Understanding growth limitation in wheat and sunflower under low phosphorus conditions

Daniel Rodríguez

Promotor: **dr. ir. J. Goudriaan**
persoonlijk hoogleraar Theoretische Productie-ecologie

Co-Promotor: **dr. ir. W.G. Keltjens**
universitair hoofddocent bij het Departement
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Propositions

1. The definition of sinks in plants, as organs that receive nutrients or sugars from other tissues (i.e. sources), is useful for modeling the response of plant growth to nutrient deficiencies.
   This thesis

2. The general response of plants to phosphorus deficiency can not be explained alone by its effects on photosynthesis.
   This thesis

3. In wheat, the larger part of the negative effects of phosphorus deficiency on total leaf area can be explained by an impaired leaf emergence and tillering, in sunflower, phosphorus deficiency also limits leaf area expansion through the impairment of photosynthesis.
   This thesis.

4. Phosphorus deficiency increases the heterogeneity in the plant population with respect to plant size and structure. A larger heterogeneity of the individuals in a plant population will increase the expression of intra- and inter- specific competition in the crop.

5. Our present understanding of plant and crop growth allows us to improve upon those simulation attempts in which the specific leaf area (SLA) is assumed to be constant.
   This thesis.

6. There is agreement on the statement that there are no models without data,... however, it seems we have forgotten that ...there are no data without experiments.

7. In transition countries, the shift from command economies to free market economies has created massive opportunities for appropriation of rents, i.e. excessive profits, and has often been accompanied by a change from a highly organized system of corruption to a more chaotic and deleterious one.

8. Rationalism is futile for the essence. It is only useful to prove theorems or invent machines.
   Ernesto Sábato. Página 12, August 31, 1998.

9. The increase in the speed of communication and transport was necessary for the globalization of the economy, but it also led to the globalization of economic crises. Unfortunately, the understanding of our societies has not evolved that fast as computers and communications. It seems that our globalized society urgently needs more globalized education.

10. Here everybody talks about the weather, but nobody does anything about it.
    Said by Mark Twain
    Unfortunately he couldn't be more mistaken, we have been changing the weather already for a long time, and it seems we will continue doing so.

11. No man qualifies as a statesman who is entirely ignorant of the problems of wheat.
    Said by Socrates.

Propositions associated with the Ph.D. thesis of Daniel Rodríguez. Understanding growth limitation in wheat and sunflower under low phosphorus conditions. Wageningen Agricultural University, October 7, 1998.

Abstract

Rodríguez, D. **Understanding growth limitation in wheat and sunflower under low phosphorus conditions.** PhD thesis, Sub-department of Theoretical Production Ecology and Soil Science and Plant Nutrition, Wageningen Agricultural University, The Netherlands. 124 pp. English, Dutch and Spanish summaries.

The study described in this thesis focuses on the understanding of growth of leaf area and biomass in wheat and sunflower under low phosphorus conditions. Chapters 2 and 3 address the question whether P-deficiency limits leaf area expansion directly by inhibiting the individual leaf expansion, or through its effects on the availability of assimilates for growth. Experimental and simulation techniques were used to find an answer to this question. It was concluded that in sunflower the effects of P on sink size and assimilate supply were important for determining plant leaf area expansion (Chapter 2). Instead, wheat responded to a low P supply mainly by reducing sink size (Chapter 3). A sensitivity analysis of the effects of different model parameters at different levels of P supply, on the simulated total leaf area, showed that the value of the phyllochron, in wheat and sunflower, and tillering in wheat were crucial in determining the total leaf area. Chapter 4 focuses on the effects of P on the generation of leaves and tillers in wheat. Here, it was shown that the emergence of main stem leaves in wheat plants grown under P-deficient conditions was delayed (higher phyllochron) as both, the rate of leaf primordia initiation in the apex, and the rate of individual leaf expansion were reduced. In Chapter 5, the effects of P and assimilate supply on tillering were studied in a field experiment. P deficiencies directly altered the normal pattern of tiller emergence by slowing down the emergence of leaves on the main stem, and by reducing the maximum rate of tiller emergence. In general terms, assimilate supply seemed to play a minor role determining tillering. Including the effects of P on the phyllochron and on the maximum tillering rate in a morphogenetic model of the generation of leaves and tillers, it was possible to explain the dynamics of tiller emergence of a field experiment. In Chapter 6, a comparative study of the response to P in wheat and sunflower is presented. The different responses of wheat and sunflower to P were explained in terms of their capacity for P uptake, root to shoot ratio, and the response of photosynthesis to P. In Chapter 7, the results obtained in this thesis are discussed with respect to our present understanding of the processes involved in crops grown under P-deficient conditions. Finally, needs of research in P-limiting growth are identified and new avenues for future work discussed.

Key words: leaf expansion, modelling, phosphorus, photosynthesis, sunflower, tillering, wheat

PREFACE

During the last three years I have received a lot of backing from many people, however, I couldn't have ever finished this work without the companionship, love and intelligence of my wife Fernanda.

Familia, gracias por el apoyo que nos brindaron, y perdón por las preocupaciones que les hayamos ocasionado con nuestros viajes y estadías en el extranjero, ustedes saben que para nosotros ...ustedes son lo mejor!!

During my struggle at Wageningen, I have got huge support from my supervisors Jan Goudriaan, and Willem Keltjens. Thank you both for your enthusiasm and critical reading of many manuscripts. I also feel I am most deeply indebted to Fernando Andrade. Thanks for trusting me! I not only miss our scientific discussions, but also the friend I believe I have made.

I feel I must mention Prof. Daniel Cogliatti. Despite you have not been directly involved in my project I had always managed to discuss my results with you, one of the most intelligent persons I have ever met. Thanks for your inspiring comments, and you know I still look forward to do projects together.

I also want to say thanks to the people of the discussion group of the C.T. de Wit Graduate School for Production Ecology. Thanks to the PhD Sandwich Program for financing part of my stay at Wageningen and my trip to U.S.A.. Thanks Mr. Van Heijst for your diligence, and interest. I am also obliged to the co-authors of my papers. Thanks María Cristina Pomar, Mercedes Zubillaga and Edy Ploschuk for your contributions and outstanding technical skills and particularly, for your friendship.

Thanks to the people of Fertilidad y Fertilizantes (FAUBA), from where I have been absent quite for a while. Here I want to particularly thank Silvana Torri, thanks for your friendship and for being understanding. *Otro,.. grande Nidia!!, gracias por lavar las raíces y por los cafecitos.*

Since 1992, I have spent a lot of time at Theoretical Production Ecology, so I have quite a lot to say. First of all sorry for my dumbness with your language (Dutch), I will keep on trying. Anyway, I think I have learned a lot from you, I admire your organization and the way you work. I believe I have also made some good friends I will never forget you Jacaranda, Eddy, Leo, Cor, Bjørn, Jacco, Shana, Anne Marie, Joost, Reinoud,.... Here I have special thanks for Gon van Laar, thanks for your help with the outline of this thesis, Gon your stampot is great!!, you should come to Argentina for some *"asado"*. Thanks Jan van Kleef, Rob Dierkx and Aad van Ast for your help in the logistics, thanks Daniel van Kraalingen for your tips with FST, thanks Jacques Withagen for your outstanding help with the statistics and the programming of Genstat. Thanks Lien and Ria for your kindness. I would also like to thank Willem Stol and Hein ten Berge for your fishing lessons in the Dutch canals...I strongly believe that if we keep on trying... we will finally catch a fish!! Many super people have

passed by during my stay at T.P.E. Among them I have made a few good friends to whom I owe their companionship, particularly during those gloomy Dutch days, so thanks Marcos Silveira Bernardes, David Casanova, Imma Farré, Francesc Domingo, Hiroshi Nakagawa.

Finding a place to live in Wageningen was not easy, and dealing with the Woningstichting and the Gemeente was even a bit grisly,… thanks Jeanine Hermans and Ankie Lambers for coming into our rescue. So,… thank you all once again.

Daniel

A mi familia

List of abbreviations

AMAX	photosynthesis rate at high radiation	$[\mu mol\ CO_2\ m^{-2}\ s^{-1}]$
DAE	days after emergence	$[d]$
dd	degree days	$[°Cd]$
DR	dark respiration	$[\mu mol\ CO_2\ m^{-2}\ s^{-1}]$
LAI	leaf area index	$[m^2\ m^{-2}]$
LDUR	duration of leaf expansion	$[d]$
Leaf P%	concentration of P in individual leaves	$[\%]$
Leaves P%	averaged concentration of P in all the plant leaves	$[\%]$
LER	leaf expansion rate during the linear expansion phase	$[cm^2\ d^{-1}]$
LLER	leaf length expansion rate	$[cm\ dd^{-1}]$
MTR	maximum tillering rate	$[dd^{-1}]$
PAR	photosynthetic active radiation	$[MJ\ m^{-2}\ d^{-1}]$
PHY	phyllochron	$[dd\ leaf^{-1}]$
PHY-Haun	phyllochron as a function of the Haun index	$[dd\ leaf^{-1}]$
PHY-Ltip	phyllochron as a function of leaf tip emergence	$[dd\ leaf^{-1}]$
PHYtt	phyllochron as a function of cumulated thermal time	$[dd\ leaf^{-1}]$
QY	apparent quantum yield	$[\mu mol\ \mu mol^{-1}]$
RLER	relative leaf expansion rate	
RUE-PAR	radiation use efficiency for PAR	$[g\ MJ^{-1}]$
SLA	specific leaf area of the canopy	$[m^2\ g^{-1}]$
SLA_{nl}	specific leaf area of recently expanded leaves	$[m^2\ g^{-1}]$
T0, T1,.., Tn	main stem tiller number	
TDUR	tillering duration	$[d]$
TRATE	tillering rate	$[tillers\ m^{-2}\ d^{-1}]$
WSC	water soluble carbohydrates	$[\%]$

Contents

Chapter 1

General introduction

Because of its relative scarcity and its essential role in energy transformations in all forms of life, phosphorus (P) occupies a key place among the major nutrients. Under low soil P availability, biological productivity is limited and P nutrition must be restored by addition of mineral fertilizers or organic manure. However, abundant use of fertilizers has created considerable negative environmental side effects in some areas, whereas in other areas, under-use of external inputs has caused the exhaustion of the natural fertility of soils, and soil degradation. In many Western countries, the use of P fertilizers has become excessive resulting in an increased leaching of P to surface waters and in environmental pollution (Breeuwsma and Silva, 1993). In less developed economies, the net financial return of P fertilization is rather low and difficult to predict. In these countries, the farmers grow crops at the expense of the natural fertility of their soils because of financial uncertainty and lack of information.

To identify whether a certain nutrient limits crop yield is quite simple, if it does, fertilization will increase yields. However it is not that simple to identify the specific physiological basis of a nutrient-induced yield limitation. Alleviating nutrient limitations with a minimum use of fertilizers requires such identification, followed by beneficial modifications of the identified processes (Radin and Lynch, 1994).

Although a considerable amount of research has already been conducted on P nutrition and P fertilizer use, the mechanisms involved in the reduction of crop growth under P deficiency remain a matter of continual study. The main reason is the complexity of the system under analysis.

When complex systems are studied, the use of simulation models in combination with experimental work has proven to be the most efficient method of integrating information and problem solving (Barber, 1984; Hoffland et al., 1990; Bastiaans and Kropff, 1993). The use of simulation models in combination with experimental work could help to understand the effects of various P nutrition levels on different plant species and to find more efficient strategies for fertilizer use.

Background and problem definition

P limitation can restrict plant biomass accumulation by several interactive mechanisms (Osman et al., 1977; Halsted and Lynch, 1996). However, at the level of carbon economy, this general statement may be reduced to two main causes, (i) a reduction in radiation interception as a consequence of a smaller photosynthetic surface area, and (ii) a reduced efficiency of the transformation of the intercepted radiation into dry matter.

Effects of P deficiency on the size of the photosynthetic surface area
Reductions in the size of the photosynthetic area as a consequence of inadequate P availability have been mainly associated to, (i) smaller size of each individual leaf

(Radin and Eidenbock, 1984), and (ii) lower number of leaves per plant, particularly in those plants that produce tillers or branches (Cromer et al., 1993).

Leaf expansion: The final size of an individual leaf depends on both, the duration and rate of leaf expansion. The duration of the period of leaf growth expressed in thermal units, was shown to be little affected by nutritional factors (Ong and Baker, 1985). As a consequence, under conditions of sub-optimal nutrient availability, the rate of individual leaf expansion will be the main determinant of the final size of each leaf. Assuming that under P-deficient conditions leaf size is reduced (Radin and Eidenbock, 1984), the question whether the rate of individual leaf expansion is directly reduced, or whether a lack of assimilates at the plant level is the driving leaf area expansion is still not answered.

