibilities are open for maximisation of photosynthesis at the canopy level and ultimately, RUE (Field and Mooney, 1983; Loomis, 1993). The nature of the photosynthesis response to irradiance indicates that the efficiency (slope) is reduced as the irradiance level is increased (Fig. 2.2). The two main parameters describing this relation are the maximum efficiency at low levels of irradiance based on incident light (EFF_i) and the rate of photosynthesis at saturating irradiance (the asymptote, A_{max}). Then, to increase RUE at the canopy level, the inefficiencies occurring in the uppermost layers exposed to high irradiance must be considered (see zone 1 in Fig. 2.2). The most frequently discussed and experimentally explored alternative has been to improve the distribution of radiation within the canopy through varying leaf angles along the plant. The ideal genotype would have more erect leaves in the top leaf layers (Duncan, 1971). As a consequence, the amount of radiation intercepted at saturating irradiance would be reduced and light penetration improved. Furthermore, the sunlit area in the bottom of the canopy would be increased, i.e. where radiation levels are lower and photosynthesis is more efficient (see zone 2 in Fig. 2.2). As a result of increasing leaf erectness the coefficient of light attenuation (k) would be reduced (Fig. 2.3a). Carvalho and Qualset (1978) have found genes having a major effect on flag leaf angle. Moreover, genetic variability for canopy photosynthesis or RUE in wheat associated with different patterns of radiation distribution within the canopy have been reported (Austin et al., 1976; Rasmusson, 1987; Aikman, 1989; Green, 1989), as well as in other crops (Kiniry et al., 1989). However, the impact of changes in leaf angle on winter cereals yields has been inconclusive. For example, Innes and Blackwell (1983) in wheat and Angus et al. (1972) in barley have reported that crops with erect upper leaves produced higher yields than those with predominantly horizontal leaves. But, Austin et al. (1976) and Tunland et al. (1987) found little evidence that erect leaf angle enhanced yield in wheat and in barley, respectively. The apparent contradiction may stem from the fact that the advantage of leaf erectness can be better expressed only at high leaf area index (LAI) and under high irradiance (Duncan, 1971; Goudriaan, 1988). This may be indirectly confirmed by the fact that in summer crops,

such as rice, a positive effect of leaf erectness on yield has been more consistently observed (e.g. Chang and Tagumpay, 1970; Trenbath and Angus, 1975; Peng et al., 1994). Then, the room for impact of leaf erectness on RUE of winter cereals grown under potential conditions may be limited to the grain filling period in crops of temperate regions, i.e. when the angle of incident irradiance is highest, or to crops grown at low latitudes.

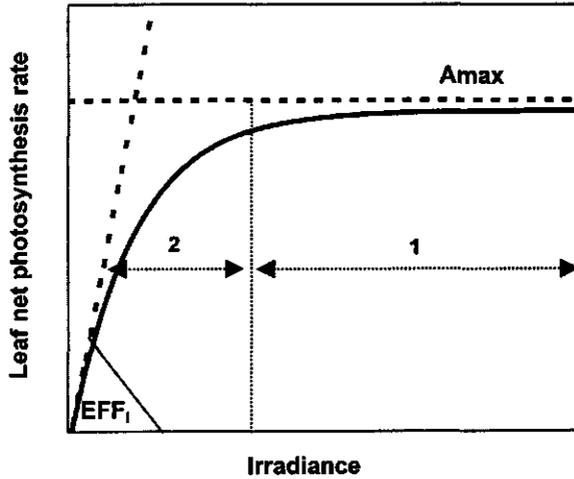


Figure 2.2. Leaf photosynthesis rate as a function of irradiance. See text for explanation of numbers.

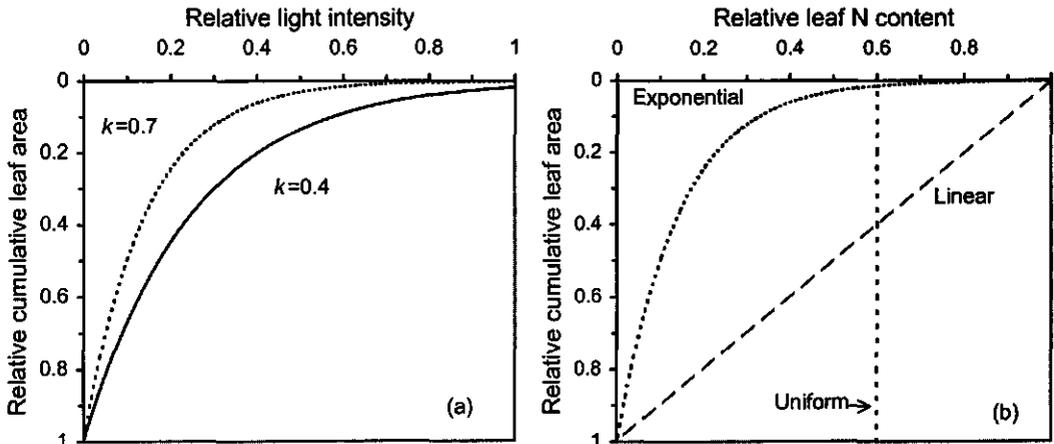


Figure 2.3. Changes in (a) light intensity for different light extinction coefficients (k) and (b) leaf N allocation for different leaf N distribution models, relative to the top of the canopy and as a function of relative cumulative leaf area (0 and 1 are the top and bottom of the canopy).

An alternative approach to increase RUE would be the maximisation of growth through the improvement of the vertical distribution of N among leaves. Leaf N content strongly determines the maximum photosynthetic rate at high irradiances (Field and Mooney, 1986; Van Keulen et al., 1989; Evans, 1989a). Therefore, theoretical studies have suggested that canopy photosynthesis would be maximised if N is preferentially allocated to the more illuminated leaves (Field, 1983; Hirose and Werger, 1987b). In this scenario, for a fixed amount of leaf N in the canopy, the possibility of increasing RUE would rely on the redistribution of canopy N from bottom to uppermost leaf layers (Fig. 2.3b). In closed canopies, leaf N gradients are frequently observed, and have been interpreted as an adaptive response to the light environment that allows a higher canopy productivity than that expected from the uniform distribution, i.e. when the N content of every leaf equals the mean N content of the canopy (Mooney and Gulmon, 1979; Mooney et al., 1981).

The leaf N vertical distribution or profile that yields maximum canopy carbon gain has been termed 'optimum profile' and can be calculated with canopy photosynthesis models that take light and leaf N gradients into consideration (Hirose and Werger, 1987a; Goudriaan, 1995; Anten et al., 1995). Application of optimisation theory and models have been formerly used to test if the naturally uneven distribution of canopy N is required to maximise growth, focusing on perennial herbs and native shrubs (Hirose and Werger, 1987a; Schieving et al. 1992a).

This approach may be helpful for assessing the impact of leaf N distribution on growth rate of agricultural crops, which are subjected to changes in canopy structure and N availability during the growing season and through management practices. Aspects of the issue whether leaf N distribution limits crop canopy photosynthesis in extensive crops have been addressed in summer crops (peanut: Wright and Hammer, 1994; Hammer and Wright, 1994; soybean: Sinclair and Shiraiwa, 1993; sunflower: Sadras et al., 1993; Connor et al., 1995, Giménez et al., 1994). For winter cereals grown in temperate areas, the topic has not been methodically addressed by any study. The reason might be that these crops are mostly exposed to relatively low levels of radiation. However, the fact that their photosynthetic rate is saturated at a lower irradiance threshold could make the hypothesis of N redistribution as contributing to maximise RUE equally applicable for winter cereals. Particularly, if we consider that a critical growth phase for biomass accumulation and yield formation in these cereals, i.e. the period of grain number definition (Fischer, 1985), occurs with increasing irradiance during spring.

Objective

The objective of this article is to review the literature on the relation between vertical leaf N distribution and canopy photosynthesis, and to speculate on the possibilities for manipulation of canopy N profiles in order to maximise growth in winter cereals. For this purpose, we will initially address the influence of leaf N content on photosynthesis and RUE and analyse the expected impact of a gradient in leaf N distribution. The mechanisms behind leaf N distribution in vegetative canopies will be

briefly considered. A following section is focused on the revision of main assumptions of models that have been used to calculate the N profile that maximises canopy photosynthesis. Then, major dynamic aspects that should be included in studies of optimisation of N distribution for crop growth are identified. Finally, possible applications of the topic in different winter cereals are discussed and areas for future research highlighted.

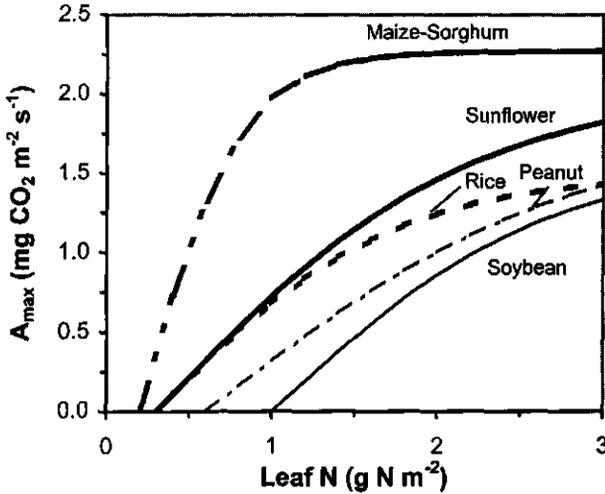


Figure 2.4. Light saturated leaf photosynthesis rate as a function of leaf N content in different crops: maize-sorghum (Muchow and Sinclair, 1994); rice and soybean (Sinclair and Horie, 1989), sunflower (Connor et al., 1993), peanut (Sinclair et al., 1993).

Leaf nitrogen, photosynthesis and radiation use efficiency

Photosynthetic response to irradiance is largely determined by leaf N content (Field and Mooney, 1986; Evans, 1989a). Since approximately 40-70 % of the soluble protein in the leaf is concentrated in the carboxylation enzymes (Evans, 1983; Terashima and Evans, 1988; Evans, 1989a), the relation between photosynthesis and leaf N is not surprising. The response of photosynthesis to N can be analysed through the effect on A_{max} and EFF_1 . In many crops, A_{max} increases asymptotically with leaf N content per unit leaf area (wheat: Evans, 1983; soybean: Sinclair and Horie, 1989; potato: Marshall and Vos, 1991; sunflower: Connor et al., 1993; peanut: Sinclair et al., 1993; Muchow and Sinclair, 1994) (see examples in Fig. 2.4). Among the factors contributing to the degree of curvature of the A_{max} -leaf N relation, the saturating relation between leaf N and the content of carboxylating enzyme and the increasing irradiance threshold needed to reach light saturation in leaves with higher leaf N, have been mentioned (Evans, 1989a). Linear increases in A_{max} with leaf N have also been reported (Peng et al., 1995; Anten et al., 1995).

At the canopy level of organisation, RUE response to N availability has been documented in many crops (Green, 1987; Muchow and Sinclair, 1994; Hall et al., 1995) and investigated with models that integrate leaf carbon assimilation over canopy architecture and environmental gradients. Based on such a model, Sinclair and Horie (1989) theoretically developed the relation between RUE and leaf N as hyperbolic, highly sensitive to low leaf N and species-dependent. Their initial calculations were performed under the assumption that leaf N was equal at any canopy height, or uniformly distributed. The observation of uneven N distribution in natural canopies led to question: to what extent could leaf N distribution affect canopy assimilation rate or RUE? Evans (1993b) calculated that daily gains in canopy photosynthesis with actual leaf N profiles could be 1-36% higher than those under uniform leaf N distribution. Whereas, the comparison between canopy photosynthesis calculated with actual vs. optimal profiles yielded up to 7% gain (Schieving et al., 1992b; Connor et al., 1995). Furthermore, it has been reported that including a leaf N gradient in the calculation of RUE could increase its value from 1-20% in peanut (Hammer and Wright, 1994) or even more in soybean (Sinclair and Shiraiwa, 1993).

The above-mentioned conclusions about estimated quantitative effects of leaf N profiles on canopy photosynthesis must be taken cautiously since they were reached accepting assumptions that may not yet be sufficiently tested. One of them is that the leaf N content does not generally affect EFF_1 . Consequently, the increase in A_{max} due to higher leaf N in the upper layers of the canopy during the hours of saturating irradiance would not be accompanied by a decrease in EFF_1 in the lower layers, and by an increased EFF_1 in the upper layers during the rest of the day. This deserves a rigorous testing before quantitative conclusions on the effect of leaf N distribution can be accepted and confidently extrapolated. The few studies in which the relationship between leaf N and EFF_1 was investigated yielded variable results. EFF_1 has been linearly (Hirose and Werger, 1987a; Dingkuhn and Kropff, 1996) or hyperbolically (Pons et al., 1989) related to leaf N in some species, while these traits appear to be independent in others (Connor et al., 1993; Anten et al., 1995). These considerations are probably of consequence for a winter cereal crop, in which a significant part of the growing cycle is exposed to low radiation and a large fraction of leaf area is shaded during most of the season.

To explore the sensitivity of daily total gross photosynthesis to the presence of a gradient in A_{max} or EFF_1 in the canopy, a simulation model was built based on subroutines of SUCROS (Goudriaan and Van Laar, 1994). Parameters for wheat were derived from Goudriaan and Van Laar (1994) and Van Heemst (1988). The model calculates daily total photosynthesis by integrating the rates of photosynthesis of sunlit and shaded leaves at different canopy depths three times a day, at gaussian intervals (Goudriaan and Van Laar, 1994). Total incident radiation is separated in direct and diffuse flux. The gradient in photosynthesis parameters for the present analysis was arbitrarily chosen within the ranges observed in the literature: A_{max} and EFF_1 decrease 25%, linearly, from top to bottom canopy layers.

Table 2.1. Relative decrease in daily total gross photosynthesis (%) by introducing a 25% linear decrease in A_{\max} or EFF_1 from top to bottom canopy layers with respect to a unique A_{\max} or EFF_1 (see text for details).

Daily Total Radiation [MJ m ⁻² d ⁻¹]	A_{\max} reduction	EFF_1 reduction
31 July, 35° lat. S		
7	5.3	21.5
14	11.3	16.4
21	12.5	15.0
15 November, 35° lat. S		
7	4.3	22.4
14	7.3	20.3
21	10.1	18.1
28	12.5	15.6

Calculations were performed for a crop with a LAI=5, grown in a temperate region in the southern hemisphere (e.g. Rolling Pampas, Argentina, at 35° lat. S), at different values of daily total radiation, for a day in the end of July and mid November. Those dates correspond roughly to the stages of tillering and mid grain filling of a crop sown under current practices. The relative decrease in canopy gross photosynthesis as a percentage of that of a canopy with a constant value of A_{\max} and EFF_1 over canopy depth are presented in Table 2.1. The outcome indicates that wheat canopy photosynthesis is likely to be more sensitive to changes in EFF_1 than in A_{\max} , but the impact is more similar at high radiation levels. As expected, the risk of overestimating photosynthesis by a constant A_{\max} value increases with increasing radiation, while the trend is inverse for EFF_1 . In conclusion, canopy photosynthesis in wheat is sensitive to changes in EFF_1 , and therefore, those factors affecting EFF_1 , such as possibly leaf N, merit further attention. Other authors have calculated that, in temperate regions, wheat dry matter production would be fairly insensitive to increases in A_{\max} and suggested that genetically manipulating EFF_1 would yield high returns on canopy photosynthesis (Day and Chalabi, 1988; Ort and Baker, 1988).

Leaf N distribution: Effect of Light Climate Within the Canopy and Leaf Age

The main proposed factors controlling leaf N allocation in dense vegetative canopies have been light gradient (Hirose et al., 1988) and leaf age (Mooney et al., 1981; Field, 1983) and both may operate simultaneously. Several authors have tried to manipulate them independently to evaluate the importance of each in the definition of leaf N gradients, using different experimental approaches. For instance, Schieving et al. (1992b), growing a monocot herb, which develops new leaves from a basal meristem and hence places them in the shade, still observed a steep decline in leaf N with depth. Burkey and Wells (1991) observed in dense soybean canopies that the leaf N gradient was largely reversible when the stand was thinned. Hikosaka et al. (1994) examined

the effects of irradiance level and age by creating two types of shade gradients on a horizontally grown vine. In one of the gradients, younger leaves received more shade, the other simulated a canopy-type shading. They concluded that the effect of radiation level on leaf N was more significant than that of age. Important quantitative effects of radiation level over age in the definition of the leaf N gradients have been also observed by Woledge (1986).

The overall conclusion of the above-mentioned experiments, that the light gradient has a pre-eminent role in the definition of leaf N has been included as the backbone criteria of optimisation of N distribution for canopy photosynthesis. Theoretically, the closer the patterns of light and N distribution, the smaller the gap between actual and maximum capacity for biomass production, under the assumption that the effect of leaf N on EFF_1 is small or negligible.

Optimisation of Leaf N distribution: revision of model assumptions

In most of the studies where photosynthetic production of the canopy under actual and optimal N allocation patterns were compared, the optimal leaf N profile was steeper than the actual, i.e. leaf N was overestimated in top and underestimated in bottom leaf layers (Hirose and Werger, 1987b; Evans, 1993b; Pons et al., 1989, Giménez et al., 1994; Anten et al., 1995). The bias was independent of the magnitude of the predicted gain in canopy photosynthesis under optimum leaf N distribution (see Giménez, Connor, and Rueda, 1994 as an example). Thus, it is possible to suspect that one or more of the criteria used to dictate the optimum N distribution are not enough robust. To draw solid conclusions about possible gains in biomass production and efficient use of light and N, the basis for prediction of the optimum leaf N profile has to be reliable. Particularly, when the benefits need to be assessed under changing N availability, canopy structure and environmental conditions, as is the case of agricultural crops. For this reason, we revised two main assumptions in models that predict canopy photosynthesis under optimum N-allocation by contrasting them with experimental results.

The pattern of leaf N distribution is determined by light distribution within the canopy

The core of optimisation models of leaf N distribution is that the optimum solution is determined by radiation distribution. The shape of the leaf N profile is usually described with the coefficient of leaf N allocation, k_N . The actual k_N values are calculated by regression of leaf N and LAI at different canopy heights. The value of k_N equals zero when the leaf N content of every leaf equals the mean canopy leaf N, uniform profile, and increases as more N is partitioned to upper leaf layers. In studies performed in native perennial species, leaf N distribution was fitted to exponential models (Hirose and Werger, 1987b; Pons et al., 1989), so canopy photosynthesis would be maximised when the leaf N allocation mimicked that of radiation distribution within the canopy ($k_N=k$).

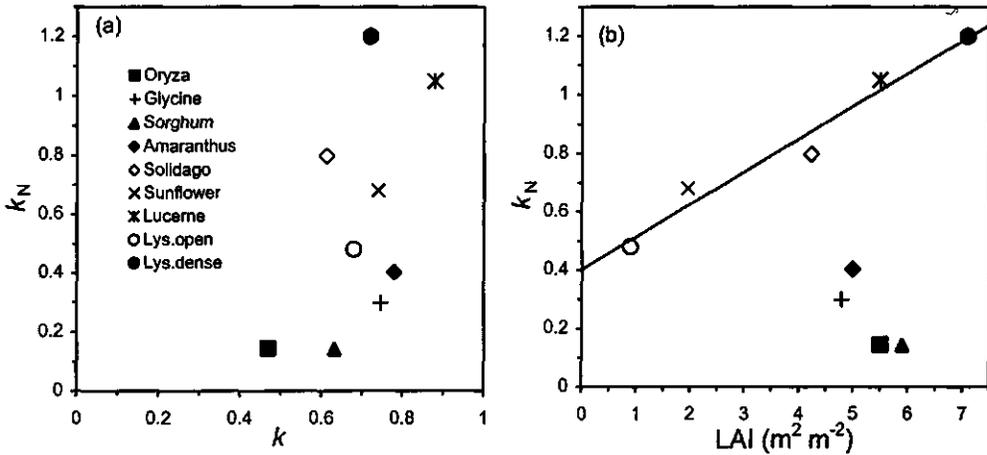


Figure 2.5. Relation between the coefficient of leaf nitrogen allocation (k_N) and (a) k , and (b) LAI for different species: *Solidago altissima* (Hirose and Werger, 1987b), *Lysimachia vulgaris* (open or dense canopy: Hirose et al., 1988), *Medicago sativa* (Lucerne: Lemaire et al., 1991); *Helianthus annuus* (Sunflower: Sadras et al., 1993); *Oryza sativa*, *Glycine max*, *Sorghum bicolor* and *Amaranthus cruentus* (Anten et al., 1995). Regression in (b) $R^2=0.95$, $P<0.005$ (Sadras et al., 1993).

For crop canopies, few and contrasting descriptions of leaf N profiles are available to date. In soybean (Shiraiwa and Sinclair, 1993) and peanut (Wright and Hammer, 1994) a linear decline in leaf N with cumulative LAI has been reported, whereas an exponential decline has been observed in a grain legume and forage sorghum (Charles-Edwards et al., 1987) and sunflower (Sadras et al., 1993). The following analysis has been restricted to those situations where an exponential decline in leaf N could be fitted.

If the radiation distribution dictates the leaf N profile then, changes in structural characteristics of the canopy are expected to influence k_N (Hirose et al., 1988; Anten et al., 1995). When differences in k among species were pictured against k_N , no clear relation emerged (Fig. 2.5a). The effect of canopy structure on leaf N profiles was also tested by manipulation of stand density by Hirose et al. (1988), who found that the distribution of leaf N was markedly more non-uniform in the dense than in the open stand. Sadras et al. (1993) pictured the relation between LAI and k_N including data from different species during vegetative growth (see data and regression line in upper part of Fig. 2.5b), and suggested that a departure from this trend could be accounted for by differences in k among species. However, data of four species from the study of Anten et al. (1995) did not fit in this regression despite the k 's of the species used were in the range of those presented by Sadras et al. (1993) (Fig. 2.5a and b). Other studies have failed to find any effect of plant density on the steepness of leaf N gradients. For instance, no consistent differences were found in k_N of the perennial

herb *Solidago altissima*, despite 53% vs. 7-10% of total incident irradiance reached the lowest branches in open vs. dense stands, respectively (Werger and Hirose, 1988). Similar results were reported for a range of population densities of sunflower (Sadras et al., 1993) and soybean (Shiraiwa and Sinclair, 1993).

If the light gradient has a regulatory role on leaf N distribution, why is k_N not always associated with the characteristics that determine the light climate within the canopy? Several reasons can be raised. On one hand, the ability to respond or not to light distribution could be species-dependent. Aerts and de Caluwe (1994) have suggested that low-productivity species have a low phenotypic plasticity to shape the vertical leaf N distribution. However, this concept can not be easily extrapolated to crops, which are usually selected under potential growing conditions and have high growth rate. Another issue is that most of the studies have been performed on closed or nearly closed canopies (Table 2.2). If the lag period between the imposition of a certain light environment and the corresponding leaf N gradient is several days long (Pons and Pearcy, 1994), actual leaf N profiles would be more uniform than expected. This effect could be clarified by performing a sequence of harvests and assessing optimality on a period of several days. The fact that leaf N profiles are usually more uniform than expected, could also originate in the limited chance to do a precise determination of the response of EFF_1 and respiration to leaf N with measurements available from portable photosynthetic systems (the error of the measurement usually increases as the CO_2 exchange decreases). If the linear responses of both respiration and EFF_1 to leaf N are underestimated, optimisation procedures will tend to allocate N in upper layers at a very low cost. Finally, although light is an important factor controlling the distribution of leaf N, the fact that its influence is only detected under certain conditions may indicate that it is not the only regulatory factor.

Does k_N increase with total leaf N content in the canopy?

In most models of optimisation of N allocation among leaves, the leaf N content is divided in two functional pools, labile metabolic N or free for mobilisation vs. structural N, as proposed by Caloin and Yu (1984). Hirose and Werger (1987b), by means of a numerical model, derived that as canopy N content increased, so did the k_N necessary to maximise canopy photosynthesis. Their explanation was that more N becomes available for translocation to more illuminated micro-sites. This notion was incorporated in the analytical calculation of the optimum N distribution developed by Anten et al. (1995), where k_N is proportional to the amount of 'leaf free' or 'photosynthetic' N integrated over leaf area. Despite the theoretical demonstrations, actual k_N values do not meet this presumption straightforward, questioning the realism of the assumption (see data from Aerts and de Caluwe, 1994; Hikosaka et al., 1994; Anten, 1995; Anten et al., 1995). In Fig. 2.6, k_N is pictured as a function of the total amount of photosynthetic N in the canopy, to allow comparison among species and growing conditions without the confounding effect of the proportion of structural N. The data presented belong to crops grown at two levels of N addition from Anten (1995).

Table 2.2. Coefficient of N allocation (k_N) and intercepted radiation at the moment of k_N determination.

Species	k_N	Intercepted Radiation	Source
- <i>Oryza</i> HN ⁽¹⁾	0.14	0.92	Anten (1995); Anten et al. (1995)
<i>Oryza</i> LN	0.17	0.87	
<i>Glycine</i> HN	0.30	0.97	
<i>Sorghum</i> HN	0.14	0.97	
<i>Sorghum</i> LN	0.22	0.94	
<i>Amaranthus</i> HN	0.40	0.97	
<i>Amaranthus</i> LN	0.49	0.81	
- <i>Solidago</i>	0.79	0.92	Hirose and Werger (1987b)
- <i>Sunflower</i>	0.68	0.76	Sadras et al. (1993)
- <i>Lucerne</i>	1.05	0.99	Lemaire et al. (1991)
- <i>Lysimachia</i> open	0.48	0.45	Hirose et al. (1988)
<i>Lysimachia</i> dense	1.20	0.99	

⁽¹⁾Legends as in Figs 2.5 and 2.6.

By contrast to model expectations, a slight negative relation between k_N and total photosynthetic N is apparent (Fig. 2.6). DeJong et al. (1989) reported that in peach, a major influence of N fertiliser was to increase the photosynthetic capacity of partially shaded leaves but not the A_{max} of highly exposed leaves. Both the data of Anten (1995) and the evidence of DeJong et al. (1989) suggest that, as total canopy N content in the leaves increases, canopy photosynthesis is fostered by keeping a more uniform leaf N profile, i.e. retaining more N in the lower leaves. These observations, agree with sensitivity studies on the introduction of leaf N gradients on RUE, which have shown that the most significant impact could be expected in canopies with low leaf N (Sinclair and Shiraiwa, 1993; Hammer and Wright, 1994).

Introducing dynamic aspects in studies of optimisation of leaf N distribution

Changes along crop ontogeny

Studies on optimisation of N allocation have generally relied on short-term observations. However, crops are exposed to a fluctuating environment and changes in source-sink relationships along development. The whole growth cycle should be considered to adequately describe the dynamics of the relationship between net photosynthesis and leaf N. This should be taken as an essential condition if the aim is to identify those phenological stages where optimisation of N distribution may have an impact on the growth rate of winter crops. The main features of the crop that should be considered in relation to its phenology are changes in light distribution and in source-sink ratios with advancing development.

The light environment changes during the growth cycle

Due to the phases of leaf expansion and senescence, the growth of reproductive organs and the evolution of total radiation during the season, the radiation environment within the canopy changes dramatically along crop ontogeny. The k may vary with canopy depth within a developmental stage and between stages as well (Russell et al., 1989; Baldocchi and Collineau, 1994; Meinke, 1996). However, the role of k in determining biomass production of winter cereals or dictating the leaf N profiles in vegetative canopies after canopy closure does not seem conclusive (see Fig. 2.5a). Instead, Meinke (1996) have alerted that ignoring the higher efficiency of light capture of wheat canopies during early development, by using a constant k , could lead to serious underestimation of biomass production at anthesis when maximum LAI is low. As pointed before, only sporadic observations about the development of leaf N profiles before canopy closure are available in the literature, and therefore its implications for early biomass production can hardly be discussed.

As the growing season advances, the proportion of senescent tissue increases and the reproductive structures grow. The role of the inflorescence in intercepting light has been minimised in natural herbaceous canopies (Werger and Hirose, 1991). However, in agricultural crops, it can exert a major difference in light climate during grain filling by reducing the incident radiation on leaves (Rosenthal et al., 1985; Yunusa et al., 1993). An extreme case would be that of a flowering canopy of oilseed rape, which reflects most of the radiation, thereby reducing light available for leaves and immature pods in a critical stage for yield formation (Yates and Steven, 1987). A final remark is that the photosynthesis of the inflorescence can make an important contribution to grain filling in several species (Rood et al., 1984; Rosenthal et al., 1985). All these aspects should be considered when analysing the potential impact of leaf N profiles development during the reproductive phase.

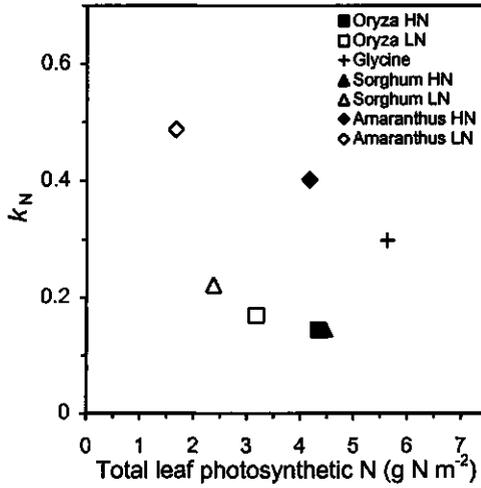


Figure 2.6. Relation between the coefficient of leaf nitrogen allocation (k_N) and total leaf photosynthetic N in the canopy of *Oryza sativa*, *Glycine max*, *Sorghum bicolor* and *Amaranthus cruentus* at high and low nitrogen availability (HN and LN, respectively) (data from Anten, 1995).

Source-sink relations during the reproductive stage

The idea that the optimum leaf N profile could change during ontogeny has been addressed by comparing late vegetative and reproductive stages. Lack of difference (Werger and Hirose, 1988; Schieving et al., 1992a) or ample contrast (Sadras et al., 1993) between leaf N profiles corresponding to those stages have been documented. In this context, the possibility that fruits with contrasting N requirements for seed formation, as oilseed rape and wheat, exert a differential influence on the optimum distribution of leaf N during grain filling has not been explored yet. If sink capacity plays a role in the regulation of photosynthetic rate (Lawlor, 1995), considering its influence will be an essential step in order to set the limits for manipulation of leaf N profiles on crop growth during grain filling.

Reproductive canopies offer an interesting opportunity for the study of the impact of leaf N profiles on biomass formation since it is possible to combine large LAI with low leaf N contents. Penning de Vries et al. (1988), for rice, have calculated with a simulation model that N redistribution from leaves to grains during grain filling could be responsible for a yield loss of up to 10% at high yield levels (ca. 10 Mg ha⁻¹). The authors proposed that yield reduction could be decreased if stems instead of the leaves provided a larger share of the N allocated to grains. However, N storage in organs of non-legume crops is mostly packaged in photosynthesis-related pigments and proteins, while the evidences about the role of storage proteins in cereals reported so far has been variable and highly dependent on the species (Williams et al., 1989; MacKown et

al., 1992). Therefore, the development of differently shaped N profiles could be regarded as a promising alternative to increase total canopy photosynthesis during grain fill for a given crop N status.

Possible applications of canopy leaf N distribution to increase grain yield and quality in winter cereals. The case of bread wheat and malting barley.

Bread wheat and barley for malting are two widespread, often fertilised, winter cereals that contrast in the quality standards required for industrialisation, specifically, high vs. low protein percentage in the grains for wheat and barley respectively. Therefore, they offer an interesting case to analyse comparatively the impact of the development of leaf N profiles on yield and quality formation. The analysis proposed here is focused on the grain filling period.

In Fig. 2.7, a diagrammatic model of the changes in leaf N profiles from anthesis to maturity is presented. During grain filling, massive leaf senescence and N translocation to the grains take place, therefore, total canopy N content in the leaves declines. The idea that genotypic differences in the pattern of N vertical distribution during this stage could have different consequences on yield and protein percentage is explored with genotypes A and B. Several assumptions have been made for this analysis. Among them, that the genotypes do not differ in the response of photosynthesis parameters to leaf N, that both crops have been grown under sufficient N provision and they have equal total N uptake during grain filling. The main difference between genotypes A and B is their capacity to develop leaf N profiles.

Genotype A can develop more non-uniform leaf N profiles than genotype B at a given total amount of leaf N in the canopy (Fig. 2.7). Thus, in A, leaf N is depleted to a greater extent from lower and intermediate leaf layers than in genotype B, and leaf N is higher at the light-saturated leaf layers. Consequently, basal leaf senescence will take place earlier in genotype A than B, and canopy photosynthesis will be concentrated on the upper leaf layers. By contrast, in genotype B, the amount of N mobilised is similar among leaf layers, thus the slope of the leaf N profile in B is only slightly altered towards maturity. Eventually, both genotypes will present a uniform profile at maturity, with the leaf content approaching the content of structural N.

The relative advantage of one genotype over the other in canopy photosynthesis will depend on the magnitude and shape of the response of A_{\max} and EFF_1 to leaf N. For instance, shortly after anthesis, leaf N content in upper leaf layers is still high and probably comprised in the plateau region of the A_{\max} -leaf N response for both genotypes (Fig. 2.4). If the response of the EFF_1 to leaf N were linear, the decline in canopy photosynthesis would be explained by the drop in EFF_1 due to N mobilisation from the light-non-saturated leaf layers. Under those conditions, genotype B may outyield canopy photosynthesis of genotype A around anthesis and early grain filling.

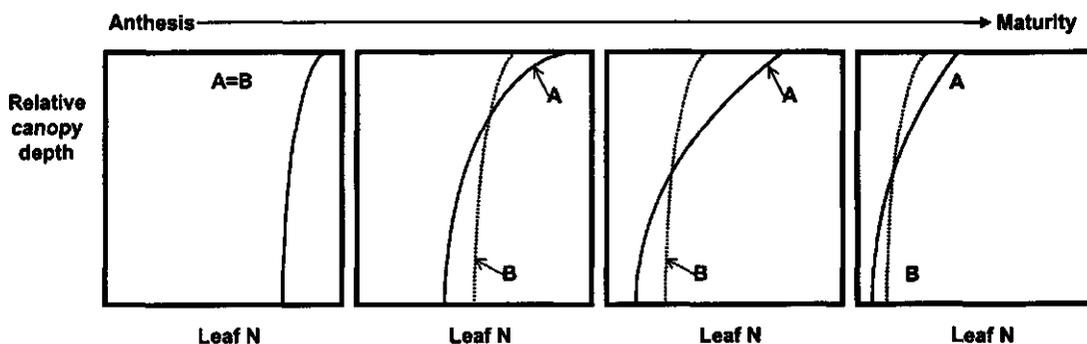


Figure 2.7. Changes in leaf N with depth in the canopy from anthesis to maturity for genotype A and B. See text for explanation.

But, as grain filling proceeds, leaf N content of light-saturated leaf layers will inevitably fall in the linear region of the response A_{\max} -leaf N, then, the maintenance of higher leaf N in upper leaf layers of genotype A may help to compensate for the general loss in potential canopy photosynthesis.

One of the assumptions of the previous analysis was that both genotypes, at any moment during grain filling, presented the same total amount of N in the leaves. However, assuming that changes in k_N have no relation with the capacity of the crop for N translocation to the grains, maybe a rather simplistic view. It is possible that genotype A also has a greater efficiency for N translocation, i.e. total leaf N in the canopy decreases to a greater extent than in genotype B. If that is the case, both genotypes may reach similar total dry matter production during grain filling with different strategies, A through a more steep leaf N profile and B keeping a higher total canopy N content in the leaves. The different strategies may have consequences on the definition of the grain protein content. In genotype B, the trend towards slow changes in the leaf N gradient maybe associated with a lower rate of leaf N turnover and mobilisation. Then, in genotype B, an increase in N availability during heading and grain filling will affect relatively more grain dry matter accumulation than protein yield, thus protein concentration will be kept low. Therefore, ideotypes alike B could be searched for in barley breeding. By contrast, genotype A, could be more suitable as a bread wheat ideotype. If canopy photosynthesis during grain filling is the main source of assimilates for the growing grains, genotype A would reach the maximum thousand grain weight, maximising protein yield and percentage in the grain.

Concluding remarks and future research

In the past, breeding and selection successfully managed to improve yield potential of winter cereals through the increase in dry matter allocation to the grains, without major changes in total biomass production. As the calculated limit in harvest index is

being approached, increases in biomass production are likely to be the route towards future higher yields. The expected increase will probably rely on the development of new insights into physiological processes at the crop level more than on empirical selection. Crop growth models may help in the achievement of this search and the definition of ideotypes. When the total amount of radiation intercepted is maximised through exploitation of the whole length of the growing season, increases in biomass will be based on gains in RUE. Such increase may probably not be expected by the improvement of a single physiological attribute but to the addition of marginal contributions achieved by modifications in several traits at time. Among them a reduction in photorespiration and maintenance respiration and an increase in canopy gross assimilation capacity have been mentioned (Slafer et al., 1994). In this paper we have focused on one of the possibilities to increase RUE and yield (if harvest index is maintained) in winter cereals, by examining the role of optimisation of vertical distribution of canopy N.

During the review and analysis of literature on the topic, several gaps of understanding and information became apparent. Firstly, the limited amount of experimentation carried out in crops, particularly winter crops. Secondly, the fact that the criteria accepted to rule N redistribution performed rather poorly when examined in the light of experimental results. More work will be needed at the interface between processes and mechanisms regulating N mobilisation in order to have sound optimisation criteria with a defined domain of applicability. For instance, when the division of N pools in the leaf is considered, all the non-structural N is assumed as equally ready for mobilisation, though there are evidences that the different proteins involved in photosynthesis may not decrease in parallel during senescence (Makino et al., 1983). In addition, for a given leaf N content photosynthetic capacity may vary with the level of irradiance during growth (Evans, 1989b).

A third point is that, to define the importance of leaf N profiles to increase biomass and yield potential, the whole growth cycle should be studied, since the impact may change among growth stages. Then, windows of opportunity could be detected, where an increase in crop growth rate has a direct consequence on yield potential. In wheat, this is likely to be the case in the period of grain number definition around anthesis, as established by Fischer (1985). In addition, since the possibilities of development of leaf N profiles and their impact may vary with growing conditions (Fig. 2.6), experimentation should include monitoring of the leaf N profiles under combination of factors such as nitrogen availability and planting density. Furthermore, the assessment should be made frequently during the stages under study.

To answer the question whether the development of different patterns of leaf N allocation within the canopy can effectively impact biomass production, research will be needed in certain basic areas. The relation between photosynthesis parameters and leaf N has been documented to change in shape and magnitude in many crops (e.g. rice: Peng et al., 1995). This variation, has often been associated to the growing conditions, such as exposure to high vs. low radiation (DeJong et al., 1989) and needs to be appraised in order to define the potential of photosynthesis parameters at

different canopy depths. Implications of such changes are expected to be important for the definition of the optimum leaf N profiles. For instance, increasing investment of leaf N in upper leaf layers may have different profit when the A_{\max} -leaf N relation is asymptotic or linear. In addition, the importance of photosynthesis at low radiation levels for total canopy assimilation has been highlighted and thus, the impact of leaf N as potentially limiting EFF_1 merits further research.

An interesting area which remains open for research is whether the impact of leaf N profiles on biomass production will be different under elevated CO_2 concentration environments, expected as a result of global change. We hope that some of the elements given in this paper may help to advance in the discussion of this topic. Finally, it is necessary to remark that we are aware of the shortcomings of optimisation theory, e.g. time constraints, oversimplifications and lack of insights as discussed by Chen et al. (1993). Nevertheless, it offers a quite simple framework to analyse exploitation of scarce resources or environmental features in agriculture (Loomis, 1993). The study of solutions originated in such analysis, followed by appropriate design of phenotypes, is possibly a valid tool to help move upwards the actual barrier of yield potential in winter cereals.

Chapter 3

wheat and oilseed rape to nitrogen supply: efficiency of radiation and nitrogen during the lining biomass and yield

of spring wheat and oilseed rape to nitrogen (N) supply, and for grain number definition and grain filling. Crops were a shelter and treated with five combinations of applied produced comparable amounts of biomass and yield when synthesis (SC). From both the responses of biomass and yield the apparent contribution of mobilised biomass to yield, it was found that oilseed rape was more limited by the capacity for assimilating N than that of wheat, particularly at the medium and high N levels. However, when equal amounts from the total available N in the soil were applied, oilseed rape showed a higher efficiency to convert absorbed N in biomass, but a lower apparent contribution of mobilised biomass to yield than wheat. Oilseed rape had similar or lower root biomass and N level, but higher root length per unit soil volume and higher absorption of N per unit root biomass during the critical period for grain number determination was similar among species. In wheat, N was used through a similar or lower reduction in radiation use efficiency than that in the cumulative amount of intercepted radiation (IPAR_c). In oilseed rape, lower growth due to N deficiency was more associated with RUE_{SC} than IPAR_c, while the contribution of mobilised biomass components contributed similarly to decreased growth. The contribution of N in photosynthetic areas decreased from flowering to grain filling, and was linearly related. Oilseed rape tended to higher RUE_{SC} than wheat during the critical period for grain number determination and lower during grain filling. Reasons for these differences and possibilities to increase yield in terms of the photosynthetic efficiency of the different components and the N:K ratio during grain filling.

Erratum

Page 25, line 14 of the Abstract, where it reads:

“The absorption of N per unit root biomass during the critical period for grain number determination was similar among species”

should read:

“The specific absorption rate of N per unit root dry weight during the critical period for grain number determination was higher in oilseed rape than wheat”

Organs and ...

Key words: biomass, yield, intercepted radiation, radiation use efficiency, nitrogen, wheat, oilseed rape.

Abbreviations: DAS, days after sowing; fIPAR, fraction of intercepted PAR; IPAR_c, cumulative intercepted PAR; LIN%, leaf and inflorescence N concentration; PAR, photosynthetically active radiation; RUE, radiation use efficiency; SC, synthesis costs.

Introduction

Nitrogen (N) fertilisation has made an unquestionable contribution to the improvement of yield and quality of winter/spring crops during this century (e.g. Bell et al., 1995). However, due to economic as well as environmental reasons, today's challenge lies in maximising production using the minimum possible amount of N fertiliser. The use of N-efficient genotypes and the improvement of crop N management can be part of the solution. Both approaches require an understanding of the contribution that different physiological processes make to biomass and yield formation under N limitation.

Wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) are frequent winter/spring components of crop rotations in temperate regions world-wide. The cereal is undoubtedly dominant (total cultivated area ca. 230×10^6 ha, FAO, 1997). The oilcrop rises as an increasingly popular alternative because it adds profitability to the traditionally cereal-based rotation (Zentner et al., 1996), helps to disrupt the cycle of soil-born pathogens (Kirkegaard et al., 1997) and is potentially suitable for double-cropped systems. Despite this growing interest, studies that comparatively assess the productivity and N use efficiency of wheat and oilseed rape have been scarce so far (Habekotté, 1997a; Hocking et al., 1997a). Changes in biomass production and yield in response to N supply have been observed both in oilseed rape (Bilborrow et al., 1993; Wright et al., 1988) and wheat (Fischer, 1993), but the species seem to differ in the magnitude of the response. It has been argued that oilseed rape has a higher critical N concentration for biomass formation than wheat (Colnenne et al., 1998), and higher doses of N fertiliser are recommended for oilseed rape than wheat (Hocking et al., 1997a). These results suggest that the crops differ in the capacity to recover N fertiliser, and/or in the efficiency to use N for biomass or yield formation. Variations in biomass production in response to N availability could rise from differences in the amount of cumulative intercepted radiation by the canopy (IPARc, MJ m⁻²), the radiation use efficiency (RUE, g MJ⁻¹), and the partitioning among different organs (Charles-Edwards, 1982). Different evidences suggest that in oilseed rape, more than in wheat, changes in biomass formation in response to N level could be linked to variation in IPARc. During vegetative stages, a higher sensitivity of leaf expansion to N shortage has been observed in dicots with respect to cereals (Radin et al., 1983). This has been mentioned as the cause of sustained leaf N status and, consequently, photosynthetic capacity and RUE in dicots (Vos and van der Putten, 1997). In addition, during reproductive growth in oilseed rape and other *Cruciferae*, leaf shedding is a widespread phenomenon that decreases the amount of light intercepted by the crop.

Direct comparisons between wheat and oilseed rape, under non-limiting water conditions, can contribute to elucidate the strategies of each species to maximise N productivity. In both crops, a period has been identified when biomass accumulation is critical for the determination of grain or seed number, i.e. the grain yield component more correlated to yield (Fischer, 1985; Mendham, 1981). Growth between the end of flowering and maturity determines mainly the remaining yield component, seed

weight. Thus, in this study, we investigated differences and similarities between wheat and oilseed rape in the response of yield, growth and N accumulation and use to N supply focusing on the critical period for grain number determination and grain filling. The results are used to identify and discuss options for improvement of biomass production and yield, N productivity and N management in these crops.

Materials and Methods

Experimental set-up

Spring wheat (*Triticum aestivum* L., var. Minaret) and spring oilseed rape (*Brassica napus* L., var. Aries) were sown on 16.4.1997 in Wageningen, The Netherlands (51°58' N, 5°40' E), in containers (0.7 (W) x 0.9(L) x 0.4 (H) m). Each container had only one species, planted in 6 rows (2 were border), 0.12 m apart. Crop rows were oriented in the direction north-south. From bottom to top, the containers were filled with: a layer of pebbles (0.05-0.06 m deep), a thin plastic net to prevent rooting below the soil layer, and sandy soil (pH= 5.9, Nt=0.34 gN kg⁻¹ soil). The initial amount of mineral N (NO₃⁻ and NH₄⁺) in the soil was 3.0 g N m⁻². A ground mixture containing the equivalent to 555 g m⁻² of dolomite, 25.4 g m⁻² triple superphosphate (43% P₂O₅), 26.4 g K (19% as K₂O and the remainder as K₂SO₄) and 8.6 g S as K₂SO₄ was added to every container. Half of the mixture was placed at 0.05 and half at 0.2 m below soil surface. A solution containing micronutrients and Fe was applied twice a week together with the watering. Pipelines for drip irrigation were installed 0.05 m below soil surface. Soil moisture was kept at 80-90% field capacity. Soil humidity was monitored with tensiometers and the containers were weighed before watering to prevent leaching. Weeds were manually removed and pests and diseases chemically controlled. The containers were placed under a shelter with open sides and polycarbonate roof (3.5 (H) m), which could be opened for ventilation. Temperature and humidity were recorded inside the shelter. Each container was surrounded by a plastic net to diminish light penetration from the sides. Sampling areas were bordered on either side by an intact row and on either end by at least 0.25m.

Treatments and experimental design

Wheat and oilseed rape were grown at different levels of N supply. The basic N doses were 2 (N1), 5 (N2) and 14 (N3) g N m⁻². Two more treatments were created by supplying 5 g N m⁻² during grain filling to N1 and N3 (N1+GF and N3+GF, respectively). With the late fertilisation we aimed to alter leaf area duration and leaf N content. The total amount of fertiliser applied during vegetative growth was split in three additions. N was added as Ca(NO₃)₂·4 H₂O dissolved in the irrigation water. The late dose was supplied to oilseed rape after the end of flowering (70 days after sowing, DAS), in view of the rapid leaf loss during flowering, and in wheat, during grain filling (86 DAS). Plant density was 300 pl. m⁻² for wheat and 150 pl m⁻² for the oilseed rape. Treatments were replicated three times and arranged in a completely randomised block design. Each replicate consisted of two containers.

Phenology, biomass and leaf area

Crop phenology was determined with the decimal codes (DC) proposed by Zadoks et al. (1974) for wheat and by Sylvester-Bradley and Makepeace (1984) for oilseed rape. Within each plot, the stages were dated when 50% of the plants reached that stage. Five harvests of above and below ground biomass were carried out at 50, 69, 85, 97 and 110 DAS. The first two were scheduled to include the critical period for grain number determination in both crops. In wheat, grain number is determined during the period that extends from early booting to almost one week after anthesis (Fischer, 1985). In oilseed rape, pod and seed number are determined during flowering (Habekotté, 1993; Tayo and Morgan, 1979). The remaining harvests took place during grain filling and at maturity. From harvests 1 to 4, 0.3 m of row was harvested per replicate, in the final harvest 0.9 m was sampled. Plants were cut at ground level and dissected in green and senescent leaves (including shed leaves in oilseed rape), stems (including tap root and inflorescence stalks in oilseed rape and leaf sheaths in wheat) and reproductive organs. Roots were collected from four core samples (50 mm diameter), two from the row and two from the interrow, in the full depth of the container. After harvesting, roots were washed from soil, and root length was assessed by the line intersect method (Tennant, 1976). The area of green leaves, stems, spikes and pods was measured with an electronic planimeter (LI-3100, LI-COR, Inc., Lincoln, Nebraska, USA). In the case of non-cylindrical organs, stems, spikes and pods this corresponds to projected area. All plant parts were oven-dried at 70°C for 48h and weighed. Grains and chaff from wheat, and pod walls and seeds of oilseed rape, were separated by manual threshing.

Estimation of glucose requirements for biomass synthesis

To evaluate biomass production of wheat and oilseed rape on a comparable basis, we corrected it by the costs of synthesis, deriving the glucose requirements for synthesis of proteins, lipids and carbohydrates from Penning de Vries et al. (1983). A fraction of seed proteins was assumed to be synthesised from amides, resulting from degradation of vegetative proteins (Flénet and Kiniry, 1995). Total N was determined in each plant part with a CHN-O analyser (Fissons Instruments, Interscience BV, Breda, The Netherlands); conversion factors for N to protein were 5.75 for wheat (Gooding and Davies, 1997) and 6.25 for oilseed rape (Uppström, 1995). Lipid content in oilseed rape seeds was analysed using a capillary Chrompack CP9000 gas chromatograph. Oil percentage in wheat grains and other plant parts in both species was set to 2% (Penning de Vries et al., 1983). The percentages of organic acids, mineral and lignins in different plant parts were set according to Penning de Vries et al. (1983). The percentage of carbohydrates was calculated by difference. Biomass values corrected by synthesis costs (SC) were expressed in g glucose m⁻².

Interception of radiation and radiation use efficiency

Photosynthetically active radiation (PAR) was measured twice a week above the canopy, below the inflorescence, above the layer of senescent leaves and at ground

level, using a linear ceptometer (SF-80, DELTA-T Devices LTD, Cambridge, England). Readings were performed within 1h of solar noon. Global radiation data were obtained from a nearby station, PAR was taken as 50% of the global radiation. The fraction of the incident PAR intercepted by the crop ($fIPAR$) was calculated above the line where most dead leaves were present. Changes in $fIPAR$ between consecutive measurements were assumed to be linear. The cumulative radiation intercepted by the crop (IPARc) from 50 DAS onwards was calculated from the measured and estimated values of daily $fIPAR$, and the data of external incident PAR. Roof transmissivity was 0.85. The extinction coefficient of light penetrating in the canopy was calculated at 50 DAS after Thorne and Pearman (1988). Radiation use efficiency was calculated on total biomass corrected by the costs of synthesis (RUE_{SC}) and expressed in g glucose MJ^{-1} PAR. RUE_{SC} was derived separately for the critical period for grain number determination and grain filling. The first one was estimated as the quotient between biomass (SC) accumulation from 50-69 DAS and the corresponding IPARc. During the grain filling period, RUE_{SC} was estimated as the slope of the linear regression between biomass accumulated after the first harvest and IPARc.

Concentration of N in photosynthetic tissues

An indicator of N concentration in leaves and inflorescence structures (i.e. pod walls and chaff) was calculated (LIN%). Its value was determined as the weighted average of N concentration in those organs multiplied by their dry weight, over the successive harvest intervals.

Mobilisation of biomass and N

The basic formula to calculate the percentage apparent mobilisation of N and biomass from different organs during grain filling was:

$$\text{Apparent mobilisation} = \frac{\text{Maximum amount} - \text{amount at maturity}}{\text{Maximum amount}} \cdot 100 \quad \text{Eq. 3.1}$$

To have a more adequate estimate of the amount of biomass mobilised, the numerator of Eq. 3.1 was modified to account for maintenance costs and conversion losses. First, biomass from non-grain organs was converted to its glucose equivalent, given the composition of each organ and the carbon content of each fraction (Goudriaan and van Laar, 1994). Biomass spent in maintenance respiration during the period in question was obtained per organ with the coefficients proposed by Penning de Vries et al. (1983). The estimate of maintenance respiration was deducted from the numerator in Eq. 3.1. Further, it was considered that only 0.947 of the remaining amount was available to be mobilised due to losses in the conversion between sugar forms (Goudriaan and van Laar, 1994). It was assumed that the N and biomass mobilised

was translocated to the grains. To calculate the contribution of mobilised biomass from different organs to yield several steps were followed. The equivalent of mobilised biomass was expressed in terms of yield by considering the assimilate requirements of the grains. Then, the percentage contribution of mobilised biomass or N to yield or grain N content, respectively, was calculated as:

$$\% \text{ Contribution} = \frac{\text{Amount mobilised}}{\text{Amount at maturity}} \cdot 100 \quad \text{Eq. 3.2}$$

Indexes of N use efficiency

N uptake was calculated as the summation of the products of N concentration and dry matter of different plant parts. N harvest index, as the ratio of N in the grain with respect to N in aboveground biomass, both at maturity, expressed as percentage. N uptake efficiency was calculated as the ratio between N uptake and available N (Huggins and Pan, 1993). Available soil N was the sum of initial amount of N in the soil, N added as fertiliser and N mineralised during the season. The last fraction was estimated as the difference between the amount of N present in the plant + soil system at maturity and the sum of N present at sowing and added as fertiliser. N use efficiency was calculated as the ratio between yield and N uptake at maturity ($\text{kg grain kg}^{-1} \text{ N}$) (Huggins and Pan, 1993). The efficiency to produce biomass from N was estimated as the slope of the regression between cumulative biomass and cumulative N uptake. Agronomic efficiency was estimated as the amount of yield formed per unit of N supplied as fertiliser ($\text{kg grain kg}^{-1} \text{ N}$ from fertiliser). The specific absorption rate of N per unit root dry weight was calculated for the period between 50 and 69 DAS as the ratio between the rate for N uptake and the mean root dry weight ($\text{gN g}^{-1} \text{ root biomass d}^{-1}$).

Statistical analysis

Treatment effects were tested by ANOVA, and differences were compared using the Least Significant Differences test (LSD, $P < 0.05$). Differences between slopes, intercepts and linear models were assessed by pairwise comparison with a t-test built in Genstat 5 (1987).

Results

Weather and phenology

Between first and last harvest, average weekly values of daily minimum and maximum temperatures were between 9.6 and 25.1 °C (Fig. 3.1). Daily temperatures were below the minimum weekly average in ten occasions (the lowest was 5.1°C) and above the weekly maximum in seven occasions (the highest was 28.6 °C). Weekly averages of global radiation varied between 11.2 and 26.7 $\text{MJ m}^{-2} \text{ d}^{-1}$.

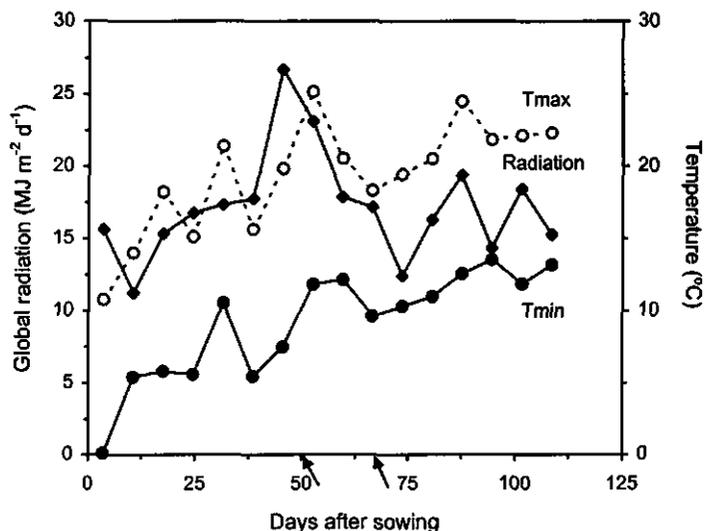


Figure 3.1. Weekly average of global radiation (closed diamond), maximum and minimum temperature since sowing (open and closed circle respectively). Arrows indicate the start and end of the critical period for grain number determination (50-69 DAS).

Both crops emerged at 9 days after sowing (DAS). At 50 DAS wheat was with the ligule of the flag leaf just visible (DC39) and oilseed rape in first flower open (DC41). Anthesis in wheat was recorded at 62 DAS. At 69 DAS, wheat was in the beginning of the grain-filling period (DC71), and oilseed rape had the lower pods longer than 2 cm (DC51). In all combinations of species and N levels, the critical period for grain number definition occurred between 50 and 69 DAS.

Biomass, yield, N uptake and harvest indexes

In both crops, dry matter production was increased by N supply (from N1 to N3) (Table 3.1). The species had similar total biomass when the costs of synthesis were considered but oilseed rape showed slightly but consistently lower values than wheat. The harvest index (HI) was significantly increased by N fertilisation, but the effect was minimised when the changes in chemical composition were taken into account (HI_{SC}). Oilseed rape had a lower proportion of biomass in the grains, expressed as HI or HI_{SC} ($P < 0.001$). Wheat was more productive in terms of yield, on a dry matter basis (Table 3.1). Once corrected for the synthesis costs, yields did not differ among species, though oilseed rape showed consistently lower values. Oilseed rape yield and biomass responded more than wheat to N addition during grain filling. For instance, whilst yield (SC) of wheat increased ca. 65 g m⁻² in response to late N application that of oilseed rape increased ca. 125 g m⁻² (in both cases averaging N1+GF and N3+GF over N1 and N3, respectively; Table 3.1). The interaction of timing of N application and species on yield and biomass, may be a reflection of the fact that oilseed rape was more source-limited than wheat during the grain filling period.

Table 3.1. Total biomass, grain yield and harvest index (HI) with respective values corrected by synthesis costs (SC), total N uptake and harvest index for N.

	Total biomass	Grain yield	Total biomass SC	Grain yield SC	HI ¹	HI _{sc} ¹	Total N absorbed	HIN ¹
	(g m ⁻²)	(g m ⁻²)	(g glucose m ⁻²)	(g glucose m ⁻²)	(%)	(%)	(gN m ⁻²)	(%)
Wheat								
N1	541	198	735	263	41	40	4.69	76
N2	842	331	1144	442	43	42	7.98	76
N3	1363	580	1863	785	45	45	14.78	78
N1+GF	562	239	771	328	45	45	6.64	79
N3+GF	1365	643	1848	848	49	48	17.97	79
Oilseed rape								
N1	406	102	630	206	27	35	4.20	69
N2	602	165	945	335	29	37	6.52	72
N3	1132	335	1804	698	30	39	11.97	73
N1+GF	472	153	761	315	34	43	7.05	79
N3+GF	1497	496	2237	841	34	38	16.54	73
Nitrogen (N)	*** ²	***	***	***	***	NS	***	NS
Species (S)	***	***	NS	NS	***	***	NS	**
N x S	***	***	**	NS	NS	NS	NS	NS
LSD (<i>P</i> =0.05)	89	39	206	171	4	7	3.73	7

¹ Referred to aboveground content (biomass, N, etc.)

² F-probability value: ***=*P*<0.001, **=*P*<0.01, *=*P*<0.05, NS = non significant.

Differences in grain yield due to N availability were linearly and significantly associated to variations in biomass production (wheat: $R^2 = 0.98$, $P < 0.001$; oilseed rape: $R^2 = 0.99$, $P < 0.001$), and not related to changes in HI. These conclusions were also valid when synthesis costs were taken into account.

Total N uptake at maturity was similar in wheat and oilseed rape (Table 3.1). In both species N absorption increased in response to N availability, including the post-anthesis applications (Table 3.1). At final harvest, 70-80% of the N was in seeds (HI_N) (Table 3.1). The HI_N increased with N, though not significantly, and was lower in oilseed rape than in wheat. N application during grain filling tended to increase the HI_N in both species.

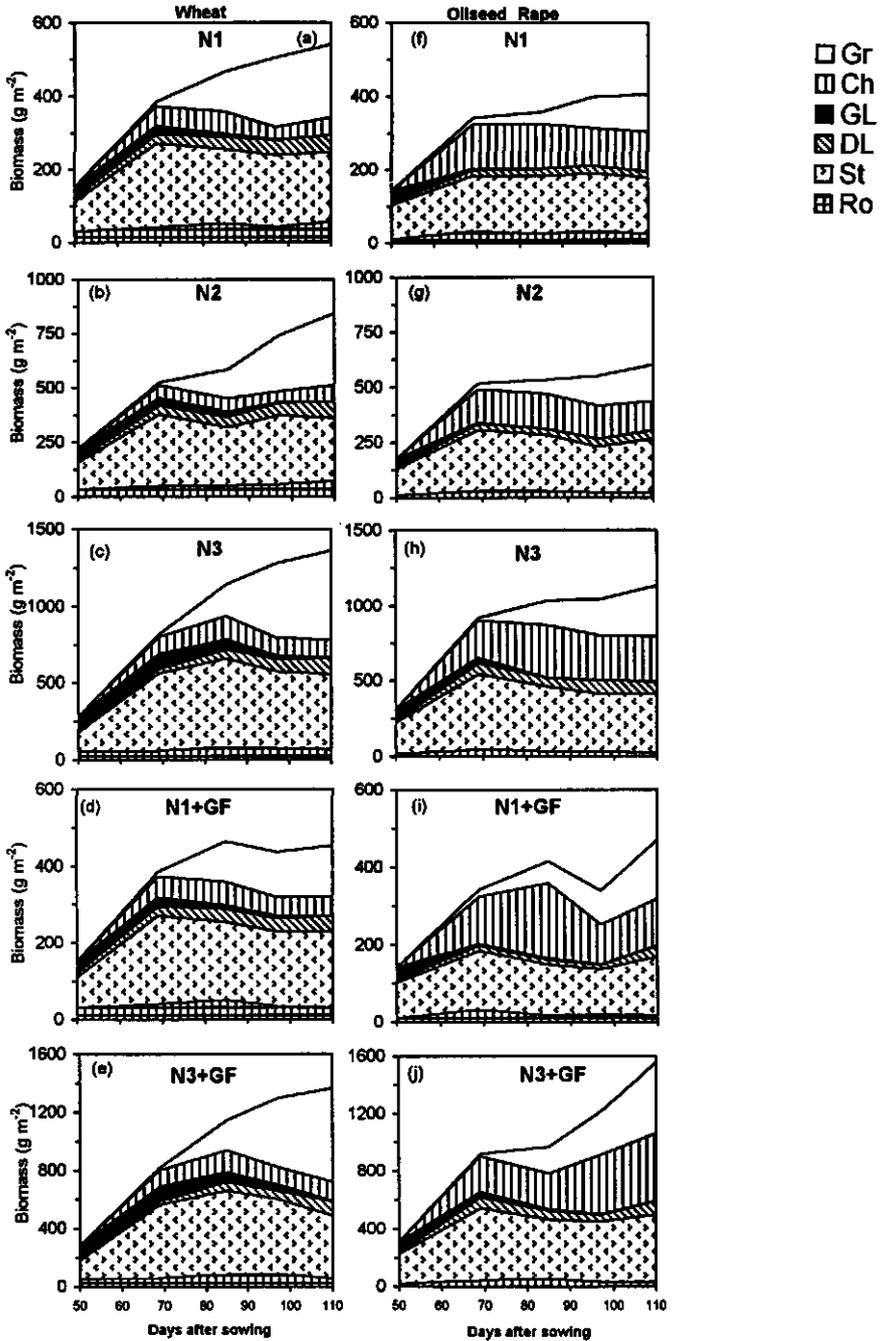


Figure 3.2. Changes in total biomass in wheat (a-e) and oilseed rape (f-j) at different N levels. Dry weight of plant organs indicated by the vertical distances between lines. Ro: roots, DL: dead leaves, Ch: chaff or pod walls, St: stem, GL: green leaves and Gr: grains. Note the different scales.

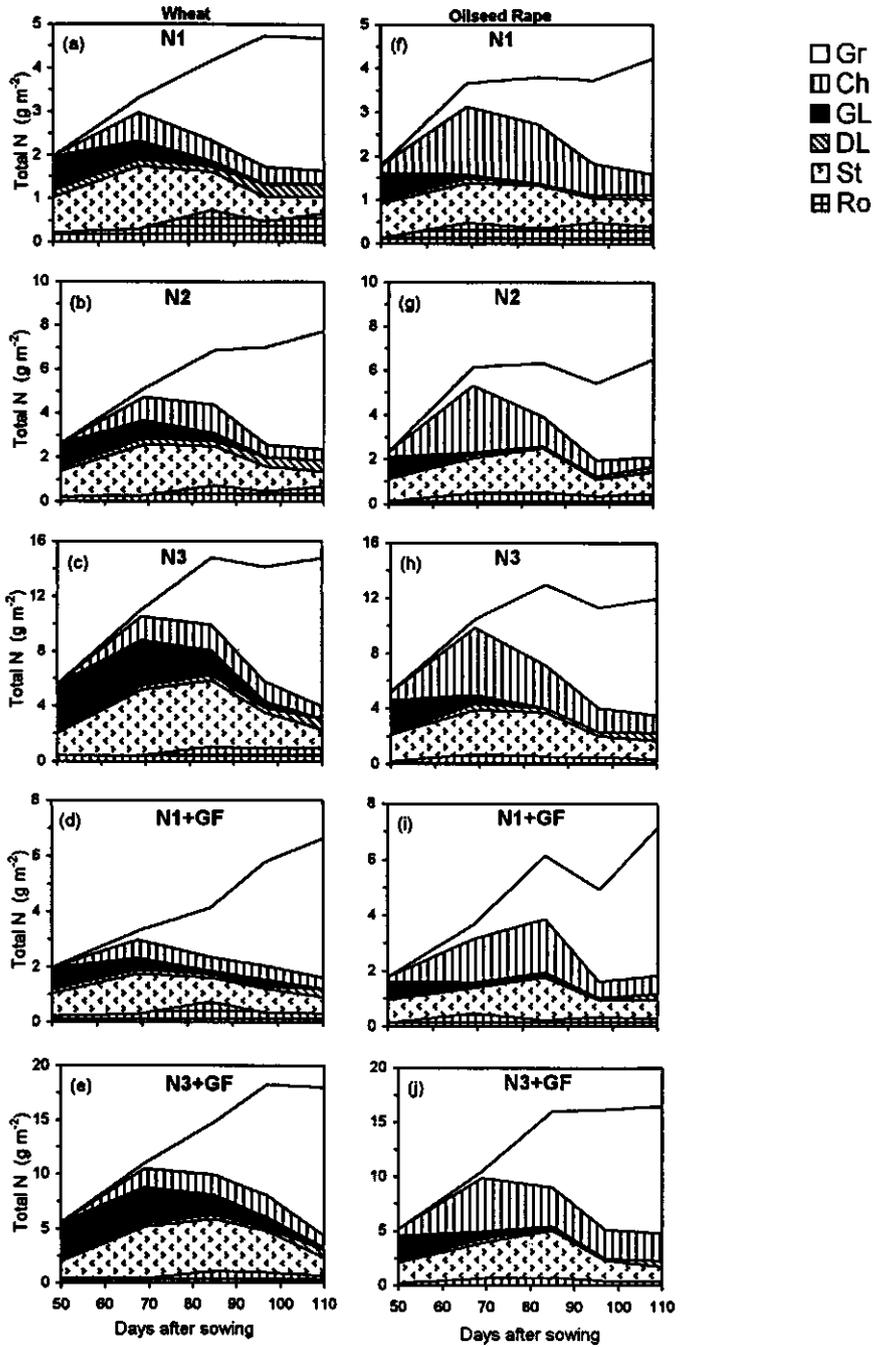


Figure 3.3. Changes in total N in wheat (a-e) and oilseed rape (f-j) at different N levels. N content of plant organs indicated by the vertical distances between lines. Ro: roots, DL: dead leaves, Ch: chaff or pod walls, St: stem, GL: green leaves and Gr: grains. Note the different scales.

Patterns of distribution, accumulation and loss of biomass and N

Between 50-69 DAS, the crops accumulated between ca. 35 to 50% of the biomass and N present at maturity (Figs. 3.2 and 3.3). The time course of dry matter and N gain and mobilisation from different organs varied with species and N level (Figs. 3.2 and 3.3). Biomass and N loss from oilseed rape leaves were observed since the beginning of flowering, whereas in wheat, they started during the period of spike growth at N1 and N2, and at the beginning of grain filling in N3. N application during grain filling retarded the decrease in green leaf mass, particularly in wheat and at low levels of N supply (Fig. 3.2). In both crops, leaves were the most active sink for the late N addition (Fig. 3.3). Together with massive leaf loss, pod growth was the most important change in oilseed rape between 50-69 DAS. Interestingly, pod walls started exporting N since the end of flowering although they were still accumulating biomass (Figs. 3.2 and 3.3). Grain growth started actively after 69 DAS in both species and proceeded at a slower rate at N1 than N3. The stems were the organs with the larger proportion of total dry matter at 50 DAS, and the increase in stem weight from 50 to 69 DAS was higher in wheat than in oilseed rape (Fig. 3.2). Root biomass was a comparatively small fraction of total biomass, generally less than 10%, and it was lower in oilseed rape than in wheat ($P < 0.001$). In both species, the root:shoot ratio decreased at higher N levels, differences were significant between N1 and N3 at 50 DAS ($P < 0.05$).

The N allocation pattern at the beginning of flowering was different from that of dry matter (Fig. 3.3). In oilseed rape and wheat, stems and leaves had a similar share of the total N at the lowest and intermediate N treatments (ca. 40%) (Fig. 3.3). In plants growing at the highest N level, green leaves contained more than 60% of the absorbed N in wheat and ca. 45% in oilseed rape (Fig. 3.3). Irrespective of N level, by the end of flowering, pods were the main reservoir of N in oilseed rape, concentrating 40-45% of the total N, while stems were second in importance (25-30% of the total N). In wheat, most of the total N in the plants at the end of the critical period for grain number determination was present in stems.

Apparent efficiency of biomass and N mobilisation and contribution to grain yield and grain N content

The apparent contribution of biomass mobilisation during grain filling to grain yield was more important in wheat than in oilseed rape at N1, the opposite trend was detected at N3 (Table 3.2). In both species, stems and chaff or pods concentrated most of the biomass at the beginning of grain filling (Table 3.2, Fig. 3.2). These organs were also the most efficient in remobilising dry matter. Stems and chaff or pods ranked first and second for the contribution of reserves to grain growth at the intermediate and high levels of N supply (Table 3.2). In oilseed rape, at N1, the contribution of pod walls exceeded that of stems. The contribution of leaves to the N or dry matter in the grains was low, since the calculations started at the beginning of grain filling, i.e. after massive leaf loss had occurred in oilseed rape (Fig. 3.2).

Table 3.2. Effect of N supply on the apparent mobilisation of biomass and N from different organs and on the estimated contribution of mobilised biomass or N to yield and grain N content.

	Wheat			Oilseed rape		
	N1	N2	N3	N1	N2	N3
Proportion of biomass mobilised (%)						
Stems	11.1	7.7	11.9	2.9	10.3	18.5
Leaves	¹	-	8.4	17.9	-	21.8
Chaff/Pods	15.0	-	16.0	8.7	12.7	11.2
Proportion of N mobilised (%)						
Stems	74.5	70.4	71.7	35.7	49.7	58.7
Leaves	49.2	52.7	78.3	31.6	22.2	47.3
Chaff/Pods	53.1	54.7	47.4	73.7	85.0	73.4
Estimated contribution of mobilised biomass to yield (%)						
Stems	10.2	6.1	9.1	2.2	6.3	10.3
Leaves	0.0	0.0	1.3	1.6	0.0	3.2
Chaff/Pods	3.7	0.0	3.2	4.9	5.9	5.8
Total	13.9	6.1	13.5	8.7	12.2	19.3
Estimated contribution of mobilised N to grain N (%)						
Stems	35.5	29.6	31.5	13.3	22.5	21.9
Leaves	9.5	11.0	26.4	2.3	1.4	6.2
Chaff/Pods	11.2	15.3	8.9	43.5	58.0	42.4
Total	56.3	55.9	66.8	59.1	81.9	70.5

¹ No change or gain in biomass.

The amount of N remaining in dead leaves of oilseed rape never exceeded 5% of the total N absorbed. Pod walls in oilseed rape and stems in wheat were the most efficient N donors (Table 3.2). In both crops, N redistribution provided half or more of the N required for protein synthesis in the grains. The apparent contribution of mobilised N to grain N increased with the N dose, more in oilseed rape than in wheat (Table 3.2).

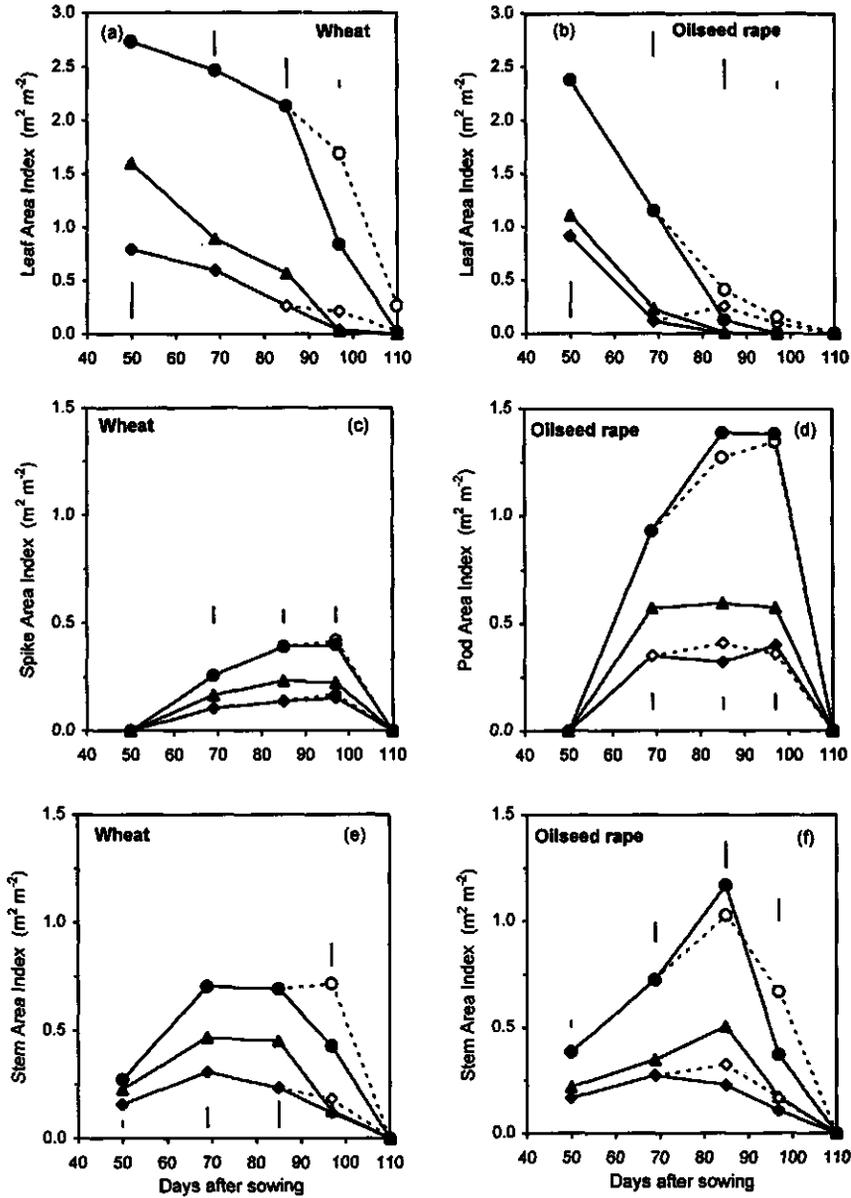


Figure 3.4. Time course of leaf area index, spike or pod area index and stem area index. Closed diamond, N1; closed triangle, N2; closed circle, N3; open diamond, N1+GF; open circle, N3+GF. Values for wheat in a-c; oilseed rape in d-f. Vertical bars are standard errors of the differences between means.

Table 3.3. Effect of N supply on the total fraction of IPAR ($fIPAR$), the $fIPAR$ done by the inflorescence, cumulative IPAR ($MJ m^{-2}$) and PAR extinction coefficient (k) at 50 DAS.

	Wheat					Oilseed rape					LSD ($P=0.05$)
	N1	N2	N3	N1+GF	N3+GF	N1	N2	N3	N1+GF	N3+GF	
Total $fIPAR$											
50 DAS	0.54	0.67	0.83			0.68	0.78	0.86			0.11
69 DAS	0.49	0.53	0.76			0.34	0.52	0.74			0.22
85 DAS	0.38	0.48	0.71			0.33	0.33	0.55	0.38	0.60	0.07
97 DAS	0.22	0.29	0.66	0.40	0.59	0.27	0.38	0.55	0.43	0.54	0.14
$fIPAR$ by the inflorescence											
69 DAS	0.07	0.09	0.18			0.18	0.32	0.46			0.10
97 DAS	0.09	0.12	0.17	0.13	0.15	0.27	0.38	0.55	0.38	0.54	0.14
Accumulated IPAR ($MJ m^{-2}$)											
50-69 DAS	75	88	115			76	97	117			21
50-85 DAS	115	134	183			107	136	177	109	176	32
50-97 DAS	140	167	239	146	230	132	165	227	143	223	35
50-110 DAS	151	181	271	166	259	146	184	249	164	250	39
k											
50 DAS	0.65	0.43	0.43			1.06	0.90	0.77			0.22

Green area, interception and accumulation of radiation

The effect of N addition on leaf area index (LAI) and fractional radiation interception ($fIPAR$) at 50 DAS was highly significant ($P < 0.001$) (Fig. 3.4, Table 3.3). At 50 DAS, the crops differed in the $fIPAR$ at the lower N levels but reached similar values at N3 (Table 3.3). By the end of flowering, leaf shedding in oilseed rape, produced a dramatic reduction in LAI, particularly in N1 and N2 (Fig. 3.4b). In oilseed rape, the inflorescence was more important for intercepting radiation than in wheat (Table 3.3). The growth of pods and inflorescence stalks increased the total green area of oilseed rape until 85 DAS (Fig. 3.4) but the capacity for interception remained similar or occasionally smaller than wheat (Table 3.3). N addition during grain filling retarded leaf area loss in both crops and N levels, with significant effect on $fIPAR$ only in low N crops (Table 3.3). The light extinction coefficient (k) at 50 DAS, was higher in oilseed rape (Table 3.3). In both crops, N fertilisation tended to decrease k . The total amount of IPAR accumulated (IPARc) per N treatment was similar for the two crops, irrespective of the period under comparison (Table 3.3).

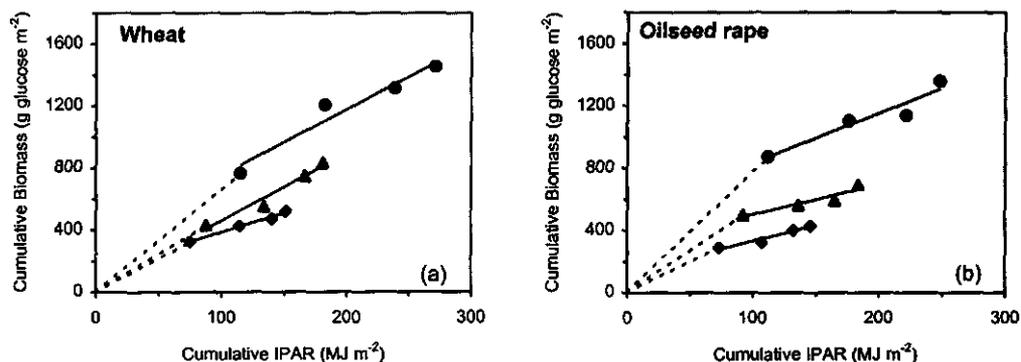


Figure 3.5. Cumulative total biomass vs. cumulative IPAR for wheat (a) and oilseed rape (b) from 50 to 69 DAS (dotted line) and from 69 to 110 DAS (solid line). Closed diamond, N1; closed triangle, N2; closed circle, N3. Details about regression lines in text.

Radiation use efficiency

The effect of the treatments on RUE_{SC} were similar when calculated on above or total biomass basis, therefore the latter are presented. RUE_{SC} was significantly affected by N availability during the critical period for grain number determination (50-69 DAS) ($P < 0.01$) or grain filling (69-110 DAS) ($P < 0.005$). RUE_{SC} values from 50 to 69 DAS in wheat were N1= 4.5, N2=5.0 and N3=6.7 and in oilseed rape N1=4.1, N2=5.4, N3=7.8 g glucose MJ⁻¹ (LSD_{0.05}=2.3) (dotted lines in Fig. 3.5). The species did not differ in RUE_{SC} from 50 to 69 DAS but it is interesting to note that the maximum value was reached by oilseed rape. The linear regressions between biomass (SC) and IPAR_c during grain filling were significant at $P < 0.05$, except for N2 in oilseed rape ($P = 0.06$) (Fig. 3.5, solid lines). The values of the slopes were in wheat N1= 2.4, N2=4.3 and N3=4.2 and in oilseed rape N1=2.0, N2=1.9, N3=3.2 g glucose MJ⁻¹. RUE_{SC} between 69 and 110 DAS was similar between species at N1 and N3, and higher in wheat at N2 compared to oilseed rape ($P = 0.02$). In the treatments with N addition during grain filling, RUE_{SC} in wheat was for N1+GF= 2.5 (s.e.= 0.6) and N3+GF= 4.5 (s.e.= 0.3), and in oilseed rape for N1+GF = 2.3 g glucose MJ⁻¹ (s.e.= 1.1) and N3+GF= 5.1 g glucose MJ⁻¹ (s.e.= 0.7). The basic N levels and their correspondent with extra dressing during grain filling were similar per species, but oilseed rape tended to increased RUE_{SC} when N was added over N3. The few harvests after the late N addition make the detection of significant differences difficult.

RUE_{SC} values for the period of 50-69 DAS and grain filling were plotted against a variable reflecting the weighed N concentration of leaves and chaff or pod (LIN%, see Materials and Methods) (Fig. 3.6). The relation between RUE_{SC} and LIN% was curvilinear. During the critical period for grain number determination, the maximum value of RUE_{SC} was slightly higher in oilseed rape than in wheat, despite the lower LIN%. Furthermore, the differences in RUE_{SC} between flowering and grain filling were higher for oilseed rape than for wheat.

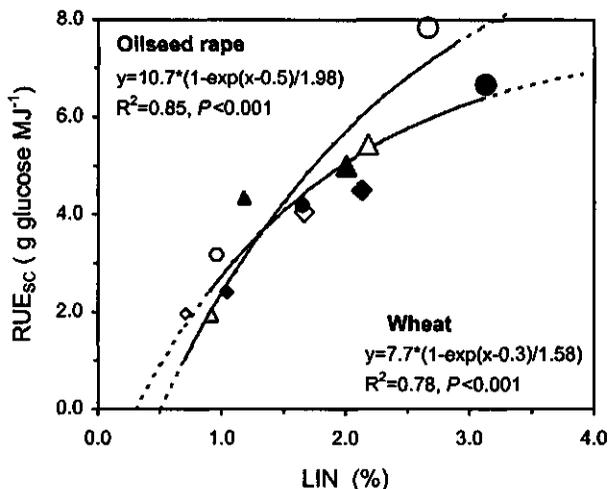


Figure 3.6. Radiation use efficiency vs. leaf and chaff or pods N concentration (LIN%). Open symbols correspond to oilseed rape, closed to wheat. Big symbols represent the period 50-69 DAS, small correspond to 69-110 DAS. Diamond, N1; triangle, N2; circle, N3. Regression lines were fit per species, covering both periods.

Indexes of N use efficiency

Both species recovered equal amounts from the total available N in the soil, the N uptake efficiency averaged 84% (Table 3.4). The gain in biomass (SC) vs. N uptake after 50 DAS was fit to a linear regression. The slope ($\text{g glucose g}^{-1} \text{N}$) represents the efficiency to use N for biomass formation, and was higher ($P < 0.05$) in oilseed rape ($y = 167.3x + 29.3$, $R^2 = 0.95$, $n = 12$) compared to wheat ($y = 136.7x + 85.6$, $R^2 = 0.98$, $n = 12$). The species had a similar N use efficiency, expressed as yield per unit of N absorbed (Table 3.4). The agronomic N use efficiency declined with increased N supply and was significantly lower in oilseed rape than wheat (Table 3.4) associated to the lower HI for biomass of oilseed rape (Table 3.1). Oilseed rape had a significantly higher amount of root length per unit soil volume and higher specific root length at 69 DAS (Table 3.4). This means that oilseed rape had either thinner or lighter roots than wheat, since its root biomass was similar or smaller than that of wheat depending on the treatment (Fig. 3.2). The specific absorption rate of N per unit root dry weight during the critical period for grain number determination was higher in oilseed rape than wheat ($P < 0.01$).

Grain quality

The protein content of the grains was significantly affected by species and N level ($P < 0.001$) and the interaction was also significant ($P < 0.001$) (Table 3.4). N supply increased the protein content in wheat, and did not alter it in oilseed rape. N addition during grain filling increased protein content in both species.

Table 3.4. Different indexes of N use, root length density, specific root length, specific N uptake rate, grain protein content and oil concentration in seeds.