Leaf emergence and final number of leaves per stem: Morphogenetic descriptions of leaf area development can be expressed in terms of plastochrons. The term plastochron is used to describe the interval (in thermal units) between the inception of two successive leaf primordia in the stem apex (Esau, 1977). In cereals, between the initiation of a leaf in the apex and its emergence through the sheath of the preceding leaf there is a period of growth whose duration depends on the leaf expansion rate of the new leaf, and on the length of the sheath of the preceding one. These factors, along with the time when elongation is initiated determine the phyllochron, the interval (in thermal units) between the emergence of two successive leaves in the main stem. The number and rate of leaf primordia initiation in the apex has been shown primarily to depend on photoperiod (Rawson, 1971; Slafer and Rawson, 1994) and on vernalization (Flood and Halloran, 1984). However, there are contrasting results with respect to the effects of mineral nutrition factors on the rate of leaf emergence. Mineral nutrition appears to have larger effects on the value of the phyllochron than on the final number of leaves per stem (Rodríguez et al., 1994; Longnecker et al., 1993). As the value of the phyllochron not only depends on the plastochron, but also on the rate of leaf expansion, and as leaf expansion is severely reduced under a low P supply, it might be expected that P will affect leaf emergence more than the final number of leaves per stem.

Leaf and tiller synchrony: In winter cereals, tillering and tiller survival not only are important in determining plants leaf area, they also establish the final number of ears per plant (Ishag and Taha, 1974).

The timing of leaf and tiller emergence are closely related (Davies and Thomas, 1983). However, the mechanism underlying such a synchrony is not completely understood (Skinner and Nelson, 1994; Skinner and Nelson, 1995). Tiller emergence may be expressed in terms of phyllochrons. Depending on the species or cultivar (Rickman and Klepper, 1995), after the first tiller has emerged, every new tiller will appear approximately at intervals of two to three phyllochrons (Rickman et al. 1983).

Under field conditions the number of tillers per unit of ground area is severely reduced by nutritional disorders (Fletcher and Dale, 1974). Nutrient limitation could slow and/or inhibit the emergence of tillers either by slowing down the rate of leaf emergence (larger phyllochron), and/or by changing the synchrony. Longnecker et al.

(1993), observed that low nitrogen supply increased the value of the phyllochron, while having little effect on the synchrony. Despite changes in the rate of leaf emergence, and/or in the leaf-tiller synchrony might account for a delay on the emergence of tillers, they are not able to explain the reductions in the number of tillers per unit of area generally observed under P-deficient conditions.

Effects of P on photosynthesis
Phosphorus shortage decreases the maximum rate of both CO_2 and light-saturated photosynthetic rates, and the carboxylation efficiency for CO_2 assimilation (Terry and Rao, 1991; Lauer et al., 1989; Brooks, 1986). These authors also showed that in P-deficient plants both the photosynthetic rate and the stomatal conductance decreased, but the former was more affected than the latter, resulting in an increase in the internal CO_2 concentration. This suggests that mesophyll factors would be more sensitive to P than stomatal factors, and that water use efficiency would decrease with severe P-deficiency. This was also observed by Lauer et al. (1989) under extreme P deficiency conditions. In these experiments, low-P plants were generally grown under extremely low-P availability, so that their growth is generally reduced by 80-90% relative to the control plants. However, no effects of P limitation on photosynthesis were found when plants were grown in the field (Colomb et al., 1995), or in pots under mild-P stress conditions (Rodríguez et al., 1994). Under those conditions plant growth was more related to leaf area expansion and assimilate partitioning than to photosynthesis rate per unit of leaf area.

The rate of net photosynthesis is usually measured in the last expanded leaf. However, it is well-known that nutrient concentrations of leaves vary widely within the plant. For mobile nutrients such as P and N, concentrations are higher in the youngest leaves. Therefore, measurements of the photosynthesis rate on young leaves will not be appropriate to assess the effects of low P on the assimilate production at a whole-plant level (Lawlor, 1993; Rodríguez et al., 1994). P concentrations, and particularly under P-deficient conditions, can be expected to be lower in the older leaves having important consequences for photosynthesis and canopy assimilation.

Why wheat and sunflower?
Various reasons make wheat and sunflower two interesting crops to be included in this study. First of all, these crops are the most important ones grown in rotation, on originally low-P soils (about 1.700.000 ha), in the south-east and south-west of the Province of Buenos Aires (Argentina). Soils in this region are Mollisols in which soil organic matter content varies from 1 to 7% with annual precipitation, varying between 500 to 900 mm from the west to the east (Atlantic coast). Important differences between these two species are not only found with respect to their leaf and root morphology (Osaky et al., 1994), but also with respect to the photosynthetic characteristics of their leaves (Connor and Sadras, 1992).

The general objective of this thesis is to improve, and further develop our capability for understanding and simulating wheat and sunflower growth for conditions of low phosphorus availability. In this thesis, special focus was made on quantifying the effects of P on:

- Leaf initiation, leaf emergence and tillering;
- Individual leaf expansion;
- Assimilate production at leaf and canopy levels.

In this work, the following scientific questions were investigated: Which mechanisms at plant and crop levels should be invoked to explain the effects of a shortfall in the supply of P on the growth of wheat and sunflower plants?; Is it possible to separately quantify direct effects of P deficiency on leaf area expansion and those on the rate of assimilate production per leaf area?; Is the extent of these effects similar in wheat and sunflower?; Why is leaf emergence usually delayed under P-deficient conditions?; What is the nature of the resources limiting tillering in a low P environment?.
Specific objectives were:

- To describe the effects of P on the photosynthetic characteristics of wheat and sunflower leaves.
- To describe the effects of P on the expansion characteristics of wheat and sunflower leaves.
- To quantify the importance of assimilate production on the individual leaf expansion of wheat and sunflower plants grown under low P conditions.
- To describe the effects of P on the generation of the leaf area, i.e. leaf initiation, leaf emergence and tillering.

Outline of the thesis
The experimental results in this thesis were obtained from experiments conducted at: Facultad de Agronomía Universidad de Buenos Aires Argentina (FAUBA), Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Regional Balcarce Argentina (INTA-Balcarce), and at the Wageningen Agricultural University (WAU).
Chapters 2 and 3 focus on the question whether P deficiency limits leaf area expansion by directly inhibiting individual leaf expansion or whether the availability of assimilates for growth was also impaired at low P. These two chapters combine experimental data and results of simulation models developed to better understand the processes experimentally studied. Data in Chapter 2 were obtained from two experiments using the same hybrid of sunflower. A first experiment was conducted at Facultad de Agronomía, Universidad de Buenos Aires (FAUBA) during 1995, and a second one at the Wageningen Agricultural University (WAU) using the installations of Research Institute for Agrobiology and Soil Fertility (AB-DLO) Wageningen, during 1996. Chapter 3 deals with the same question in wheat. The experimental data in Chapter 3 were obtained during the experimental period at WAU, during 1996. Chapter 4 goes in more detail trying to understand the effects of P deficiency on leaf emergence and tillering in wheat. Data for Chapter 4 were obtained from two simultaneous experiments that were done at FAUBA in 1995. Chapter 5 describes results from a field study in which wheat was grown under different levels of P and assimilate supply, by varying the levels of P fertilization and incident radiation. The experiment in Chapter 5 was done at INTA-Balcarce during 1997. In chapter 6 a comparative study described the effects of P deficiency in the two crops taken as case study. Data for Chapter 6 were taken from the two experiments done at WAU in 1996. Finally Chapter 7 presents a general discussion of the main results obtained in these

experiments in the context of our present understanding of the processes involved in crops grown under P-deficient conditions. The FST source code, of the simulation models of Chapters 2, 3, and 5, are listed in the Appendix.

Chapter 2

Leaf area expansion and assimilate production in sunflower (*Helianthus annuus* L.) growing under low phosphorus conditions

Abstract   Reductions in leaf area and plant growth as a consequence of phosphorus (P) limitations have been attributed both to direct effects of P shortage on leaf expansion rate and to a reduced production of assimilates required for growth. Canopy assimilation and leaf area expansion are closely interrelated processes. In this work, experimental and simulation techniques were used to identify and study their importance in determining leaf area on sunflower (*Helianthus annuus* L.) growing under P-deficient conditions. Experiment 1 was done outdoors, in Buenos Aires, Argentina, and Experiment 2 in a glasshouse in Wageningen, The Netherlands. In both experiments, the effects of soil P addition on leaf emergence, leaf expansion, dry matter accumulation, and leaf photosynthesis of non water stressed plants grown in pots containing a P-deficient soil were studied. Before sowing the equivalent amounts of 0 to 600 kg of super phosphate ha^{-1} were added to the pots. Phosphorus deficiency delayed leaf emergence increasing the value of the phyllochron (PHY) by up to 76%, and the rate of leaf area expansion during the *quasi*-linear phase of leaf expansion (LER) was reduced by up to 74%, with respect to high-P plants. Phosphorus deficiency reduced the rate of light saturated photosynthesis per leaf area (AMAX) in recently expanded leaves by up to 50%, while at low levels of leaf insertion in the canopy AMAX was reduced by up to 85%, with respect to high P plants. The values of LER were related (r=0.56, P<0.05) to the mean concentration of P in all the leaves (Leaves P%) and not to the concentration of P at the individual leaf where LER was determined (r=0.22, P<0.4) suggesting that under P deficiency individual leaf expansion was not likely to be regulated by the total P concentration at leaf level. The values AMAX showed an hyperbolic relationship with Leaf P% (R^2=0.73, P<0.01, n=53) that saturated with values of Leaf P % higher than 0.22. A morphogenetic model of leaf area development and growth was developed to quantify the effect of assimilate supply at canopy level on total leaf area expansion. With such a model, the existence of direct effects of P deficiency on individual leaf area expansion were identified. However, it was calculated that under mild P stress conditions up to a 83% of the reduction in the observed leaf area was explained by the particular effects of P: on the rate of leaf emergence, on the duration of the linear period of leaf expansion, and on the value of AMAX. It was also calculated that the effects of P deficiency on the value of AMAX alone, explained up to a 41% of the observed reductions in total leaf area between the highest and intermediate P level at Exp. 2. Possible mechanisms of action of the direct effects of P on individual leaf expansion are discussed in this work.

Abbreviations: AMAX - Photosynthesis rate per unit of leaf area at high radiation, DAE - days after emergence, LDUR - duration of leaf expansion, Leaf P% - individual leaf P concentration, Leaves P% - averaged P concentration in all the plant leaves, LER - leaf expansion rate during the linear expansion phase, PHY - phyllochron, RLER - relative leaf expansion rate, SLA - specific leaf area considering all plant leaves

Introduction

Phosphorus is the main environmental factor controlling crop growth and yield in many regions of the world (Constant and Sheldrick, 1991). Phosphorus deficiency reduces plant growth by reducing leaf emergence (Cromer et al., 1993; Rodríguez et al., 1994), leaf expansion (Cromer et al., 1993;) and the light-saturated rate of photosynthesis per unit leaf area (Rao and Terry, 1989; Jacob and Lawlor, 1991; Kirschbaum and Tompkins, 1990). When phosphorus availability to the plant is limited, growth is generally more reduced than the photosynthesis rate per unit leaf area (Terry and Rao, 1991). This would suggest that under P-limiting conditions, the availability of assimilates, at least at the leaf level, might not be the main factor responsible for reductions in leaf area and plant growth. Direct effects of P supply on leaf expansion have also been postulated. Radin and Eidenbock (1984) observed that P deficiency decreased the hydraulic conductance of cotton roots, and suggested that this would lead to reduced cell turgor and inhibited leaf area expansion. Alternatively, Fredeen et al. (1989) suggested that reduced leaf expansion under conditions of P deficiency might be related to a specific effect of phosphate on the expansion of epidermal cells.

The final area of each individual leaf of a plant is the integral of the leaf expansion rate over the duration of expansion. Under conditions of P deficiency, reduction in leaf area in seedling trees of gamelia, was primarily mediated through limitations of the rate, while the duration of leaf expansion changed little (Cromer et al., 1993). Similar results were observed in sunflower growing under low nitrogen conditions (Trapani and Hall, 1996).