	N uptake efficiency	N use efficiency	Agronomic efficiency	Root length density 69 DAS	Specific root length 69 DAS	Specific N uptake rate 50-69 DAS	Grain protein content	Seed oil
	(%) kg N absorbed kg N ⁻¹ available	kg yield (glucose) kg N absorbed ⁻¹	kg yield (glucose) kg N ⁻¹ applied	cm cm ⁻³	km g ⁻¹	gN (g d) ⁻¹	(%)	(%)
Wheat								
N1	82	56	132	5.83	0.149	0.002	8.9	- ¹
N2	91	55	88	8.25	0.168	0.003	9.4	-
N3	95	53	71	8.46	0.150	0.005	10.7	-
N1+GF	67	49	47	NA ²	NA	NA	12.1	-
N3+GF	95	47	53	NA	NA	NA	12.8	-
Oilseed rape								
N1	84	49	103	9.24	0.308	0.005	16.2	34.5
N2	81	52	67	9.95	0.368	0.011	16.6	35.8
N3	86	59	64	18.61	0.425	0.010	15.8	38.9
N1+GF	71	45	45	NA	NA	NA	21.7	33.5
N3+GF	87	51	53	NA	NA	NA	17.5	33.3
LSD (<i>P</i> =0.05)	20	9	18	3.7	0.11	0.006	1.40	8.2

¹Oil content was not determined in wheat.²Not applicable.

The effect of N supply on the oil content of oilseed rape seeds was not significant (Table 3.4), but the ratio of final oil to protein was lower when N was added during grain filling.

Discussion

The timing of the critical period for grain number determination and the duration of the total growth cycle were similar for the genotypes of wheat and oilseed rape tested. This synchronicity set the framework for a straightforward comparison of N effects among species.

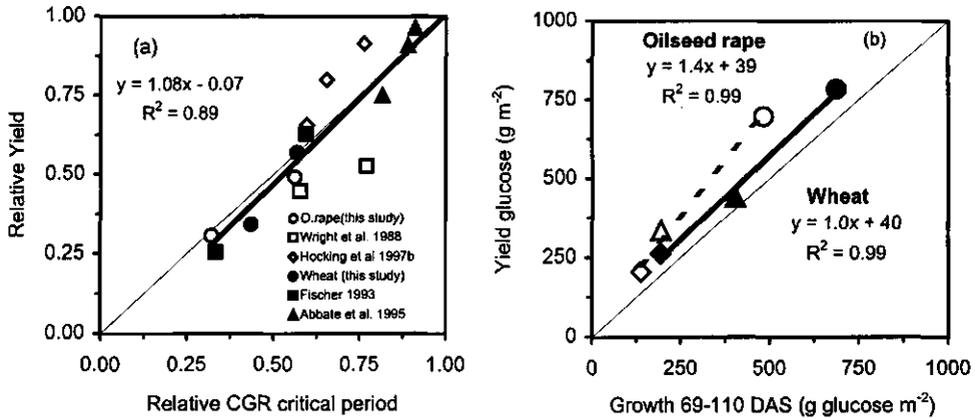


Figure 3.7. (a) Yield vs. crop growth between 50-69 DAS, both variables relative to the highest N level, open symbols for oilseed rape, closed for wheat; (b) Yield vs. crop growth during grain filling. In (b) open symbols and dotted line for oilseed rape, closed symbols and solid line for wheat; N1, diamond; N2, triangle; N3, circle. The studies were carried out under irrigation, except for Hocking et al. (1997b) in oilseed rape.

Yield, harvest index and grain quality

The increase in yield with N availability was associated to the impact on biomass accumulation, particularly during the critical period for grain number determination. Different studies, representing the changes in the crop growth rate during the critical period for grain number determination against yield, agree with this trend (Fig. 3.7a). We did not analyse yield components, but in the data from other studies depicted in Fig. 3.7a grain number was the variable more associated to grain yield. The comparison of growth after flowering vs. yield gives a rough indication of the degree of source-sink limitation during grain filling. In general, wheat was near the 1:1 line, suggesting co-limitation by sink and source, while oilseed rape was increasingly source limited from low to high N supply (Fig. 3.7b). The inter-specific differences in source-sink limitation were consistent with the observed apparent contribution of mobilised biomass to yield (Table 3.2), which increased with N availability in oilseed rape, while in wheat it was similar at high or low N supply. As a whole, the results of this study share the view that during grain filling, wheat yield is either sink-limited or co-limited (Slafer and Savin, 1994), whereas pod growth and yield are more frequently source-limited in oilseed rape (Pechan and Morgan, 1985; Wright et al., 1988). Adding N during grain filling in oilseed rape partly relieved the source limitation, but the impact was bigger on total biomass production than on yield (Table 3.1). This indicates the need for increasing simultaneously the source and sink capacity to effectively raise yield potential (Habekotté, 1997b). The small impact of N supply during grain filling on wheat yields was probably not related to the later moment of N addition compared to oilseed rape. Fischer et al (1993), have observed

that an extra N addition at stem elongation (DC42), i.e. earlier than in our study, increased yield only slightly.

Oilseed rape had a slightly lower harvest index (SC) than wheat, others have reported similar values (Angus et al., 1991; Hocking et al., 1997a). We suggest that an increase in yield potential through harvest index could be achieved in oilseed rape if pod walls had a lower proportion of structural dry matter without losing shattering resistance. In oilseed rape the proportion of biomass partitioned to pod walls was higher than that invested in chaff in wheat (25 vs. 9%, this study), and even a higher percentage of dry matter in pod walls than grains at maturity has been reported (Hocking et al., 1997b). The N harvest index, was slightly but consistently lower in oilseed rape than in wheat (shed leaves were included in the calculations). During grain filling, oilseed rape stems and leaves made a smaller contribution to the N pool in the seeds and showed a lower efficiency for remobilising N than in other experiments (Hocking et al., 1997b; Schjoerring et al., 1995) or in wheat. It is possible that N in the leaves at the beginning of flowering might have been translocated to the pods (Zhang et al., 1991). Still, more grain N was derived from mobilisation during grain filling in oilseed rape than in wheat (Table 3.2). The main N reservoirs were pods and stems in oilseed rape and stems and leaves in wheat (this paper, Hocking and Mason, 1993; Schjoerring et al., 1995; Spiertz and Vos, 1983). Finally, the changes in grain quality in response to N were modest in comparison with the variations in yield. In oilseed rape, the increase in the contribution of biomass and N from mobilisation with N level, may have buffered the protein content between the lowest and highest N doses (Table 3.1). Other authors have reported that N fertilisation increased protein content at the expense of oil concentration (Andersen et al., 1996; Taylor et al., 1991). In this study that was only the case when N was added during grain filling. Nevertheless, due to the positive effect of late N addition on yield, the yield of oil per unit area increased with N availability. In both crops, N addition during grain filling had a positive impact on grain protein concentration, because N uptake was increased more than growth.

Interception of radiation and radiation use efficiency

Wheat and oilseed rape growing at high N availability produced comparable amounts of biomass (SC) from the start of the critical period for grain number determination to maturity (50-110 DAS). In addition, in both crops low N availability similarly reduced growth until 50 DAS and from 50 to 110 DAS. However, for each species, the reduction between 50 and 110 DAS was built on processes affected to a different degree. N limitation affected wheat growth through a similar (50-69 DAS) or lower (69-110 DAS) reduction in RUE_{SC} than in $IPAR_c$ (Fig. 3.8a and c). While in oilseed rape, lower biomass accumulation in response to N shortage was, during flowering, more associated to lower RUE_{SC} than to lower $IPAR_c$ (Fig. 3.8b). However, during grain filling, both components contributed similarly to the decreases in growth (Fig. 3.8d). Changes in light interception from 50-110 DAS were related to initial canopy size, the growth of the inflorescence and leaf senescence.

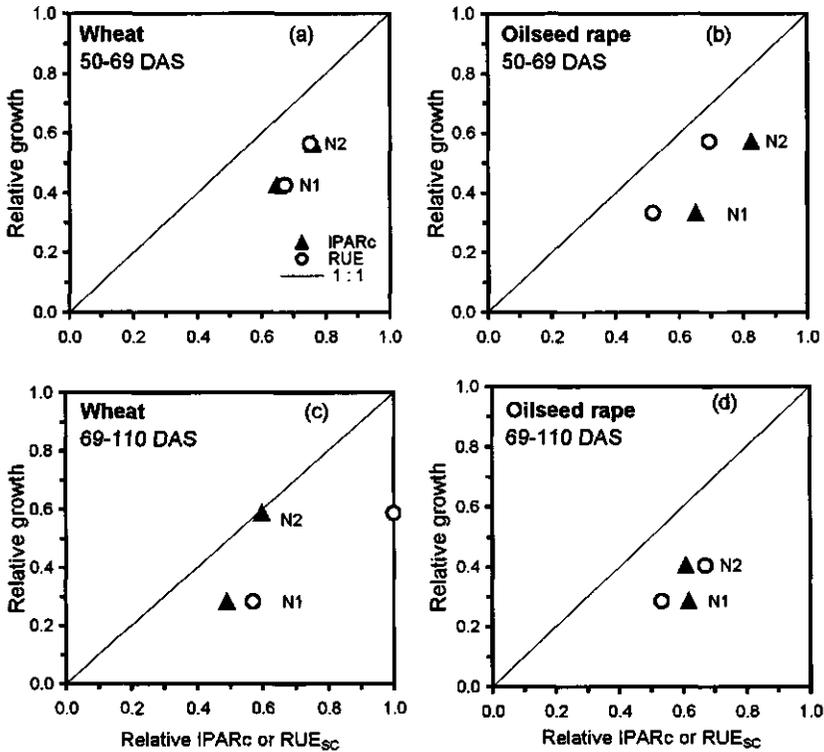


Figure 3.8. Crop growth between 50 and 69 DAS vs. cumulative IPAR (closed triangle) or RUE_{sc} (open circle), all variables relative to the highest N level. Wheat and oilseed rape at 50-69 (a and b) or 69-110 DAS (c and d).

Low N availability resulted in lower leaf area at 50 DAS in both wheat and oilseed rape. Radin et al. (1983) have reported higher sensitivity of leaf expansion to N supply in broad-leaved species than monocots. However, at the canopy level, the large effect of low N on wheat tillering (Fischer, 1993) may have accounted for most of the reduction in leaf area. In both species, k values at the beginning of flowering were in the range observed by other authors (wheat: Fischer, 1993; oilseed rape: Andersen et al., 1996). The trend towards a more planophile canopy observed at low N, may be related to smaller and more rigid laminae (van Arendonk et al., 1997).

RUE_{sc} increased with N availability during the period of grain number definition and grain filling. In wheat, similar responses were observed by Green (1987) and Fischer (1993). Instead, in oilseed rape, Wright et al. (1988) reported no effect of N on RUE measured at rosette and early flowering stage when the leaf N concentration was between 4-5%, a range at which hardly any effects on photosynthesis can be expected. The dependence of RUE_{sc} on N concentration in photosynthetic areas was established with close patterns between species (Fig. 3.6). The sharper response of RUE_{sc} to LIN% during flowering in oilseed rape could be related to differences between species

in leaf photosynthesis rate per unit leaf N (Sinclair and Horie, 1989), the capacity to distribute N to the more-illuminated canopy layers (Hammer and Wright, 1994) or the change in irradiance environment over the leaves during inflorescence growth. The lower RUE_{SC} observed in oilseed rape compared to wheat during grain filling, may be associated with the high mobilisation of N from leaves and pods (Table 3.4) but also with the shift from leaves to pods as the main site for CO₂ assimilation (Rood et al., 1984). Pods have a lower photosynthetic capacity than leaves (Gammelvind et al., 1996). When N was added during grain filling in N3, RUE was increased, because although the change in LIN% (pods + leaves) was small (N3=0.96 vs. N3+GF=1.09 N%) it included a sharp increase in leaf N concentration in the small pool of leaves. Wright et al. (1988) have also shown that, under irrigation, in crops receiving 100 vs. 200 kg N ha⁻¹ (split), yield could be increased from ca. 2.4 to 3.6 tn ha⁻¹ with a similar leaf area duration during pod filling.

During the critical period for grain number determination, RUE in high N crops was 4.6 for wheat and 5.6 g total biomass MJ PAR⁻¹ for oilseed rape, which expressed on aboveground biomass and absorbed PAR (90%) would be 4.6 for wheat and 5.8 g MJ⁻¹ PAR for oilseed rape. These values compare favourably with maximum values recorded in the field, e.g. 3.8 g MJ⁻¹ PAR for wheat (see for review Sinclair et al., 1999). Our estimates may be higher because radiation levels under the shelter are non-saturating and more diffuse (Hammer and Wright, 1994). We can not rule out light penetration from the plot sides at low solar angles, but took every precaution to minimise it (with a border row of crop and net). In oilseed rape, RUE estimates during the critical period may be even higher if we consider that the flower layer may reflect up to 20% of the incoming irradiance when the flower cover is maximum (Yates and Steven, 1987).

N absorption and use

Wheat and oilseed rape had different strategies to capture and use N. Our estimates of N uptake efficiency could be high because N was applied in solution and in split doses, in a sandy soil, and we did not subtract an estimate of absorption at null N dose. Depending on N doses and time of application, values from 40 to 80% have been reported for wheat in irrigated field experiments (Fischer et al., 1993; Smith et al., 1989; Smith and Whitfield, 1990) and from 45 to 90% for oilseed rape (Hocking et al., 1997b; Schjoerring et al., 1995; Smith et al., 1989). Despite the fact that the ranges of N uptake efficiencies are broad and overlap between species, many authors argue that oilseed rape has a lower efficiency of N uptake than cereals (Hocking et al., 1997a; Schjoerring et al., 1995). Such differences in field experiments might be related to a higher sensitivity of dicots to soil compaction, observed as a slower rate of penetration (Gregory and Eastham, 1996) and decreased diameter of root laterals (Bennie, 1991) compared to a monocot. Judging from the values of N uptake efficiency and total N uptake (Table 3.1), the slightly lower root:shoot ratio in oilseed rape was more than compensated by its consistently higher root density, specific root length and specific N uptake rate. Barraclough (1989) also observed that winter oilseed rape growing in the field had higher specific length in fibrous roots than winter wheat. Higher total N

uptake has been observed in winter genotypes of wheat than oilseed rape (Barraclough, 1986 and 1989), such differences are not so evident in spring type crops (wheat: Fischer, 1993; Stapper and Fischer, 1990; oilseed rape: Hocking et al., 1997b; Taylor et al., 1991). The capacity to form yield (SC) per unit N absorbed was similar in wheat and oilseed rape (see also Hocking et al., 1997b), but constructed by each species in a different way. Oilseed rape had a higher capacity to produce biomass per unit N absorbed than wheat but a lower HI (Table 3.1).

Conclusions

The results of this study suggest that efforts to increase yield potential in wheat should be directed to increase biomass production during the period of grain number definition, to improve both the capacity to produce and fill more grains (Slafer et al., 1994). Similar efforts in oilseed rape are likely to be counterbalanced by concomitant reductions in grain weight, unless the capacity for assimilate production during grain filling were also improved. Oilseed rape genotypes with the ability to maintain active leaves during flowering and grain filling are likely to succeed in raising yield potential because leaves have higher photosynthetic rate than pods and are exposed to lower levels of radiation, thus photosynthesising more efficiently. Besides, leaf area duration will be increased. Further, leaf retention could contribute to continued N uptake (Schenk, 1996). This characteristic will not have a negative effect on the ratio between oil and protein content in the seeds, provided the sink capacity is increased in response to higher availability of assimilates.

From the point of view of the management, our results agree that in both crops, N fertilisation should aim to maximise cumulative light absorption during the critical period for seed formation and set (Andersen et al., 1996; Fischer, 1993; Habekotté, 1997b). But, particularly in oilseed rape, it should also aim to enhance leaf photosynthetic capacity during grain filling. Provided soil compaction is not limiting and the duration of crop cycles are similar, the decision of which N doses to apply will differ among species according to the targeted yield, amount of proteins in the grains and the harvest indexes of the varieties. Under the last-mentioned conditions, the capacities for N recovery and total uptake can be assumed as fairly similar among species.

Chapter 4

Radiation and nitrogen use at the leaf and canopy level by wheat and oilseed rape during the critical period for grain number definition

Abstract

Growth analysis during the critical period for grain number definition indicated that biomass production per unit absorbed irradiance (RUE_A) in oilseed rape was more sensitive to N supply than wheat and, reached a higher value at high N (Chapter 3). The reasons behind this response were investigated by combining experimental and modelling work to distinguish between process dynamics at leaf and canopy levels. Oilseed rape and wheat were grown in containers under a shelter at three different levels of nitrogen supply, combined with two levels of plant density at high N supply. Photosynthetically active radiation, photosynthesis light-response curves, leaf N, chlorophyll content and leaf absorptance were measured at different heights within the canopy. The photosynthetic capacities at high and low light and dark respiration were dependent on leaf N content in both species. Canopy photosynthesis and daytime RUE_A were calculated with a model based on N-dependent leaf photosynthesis and intra-canopy light and vertical leaf N distribution. RUE_A was higher in oilseed rape than wheat, and the sensitivity to the average canopy leaf N content increased in oilseed rape from the start to the end of the critical period. This was associated to the fact that oilseed rape had a higher leaf photosynthetic capacity per unit leaf N than wheat and was increasingly shaded by the growth of the inflorescence. The vertical distribution of leaf N was close to that optimising canopy photosynthesis and was similar between species for the extreme levels of N. The model was used for exploring the sensitivity of canopy photosynthesis and RUE_A to different crop characteristics and the results are discussed in relation to possibilities of improvement of productivity in wheat and oilseed rape.

Keywords: radiation use efficiency, leaf photosynthesis, nitrogen use efficiency, period of grain number definition, wheat, oilseed rape.

Abbreviations: see Table 4.1.

Introduction

Optimisation of use of limiting resources, such as nitrogen (N), is one of the most distinctive objectives of contemporary agriculture. To pursue this goal, it is important to acknowledge that plant carbon and N acquisition, and ultimately crop production are regulated by processes occurring at many different levels of organisation. Two distinctive ones are the leaf and the canopy level. The amount of canopy photosynthesis or biomass produced per unit absorbed irradiance, also called radiation use efficiency (RUE_A), can be used as an estimate of crop productivity. RUE_A is affected by N supply via the interplay of effects at the leaf and at the canopy level (Sinclair and Horie, 1989; Muchow and Sinclair, 1994; Hall et al., 1995). At the leaf level, photosynthesis is strongly dependent on leaf N (Evans, 1989a). About three quarters of the leaf N are invested in proteins with different photosynthetic functions (Evans, 1989b) and the partitioning of N between these fractions regulates the photosynthetic capacity at low and high light. In a coarse division, the amount of N invested in pigments determines the efficiency for photosynthesis per unit incident irradiance at low light (EFF_l), whereas the amount of leaf N in electron transport and carboxylation compounds determines the maximum photosynthetic capacity at high light (A_{max}) (Evans, 1989b; Hikosaka and Terashima, 1998).

At the canopy level, N shortage can restrict light interception by reducing leaf area expansion and branching (Fischer, 1993). In some crops, such as oilseed rape, light interception by the leaves can also be reduced at high N supply due to exuberant development of the inflorescence (Chapter 3). These changes in canopy architecture affect the light distribution in the canopy and the average degree of light-saturation of the leaves. The impact that N-induced changes on the light environment have on RUE_A will depend on the vertical distribution of leaf N (Shiraiwa and Sinclair, 1993; Hammer and Wright, 1994). The distribution of leaf N in the canopy affects canopy photosynthesis because processes such as light absorption, regeneration of the activity of enzymes determining CO_2 fixation (i.e. ribulose 1,5-bisphosphate carboxylase /oxygenase) are dependent on both light and leaf N. In fact, for a given light distribution in the canopy, a matching leaf N profile can be calculated that maximises canopy photosynthesis (Field, 1983; Hirose and Werger, 1987b; see Dreccer et al., 1998 for review).

Variability between species has been reported in the response of leaf and canopy photosynthesis to leaf N (Evans, 1989a; Chapter 3). Knowledge on the reasons for differences in the use of N for canopy photosynthesis could help to pinpoint desirable characteristics for the design of more efficient crop ideotypes and improve timing of N-supply. Wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.) are alternative crops in rotations of temperate regions. In both crops, biomass accumulation during the critical period for grain number definition, i.e. around flowering, is crucial for the final yield (Fischer, 1985; Habekotté, 1993; Tayo and Morgan, 1979). In a previous study we observed that growth of oilseed rape during

this period was more sensitive to N shortage than that of wheat due to a greater negative impact on canopy radiation use efficiency (Chapter 3). In addition, at high rates of N supply, the radiation use efficiency was higher in oilseed rape than in wheat. The present contribution aims to elucidate the physiological principles behind the differential response of radiation use efficiency to N observed between wheat and oilseed rape with growth analysis (Chapter 3). Experimental and modelling work were combined to distinguish between process dynamics at leaf and canopy levels. Canopy photosynthesis and daytime RUE_A were calculated with a model based on N-dependent leaf photosynthesis and intra-canopy light and leaf N distribution. The model was also used for exploring the sensitivity of canopy photosynthesis and RUE_A to different crop characteristics. The experimental set-up included N treatments and density levels in order to create a range of light interception and leaf N contents.

Materials and Methods

Experimental set-up, treatments and statistical analysis

Spring wheat (*Triticum aestivum* L., var. Minaret) and spring oilseed rape (*Brassica napus* L., var. Aries) were sown on 16.4.1997 in Wageningen, The Netherlands (51°58' N, 5°40' E), in containers (0.7 (W) x 0.9 (L) x 0.4 (H) m), under an open-sided shelter of transparent plastic roof (3.5 (H) m). Soil moisture was kept at 80-90% field capacity by weighing the containers three times a week. A detailed description of the experimental set-up is given in Chapter 3. The treatments consisted in adding N at the rates of 2 (N1), 5 (N2) and 14 (N3) g N m⁻² to a soil originally containing 3.0 g mineral N m⁻². N was added as Ca(NO₃)₂·4 H₂O in the irrigation water, and split in three additions evenly timed during vegetative growth to prevent depletion effects. Macro-nutrients other than N and micro-nutrients were supplied in non-limiting amounts. Plant density was 300 pl m⁻² for wheat and 150 pl m⁻² for the oilseed rape (D2). At the highest N level, additional lower densities were tested (D1), 150 pl m⁻² in wheat and 75 pl m⁻² in oilseed rape. Treatments were replicated three times and arranged in a completely randomised block design. Two containers were available per replicate. The containers were surrounded by a net to prevent light penetration from the sides. The sampling area within each container was surrounded by a border row and a plastic net, samples were bordered on either side by an intact row and on either end by at least 0.25 m of crop row. Treatment effects were tested by ANOVA. Differences between slopes, intercepts and linear models were assessed by pairwise comparison with a t-test built in Genstat 5 (1987).

Biomass, leaf area and chemical analyses

Crop phenology was described with the decimal codes (DC) proposed by Zadoks et al. (1974) for wheat and by Sylvester-Bradley and Makepeace (1984) for oilseed rape. Harvests of biomass were carried out at 50 (5.6.1997) and 69 (24.6.1997) days after sowing (DAS), collecting 0.3 m of row per replicate. Plants were dissected at height

intervals of 0.05 m, from which green and senescent leaves were separated. At each interval, the area of green leaves was measured with an electronic planimeter (LI-3100, LI-COR, Inc., Lincoln, Nebraska, USA). Samples were oven-dried at 70°C for 48 h, weighed and analysed for total N content with a CHN-O analyser (Fissons Instruments, Interscience BV, Breda, The Netherlands).

Leaf gas exchange, chlorophyll and absorptance

Leaf photosynthesis response to irradiance was measured in an open gas exchange system, the concentrations of CO₂ and H₂O in the ingoing reference air and the outgoing air stream air were measured with infrared gas analysers (ADC The Analytical Development Co. Ltd, UK, model 225 MK3). Air was passed through sodalime to remove CO₂ and pure CO₂ was injected using mass-flow controllers (model 5850, Brooks Instrument B.V., Veenendaal, The Netherlands) to adjust the concentration to 350 ppm CO₂. Air humidity of the ingoing air stream was set to a relative humidity of 70 % by passing dry air through a temperature-controlled humidifier. Measurements were made on leaves number 5 to 8 in wheat and 2 to 8 in oilseed rape, not all leaves were present in all treatments. Photosynthetic rates were measured on detached leaves at five levels of photosynthetic active radiation (PAR), starting at 1500 and down to 0 μmol m⁻² s⁻¹, supplied by a halogen lamp (Philips type 6423, 15V/150W) with an infrared filter. Time for equilibration was allowed between consecutive irradiance levels. Leaves were cut under water to prevent airlock and stomatal closure. From the light response curve, the net photosynthetic rate at light saturation ($A_{\max,n}$, μmol CO₂ m⁻² s⁻¹), the initial slope at low light or quantum efficiency based on incident light (EFF_i , mol CO₂ mol⁻¹ quanta) and the respiration in the dark (R_d , μmol CO₂ m⁻² s⁻¹) were derived. The gross rate of photosynthesis at light saturation ($A_{\max,g}$) was calculated by adding the value of R_d to the $A_{\max,n}$. The level of PAR at which leaves were light saturated was taken as that for 95% of maximum leaf photosynthesis. From the leaves where photosynthesis was measured leaf discs were taken for determination of chlorophyll *a* and *b* (Inskeep and Bloom, 1985) and N was determined as explained. On a separate set of leaves representing all leaf positions, reflectance and transmittance were measured between 400 to 700 nm wavelength with a spectroradiometer connected to a Taylor-type integrating sphere (LI-COR, Inc., Lincoln, Nebraska, USA) and chlorophyll was determined both by reading with a hand-held chlorophyll meter (SPAD-502, Minolta Camera CO., Japan) and chemically as explained.

Interception of radiation: measurements and calculations

PAR was measured twice a week above the canopy, below the inflorescences, inside the canopy every 0.05 m height until the layer of senescent leaves and at ground level, using a linear ceptometer (SF-80, DELTA-T Devices LTD, Cambridge, England). Readings were performed within 1h of solar noon. Global radiation data were obtained from a nearby station, PAR was taken as 50% of the global radiation. Roof

transmissivity was 0.85. The extinction of PAR through the canopy was fit to an exponential model (Monsi and Saeki, 1953). On this basis, the mean level of PAR absorbed per unit leaf area at any height (I_{AL} , $\mu\text{mol quanta m}^{-2} \text{ leaf}$) was calculated as:

$$I_{AL} = I_0 k \exp(-k \text{ LAI}) / (1 - t) \quad \text{Eq. 4.1}$$

where I_0 is the PAR level at the top of the canopy ($\mu\text{mol quanta m}^{-2} \text{ ground}$), measured on a horizontal plane. The k represents the coefficient of light extinction in the canopy, calculated as the slope of the linear regression between the log-transformed ratio of PAR incident on a horizontal plane at a certain height and I_0 vs. the leaf area index accumulated above the height of measurement (LAI, $\text{m}^2 \text{ m}^{-2}$). The transmission coefficient of the leaves, t , was taken as 0.05 (Goudriaan and van Laar, 1994).

Model for calculation of actual and optimal canopy photosynthesis

A static explanatory model was developed to calculate daytime total net CO_2 assimilation by the canopy (DTNA, $\text{mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$) and explore its sensitivity to different crop characteristics. The model calculates DTNA at the measured radiation, total leaf N content and observed vertical leaf N distribution. Green leaves were the only organs considered for contribution to photosynthesis. In the model, incident PAR on sunlit and shaded leaves, light absorption and photosynthetic response to irradiance are calculated in a multilayer canopy (every 10^{th} of total LAI), and integrated five times during the day to yield DTNA, by modification of subroutines of the model SUCROS (Goudriaan and Van Laar, 1994). According to observed data, radiation is exponentially extinguished with cumulative leaf area, while leaf N declines linearly with it. The leaf net photosynthetic response to irradiance (P_n , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) is described in terms of $A_{\text{max},g}$, the photosynthetic efficiency based on absorbed irradiance (EFF_A) and the R_d . We assume an asymptotic negative exponential function with incoming irradiance (Goudriaan and van Laar, 1994):

$$P_n = A_{\text{max},g} (1 - \exp(-\text{EFF}_A I_L / (A_{\text{max},g}))) - R_d \quad \text{Eq. 4.2}$$

Our experimental data on $A_{\text{max},g}$, R_d and EFF_1 were dependent on leaf N content per unit leaf area ($\text{mmol N m}^{-2} \text{ leaf}$). $A_{\text{max},g}$ and R_d followed a linear equation, in accordance with Hirose and Werger (1987a). An asymptotic curve was fit to the relation between EFF_1 and leaf N (Pons et al., 1989). EFF_A was calculated from EFF_1 by dividing by the corresponding absorbance value. The sensitivity of DTNA to changes in the vertical leaf N distribution was studied by simulations for a wide range of vertical leaf N distributions, from which the optimal, i.e. that maximising DTNA, was identified. The total amount of N in the canopy and LAI were kept as observed and the radiation level was the average of the four preceding days. In the model, leaf layers could loose N until its content became equal to that at which $A_{\text{max},g}=0$.

Radiation and N use efficiency

The radiation use efficiency at the canopy level is the ratio between calculated daytime net canopy photosynthesis and the daily absorbed PAR, both on a ground area basis (RUE_A , $\text{mol CO}_2 \text{ mol quanta}^{-1}$). Photosynthetic N use efficiency at the leaf level (leaf-PNUE, $\mu\text{mol CO}_2 \text{ mmol N s}^{-1}$) was defined as the ratio of leaf net photosynthesis (P_n) and the leaf N content ($\text{mmol N m}^{-2} \text{ leaf}$). Canopy photosynthetic N use efficiency is the ratio between daytime canopy photosynthesis and N content in green leaves, both evaluated on a ground area basis (canopy-PNUE, $\text{mol CO}_2 \text{ mol N d}^{-1}$). A summary of acronyms and units is given in Table 4.1.

Table 4.1. List of acronyms.

Acronyms		Units
$A_{\text{max},n}$	Leaf net photosynthesis at light saturation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
$A_{\text{max},g}$	Leaf gross photosynthesis at light saturation	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
EFF_I	quantum efficiency based on incident light	$\text{mol CO}_2 \text{ mol}^{-1} \text{ quanta}$
EFF_A	quantum efficiency based on absorbed light	$\text{mol CO}_2 \text{ mol}^{-1} \text{ quanta}$
DTNA	daytime total net CO_2 assimilation by the canopy	$\text{mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$
Canopy-PNUE	Canopy photosynthetic N use efficiency of PAR absorbed per unit leaf area	$\text{mol CO}_2 \text{ mol N d}^{-1}$
I_{AL}		$\mu\text{mol quanta m}^{-2} \text{ leaf}$
k	coefficient of light extinction	
LAI	leaf area index	$\text{m}^2 \text{ m}^{-2}$
Leaf N	Leaf N content	$\text{mmol N m}^{-2} \text{ leaf}$
Leaf-PNUE	Leaf photosynthetic N use efficiency	$\mu\text{mol CO}_2 \text{ mmol N s}^{-1}$
R_d	Respiration in the dark	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
RUE_A	Canopy radiation use efficiency on absorbed PAR	$\text{mol CO}_2 \text{ mol quanta}^{-1}$
P_n	Leaf net photosynthesis rate	$\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$
t	Light transmission coefficient	

Results*General observations*

The observations were concentrated on the period of grain number determination. At 50 DAS the ligule of the flag leaf was just visible (DC39) in wheat, and in oilseed rape the first flower was fully open (DC41). At 69 DAS, wheat was in the beginning of the grain-filling period (DC71), and in the oilseed rape the lower pods were longer than 2 cm (DC51). Between 50 and 69 DAS, average weekly values of daily temperature were in the range of 9.6 to 12.1°C for the minimum and 19.4 to 25.1°C for the maximum. During the same period, the weekly mean global radiation decreased from 27.1 to 14.2 $\text{MJ m}^{-2} \text{ d}^{-1}$.

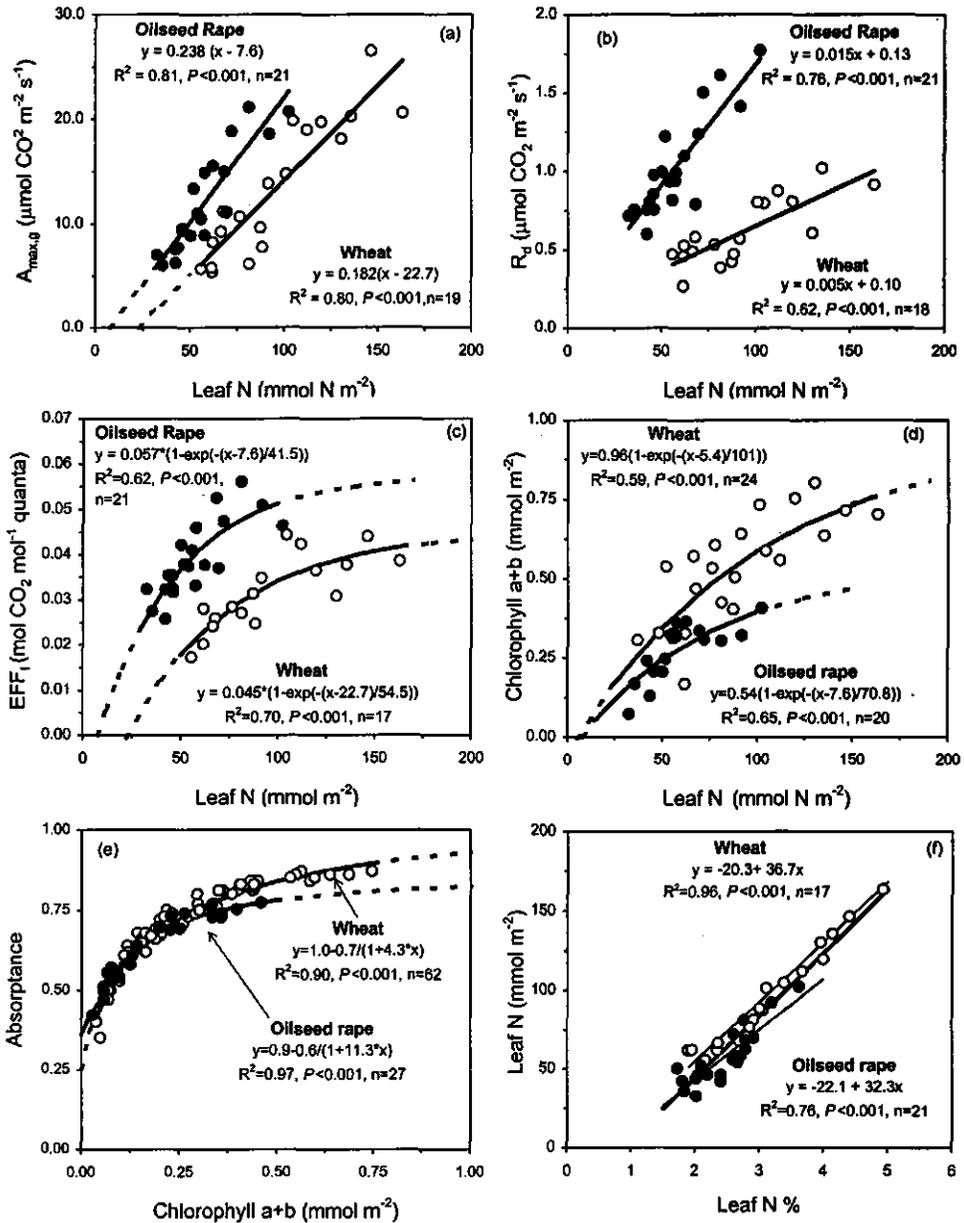


Figure 4.1. Variation in light-saturated gross photosynthesis rate (a), respiration in the dark (b), photosynthetic efficiency on incident irradiance (c) and leaf chlorophyll content with leaf N content; light absorbance as a function of chlorophyll content (e) and leaf N on area vs. dry matter basis (f). Closed circles for oilseed rape, open for wheat. In (f) thin lines indicate individual crop regressions and thick line is combined regression for both crops. Each data is mean of three replicates.

Leaf photosynthesis

Oilseed rape and wheat differed in the response of photosynthesis to leaf N. The relation between the light-saturated gross photosynthetic capacity ($A_{\max,g}$) and leaf N was linear and significantly different in both species (Fig. 4.1a). The slopes were similar between species ($P=0.13$), but the intercept on the abscissa, representing the amount of 'non-photosynthetic-N', was lower in oilseed rape than wheat ($P<0.001$). Dark respiration (R_d) was also linearly related to leaf N (4.1b). The N effect on R_d was stronger for oilseed rape than for wheat, as shown by the higher slope value ($P<0.001$). The photosynthetic efficiency based on incident light (EFF_I) was an asymptotic function of leaf N (Fig. 4.1c). EFF_I in oilseed rape was significantly higher than in wheat over the whole leaf N range ($P<0.001$). The photosynthetic efficiency based on absorbed light (EFF_A) was related to leaf N content similarly to EFF_I , with higher values (oilseed rape: $y=0.068x(1-\exp(-(x-7.6)/30.3))$, $R^2=0.49$, $P<0.001$, $n=19$; wheat: $y=0.048x(1-\exp(-(x-22.7)/43.4))$, $R^2=0.60$, $P<0.001$, $n=17$).

The species differed in the proportion of leaf N invested in chlorophyll and in the maximum absorbance of light per unit chlorophyll. Leaf chlorophyll content increased curvilinearly with leaf N in both species, but wheat leaves had a higher chlorophyll content than oilseed rape leaves (Fig. 4.1d). Light absorbance was asymptotically related to leaf chlorophyll content (Fig. 4.1e). At high leaf chlorophyll content, wheat leaves reached a slightly but significantly higher absorbance plateau ($P<0.05$). The ranges of leaf N contents differed for the two species (Fig. 4.1f), but they coincided between 50 and 100 mmol N m⁻², where most of the differences in assimilation were found. Differences in leaf N on an area basis between the two species were mainly caused by concomitant changes in leaf N concentration (Fig. 4.1f). Although oilseed rape tended to have slightly thinner leaves than wheat, as indicated by the slope of the regressions per species in Fig. 4.1f, the leaf N content expressed on an area and mass basis were equally related in both crops ($y=-35.1+39.6x$; $R^2=0.90$, $P<0.001$, $n=39$).

The response of leaf CO₂ assimilation to leaf N per species (calculated using Eq. 4.2) was compared in Fig. 4.2a, for a range of leaf N contents similar to that observed in the experiment and at a non-saturating and a saturating level of PAR (300 and 1000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Oilseed rape had a higher leaf net photosynthesis than wheat over the whole leaf N range. The ratio of photosynthesis values between species decreased from 2.0 to 1.3-1.4 from 50 to 150 mmol N m⁻².

The N use efficiency at the leaf level (leaf-PNUE), reflecting the return in photosynthesis per unit N invested in the leaves, was higher in oilseed rape than in wheat (Fig. 4.2b). A drop in leaf-PNUE with leaf N was noticed when leaf photosynthesis was calculated for low irradiance levels, and more marked in oilseed rape. Instead, leaf-PNUE was relatively independent of leaf N at high irradiance levels.

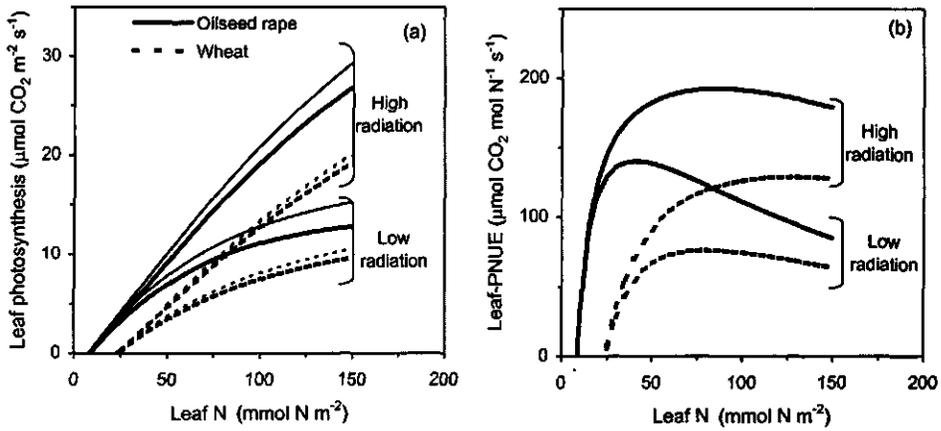


Figure 4.2. Model calculated leaf photosynthesis (a) and leaf photosynthesis per unit leaf N (b). Solid line for oilseed rape, dotted for wheat. In (a) thick lines represent gross photosynthesis and thin net photosynthesis. Low radiation was $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$, high radiation was $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

Canopy photosynthesis

Daytime canopy photosynthesis (DTNA) was higher in oilseed rape than in wheat at 50 DAS (Table 4.2). The differences between crops were maximum at the intermediate level of N supply and higher at high than low N levels. At 50 DAS, the amount of radiation absorbed by each crop canopy was quite similar per N level (Table 4.2). Therefore, the higher DTNA of oilseed rape vs. wheat was associated with differences in radiation use efficiency (RUE_A). By contrast, at 69 DAS, DTNA in oilseed rape was about half of that in wheat. This drop in time was due to the decrease in the total amount of absorbed radiation by oilseed rape, because during the same period, RUE_A was higher in oilseed rape than wheat for all combinations of treatments and dates. The N productivity of the canopy, expressed in photosynthesis per unit N invested in leaves (canopy-PNUE) was higher in oilseed rape than wheat (Table 4.2). The constancy of canopy-PNUE from 50 to 69 DAS, is presumably due to the fact that the reduction in DTNA kept pace with the loss of leaf N between the beginning and end of the critical period (Table 4.2).

RUE_A increased from low to high N supply (Table 4.2). Under N limiting conditions, the decline in RUE_A with low canopy leaf N-content is an important determinant of crop productivity. RUE_A was linearly related to the average leaf N content of the canopy for both species and harvesting dates (Fig. 4.3).

Table 4.2. Leaf area index, total amount of N in green leaves, model calculated daily total net photosynthesis by the canopy (DTNA), ratio between DTNA of wheat and oilseed rape, canopy daily absorption of radiation, canopy radiation use efficiency (RUE_A), ratio between RUE_A and the maximum leaf photosynthetic efficiency, daily photosynthetic N use efficiency by the canopy.