During the period of *quasi*-linear increase in leaf area, the rapid expansion of cells is mainly driven by the accumulation of water in the vacuoles, the synthesis of osmotically active compounds, and constituents of cell walls, membranes and organelles. Particularly during the initial phase of leaf unfolding, most of the required substrate for leaf growth is imported into the newly developing leaves from expanded leaves (Kriedemann, 1986). Lack of assimilates reduced the number of cells per leaf, and consequently the potential leaf area in beans (Dale, 1976), barley (Gallagher, 1985) and sunflower (Takami et al., 1982). Consequently, in addition to any direct effect of P on leaf expansion, its deficiency will also limit leaf area expansion through an inadequate supply of substrate for structural components of the developing leaves.

The most important constraint to the development of comprehensive simulation models that predict plant growth under conditions of P limitation has been to identify and to quantify the processes affected. Particularly, little is known about the relative effect of a lack of assimilates due to the impairment of photosynthesis under P limitation, and the direct effect of P shortage on leaf expansion. The aim of this work was (i) to describe the effects of P shortage on leaf area expansion of sunflower plants, and (ii) to determine the proportion of the observed reduction in leaf area that can be explained by a limited availability of assimilates, and/or by direct effects of P deficiency on the characteristics of leaf expansion of sunflower plants.

Material and methods

Two experiments were carried out with sunflower (*Helianthus annuus* L.) using the commercial hybrid Paraiso 5 of Nidera S.A. Experiment 1 was conducted in Buenos Aires, Argentina, while Experiment 2 was carried out in Wageningen, The Netherlands. In both experiments, the effects of plant P nutrition on leaf emergence, leaf growth, and leaf photosynthesis were determined. A simulation model was developed to simulate the results and to study the importance of different processes affected by P deficiency on leaf area expansion and dry matter production.

Experiment 1
Cultural techniques and growth conditions
Seeds of sunflower were sown on 14.12.95 and grown outdoors in pots at the Facultad de Agronomía, Universidad de Buenos Aires, Argentina (34°35' S, 58°29' W). Plants were grown in 100 L containers (0.58 m diameter and 0.40 m deep), filled with a (1:1; v/v) mixture of a sandy soil containing 17 mg P kg⁻¹ (Bray and Kurtz, 1945) and washed sand containing a negligible amount of P. Temperature and radiation data during the experiment were obtained from a meteorological station located at the experimental site.

Treatments and experimental set-up: Equivalents of 0 (P1), 15 (P2), 30 (P3), 90 (P4), 150 (P5) and 600 (P6) kg of super phosphate ha⁻¹ were thoroughly mixed with soil within the upper 10 cm of each container before sowing. In addition to the P fertilization, all pots received the equivalent of 600 kg N ha⁻¹ (as urea) split in two applications, half at sowing and half when the plants had three expanded leaves. All containers were watered daily. Other macro and micro elements were applied once at sowing. Treatments were randomized within each of three blocks. Containers were arranged 1 m apart from each other. Within each container three plants were grown individually at 0.3 m distance.

Determinations and measurements: Emergence of new leaves (leaf area > 2 cm²) was recorded every two days. The phyllochron (PHY) for each treatment was calculated from the relationship between cumulative number of leaves (> 2cm²) and cumulated thermal time using a base temperature of 4°C (Connor and Sadras, 1992). Leaves were numbered upwards from 1, oldest, to the last appeared leaf, youngest. Width (w, cm) of two leaves per plant, leaves 5-6, 11-12 and 19-20, were recorded daily and the corresponding individual leaf areas (A, cm²) were calculated by using the relationship given by Pereyra et al. (1982).

$$A = 0.80 + 0.69 \bullet w^2 \qquad \text{if } w \leq 21\text{cm} \qquad (2.1a)$$

$$A = 4.297 \bullet w + 0.565 \bullet w^2 - 15 \qquad \text{if } w > 21\text{cm} \qquad (2.1b)$$

When the leaves reached 90-100% of their final area, i.e. 17 days after emergence (DAE) for leaves 5-6, 37 DAE for leaves 11-12, and 56 DAE for leaves 19-20, the light-saturated photosynthetic rate (AMAX) in those leaves were measured. The rate of

photosynthesis at light saturation (1900-2000 μmol m^{-2} s^{-1} PAR) was measured, using a portable photosynthetic system Licor 6200 (Li-Cor, Nebraska, USA). Subsequently, the shoot part of the plants was harvested. At each harvest, the pair of leaves where AMAX was measured was separated for P analysis, while the remaining tissue was divided into stems plus petioles, leaves and heads. Then, leaf area and dry matter were determined.

The leaf expansion rate during the *quasi*-linear expansion phase (LER) was calculated by linear regression from plots presenting leaf area versus days after leaf emergence (> 2cm^2). Total-P concentration in the leaves was determined by the colorimetric molybdenum-blue method after wet digestion in a H_2SO_4-Se-salicylic acid mixture with addition of H_2O_2.

Experiment 2
Cultural techniques and conditions
Plants of sunflower were grown in pots with natural radiation in a glasshouse in Wageningen, The Netherlands (51° 58' N, 5° 40' E). The 7.5 L pots (0.19 m diameter and 0.27 m deep) were filled with a sandy soil containing 3 mg P kg^{-1} (Bray and Kurtz, 1945). The centres of the pots were 0.33 m apart on the same line and 0.5 m apart between lines, simulating a canopy of a plant density of 6 plants m^{-2}. Test plants were surrounded by plants of the same treatment as a border. Three seeds were sown per pot on 29 May 1996 and thinned to one at the two-leaf stage. Plants were watered twice or three times a week to maintain an adequate soil moisture level in the soil throughout the experimental period. Daily maximum and minimum temperatures were measured inside the glasshouse, and radiation data were obtained from a meteorological station located 200 m from the experimental site.

Treatments and experimental set-up: Three levels of P, equivalent to 15 (P1), 60 (P2), and 300 (P3) kg ha^{-1}, were applied as super-phosphate (ground in mortar). With the two lowest levels, P was incorporated at once into each pot before sowing as in Exp. 1. The highest doses (P3) was split, half applied and incorporated before sowing and half applied on the soil surface at the 5 leaf stage. All pots received a dressing of macro and micronutrients at the time of sowing. The equivalent of 400 kg N ha^{-1} (as NH_4NO_3) was distributed during the development of the plants. Treatments were randomized within each of the three blocks.

Determinations and measurements: During the growing period, leaf emergence was recorded at two-day intervals, and the leaf area and LER of individual leaves was determined as in Exp.1. Leaf expansion rate was monitored with leaves 7, 8, 11, 12, 15 and 16. As in Exp. 1, AMAX was determined when each of those leaves reached their maximum size, then the plants were harvested immediately for dry matter and leaf-P analysis. At each harvest, the leaf area of each individual leaf was determined using a LI-3100 area meter (Li-Cor, Nebraska, USA). Additionally leaf photosynthesis and leaf P content were determined in those leaf insertion numbers where LER and AMAX had been measured in previous harvests, i.e. AMAX and leaf P were measured in leaves 7 and 8 at harvest 1, leaves 7, 8, 11 and 12 at harvest 2, and leaves 7, 8, 11, 12, 15 and 16 at harvest 3. AMAX was determined using a portable photosynthesis system

LCA-2 System ADC (Analytical Development Co. Ltd.) in combination with a lamp (Philips Projection Lamp 6853, 75 W) installed over the leaf chamber resulting in a PAR of 2000 μmol m^{-2} s^{-1}.

In this experiment, LER and duration of leaf expansion were calculated using an optimization model (eq. 2.2) that fitted the experimental data iteratively by means of curve-fitting software (Jandel Scientific, Erkrath, Germany),

$$y = a + b \bullet x \qquad \text{if } x \leq c \qquad (2.2a)$$
$$y = a + b \bullet c \qquad \text{if } x > c \qquad (2.2b)$$

where y is total leaf area [cm^2], a the y-axis intercept, b the value of LER [cm^2 d^{-1}], x is the time since leaf emergence [d], and c the time when leaf expansion stopped [d]. The duration of the *quasi*-linear leaf expansion (LDUR, d), was calculated as:

$$LDUR = c + (a/b) \qquad (2.3)$$

Leaves 7-8 were separately harvested at 46 DAE in all treatments and also at harvests 2 and 3. Due to observed differences in leaf emergence and the time the leaves reached final size among the P treatments, leaves 11-12 had to be harvested at 60 DAE in P1, and at 53 DAE in P2 and P3, and leaves 15-16, at 66 DAE in P1 and at 60 DAE in P2 and P3. Dried plant material was wet digested in a H_2SO_4 / salicylic acid / H_2O_2 / selenium mixture concentrations of total N and P were colorimetrically measured in the digests using an automated continuous-flow system.

Differences among values of PHY and final leaf number were tested by the Tuckey test (P=0.05) after ANOVA. Treatment effects on LER were tested by ANOVA using Sigma Stat (Jandel Scientific, Erkrath, Germany).

Simulation model
The model calculates actual leaf area expansion by comparing the potential plant leaf expansion rate (cm^2 plant^{-1} d^{-1}) with the actual availability of assimilates for leaf dry weight growth. The potential plant leaf expansion rate is calculated morphogenetically as the sum of the expansion rates of individual leaves that are expanding at any time (LER, cm^2 leaf^{-1} d^{-1}). The model identifies three groups of leaves: (i) leaves being in the lag phase of expansion, (ii) leaves that are expanding rapidly (*quasi*-linear phase), and (iii) fully expanded leaves. After emergence leaves expand slowly (2 cm^2 day^{-1}) during the lag phase that lasts for 100 degree days (dd), then a rapid *quasi*-linear expansion starts. During this last period LER is calculated as a function of the potential for expansion of every leaf at optimum temperatures (24°C) according to leaf position, and the mean daily air temperature (Villalobos et al., 1996).

Maximum and minimum values of specific leaf area for the newly expanding leaves (SLA$_{nl}$, m^2 g^{-1}) were tabulated to calculate the minimum and maximum leaf growth, respectively (Villalobos et al., 1996). Minimum values of SLA$_{nl}$ were taken from observed values of high P plants, and maximum values of SLA$_{nl}$ were considered to be 30% higher, as observed at Exp. 2.

Assimilate production is calculated using the subroutines of the model SUCROS (Goudriaan and van Laar, 1994), modified to account for a gradient of AMAX within the canopy as a function of cumulative leaf area index (LAI). The cumulative leaf area was calculated by adding the areas of all the leaves downwards from the top of the canopy, at each harvest. In the model, P limitation is assumed to affect canopy assimilate production by affecting AMAX. The effects of P shortage on AMAX at different heights within the canopy were included in the model from observed AMAX as a function of the cumulative LAI from the top of the canopy.

In the model, the apparent quantum yield (QY, μmol CO_2 m^{-2} s^{-1} (J m^{-2} s^{-1})$^{-1}$), was assumed not to be affected by P, and was taken from Connor and Sadras (1992). Scattering coefficient varies with LAI as in Villalobos et al. (1996). Coefficients for maintenance respiration and effects of temperature on photosynthesis were taken from Horie (1977), coefficients for growth respiration were taken from Penning de Vries et al. (1989). Growth and maintenance respiration are assumed to be not affected by P-deficiency.

The model also calculates the intensity of incident radiation, and the proportion of incident radiation that is diffuse at different depths within the canopy. Canopy assimilation rate is calculated by accumulating the instantaneous assimilation rates over three layers and integrating the instantaneous rates over the day (Hammer and Wright, 1994), by using the three-point Gaussian method (Goudriaan, 1986). Partitioning coefficients and the fraction of leaves that are senescent were introduced as forcing functions from the observed data of each P treatment. The model was written using the programming environment denominated Fortran Simulation Translator 2.0 (Rappoldt and van Kraalingen, 1996), and has a daily time step of integration. Inputs include a function for the reduction of AMAX with cumulative LAI, PHY, LDUR, forcing functions for partitioning and senescent leaf area, daily maximum and minimum temperatures, daily total radiation and latitude. Some of the outputs of the simulation program are, plant leaf area (TLA, cm^2 pl^{-1}), plant dry weight (TDW, g pl^{-1}), specific leaf area of the whole canopy (SLA, m^2 g^{-1}), and radiation-use-efficiency (RUE, g MJ^{-1}).