		LAI ¹	N in green leaves ¹	DTNA	Ratio DTNA Wheat/O.Rape	Canopy absorbed PAR	RUE _A	RUE _A /EFF _A	Canopy-PNUE
		m ² m ⁻²	mmol N m ⁻² soil	mol CO ₂ m ⁻² d ⁻¹		mol m ⁻² d ⁻¹	mol mol ⁻¹		mol CO ₂ mol ⁻¹ N d ⁻¹
50 DAS									
Wheat	N1D2	0.8	56.7	0.246	0.56	20.1	0.0122	0.38	4.33
	N2D2	1.5	70.5	0.188	0.36	23.5	0.0080	0.34	2.67
	N3D2	2.7	255.7	0.664	0.70	35.3	0.0188	0.50	2.60
	N3D1	2.0	182.1	0.591	0.77	33.9	0.0174	0.48	3.25
O.Rape	N1D2	0.9	57.4	0.442		23.9	0.0185	0.35	7.69
	N2D2	1.0	75.3	0.524		24.7	0.0212	0.37	6.96
	N3D2	2.2	177.9	0.954		37.6	0.0254	0.42	5.36
	N3D1	2.2	152.3	0.767		35.4	0.0216	0.37	5.03
	s.e.d.N ²	0.3	24.7						
	s.e.d.D ²	0.4	40.7						
69 DAS									
Wheat	N1D2	0.6	31.7	0.109	2.53	8.8	0.0123	0.48	3.37
	N2D2	0.9	79.5	0.146	2.31	8.1	0.0181	0.58	2.44
	N3D2	2.5	239.3	0.415	2.11	15.3	0.0271	0.68	1.66
	N3D1	2.2	218.5	0.383	1.91	14.2	0.0270	0.68	1.72
O.Rape	N1D2	0.1	5.5	0.043		2.4	0.0180	0.35	7.75
	N2D2	0.23	9.2	0.063		3.3	0.0193	0.40	6.62
	N3D2	1.2	61.3	0.197		6.1	0.0322	0.57	2.84
	N3D1	0.80	45.2	0.201		6.2	0.0321	0.59	3.56
	s.e.d.N ²	0.23	25.5						
	s.e.d.D ²	0.23	26.6						

¹ Data from Chapter 3.

² s.e.d, standard error of the difference between means, among nitrogen (N) or density (D) levels.

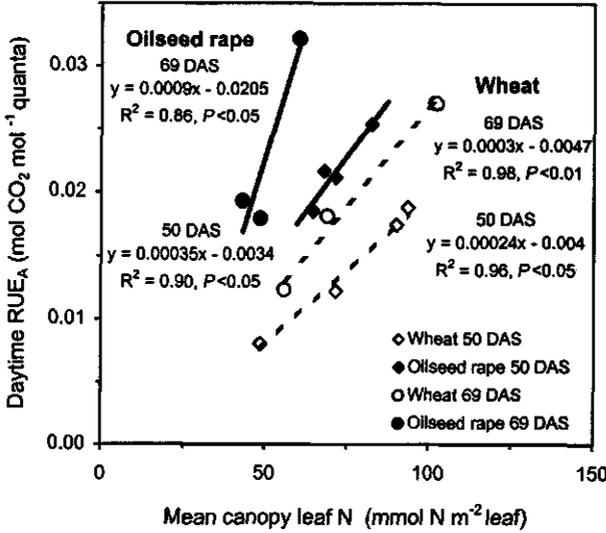


Figure 4.3. Daytime calculated radiation use efficiency as a function of average leaf N content in the canopy. Diamonds are 50 DAS and circles 69 DAS. Wheat identified by open symbols and dotted lines, oilseed rape by closed symbols and solid lines. All regressions with $n=4$.

The slopes of the linear regressions show that the N-dependent changes of RUE_A were similar for wheat and oilseed rape at 50 DAS. Higher values of RUE_A and more sensitivity to N were measured for oilseed rape in the last harvest at 69 DAS. Why was RUE_A higher in oilseed rape than wheat and why the relation with leaf N changed over time? One of the reasons, interspecific differences in the leaf photosynthesis response to N, has been treated in the previous section. In the following sections, two other aspects that can contribute to the differences are explored, i.e. the degree of canopy saturation by irradiance and the vertical distribution of leaf N.

Characterisation of the degree of canopy saturation by irradiance

To evaluate how the oilseed rape and wheat canopies were saturated by irradiance, we compared the amount of PAR absorbed per unit leaf surface per leaf layer (I_{AL}) with the level of PAR at which photosynthesis of leaves of different N contents is saturated. The comparison was carried out at noon of an average day at the beginning and end of the period under study (Fig. 4.4, data points above the $x=y$ line indicate saturation). The PAR saturation threshold ($y, \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) varied with leaf N level ($x, \text{mmol N m}^{-2}$) in both species (oilseed rape: $y = 1568 - 1459x0.992^x, R^2=0.99$; wheat: $y = 1548 - 1142x0.993^x, R^2=0.99$). At 50 DAS, in the low N treatments, photosynthesis in all leaf layers was light-saturated, particularly in oilseed rape, whereas at high N, only the top layers of oilseed rape were saturated (Fig. 4.4a).

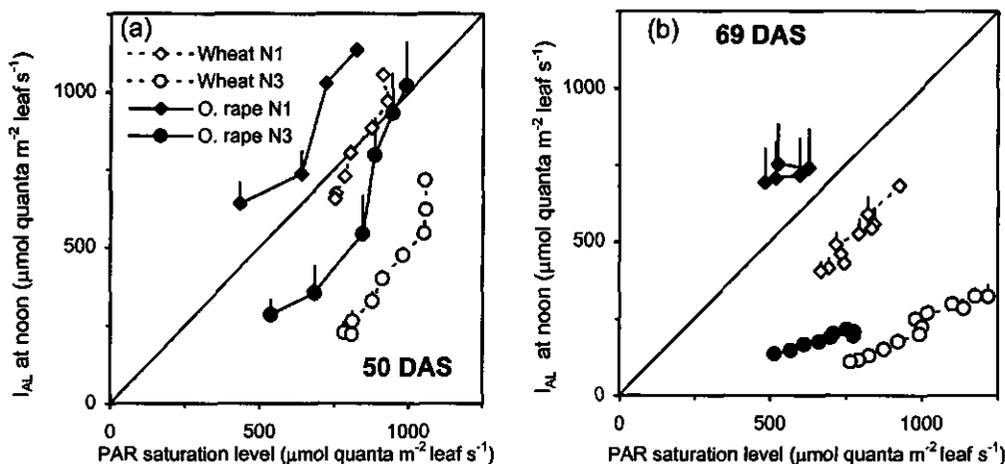


Figure 4.4. PAR level absorbed per leaf layer at noon vs. PAR level of light-saturated photosynthesis, at 50 DAS (a) or 69 DAS (b). Wheat identified by open symbols and dotted lines, oilseed rape by closed symbols and solid lines. Diamonds are low N (N1D2) and circles high N supply (N3D2). Bars are standard errors.

Later in time, at 69 DAS, both crops were less saturated, except in the oilseed rape growing at low N (Fig. 4.4b). The de-saturation of the canopies with time was a consequence of the growth of the inflorescence together with the decline in incident radiation. At 69 DAS, the fraction intercepted by the spikes in wheat was between 0.07 and 0.18 and in oilseed rape between 0.18 to 0.46 for the lowest and highest N treatments respectively (Chapter 3).

To further evaluate the extent to which the rather big changes in the radiation environment affected canopy photosynthesis, we compared the RUE_A with the maximum possible efficiency, EFF_A , calculated for the average leaf N of the canopy (Table 4.2). The higher values of the ratio between RUE_A and EFF_A at 69 DAS confirmed that in both crops, the proximity to maximum efficiency was closer than at 50 DAS (Table 4.1). In addition, at 50 or 69 DAS, the canopy of wheat was closer to operating at maximum efficiency than that of oilseed rape.

Vertical distribution of leaf N in relation to absorbed irradiance

All canopies partitioned leaf N preferentially to the upper, more illuminated leaf layers. The distribution pattern of leaf N was proportional to the ratio between the absorbed irradiance at a certain leaf level and the incoming PAR flux at the top of the canopy (Table 4.3, Fig. 4.5). Leaf N distribution, as judged by the slope of the leaf N partitioning along the light gradient, became steeper from 50 to 69 DAS in the majority of treatments.

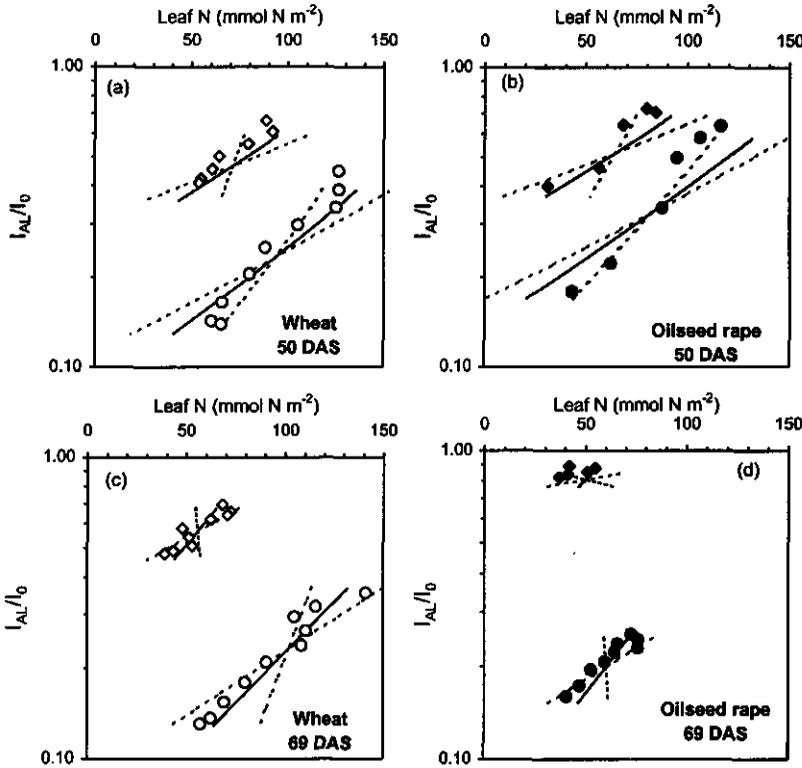


Figure 4.5. Leaf N as a function of the absorbed relative to incoming PAR level. Wheat at 50 (a) or 69 DAS (c), oilseed rape at 50 (b) or 69 DAS (d). Open symbols for wheat, closed for oilseed rape. Diamonds are low N (N1D2) and circles are high N supply (N3D2). Solid lines represent model-calculated optimal leaf N distribution, dotted lines represent a 1% deviation from optimal.

The effect of N nutrition on the slope of the relation between leaf N and absorbed light, was transient. The steepest N distribution that was observed at 50 DAS at the lowest (N1D2) with respect to the highest N treatment (N3D2) was not detectable at 69 DAS. This trend towards parallel profiles of leaf N was stronger in wheat than oilseed rape. Both crops had similar leaf N distributions with respect to the absorbed irradiance in the lowest or the highest N treatments (Table 4.3). No differences were found in the N distribution at high (D2) or low-density (D1) (Table 4.3). The actual patterns of leaf N distribution were in most of the cases within 1-2 % of those that would maximise canopy photosynthesis as calculated with the model (Fig. 4.5). Expressing the leaf N distribution as a function of the absorbed light gradient helped to combine characteristics of canopy architecture of each species that were seemingly different. The species differed in LAI (Table 4.2) and in leaf orientation due to N treatment. The extinction coefficient for PAR at 50 DAS in wheat was 0.65 at low and

0.44 at high N, and in 0.73 at low and 0.63 at high N in oilseed rape (s.e.d.= 0.07). In both crops, leaf N decreased linearly with cumulative LAI (Fig. 4.6). All regressions were significant at least at $P<0.01$, except in oilseed rape at 69 DAS in those treatments where LAI was extremely reduced (N1D2 and N2D2, non-significant and $P<0.05$, respectively). Some examples are given in Fig. 4.6.

Table 4.3. Linear regressions between leaf N content per layer (mmol N m^{-2}) and PAR absorbed per unit leaf area relative to above-canopy incident PAR (I_{AL}/I_0 , after logarithmic transformation).

		Leaf N = Intercept-Slope x $\ln(I_{AL}/I_0)$					Among Slopes	Among lines
		Intercept	Slope	<i>n</i>	r^2	<i>P</i>		
50 DAS								
Wheat	N1D2	127.2	83.5	7	0.927	<0.001	** ¹	
	N2D2	106.0	47.9	6	0.958	<0.001		
	N3D2	182.3	62.5	9	0.948	<0.001		
Oilseed rape	N1D2	105.0	73.5	5	0.884	0.011		
	N2D2	134.5	87.1	6	0.920	0.002		
	N3D2	136.3	51.2	6	0.958	<0.001		
69 DAS								
Wheat	N1D2	106.7	89.8	10	0.891	<0.001	NS	***
	N2D2	184.4	135.8	11	0.902	<0.001		
	N3D2	250.1	98.1	13	0.857	<0.001		
Oilseed rape	N1D2	65.1	126.0	5	0.104	0.313		
	N2D2	106.4	112.0	6	0.488	0.074		
	N3D2	180.8	76.5	9	0.899	<0.001		
50 DAS								
Wheat	N3D1	156.2	60.0	9	0.859	<0.001	NS	**
	N3D2	182.3	62.5	9	0.948	<0.001		
Oilseed rape	N3D1	122.4	47.5	8	0.889	<0.001		
	N3D2	136.3	51.2	6	0.958	<0.001		
69 DAS								
Wheat	N3D1	219.8	79.3	12	0.963	<0.001	NS	**
	N3D2	250.1	98.1	13	0.857	<0.001		
Oilseed rape	N3D1	57.2	125.5	8	0.764	0.003		
	N3D2	180.8	76.5	9	0.899	<0.001		

¹ F-probability value: ***= $P<0.001$, **= $P<0.01$, *= $P<0.05$, NS = non significant ($P>=0.05$)

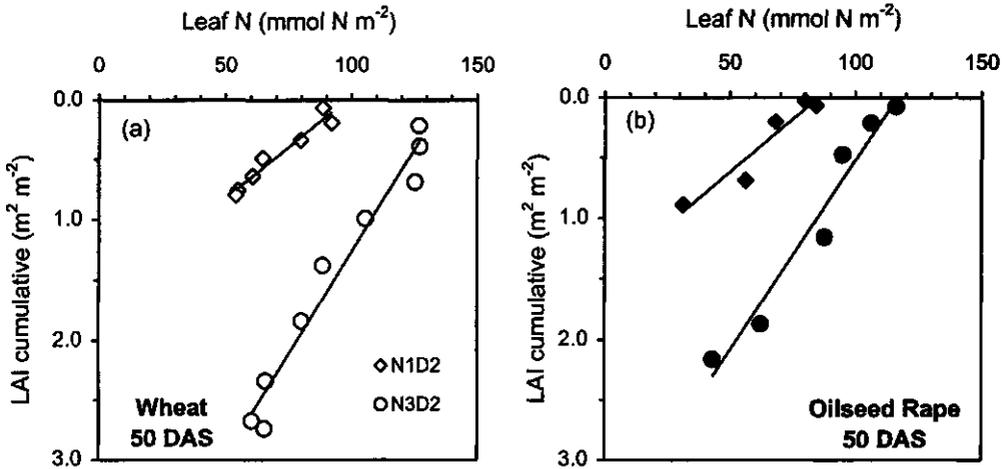


Figure 4.6. Leaf N as a function of LAI at 50 DAS in wheat (a, open symbols) or oilseed rape (b, closed symbols). Diamonds are low N (N1D2) and circles are high N supply (N3D2). Regressions for each treatment, where y is leaf N and x is cumulative LAI were as follows: wheat at low N, $y = 96.2 - 54.7x$, $R^2 = 0.93$, $P < 0.001$, $n = 8$; wheat at high N, $y = 135.3 - 28.2x$, $R^2 = 0.96$, $P < 0.001$, $n = 9$; oilseed rape at low N, $y = 83.3 - 51.6x$, $R^2 = 0.88$, $P < 0.05$, $n = 5$; oilseed rape at high N, $y = 115.2 - 30.4x$, $R^2 = 0.95$, $P < 0.001$, $n = 6$.

Discussion

Oilseed rape was more efficient than wheat in converting radiation or N into canopy photosynthesis and it was also more responsive to changes in N nutrition during the critical period for grain number determination. Factors involved both at the canopy and the leaf levels were responsible for the observed differences in canopy photosynthesis and canopy-PNUE.

RUE and canopy characteristics

The relation between daytime RUE_A and canopy N, observed in many other crops (e.g. Sinclair and Horie, 1989; Sinclair et al., 1992), shifted in a considerably short but crucial period of the crop cycle. The development of the inflorescence introduced a change in canopy architecture that, together with the decrease in global irradiance, explained the higher values of RUE_A at the end of the critical period for grain number determination (see upward shift in slopes in Fig. 4.3). Radiation use efficiency increases with a low level of irradiance, due to a more efficient use of light by sunlit leaves, and with a high proportion of diffuse radiation (Sinclair et al., 1992; Hammer and Wright, 1994; Bange et al., 1997). The restriction imposed by the degree of canopy saturation on RUE_A was particularly evident in oilseed rape at high N supply,

its value was fairly increased from 50 to 69 DAS, despite the decrease in leaf N content in the canopy (Fig. 4.3). In leaves with low N content, the size of the antennae and electron transport chain limit the capacity for processing incoming radiant energy (Hikosaka and Terashima, 1996; Hirose and Werger, 1987a). For this reason, N loss from leaves, partially counteracted the beneficial effect of lower irradiance due to inflorescence growth, and drifted the low N canopy towards saturation (Table 4.2 and Fig. 4.4b).

One interesting feature of the comparison of RUE_A between species was that, before the change in the light environment due to the inflorescence growth, wheat and oilseed rape were similarly sensitive to the changes in canopy N (see slope of regressions at 50 DAS in Fig. 4.3). It has been proposed that broad-leaf crops subjected to low N supply, minimise the effects of N deficiency on RUE due to a severe reduction in leaf expansion that helps to maintain the photosynthetic capacity per unit leaf N constant (Vos and Van der Putten, 1997). In our study, the range of leaf N contents and LAI was narrower for oilseed rape than wheat. Nevertheless, in both species the magnitude of change in RUE_A with canopy N was similar at the beginning of the critical period for grain number determination.

In wheat and oilseed rape, the vertical leaf N distribution was close to the theoretical optimal, therefore aiding to maximise canopy photosynthesis and RUE_A in both species. In addition, the leaf N profile was similar between species for extreme combinations of N supply and over harvests. A steeper distribution of leaf N over cumulative leaf area has been reported for dicots vs. monocot species (Anten et al., 1995). By relating the leaf N profiles to the gradient of absorbed irradiance we combined the differences in LAI and extinction coefficient inherent to the treatments and formed a more straightforward basis for comparison between species across N levels. The distribution of leaf N with absorbed irradiance, changed to steeper values as the amount of irradiance over the leaves decreased (from 50 to 69 DAS) or at low vs. high N supply. Steeper leaf N profiles at low N supply are likely to be associated with increasing N remobilisation from the leaves to the growing inflorescence (Chapter 3). The trend to steeper leaf N profiles at low N supply has also been observed in vegetative herbaceous (Hikosaka et al., 1994) or tree canopies (DeJong et al., 1989). Simulation studies have confirmed that the gains of redistributing N as a function of irradiance are bigger at low than high foliage N content (Leuning et al., 1995). Finally, in other studies comparing crop canopies, the calculated optimal distribution had been steeper than the actual one (e.g. Anten et al., 1995). This difference with our work could be related to their assumption that the photosynthetic efficiency at low light is independent of leaf N content, then N can be allocated to the top of the canopy without apparent loss for photosynthesis of the less-illuminated leaf layers (but see Fig. 4.1c in the present study).

Table 4.4. Calculated canopy daytime photosynthesis ($\text{mol m}^{-2} \text{d}^{-1}$) for optional canopy characteristics compared to observed ones. In each run with an 'optional' characteristic only the one indicated in the text is changed, the others remain as observed.

OPTIONS	Wheat			Oilseed rape			Ratio Oilseed rape with option/ Wheat observed
	Observed	Changed by option	Ratio Option/ Observed	Observed	Changed by option	Ratio Option/ Observed	
High N at 50 DAS							
1. Wheat with leaf photosynthesis- N relations of oilseed rape	0.667	1.012	1.52				
2. Wheat with 'non- photosynthetic' leaf N equal to oilseed rape	0.667	0.768	1.15				
69 DAS							
3a. At high N (N3), oilseed rape keeps the LAI and total leaf N in canopy from 50 until 69 DAS	0.412			0.197	0.393	2.0	0.95
3b. At low N (N1), oilseed rape keeps the LAI and total leaf N in canopy from 50 until 69 DAS	0.109			0.043	0.220	5.1	2.02

RUE and leaf photosynthesis

Wheat and oilseed rape differed in the leaf photosynthesis at high and low light and its response to leaf N content with consequences for the canopy photosynthesis and hence RUE_A (Figs. 4.1, 4.2). We appraised the impact of leaf characteristics on canopy photosynthesis by using the model. We calculated that transposing the leaf photosynthetic N-dependent characteristics of oilseed rape to wheat could increase canopy photosynthesis and RUE_A by 50% (Option 1, Table 4.4). This effect of increased photosynthetic capacity on RUE_A is expected to decline when N content of the canopy increases (Sinclair and Horie, 1989).

The components of leaf photosynthesis, i.e. maximum photosynthetic capacity, dark respiration and photosynthetic efficiency were related to leaf N and were higher in oilseed rape than wheat. Variability in maximum photosynthesis or respiration at similar leaf N content has been observed between species (Evans, 1989a). However, changes in the quantum yield for absorbed light in response to N are not frequently reported. The photosynthetic rate per unit leaf N (leaf-PNUE) was higher in oilseed

rape than wheat and changed with irradiance and leaf N content as observed by Evans and Terashima (1988). A number of interspecific differences in the chain of events leading to CO₂ assimilation, and most likely not a single one at a time, may contribute to explain the differences in leaf photosynthesis per unit leaf N (Fig. 4.2b) (Makino et al., 1988; Poorter and Evans, 1998). These may range from the capacity for light absorption (Evans, 1989b) and stomatal conductance for CO₂ (Henson et al., 1990) to the activation state of Rubisco (Makino et al., 1988). We will limit to discuss the role of leaf thickness and leaf N investment in pigments in relation to the observed differences in leaf-PNUE between wheat and oilseed rape.

Inspecting a number of species with broad variation in leaf mass per unit area, Poorter and Evans (1998) observed that high leaf-PNUE was correlated with thinner leaves. In our study, the variation in leaf mass per unit area was minimal between species compared to changes in leaf N content (cf. Fig. 4.1f), and not likely to be the main cause of higher leaf-PNUE in oilseed rape. Nevertheless, the slightly thinner leaves of oilseed rape with respect to wheat may help to explain its lower amount of non-photosynthetic N, the fraction of N invested in support and vascular tissue, etc. (abscissa intercept in Fig. 4.1a). The persistence of interspecific differences in the fraction of non-photosynthetic leaf N is questionable when the fraction is expressed on a leaf mass basis (Niinemets and Tenhunen, 1997). For the crops under study, calculations with the model indicated that decreasing the non-photosynthetic leaf N of wheat to the level of oilseed rape, could explain only a small part of the differences in daytime calculated photosynthesis between the species (Option 2, Table 4.4).

In the leaf N range of response of photosynthesis, oilseed rape had ca. 70% of the chlorophyll content of wheat and 90% of its absorptance capacity. The higher chlorophyll to leaf N ratio in wheat can be seen as an adaptation to the relatively low levels of irradiance (Evans, 1996 and references therein) normally incident on more erectophile crops. We interpret that the low leaf-PNUE of wheat may be connected to the higher investment of leaf N in pigments, at the expense of electron transport carriers and Rubisco, in order to increase the absorptance capacity in a canopy where the average degree of light saturation is low. The higher absorptivity per unit chlorophyll of oilseed rape compared to wheat could be related to the presence of spongy mesophyll in the leaf of the dicot, that enhances light scattering (Vogelmann et al., 1996).

Options for improving canopy photosynthesis per unit N and conclusions

Growth analysis during the critical period for grain number determination indicated that biomass production per unit intercepted irradiance in oilseed rape was more sensitive to N supply than wheat and, could achieve a higher potential at high N (Chapter 3). Integrating leaf photosynthesis with the distribution of light and leaf N in the canopy helped to explore the reasons behind this behaviour. Oilseed rape had a higher leaf photosynthetic capacity per unit leaf N than wheat and its leaves were increasingly shaded by the growth of the inflorescence, particularly at high N

availability. Both factors contributed to explain the higher RUE_A of the oilseed and its increasing sensitivity to canopy N content in time.

Options, which emerged from the present study, for improving biomass production per unit canopy N during the critical period for grain number determination will be different for each species. Leaf-PNUE has been recognised as a major determinant of the capacity for biomass production per unit plant N (Garnier et al., 1995) and was certainly a factor behind the higher canopy-PNUE of oilseed rape. However, the increase in RUE_A of oilseed rape by the end of the critical period, did not translate into higher canopy photosynthesis because of the lower amount of PAR absorbed due to leaf shedding and the shading by the inflorescence. Maintaining the LAI from the beginning to the end of the critical period would be clearly advantageous (Option 3 in Table 4.4), oilseed rape could equal or duplicate the photosynthesis of wheat depending on the N supply. Cultural practices that ensure reaching high LAI at flowering, even above critical, and keeping it during grain filling will help to overcome source-limited yields in this crop (Chapter 3; Rood and Major, 1984). Making N available near flowering could increase leaf area duration. Since applications at that stage are difficult to implement, use of slow release products supplied at rosette stage could be an option to try. Otherwise, breeding genotypes that can keep the leaves active during grain filling could help to raise yields in oilseed rape. At the crop scale, retention of leaves would also contribute to increase canopy-PNUE. Particularly if we consider that pods, the main sink for N from the end of flowering to maturity have about three times lower maximum photosynthetic capacity per unit N than leaves (Gammelvind et al., 1996). In wheat, the options for increasing biomass production will have to rely more in breakthroughs of photosynthesis at the leaf level (see Lawlor, 1995), which can be translated at the canopy level (Option 1, Table 4.4). Promising examples, of higher yields related to higher stomatal conductance have been detected among cultivars released from 1962 to 1988 at CIMMYT (Fischer et al., 1998). Finally, it is important to remember that to translate any increase in biomass into yield, the development and maintenance of the sink capacity has to keep pace with it. This will in turn ensure that photosynthesis is not down-regulated and N use efficiency can be raised.

Chapter 5

Light-associated dynamics of leaf nitrogen allocation in vegetative wheat canopy. Impact on canopy photosynthesis.

Abstract

The development of canopy gradients of leaf N has been reported as an adaptation to the light gradient that helps to maximise canopy photosynthesis. In this study, we report the dynamics of vertical leaf N distribution during vegetative growth of wheat in response to changes in N availability and canopy structure. The question to what extent the observed leaf N distribution maximised canopy photosynthesis was addressed with a multilayered model of canopy photosynthesis that integrates leaf photosynthesis-N dependent relations according to the light and leaf N distribution. Plants were grown hydroponically and N was supplied in proportion to expected growth in order to avoid time-dependent effects of N availability, and keep the plant N concentration steady. The photosynthetic rate at light saturation was associated to leaf N. N-independent age effects on the photosynthesis rate were only noticeable in old leaves at low N supply. The leaf N distribution changed dynamically during crop growth in response to the light environment and was regulated by N supply with negligible effects of sowing density. At high N supply, the leaf N profiles were stable in time. At low N supply, the leaf N profiles fluctuated from uniform to steeper values than those found at high N. These fluctuations were associated to reduced leaf expansion and tillering and increasing N remobilisation from lower leaf layers. Thus, the local light environment played a role in N distribution, but the ultimate regulation of N allocation was co-ordinated at the plant level. The distribution of leaf N with respect to the gradient of absorbed irradiance was close to the theoretical optimum. By means of a sensitivity analysis we conclude that plants keep the optimal vertical leaf N distribution by balancing the capacity for photosynthesis at high and low light.

Key words: leaf N distribution, canopy photosynthesis, nitrogen, wheat.

Abbreviations: $A_{\max,n}$, net rate of photosynthesis at light saturation; $b_{N,LAI}$ slope of vertical leaf N distribution vs. cumulative LAI; DAG, days after germination; DTNA, daytime total net CO₂ assimilation by canopy; EFF_A , EFF_I photosynthetic efficiency at low light based on absorbed or incident light respectively; $fIPAR$, fraction of PAR intercepted; I_{AL} , PAR absorbed per unit leaf area; k , the coefficient of light extinction; PAR, photosynthetically active radiation.

Introduction

Vertical gradients of leaf nitrogen (N) content are a common feature in canopies of crops (Connor et al., 1995; Shiraiwa and Sinclair, 1993), natural vegetation (Field, 1983; Hirose and Werger, 1987a) or trees (DeJong and Doyle, 1985; Hollinger, 1996). This variation corresponds well to that of irradiance within the canopy, in terms of amount (Hirose and Werger, 1987b) or spectral quality (Rousseaux et al., 1999). At the leaf level, photosynthesis increases with the amount of irradiance absorbed and the leaf N content (Evans, 1989a). Therefore, the preferential allocation of leaf N to the more illuminated canopy layers has been interpreted as a pathway to maximise canopy photosynthesis and optimise the use of N (Mooney and Gulmon, 1979).

In field crops such as wheat, maximising biomass production with an efficient use of N is particularly relevant at early vegetative stages, when the crop is very sensitive to N shortage, and rapid canopy expansion is crucial for intercepting radiation and competing against weeds. During this period, the canopy undergoes radical changes in its structure, given by leaf area and leaf orientation (Meinke, 1996). In addition, during the whole crop cycle, plant N concentration declines, as plant biomass and the proportion of supporting tissues (stems) increase (Lemaire and Gastal, 1997). Thus, leaf N distribution in wheat is likely to change from emergence to full canopy cover, as shown by Bindraban (1999). The question whether actual leaf N distribution during this period of the wheat cycle is close to optimal has not been addressed before. Calculated gains in canopy photosynthesis, if the actual N profile were optimal, range from 0 to 12% in other species (Schieving et al., 1992a; Evans, 1993a; Connor et al., 1995; Anten et al., 1995). These estimates are done by calculating daily canopy photosynthesis for a particular light distribution, based on N-dependent leaf photosynthetic rates (Hirose and Werger, 1987b).

In theory, for a fixed leaf area, the optimal leaf N profile would be more steep in a canopy growing at high vs. low N supply because there is more N available for redistribution to more illuminated leaf layers (Hirose and Werger, 1987b). However, plants growing at low N supply usually present a more steep N distribution than at high N (Hikosaka et al., 1994). Low N nutrition, by accelerating basal leaf senescence, adds another driving force to the determination of the leaf N profiles. Finally, leaf age may also contribute to the formation of leaf N gradients (Mooney et al., 1981), and its contribution increases at low N nutrition (Hikosaka et al., 1994). It remains a question whether at low N availability, the interaction between accelerated leaf N remobilisation and leaf age limits the development of light-associated leaf N profiles. This question is addressed in this paper where we report how, during different stages of wheat vegetative growth, the light and leaf N distribution change within the canopy and analyse the extent to which actual leaf N profiles differ from calculated optimal ones. In order to break the normally strong correlation between leaf age vs. light interception and leaf age vs. N content we used different levels of sowing density and N supply. Furthermore, to overcome complex time-dependent effects of N availability and dilution of N in plant biomass, plants were grown hydroponically and N was provided in proportion to the expected growth to keep the plant N concentration steady.

Materials and Methods

Plant material and growing conditions

Spring wheat (*Triticum aestivum* L., var. Minaret) was grown on a hydroponics system in a greenhouse during winter months in Wageningen, The Netherlands (51°58' N, 5°40' E). The system consisted of four rows of 16 containers connected by a pipeline. Nitrogen addition was independently controlled per row (Schapendonk et al., 1990). Plants were arranged in three rows distant 0.09 m, simulating a canopy in each container. The containers had 25 L capacity and 0.145 m² surface (0.54 (L) x 0.27 (W) m) and were shielded with an aluminium screen. Seedlings were transplanted on 15 November 1996, i.e. 10 days after germination (DAG). The pH of the solution was monitored every minute and adjusted to 5.5 by computer controlled addition of H₂SO₄. Photoperiod was 13 h, relative humidity 70%, average day/night temperatures were 15/10°C. Natural irradiance was supplemented with high pressure sodium lamps (Phillips SON-T) supplying 80 μmol m⁻² s⁻¹ in the range of photosynthetically active radiation (PAR, 400-700 nm). PAR was measured at the top of the plants every minute by two linear 1.1 m sensors (TFLD, Wageningen, The Netherlands).

Until 20 DAG, the plants received Steiner nutrient solution (1984) prepared without N. Later on, N was added as NO₃⁻ on an hourly basis depending on crop demand. N addition was based on calculations of actual growth rate from intercepted radiation on a minute basis, assuming exponential light extinction (Schapendonk et al., 1990). N was supplied in order to keep the potential growth rate (high N, HN) or ca. 50% of its value (low N, LN). Radiation use efficiency was taken as 5.0 g MJ⁻¹(PAR) for the potential growth rate (HN). Partitioning coefficients for biomass, initial values for leaf area, specific leaf area, target N concentrations in the different tissues were model inputs. Calculated leaf area was frequently updated by non-destructive leaf area measurements and after each harvest.

Experimental design and statistical analysis

Plants were grown at two levels of N supply, high and low as explained above, combined with two levels of plant density. Plant density was 165 (low density, LD) or 330 (high density, HD) pl m⁻². Treatments were arranged in a nested design in randomised blocks. N level was randomised over two main blocks, consisting of two rows of containers each. Within each row of containers, density levels were randomised over two blocks. Therefore each combination of N and density level was replicated 4 times in the whole experimental set up. Treatment effects were tested by ANOVA, when F-tests were significant ($P < 0.05$) means were compared by the Least Significant Differences test (LSD, $P = 0.05$). Differences between the slopes of individual lines were tested by pairwise comparison with a *t*-test (Genstat 5, 1987).

Measurements

Harvests of total biomass were scheduled on 42, 64, 78 and 92 DAG, i.e. between beginning of tillering and advanced stem elongation, DC21 and DC38 according to Zadoks et al. (1974). At each date, samples of 0.054 m² were taken. Plants were dissected at height intervals of 0.05 m, from which green and senescent leaves, and stems (including leaf sheaths) were separated. Leaf area was measured with an electronic planimeter (LI-3100, LI-COR, Inc., Lincoln, Nebraska, USA) after leaf discs were taken for determination of chlorophyll (Inskeep and Bloom, 1985). Roots were also collected. All plant parts were oven-dried at 70°C for 48 h and weighed. N content was analysed with a CHN-O analyser (Fissons Instruments, Interscience BV, Breda, the Netherlands). Three times a week, PAR level was measured inside the canopy every 0.05 m height, above the canopy and at ground level, using a linear ceptometer (SF-80, DELTA-T Devices LTD, Cambridge, England). Readings were performed within 1h of solar noon. Global radiation data were obtained from a nearby station, PAR was taken as 50% of the global radiation.

The net rate of photosynthesis at saturating irradiance ($A_{\max,ns}$, $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was measured in different leaves on the main stem, using a portable open gas exchange system (ADC-LCA2, The Analytical Development Co. Ltd, UK) at constant air composition (350 ppm CO₂). Leaves number 3 to 5 were measured at 42 DAG, 3 to 7 at 64 DAG, 5 to 7 at 78 DAG and 5 to 8 at 92 DAG. On these leaves, leaf N content was determined as explained. Leaf absorptance of PAR was calculated from measurements of reflectance and transmittance with a spectroradiometer connected to a Taylor-type integrating sphere (LI-COR, Inc., Lincoln, Nebraska, USA).

Radiation distribution within the canopy

The extinction of PAR through the canopy was fit to an exponential model (Monsi and Saeki, 1953). The mean level of PAR absorbed per unit leaf area at a given height (I_{AL} , $\mu\text{mol quanta m}^{-2} \text{ leaf}$) was calculated after Thornley and Johnson (1990) as:

$$I_{AL} = I_0 k \exp(-k \text{ LAI}) / (1 - t) \quad \text{Eq. 5.1}$$

where I_0 is PAR measured on a horizontal plane at the top of the canopy ($\mu\text{mol m}^{-2} \text{ s}^{-1}$). The k is the coefficient of light extinction and LAI calculated after Thornley and Johnson (1990). LAI is the leaf area index ($\text{m}^2 \text{ m}^{-2}$) accumulated at the height of measurement. The fraction of PAR intercepted by the crop ($f\text{IPAR}$) was calculated as 1 minus the fraction not intercepted, i.e. the ratio between PAR at the senescence line to the top of the canopy. The transmission coefficient, t , was taken as 0.05 (Goudriaan and Van Laar, 1994).

Model for calculation of actual and optimal canopy photosynthesis

A static explanatory model was developed with the aim of calculating the impact of different leaf N profiles on daytime canopy net CO₂ assimilation (DTNA, $\text{mol CO}_2 \text{ m}^{-2}$

d^{-1}). The model calculates DTNA at the measured radiation, total leaf N content and observed vertical leaf N distribution. Intercepted PAR by sunlit and shaded leaves, light absorbance and photosynthetic response to irradiance were calculated every 10^{th} of total LAI, and integrated five times during the day to yield DTNA, by modification of subroutines of the model SUCROS (Goudriaan and van Laar, 1994). The photosynthetic response to irradiance was described in terms of the net rate of photosynthesis at light saturation ($A_{\text{max},n}$) and the photosynthetic efficiency measured at low light based on absorbed light (EFF_A , $\text{mol CO}_2 \text{ mol}^{-1} \text{ quanta}$) with an asymptotic negative exponential equation (Goudriaan and van Laar, 1994).

Following observed data, leaf N ($\text{g N m}^{-2} \text{ leaf}$) declined linearly with cumulative leaf area. $A_{\text{max},n}$ was linearly related to leaf N, after Hirose and Werger (1987a). The photosynthetic efficiency on incident PAR basis (EFF_I) was calculated from the observed leaf N content, using the equation reported for the same cultivar in Chapter 4, $\text{EFF}_I = 0.045 (1 - \exp(\text{Leaf N} - 0.32)/0.76)$, $P < 0.001$, $R^2 = 0.70$, $n = 17$. EFF_A was calculated from EFF_I and the corresponding absorbance value. The data of leaf absorbance were fit to the observed chlorophyll content ($\text{mmol m}^{-2} \text{ leaf}$) with a rectangular hyperbola. The model calculates DTNA for a range of leaf N distributions with the observed LAI, total leaf N content, and the average radiation level from the four preceding days, with the condition that the minimum amount of N possible in any leaf layer is that at which $A_{\text{max},n} = 0$. From these simulations, the optimal leaf N distribution, i.e. that maximising DTNA, was identified.

The sensitivity of DTNA to changes of 1% in the slope of leaf N vs. cumulative LAI ($b_{N,\text{LAI}}$) was assessed by:

$$S_{\text{DTNA},b_{N,\text{LAI}}} = ((\text{DTNA}_{1\%} - \text{DTNA}_{\text{opt}})/\text{DTNA}_{\text{opt}})/((b_{N,\text{LAI } 1\%} - b_{N,\text{LAI opt}})/b_{N,\text{LAI opt}}) \quad \text{Eq. 5.2}$$

The level of 1% was chosen because within that range, the changes in photosynthetic parameters as a function of leaf N can be assumed to be linear.

Results

General

The PAR level over the canopy throughout the experimental period increased from 1.2 to $1.6 \text{ MJ m}^{-2} \text{ d}^{-1}$.

Biomass, N uptake, canopy structure and interception of radiation

The N concentration in the whole plant and particularly in the foliage was relatively constant during the experiment. On average for the whole sampling period, leaf N on a mass basis in the pool of green leaves was 1.72 % (s.e.=0.10) for low N and 2.47 % (s.e.=0.06) for high N. Biomass production and N uptake were more responsive to N regime than to density levels (Fig. 5.1a and b), and were linearly related.

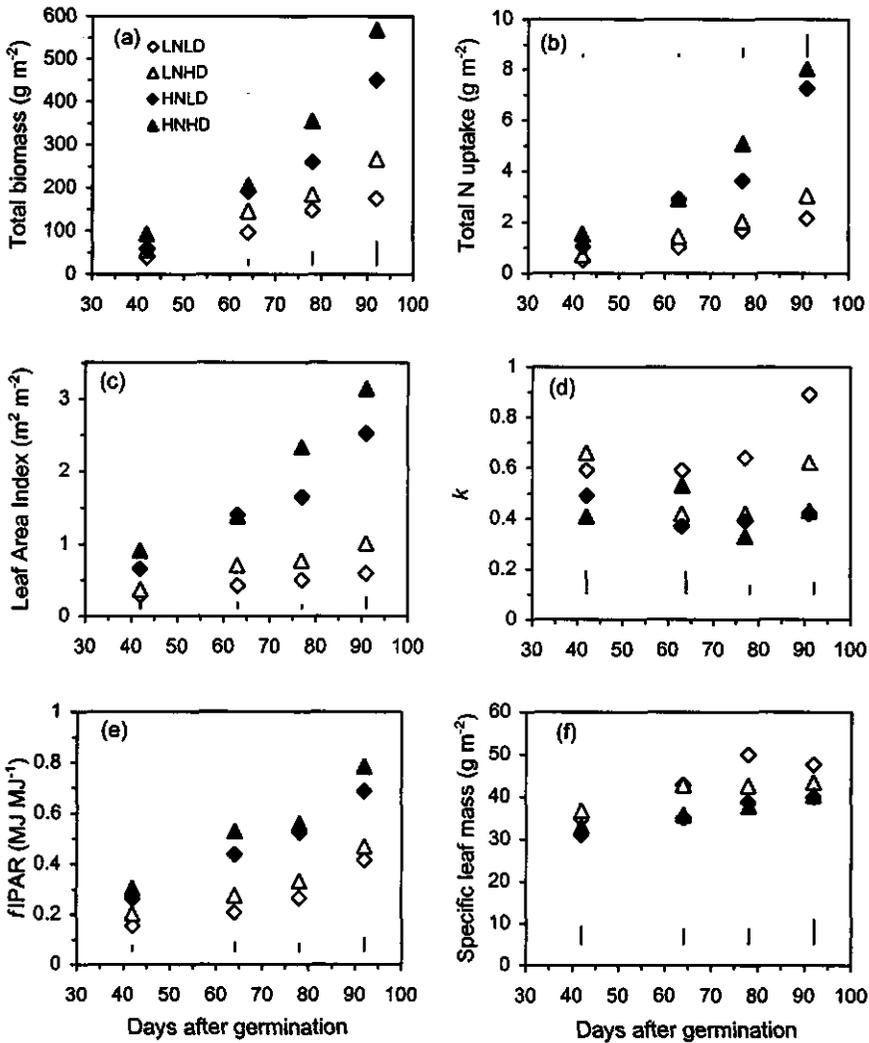


Figure 5.1. Dynamics of (a) total biomass, (b) total N uptake, (c) leaf area index, (d) light extinction coefficient, (e) fraction of intercepted PAR and (f) specific leaf mass. Treatment symbols are: open for LN and closed for HN, diamond for LD, triangle for HD. Vertical bars indicate the standard error of the differences between means.