Results

Weather
Figure 2.1 summarizes solar radiation and temperature during the periods of Exp. 1 and Exp. 2. During Exp. 1 the mean daily temperature was 24.4 °C with an absolute maximum of 37 and minimum of 9 °C. Average daily total radiation was 21.6 MJ m^{-2} d^{-1}. In Exp. 2 the mean daily temperature in the glasshouse was 20.4°C with extreme temperatures of 38 and 6°C; the mean daily radiation outside the glasshouse was 18.2 MJ m^{-2} d^{-1}, with a measured transmission through the roof of 80%. The roof of the glasshouse did not change the quality of the light.

(a)

(b)

Days after emergence [days]
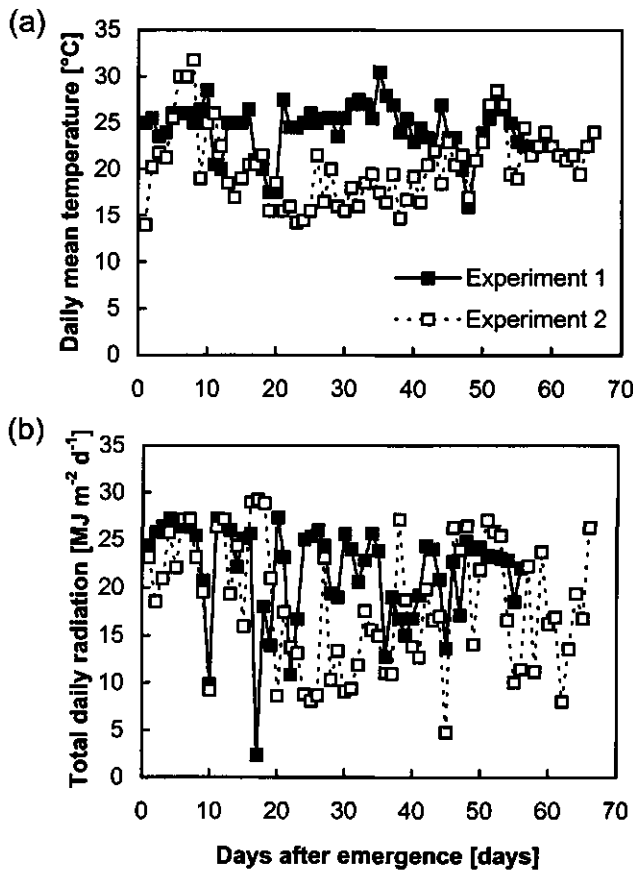
Figure 2.1 Daily mean temperature (a) and total daily radiation (b) during Exp. 1 (Argentina, outdoors), and Exp. 2 (The Netherlands, glasshouse).

Plant growth
In both experiments, the plants reached the stage of bud visible. Experiments 1 and 2 differed mainly with respect to the degree of P shortage developed by the plants. In Exp.1, P deficiency was mild and differences among P treatments in plant shoot dry weight and total leaf area were statistically significant ($P<5\%$) only between P6 and P1 at the first harvest (Fig. 2.2a, c). In Exp. 2 differences among P treatments were significant at all three harvests (Fig. 2.2b, d). Therefore focus will be on the results from Exp. 2, and data from Exp.1 will be introduced to give a broader range of results when required.

At harvest 3 in Exp. 1, plant leaf area and shoot dry weight at the lowest level of P supply were, respectively, 9 and 13% lower than the control plants (P6). At harvest 3 in Exp. 2, total leaf area and shoot dry weight of P2 plants were, respectively, 48 and 58% less than the control plants (P3), and P1 plants were 77 and 81% less than P3 plants. In Exp. 2, plants reached LAI values of 1.5 at P3, 0.7 at P2, and 0.34 at P1.

(a)

(b)

(c)

(d)
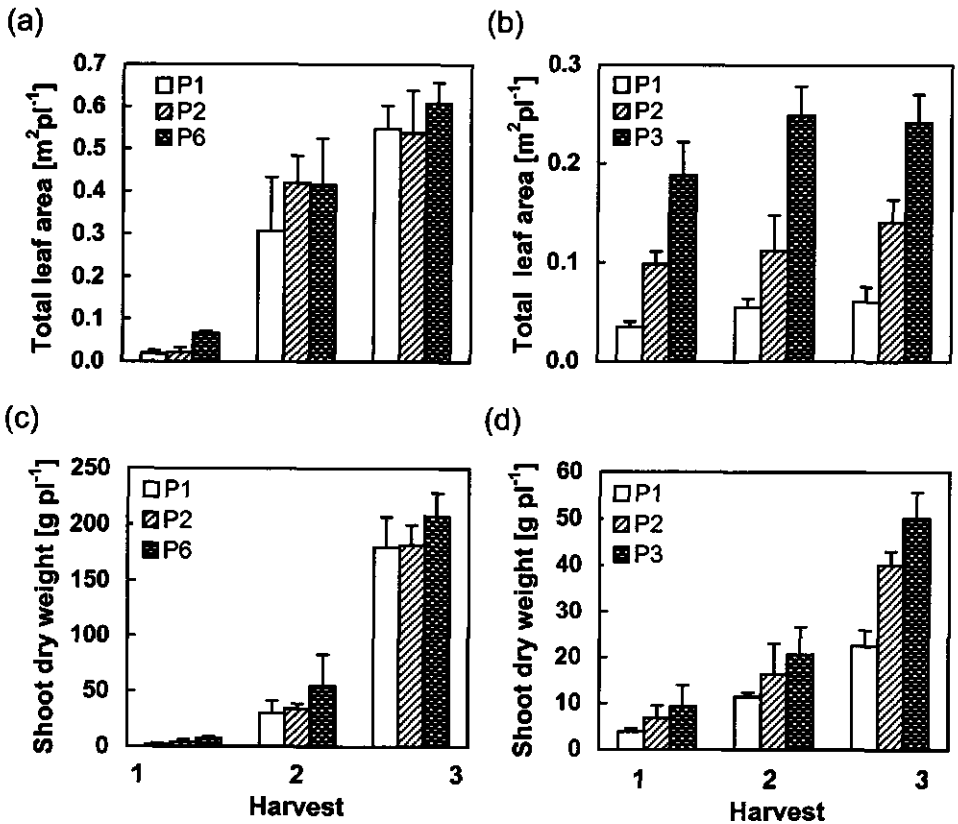
Figure 2.2 Plant leaf area (a, b), and shoot dry weight (c, d) of treatments P1, P2 and P6 in Exp. 1(a, c), and for treatments P1, P2 and P3 in Exp. 2 (b, d). Bars represent standard errors.

Leaf area development
In Exp. 1, the P treatments did not significantly affect the PHY after leaf 5 had appeared or the total number of leaves per plant (Table 2.1). In Exp. 2, PHY was significantly longer in conditions of P deficiency, and the number of leaves for P3 and P2 plants was higher than at P1. In Exp. 2, in P1 PHY was 21% longer than in P2 plants, and 76% longer than in control plants (P3). Furthermore, on average the first five leaves showed a longer PHY than did subsequent leaves, e.g. 44 and 50 degree-days in Exp. 1 and Exp. 2, respectively. The longer of PHY for P1 plants in Exp. 2 delayed the time of harvest for that treatment. On average the final number of leaves per plant in Exp. 1 was 27, while in Exp. 2, the P2 and P3 treatments produced more leaves than did P1 plants.

Individual leaf expansion
Area of individual leaves increased with time, and their final size was strongly affected by the P level, particularly in Exp. 2 (Fig. 2.3). Despite of differences in environmental and experimental conditions between the two experiments, the values of LER for corresponding leaves were relatively similar (Table 2.2).

Table 2.1 Value of the phyllochron after emergence of leaf five, and the total number of leaves per plant at the final harvest. Different letters within the same experiment indicate significant differences by a Tuckey test (*P<0.05*) after ANOVA.

	Experiment 1		Experiment 2		
	P1	P6	P1	P2	P3
Phyllochron [degree days]	30.3 a	26.6 a	68.2 b	47.0 a	38.6 a
Final number of leaves	24.0 a	29.0 a	21.0 b	24.3 a	26.0 a
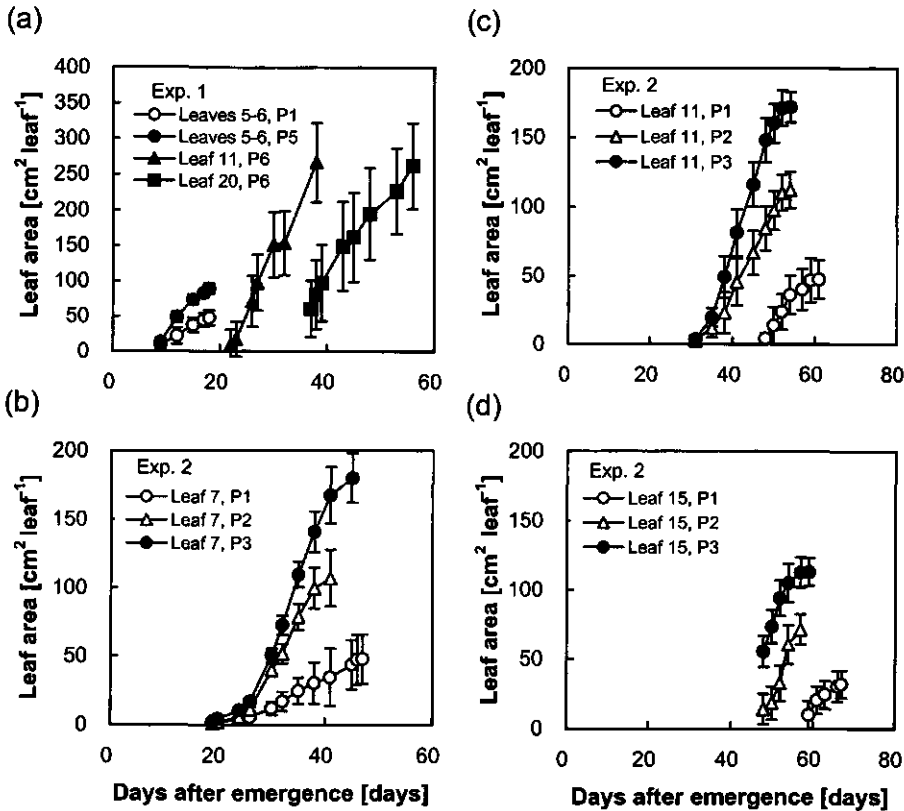
Figure 2.3 Areas of individual leaves in Exp. 1 (a) and Exp. 2 (b, c and d). Bars indicate standard errors.

In both experiments, maximum LERs were observed for leaves 11 and 12. In Exp. 1, LER was significantly reduced by P limitation in leaves 5 and 6 (*P<0.05*), their values are presented as an average, (Table 2.2). In Exp. 2, differences in LER among P treatments were statistically significant for leaves 7 (*P<0.01*), 8 (*P<0.05*), 11 (*P<0.05*), and 15 (*P<0.01*). In Exp. 2, the duration of the lag phase prior to the rapid linear growth phase lasted 5 days (c.v.=30%) and was not affected by the P treatments. However, P deficiency significantly decreased the duration of the *quasi*-linear leaf

expansion phase (LDUR) for leaves 11 and 12 (Table 2.2). In Exp. 1, LDUR was not calculated because the leaves were harvested before reaching 100% of their final size.

Table 2.2 Leaf expansion rate (LER; cm^2 d^{-1}) and duration of the *quasi*-linear leaf expansion phase (LDUR; days), in sunflower growing at different levels of P supply.

	Leaf expansion rate (LER; cm^2 day^{-1})					
Exp.1						
	Leaves 5-6	Leaf 11	Leaf 12	Leaf 19	Leaf 20	
P1	3.4	19.0	26.3	20.5	16.8	
P2	8.2	19.4	26.9	20.2	20.2	
P6	9.8	16.4	15.2	16.8	16.1	
s.e.m.	1.5	3.6	5.7	5.2	5.1	
Exp. 2						
	Leaf 7	Leaf 8	Leaf 11	Leaf 12	Leaf 15	Leaf 16
P1	2.9	3.2	10.6	-	8.4	3.0
P2	7.9	7.1	15.9	20.0	23.4	9.4
P3	11.2	10.1	26.0	24.8	17.3	9.6
s.e.m.	0.9	1.6	1.8	2.5	0.2	3.1

	Duration of the *quasi*-linear expansion phase (LDUR; days)					
Exp. 2						
	Leaf 7	Leaf 8	Leaf 11	Leaf 12	Leaf 15	Leaf 16
P1	13.7	15.1	8.0	6.9	-	-
P2	12.4	11.1	11.0	8.1	-	-
P3	15.9	14.1	16.0	13.7	-	-
s.e.m.	1.9	2.7	1.2	0.8	-	-

Leaf photosynthesis
In Exp. 1, the values of AMAX were not affected by P treatments and were somewhat higher than those observed in high P plants of Exp. 2. In Exp. 2, the imposed P treatments strongly affected the photosynthetic activity of the leaves, particularly in those leaves low in the canopy (Fig. 2.4). Relative to the AMAX at the top of the canopy in P3 plants, AMAX at the top of the canopy was reduced by 10% in P2 plants, and by 50% in P1 plants. Furthermore, AMAX decreased much faster from the top to the bottom leaves in P-deficient than in control plants (Fig. 2.4). At low levels of leaf insertion in the canopy the reduction in AMAX was 85% for P2 and 46% in P1 plants, compared to corresponding leaves of P3 plants. Interestingly, the relationship between the relative leaf P concentrations and the relative values of AMAX followed a similar pattern for the three levels of P supply (Fig. 2.4).

Phosphorus nutrition in relation to LER and AMAX

Correlation analysis: The correlation analysis presented in Table 2.3 indicates a strong positive relationship between AMAX and the P status of individual leaves, both on a weight (Leaf P%), and on a leaf area basis (SLP, μg P cm^{-2}). LER was not related to the P concentration of the individual leaf (Leaf P%), but was to SLP ($P<0.05$) and the mean P concentration of all plant leaves (Leaves P%) ($P<0.05$).
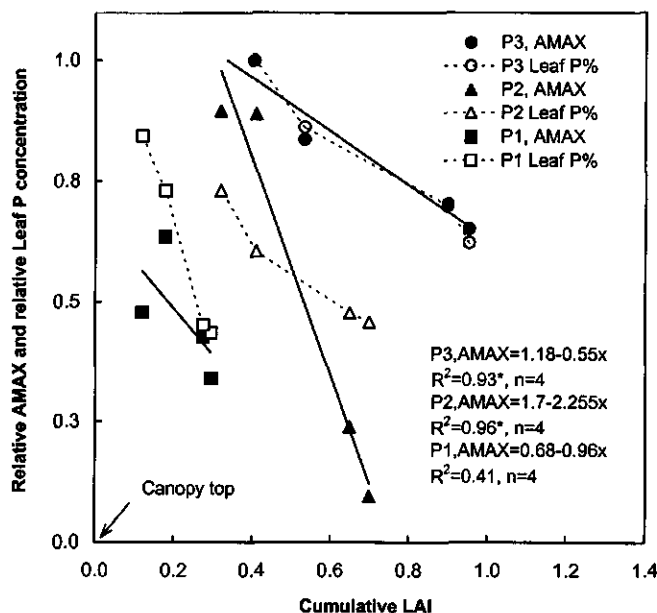
Figure 2.4 Relative AMAX (solid lines) and Leaf P% (dotted lines) with respect to their values at the top of the canopy in P3 plants, versus the cumulative LAI from the top of the canopy for treatments P1, P2 and P3. Data from Exp. 2.

The specific leaf area of the newly expanded leaves (SLA_{nl}), was higher for plants with P deficiency and as a consequence this leaf characteristic was negatively related to the P status of the plant. AMAX of a given leaf was not related to values of SLA_{nl} for the same leaf.

The value of LER was associated with Leaves P%, but different leaf cohorts showed different responses to this variable (Fig. 2.5a). When the values of LER were plotted relative to the LER of P3 plants of each leaf cohort (*RLER*), a significant relationship was obtained with Leaves P%, (Fig. 2.5b, eq. 2.4). The data point for leaf 16 at 0.06%P in Figure 2.5b is difficult to explain and, given the position of surrounding points for all treatments it was chosen to ignore it when fitting the response curve of RLER to Leaves P%.

$$RLER = 1 - \{\exp[-24.8 \pm 3.4(\text{Leaves P}\% - 0.046 \pm .003)]\} \qquad (2.4)$$

$$R^2 = 0.94**, n = 13$$

From the fitted function RLER is zero when the threshold value of Leaves P% was 0.046%P, and the critical Leaves P% for RLER, i.e. the Leaves P% where RLER is 90% of the maximum, was 0.14%.

Taking into account the data from Exp. 2, in recently expanded leaves, there was little variation in Leaf P% among P treatments, e.g. from 0.12 to 0.23% (Fig. 2.6a), while Leaves P% varied fourfold, e.g. from 0.05 to 0.2% (Fig. 2.5). In older and senescent leaves, leaves were AMAX was measured in previous harvests, Leaf P% varied among treatments with a factor of 2, e.g. from 0.075 to 0.15 % (Fig. 2.6a).

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Combining the data from both experiments and including recently expanded and older leaves, AMAX showed an hyperbolic relationship with Leaf P% (Fig. 2.6a, and eq. 2.5). From the fitted function AMAX was zero when the threshold value of Leaf P% was 0.08%P, and the critical Leaf P% for AMAX, i.e. the Leaf P% where AMAX is 90% of the maximum, was 0.22%.

$$AMAX = 37.3 \pm 1.6\{1 - \exp[-16.9 \pm 3.6(LeafP\% - 0.08 \pm 0.006)]\} \qquad (2.5)$$
$$R^2 = 0.73**, n = 53$$

Specific leaf nitrogen (SLN) in recently expanded and older leaves was between 0.17 and 0.24 mg N cm^{-2}. No relationship was observed between AMAX and SLN (Fig. 2.6b).

Modelling
A simulation model was developed to simulate the results of Exp. 2, and to test whether leaf expansion was reduced by direct effects of P limitation, by a lack of assimilates required for leaf growth or both. In order to test the performance of the morphogenetic model the rate of plant leaf area expansion (cm^2 $plant^{-1}$ d^{-1}) was calculated and daily integrated, by using the observed values of LER for the individual leaves as an input. When the observed LER was used as an input, the model described well the observed values of leaf area, particularly with P3 and P2 (dashed lines in Fig. 2.7a). When the model calculated plant leaf area as a function of the potential leaf expansion rate and the supply of assimilates from photosynthesis, simulated results also agreed well with observed results at P3 and P2, but overestimated the values with P1. At P3 the model also explained well the production of total dry matter and its partitioning among the different plant organs (Fig. 2.7b). Furthermore, for both P3 and P2 treatments, the model satisfactorily described the observed specific leaf area at canopy level (SLA, m^2 g^{-1}) (Fig. 2.7c). With P1 the simulation model overestimated the final leaf area by about a 60% (continuous lines in Fig. 2.7a).

The simulation model was used to estimate the proportion of the reduction in total leaf area in treatment P2 at Exp. 2, that could be explained by a lack of assimilates and by direct effects of P limitation on leaf expansion. Table 2.4 presents the observed total leaf area at P3 and P2 (Exp. 2), and the simulated total leaf area in P2 plants calculated as a function of the effects of P deficiency on AMAX, PHY and LDUR (P2[a]), and as a function of the effects of P deficiency only on AMAX (P2[b]).

Table 2.4 shows that the effects of P on AMAX explained 42% of the observed reduction in total leaf area between P3 and P2 plants, it was also calculated that direct effects of P deficiency on leaf area expansion explained a 17% of the observed reduction in total leaf area between P3 and P2 plants.

Table 2.3 Correlation coefficients between different plant characteristics.

	Leaves P%	Leaf P%	SLP	SLA$_{nl}$	AMAX	LDUR
LER[1]	0.56	0.22	0.54	-0.62	-0.177	-0.02
p.[2]	0.019	0.4	0.025	<0.01	0.49	0.9
n.[3]	17	17	17	17	17	11
LDUR[4]	0.52	0.16	0.24	-0.13	0.59	1
p.	0.078	0.62	0.45	0.66	0.04	
n.	12	11	12	12	12	
AMAX[5]	0.3	0.79	0.69	0.21	1	
p.	0.25	<0.01	<0.01	0.41		
n.	17	30	30	17		
SLA$_{nl}$[6]	-0.63	-0.71	-0.76	1		
p.	<0.01	<0.01	<0.01			
n.	17	17	17			
SLP[7]	0.5	0.5	1			
p.	0.03	0.03				
n.	17	17				
Leaf P%	0.29	1				
p.	0.25					
n.	17					

[1] LER   = Leaf expansion rate during quasi-linear phase [cm^2 leaf^{-1} day^{-1}]
[2] p.   = Level of significance
[3] n.   = number of data points
[4] LDUR   = Duration of the quasi-linear phase of leaf expansion [days]
[5] AMAX = Photosynthesis at high radiation [μmol CO_2 m^{-2} s^{-1}]
[6] SLA$_{nl}$ = Specific leaf area of recently expanded leaves.
[7] SLP   = Specific leaf phosphorus in the leaves [μg P cm^{-2}]

Sensitivity analysis

An additional study of several model variables related to leaf area expansion and crop dry matter accumulation was performed through an analysis of sensitivity. The sensitivity of three output variables: total leaf area (TLA, cm^2 plant^{-1}), total dry weight (TDW, g plant^{-1}), and specific leaf area (SLA) to $\pm10\%$ changes in the value of the parameters (i) AMAX at the top of the canopy, (ii) the apparent quantum yield (QY), (iii) the duration of the linear expansion phase (LDUR) and (iv) the phyllochron (PHY) was performed for treatments P3 and P2 (Table 2.5). A sensitivity coefficient was calculated as $SC = (\Delta V/V) / (\Delta p/p)$, where V and p are the output variables and parameters, respectively (Thornley and Johnson, 1990). Values of SC higher than 1 or lower than -1 indicate a high sensitivity, while a SC between 0.5 and -0.5, would indicate a low sensitivity of the variable to changes in a certain parameter.
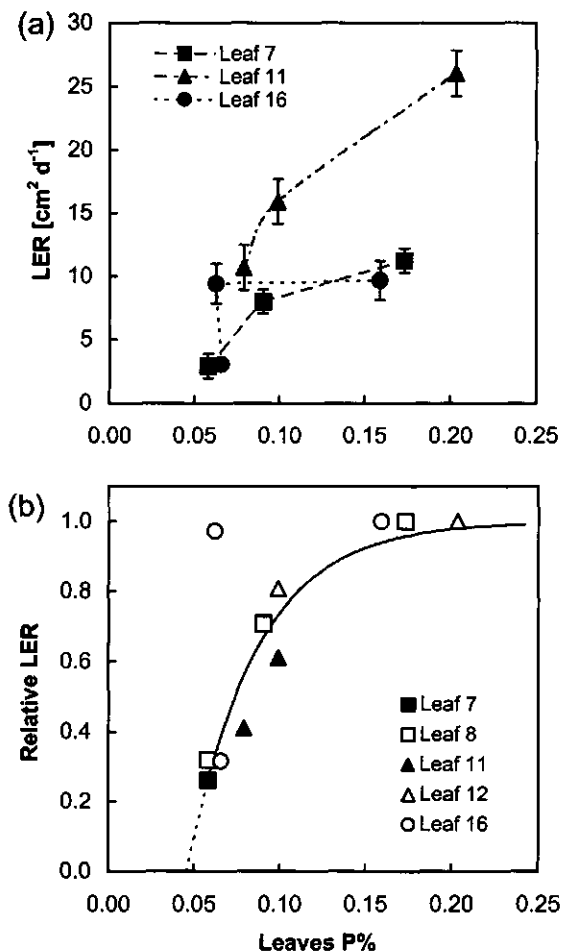
Figure 2.5 Rate of leaf expansion (LER) (a), and relative rate of leaf expansion (b) with respect to values of LER of the same leaf cohort in P3 plants, during the quasi-linear phase for different leaves from Exp. 2, versus the value of Leaves P%. Bars in (a) indicate standard errors.