The efficiency of total biomass formation per unit N absorbed ($\text{g biomass g}^{-1} \text{N}$) was significantly higher at low than high N ($P < 0.05$), with no effects of density. The functions were for low N, $y = 0.44 + 87.9x$ ($R^2 = 0.98$, $P < 0.001$, $n = 8$) and for high N, $y = -4.0 + 68.6x$ ($R^2 = 0.99$, $P < 0.001$).

LAI increased with high levels of N and density, resulting in an increase in the fraction of radiation intercepted (Fig. 5.1c and e). The extinction coefficient for light (k) did not vary along canopy height, as indicated by the strong linear relation between the log-transformed data of I/I_0 and LAI ($P < 0.001$). The k values were slightly higher

at low than at high N and, in some cases, at low than at high density ($P < 0.05$ at 64 DAG, Fig. 5.1d).

Light and leaf N profiles

The amount of PAR absorbed per unit leaf area relative to that incident at the top of the canopy (I_{AL}/I_0) decreased with depth in the canopy (Fig. 5.2 a-d). In general, the amount of PAR absorbed per leaf layer was higher at low than high N supply, and within the low N level at low than high sowing density. This is a consequence of the lower LAI and more planophile leaf position of the low N treatment (Fig. 5.1 c and d). Differences between treatments increased from 42 to 92 DAG.

The leaf N content per leaf strata was linearly related to I_{AL}/I_0 (after transformation with natural logarithm) (Fig. 5.2 e-h, Table 5.1). Similar relations were obtained when leaf N was expressed on a dry matter basis, since the differences in leaf thickness (specific leaf mass, g leaf m^{-2} leaf) were small among treatments (Fig. 5.1f), and did not vary systematically through the canopy (not shown). The slope of the regression between leaf N and I_{AL}/I_0 (log-transformed) changed with time and with N availability. Differences between density levels were occasional and did not follow a pattern, e.g. within low N at 92 DAG ($P < 0.001$). Thus we concentrated on comparing the N levels calculating regressions with pooled data across densities (Table 5.1, Fig. 5.2e - h). The dynamics of the light-associated leaf N profiles was different at high and low N supply. Judging from the slope values, the distribution of leaf N was more stable in time for high than low N supply, after the first harvest. (Fig. 5.2e-h). In low N canopies, the slope fluctuated from more to less steep (Table 5.1). As a consequence, the distribution of leaf N with the gradient of absorbed PAR between N treatments was different at 64 DAG, similar at 78 DAG and different at 92 DAG. At low N, the N remobilised from leaves that ultimately senesced (Fig. 5.3) in combination with the reduced leaf expansion (Fig. 5.1c), led to temporary increases in canopy N content expressed per unit leaf area.

Light saturated assimilation rate in relation to leaf N and leaf position

The maximum leaf photosynthetic rate at saturating irradiance ($A_{max,n}$) was linearly related to the leaf N content (Fig. 5.4). The spread in the y-axis suggested a possible effect of leaf position on $A_{max,n}$. This effect can be related to leaf age or to acclimation to the PAR level incident on each leaf, progressively declining as the canopy grows. An increase in the proportion of chlorophyll per unit leaf N or a decrease in the chlorophyll a/b may be indicative of acclimation of photosynthesis to low light levels. In this experiment, the prevailing PAR level was low and we did not detect a N-independent change in $A_{max,n}$ with I_{AL} . The amount of chlorophyll per unit leaf N was described with a single slope for all N treatments, canopy depths and harvests (Fig. 5.5). The chlorophyll a/b ratio was low and varied from 4.4 (s.e.=0.12) at low N to 4.8 (s.e.=0.04) at high N, but not systematically related to canopy depth or time.

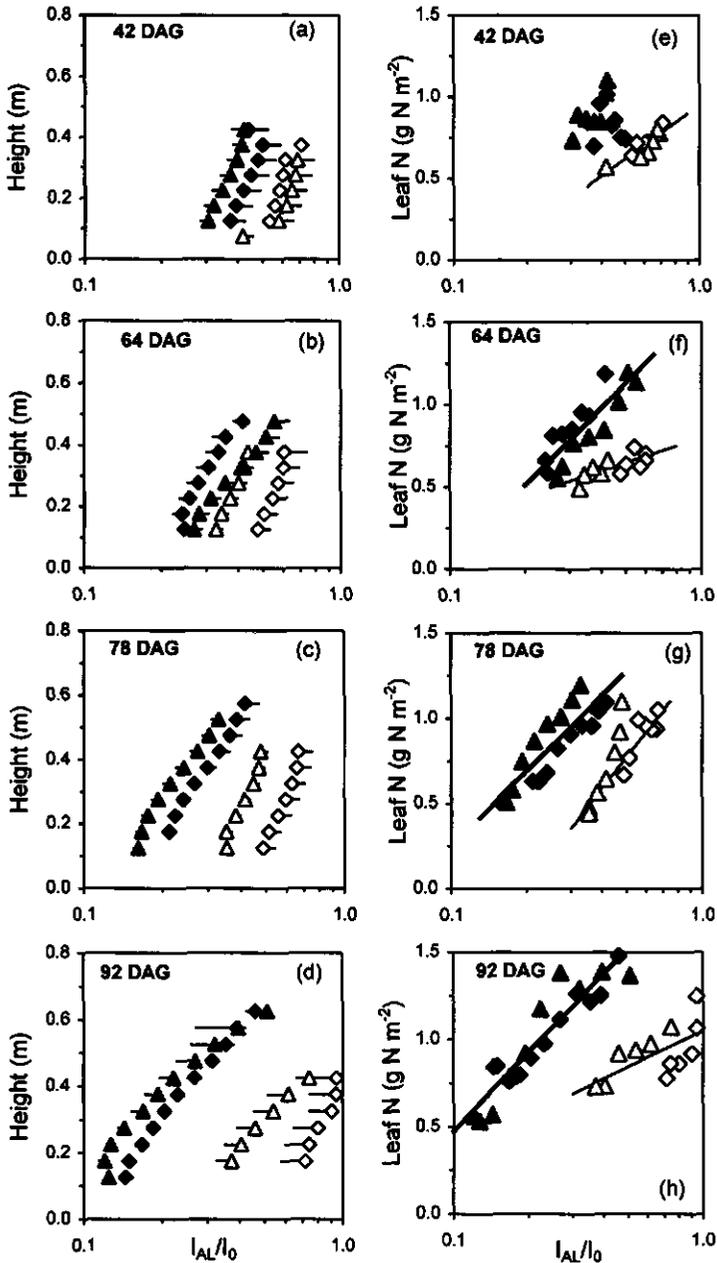


Figure 5.2. Changes in absorbed PAR per unit leaf area relative to incident PAR above the canopy (I_{AL}/I_0), with plant height (a-d), and leaf N content as a function of I_{AL}/I_0 (e-h). Treatment symbols as in Fig. 5.1. Bars are standard errors. Regression lines for N levels in Table 5.1.

Table 5.1. Linear regressions between leaf N content (g N m^{-2} ; dependent variable) and relative incident PAR per unit leaf area (I_{AL}/I_0 after logarithmic transformation) at different days after germination.

		Intercept	Slope	R ²	P	Among Slopes	Among lines
42 DAG	N1D1	1.04	0.65	0.719	0.021	NS	***
	N1D2	0.92	0.43	0.725	0.020		
	N2D1	0.60	-0.29	²	NS		
	N2D2	1.67	0.77	0.528	0.039		
	N1	0.95	0.48	0.717	<0.001	NS	***
	N2	0.92	0.05	-	NS		
64 DAG	N1D1	0.85	0.32	0.090	NS	NS	***
	N1D2	1.06	0.47	0.544	0.096		
	N2D1	1.93	0.90	0.868	<0.001		
	N2D2	1.65	0.82	0.932	<0.001		
	N1	0.80	0.22	0.483	0.011	**	
	N2	1.59	0.67	0.734	<0.001		
78 DAG	N1D1	1.39	0.91	0.653	0.017	***	
	N1D2	2.31	1.81	0.902	<0.001		
	N2D1	1.72	0.70	0.956	<0.001		
	N2D2	2.29	0.97	0.970	<0.001		
	N1	1.34	0.82	0.663	<0.001	NS	***
	N2	1.72	0.64	0.727	<0.001		
92 DAG	N1D1	1.17	1.19	0.651	0.032	NS	***
	N1D2	1.22	0.49	0.868	0.004		
	N2D1	1.89	0.61	0.918	<0.001		
	N2D2	2.04	0.69	0.852	<0.001		
	N1	1.05	0.30	0.875	<0.001	***	
	N2	1.97	0.65	0.874	<0.001		

¹ F-probability value: ***= $P<0.001$, **= $P<0.01$, *= $P<0.05$, NS = non significant ($P>0.05$)

² Residual variance exceeds variance of y variate.

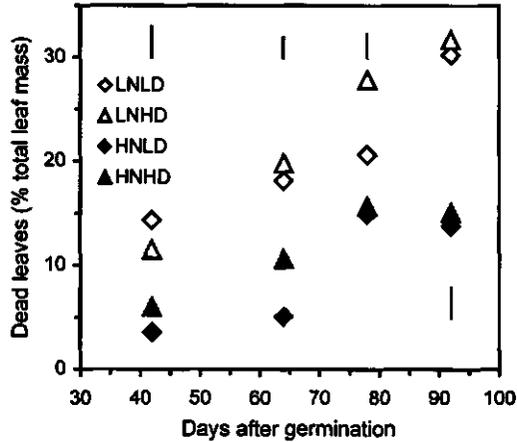


Figure 5.3. Changes in the proportion of dead leaves (with respect to total biomass of leaves) with time. Bars indicate standard error of the differences between means.

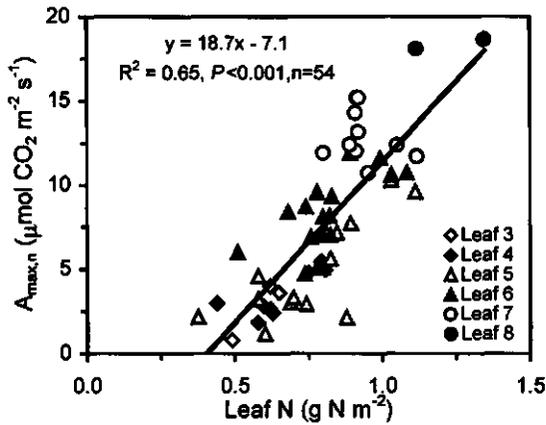


Figure 5.4. Leaf photosynthetic rate at light saturation ($A_{\max,n}$) as a function of leaf N content, all harvests, leaf positions and treatments included.

Thus, we assumed the variation in $A_{\max,n}$ not related to leaf N was linked to leaf age. In order to appraise the magnitude of the effects of leaf N and age separately, $A_{\max,n}$ was plotted vs. the time after leaf emergence (DALE, days) and the leaf N, for leaves number 5 and 6 (Fig. 5.6). These were the only positions where photosynthesis was measured at the four harvest dates; by 42 DAG leaf number 6 was recently fully expanded. The effect of leaf age on $A_{\max,n}$ was smaller than that of leaf N, and was only detectable after 40-45 DALE. For instance, from 10 to 40 DALE a change in leaf N from 0.5 to 1.0 g N m⁻² represented an increase in $A_{\max,n}$ of 60-70%. Only at 50 DALE, the gain in $A_{\max,n}$ by duplicating leaf N increased to 90%, indicating that the effects of age were more visible in leaves of lower N content.

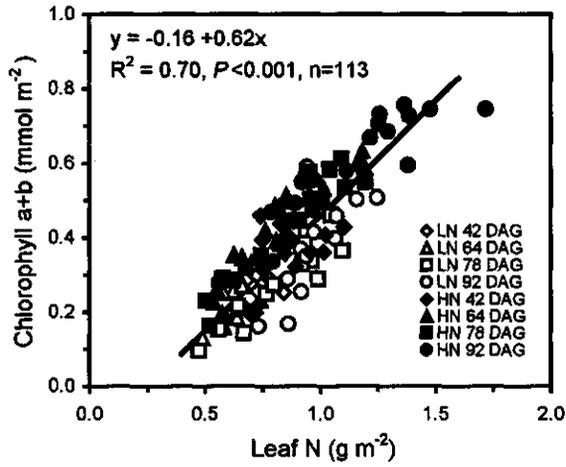


Figure 5.5. Chlorophyll vs. leaf N content at different dates and nitrogen levels.

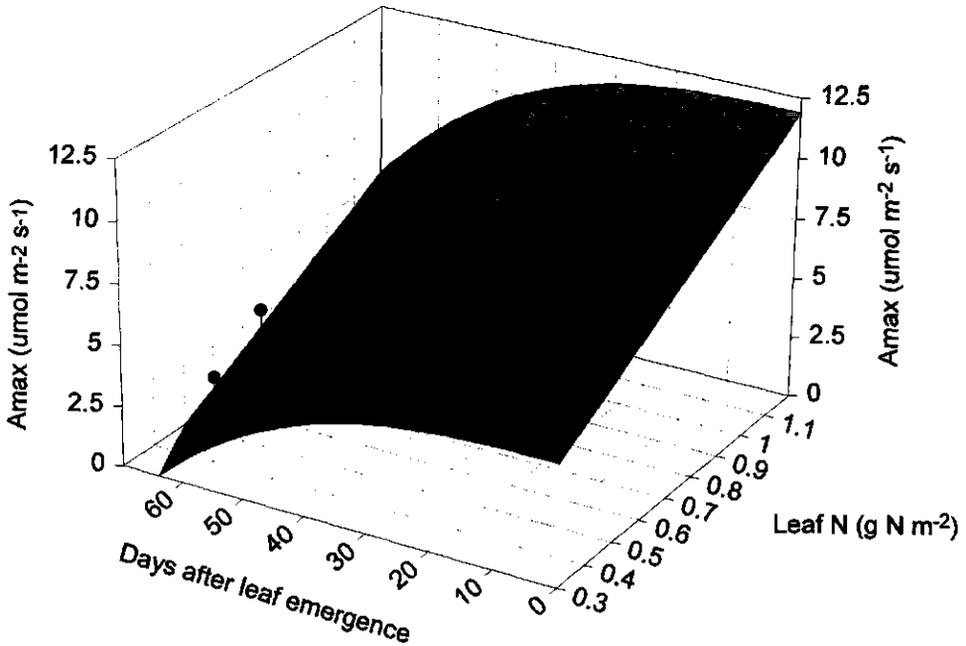


Figure 5.6. Changes in $A_{max,n}$ with leaf N content and days after leaf emergence, for leaves number 5 and 6.

Leaf N vertical distribution and canopy photosynthesis

Unlike radiation levels in the canopy, which were exponentially related to LAI, leaf N distribution was linearly related to LAI (Fig. 5.7). Based on the small effects of leaf age on photosynthesis, we calculated canopy photosynthesis assuming that changes in $A_{\max,n}$ in space and time were mainly a response to leaf N (Fig. 5.4). Leaf absorptance was a function of leaf chlorophyll content (mmol m^{-2}) ($y=0.97-0.86/(1+11.3x)$); $P<0.001$, $R^2=0.96$, $n=62$), which in turn was a function of leaf N (Fig. 5.5).

Regression lines in Fig. 5.7 are calculated with the simulation model and depict the leaf N distribution that maximised canopy photosynthesis (DTNA). Across harvests, and for most of the treatments, the actual leaf N distribution was within 1% of the optimal. When $\text{LAI}<1$ (e.g. all harvests of low N and low density or high N at 42 DAG), DTNA was within 1% of the optimal. Calculated DTNA with actual leaf N profiles was, across harvests, $0.02\text{--}0.08 \text{ mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$ at low N and density and 0.05 to $0.19 \text{ mol CO}_2 \text{ m}^{-2} \text{ d}^{-1}$ at high N and density. For the range of situations explored, the advantage in DTNA of the optimal profile over the uniform distribution never exceeded 2%, except at 64 DAG at high N and density when the advantage was 7%.

Sensitivity analysis of canopy photosynthesis

Two questions remained after running the model. The first one was to what extent treatment differences in maximum DTNA and the associated optimal leaf N profiles would change at irradiance levels higher than those of the experiment. This was analysed by running the model for extreme treatments in LAI and N content (LNLD vs. HNHD) on February 5 (92 DAG, $2.9 \text{ MJ m}^{-2} \text{ d}^{-1}$) and a clear summer day (July 15, $17 \text{ MJ m}^{-2} \text{ d}^{-1}$). In February, DTNA was 2.3 times higher at HNHD than LNLD (0.180 vs. $0.077 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), while in July the difference rose to 2.6 (0.444 vs. $0.168 \text{ mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). To achieve the maximum DTNA, the slope of the optimum leaf N distribution vs. cumulative LAI was steeper at high than low irradiance, and higher at LNLD than HNHD (0.55 vs. 0.27 in February, 0.81 vs. 0.35 in July).

The second issue was if at different irradiance levels, the optimum leaf N distribution was associated to differential changes in the photosynthetic capacity at low (EFF_A) and high light ($A_{\max,n}$). We explored this by running the model for a canopy with the characteristics of HNHD on February 5 and July 15. To quantitatively assess the importance of EFF_A and $A_{\max,n}$ in defining the maximum DTNA, the model was run either with a fixed EFF_A profile corresponding to the optimal leaf N profile, while $A_{\max,n}$ changed as a function of leaf N and alternatively with a fixed $A_{\max,n}$ profile corresponding to the optimal leaf N profile, while the EFF_A changed as a function of leaf N (Fig. 5.8a-b). DTNA was more sensitive to a change in the slope of the leaf N distribution at high than low light. Variations in EFF_A with leaf N explained closely the pattern in DTNA at low irradiance, while changes in $A_{\max,n}$ did at high irradiance (Fig. 5.8a and b). At low and more evidently at high light, maximum DTNA was reached when leaf N distribution was between the optimum for $A_{\max,n}$ and EFF_A . Around the

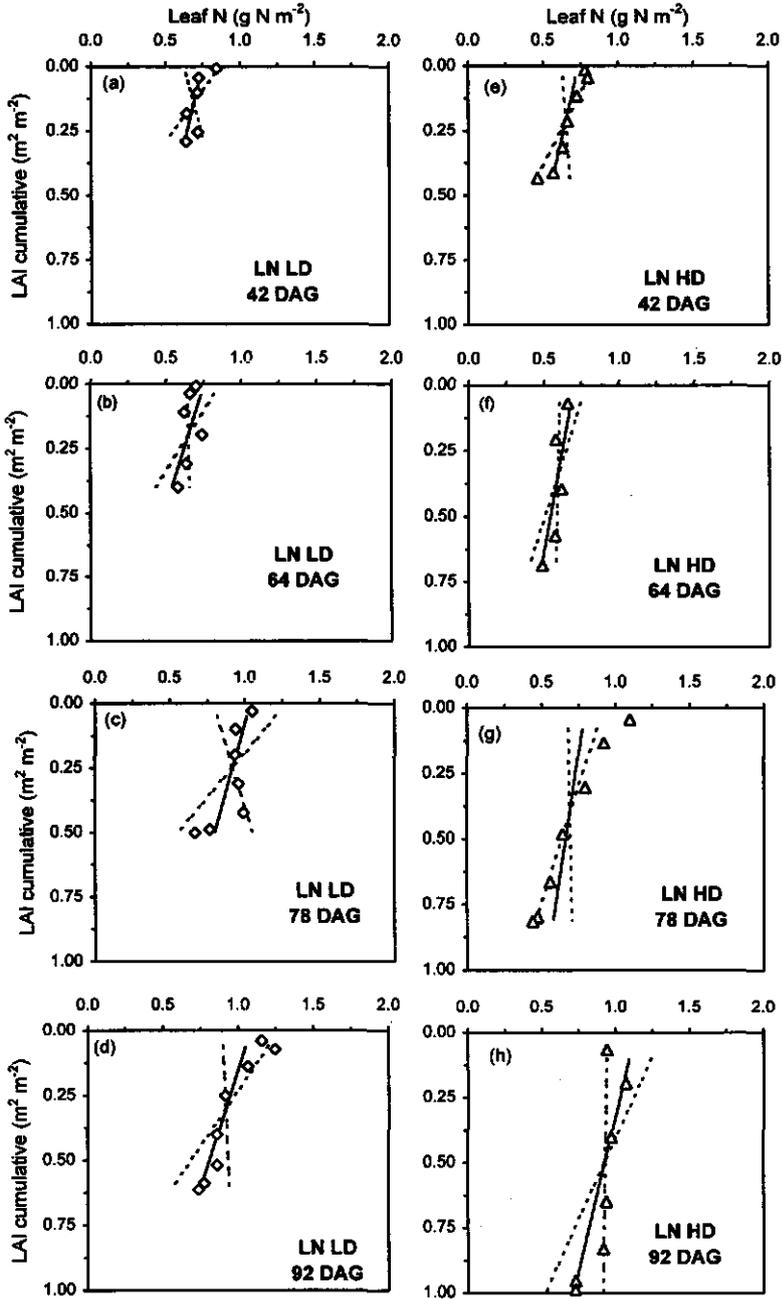
optimum, a change in 1% in slope of the leaf N resulted in a similar drop in DTNA due to changes in either EFF_A or $A_{max,n}$ as a function of leaf N (Fig. 5.8c-d).

Discussion

Light-associated leaf N distribution changes dynamically, depends on N supply and is close to optimal

During vegetative growth of wheat, the vertical distribution of leaf N changed dynamically with the gradient of absorbed irradiance (Fig. 5.2 e-h, Table 5.1). This relation was responsive to N supply. At high N supply, the leaf N profiles were quite stable in time, as observed in other vegetative canopies (Evans, 1993a). In a canopy in active expansion (Fig. 5.1c) this means that the loss of N from lower leaf layers kept a proportionality with the decrease in irradiance as leaves were progressively shaded. By contrast, at low N supply, the relation between leaf N distribution and absorbed irradiance fluctuated in time from steeper to more uniform than that observed at high N supply, even when changes in the radiation profiles were small. This suggests that other processes, affected by N shortage, played a role in the definition of the vertical leaf N distribution. Leaf expansion, is severely limited at low N supply (Gastal et al., 1992). The restriction favours the maintenance of a high leaf N content, and hence photosynthetic activity, at the sites of active expansion (Vos and Van der Putten, 1997). In wheat, low N supply also restricts tillering (Fischer, 1993), thus changing the proportion of younger vs. older shoots per plant. At the same time, at low N supply, lower leaves reach a low N content earlier than at high N, because even if the export rate is the same, the initial N concentration is lower (Pons and Pearcy, 1994). Ultimately, leaf senescence of lower leaf layers can lead to a momentarily more uniform leaf N profile at low N supply. Thus, in a crop growing under N deficiency, the balance between reduced area expansion and branching and N mobilisation will define the leaf N distribution of the canopy. The interaction of these processes enhances plant photosynthetic performance at low N. Leuning et al. (1995) calculated that at low N supply, canopy photosynthesis was significantly increased if leaf N followed light attenuation compared to a uniform pattern, while gains were negligible at high N. Our sensitivity analysis also indicates that the increase in slope of leaf N distribution at low N supply helped to narrow the gap in maximum canopy photosynthesis compared to the high N treatment, particularly at high irradiance. Finally, plant density had a negligible effect on the distribution of leaf N (see also Shiraiwa and Sinclair, 1993; Del Pozo and Dennett, 1999) probably related to a compensation in canopy expansion that led to a close radiation distribution between densities.

At the prevailing low light level, broad oscillations in leaf N profiles kept the actual canopy daytime assimilation within 1% of the optimum (Fig. 5.7). Independently of the magnitude of gain in canopy photosynthesis, the leaf N distribution is generally found to be more uniform in the actual than the optimal pattern (e.g. Anten et al., 1995). In the first place, leaf N distribution may be more uniform than expected because it responds to other goals than optimisation of photosynthesis, such as maximum growth capacity after grazing.



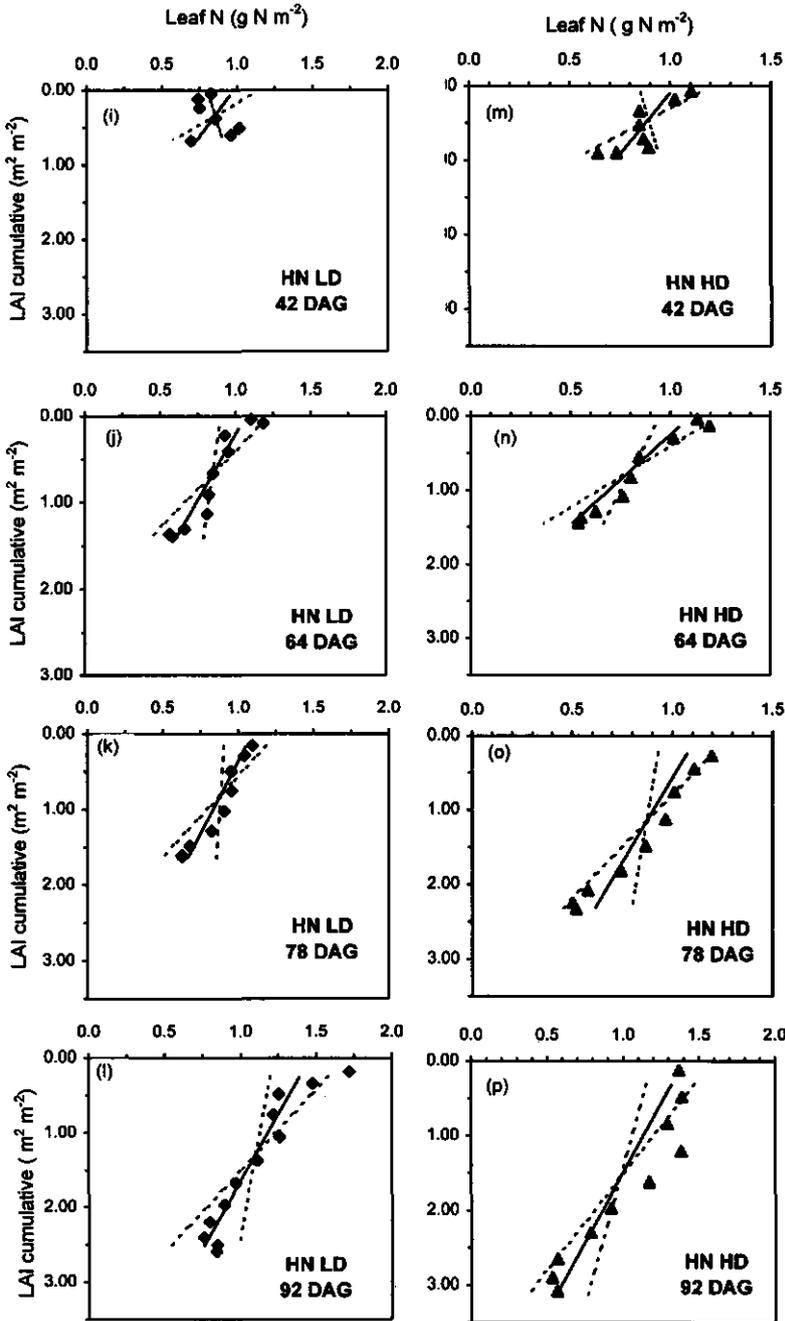


Figure 5.7. Variation in leaf N content per unit area with LAI cumulative from the top of the canopy. Treatment symbols as in Fig. 5.1. Solid line indicates model calculated distribution for maximum canopy photoperiodic net assimilation. Dotted lines indicate deviation of 1% from the maximum.

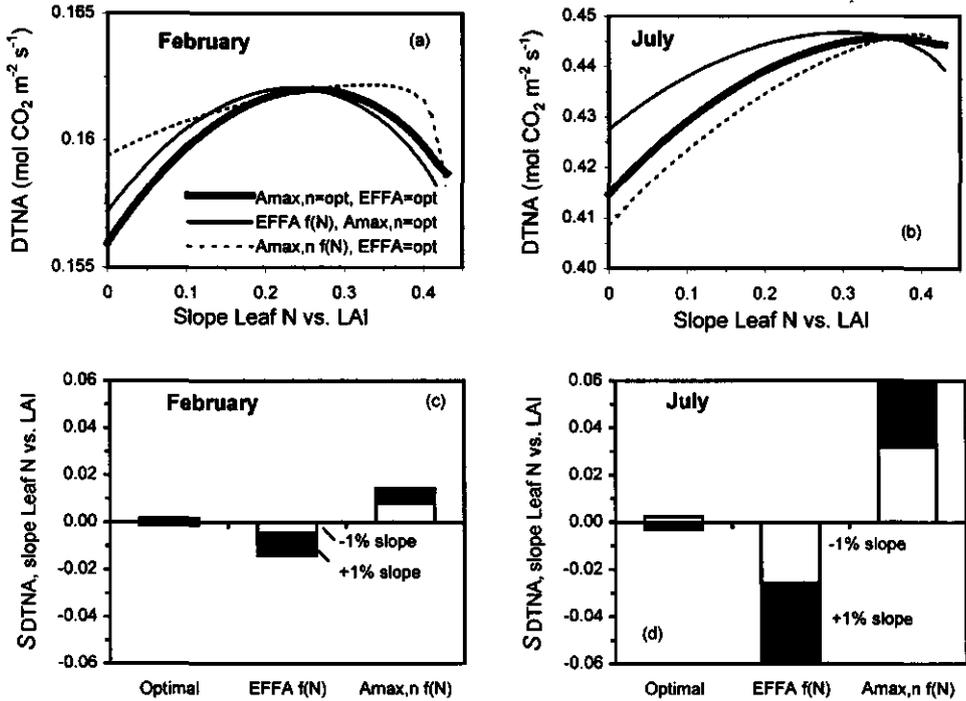


Figure 5.8 Changes in canopy photosynthesis (DTNA) with the slope of the leaf N distribution within the canopy ($b_{N,LAI}$) for a day in (a,c) summer (February) and (b,d) winter (July). In (a) and (b) thick lines are DTNA when $A_{max,n}$ and EFF_A vary with leaf N; thin lines are DTNA for $A_{max,n}$ as in optimum profile and EFF_A as a function of leaf N; dotted lines are for $A_{max,n}$ as a function of leaf N and EFF_A as in optimum profile. In (c) and (d) the sensitivity of DTNA to $\pm 1\%$ change in $b_{N,LAI}$ around the optimum (see Materials and Methods) when $A_{max,n}$ or EFF_A are a function of leaf N.

Alternatively, the simulation of leaf N distribution could be incorrect because in attempting to simplify, it fails to account for relevant processes such as leaf aging, acclimation of photosynthesis to low light (Pons et al., 1989), etc. According to the results of this study (Fig. 5.6) and others (Hikosaka et al., 1994), considering N-independent effects of leaf age on photosynthesis is not likely to improve dramatically the prediction of the distribution of photosynthetic capacity in the canopy. The effects of leaf aging on maximum photosynthetic capacity were low, only noticeable when leaves were relatively old, and the pattern seemed independent of leaf position, as reported by Rawson and Hackett (1974). On the other hand, N-independent effects of light level on photosynthesis could not be distinguished. When leaves acclimate to low irradiance, light absorption is increased vs. photosynthetic capacity, so that the photosynthesis per unit leaf N is maintained nearly constant (Evans, 1989b). Most likely, the whole canopy was adapted to the low irradiance as judged by the absence of variation in morphological (specific leaf mass) or physiological features (ratio of chlorophyll vs. leaf N or chlorophyll a/b) that typically change with increasing shade

in canopy depth (Evans, 1996). Evans (1993a) reported that accounting for acclimation of canopy photosynthesis to low light contributed only a few per cent to the potential canopy photosynthesis of lucerne.

Leaf N content accounted for most of the variance in maximum photosynthetic capacity, in line with previous observations (Evans, 1989a). Because there were no light acclimation effects in the canopy profile and leaf age effects were small, accounting for metabolic costs of N allocation seems the most important point missing in our analysis (Field, 1983). We propose that in our study the inclusion of a N-dependent change in photosynthetic efficiency at low light helped to narrow the discrepancy between optimal and actual N profiles, compared to approaches where only the maximum photosynthetic capacity is dependent on leaf N (Anten et al., 1995).

Optimal leaf N profiles and trade-offs between photosynthetic parameters

Canopy photosynthesis was equally sensitive to changes in the slope of leaf N distribution at high than low N. In addition, the sensitivity analysis showed that plants respond to changes in the environment by keeping a leaf N distribution that balances the capacity for photosynthesis at high light and low light (Fig. 5.8). The maximum photosynthetic capacity is determined by the amount and activity of carboxylating enzymes, the diffusion of CO₂ to the site of carboxylation and the rate of regeneration of acceptor for carboxylation, usually limited by the maximum rate of electron transport (Lawlor, 1993). The photosynthetic efficiency at low light is determined by leaf absorptance and the efficiency of transduction of the light intercepted by the leaf (Lawlor, 1993). Based on the photosynthesis model of Farquhar and colleagues (1980), Chen et al. (1993) proposed that plants allocate N to maintain the balance between the Rubisco-limited rate of carboxylation and the electron transport limited rate of carboxylation, dictated by the irradiance. However, a major constraint in the Farquhar model is that the efficiency of electron transport limited carboxylation is not dynamically considered. This is a major constraint, because then changes in the photosynthetic efficiency at low light with leaf N, as seen in our study, can not be simulated. The issue is particularly relevant considering that crop CO₂ uptake is only more sensitive to the light-saturated photosynthetic capacity than to the initial photosynthetic efficiency under high radiation level and when leaf area is small (Ort and Baker, 1988). Our results echo at the canopy level, the findings of Hikosaka and Terashima (1995) with respect to N partitioning within the leaf, that there is a trade-off relationship between the maximum rate and the initial slope of the light response curve of photosynthesis.

Conclusions

In a vegetative wheat canopy, leaf N and photosynthetic capacity were allocated in relation to the light gradient. This association was more stable under high than low N supply. At low N supply, restricted canopy expansion and N remobilisation were also driving forces in the definition of the vertical leaf N distribution. This indicates that

although local light environment plays a major role in dictating leaf N distribution (Drouet and Bonhomme, 1999), the ultimate regulation of resource allocation is coordinated at the plant level. The study of the signals and processes involved in this regulation is at the moment rather fragmentary. A role for spectral light quality (Rousseaux et al., 1999) and light intensity (Wingler et al., 1998) on the regulation of leaf senescence has been established. Additionally, there are ideas about the role of cytokinins in regulating biomass allocation under low N supply (Van der Werf and Nagel, 1996) and redistribution of N in relation to irradiance level (Pons and Jordi, 1997). The interaction between these fields deserves more attention.

Finally, both at high and low N, the actual leaf N distribution was nearly optimal. The sensitivity analysis indicated that fluctuations in the vertical allocation of leaf N around maximum canopy photosynthesis entail changes of similar magnitude in the capacity for photosynthesis at high and low light. This highlights the need for inclusion of more dynamic modelling of the photosynthetic efficiency at low light in response to different stresses, as opposed to current models that consider it constant.

Chapter 6

Photosynthetic acclimation to high atmospheric CO₂ in a wheat canopy supplied with N in proportion to growth

Abstract

A decrease in leaf tissue N and acclimation (depression) of photosynthetic capacity have been reported in plants exposed to high atmospheric CO₂ concentrations for a long time. This phenomenon has been interpreted as a consequence of sink limitation for carbohydrates at the plant level, and been linked to N reallocation within the photosynthetic apparatus and within the plant. The interaction between CO₂ level and N availability was studied in wheat during a critical phase for yield definition, both at the canopy and the leaf level. To isolate the effects of CO₂ on N concentration from those of N availability or ontogenetic decline, N was supplied in proportion to growth to keep the plant N concentration constant. At the leaf level, we studied the relation between tissue composition and photosynthesis at different leaf positions and harvest times. At the canopy level, growth, light and leaf N distribution patterns were characterised for main stem and tillers. Observed differences in biomass and LAI due to high CO₂ level were regulated by the effect of N on tiller survival and growth. The light-associated patterns of leaf N distribution were similar at high and low CO₂ and only affected by N availability. Leaf photosynthetic acclimation to high CO₂, absent directly after full leaf expansion, appeared in time and seemed independent of leaf N content and accumulation of soluble carbohydrates.

Key words: photosynthetic acclimation, leaf N distribution, canopy photosynthesis, CO₂, nitrogen, wheat.

Abbreviations: A_{sat} , gross rate of photosynthesis at CO₂ and light saturation; C_i , internal CO₂ concentration; DAG, days after germination; EFF_A , EFF_I photosynthetic efficiency at low light based on absorbed or incident light, respectively; I_{AL} , PAR absorbed per unit leaf area; k , coefficient of light extinction; k_m , affinity of Rubisco for CO₂; PAR, photosynthetically active radiation; Γ_* , CO₂-compensation point; $V_{c,max}$, maximum rate of carboxylation; R_d , respiration in the dark.

Introduction

Exposure of C_3 plants to high atmospheric CO_2 concentrations usually results in an increase in photosynthetic rates and growth (Kimball et al., 1993). However, the initial effect of CO_2 on photosynthesis can decline, after several weeks of exposure, to values similar than those found at ambient CO_2 concentration (Sage, 1994). One explanation for this phenomenon is that accumulation of sugars can lead, through a cascade of metabolic steps, to a decrease in leaf N and ribulose bis-phosphate carboxylase/oxygenase (Rubisco) content (Stitt, 1991; Van Oosten et al., 1994). The phenomenon of decrease in photosynthetic rate accompanied by a compositional change at the leaf/plant level is called acclimation to high CO_2 (Sage, 1994). The presence of active sinks for carbohydrates however, could annihilate the feedback on photosynthesis (Woodrow, 1994).

Nitrogen (N) availability stimulates biomass production through direct effects both on the source of carbohydrates, i.e. leaf photosynthesis (Evans, 1989a) and on the sink, i.e. leaf area expansion and formation of branches or tillers (Fischer, 1993). Therefore interactive effects of N availability with the level of atmospheric CO_2 can be expected. High N supply helped to prevent photosynthetic acclimation to high CO_2 in wheat (Rogers et al., 1996) and rice (Ziska et al., 1996) by stimulating tiller growth and partially eliminating the drop in tissue N concentration often observed at high CO_2 . These findings led to the conclusion that acclimation to high CO_2 was primarily a response to N availability. Others suggest that the way in which the nutrient limitation is imposed influences the response of growth to elevated CO_2 . Accordingly, acclimation of photosynthesis to high CO_2 was prevented in the last expanded leaf of young wheat plants (Farage et al., 1998) by supplying sub-optimal amounts of N at a rate that stabilises the N concentration in the plant. However, predictions from measurements on the last fully expanded leaf may not necessarily hold at the canopy level. There is evidence that in field-grown wheat under elevated CO_2 , photosynthetic acclimation occurred mainly in older leaves (Nie et al. 1995; Osborne et al., 1998). The heterogeneity in leaf response is worth assessing because it will have consequences for canopy photosynthesis. Under ambient CO_2 concentrations, canopy photosynthesis depends on the attenuation of light through the canopy and the vertical leaf N distribution associated to it (Field, 1983). In a CO_2 enriched environment, the reported variation in acclimation capacity along the plant could alter the relation between the distribution of N and photosynthetic capacity for a given light environment. In addition, the photosynthetic efficiency at low light increases at high CO_2 (Long and Drake, 1991). Thus, alternatively, in closed canopies at high CO_2 , higher investment of N in lower leaf layers, particularly as pigments and energy processing compounds, could help to take advantage of the shift in the light compensation point. Since higher wheat yields at high CO_2 have been related to the canopy gross photosynthesis per unit absorbed irradiance (Monje and Bugbee, 1998), factors that could affect it deserve particular attention.

The objective of this work was to test whether the phenomenon of acclimation to CO_2 is a simple response to changes in the plant N status. To isolate the effects of CO_2 on

N concentration from those of N availability or ontogenetic decline, N was supplied in proportion to growth so that the N concentration in the plant remained constant. At the leaf level, we studied the relation between tissue composition and photosynthesis at different leaf positions and harvest times. At the canopy level, growth, light and leaf N distribution patterns were characterised for main stem and tillers. Labelling with ¹⁵N was used to evaluate if the investment of recently absorbed N differed at high and low CO₂ levels. We have focused on the period between boot stage and heading because of its crucial importance in the determination of the grain number in wheat (Fischer, 1985).

Materials and Methods

Growth conditions and characteristics of the hydroponics system

Spring wheat (*Triticum aestivum* L., var. Minaret) was grown in nutrient solution at 350 or 700 μ bar CO₂ combined with two levels of N availability. Seeds were germinated at room temperature and ambient CO₂ concentration. After germination seedlings were placed in rockwool cylinders and transplanted to containers with aerated nutrient solution, placed in growth chambers (10 m² each). Day and night temperatures were kept at 17/12°C, relative air humidity 70-80% and the level of photosynthetically active radiation (PAR, 400-700 nm) was 350 μ mol quanta m⁻² s⁻¹ at the top of the plants during 18 h a day, i.e. ca. 5 MJ m⁻² d⁻¹. The CO₂ was supplied from a compressed gas cylinder with pure CO₂ (3 bar), through a flow meter and a valve that regulated the concentration in the chambers at either 350 \pm 30 or 700 \pm 60 μ bar CO₂. The aperture of the valve was controlled by measurement of actual CO₂ concentration in the chambers with an infrared gas analyser.

The hydroponics system consisted of containers of 45 L capacity and 0.264 m² surface (0.55 x 0.48 m). The nutrient solution was re-circulated with a pump between the container and its individual reservoir tank (30 L). The solutions were continually aerated. In each container, plants were arranged in a canopy of 318 pl. m⁻² in rows 0.09 m apart. Rows consisted of perforated foam strips where the seedlings were inserted, the canopy was constructed by placing 6 strips next to each other per container. The surface of the containers was fully covered by strips and kept free from light to prevent algae growth.

Nutrient solution

The two N treatments were a free-access level or 'high N' and a growth-limiting level or 'low N'. The high N consisted in the full Steiner solution (1984) (12mM NO₃⁻). At low N availability, N was supplied in order to reduce 50% the expected potential growth rate. Potential growth was calculated based on measured intercepted irradiance and radiation use efficiency. Radiation use efficiency was taken as 5.5 g MJ⁻¹ at 350 μ bar CO₂, based on previous experience with this cultivar growing under artificial light. At 700 μ bar CO₂, an increase in radiation use efficiency of 20% was assumed based on literature (Mulholland et al., 1998; Ewert et al., 1999). To control the

expected vs. achieved biomass, 3 plants per treatment were collected every two days from additional containers. N was supplied every two days. Before every N addition, NO_3^- level in the solution was checked with NO_3^- test-strips (Merckoquant 10020, Merck, Darmstadt, Germany) to ensure that all the N applied had been taken up. At high and low N, micronutrients and iron were added. In the low N treatment, NO_3^- was replaced with Cl⁻ to keep the electrical potential of the solution balanced. The pH was kept at 6 by daily measure and correction. After the second harvest, the solution was renewed. Since then, N was supplied as enriched to 1.00 atom % ^{15}N , using 99 atom % $^{15}\text{N-KNO}_3$ and $-\text{Ca}(\text{NO}_3)_2$ (Isotec Inc., Miamisburg, Ohio, USA).