Total dry weight was the most sensitive variable to changes in AMAX, EFF, and PHY, particularly for treatment P2. The total leaf area showed relatively more sensitivity to changes in AMAX and QY, while the SLA showed little sensitivity to changes in the model parameters.
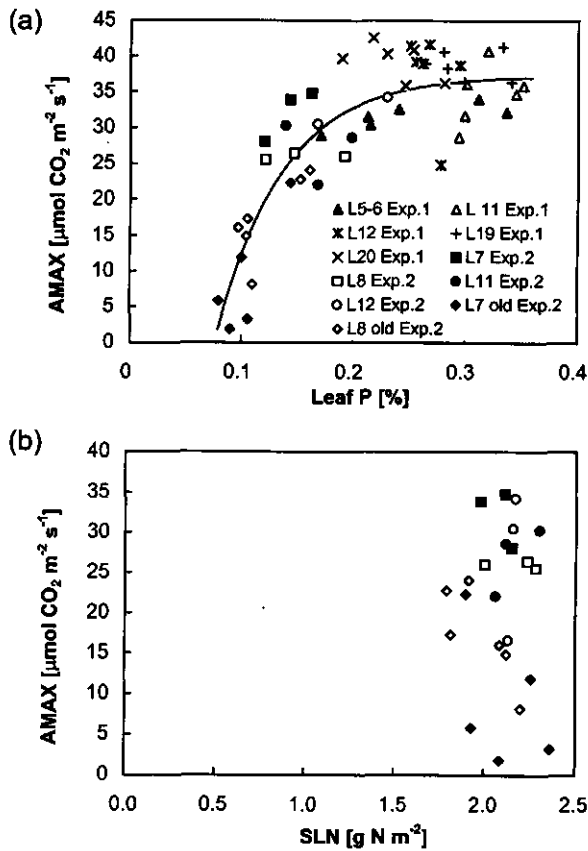
Figure 2.6 Values of light-saturated photosynthesis rate per unit of area (AMAX) of different leaves in Exps. 1 and 2 as a function of Leaf P% (a), and (b) specific leaf nitrogen concentration in Exp. 2.

Table 2.4 Observed total leaf area on treatments P3 and P2 at Exp. 2 and % of change in total leaf area between P3 and P2. Simulated total leaf area and percentage of the reduction in total leaf area of P2 plants explained by the models taking into account: the effects of P deficiency on AMAX, PHY and LDUR (P2[a]), and taking into account only the effects of P deficiency on AMAX (P2[b]). Estimated direct effects of P deficiency on leaf expansion.

Observed total leaf area			Simulated total leaf area		
P3	P2	% Change	P2[a]	P2[b]	Direct effects
2718.3	1407.2	-48	1611.2	2169.5	
			83%	42%	17%

Discussion

The effects of P limitation on the rate of leaf emergence and the final number of leaves are consistent with previous results using soybean (Fredeen et al., 1989), gamelia (Cromer et al., 1993), and wheat (Rodríguez et al., 1994). Differences in the final number of leaves between the two experiments were expected since the daylength was 2 h shorter in Exp. 1 than in Exp. 2 (Marc and Palmer, 1981; Goyne and Hammer, 1982; Sadras and Villalobos, 1993).

The values of AMAX found in these experiments are consistent with those in other studies (English et al., 1979; Connor and Sadras, 1992; Diepenbrock and Pasda, 1995). The smaller values of AMAX obtained in Exp. 2 with respect to those in Exp. 1 (Fig. 2.6a), were probably due to an acclimation of the plants to a lower radiation environment in Exp. 2 (Baker and Bowyer, 1994), and to different values of SLA (Chapin and Wardlow, 1988).

Variations in leaf P and consequently in their photosynthetic characteristics were magnified by the effects of age and position (Fig. 2.4). The value of AMAX in recently expanded leaves was reduced by P limitation by 10-50%, while lower in the canopy reductions in AMAX ranged from 46-85%, always compared with leaves of P3 plants. The nitrogen concentration of recently expanded and older leaves did not vary much, and their light saturated assimilation was not related to their SLN (Fig. 2.6b). Furthermore, leaves of different position and age showed the same response to Leaf P%. Therefore, it was considered that an important cause of variation in AMAX among treatments, in recently expanded and older leaves, was the level of P nutrition of the plant. In this work it has been shown that AMAX and Leaf P% presented a linear decline with cumulative LAI, and that the decline was more important under P-limiting conditions.

In peanut grown under non-limiting nitrogen supply, Wright and Hammer (1994) found that the specific leaf nitrogen concentration (SLN) decreased 0.14 units per each unit of cumulative LAI, downwards from the top of the canopy. In vegetative canopies of sunflower, Sadras et al. (1993) found that the vertical gradient of SLN matched the profile of light distribution within the canopy, so that the actual nitrogen partitioning approached an optimal pattern for canopy photosynthesis. In this work, AMAX and Leaf P% decreased faster in plants having a smaller LAI, this and the fact that the crop did not reach full cover indicate that in this experiment light was not the main factor determining the actual partitioning of P within the canopy.

The effects of P on AMAX are consistent with those observed by other authors (Table 2.6). When the data of both experiments were combined, AMAX presented a significant non-linear response to Leaf P% in the range of 0.08 to 0.38%. It is known that only 5-15% of leaf P is involved in leaf photosynthesis (Bieleski, 1973). Accordingly, total-P concentration as reported here would comprise both inorganic-P (largely vacuolar) plus a variety of different organic forms, of which only some are directly involved in CO_2 assimilation. Since P in the vacuole is not directly involved in photosynthetic reactions, increases in the internal P concentration above a certain threshold would not be expected to result in further increases in assimilation rate.
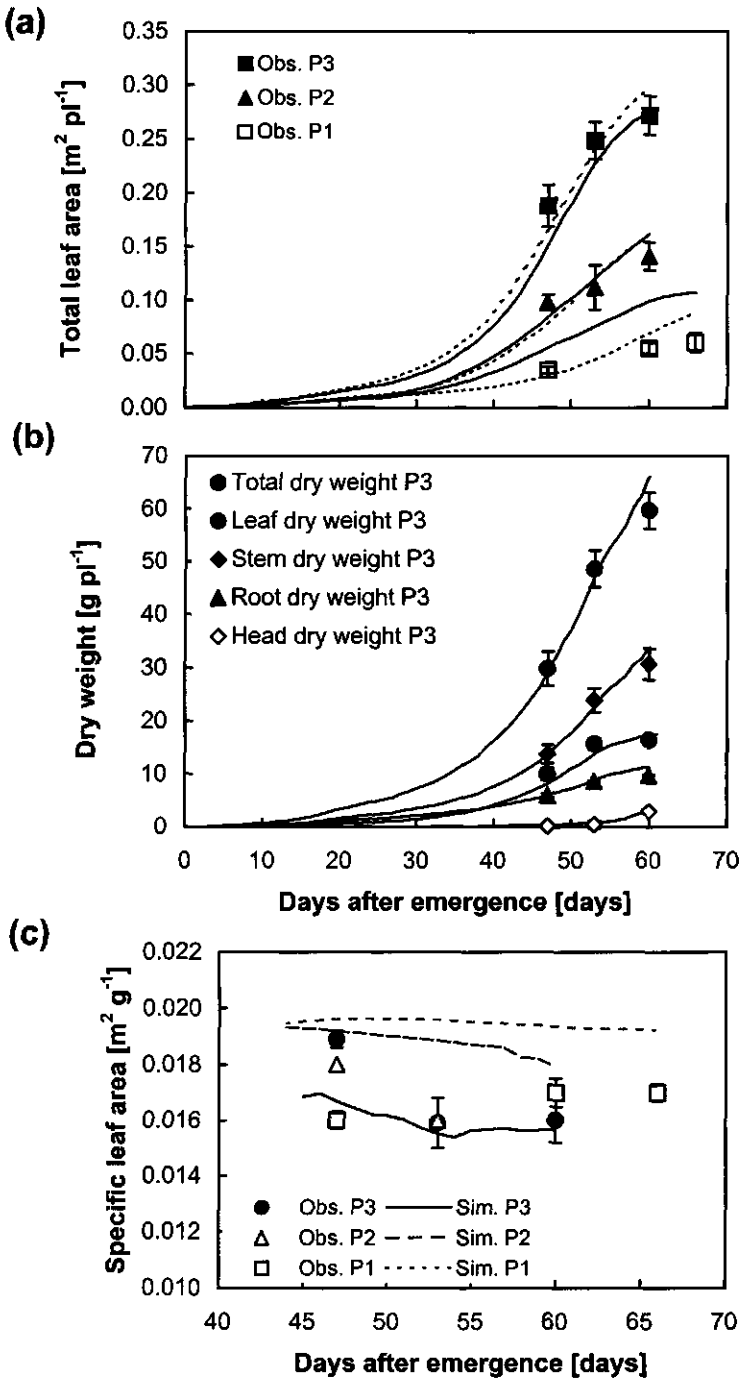
Figure 2.7 Observed (symbols), and simulated (solid lines) plant leaf area (a), plant dry matter (b) and specific leaf area (c) versus days after emergence. Dotted lines in (a) are results of the simulations when the observed LER of individual leaves was used as input in the model. Bars indicate standard errors.

Table 2.5. Sensitivity coefficients for different calculated variables.

Parameter		Sensitivity coefficients					
		Positive change			Negative change		
	Level of P	TLA[5]	TDW[6]	SLA[7]	TLA[5]	TDW[6]	SLA[7]
AMAX[1]							
	P3	0.01	0.45	-0.34	0.03	0.42	-0.48
	P2	0.59	0.94	-0.15	0.67	0.87	0
QY[2]							
	P3	0.05	0.51	-0.20	0.09	0.56	-0.72
	P2	0.46	0.80	-0.16	0.55	0.75	0
LDUR[3]							
	P3	0.47	0.15	-0.02	0.60	0.24	0.49
	P2	0.31	0.14	-0.09	0.25	0.12	-0.03
PHY2[4]							
	P3	-0.61	-0.48	0.16	-0.30	-0.52	-0.50
	P2	-0.57	-0.86	-0.16	0.05	-0.40	0.22

[1] Light saturated photosynthesis (AMAX). The value of AMAX at the top of the canopy was 40 and 35 μmol CO_2 m^{-2} s^{-1} for P3 and P2, respectively.

[2] Apparent quantum yield (QY). The value of QY was 0.06 μmol CO_2 μmol PAR^{-1}, for both P3 and P2.

[3] Duration of the *quasi*-linear leaf expansion phase (LDUR). The value of LDUR was 250 and 200 dd for P3 and P2, respectively. The duration of the lag phase of leaf expansion after leaf appearance, was 100 dd for both P3 and P2.

[4] Value of the phylochron after leaf 5 appearance (PHY2). The value of PHY2 was 38 and 47 dd for P3 and P2, respectively.

[5] Calculated total leaf area (TLA).

[6] Calculated total dry weight (TDW).

[7] Calculated specific leaf area for the whole canopy.

Hyperbolic relationships between AMAX and Leaf P% and the critical value of Leaf P% (0.22%) for AMAX, agrees well with the results obtained from field experiments with sunflower (Spencer and Chan, 1981), and with those reported for other species (Cromer et al., 1993; Kirschbaum and Tomkins, 1990).

In Exp. 2, P deficiency, though not always statistically significant, reduced the duration of the *quasi*-linear leaf expansion phase by 33%, compared with treatment P3. LDUR was significantly ($P<0.05$) and positively related to the value of AMAX (Table 2.3). Consequently changes in LDUR under P deficiency, might be related to the capacity of the plant to produce assimilates for the newly developing leaves. Kriedemann (1986) stated that a limited supply of photo-assimilates constitutes a stronger restriction of division than enlargement of primordial leaf cells in sunflower. Therefore, it is most probable that leaves having fewer cells will achieve their final size sooner than leaves with a higher number of cells.

Table 2.6 Percentage of change in shoot dry weight (ΔSDW), leaf area (ΔLA), AMAX (ΔAMAX), initial quantum yield (ΔQY), respiration rate (ΔR), and SLA (ΔSLA), when growing at low P nutrition compared to controls at high P nutrition.

Crop	ΔSDW	ΔLA	ΔAMAX	ΔQY	ΔR	ΔSLA	Author
Sunflower	-13	-9	0	--	--	0	Exp. 1 this work
Sunflower	-81	-77	-50	--	--	14	Exp. 2 this work
Soybean	-78	-85	-46	0	0	-20	Fredeen et al., 1989
Sugar beet	-77	-80	-32	-6	--	-22	Abadia et al., 1987
Sugar beet	-60	-76	-35	0	-12	-22	Rao & Terry, 1989

Leaf expansion rates during the *quasi*-linear growth phase were correlated to Leaves P% (Table 2.3), but different leaf cohorts presented a different response to Leaves P% (Fig. 2.5a). This was expected since different leaf cohorts usually present a different potential for leaf expansion (Rawson and Hindmarsh, 1982; Rawson and Dunstone, 1986; Villalobos et al., 1996). Nevertheless, by using relative values of LER with the highest value for each treatment being 1.0, an unique non-linear relationship with Leaves P% was obtained (eq. 2.3, and Fig. 2.5b). The absence of a relationship between LER and Leaf P% during expansion and the observation that LER seems to be related to Leaves P% indicates that the rate of expansion of new leaves under low P conditions, might not be regulated at leaf level. Alternatively, LER could be driven by (i) effects of the overall plant-P status on the hydraulic conductivity of the root system and consequently on the required turgor for cell expansion (Radin and Eidenbock, 1984); (ii) the availability of assimilates required for leaf growth at canopy level (Takami et al., 1982; Gallagher, 1985); or (iii) unknown leaf epidermal properties that may change in response to a low plant P status (Fredeen et al., 1989).

Direct measurements of cell turgor, made using a miniaturised cell pressure probe (Palmer et al. 1996), showed that low nitrogen did not reduce leaf area expansion through a reduced turgor pressure. They suggested that reduced cell wall expansion was most probably due to changes in cell wall properties. This hypothesis is in agreement with studies indicating that certain cell wall properties limit expansion (Pritchard et al., 1991). Since nitrogen has an effect similar to P on root hydraulic conductivity and leaf expansion (Radin and Boyer, 1982), it is unlikely, according to the results of Palmer et al. (1996), that in low-P plants turgor would play a role in leaf expansion. Cell division is not confined to primordial phases of leaf growth. Particularly in dicots 90% of cell population within a mature leaf can originate subsequent to unfolding (Kriedemann, 1986). Cell enlargement also involves a substantial synthesis of new cellular materials. For example, Wenkert et al. (1978) observed that exclusion of morning sunlight in soybean resulted in zero leaf area growth that same afternoon.

In this work, when the effects of P deficiency on leaf emergence, duration of the *quasi*-linear phase of leaf expansion, and leaf photosynthetic activity were included in a simulation model it was possible to explain most of the observed characteristics of sunflower plants growing under a moderate P deficiency (treatment P2). In P2 plants, it was calculated that the lack of assimilates as a consequence of reduced AMAX explained a 42% of the observed reduction in leaf area while the direct effect of P deficiency on leaf expansion accounted only for 17% (Table 2.4). This and the results

of the sensitivity analysis indicate that the effects of P deficiency on assimilate production and on the dynamics of leaf emergence (PHY) and expansion (LDUR) explained most of the characteristics of sunflower plants grown under low P conditions. Under extreme P starvation, e.g. treatment P1 in Exp. 2, even when the observed LER values were given as an input, the model failed to reproduce the observed leaf area of the plant. This indicates that under extreme P shortage, both the morphogenetic model was not completely correct and leaf area expansion overestimated (Fig. 2.7a).

The overestimation in total leaf area by the morphogenetic model after about 50 DAE (dotted lines in Fig. 2.7a), could result since it was assumed that LDUR did not change with time within each P treatment, and LDUR for leaves 15 and 16 was not calculated, because of few observations (Fig. 2.3d). However, the overestimation of the leaf area expansion calculated as a function of the supply of assimilates, started much earlier, i.e. 30 DAE, (solid lines in Fig. 2.7a), and should be attributed to other factors that were active at early stages and not taken into account by the model, e.g. direct effects of P limitation on leaf area expansion. Other factors not taken into account by the model include: a limitation of the initial apparent quantum yield of individual leaves and changes in the maintenance requirement under P limitation. Phosphorus shortage generally affects photosynthesis much less at low than at high radiation (Terry and Rao, 1991), because at low radiation photosynthesis is limited by the energy input and not by the carboxylase activity (Connor et al., 1993). Photosynthesis at very low radiation, such as that used to measure quantum yield, was virtually unaffected by low P (Abadia et al., 1987). In sugar beet and soybean, P deficiencies that reduced dry matter accumulation by 60-78% had up to a 6% decrease in apparent quantum yield (ΔQY) (Table 2.6). In this work, a 10% variation in the value of QY in P2 plants had a relatively important effect on the simulated total dry weight as indicated the sensitivity analysis (*SC*=0.8). Therefore, I think that information on the effects of low P on QY particularly in lower shaded leaves is still required for a better understanding of the effects of plant growth under P-deficient conditions. Dark respiration was not affected by P deficiency, even when P deficiency decreased the value of SLA by 20% compared to high P plants (Table 2.6). This indicates that any decrease in SLA as a consequence of low P nutrition is probably related to the accumulation of storage sugars or cell wall materials, which have a very low maintenance requirement. In this work, SLA differed slightly among treatments, and tended to increase under P limitation (Fig, 2.7c), mainly at early growth stages. Under these conditions, it would be even much more unlikely that maintenance respiration would be affected. Furthermore, increases in SLA of cereals have been associated with a lack of assimilates required for growth (Kemp, 1981). In general terms in this work, lack of assimilates seemed to play an important role in the dry weight increase of the plants, particularly since in both experiments leaf area expansion was less sensitive to P limitation than was dry matter accumulation.

Conclusions

Phosphorus limitation decreased the rates of both leaf expansion and the light-saturated photosynthesis per unit of leaf area. Leaf expansion rate during the *quasi*-linear phase of

leaf expansion was related to averaged P% of all the leaves, while the photosynthesis rate at high radiation was associated with the P concentration of the individual leaf. In this work by means of experimental and simulation techniques, the existence of direct effects of P deficiency on individual leaf area expansion was identified. However, under P-deficient conditions leaf area expansion depended mainly on the particular effects of P on: the rate of leaf emergence, duration of the linear leaf expansion period, and on the effects of P on the rate of leaf photosynthesis. In order to clearly differentiate direct effects of P on leaf expansion, from those related to the lack of assimilates, more detailed research in conjunction with the use of simulation techniques would be required. Particularly, more information is needed regarding the effects of P on cell wall properties especially those affecting cell division and cell wall expansion.

Chapter 3

Plant leaf area expansion and assimilate production in wheat (*Triticum aestivum* L.) growing under low phosphorus conditions

Abstract   Under phosphorus deficiency reductions in plant leaf area have been attributed to both direct effects of P on the individual leaf expansion rate and to a reduced availability of assimilates for leaf growth. In this work, experimental and simulation techniques were used to identify and quantify these processes in wheat plants growing under P-deficient conditions. In a glasshouse experiment, the effects of soil P addition (0 - 138 kg P_2O_5 ha^{-1}) on tillering, leaf emergence, leaf expansion, plant growth, and leaf photosynthesis of non water stressed wheat plants (cv. INTA Oasis) were studied. Plants were grown in pots containing a P-deficient (3 mg P g $soil^{-1}$) sandy soil. Sowing and pots were arranged to simulate a crop stand of 173 pl m^{-2}. Experimental results were integrated in a simulation model to study the relative importance of each process in determining the plant leaf area during vegetative stages of wheat. Phosphorus deficiency significantly reduced plant leaf area and dry weight production. Under P-deficient conditions the phyllochron (PHY) was increased up to a 32%, with respect to high P plants. In low P plants the rate of individual leaf area expansion during the *quasi*-linear phase of leaf expansion (LER) was significantly reduced. The effect of P deficiency on LER was the main determinant of the final size of the individual leaves. In recently expanded leaves, phosphorus deficiency reduced the photosynthesis rate per unit of leaf area at high radiation (AMAX), up to a 57%. Relative values of AMAX showed an hyperbolic relationship with leaf P% saturating at 0.27%. Relative values of the tillering rate showed an hyperbolic relationship with the shoot P% saturating at values above 0.38%. The value of LER was not related to the concentration of P in leaves or shoots. A morphogenetic model of leaf area development and growth was developed to quantify the effect of assimilate supply at canopy level on total leaf area expansion, and to study the sensitivity of different model variables to changes in model parameters. Simulation results indicated that under mild P stress conditions up to 80 % of the observed reduction in plant leaf area was due to the effects of P deficiency on leaf emergence and tillering. Under extreme P-deficient conditions the simulation model failed to explain the experimental results indicating that other factors not taken into account by the model e.g. direct effects of P on leaf expansion, must have been active. Possible mechanisms of action of the direct effects of P on individual leaf expansion are discussed in this work.

Abbreviations: AMAX - photosynthesis at high radiation, DR - dark respiration, DUR - duration of leaf expansion, LER - leaf expansion rate, PHY - phyllochron, QY - apparent quantum yield, TDUR - tillering duration, TRATE - tillering rate, SLA_{nl} - limit values for the specific leaf area of recently expanded leaves, SLA - calculated specific leaf area of recently expanded leaves

Introduction

Phosphorus (P) has been recognized as an important environmental factor limiting crop growth and production (Rahman and Wilson, 1977; Batten et al., 1984). Phosphorus deficiency limits crop growth by reducing the size of the leaf area of the plants and consequently limiting light interception (Radin and Eidenbock, 1984; Cromer et al., 1993), and by reducing the photosynthetic capacity of leaves (Rao and Terry, 1989; Jacob and Lawlor, 1991). In wheat, P deficiency reduces plant leaf area by producing less and smaller leaves. Under low P supply, the number of leaves per plant is reduced due to a lower emergency of tillers (Sato et al., 1996), and to a slower rate of leaf emergence per stem (Rodríguez and Goudriaan, 1995). Low P reduces the size of individual leaves by limiting their rate of expansion, while the duration of leaf expansion is hardly affected (Kirschbaum and Tompkins, 1990; Cromer et al., 1993).

When phosphorus availability is limited, growth is generally more reduced than the rate of photosynthesis per unit of leaf area in recently expanded leaves (Terry and Rao, 1991; Jacob and Lawlor, 1991). This would suggest that under P-limiting conditions the availability of assimilates at leaf level might not be the main responsible factor for the reduction in leaf area expansion and plant growth. Direct effects of P on plant leaf area expansion have been proposed before by other researchers. Radin and Eidenbock (1984) observed that leaf area expansion in cotton grown at low P or low nitrogen was driven by a lack of turgor in cells. Direct effects of P have also been mentioned to act on tillering, in wheat (Sato et al., 1996) and in rice (Hanada, 1995).

Despite the fact that direct effects of P deficiency on individual leaf expansion and tillering have been found, little is known on their relative importance in determining plant leaf area expansion and growth of wheat. This paper focuses on the effects of P nutrition on the production of assimilates at canopy level and their importance on the growth of the plant leaf area, and on the existence and significance of direct effects of P on plant leaf area expansion in wheat. This work aim to (i) describe the effects of P deficiency on leaf area expansion and leaf photosynthesis of wheat plants, and (ii) to test whether it is possible to simulate leaf area expansion of vegetative wheat plants under conditions of low P supply, taking into account the effects of P nutrition on leaf emergence, tillering and assimilate production at canopy level.

Material and methods

This work presents combined results, partly from one experiment, partly from a simulation model developed to understand the processes involved in the expansion of leaf area and growth of wheat plants grown under conditions of low P availability. The experiment was carried out with wheat (*Triticum aestivum* L. cv. INTA-Oasis), and the performance of the plants was evaluated by measuring the effects of various levels of P supply on leaf emergence, tillering, leaf growth and leaf photosynthesis.

Cultural techniques and conditions
Plants of wheat were grown in pots (4 litre content) in a glasshouse under natural radiation, in Wageningen, The Netherlands (51°58' N, 5°40' E). The pots were arranged to form a canopy of 173 pl. m^{-2}. At sowing on 29 May1996, seeds were

placed in a line through the center of the pots (0.17 m diameter and 0.25 m deep). There were five homogeneous plants per pot. Test plants were surrounded by plants of the same treatment as a border. The pots were filled with a sandy soil containing 3 mg P kg^{-1} P (Bray and Kurtz, 1945). Adequate soil moisture level in the pots was maintained throughout the experimental period. Daily maximum and minimum temperatures were measured inside the glasshouse, and radiation data was obtained from a meteorological station located at the experimental site.

Treatments

Treatments consisted of four levels of P supply equivalent to 7 (P1), 15 (P2), 60 (P3), 300 (P4) kg ha^{-1} of ground super-phosphate (46% P_2O_5). With the three lowest levels, all P was incorporated before sowing. The highest doses (P4) was splitted, half applied and incorporated before sowing and half surface applied at the three leaves stage. All pots received a dressing of macro and micronutrients, including 400 kg N ha^{-1}, as NH_4NO_3, distributed during the experimental period. Treatments were randomized within each of three blocks.