Canopy biomass, leaf area and chemical analysis

Phenological stages were characterised with the decimal code scale of Zadoks et al. (1974). At 52 and 60 days after germination (DAG) 0.03 m^2 were harvested from a strip in the centre of the containers. After the harvest at 52 DAG the remaining strips were moved to the centre to close the gap and the border of the container was covered with a non-perforated strip. Roots and shoots were separated, shoots were clipped at height intervals of 0.10 m. Green and senescent leaves, stem+sheaths and spikes were separated. This procedure was followed separately for the main stem and the tillers. Leaf area per canopy layer was measured with an electronic planimeter (LI-3100, LI-COR, Inc., Lincoln, Nebraska, USA). Samples were oven-dried at 70°C during 48 h and weighed.

Tissue content of N and atom % ^{15}N were determined using an isotope-ratio mass-spectrometer with on-line sample preparation (Roboprep TCD-Tracermass; Europa Scientific, Crewe, UK). International standard-gauged laboratory standards were used as references (cf. Schnyder 1992). The amount of ^{15}N in leaf tissue, referred to as 'new N', was calculated as in Deléens et al. (1994), considering natural abundance of ^{15}N in nitrate as 0.368 atom % (Handley and Raven, 1992). Water-soluble carbohydrates (WSC), including fructans and amylose, were determined in leaf 7 and 5 and in stems+sheaths of main stem and tillers at 60 DAG. The samples were extracted in boiling demineralised water (10') and the amount of reducing monosaccharides were determined colorimetrically with ferricyanide after addition of H_2SO_4 (0.26 N).

Canopy light environment

PAR attenuation through the canopy was measured at height intervals of 0.10 m on the harvest days using a linear ceptometer (SF-80, DELTA-T Devices LTD, Cambridge, England). The extinction of PAR through the canopy was described with an exponential model (Monsi and Saeki, 1953), and the mean level of PAR absorbed per unit leaf area at any height (I_{AL} , $\mu\text{mol quanta m}^{-2} \text{ leaf}$) was calculated as:

$$I_{AL} = I_0 k \exp(-k LAI)/(1 - t)$$

Eq. 6.1

where I_0 is the PAR level at the top of the canopy ($\mu\text{mol quanta m}^{-2}$ ground), measured on a horizontal plane. k is the coefficient of light extinction in the canopy calculated as the slope of the linear regression between the log-transformed ratio of PAR incident on a horizontal plane at a certain height and I_0 vs. the cumulative leaf area index (LAI, $\text{m}^2 \text{m}^{-2}$). The transmission coefficient of the leaves, t , was taken as 0.05 (Goudriaan and Van Laar, 1994).

Gas exchange measurements

Leaf photosynthesis was measured in an open gas exchange system, the concentrations of CO₂ and H₂O vapour in the ingoing reference air and the outgoing air stream air were measured with infrared gas analysers (ADC The Analytical Development Co. Ltd, UK, model 225 MK3). Air passed through sodalime to remove CO₂ and pure CO₂ was injected using mass-flow controllers (model 5850, Brooks Instrument B.V., Veenendaal, The Netherlands) to adjust the CO₂ concentration. Air humidity of the ingoing air stream was set to a relative humidity of 70 % by passing dry air through a temperature-controlled humidifier. The photosynthesis-light response curves were measured in both leaves 7 and 5 at 52 DAG and 60 DAG, at the same CO₂ concentration that the plants were growing, starting from 1500 down to 0 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ PAR supplied by a halogen lamp (Philips type 6423, 15V/150W) with an infrared filter. The photosynthesis-CO₂ response curves were measured in leaves 7 and 5 at 52 DAG and in leaf 7 at 60 DAG, measurements were done at 350, 700, 1300 and 180 $\mu\text{bar CO}_2$.

The photosynthetic efficiency based on incident light was calculated from the initial slope of the light response curve at low light (EFF_I , $\text{mol CO}_2 \text{ mol}^{-1} \text{ quanta}$). Leaf absorbance was calculated knowing the chlorophyll content as in Chapter 4 (Absorbance = $1.0 - 0.7 / (1.0 - 4.3 \text{ Chlorophyll [mmol m}^{-2}\text{]})$). EFF_I was converted to the efficiency of absorbed light (EFF_A). The initial slope of the photosynthesis vs. internal CO₂ concentration curve (A/C_i) was determined between 180 $\mu\text{bar CO}_2$ and the x-intercept that represents the CO₂-compensation point, estimated at 35.5 $\mu\text{bar CO}_2$ (Azcón-Bieto et al., 1986). The maximum rate of carboxylation, $V_{c,\text{max}}$, was derived from the slope of the A/C_i curve and the affinity of Rubisco, i.e. $k_m = 527.6 \text{ mmol mol}^{-1}$ according to Evans and Farquhar (1991). The gross rate of photosynthesis at light and CO₂ saturation (A_{sat}) was calculated by adding up the dark respiration (R_d , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) to the net photosynthetic rates. R_d was measured after the plants were in the dark for 20'. The calculated EFF_A was related to leaf N content with an asymptotic exponential curve as reported in Chapter 4 ($\text{EFF}_A = a (1 - \exp(-(\text{Leaf N} - b)/c))$). The values of A_{sat} and $V_{c,\text{max}}$ were fit to the leaf N content with a linear regression (see Osborne et al., 1998).

Leaf chemical analysis and relation with photosynthetic parameters

At each harvest, middle portions of leaves number 5 and 7 on the main stem were frozen in liquid N₂ and stored at -80°C for determination of Rubisco and chlorophyll (Inskeep and Bloom, 1985). Rubisco content was determined after grinding leaves in a

precooled mortar with liquid N₂. Protein patterns were visualised in 12% SDS polyacrilamide gel after dyeing with Coomassie brilliant blue (R250) and quantified by densitometry using bovine serum albumin as standard. WSC and leaf N and ¹⁵N content were determined as explained. 'Apparent' structural leaf weight was calculated as total dry weight minus WSC. Since starch content was not determined, this constitutes an approximation to the actual structural leaf weight. The leaf N content expressed on an area basis for leaf 5 and 7 is based on the N content expressed on the apparent structural dry mass basis.

Statistical analysis

The results were analysed with ANOVA as a split-plot design with 2 chambers and 4 blocks in each chamber, within each block N-treatments were randomised. Since each CO₂ level was assigned to a different chamber caution is advised in the interpretation of the terms connected to CO₂. The chambers were checked for uniformity in temperature, humidity and light intensity before starting and behaved accordingly during the experimental period. Differences between regressions were assessed by pairwise comparison with a *t*-test build in Genstat 5 (1987).

Results

At the first harvest (52 DAG) the plants were in boot stage (DC 43), at the second one (60 DAG) they were at heading (DC 59). There were no significant effects of CO₂ or N level on the crop developmental stages.

Growth, leaf area and N uptake

Tissue N concentration at the whole plant level or in the leaves was relatively constant between 52 and 60 DAG, significantly lower at low N availability, and the CO₂ level had no effect on it (Table 6.1). During the period of observation two important events were taking place in the crop, the fast accumulation of weight by the spike and tiller death. The proportion of total biomass partitioned to spikes from 52 to 60 DAG was independent of N or CO₂ level, ca. 23%. The increase in total or aboveground biomass due to high CO₂ was relatively higher at high than low N availability (Table 6.1, Fig. 6.1). The tillers were the plant organs accounting for most of the change in canopy biomass and LAI (Table 6.2, Fig. 6.1). The interactive effect of CO₂ and N was evident on the net decrease in tiller number per unit surface between 52 and 60 DAG. The decrease was lowest at elevated CO₂ and high N availability (Table 6.2, Fig. 6.1). At heading, the weight per tiller was similar at low and high CO₂, but the tiller number per plant was higher at high CO₂. The limited stimulation of growth due to CO₂ at low N supply could be associated with sink-limitation in the low N plants. This is indicated by the lower tiller number at 52 DAG, the lower specific leaf area, the higher root:shoot ratio and the higher accumulation of WSC in the stems of tillers at 60 DAG compared to plants growing at high N availability (Tables 6.1 and 6.2).

Table 6.1. Plant and leaf N concentration, total biomass, root:shoot ratio, total N uptake, Leaf Area Index, and specific leaf area

Date	CO ₂	N	Plant N	Green	Total	Root:	Total N	LAI	Specific
	[μbar]		[mgN g ⁻¹]	Leaves N	biomass	Shoot	Uptake	[m ² m ⁻²]	Leaf
				[mgN g ⁻¹]	[g m ⁻²]	ratio	[gN m ⁻²]		Area
						[%]			[m ² kg
52 DAG	350	Low	25.2	37.7	517	11.6	12.9	5.4	34.8
		High	30.6	48.5	718	11.5	21.7	10.8	38.9
	700	Low	24.2	41.1	591	17.7	14.2	5.9	32.6
		High	27.4	48.5	887	12.4	24.7	12.2	35.8
60 DAG	350	CO ₂	NS ¹	NS	NS	NS	NS	NS	*
		N	**	***	**	*	***	***	***
		CO ₂ *N	NS	NS	NS	*	NS	NS	NS
		Low	18.3	37.8	809	9.4	14.8	5.4	30.9
	700	High	27.0	44.0	1118	7.6	29.5	10.7	33.1
		Low	20.4	35.0	938	15.2	19.1	6.0	31.2
		High	24.7	44.0	1562	10.5	38.6	13.9	34.2
		CO ₂	NS	NS	**	*	**	*	NS
CO ₂ *N	N	***	***	***	**	***	***	***	*
	CO ₂ *N	*	NS	*	*	NS	NS	NS	NS

¹ F-probability value: ***=P<0.001, **=P<0.01, *=P<0.05, NS = non significant, P>=0.05).

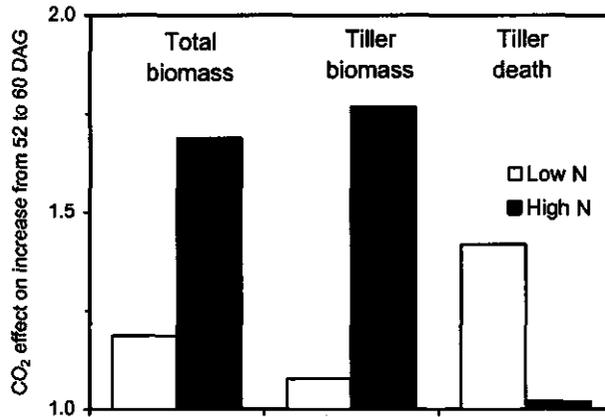


Figure 6.1. Increase in total biomass, aboveground tiller biomass and number of dead tillers between 52 and 60 DAG, expressed as ratio between 700 vs. 350 μ bar, at low (open bars) and high (closed bars) N supply.

Table 6.2. Tiller characteristics, i.e. number, biomass, average individual weight and associated proportion of canopy aboveground biomass and LAI at 52 and 60 DAG; concentration of total non-structural carbohydrates (WSC) in stem+sheaths of main stem or tillers at 60 DAG.

Date	CO ₂	N	Tiller number	Tiller biomass	Weight per Tiller	Biomass in tillers	LAI in tillers	WSC in main stem	WSC in tillers
	[μ bar]		[# m ⁻²]	[g m ⁻²]	[g tiller ⁻¹]	(% of above)	(% of total)	[mg g ⁻¹]	[mg g ⁻¹]
52 DAG	350	Low	1047	179	0.17	39	64	-	-
		High	1252	405	0.33	63	76	-	-
	700	Low	981	249	0.25	51	69	-	-
		High	1431	523	0.37	67	79	-	-
		CO ₂	NS ¹	0.05	*	**	NS		
		N	*	***	***	***	***		
	CO ₂ *N	NS	NS	NS	NS	NS			
60 DAG	350	Low	941	358	0.39	49	69	25.8	26.9
		High	1193	677	0.57	65	75	21.0	18.9
	700	Low	1140	442	0.39	55	70	27.7	23.1
		High	1630	1004	0.64	72	83	18.1	17.3
		CO ₂	0.07	**	NS	*	**	NS	NS
		N	***	***	***	***	***	NS	*
	CO ₂ *N	NS	*	NS	NS	*	NS	NS	

¹ F-probability value: ***= $P < 0.001$, **= $P < 0.01$, *= $P < 0.05$, NS = non significant, $P > 0.05$).

LAI values were maintained in all treatments from 52 to 60 DAG, except at high N and elevated CO₂, where a net expansion was detected (Table 6.1). Light attenuation (k), was higher at low than high N availability ($P < 0.001$), independent of the CO₂ environment and did not change over time. Average values for k across harvests and CO₂ levels were 0.27 for the low and 0.17 for the high N levels.

Leaf composition and photosynthetic acclimation to CO₂

The question if photosynthesis was down-regulated at high CO₂ and its relation with leaf composition was addressed by comparing treatment effects on leaves 7 and 5 at 52 DAG and following leaf 7 from 52 to 60 DAG. At 52 DAG, the ligule of leaf 7 was recently emerged and leaf 5 was at ca.12 days after full size. Leaf 7 had a consistently higher leaf N content per unit leaf area and lower specific leaf area than leaf 5 (Table 6.3). For each leaf position, leaf N on an area basis was similar among treatments due to a combination of higher leaf N on mass basis and thinner leaves at high N availability. No systematic effects of CO₂ on leaf N or WSC content were detected. The ratio between the content of ¹⁵N vs. total leaf N was not affected by treatments and varied from 0.20 (s.e.=0.02) for leaf 5 to 0.28 (s.e.=0.04) for leaf 7.

A_{sat} and $V_{\text{c,max}}$ from all leaf positions and dates could be explained with a unique regression as a function of leaf N (Fig. 6.2). Variation in the values of photosynthesis parameters was bigger between leaf positions than due to treatment effects. At 52 DAG, within each leaf position, A_{sat} and $V_{\text{c,max}}$ at elevated CO₂ were higher or similar to low CO₂ (Fig. 6.3 a and b). Between 52 and 60 DAG, elevated CO₂ accelerated the rate of leaf senescence in leaf 7. At high N supply, $V_{\text{c,max}}$ dropped by 50% and A_{sat} by 42% from 52 to 60 DAG (Fig. 6.3 a and b). A_{sat} remained constant at low CO₂ (Fig. 6.3a, c, d). Thus, when measured just after full expansion (52 DAG), leaf 7 showed no symptoms of photosynthetic acclimation to high CO₂ but its photosynthetic capacity declined in time at high CO₂ ($P < 0.001$), particularly at high N availability (the interaction CO₂ x N was significant at $P < 0.05$). This decline coincided with a decrease in the amount of irradiance absorbed at the layer where the middle-portion of leaf 7 was situated (Table 6.3, Fig. 6.5) and an increase in the proportion of chlorophyll per unit Rubisco at 60 DAG (Fig. 6.4 d). The comparison of the A/C_i curves (Fig. 6.3a and b) together with the analysis of Rubisco content (Fig. 6.4a) suggests that leaf 7 achieved earlier its potential photosynthetic capacity at elevated than low CO₂.

Leaf N distribution in the canopy

Leaf N decreased from top to bottom of the canopy, in proportion to the ratio between the absorbed irradiance per leaf layer and the incoming radiation at the top of the canopy (I_{AL}/I_0). This trend was present in main stems as well as in the tillers (Fig. 6.6). Note that for the leaf N profiles, leaf N was not corrected by WSC content and the upward shift in the regressions of the main stem at low CO₂ may be explained by higher specific leaf area.

Table 6.3. Specific leaf area, total non-structural carbohydrates, N content on structural mass basis and on leaf area basis and PAR absorbed per unit leaf area relative to above-canopy incident PAR (I_{AI}/I_0) at the height of the canopy corresponding to leaf 7 and 5.

Leaf and Date	CO ₂ [μbar]	N	Specific leaf area [m ² kg]	WSC [mg g ⁻¹]	Leaf N [mg g ⁻¹ structural d.wt.]	Leaf N [mmol N m ⁻² leaf]	I_{AI}/I_0
52 DAG							
Leaf 7	350	Low	24.8	28.9	48.2	140.2	0.16
		High	30.8	29.3	53.0	127.7	0.11
	700	Low	25.4	33.2	48.8	144.4	0.15
		High	28.8	36.0	55.5	140.6	0.11
CO ₂ N LSD ¹			4.1	6.4	6.1	22.7	0.02
Leaf 5	350	Low	47.6	40.0	31.8	49.1	0.07
		High	52.8	31.8	43.7	63.1	0.02
	700	Low	46.9	32.7	30.7	49.3	0.07
		High	50.5	40.0	39.5	60.0	0.03
CO ₂ N LSD			11.3	6.2	6.4	19.2	0.01
60 DAG							
Leaf 7	350	Low	23.8	56.3	31.8	94.0	0.15
		High	31.7	52.3	43.6	104.0	0.11
	700	Low	28.2	39.7	35.6	108.0	0.14
		High	29.4	41.4	44.7	109.0	0.07
CO ₂ N LSD			13.9	11.7	10.4	64.5	0.02

¹ LSD stands for Least Significant Difference and corresponds to $P=0.05$

Differences in the slope of the relation between leaf N distribution vs. irradiance, at high vs. low CO₂, may be indicative of an acclimatory effect at the canopy level. Changes in the slope were only observed in time or in response to N supply, independently of CO₂ level (Table 6.4). In general, the main stem, which is the older shoot, showed the steepest N profile due to low N availability earlier than the tillers (main stem at 52 DAG: $P<0.01$; tillers at 60 DAG: $P<0.01$). In the tillers, at 52 DAG, the top layer of some of the treatments had lower leaf N content than the consecutive ones in depth, presumably due to the presence of leaves in expansion (these data were excluded from the regressions reported in Table 6.4).

The assimilation of N at different heights in the canopy between 52 and 60 DAG, was studied with ¹⁵N, and expressed as the fraction of 'new' or labelled N assimilated with respect to the total leaf N content (Fig. 6.6e and f). Plants growing at low N availability showed a lower proportion of labelled N than those at high N availability. At low N, all leaf positions incorporated N in proportion to their N status at the start of labelling, particularly in the main stem.

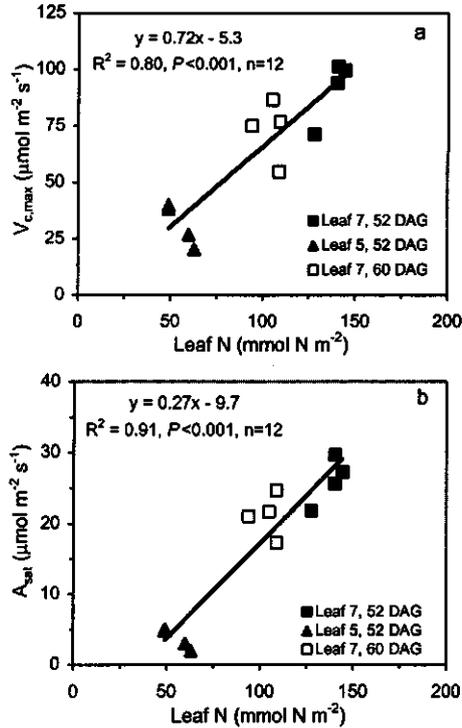


Figure 6.2. $V_{c,max}$ (a) and A_{sat} (b) against leaf N (on 'apparent' structural biomass) for leaf 7 at 52 DAG (closed squares), leaf 7 at 60 DAG (open squares) and leaf 5 at 52 DAG (closed squares).

By contrast, at high N supply, where leaf N distribution was more uniform at the start of the labelling period, leaves in the upper canopy layers were a stronger sink for new N than those at the bottom.

Photosynthetic efficiency at low light and dark respiration

The photosynthetic efficiency based on absorbed light increased with leaf N, and fit an asymptotic exponential function (Fig. 6.7a). The asymptote values were significantly different ($P < 0.01$). The rate of respiration was very low and no clear association with leaf N was detected, the average values were $0.37 \mu\text{mol m}^{-2} \text{s}^{-1}$ (s.e.=0.01) for low CO₂ and $0.50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (s.e.=0.05) for elevated CO₂ (Fig. 6.7b).

Discussion

Growth at high CO₂ depends on N availability

CO₂ effects on biomass and LAI were regulated by N availability and its effects on tiller survival and growth.

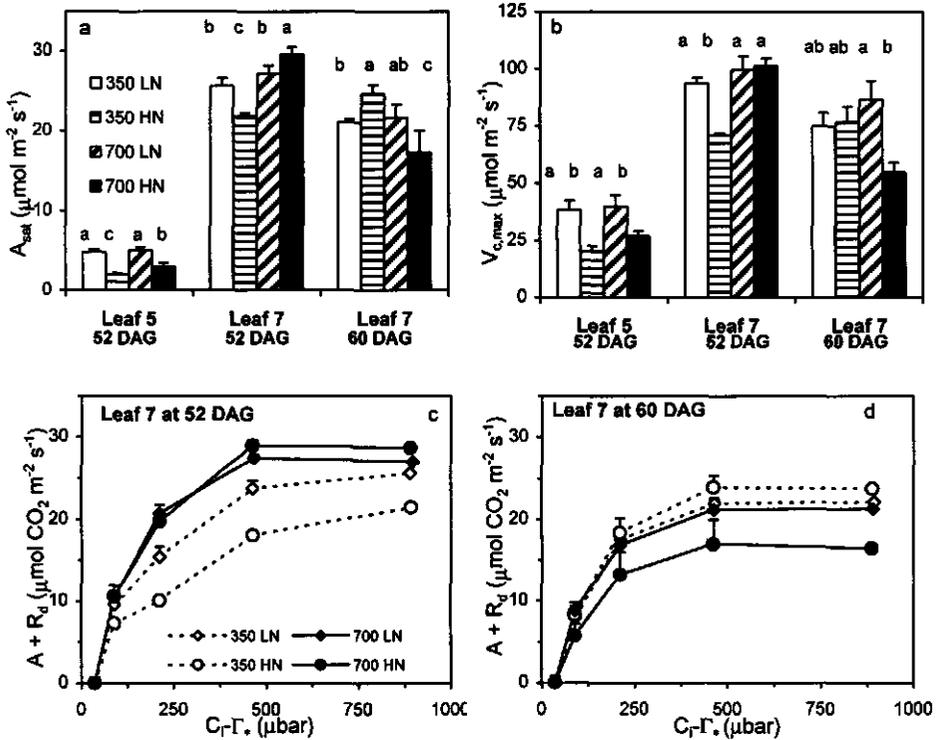


Figure 6.3. A_{sat} (a) and $V_{c,max}$ (b) for leaf 7 at 52 and 60 DAG and leaf 5 at 52 DAG, at 350 μbar and low N (open), 350 μbar and high N (horizontal stripes), 700 μbar and low N (diagonal stripes) and 700 μbar and low N supply (closed). Gross photosynthetic rate vs. internal CO_2 concentration minus the CO_2 compensation point for leaf 7 at 52 DAG (c) and 60 DAG (d), at 350 μbar and low N (open diamond), 350 μbar and high N (open circle), 700 μbar and low N (closed diamond) and 700 μbar and low N supply (closed circle). Vertical bars are s.e. In (a) and (b) differences among treatments by LSD ($P=0.05$) within each combination leaf \times date are indicated with different letters.

A high CO_2 level enhanced biomass production of the whole canopy by shifting the balance of the competition between tillers in a very narrow window of opportunity, corresponding to the period of fast growth of the spike. In a study with small wheat plants during exponential growth under steady-state N nutrition, Farage et al. (1998) attributed the higher plant mass at high CO_2 to a very early and transient stimulation of growth. Interestingly, in our study the effect of elevated CO_2 on canopy biomass was expressed later in time, during the linear growth phase. By boot stage, plants at elevated CO_2 had only a slight advantage in biomass (Table 6.1) but higher average individual tiller weight than at low CO_2 (Table 6.2). During the period from boot stage to heading, the fast growing spike competes for assimilates with the elongating stem (Fischer, 1993). At the same time tiller death, which has started with stem elongation, is still taking place. The reasons for higher tiller survival under the influence of high CO_2 maybe two-fold.

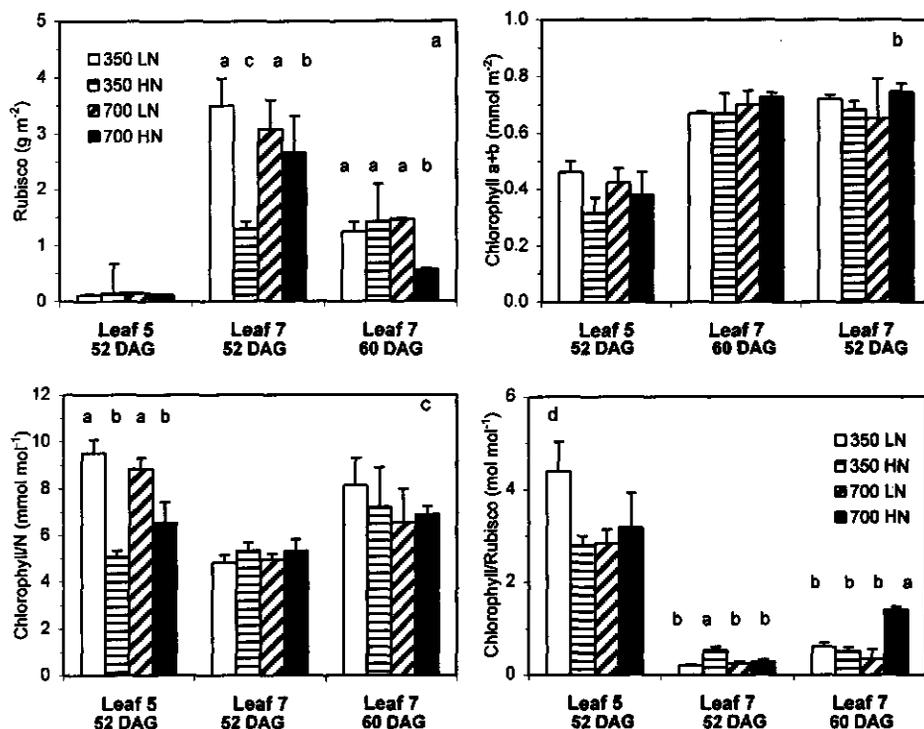


Figure 6.4. Rubisco (a) and chlorophyll content (b), chlorophyll vs. leaf N (c) and chlorophyll vs. Rubisco (d) for leaf 7 at 52 and 60 DAG and leaf 5 at 52 DAG. Symbols and statistics as in Figure 3 a and b. Note: leaf N calculated on 'apparent' structural dry matter.

The weight per tiller was higher at high CO₂, thus probably more tillers were above the minimum weight postulated for tiller survival (Roy and Gallagher, 1984). In addition, high order tillers (younger) could probably take advantage of the extra availability of carbohydrates at high CO₂. This pattern of change is consistent with recent findings that early-formed tillers are less responsive to CO₂ than later formed ones (Li et al., 1999). Thus, tillers played a role in amplifying the response to high CO₂ during a stage crucial for yield formation, when intra-plant competition is strong.

The response of tillering to high CO₂ was severely limited at low N availability, in agreement with other authors (Rogers et al., 1996). Low N plants had initially a lower sink capacity given by the lower amount of tillers per plant. Other indications of limited sink-capacity at low N are the increase in root-shoot ratio and the small accumulation of WSC in the stems at heading. We interpret that tiller growth at high CO₂ and low N availability was not impaired by the availability of carbohydrates but instead, by a direct effect of N on sink size, as observed for other nutrients (Rodríguez et al., 1999).

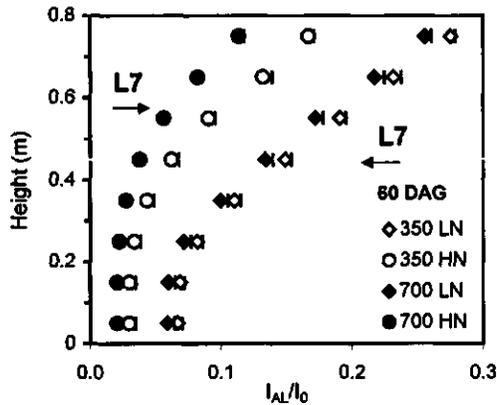


Figure 6.5. Changes in the amount of PAR absorbed per unit leaf area relative to the PAR incident on a horizontal plane at the top of the canopy (I_{AL}/I_0) with plant height at 60 DAG. Horizontal bars are standard errors. The heights occupied by the middle portions of leaf 7 or 5 are indicated with arrows. Symbols as in Figure 6.3c and d.

N availability regulated leaf area production and vertical leaf N distribution at high CO_2

Based on the increase in photosynthetic efficiency and the decrease of the light compensation point for photosynthesis at high CO_2 (Long and Drake, 1991), an increase in LAI has been postulated, assuming that more leaves can be retained in shaded micro-environments of the canopy (Hirose et al., 1996). Our results seem to agree with this prediction, more LAI was found at high N and high CO_2 (Table 6.1), though this is not a frequent case in field experiments with wheat (Pinter et al., 1996) or dicot species (Hirose et al., 1996; Sims et al., 1999). Hirose et al. (1996) have proposed that the restricted LAI development at high CO_2 is due to N limitation. At high CO_2 , leaves have higher photosynthetic efficiency (Fig. 6.7), thus at the same level of illumination they become more light saturated and will require more N in order to operate near the maximum efficiency (Goudriaan, 1995; Hirose et al., 1997). Thus, mobilisation of N to the top layers could counteract the improved carbon balance hypothesis under shade (Hirose et al., 1997). Alternatively, we propose that in wheat supplied with N in proportion to growth, LAI at high CO_2 and low N availability was restricted through a direct effect of N on leaf area expansion and the number of growing points (tiller survival). Although steeper leaf N profiles were observed at low N supply, the distribution was similar at high and low CO_2 . In addition, N is constantly re-circulating (Simpson et al., 1982) and young and old leaves had a similar capacity to assimilate new N, proportional to N content (Fig. 6.4e-f). This sheds a different light on the $CO_2 \times N$ interaction because so far, secondary effects of CO_2 on morphology and concomitant sink activity were out of scope, and most attention was directed towards primary effects on the source side of the balance.

Table 6.4. Linear regressions between leaf N content per leaf layer (mmol N m⁻²) or proportion of ¹⁵N (new N) vs. total leaf N and PAR absorbed per unit leaf area relative to above-canopy incident PAR (I_{AL}/I_0 , after logarithmic transformation).

Date and stem category	CO ₂ [μ bar]	N	Leaf N = Intercept-Slope * $\ln(I_{AL}/I_0)$					Among Slopes
			Intercept	Slope	n	R ²	P	
52 DAG								
Main Stem	350	Low	278.2	62.5	8	0.82	<0.001	***
		High	226.1	31.6	8	0.99	<0.001	
	700	Low	353.2	77.6	8	0.97	<0.001	
		High	258.0	34.1	8	0.87	<0.001	
Tillers	350	Low	211.3	59.0	7	0.79	<0.01	***
		High	240.4 ²	46.2	6	0.99	<0.001	
	700	Low	282.5 ²	76.5	6	0.97	<0.001	
		High	268.7	51.3	7	0.89	<0.001	
60 DAG								
Main Stem	350	Low	325.3	75.9	8	0.97	<0.001	***
		High	245.1	38.9	7	0.92	<0.001	
	700	Low	241.9	47.1	7	0.80	<0.005	
		High	261.7	35.9	7	0.95	<0.001	
Tillers	350	Low	386.4	115.4	7	0.80	<0.005	**
		High	324.6	72.8	8	0.97	<0.001	
	700	Low	315.4	88.0	7	0.86	<0.005	
		High	274.4	56.4	7	0.99	<0.001	
60 DAG New/N total								
Main Stem	350	Low	0.36	0.04	8	0.50	<0.05	**
		High	0.60 ²	0.07	6	0.77	<0.05	
	700	Low	0.16	-0.01	7	0.13	NS	
		High	0.67	0.08	7	0.85	<0.01	
Tillers	350	Low	0.61	0.13	7	0.67	<0.05	NS
		High	1.10	0.21	8	0.97	<0.001	
	700	Low	0.58	0.11	7	0.40	NS	
		High	1.19	0.23	7	0.79	<0.01	

¹ F-probability value: ***= $P<0.001$, **= $P<0.01$, *= $P<0.05$, NS = non significant ($P>=0.05$).

² Top canopy value in Fig. 6.6 was not considered for regression.

Finally, the increase in LAI at high N and high CO₂ did not lead to changes in the vertical distribution of LAI (data not shown) or leaf N (Table 6.4) with respect to the low CO₂. Changes in leaf N distribution at high CO₂ have not been observed in pot experiments fertilised with a fixed rate of N (Hirose et al., 1996), or in our study supplying N in proportion to growth.

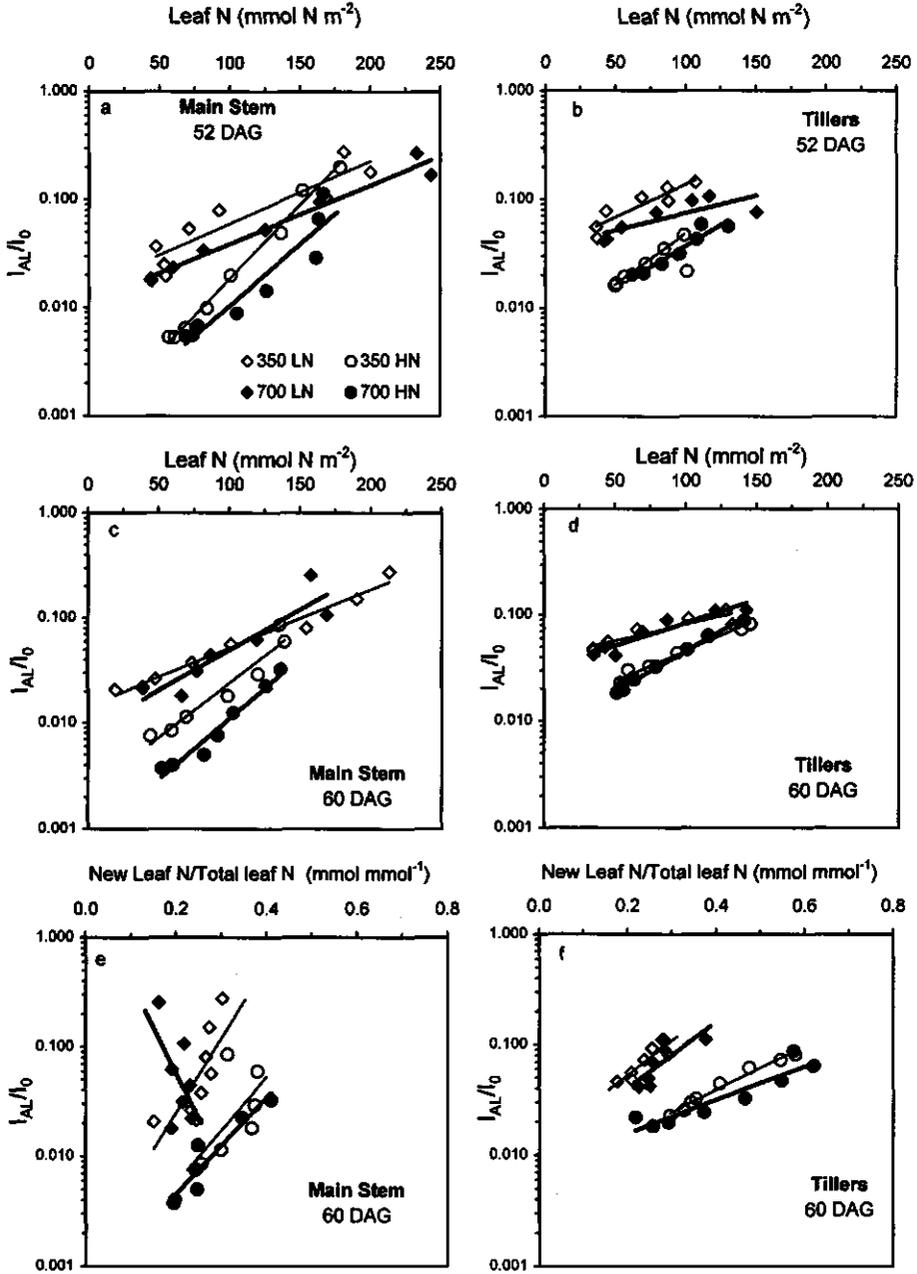


Figure 6.6. Leaf N against the amount of PAR absorbed per unit leaf area relative to the PAR incident on a horizontal plane at the top of the canopy (I_{AL}/I_0) for main stem at 52 (a) and 60 (c) DAG and tillers at 52 (b) and 60 (d) DAG. Regression characteristics in Table 6.4. Proportion of new (^{15}N) vs. total leaf N I_{AL}/I_0 in main stem (e) or tillers (f) at 60 DAG. Note: leaf N has not been calculated on a structural dry matter basis.

Predictions with a simulation model suggest that the optimal distribution of leaf N is similar at high and low CO₂ because CO₂ has very little effect on the shape of the response curve of photosynthesis to N (Hikosaka and Hirose, 1998).

Acclimation of photosynthesis

In several species, in experiments with fixed addition of N, the expression of acclimation to high CO₂ depends on leaf position and phenological stage (Xu et al., 1994; Nie et al., 1995; Osborne et al., 1998; Sims et al., 1999). We provided evidence that, when supplying N in proportion to growth, photosynthetic acclimation to high CO₂ can appear in time, but is not directly linked to the leaf N content or short-term changes in the source-sink balance evaluated through the WSC content (Fig. 6.3 and 6.4).

In wheat, leaf photosynthesis, rises after ligule emergence, then reaches a plateau followed by a linear decline with age (Rawson et al., 1983). Leaves from high and low CO₂ treatments were not synchronised with respect to these stages. At boot stage, the low CO₂ level showed lower but increasing carboxylation rates than the high CO₂ treatment for a broad range of internal CO₂ concentrations (Fig. 6.3c). But at the time of heading, both CO₂ treatments reached a plateau at similar internal CO₂ concentrations (Fig. 6.3d). The maximum photosynthetic capacity (A_{sat}) was relatively constant over time at low CO₂, whereas a drop occurred at high CO₂. In tomato, Besford et al. (1990) reported that photosynthesis of leaves exposed to high or ambient CO₂ reached the same maximum value during leaf development, but at high CO₂ leaves developed faster and reached the maximum sooner. Miller and colleagues (1997) have proposed that in tobacco, an increase in the availability of sugars at high CO₂ accelerates leaf maturation and results in photosynthetic acclimation to high CO₂. To evaluate this hypothesis, we would need to complete our data set with a determination of starch, since no differential accumulation of WSC was observed between high and low CO₂, in agreement with Tuba et al. (1994). Besides, recent evidences indicate that a decline in photosynthetic capacity is not directly linked to sugar accumulation in the source leaf, but rather to changes in the ratios between sugars (Sims et al., 1999).

In addition to the effects on leaf photosynthesis, high CO₂ increased leaf area production at high N availability. This resulted in a reduction of the light level impinging on leaf 7 from boot stage to heading at high N and high CO₂ compared to low CO₂ (Fig. 6.5). At low light intensity, down-regulation of photosynthesis occurs (Evans, 1996). This may help to explain why, although down-regulation of photosynthesis occurred at both N levels when CO₂ was high, it was more pronounced at high N availability (Fig. 6.3). The information provided on changes in leaf composition may not be enough to distinguish between the effects of light and CO₂, since increases in the ratio between pigments and energy-compounds vs. Rubisco or soluble proteins have been observed in response to high CO₂ (van Oosten et al., 1995; Osborne et al., 1998) and low light levels (Evans, 1996). However, independent

acclimation of photosynthesis to CO_2 in different light environments has also been observed in other species (Sims et al., 1999).

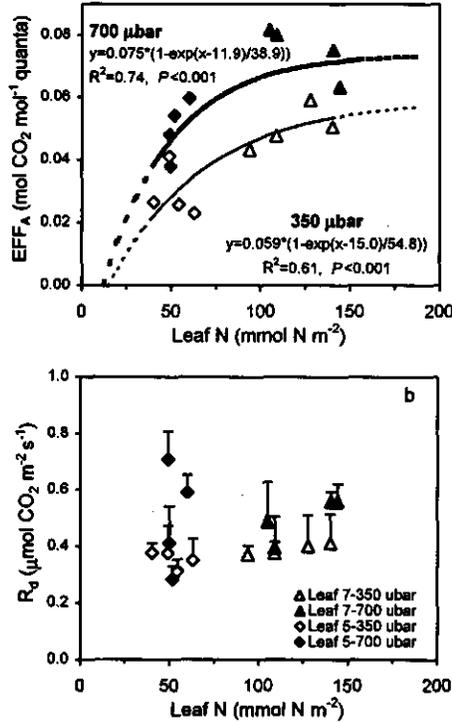


Figure 6.7. Photosynthetic efficiency at low light on absorbed light basis (a) and respiration in the dark (b) against leaf N (on structural dry matter basis) at 700 μbar (closed symbols) or 350 μbar (open symbols) for leaf 7 (triangle) and leaf 5 (diamond) at 52 and 60 DAG.

Conclusions

Applying N in proportion to growth helped to avoid high CO_2 -induced dilution of plant N in plant biomass (N concentration), and had favourable consequences for biomass production in a crucial period for the definition of wheat yield. An increase in total biomass at heading of 40% at high N and 15% at low N was achieved mainly connected to the plasticity of tiller growth. The response was of considerable magnitude given the low light level compared to the field, where responses are in the order of 20-50%. However, supplying N in proportion to growth could not prevent photosynthetic acclimation in response to CO_2 with leaf age. The results suggest that CO_2 plays a direct role in the down-regulation of photosynthesis and is independent of leaf N content and accumulation of soluble carbohydrates.

Chapter 7

General discussion

Light acts both as a signal and a source of energy for plants, regulating the co-ordinated performance between the carbon and N metabolisms. Given the stationary habit of plants and the scarcity of N in many ecosystems, plants have adapted to cope with limiting nutrient availability by adjusting the distribution of biomass between organs, the light-intercepting area and the photosynthetic machinery. The capacity to adjust leaf N contents to different light conditions in the canopy prevents damage and optimises plant performance. Understanding that plants act as information-acquiring systems and apply that information to control their physiology (Aphalo and Ballaré, 1995) is essential to those who aim to manipulate plant performance. Particularly regarding further improvement of crops that have already been through a severe selection pressure. This thesis aimed to gain insight in the physiological bases of biomass production per unit absorbed radiation and per unit N taken up in wheat and oilseed rape. Emphasis was put on the period of grain number definition, as critical for the determination of final grain yield. Experimental and modelling work were combined to distinguish between process dynamics at leaf and canopy levels. The potential application of the results is discussed in relation to crop breeding and crop management.

Insight

Growth response to N availability differs in wheat and oilseed rape

The development of N use efficient genotypes requires understanding the contribution that different physiological processes make to biomass and yield formation under limited N availability. It was shown that oilseed rape had a lower harvest index for biomass than wheat, and its yield was more limited by the capacity for assimilate production than that of wheat (Chapter 3). Instead, total N uptake and N use efficiency, expressed as yield per unit of N absorbed, were similar between these species. Growth analysis, during the critical period for grain number determination, indicated that biomass production per unit intercepted irradiance (RUE) in oilseed rape was more sensitive to N supply than wheat, and reached higher values at high N (Chapter 3). The higher responsiveness of RUE to leaf N in oilseed rape was not anticipated, because dicots severely restrict leaf expansion and maintain a high leaf photosynthetic capacity (Radin, 1983; Vos and Van der Putten, 1998). The reasons for inter-specific differences in growth sensitivity to N availability were attributed to characteristics at the leaf level and linked to canopy structure (Chapter 4). Evidence was presented that leaf photosynthesis at high (A_{max}) and low light (EFF_A) was related to leaf N content in both species. In addition it was shown that leaf net photosynthesis was higher in oilseed rape than in wheat for a wide range of irradiances and leaf N contents. Integrating leaf photosynthesis with the distribution of light and leaf N in the

canopy by means of modelling helped to explore further the higher RUE of oilseed rape (Chapter 4). An important factor affecting RUE in oilseed rape was the growth of the inflorescence, which introduced a change in canopy architecture so that leaves were progressively more shaded from the start to the end of flowering. This effect was more pronounced at high than low N availability and resulted in an increase in RUE because leaves were on average less light-saturated, the radiation being lower and more diffuse. Thus, the higher leaf photosynthesis and the shade cast by the inflorescence contribute to the higher RUE of the oilseed compared to wheat. The positive effect of N on the size of the inflorescence (and its shade) and on photosynthetic capacity explain why RUE was also more sensitive to N availability in oilseed rape compared to wheat (Chapter 4).

Light-associated leaf N profiles change dynamically and depend on N availability

Most studies on maximisation of canopy photosynthesis in relation to partitioning of N between leaf layers provide limited information because (i) they are carried out at a single moment of crop development, (ii) they assume that photosynthetic performance of plants is dependent on leaf N content in high light environments only (Chapter 2). In addition, these studies generally conclude that the optimal profiles of leaf N distribution are steeper than the measured profiles (Chapter 2). In wheat and oilseed rape, vertical leaf N distribution was correlated to the gradient of absorbed irradiance, i.e. more leaf N in the upper, more-illuminated leaf layers (Chapters 4, 5, 6). The leaf N distribution changed dynamically during crop growth in response to the light environment. Furthermore, leaf N distribution was regulated by N availability but not affected by the CO₂ environment. At high N supply, the leaf N profiles were stable in time (Chapter 4, 5, 6). At low N supply, the leaf N profiles drifted from uniform to steeper gradients than those found at high N. These fluctuations were associated with reduced leaf expansion and tillering and increasing N remobilisation from lower leaf layers (Chapter 4, 5, 6). Showing that the light-associated N profiles change with N availability, this work has provided evidence that although the local light environment plays an important role in N distribution the ultimate regulation of N and C allocation is co-ordinated at the plant level.

The initial slope of the photosynthesis light response curve at low light, i.e. quantum efficiency based on incident or absorbed light (EFF_A or EFF_I), increased with leaf N content (Chapter 4). It is generally assumed that N availability affects the quantum efficiency for incident light through the chlorophyll content and thus leaf absorptance (Hikosaka and Terashima, 1995). The finding of a decrease in quantum efficiency for absorbed light at low leaf N content (Chapter 4) is less frequent, but may be linked to an increase in photorespiration at low N nutrition (Schapendonk et al., 1999). It has been observed that at low N availability leaves have a more compact mesophyll and lower proportion of intercellular spaces (van Arendonk et al., 1997), this structural change could hamper CO₂ more than O₂ diffusion to the sites of carboxylation and increase photorespiration. This hypothesis needs to be investigated further given the quantitative importance of the quantum efficiency for canopy radiation use efficiency and biomass production (Ort and Baker, 1988; Chapters 2 and 4).

Furthermore it was shown that plants keep the shape of the vertical leaf N distribution such that the capacity for photosynthesis at high and low light are in balance (Chapter 5). At the leaf level, a trade-off in N partitioning between the capacity for photosynthesis at high and low light has been postulated (Chapter 2; Hikosaka and Terashima, 1995). Under the conditions studied, the distribution of leaf N with respect to the gradient of absorbed irradiance was close to the theoretical optimum (Chapter 4 and 5) and did not differ remarkably between species (Chapter 4). It is plausible that the predictions of many studies about the benefit for canopy photosynthesis of a steeper leaf N profile than the measured one are due to the presumption of constant quantum efficiency. In that case, N can be allocated to the top of the canopy without loss of photosynthesis in the lower less illuminated leaf layers. Considering N to be allocated as a function of irradiance (Chapters 4, 5, 6) seems a good approximation to predict vertical leaf N distribution in a canopy at high N availability, when the dependency of photosynthesis both at high and low light on leaf N content is taken into account. A more mechanistic approach than the one used here, would describe N allocation accounting for the costs associated with N acquisition, remobilisation and leaf construction (Mooney and Gulmon, 1979) and consider the occurrence of photoinhibition and sunflecks. Photoinhibition leads to lower photosynthesis rates in top canopy layers, thus from the point of view of optimisation of N use, lower leaf N investment would be necessary in the more-illuminated leaves. Accounting for sunflecks, i.e. the occurrence of brief episodes of direct light penetrating to basal leaves, could lead to maximisation of canopy photosynthesis through higher investment of N in lower leaf layers, assuming that the sunfleck occurrence is frequent and most days are sunny.

N increases sink capacity and growth at high CO₂ but does not prevent leaf photosynthetic acclimation

When the supply of N was abundant, CO₂ enrichment increased wheat growth due to a higher tiller survival (Chapter 6). Tillers formed at high N supply, that died after canopy closure at ambient CO₂, could be maintained and grow at high CO₂. Instead at low N availability and high CO₂, tiller number was initially lower and declined independently of carbohydrate availability. At low N availability, a direct effect of N on sink size is postulated, as observed for other nutrients (Rodríguez et al., 1999). At the canopy level, the leaf N vertical distribution was associated with the light gradient and it was responsive to N availability but not to the CO₂ level.

Under long term exposure to high CO₂ concentrations, a decrease in photosynthesis due to loss of activity and/or amount of photosynthetic enzymes, also called acclimation, has been observed (Sage, 1994). This phenomenon has been linked to sink limitation for carbohydrates at the plant level, and leads to N re-allocation within the photosynthetic machinery and between organs (Bowes, 1996). Photosynthetic acclimation in the last fully expanded leaf was prevented at low N availability when N was given in proportion to expected growth, in agreement with Farage et al (1998). However, this methodology of N supply could not prevent photosynthetic acclimation in response to CO₂ with leaf age. The results suggest that CO₂ plays a direct role in the

down-regulation of photosynthesis and is independent of leaf N content and the accumulation of soluble carbohydrates.

Applicability of the results

In relation to crop breeding

A rational attempt to improve crop productivity in a rapid and cost-effective way requires the identification of key-limiting processes. Based on the results of this study, desirable traits can be pinpointed to improve the potential yield of wheat and oilseed rape while maximising the N use efficiency. While yield in modern wheat cultivars is co-limited by sink and source during grain filling (Chapter 3, Slafer and Savin, 1994), oilseed rape yield is more source-limited (Chapter 3; Wright et al., 1988). Therefore, efforts to raise yield potential in wheat should be directed to increase biomass production during the period of grain number definition, to improve both the number of grains and the capacity to produce assimilates to fill them (Slafer et al., 1994). In oilseed rape efforts to produce genetic gains in number of seeds per unit land area are likely to be counterbalanced by concomitant reductions in grain weight, unless the capacity for assimilate production during grain filling is also improved (Chapter 4).

From the results of this study it seems evident that in wheat yield progress associated to increased biomass production will have to rely in breakthroughs on the potential of photosynthesis at the leaf level, which can then be translated to the canopy level (Chapter 4). Higher wheat yields in cultivars released by CIMMYT from 1962 to 1988 have been associated to higher stomatal conductance, increasing internal CO₂ concentrations (C_i) and also C_i-independent photosynthetic capacity (Fischer et al., 1998). The underlying causal relation linking these changes are, however, not clear. According to Lawlor (1995), increased photosynthesis will come from altering the potential for light harvesting, electron transport or synthesis of energy rich compounds (e.g. ATP), which are already largely flexible processes. Selection for lower respiration rates has been made in *Lolium* (Wilson, 1982), but higher yields resulting from it could not be reproduced under a variety of conditions (Kraus et al., 1993). In many cases, different respiration rates between cultivars can be due to differences in developmental stage (Bouma, 1995). These efforts may not be successful if they result in a reduced ability of plants to adapt to fluctuating environments. Photorespiration and the glycolate pathway represent a drain of assimilated CO₂ and energy. However, they perform also protective functions, decreasing the energy burden on photosystem II, sensitive to photoinhibition, and consuming reductant compounds, which may be important when photosynthesis is severely restricted (e.g. by water stress) (Lawlor, 1993). The fact that a drop in photorespiration results in increased productivity is evident from CO₂-enriched studies. But even if proper selection tests for reduced photorespiration under ambient CO₂ become available, selecting against this process may lead to unstable yields if crops are grown at high light intensities, e.g. in the tropics, or under severe water or N limitation. Finally, it is worth considering the weight of these changes from the perspective of the predicted increase in CO₂ concentration with global climate change. If CO₂ emissions continue at present rates,

1.5 $\mu\text{bar y}^{-1}$ (Keeling et al., 1995), the CO_2 concentration will reach ca. 450 μbar within 50 years. This would mean that the added value of photosynthetic changes related to increase the affinity of Rubisco for CO_2 (Lawlor, 1995), will become smaller, because photorespiration will be already low due to high CO_2 concentration. However, taking into account that releasing a wheat cultivar selected for a particular attribute can take between 6-15 years (depending on breeding methods and possibilities of multiplication), several cycles of selection for these attributes could still give successful results in terms of increase in yield potential. In Chapter 6, the importance of sink capacity in preventing photosynthetic acclimation to high CO_2 was discussed. Thus, whatever the path chosen to increase photosynthesis rates, breeders should consciously select for cultivars with a high tillering capacity. Otherwise the effects of photosynthetic acclimation to high CO_2 may offset all the effort put in selecting for higher photosynthetic capacity.

An increased photosynthetic rate is very hard to define because the process of photosynthesis is subjected to various feedback control systems ranging from regulation at the thylakoid level to the acclimated genetic control. Thus increasing the source capacity by manipulating photosynthesis is a long-term project. In oilseed rape, several crop characteristics can be targeted to raise yield potential before an improvement in potential photosynthetic capacity is needed. It is essential that the crop reaches canopy cover at flowering and can maintain full light interception by the leaves through the seed filling period to increase the source capacity. Preventing or slowing leaf senescence has also been pointed as a key attribute for high yielding rice (Mae, 1997). This status, that in other crops can be obtained through management under optimal conditions, seems more difficult to attain in oilseed rape, given the characteristic leaf shedding associated to reproductive stages observed in *Cruciferae*. Additionally, in oilseed rape, the inflorescence is the organ contributing most to the light interception and assimilates production from flowering to the end of grain filling. Genotypes that can maintain active leaf area during flowering and seed filling will benefit two-fold, from an increase in the radiation use efficiency and in light interception (Chapters 3 and 4). Radiation use efficiency will be increased because leaves have ca. three times higher photosynthetic rate per unit N than pods (Gammelvind et al., 1996) and are exposed to lower levels of radiation due to the presence of the inflorescence, thus photosynthesising more efficiently (Bange et al., 1997). In addition, leaf area duration will be increased, extending the source capacity, proved limiting in this crop (Chapter 3; Rood and Major, 1984). Further, leaf retention could contribute to continued N uptake from the soil (Schenk, 1996). Provided sink capacity is increased in response to higher availability of assimilates, quality aspects of the crop will not be affected, i.e. more protein will be diluted in higher grain weight and probably the ratio between oil and protein content will not be affected. Finally, the harvest index of oilseed rape is low compared to wheat (Chapter 4; Habekotté, 1997a). In this crop, the proportion of biomass in non-grain inflorescence structures is very high (e.g. more than 25% vs. 10% in wheat). Decreasing the amount of structural carbohydrates in pod walls, without losing shattering resistance, might be a direct way to increase the harvest index of this crop.

In relation to crop management

From the point of view of the management, in both crops, N fertilisation should aim to maximise cumulative light absorption during the critical period for seed formation and set (Andersen et al., 1996; Fischer, 1993; Habekotté, 1997b). But, particularly in oilseed rape, it should also aim at enhanced leaf photosynthetic capacity during grain filling. The doses and technique of fertiliser application should be tuned to make an optimal use of the nutrient. Split-doses of N fertiliser are already used in both crops with variable degree of success depending on other environmental conditions (Chalmers and Darby, 1992; Asseng et al, 1998). Optimal N application for a high-yielding high-quality wheat crop can combine a split N dosis with foliar N application (Gooding and Davies, 1997). In oilseed rape, the latest moment in the split application is generally the rosette stage (e.g. Wright et al., 1988; Taylor et al, 1991), still far from the beginning of flowering. Coated fertilisers or other slow-release formulations, and the use of nitrification inhibitors, could improve the availability of N in the proximity of flowering in oilseed rape. The possibility of implementing foliar N applications in oilseed rape seems more time-constrained than in wheat. As soon as the growth of the inflorescence starts, it will not be possible to apply the fertiliser uniformly over the leaves. Until now, this technique has not been tried out. So far, concern about the impact on grain quality has been the more important issue restricting N fertilisation in oilseed rape. A negative relation between yield and nitrogen concentration in the grain is generally reported (Andersen et al., 1996). However, even when oil concentration declines, yield increase due to N fertilisation may result in a higher oil yield per m². Finally, the literature is contradictory regarding the effect of N fertilisation on the concentration of glucosinolates, compounds that are hydrolysed into toxic products during seed crushing (Joseffson, 1970; Holmes, 1980; Bilsborrow et al., 1993). In this respect, there are already available genotypes with low synthesis of glucosinolates, a complete inhibition of the synthesis would aid in the implementation of more safe fertilisation schemes.

Bearing in mind the considerations made above, the amount of N to apply to wheat or oilseed rape will depend on the targeted yield, amount of proteins in the grains and the harvest indexes of the varieties. In general terms, the capacities for N recovery and total uptake can be assumed as fairly similar among species. Finally, fertilisation technology for an efficient use of N and potential yields under high-CO₂ should aim at several N additions during the season, to support the development of sink capacity. This will also help to avoid late N deficiency due to accelerated growth and uptake in the beginning of the season.

Conclusions and areas for further research

Physiological insight into the possibilities to manipulate biomass production in wheat and oilseed rape was gained by complementing traditional experimentation with integration of different scales of observation through modelling. Conclusions and main areas of research to make further progress are indicated below.

- *Wheat and oilseed rape yield potential*

To increase yield potential in wheat and oilseed rape through higher biomass production, different physiological attributes can be exploited. In *wheat*, there are strong evidences that the increase will have to rely on breakthroughs in the photosynthetic capacity at the leaf level. Increasing stomatal conductance (Fischer et al., 1998) may be one possibility, but the advantage has yet to be tested in a wider range of environments, particularly under water stress. In *oilseed rape*, increasing leaf area duration is crucial for improving radiation use efficiency and interception during flowering and grain filling. The advantage of oilseed rape genotypes that can maintain their leaves active for a longer period is that they will be able to increase source capacity with a more efficient use of N, particularly because leaves have a higher photosynthesis rate per unit N than pods (normally the main photosynthesising organs during grain filling).

For both crops, in the context of a future high-CO₂ world, it is crucial to remember that only genotypes with high capacity for sink development will allow the expression of potential production.

- *Photosynthesis at low light and nutrient availability*

The value of the leaf quantum efficiency is of paramount importance for the total canopy photosynthesis, playing an important role in the definition of the vertical allocation of N associated to the intra-canopy light gradient. In *wheat and oilseed rape*, the sensitivity of quantum efficiency to N-limitation deserves further investigation. The hypothesis that the decrease in quantum efficiency on absorbed irradiance, at low N, is due to an increase in photorespiration linked to changes in the structure of the leaf that leads to lower concentrations of CO₂ at the site of carboxylation needs further exploration. In addition, efforts should be made to model the efficiency of electron transport-limited carboxylation in a dynamic fashion.

- *Light and leaf nitrogen allocation in the canopy*

In this study, leaf N distribution within the canopy was nearly optimal for canopy photosynthesis, indicating a co-ordination between the carbon and N metabolisms. The study of the signals, processes and metabolic pools involved in this regulation is at the moment rather fragmentary. Spectral light quality, light intensity and cytokinin levels play apparently an important role in defining leaf N allocation (see discussion in Chapter 5 and references therein). The integration between these fields deserves more attention.

- *Source-sink relations, acclimation and temporal up-scaling*

Most of the scientific effort directed to predict crop productivity has been so far devoted to describe, understand and model how physiological processes vary spatially within plant canopies starting from single leaf measurements and scaling-up to the

crop level. This and previous works trying to link the intra-canopy gradients of leaf N and light to canopy photosynthesis are an example. It was shown that light-associated leaf N profiles are regulated by N availability (Chapters 4, 5, 6) and that CO₂ effects on growth are dependent on the plant source-sink ratio (Chapter 6). Thus modelling processes such as photosynthesis on a seasonal scale using time-invariant characteristics is not advisable. The incorporation of feedback or regulatory mechanisms can help to tackle the response to multiple environmental variables in a more realistic way. This will improve the predictability of yield under complex conditions, e.g. high CO₂ and low nutrient availability, but also contribute to our understanding of the behaviour of more integrative variables, such as radiation use efficiency.

In the particular case of modelling growth at high CO₂ and limited nutrient availability, future efforts should be concentrated on :

- Describing the sink term*, given the fact that low N availability restricted tiller growth at high CO₂ independently of assimilate availability (Chapter 6).
- Understanding how sink growth regulates leaf photosynthesis*. Although carbohydrates may be the information-link between sources and sinks, accumulation does not seem to be the mechanism triggering acclimation, as discussed in Chapter 6. Other modes of action, related to the export, transport or import of sugars are worth exploring.

Summary

Raising the yield potential of main agricultural crops with an efficient use of nutrients is imperative, given the prospects of increase in world population and the environmental problems associated with high input agriculture. Crop yields are associated to biomass formation under a wide range of environmental conditions. Crop biomass production depends on the capture of radiation and the efficiency of use of that radiation to produce biomass, also called radiation use efficiency (RUE). Both attributes are the outcome of several processes, which range from canopy architecture and leaf optical properties to the potential capacity for leaf photosynthesis as affected by light, temperature and CO₂ level. RUE has been indicated as a physiological attribute that limits yield potential in different crops (Chapter 2) and is known to be affected by N availability. This thesis was conducted to gain insight in the physiological bases of RUE, with the aim of evaluating different possibilities of manipulation to increase the yield potential in wheat (*Triticum aestivum* L.) and oilseed rape (*Brassica napus* L.). The issue was addressed by combining traditional experimentation at different scales of observation (mainly leaf, plant and canopy) with modelling. Wheat and oilseed rape were chosen as target crops for the study because they are the most important winter-spring components of cereal-oilseed rotations of temperate regions. The ecophysiology of these two crops was compared in terms of yield, capacity to intercept radiation, RUE, photosynthesis and N use efficiency during the critical period for grain number definition and grain filling (Chapters 3 and 4).

Oilseed rape had a lower harvest index than wheat. In addition its yield was comparatively more limited by the capacity for assimilate production than that of wheat (Chapter 3). Growth analysis, during the critical period for grain number definition, indicated that RUE in oilseed rape was more sensitive to N availability than wheat, and reached higher values at high N (Chapter 3). The reasons for inter-specific differences in growth sensitivity to N availability originated at the leaf and canopy level (Chapter 4). Leaf net photosynthesis per unit leaf N was higher in oilseed rape than in wheat for a range of irradiances. Additionally, the growth of the inflorescence in oilseed rape increasingly shaded the leaf strata, particularly at high N supply. This resulted in an increase in RUE because leaves were on average less light-saturated, the radiation being lower and more diffuse. Thus the higher leaf photosynthesis and the shade cast by the inflorescence contributed to explain the higher RUE of the oilseed compared to wheat and its increasing sensitivity to canopy N content in time (Chapter 4).

The vertical pattern of leaf N distribution determines canopy photosynthesis and manipulating it has been suggested as a route to maximise RUE (Chapter 2). In the observed canopies of wheat and oilseed rape, leaf N was partitioned in relation to the gradient of absorbed irradiance (Chapters 4, 5, 6). The light-associated N distribution changed dynamically during crop growth and was regulated by N availability. Light-associated leaf N profiles were stable in time at high N, but fluctuated at low N availability (Chapter 4, 5, 6), indicating that although the local light environment plays an important role in N distribution the ultimate regulation of N and C allocation

is co-ordinated at the plant level. Furthermore, it was shown that the photosynthetic efficiency (EFF_A), which is quantitatively important in the definition of canopy photosynthesis (Chapters 2, 4) responds to leaf N content (Chapter 4). The canopies had a vertical leaf N distribution that kept the capacity for photosynthesis at high and low light in balance (Chapter 5). Under the conditions explored the distribution of leaf N with respect to the gradient of absorbed irradiance was close to the theoretical optimum (Chapter 4 and 5) and did not differ remarkably between species (Chapter 4).

Finally, the response of canopy photosynthesis to N availability could change under high atmospheric CO_2 if acclimation occurs. Acclimation has been observed in plants exposed for a long-term to high CO_2 , as a decrease in photosynthesis and the synthesis of photosynthetic enzymes, accompanied by N re-allocation within the photosynthetic machinery and the plant. For this reason, the interaction between CO_2 level and N availability was studied in wheat during the critical period for grain number determination. At high N availability high CO_2 resulted in increased biomass production due to enhanced tiller growth. The light-associated patterns of leaf N distribution were similar at high and low CO_2 and only affected by N availability. Leaf photosynthetic acclimation to high CO_2 , absent directly after full leaf expansion, appeared with leaf age. This response could not be prevented by supplying N in proportion to growth and seemed independent of the leaf N content (Chapter 6).

The contribution of the results to the existing knowledge and its applicability are discussed further in the context of genetic crop improvement and N fertilisation in Chapter 7. It is concluded that improvement of wheat yield through increased biomass production linked to RUE by crop breeding will have to rely on breakthroughs of photosynthesis at the leaf level. In oilseed rape there is scope for raising yields by prolonging the duration of leaf area during reproductive stages by breeding or with appropriate fertilisation techniques. In both crops, selecting genotypes with high sink capacity is essential for the expression of potential production in a high CO_2 -world.

Samenvatting

Vanwege de drastische bevolkingstoename en de beperkte beschikbaarheid van landbouwgrond, water en meststoffen, is het noodzakelijk om de opbrengstpotentie van de belangrijkste voedselgewassen in de wereld te verhogen en de efficiëntie van de benutting van plantenvoedingsstoffen en water te verbeteren. Alleen dan kan de groei van de voedselproductie gelijke tred houden met de groei van de voedselbehoefte en kunnen de negatieve milieueffecten worden beperkt. Verhoging van de lichtonderschepping door het gewas en een toename van de geproduceerde biomassa per eenheid van onderschepte straling (RUE) zijn essentieel om dit doel te realiseren. Lichtonderschepping en RUE zijn de resultanten van fysiologische en morfologische eigenschappen en ontwikkelingskenmerken, die de groeisnelheid en de productiviteit van gewassen bepalen, in afhankelijkheid van temperatuur, licht, atmosferische koolzuurconcentratie en beschikbare nutriënten (Hoofdstuk 2). In dit proefschrift gaat de aandacht speciaal uit naar de fysiologische basis van RUE met name de interactie met stikstofbeschikbaarheid. Het doel is om kennis te verwerven voor verbetering van het opbrengstpotentieel van tarwe (*Triticum aestivum* L.) en van koolzaad (*Brassica napus* L.). Voor het onderzoek zijn tarwe en koolzaad gekozen omdat ze in gematigde gebieden de belangrijkste gewassen zijn in een zomer-winter rotatie. De studie bestaat uit een combinatie van experimenten op blad-, plant- en gewasniveau. De resultaten van de experimenten zijn gebruikt voor de formulering van simulatiemodellen, die het mechanisme van RUE onderbouwen. Daarbij staan fotosynthese en de stikstofbenuttings-efficiëntie in relatie tot plantarchitectuur centraal, met name gedurende een kritiek ontwikkelingsstadium waarin het aantal korrels en zaden c.q. de vulling ervan wordt gerealiseerd (Hoofdstukken 3 en 4).

Koolzaad heeft een lagere oogstindex dan tarwe. Daarnaast wordt de opbrengst van koolzaad meer gelimiteerd door de hoeveelheid beschikbare assimilaten dan bij tarwe (Hoofdstuk 3). Groeianalyses gedurende het eerder genoemde ontwikkelingsstadium, toonden aan dat RUE van koolzaad gevoeliger is voor stikstofbeschikbaarheid, dan die van tarwe. De RUE voor koolzaad blijkt hoger te zijn bij hoge stikstofniveaus. De oorzaken voor de verschillen tussen tarwe en koolzaad waren zowel gelegen in processen op blad- als op gewasniveau (Hoofdstuk 4). Bij verschillende stralingsintensiteiten bleek de netto bladfotosynthese per eenheid bladstikstof van koolzaad hoger te zijn dan van tarwe. Een belangrijk bijkomend effect was de sterke beschaduwning van de bladeren door de bloemen en de peulen. Vooral bij een hoog stikstofaanbod had dit een grote invloed op de stralingsverdeling in het gewas. Daardoor waren de bladeren gemiddeld genomen minder licht-verzadigd, wat resulteerde in een toename van de RUE. De hogere bladfotosynthese en de beschaduwning van de bladeren door de bloeiwijze droegen gezamenlijk bij aan de verklaring voor de hogere RUE van koolzaad in vergelijking tot die van tarwe en de toenemende gevoeligheid van koolzaad voor het stikstofgehalte in de tijd (Hoofdstuk 4).

Het verticale patroon van de stikstofverdeling over de plant is belangrijk voor de optimalisatie van de gewasfotosynthese. Beïnvloeding van dit patroon bleek theoretisch gezien een mogelijkheid om de RUE te maximaliseren (Hoofdstuk 2). In tarwe en koolzaad werd de bladstikstof verdeeld volgens de gradiënt van geabsorbeerde instraling in het gewas (Hoofdstuk 4, 5, 6). De door lichtintensiteit bepaalde stikstofverdeling veranderde dynamisch gedurende de ontwikkeling van het gewas, in afhankelijkheid van de stikstofbeschikbaarheid. De stikstofverdeling bleek stabiel te blijven bij een hoog stikstofniveau maar bleek te fluctueren bij lagere stikstofniveaus. Dit gaf aan dat de lichtgradiënt weliswaar een belangrijke rol speelde bij de stikstofverdeling maar dat de uiteindelijke verdeling van koolstof- en stikstofverdeling op plantniveau werd bijgesteld. De fotosynthetische efficiëntie (EFF_A), bleek van grote kwantitatieve betekenis te zijn voor de totale gewasfotosynthese (Hoofdstuk 2 en 4) en was sterk afhankelijk van de stikstofinhoud van de bladeren. De verticale bladstikstof-verdeling was aangepast aan de behoefte van de fotosynthese componenten, zowel bij hoge als bij lage lichtintensiteiten, dus zowel boven als onder in het gewas. Daardoor was de verdeling van de bladstikstof in relatie tot de geabsorbeerde inkomende straling dichtbij het theoretische optimum. Met betrekking tot deze eigenschap waren geen grote verschillen tussen de soorten (Hoofdstuk 4 en 5).

Tenslotte is nagegaan wat de gevolgen van CO_2 verhoging in de atmosfeer zouden kunnen zijn voor gewasfotosynthese in afhankelijkheid van stikstofbeschikbaarheid (Hoofdstuk 6). In het algemeen vertonen bladeren die lange tijd aan hoge CO_2 -gehalten worden blootgesteld een afname van de fotosynthese en een geringere synthese van fotosynthese enzymen. Dit gaat gepaard met een herverdeling van stikstof over verschillende componenten van het fotosynthese-apparaat en andere eiwitten in de plant. Dit proces, acclimatie genoemd, is in het algemeen sterker bij stikstoflimitering omdat er dan een relatief overschot is aan assimilaten waardoor een negatieve terugkoppeling op het fotosynthese apparaat een logische consequentie is. Daarom bestudeerden we de CO_2 in relatie tot stikstofbeschikbaarheid in tarwe gedurende de kritische ontwikkelingsfase, waarin herverdeling van koolstof en stikstof van groot belang zijn. Bij hoge stikstofbeschikbaarheid werd bij hoge CO_2 -concentratie een hogere totale biomassa gerealiseerd, als gevolg van een toename van de spruitgroei. Gedurende de ontwikkeling werden de stikstofgehalten in de bladeren van tarwe op een constant optimaal of sub-optimaal niveau gehouden door middel van een "steady-state" doseertechniek. Er bleek nauwelijks een effect te zijn van de CO_2 -concentratie op de stikstofverdeling in de plant. Er was wel sprake van acclimatie van de fotosynthese ten gevolge van een hoge CO_2 -concentratie, maar die trad pas op nadat de bladeren volledig volgroeid waren.

De bijdrage van de resultaten van dit onderzoek aan de bestaande kennis en de toepasbaarheid daarvan in de context van genetische verbetering en stikstofbemesting worden besproken in Hoofdstuk 7. De conclusie is dat in tarwe alleen een verbetering van RUE kan worden gerealiseerd als er een doorbraak plaats vindt op het fotosyntheseniveau. In koolzaad daarentegen kan door verlenging van de levensduur van bladeren gedurende de reproductieve stadia nog winst worden geboekt.

Plantenveredeling en een adequate bemestingstechnieken kunnen daar beide aan bijdragen. In beide gewassen is het essentieel om genotypes te selecteren die een grote sink capaciteit bezitten omdat alleen daarmee de potentiële productie in een hoge CO₂-omgeving kan worden gerealiseerd.

Resumen

Aumentar el rendimiento potencial de los cultivos y la eficiencia de uso de los nutrientes es imperativo, dada la perspectiva de aumento en el número de habitantes a nivel mundial y la necesidad de disminuir la polución frecuentemente asociada a los sistemas agrícolas de altos insumos. El rendimiento de los cultivos se asocia a la producción de biomasa en un amplio rango de condiciones ambientales. La producción de biomasa depende de la captura de radiación y la eficiencia con que la radiación es utilizada para formar biomasa (EUR). Ambos atributos resultan de la combinación de diferentes procesos, que van desde la estructura del cultivo y sus propiedades ópticas hasta la capacidad fotosintética a nivel de hoja afectada por el nivel de radiación, la temperatura y el nivel de CO₂ atmosférico. La EUR ha sido señalada como un atributo fisiológico que limita el rendimiento potencial en diferentes cultivos (Capítulo 2) y es afectada por la disponibilidad de nitrógeno (N). El objetivo de esta tesis fue tratar de comprender las bases fisiológicas de la EUR y su respuesta al N, y evaluar posibilidades de manipulación que permitan incrementar la biomasa y el potencial de rendimiento en trigo (*Triticum aestivum* L.) y colza (*Brassica napus* L.). La metodología utilizada combinó experimentación tradicional y observaciones a escala de hoja, planta y canopeo con la utilización de modelos de simulación. El estudio se concentró en trigo y colza por ser los cultivos de invierno más importantes en las rotaciones de zonas templadas que involucran cereales y oleaginosas.

El rendimiento de la colza estuvo limitado por la capacidad de producir fotoasimilados en durante el llenado de granos y su índice de cosecha fue menor que el del trigo (Capítulo 3). El análisis de crecimiento indicó que en la colza la EUR durante el período crítico de formación del número de granos fue más sensible a cambios en la nutrición nitrogenada y alcanzó valores más altos a alta disponibilidad de N (Capítulo 3). Las diferente sensibilidad entre trigo y colza en la respuesta a la disponibilidad de N tuvo origen en procesos a nivel de hoja y cultivo (Capítulo 4). Por un lado, la tasa de fotosíntesis neta de la hoja por unidad de N foliar fue mayor en colza que en trigo en un amplio rango de nivel de irradiancia. Por otro lado, el crecimiento de la inflorescencia en la colza sombreó los estrato foliares, particularmente a alto nivel de N. Esto contribuyó a incrementar la EUR porque las hojas están en promedio menos saturadas por la irradiancia y reciben más radiación difusa. Así la mayor tasa fotosintética y la sombra producida por la inflorescencia explicaron la mayor EUR en la colza con respecto al trigo y el incremento en la respuesta al nivel de N en el tiempo (Capítulo 4).

La distribución vertical de N foliar en un canopeo determina su capacidad fotosintética y su manipulación ha sido sugerida como una vía posible para maximizar la EUR (Capítulo 2). En los canopeos de trigo y colza bajo estudio, el N foliar se particionó en relación al gradiente de radiación absorbida (Capítulos 4,5,6). La distribución de N foliar asociada a la radiación cambió dinámicamente durante el ciclo del cultivo y estuvo regulada por la disponibilidad de N. A alto N, los perfiles de N foliar asociados al gradiente de radiación se mantuvieron estables en el tiempo, mientras que su forma fluctuó a bajo N (Capítulos 4,5,6). Estos resultados ponen en

relevancia que si bien el nivel de radiación local sobre las hojas tiene un rol en la determinación de su contenido de N, la regulación última de la partición de carbono y N está coordinada a nivel de planta. Por otro lado se mostró que la fotosíntesis por unidad de radiación absorbida a baja irradiancia, o eficiencia fotosintética absoluta, es cuantitativamente importante en la definición de la fotosíntesis del canopeo (Capítulos 2 y 4) y responde al nivel de N (Capítulo 4). A través de un análisis de sensibilidad se demostró que las distribuciones de N foliar observadas en el canopeo contribuyen a mantener un balance entre la capacidad fotosintética a alta y baja irradiancia (Capítulo 5). En el rango de condiciones bajo estudio, la distribución vertical de N foliar con respecto al gradiente de radiación absorbida estuvo cercana al óptimo teórico que maximiza la fotosíntesis del canopeo (Capítulos 4 y 5) y no difirió remarcablemente entre especies (Capítulo 4).

La respuesta de la fotosíntesis del canopeo a la disponibilidad de N podría diferir a altas concentraciones ambiente de CO₂ si la fotosíntesis sufre aclimatación. La aclimatación fotosintética a exposiciones prolongadas a alto CO₂ se ha observado como una reducción en la tasa fotosintética y la síntesis de enzimas fotosintéticas, dilución del N foliar, y realocación de N dentro del aparato fotosintético y la planta. Por esta razón, se estudió la interacción entre el nivel de CO₂ y la disponibilidad de N durante el período crítico para la determinación del número de granos en trigo. Altos niveles de CO₂ y N dieron como resultado mayor producción de biomasa debido al incremento en el crecimiento de los macollos. El N se suministró en proporción al crecimiento esperado para estabilizar la concentración de N en la planta a través del tiempo. Sin embargo no se pudo prevenir la aclimatación fotosintética al nivel de CO₂ con la edad de la hoja (Capítulo 6).

La contribución de los resultados de este trabajo al conocimiento general sobre el tema y su aplicabilidad se discuten en el contexto del mejoramiento genético y los esquemas de fertilización (Capítulo 7). Se concluye que aumentos del rendimiento de trigo ligados al incremento de biomasa a través de la EUR por mejora genética deberán basarse en incrementos de la tasa fotosintética a nivel de hoja. En cambio en colza, hay muchas posibilidades de incrementar el rendimiento potencial a través de prolongar el funcionamiento y por ende la duración del área foliar, a través de mejora genética o técnicas de fertilización apropiadas. En ambos cultivos, seleccionar genotipos con una alta capacidad para formar destinos es esencial para la expresión del potencial de producción en un futuro con altas concentraciones de CO₂.

References

- Abbate P, Andrade FH, Culot JP. 1995. The effects of radiation and nitrogen on number of grains in wheat. *Journal of Agricultural Science* 124, 351-360.
- Aerts R, de Caluwe H. 1994. Effects of nitrogen supply on canopy structure and leaf nitrogen distribution in *Carex* species. *Ecology* 75, 1482-1490.
- Aikman DP. 1989. Potential increase in photosynthetic efficiency from the redistribution of solar radiation in a crop. *Journal of Experimental Botany* 40, 855-864.
- Andersen MN, Heidman T, Plauborg F. 1996. The effects of drought and nitrogen on light interception, growth and yield of winter oilseed rape. *Acta Agricultura Scandinavica, Section B* 46, 55-67.
- Angus JF, Jones R, Wilson JH. 1972. A comparison of barley cultivars with different leaf inclination. *Australian Journal of Agricultural Research* 23, 945-957.
- Angus JF, Van Herwaarden AF, Howe GN. 1991. Productivity and break crop effects of winter-growing oilseeds. *Australian Journal of Experimental Agriculture* 31, 669-677.
- Anten NPR. 1995. Canopy structure and patterns of leaf nitrogen distribution in relation to carbon gain. PhD Thesis, University of Utrecht, The Netherlands.
- Anten NPR, Schieving F, Werger MJA. 1995. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C₃ and C₄ mono- and dicotyledoneous species. *Oecologia* 101, 504-513.
- Aphalo PJ, Ballaré CL. 1995. On the importance of information-acquiring systems in plant-plant interactions. *Functional Ecology*, 9, 5-14.
- Asseng S, Fillery IRP, Anderson GC, Dolling PJ, Dunning FX, Keating BA. 1998. Use of APSIM wheat model to predict yield, drainage, and NO₃⁻ leaching for a deep sand. *Australian Journal of Agricultural Research* 49, 363-377.
- Austin RB. 1989. Genetic variation in photosynthesis. *Journal of Agricultural Science* 112, 287-294.
- Austin RB. 1992. Can we improve on nature? Abstracts of the First International Crop Science Congress, Ames, IA, pp. 29.
- Austin RB, Ford MA, Edrich JA, Hooper BE. 1976. Some effects of leaf posture on photosynthesis and yield in wheat. *Annals of Applied Biology* 83, 425-446.
- Austin RB, Bingham J, Blackwell RD, Evans LT, Ford MA, Morgan CL, Taylor M. 1980. Genetic improvement in winter wheat yield since 1900 and associated physiological changes. *Journal of Agricultural Science* 94, 675-689.
- Austin RB, Morgan CL, Ford MA, Bhagwat SC. 1982. Flag leaf photosynthesis of *Triticum aestivum* and related diploid and tetraploid species. *Annals of Botany* 49, 177-189.
- Austin RB, Ford MA, Morgan CL. 1989. Genetic improvement in the yield of winter wheat: A further evaluation. *Journal of Agricultural Science*, 112-295.
- Azcón-Bieto J. 1986. Effect of oxygen on the contribution of respiration to the CO₂ compensation point in wheat and bean leaves. *Plant Physiology* 81, 379-382.
- Baldocchi D, Collineau S. 1994. The physical nature of solar radiation in heterogeneous canopies: spatial and temporal attributes. In *Exploitation of environmental heterogeneity by plants: ecophysiological processes above and belowground*. eds. MM Caldwell, RW Pearcy. San Diego, CA, Academic Press, pp. 21-71.

- Bange MP, Hammer GL, Rickert KG. 1997. Effect of specific leaf nitrogen on radiation use efficiency and growth of sunflower. *Crop Science* 37, 1201-1207.
- Barraclough PB. 1986. The growth and activity of winter wheat roots in the field: nutrient uptakes of high-yielding crops. *Journal of Agricultural Science* 106, 45-52.
- Barraclough PB. 1989. Root growth, macro-nutrient uptake dynamics and soil fertility requirements of a high-yielding winter oilseed rape crop. *Plant and Soil* 119, 59-70.
- Bell MA, Fischer RA, Byerlee D, Sayre K. 1995. Genetic and agronomic contributions to yield gains: a case study for wheat. *Field Crops Research* 44, 55-65.
- Bennie ATP. 1991. Growth and mechanical impedance. In *Plant roots: the hidden half*. eds. Y Waisel, A Eshel, U Kafkafi. Marcel Dekker, Inc., New York, pp. 393-414.
- Besford RT, Ludwig LJ, Withers AC. 1990. The greenhouse effect: acclimation of tomato plants in high CO₂, photosynthesis and ribulose-1,5-biphosphate carboxylase protein. *Journal of Experimental Botany* 41, 925-931.
- Bhagsari AS, Brown RH. 1986. Leaf photosynthesis and its correlation with leaf area. *Crop Science* 26, 127-131.
- Bilsborrow PE, Evans EJ, Zhao FJ. 1993. The influence of spring nitrogen on yield, tield components and glucosinolate content of autumn-sown oilseed rape (*Brassica napus*). *Journal of Agricultural Science* 120, 219-224.
- Bindraban PS. 1999. Impact of canopy nitrogen profile in wheat on growth. *Field Crops Research* 63, 63-77.
- Bouma TJ. 1995. Utilisation of respiratory energy in higher plants. Requirements for maintenance and transport processes. PhD Thesis, Wageningen Agricultural University, The Netherlands.
- Bowes, G. 1996. Photosynthesis responses to changing atmospheric CO₂ concentrations. In *Photosynthesis and the environment*. ed. NR Baker. Academic Publishers, pp. 387-407.
- Bulman P, Mather DE, Smith DL. 1993. Genetic improvement of spring barley cultivars grown in eastern Canada from 1910 to 1988. *Euphytica* 71, 35-48.
- Burkey KO, Wells R. 1991. Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. *Plant Physiology* 97, 245-252.
- Calderini DF, Dreccer MF, Slafer GA. 1995. Genetic improvement in wheat yield and associated traits. A re-examination of previous results and latest trends. *Plant Breeding* 114, 108-112.
- Caloin M, Yu O. 1984. Analysis of the time course of change in nitrogen content in *Dactylis glomerata* L. using a model of plant growth. *Annals of Botany* 44, 523-535.
- Carvalho FIF, Qualset CO. 1978. Genetic variation for canopy architecture and its use in wheat breeding. *Crop Science* 18, 561-567.
- Carver BF, Nevo E. 1990. Genetic diversity of photosynthetic characters in native populations of *Triticum dicoccoides*. *Photosynthesis Research* 25, 119-128.

- Carver BF, Johnson RC Rayburn AL. 1989. Genetic analysis of photosynthetic diversity in hexaploid and tetraploid wheat and their interspecific hybrids. *Photosynthesis Research* 20, 105-118.
- Chalmers AG, Darby RJ. 1992. Nitrogen application to oilseed rape and implications for potential leaching loss. *Aspects of Applied Biology* 30, 425-430.
- Chang TT, Tagumpay O. 1970. Genotypic association between grain yield and six agronomic traits in a cross between rice varieties of contrasting plant types. *Euphytica* 19, 356-363.
- Charles-Edwards DA. 1982. *Physiological determinants of crop growth*. Academic Press, North Ryde, North South Wales, Australia, 161 pp.
- Charles-Edwards DA, Stutzel H, Ferraris R, Beech DF. 1987. An analysis of spatial variation in the nitrogen content of leaves from different horizons within a canopy. *Annals of Botany* 60, 421-426.
- Chen JL, Reynolds JF, Harley PC, Tenhunen JD. 1993. Coordination theory of leaf nitrogen distribution in a canopy. *Oecologia* 93, 63-69.
- Colnenne C, Meynard JM, Reau R, Justes EJ, Merrien A. 1998. Determination of a critical nitrogen dilution curve for winter oilseed rape. *Annals of Botany* 81, 311-317.
- Connor DJ, Hall AJ, Sadras VO. 1993. Effect of nitrogen content on the photosynthetic characteristics of sunflower leaves. *Australian Journal of Plant Physiology* 20, 251-263.
- Connor DJ, Sadras VO, Hall AJ. 1995. Canopy nitrogen distribution and the photosynthetic performance of sunflower crops during grain filling- a quantitative analysis. *Oecologia* 101, 274-281.
- Day W, Chalabi ZS. 1988. Use of models to investigate the link between the modification of photosynthetic characteristics and improved crop yields. *Plant Physiology and Biochemistry* 26, 511-517.
- DeJong TM, Doyle JF. 1985. Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (*Prunus persica*). *Plant, Cell Env.* 8, 701-706.
- DeJong TM, Day KR, Johnson RS. 1989. Partitioning of leaf nitrogen with respect to within canopy light exposure and nitrogen availability in peach (*Prunus persica*). *Trees* 3, 89-95.
- Deléens E, Cliquet JB, Prioul JL. 1994. Use of ¹³C and ¹⁵N plant label near natural abundance for monitoring carbon and nitrogen partitioning. *Australian Journal of Plant Physiology* 21, 133-146.
- Del Pozo A, Dennett MD. 1999. Analysis of the distribution of light, leaf nitrogen, and photosynthesis within the canopy of *Vicia faba* L. at two contrasting plant densities. *Aust. J. Agric. Res.* 50, 183-9.
- Dingkuhn M, Kropff MJ. 1996. Rice. In *Photoassimilate distribution in plants and crops. Source-sink relationship*. eds. E Zamski, AA Schaffer. New York, NY: Marcel Dekker Inc., pp. 519-547.
- Dreccer MF, Grashoff C, Rabbinge R. 1997. Source-sink ratio in barley (*Hordeum vulgare* L.) during grain filling: effects on senescence and grain protein concentration. *Field Crops Research* 49, 269-277.

- Drouet JL, Bonhomme R. 1999. Do variations in local leaf irradiance explain changes to leaf nitrogen within row maize canopies? *Annals of Botany* 84, 61-69.
- Duncan WG. 1971. Leaf angles, leaf area, and canopy photosynthesis. *Crop Science* 11, 482-485.
- Evans JR. 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L). *Plant Physiology* 72, 297-302.
- Evans JR. 1989. Partitioning of nitrogen between and within leaves grown under different irradiances. *Australian Journal of Plant Physiology* 16, 533-548.
- Evans JR. 1989a. Photosynthesis and nitrogen relationships in leaves of C₃ plants. *Oecologia* 78, 9-19.
- Evans JR. 1989b. Photosynthesis - The dependence on nitrogen partitioning. In *Causes and consequences of variations in growth rate and productivity of higher plants*. eds. H Lambers, ML Cambridge, H Konings, TL Pons. The Hague: SPB Academic Publishing, pp. 159-174.
- Evans JR. 1993a. Photosynthetic acclimation and nitrogen partitioning within a lucerne canopy. II Stability through time and comparison with a theoretical optimum. *Australian Journal of Plant Physiology* 20, 55-67.
- Evans JR. 1996. Developmental constraints on photosynthesis: effects of light and nutrition. In *Photosynthesis and the environment*. ed. NR Baker. Academic Publishers, pp. 281-304.
- Evans JR, Terashima I. 1988. Photosynthetic characteristics of spinach leaves grown with different nitrogen treatments. *Plant Cell Physiol.* 29, 157-165.
- Evans JR, Farquhar GD. 1991. Modeling canopy photosynthesis from the biochemistry of the C₃ chloroplast. In *Modeling crop photosynthesis-from biochemistry to canopy*. eds. KJ Boote, RS Loomis. Crop Science Society of America, Madison, 141 pp.
- Evans LT. 1990. Assimilation, allocation, explanation, extrapolation. In *Theoretical Production Ecology: reflections and prospects*. eds. R Rabbinge, J Goudriaan, H Van Keulen, FWT Penning de Vries, HH Van Laar. Wageningen: PUDOC, pp. 77-87.
- Evans LT. 1993b. Processes, genes and yield potential. In *International Crop Science I*. Crop Science Society of America. Madison, WI, pp. 583-588.
- Ewert F, Van Oijen M, Porter JR. 1999. Simulation of growth and development processes of spring wheat in response to CO₂ and ozone for different sites and years in Europe using mechanistic crop simulation models. *European Journal of Agronomy* 10, 231-247.
- FAO. 1997. Production Yearbook vol. 50 1996. FAO Statistics series no. 135. Food and Agricultural Organization of the United Nations, Rome, 235 pp.
- Farage PK, McKee IF, Long SP. 1998. Does a low N supply necessarily lead to acclimation of photosynthesis to elevated CO₂? *Plant physiology* 118, 573-580.
- Farquhar GD, Von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149, 78-90.
- Field C. 1983. Allocating leaf nitrogen for the maximization of carbon gain: leaf age as a control on the allocation program. *Oecologia* 56, 341-347.
- Field C, Mooney HA. 1983. Leaf age and seasonal effects on light, water, and nitrogen use efficiency in a Californian shrub. *Oecologia* 56, 348-355.

- Field C, Mooney HA. 1986. The photosynthesis-nitrogen relationship in wild plants. In *On the economy of Plant Form and Function*. ed. TJ Givnish. Cambridge, Cambridge University Press, pp. 25-55.
- Fischer RA. 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science* 105, 447-461.
- Fischer RA. 1993. Irrigated spring wheat and timing and amount of nitrogen fertiliser. II. Physiology of grain yield response. *Crop Research* 33, 57-80.
- Fischer RA, Howe GN, Ibrahim Z. 1993. Irrigated spring wheat and timing and amount of nitrogen fertiliser. I Grain yield and protein content. *Field Crops Research* 33, 37-56.
- Fischer RA, Rees D, Sayre KD, Lu ZM, Condon AG, Larque Saavedra A. 1998. Wheat yield progress associated with higher stomatal conductance and photosynthetic rate, and cooler canopies. *Crop Sci.* 38, 1467-1475.
- Flénet F, Kiniry JR. 1995. Efficiency of biomass accumulation by sunflower as affected by glucose requirement of biosynthesis and leaf nitrogen content. *Field Crops Research* 44, 119-127.
- Flood RG, Halloran GM. 1986. The influence of genes for vernalisation response on development and growth in wheat. *Annals of Botany* 58, 505-508.
- Gallagher JN, Biscoe PV. 1978. Radiation absorption, growth and yield of cereals. *Journal of Agricultural Science* 105, 447-461.
- Gammelvind LH, Schjoerring JK, Mogensen VO, Jensen CR, Bock JHG. 1996. Photosynthesis in leaves and siliques of winter oilseed rape (*Brassica napus* L.). *Plant and Soil* 186, 227-236.
- Garnier E, Gobin O, Poorter H. 1995. Nitrogen productivity depends on photosynthetic nitrogen use efficiency and on nitrogen allocation within the plant. *Annals of Botany* 76, 667-672.
- Gastal F, Belanger G, Lemaire G. 1992. A model of the leaf extension rate of tall fescue in response to nitrogen and temperature. *Annals-of-Botany* 70, 437-442.
- Genstat 5 Committee. 1987. *Genstat 5 Reference Manual*. Clarendon Press, Oxford, 300 pp.
- Giménez C, Connor DJ, Rueda F. 1994. Canopy development, photosynthesis and radiation use efficiency in sunflower in response to nitrogen. *Field Crops Research* 38, 15-27.
- Gooding MJ, Davies WP. 1997. *Wheat production and utilization. Systems, quality and the environment*. CAB International, Wallingford, Oxon, UK, 355 pp.
- Goudriaan J. 1988. The bare bones of leaf-angle distribution in radiation models for canopy photosynthesis and energy exchange. *Agricultural and Forest Meteorology* 43, 155-169.
- Goudriaan J. 1995. Optimization of nitrogen distribution and of leaf area index for maximum canopy assimilation rate. In *SARP Research Proceedings: Nitrogen management studies in irrigated rice*. eds. TM Thiyagarajan, HFM Ten Berge, MCS Wopereis. Wageningen: AB-DLO, TPE-WAU and Los Baños: IRRI, pp. 85-97.
- Goudriaan J, Van Laar HH. 1994. *Modelling potential crop growth processes. Textbook with exercises*. Kluwer Academic Publishers, Dordrecht, 238 pp.

- Green CF. 1987. Nitrogen nutrition and wheat in growth in relation to absorbed solar radiation. *Agricultural and Forest Meteorology* 41, 207-248.
- Green CF. 1989. Genotypic differences in the growth of *Triticum aestivum* in relation to absorbed solar radiation. *Field Crops Research* 19, 285-295.
- Gregory PJ, Eastham J. 1996. Growth of shoots and roots, and interception of radiation by wheat and lupin crops on a shallow, duplex soil in response to time of sowing. *Australian Journal of Agricultural Research* 47, 427-447
- Habekotté B. 1993. Quantitative analysis of pod formation, seed set and seed filling in winter oilseed rape (*Brassica napus* L.) under field conditions. *Field Crops Research* 35, 21-33.
- Habekotté B. 1997a. Identification of strong and weak yield determining components of winter oilseed rape compared with winter wheat. *European Journal of Agronomy* 7, 315-321.
- Habekotté B. 1997b. Options for increasing seed yield of winter oilseed rape (*Brassica napus* L.): a simulation study. *Field Crops Research* 54, 109-126.
- Hall AJ, Connor DJ, Sadras VO. 1995. Radiation use efficiency of sunflower crops: effects of specific leaf nitrogen and ontogeny. *Field Crops Research* 41, 65-77.
- Hammer GL, Wright GC. 1994. A theoretical analysis of nitrogen and radiation effects on radiation use efficiency in peanut. *Australian Journal of Agricultural Research* 45, 575-589.
- Handley LL, Raven JA. 1992. The use of natural abundance of nitrogen isotopes in plant physiology and ecology. *Plant Cell and Environment* 15, 965-985.
- Henson IE, Jensen CR, Turner NC. 1990. Influence of leaf age and light environment on the gas exchange of lupins and wheat. *Physiologia Plantarum* 79, 15-22.
- Hikosaka K, Terashima I. 1995. A model of the acclimation of photosynthesis in the leaves of C₃ plants to sun and shade with respect to nitrogen use. *Plant Cell and Environment* 18, 605-618.
- Hikosaka K, Terashima I. 1996. Nitrogen partitioning among photosynthetic components and its consequence in sun and shade plants. *Functional Ecology* 10, 335-343.
- Hikosaka K, Hirose T. 1998. Leaf and canopy photosynthesis of C₃ plants at elevated CO₂ in relation to optimal partitioning of nitrogen among photosynthetic components: theoretical prediction. *Ecological Modelling* 106, 247-259.
- Hikosaka K, Terashima I, Katoh S. 1994. Effects of leaf age, nitrogen nutrition and photo flux density on the distribution of nitrogen among leaves of a vine (*Ipomoea tricolor* Cav.) grown horizontally to avoid mutual shading of leaves. *Oecologia* 97, 451-457.
- Hirose T, Werger MJA. 1987a. Nitrogen use efficiency in instantaneous and daily photosynthesis of leaves in the canopy of *Solidago altissima* stand. *Physiologia Plantarum* 70, 215-222.
- Hirose T, Werger MJA. 1987b. Maximizing daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* 72, 520-526.
- Hirose T, Werger MJA, Pons TL, Van Rheenen JWA. 1988. Canopy structure and leaf nitrogen distribution in a stand of *Lysimachia vulgaris* L. as influenced by stand density. *Oecologia* 77, 145-150.

- Hirose T, Ackerly DD, Traw MB, Ramseier D, Bazzaz FA. 1996. Effects of CO₂ elevation on canopy development in the stands of two co-occurring annuals. *Oecologia* 108, 215-223.
- Hirose T, Ackerly DD, Traw MB, Ramseier D, Bazzaz FA. 1997. CO₂ elevation, canopy photosynthesis, and optimal leaf area index. *Ecology* 78, 2339-2350.
- Hocking PJ, Mason L. 1993. Accumulation, distribution and redistribution of dry matter and mineral nutrients in fruits of canola (oilseed rape), and the effects of nitrogen fertiliser and windrowing. *Australian Journal of Agricultural Research* 44, 1377-1388.
- Hocking PJ, Kirkegaard JA, Angus JF, Gibson AH, Koetz EA. 1997a. Comparison of canola, Indian mustard and linola in two contrasting environments. I. Effects of nitrogen fertiliser on dry-matter production, seed yield and seed quality. *Field Crops Research* 49, 107-125.
- Hocking PJ, Randall PJ, DeMarco D. 1997b. The response of dryland canola to nitrogen fertilizer: partitioning and mobilization of dry matter and nitrogen, and nitrogen effects on yield components. *Field Crops Research* 49, 201-220.
- Hollinger DY. 1996. Optimality and nitrogen allocation in a tree canopy. *Tree Physiology* 16, 627-634.
- Holmes MRJ. 1980. *Nutrition of the oilseed rape crop*. Applied scientific Publishers. London.
- Huggins DR, Pan WL. 1993. Nitrogen efficiency component analysis: an evaluation of cropping system differences in productivity. *Crop Science* 85, 898-905.
- IFPRI. 1995. A 2020 vision for food, agriculture and the environment. The vision, challenge and recommended action. The International Food Policy Research Institute, Washington, DC, 25 pp.
- Innes P, Blackwell RD. 1983. Some effects of leaf posture on the yield and water economy of winter wheat. *Journal of Agricultural Science* 101, 367-376.
- Inskeep WP, Bloom PR. 1985. Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. *Plant Physiology* 77, 483-485.
- Jensen NF. 1978. Limits to growth in world food production. Ceilings for wheat yields are coming in developed countries. *Science* 201, 317-320.
- Johnson RC, Kebede H, Mornhinweg DW, Carver BF, Rayburn AL, Nguyen HT. 1987. Photosynthetic differences among *Triticum* accessions at tillering. *Crop Science* 27, 1046-1050.
- Joseffson E. 1970. Glucosinolate content and amino acid composition of rapeseed (*Brassica napus* L.) meal as affected by sulphur and nitrogen nutrition. *Journal of the Science of Food and Agriculture* 21, 98-103.
- Keeling CD, Whorf TP, Wahlen M, Van der Plicht J. 1995. Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980. *Nature* 375, 666-670.
- Kimball BA, Mauney, JR, Nakayama FS, Idso SB. 1993. Effects of increasing atmospheric CO₂ on vegetation. *Vegetatio* 104/105, 65-75.
- Kiniry JR, Jones CA, O'Toole JC, Blanchet R, Cabelguenne M, Spanel DA. 1989. Radiation-use efficiency in biomass accumulation prior to grain-filling for five grain-crop species. *Field Crops Research* 20, 51-64.
- Kirkegaard JA, Hocking PJ, Angus JF, Howe GN, Gardner PA. 1997. Comparison of canola, Indian mustard and linola in two contrasting environments. II. Break-crop

- and nitrogen effects on subsequent wheat crops. *Field Crops Research* 52, 179-191.
- Kraus E, Aydemir Y, Duin S, Kolloffel C, Lambers H. 1993. Yield advantage of a "slow-" over a "fast-" respiring population of *Lolium perenne* cv. S23 depends on plant density. *New Phytologist* 123, 43-54.
- Lawes DA. 1977. Yield improvement in spring oats. *Journal of Agricultural Science* 89, 751-757.
- Lawlor DW. 1993. *Photosynthesis: molecular, physiological and environmental processes*. Longman Scientific & Technical, Harlow, 318 pp.
- Lawlor DW. 1995. Photosynthesis, productivity and environment. *Journal of Experimental Botany* 46, 1449-1461.
- Lemaire G, Gastal F. 1997. N uptake and distribution in plant canopies. In *Diagnosis of the Nitrogen status in crops*. ed. G Lemaire. Springer-Verlag, Berlin, pp. 239.
- Lemaire G, Onillon B, Gosse G, Chartier M, Allirand JM. 1991. Nitrogen distribution within a lucerne canopy during regrowth: relation with light distribution. *Annals of Botany* 68, 483-488.
- Leuning R, Kelliher FM, De Pury GG, Schulze ED. 1995. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves canopies. *Plant, Cell and Environment* 18, 1183-1200.
- Li A, Wall GW, Trent A, Hou Y. 1999. Free-air CO₂ enrichment effects on apex and dimensional growth of spring wheat. *Crop Science* 39, 1083-1088.
- Long SP, Drake BG. 1991. Effect of the long-term elevation of CO₂ concentration in the field on the quantum yield of photosynthesis of the C₃ sedge, *Scirpus olneyi*. *Plant Physiology* 96, 221-226.
- Loomis RS. 1993. Optimization theory and crop improvement. In *International Crop Science I*, Crop Science Society of America. Madison, WI, pp. 583-588.
- Loss SP, Siddique KHM. 1994. Morphological and physiological traits associated with wheat yield increases in Mediterranean environments. *Advances in Agronomy* 52, 229-276.
- Mae T. 1997. physiological nitrogen efficiency in rice: Nitrogen utilisation, photosynthesis and yield potential. *Plant and Soil* 196, 201-210.
- MacKown CT, Van Sanford DA, Zhang N. 1992. Wheat vegetative nitrogen compositional changes in response to reduced reproductive sink strength. *Plant Physiology* 99, 1469-1474.
- Makino A, Mae T, Ohira K. 1983. Photosynthesis and ribulose 1,5-bisphosphate carboxylase in rice leaves. *Plant Physiology* 73, 1002-1007.
- Makino A, Mae T, Ohira K. 1988. Differences between wheat and rice in the enzymatic properties of ribulose-1,5-biphosphate carboxylase/oxygenase and the relationships to photosynthetic gas exchange. *Planta* 174, 30-38.
- Marshall B, Vos J. 1991. The relation between the nitrogen concentration and photosynthetic capacity of potato (*Solanum tuberosum* L.) leaves. *Annals of Botany* 68, 33-39.
- Meinke H. 1996. Improving wheat simulation capabilities in Australia from a cropping systems perspective. PhD Thesis, Wageningen Agricultural University, The Netherlands.

- Mendham NJ, Shipway PA, Scott RK. 1981. The effects of seed size, autumn nitrogen and plant population on the response to delayed sowing in winter oilseed rape (*Brassica napus*). *Journal of Agricultural Science* 96, 417-428.
- Miller A, Tsai CH, Hemphill D, Endres M, Rodermeil S, Spalding M. 1997. Elevated CO₂ effects during leaf ontogeny. A new perspective on acclimation. *Plant Physiology* 115, 1195-1200.
- Monje O, Bugbee B. 1998. Adaptation to high CO₂ concentration in an optimal environment: radiation capture, canopy quantum yield and carbon use efficiency. *Plant, Cell and Environment* 21, 315-324.
- Monteith JL. 1977. Climate and the efficiency of crop production in Britain. *Philosophical Transactions of Royal Society of London B* 281, 277-294.
- Monsi M, Saeki T. 1953. Ueber den Lichtfaktor in den Pflanzengesellschaften und seine Bedeutung für die Stoffproduktion. *Japanese Journal of Botany* 14, 22-52.
- Mooney HA, Gulmon SL. 1979. Environmental and evolutionary constraints on the photosynthetic characteristics of higher plants. In *Topics in Plant population biology*. eds. OT Solbrig, S Jain, GB Johnson, PH Raven. New York, NY: Columbia University Press, pp. 316-337.
- Mooney HA, Field C, Gulmon SL, Bazzaz FA. 1981. Photosynthetic capacity in relation to leaf position in desert versus old-field annuals. *Oecologia* 50, 109-112.
- Muchow RC, Sinclair TR. 1994. Nitrogen response of leaf photosynthesis and canopy radiation use efficiency in field grown maize and sorghum. *Crop Science* 34, 721-727.
- Mulholland B, Craigon J, Black CR, Colls JJ, Atherton J, Landon G. 1998. Growth, light interception and yield responses of spring wheat (*Triticum aestivum* L.) grown under elevated CO₂ and O₃ in open-top chambers. *Global Change Biology* 4, 121-130.
- Nie GY, Long SP, García RL, Kimball BA, LaMorte RL, Pinter PJ, Wall GW, Webber AN. 1995. Effects of free-air CO₂ enrichment on the development of the photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. *Plant, Cell and Environment* 18, 855-864.
- Niinemets U, Tenhunen JD. 1997. A model separating leaf structural and physiological effects on carbon gain along light gradients for the shade-tolerant species *Acer saccharum*. *Plant, Cell and Environment* 20, 845-866.
- Ort DR, Baker NR. 1988. Consideration of photosynthetic efficiency at low light as a major determinant of crop photosynthetic performance. *Plant Physiology and Biochemistry* 26, 555-565.
- Osborne CP, LaRoche J, Garcia RL, Kimball BA, Wall GW, Pinter PJ, LaMorte RL, Hendrey GR, Long SP. 1998. Does leaf position within the canopy affect acclimation of photosynthesis to elevated CO₂. *Plant Physiology* 117, 1037-1045.
- Pechan P A and Morgan D G 1985 Defoliation and its effects on pod and seed development in oilseed rape (*Brassica napus* L.). *Jouranal of Experimental Botany* 36, 458-468.
- Peltonen-Sainio P. 1990. Genetic improvements in the structure of oat plants in northern growing conditions during this century. *Plant Breeding* 104, 340-345.

- Peltonen-Sainio P. 1994. Productivity of oats: genetic gains and associated physiological changes. In *Genetic improvement of field crops*. ed. GA Slafer, New York, NY: Marcel Dekker Inc, pp. 69-94.
- Peng S, Khush GS, Cassman KG. 1994. Evolution of the new plant ideotype for increased yield potential. In *Breaking the yield barrier: Proceedings of a Workshop on Rice Potential in favourable environments*. ed. KG Cassman. Los Baños: IRRI, pp. 5-20.
- Peng S, Cassman KG, Kropff MJ. 1995. Relationship between leaf photosynthesis and nitrogen content of field-grown rice in the tropics. *Crop Science* 35, 1627-1630.
- Penning de Vries FWT, Van Laar HH, Chardon MCM. 1983. Bioenergetics of growth of seed, fruits and storage organs. In *Potential productivity of field crops under different environments*. eds. WH Smith, SJ Banta. International Rice Research Institute, Los Baños, pp. 37-60.
- Penning de Vries FWT, Van Keulen H, Alagos JC. 1988. Nitrogen redistribution and potential production in rice. In *Proceedings of the International Congress of Plant Physiology*. New Delhi, India, February 15-20, pp. 511-520.
- Petersen CT, Jorgensen U, Svedensen H, Hansen S, Jensen HE, Nielsen NE. 1995. Parameter assessment for simulation of biomass production and nitrogen uptake in winter rape. *European Journal of Agronomy* 4, 77-89.
- Pettersson R, MacDonald AJS. 1994. Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO₂. *Photosynthesis Research* 39, 389-400.
- Pinter PJ Jr, Kimball BA, García RL, Wall GW, Hunsaker DJ, LaMorte RL. 1996. Free-air CO₂ reponses of cotton and wheat crops. In *Carbon dioxide and terrestrial ecosystems*. eds. HA Mooney, GW Koch. Academic Press, Orlando, FL, pp. 215-249.
- Pons TL, Pearcy RW. 1994. Nitrogen reallocation and photosynthetic acclimation in response to partial shading in soybean plants. *Physiologia Plantarum* 92, 636-644.
- Pons TL, Jordi W. 1997. Induction of leaf senescence and shade acclimation in leaf canopies-variation with leaf longevity. In *Inherent variation in plant growth. Physiological mechanisms and ecological consequences*, eds. H Lambers, H Poorter, MMI Van Vuuren. Backhuys Publishers, Leiden, pp. 121-137.
- Pons TL, Schieving F, Hirose T, Werger MJA. 1989. Optimization of leaf nitrogen allocation for canopy photosynthesis in *Lysimachia vulgaris*. In *Causes and consequences of variations in growth rate and productivity of higher plants*. eds. H Lambers, ML Cambridge, H Konings, TL Pons. The Hague: SPB Academic Publishing, pp. 173-186.
- Poorter H, Evans JR. 1998. Photosynthetic nitrogen-use efficiency in species that differ inherently in specific leaf area. *Oecologia* 116, 26-37.
- Radin JW. 1983. Control of plant growth by nitrogen: differences between cereals and broadleaf species. *Plant Cell and Environment* 6, 65-68.
- Rasmusson DC. 1987. An evaluation of ideotype breeding. *Crop Science* 27, 1140-1146.
- Rawson HM, Hackett C. 1974. An exploration of the carbon economy of the tobacco plant. III Gas exchange of leaves in relation to position on the stem, ontogeny and nitrogen content. *Australian Journal of Plant Physiology* 1, 551-560.

- Rawson HM, Hindmarsh JH, Fischer RA, Stockman YM. 1983. Changes in leaf photosynthesis with plant ontogeny and relationships with yield per ear in wheat cultivars and 120 progeny. *Australian Journal of Plant Physiology* 10, 503-514.
- Reynolds MP, Rajaram S, McNab A. 1996a. *Increasing Yield Potential in Wheat: Breaking the Barriers*, CIMMYT, Mexico DF, 238 pp.
- Reynolds MP, Van Beem J, Van Ginkel M, Hoisington D. 1996b. Breaking the yield barriers in wheat: a brief summary of the outcomes of an international consultation. In *Increasing Yield Potential in Wheat: Breaking the Barriers*. eds. MP Reynolds, S Rajaram, A McNab. CIMMYT, Mexico DF, pp. 1-10.
- Richards R. 1992. The effect of dwarfing genes in spring wheat in dry environments. I. Agronomic characteristics. *Australian Journal of Agricultural Research* 43, 517-523.
- Riggs TJ, Hanson PR, Start ND, Miles DM, Morgan CL, Ford MA. 1981. Comparison of spring barley varieties grown in England and Wales between 1880 and 1980. *Journal of Agricultural Science* 97, 599-610.
- Rodríguez D, Andrade FH, Goudriaan J. 1999. Effects of phosphorus nutrition on tiller emergence in wheat. *Plant and Soil* 209, 283-295
- Rogers GS, Milham PJ, Gillings M, Conroy JP. 1996. Sink strength may be the key to growth and nitrogen responses in N-deficient wheat at elevated CO₂. *Australian Journal of Plant Physiology* 23, 253-264.
- Rood SB, Major DJ. 1984. Influence of plant density, nitrogen, water supply and pod or leaf removal on growth of oilseed rape. *Field Crops Research* 8, 323-331.
- Rood SB, Major DJ, Charnetski WA. 1984. Seasonal changes in ¹⁴CO₂ assimilation and ¹⁴C translocation in oilseed rape. *Field Crops Research* 8, 341-348.
- Rosenthal WD, GF Arkin, TA Howell. 1985. Transmitted and absorbed photosynthetically active radiation in grain sorghum. *Agronomy Journal* 77, 841-845.
- Rousseaux MC, Hall AJ, Sánchez RA. 1999. Light environment, nitrogen content, and carbon balance of basal leaves of sunflower canopies. *Crop Sci.* 39, 1093-1100.
- Roy SK, Gallagher JN. 1984. Production and survival of wheat tillers in relation to plant growth and development. In *Wheat growth and modelling*. eds. W Day, RK Atkin. NATO ASI Series Vol. 86, pp. 59-67.
- Russell G, PG Jarvis, JL Monteith. 1989. Absorption of radiation by canopies and stand growth. In *Plant canopies: their growth, form and function*. eds. G Russell, LB Marshall, PG Jarvis. Cambridge: Cambridge University Press, pp. 21-39.
- Sadras VO, AJ Hall, DJ Connor. 1993. Light-associated nitrogen distribution profile in flowering canopies of sunflower (*Helianthus annuus* L.) altered during grain growth. *Oecologia* 95, 488-494.
- Sage RF. 1994. Acclimation of photosynthesis to increasing CO₂: The gas exchange perspective. *Photosynthesis Research* 39, 351-368.
- Sandars DL. 1995. The future development of oilseed rape. In *New horizons for oilseed rape*. eds. DH Scarisbrick, AJ Ferguson. Semundo Ltd., Cambridge, pp. 68-104.
- Scarisbrick DH, Ferguson AJ. 1995. Oilseed rape varieties and production. In *New horizons for oilseed rape*. eds. DH Scarisbrick, AJ Ferguson. Semundo Ltd, Cambridge, pp. 9-30.

- Schapendonk AHCM, Spitters CJT, de Vos ALF. 1990. Comparison of nitrogen utilisation of diploid and tetraploid perennial ryegrass genotypes using a hydroponic system. In *Genetic aspects of plant mineral nutrition*. ed. N El Bassam. Kluwer Academic Publishers, pp. 299-306.
- Schapendonk AHCM, Van Oijen M, Pot SC, Van der Boogaard R, Harbison J. 1999. Nitrogen shortage in a tomato crop; scaling up from effects on electron-transport rate to plant productivity. *Z. Naturforsch* (in press).
- Schenk MK. 1996. Regulation of nitrogen uptake on the whole plant level. *Plant Soil* 181, 131-137.
- Schieving F, Werger MJA, Hirose T. 1992a. Canopy structure, nitrogen distribution and whole canopy photosynthetic carbon gain in growing and flowering stands of tall herbs. *Vegetatio* 102, 173-181.
- Schieving F, Werger MJA, Hirose T. 1992b. The vertical distribution of nitrogen and photosynthetic activity at different plant densities in *Carex acutiformis*. *Plant and Soil* 14, 9-17.
- Schjoerring JK, Bock JGH, Gammelvind L, Jensen CR, Mogensen VO. 1995. Nitrogen incorporation and remobilization in different shoot components of field-grown winter oilseed rape (*Brassica napus* L.) as affected by rate of nitrogen application and irrigation. *Plant Soil* 177, 255-264.
- Shiraiwa T, Sinclair TR. 1993. Distribution of nitrogen among leaves in soybean canopies. *Crop Science* 33, 804-808.
- Shorter R, Lawn RJ, Hammer GL. 1991. Improving genotypic adaptation in crops - a role for breeders, physiologists and modellers. *Experimental Agriculture* 271, 155-175.
- Siddique KHM, Belford RK, Perry MW, Tennant D. 1989. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. *Australian Journal of Agricultural Research* 40, 473-487.
- Simpson RJ, Lambers H, Beilharz VC, Dalling MJ. 1982. Translocation of nitrogen in a vegetative wheat plant (*Triticum aestivum*). *Physiologia Plantarum* 56, 11-17.
- Sims DA, Cheng W, Luo Y, Seemann JR. 1999. Photosynthetic acclimation to elevated CO₂ in a sunflower canopy. *Journal of Experimental Botany* 50, 645-653.
- Sinclair TR, Horie T. 1989. Leaf nitrogen, photosynthesis and crop radiation use efficiency: A review. *Crop Sci.* 29, 90-98.
- Sinclair TR, Shiraiwa T. 1993. Soybean radiation use efficiency as influenced by non-uniform specific leaf nitrogen distribution and diffuse radiation. *Crop Science* 33, 808-812.
- Sinclair TR, Shiraiwa T, Hammer GL. 1992. Variation in crop radiation use efficiency in response to increased proportion of diffuse radiation. *Crop Sci.* 32, 1281-1284.
- Sinclair TR, Bennet JM, Boote KJ. 1993. Leaf nitrogen content, photosynthesis and radiation use efficiency in peanut. *Peanut Science* 20, 40-43.
- Slafer GA, Andrade FH. 1989. Genetic improvement in bread wheat (*Triticum aestivum* L.) yield in Argentina. *Field Crops Research* 21, 351-367.
- Slafer GA, Andrade FH. 1991. Changes in physiological attributes of the dry matter economy of bread wheat (*Triticum aestivum*) through genetic improvement of grain yield potential at different regions of the world. A review. *Euphytica* 58, 37-49.

- Slafer GA, Savin R. 1994. Source-sink relationships and grain mass at different positions within the spike in wheat. *Field Crops Res.* 37, 39-49.
- Slafer GA, Araus JL. 1998. Improving wheat responses to abiotic stresses. *Proceedings of the 9th International Wheat Genetics Symposium.* ed. AE Slinkard, University of Saskatchewan Extension Press, Saskatoon, Canada; Vol. 1, pp. 201-213.
- Slafer GA, Satorre EH, Andrade FH. 1994. Increases in grain yield in bread wheat from breeding and associated physiological changes. In *Genetic improvement of field crops.* ed. GA Slafer. Marcel Dekker, Inc., New York, pp. 1-68.
- Slafer GA, Calderini DF, Miralles DJ. 1996. Generation of yield components and compensation in wheat: opportunities for further increasing yield potential. In *Increasing the Yield Potential in Wheat: Breaking the Barriers.* eds. S Rajaram, M Reynolds. Mexico: CYMMYT, pp. 101-133.
- Slafer GA, Araus JL, Richards RA. 1999. Physiological traits to increase the yield potential of wheat. In *Wheat: Ecology and Physiology of Yield Determination.* eds. EH Satorre, GA Slafer. Food Product Press, New York. (in press)
- Smith CJ, Whitfield DM. 1990. Nitrogen accumulation and redistribution of late applications of ¹⁵N-labelled fertiliser by wheat. *Field Crops Res.* 24, 211-226.
- Smith CJ, Wright GC, Woodroffe MR. 1988 The effect of irrigation and nitrogen fertiliser on rapeseed (*Brassica napus*) production in south eastern Australia II. Nitrogen accumulation and oil yield. *Irrig. Sci.* 9, 15-25.
- Smith CJ, Whitfield DM, Gyles OA, Wright GC. 1989 Nitrogen fertiliser balance of irrigated wheat grown on a red brown earth in southeastern Australia. *Field Crops Res.* 21, 265-275.
- Spieritz JHJ, de Vos NM. 1983. Agronomical and physiological aspects of the role of nitrogen in yield formation of cereals. *Plant Soil* 75, 379-391.
- Stapper M, Fischer RA. 1990. Genotype, sowing date and planting spacing influence on high yielding irrigated wheat in southern New south Wales. II Growth, yield and nitrogen use. *Aust. J. Agric. Res.* 41, 1021-1041.
- Steiner AA. 1984. The universal nutrient solution. *Proceedings of the VI International Congress on soilles culture, Lunteren, ISOSC, Wageningen,* pp. 633-650.
- Stitt M. 1991. Rising CO₂ levels and their potential significance for carbon flow in photosynthetic cells. *Plant, Cell and Environment* 14, 741-762.
- Sylvester-Bradley R, Makepeace RJ. 1984. A code for stages of development in oilseed rape (*Brassica napus* L.). *Asp. Appl. Biol.* 6, 399-419.
- Taylor AJ, Smith CJ, Wilson IB. 1991 Effect of irrigation and fertiliser on yield, oil content, nitrogen accumulation and water use of canola (*Brassica napus* L.). *Fert. Res.* 29, 249-260.
- Tayo TO, Morgan DG. 1979. Quantitative analysis of the growth, development and distribution of flowers and pods in oilseed rape (*Brassica napus* L.). *J. Agric. Sci. Camb.* 85, 103-110.
- Tennant D. 1976. A test of a modified line intersect method of estimating root length. *J. Ecol.* 63, 995-1001.
- Terashima I, Evans JR. 1988. Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. *Plant and Cell Physiology* 29, 143-155.

- Thorne GN, Pearman I, Day W, Todd AD. 1988 Estimation of radiation interception by winter wheat from measurements of leaf area. *J. Agric. Sci. Camb.* 110, 101-108.
- Thornley JHM, Johnson IR. 1990. *Plant and crop modelling: a mathematical approach to plant and crop physiology*. Oxford University Press, Oxford, 669 pp.
- Trenbath BR, Angus JF. 1975. Leaf inclination and crop production. *Field Crop Abstracts* 28, 231-244.
- Tuba z, Szente K; Koch J. 1994. Response of photosynthesis, stomatal conductance, water use efficiency and production to long-term elevated CO₂ in winter wheat. *Journal-of-Plant-Physiology* 144, 661-668.
- Tungland L, Chapko LB, Wiersma JV, Rasmusson DC. 1987. Effect of leaf angle on grain yield in barley. *Crop Science* 27, 37-40.
- Uppström B. 1995. Seed chemistry. In *Brassica oilseeds. Production and utilization*. eds. D Kimber, DI McGregor. CAB International, Wallingford, pp. 217-264.
- Van Arendonk JJCM, Niemann GJ, Boon JJ, Lambers H. 1997. Effects of nitrogen supply on the anatomy and chemical composition of leaves of four grass species belonging to the genus *Poa*, as determined by image-processing analysis and pyrolysis-mass spectrometry. *Plant Cell Environ.* 20, 881-897.
- Van Heemst HDJ. 1988. *Plant data values required for simple crop growth simulation models: review and bibliography*. Wageningen: Simulation Report CABO-TT.
- Van Keulen H, Goudriaan J, Seligman NG. 1989. Modelling the effects of nitrogen on canopy development and crop growth. In *Plant canopies: their growth, form and function*. eds. G Russell, LB Marshall, PG Jarvis. Cambridge: Cambridge University Press, pp. 83-104.
- Van Oosten JJ, Besford RT. 1994. Sugar feeding mimics the effect of acclimation to high CO₂ rapid down-regulation of Rubisco small sub-unit transcripts but not of the large sub-unit transcripts. *Journal of Plant Physiology* 143, 306-312.
- Van Oosten JJ, Wilkins D, Besford RT. 1995. Acclimation of tomato to different carbon dioxide concentrations. Relationships between biochemistry and gas exchange during leaf development. *New Phytologist* 130, 357-367.
- Van der Werf A, Nagel OW. 1996. Carbon allocation to shoots and roots in relation to nitrogen supply is mediated by cytokinins and sucrose:opinion. *Plant and Soil* 185, 21-32.
- Verheijen JAG, Jimmink CJ. 1995. *The oilseed complex*. Rabobank Nederland, Utrecht, 72 pp.
- Vogelmann TC, Nishio JN, Smith W. 1996. Leaves and light capture: light propagation and gradients of carbon fixation within leaves. *Trends in Plant Science* 1, 65-70.
- Vos J, Van der Putten PEL. 1998. Effect of nitrogen on leaf growth, leaf nitrogen economy and photosynthetic capacity. *Field Crops Res.* 59, 63-72.
- Waddington SR, Ormanzai M, Yoshida M, Ransom JK. 1987. The yield of durum wheats released in Mexico between 1960 and 1984. *Journal of Agricultural Science* 108, 469-477.
- Walker K. 1995. Biodiesel. In *New horizons for oilseed rape*. eds. DH Scarisbrick, AJ Ferguson. Semundo Ltd., Cambridge, pp. 105-114.
- Werger MJA, Hirose T. 1988. Effects of light climate and nitrogen partitioning on the canopy structure of stands of a dicotyledoneous, herbaceous vegetation. In *Plant*

- form and vegetation structure*. eds. MJA Werger, T Hirose. The Hague: SPB Academic Publishing bv, pp. 171-181.
- Werger MJA, Hirose T. 1991. Leaf nitrogen distribution and whole canopy photosynthetic carbon gain in herbaceous stands. *Vegetatio* 97, 11-20.
- Williams ML, Farrar JF, Pollock CJ. 1989. Cell specialization within the parenchymatous bundle sheath of barley. *Plant Cell Environment* 12, 909-918.
- Wilson D. 1982. Response to selection for dark respiration rate of mature leaves in *Lolium perenne* L. and its effects on growth of young plants and simulated swards. *Annals Botany* 49, 303-312.
- Wingler A, Von Schaewen A, Leegood R, Lea P, Quick P. 1998. Regulation of leaf senescence by cytokinin, sugars, and light. Effects on NADH-dependent hydroxypyruvate reductase. *Plant Physiol.* 116, 329-335.
- Woledge J. 1986. The effect of age and shade on the photosynthesis of white clover leaves. *Annals of Botany* 57, 257-262.
- Woodrow IE. 1994. Optimal acclimation of the C₃ photosynthetic system under enhanced CO₂. *Photosynthesis Research* 39, 401-412.
- Wright GC, Hammer GL. 1994. Distribution of nitrogen and radiation use efficiency in peanut canopies. *Australian Journal of Agricultural Research* 45, 565-574.
- Wright GC, Smith CJ, Woodroffe MR. 1988. The effect of irrigation and nitrogen fertiliser on rapeseed (*Brassica napus* L.) production in southern Australia. I. Growth and seed yield. *Irrig. Sci.* 9, 1-13.
- Xu DQ, Gifford RM, Chow WS. 1994. Photosynthetic acclimation in pea and soybean to high atmospheric CO₂ partial pressure. *Plant Physiology* 106, 661-671.
- Yates DJ, Steven MD. 1987. Reflection and absorption of solar radiation by flowering canopies of oilseed rape (*Brassica napus* L.). *Journal of Agricultural Science* 109, 409-502.
- Yunusa IAM, Siddique KHM, Belford RK, Karimi MM. 1993. Effect of canopy structure on efficiency of radiation interception and use in spring wheat cultivars during the pre-anthesis period in a mediterranean environment. *Field Crops Research* 35, 113-122.
- Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth of cereals. *Weed Research* 14, 415-421.
- Zentner RP, Brandt SA, Campbell CA. 1996. Economics of monoculture cereal and mixed oilseed-cereal rotations in west-central Saskatchewan. *Canadian Journal of Plant Science* 76, 393-400.
- Zhang QZ, Kullmann A, Geisler G. 1991. Nitrogen transportation in oilseed rape (*Brassica napus* L.) plant during flowering and early siliqua developing. *J. Agron. Crop Sci.* 167, 229-235.

Curriculum vitae

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