Determinations and measurements

Plants were grown until the leaf 7 was fully expanded, Feekes 3 (Feekes, 1941). During the experimental period, leaf and tiller emergence (leaf or tiller prophyl length > 1cm) were recorded once every two days. The value of the phyllochron for each treatment was calculated from the relationship between leaf number and cumulated thermal time using a base temperature of 0°C. Leaves were numbered upwards from the oldest (1), to the last appeared leaf, youngest. Tillers were identified as by Klepper et al. (1982), where each tiller on a plant is uniquely named by the number of the leaf axil it originates from. Tiller emergence was monitored in 20, 15 and 10 tagged plants per treatment per block till the first, second and third harvests respectively. The width (w) and length (l) of leaves 4, 5, 6 and 7 were recorded daily and the individual leaf area (A, cm^2) was calculated as the product of leaf width, leaf length and a shape factor (0.695). The value of the shape factor was calculated at each harvest by measuring the area of individual leaves with a leaf area meter LI-3100 (Li-Cor, Nebraska, USA). Leaf expansion rates during the *quasi*-linear phase (LER, cm^2 leaf^{-1}d^{-1}), and the duration of the *quasi*-linear phase of leaf expansion (DUR, days) were calculated using an optimization model (eq. 3.1) that fitted the experimental data iteratively by means of curve-fitting software (Jandel Scientific, Erkrath, Germany),

$$y = a + b \bullet x \qquad \text{if } x \leq c \qquad (3.1a)$$
$$y = a + b \bullet c \qquad \text{if } x > c \qquad (3.1b)$$

where *y* is the individual leaf area [cm^2 leaf^1], *a* the y-axis intercept, *b* the value of LER, *x* is the time since leaf emergence [d], and *c* the time when leaf expansion stopped [d]. The duration of the *quasi*-linear leaf expansion period (DUR) was calculated as *c+(a/b)*, [d]. The same model (eq. 3.1) was used, to quantify the rate of first order tillers emergence per unit of ground area [TRATE, tillers m^{-2} d^{-1}], and the period of time it took to each tiller order (T1, T2, and T3), to emerge in 100% of the tested plants (TDUR, d).

When leaves 4, 5, 6 and 7 reached 100% of their final size, their rate of photosynthesis at high radiation (AMAX, μmol CO_2 m^{-2} s^{-1}) was determined, and subsequently the plants were harvested for determinations of dry matter yield and leaf P analysis. For leaves 4 and 5 a light response curve of photosynthesis was constructed for each treatment. At each harvest time, leaves used for photosynthesis and LER determinations were separately harvested for P and nitrogen assay. Plant material was further divided in roots, stems and leaves for dry weight and P determinations. Leaf area was measured by using an area meter LI-3100 (Li-Cor, Nebraska, USA). Leaf photosynthesis was measured not only in the leaves used for LER analysis but also in those leaves numbers where LER and AMAX were measured in previous harvests, i.e. in leaf 4 at harvest 1, leaves 4 and 5 in harvest 2, leaves 4, 5, 6 in harvest 3 and in leaves 4, 5, 6, and 7 in harvest 4. Photosynthesis was measured with an open photosynthesis system LCA-2 System ADC (Analytical Development Co.Ltd., Herts, England) in combination with a lamp (Philips Projection Lamp 6853, 75 W) installed over the leaf chamber. Light intensity could be varied from 0 to 1800 μmol m^{-2} s^{-1} PAR, without altering the environmental conditions inside the leaf chamber. Data from the light response curves were fitted by a negative exponential equation (eq. 3.2), using the FITNONLINEAR procedure from Genstat 3.1 (1987).

$$A = AMAX + (DR - AMAX)e^{PAR \bullet QY/(DR-AMAX))} \qquad (3.2)$$

Where, A is the photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), AMAX the photosynthetic rate at high radiation (μmol CO_2 m^{-2} s^{-1}); DR the rate of dark respiration (μmol CO_2 m^{-2} s^{-1}); PAR the incident photosynthetic active radiation (μmol m^{-2} s^{-1}) and QY the apparent quantum yield (μmol CO_2 μmol^{-1} PAR).

Due to significant differences among P treatments, in leaf emergence and the time leaves reached their final size, leaf 4 was harvested at 19 DAE in P4 and P3 and at 20 DAE in P2 and P1, leaf 5, at 25 DAE in P4 and P3, at 28 DAE in P2 and at 30 DAE in P1; leaf 6, at 31 DAE in P4, 38 DAE in P3, and 42 DAE in P2 and P1; leaf 7 at 38 DAE in P4 and P3, and at 45 DAE in P2 and P1. Following this schedule, the leaves were harvested at the same ontogenetic stage among the P treatments, i.e. the harvests were made at the time the tested leaves reached 100% of their final size.

After wet digestion of the dried plant material in a H_2SO_4 / salicylic acid / H_2O_2 / selenium mixture, concentrations of total N and P were colorimetrically measured in the digests using an automated continuous-flow system.

Differences among values of PHY and final number of leaves per plant were tested by Tukey ($P<0.05$) after ANOVA. Treatment effects on LER and other plant characteristics were tested by ANOVA using Sigma Stat (Jandel Scientific, Erkrath, Germany). Results from the light response curves were tested by F tests by using the Fitnonlinear procedure from Genstat 3.1 for Windows (1987).

Simulation model
A simulation model was developed in order to study the effects of P deficiency on leaf area expansion during vegetative stages of wheat plants. The model calculates actual

leaf area expansion by comparing the potential plant leaf expansion rate (cm^2 plant^{-1} d^{-1}) with the amount of assimilates available for leaf growth. The potential plant leaf expansion rate (cm^2 plant^{-1} d^{-1}), is calculated morphogenetically, as the sum of the potential expansion rates of the individual leaves that are expanding at any time (LER, cm^2 leaf^{-1} d^{-1}). The phyllochron determines the time interval for leaf emergence, and this is assumed to be modified by P deficiency (Rodríguez et al., 1994). The model identifies three groups of leaves: (i) leaves in the initial exponential phase of expansion (lag phase), (ii) leaves that are expanding rapidly (*quasi*-linear phase), and (iii) fully expanded leaves. During the lag phase, it was assumed that each leaf expands at half the rate of the linear phase. The duration of the lag phase lasts 35 degree-days (dd). The *quasi*-linear phase of leaf expansion usually accounts for 90-95% of the final individual leaf size (Ong and Baker, 1985). During this period, LER is calculated as a function of the potential LER of each leaf according to leaf position. The data on potential LER as a function of leaf position was obtained from plants growing under the highest P level (P4, control plants) in the experiment.

After a plant has three main stem leaves it is assumed that tillers start emerging (Porter, 1984). The rate of tillering for each tiller order depends on the P nutrition of the plant (Sato et al., 1996). In the model, TRATE depends on the concentration of P in the leaves and on the tiller order, and TDUR depends on the value of TRATE. It was assumed that a tiller order has emerged when it has appeared at least in 50% of the entire plant population. After a tiller emerges it produces leaves at the same rate as the main stem (Kirby et al., 1985).

Assimilate production is calculated by using the subroutines TOTASS, ASTRO and ASSIM of the model SUCROS (Goudriaan and van Laar, 1994). After accounting for reflection, the model calculates the level of incident radiation, and the proportion of incident radiation that is diffuse at different depths within the canopy. Substitution into the photosynthesis light response curves of single leaves gives the assimilation rate per unit leaf area at selected depths within the canopy. Canopy assimilation rates are calculated by accumulating the instantaneous assimilation rates over canopy depths and integrating the instantaneous rates over the day, by using the three-point Gaussian method (Goudriaan, 1986). Based on the experimental results it was assumed that P deficiency affects assimilate production by reducing the rate of photosynthesis at light saturation. Partitioning coefficients for dry matter and the fraction of leaves that are senescent were introduced as forcing functions from the observed data of each P treatment.

Maximum and minimum values of specific leaf area for the newly expanding leaves (SLA$_{nl}$) were tabulated to calculate the actual minimum and maximum leaf growth, respectively, as in Villalobos et al. (1996). Minimum and maximum values of SLA$_{nl}$ were taken from observed values and set to 0.02 and 0.03 m^2 g^{-1}, respectively. A discrepancy between supply and demand of assimilates is buffered by adaptation of the calculated SLA in newly expanding leaves. When there is a shortage of assimilates, the leaves will be thinner and will present a larger SLA. Conversely, when there is an excess of assimilates the leaves will get thicker and will present a smaller SLA. When the maximum SLA$_{nl}$ is reached, the leaves cannot get any thinner, and expansion will slow down due to lack of assimilates. On the other hand, when there is a surplus of assimilates and the minimum value of SLA$_{nl}$ is reached, any surplus of assimilates is

allocated in equal amounts to stems and roots. The model was written in FST (Rappoldt and van Kraalingen, 1996), and has a daily step of integration. Required inputs are values of PHY, the average concentration of P in all plant leaves (Leaves P%), forcing functions for partitioning and senescent leaf area, daily maximum and minimum temperatures, daily total radiation and latitude.

Results

Weather
Figure 3.1 summarizes solar radiation and temperature during the experimental period. Daily mean temperature inside the glasshouse was 20.4°C with an absolute maximum of 32 and a minimum of 6°C. Average daily total radiation outside the glasshouse was 18.2 MJ m^{-2} d^{-1}, with a roof transmission of 80%.
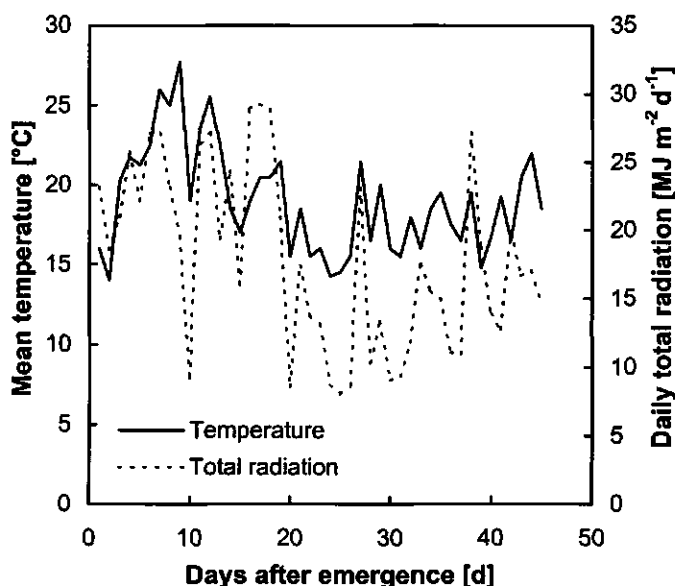
Figure 3.1 Mean temperature [°C] inside the glasshouse, and mean daily total radiation [MJ m^{-2} d^{-1}] versus days after emergence [d] during the experimental period.

Plant growth
Phosphorus significantly promoted plant growth (Table 3.1). At the final harvest total plant dry weight and total leaf area were respectively increased by a factor of 2.6 and 3.4 relative to the P1 plants. The partitioning of biomass between shoot and root was affected by the treatments. At the beginning of the growth cycle low P plants partitioned a greater proportion of their total biomass to roots, e.g. differences among treatments were statistically significant ($P<0.05$) at harvests 1 and 2. Treatments P4 and P3 reached a LAI equal or higher than 3 at the third harvest, while LAI in treatments P2 and P1 were lower than 3 during the whole experimental period.

Table 3.1 Total plant dry weight , ratio of root to total plant dry weight (Root Ratio), total leaf area and leaf area index, of wheat plants grown at different levels of P supply.

		Harvest 1	Harvest 2	Harvest 3	Harvest 4
Total Dry Weight [g pl^{-1}]					
P1		0.26	0.44	0.78	0.93
P2		0.34	0.55	1.29	1.36
P3		0.59	0.79	2.12	1.77
P4		0.49	0.83	1.45	2.42
	Prob.	$P<0.05$	$P<0.001$	$P<0.001$	$P<0.01$
	s.e.m.	0.05	0.021	0.07	0.14
Root Ratio					
P1		0.55	0.49	0.38	0.31
P2		0.54	0.52	0.38	0.34
P3		0.32	0.43	0.35	0.37
P4		0.38	0.35	0.33	0.33
	Prob.	$P<0.05$	$P<0.001$	$P<0.05$	n.s.
	s.e.m.	0.039	0.01	0.016	0.011
Total Leaf Area [cm^2 pl^{-1}]					
P1		18.7	42.1	70.6	70.8
P2		24.8	48.0	117.8	110.4
P3		32.9	76.6	174.7	142.3
P4		43.6	93.7	198.8	244.1
	Prob.	$P<0.01$	$P<0.001$	$P<0.01$	$P<0.01$
	s.e.m.	2.4	2.38	13.2	14.0
Leaf Area Index					
P1		0.32	0.72	1.21	1.38
P2		0.43	0.83	2.04	1.91
P3		0.57	1.32	3.00	2.45
P4		0.75	1.62	3.44	4.22
	Prob.	$P<0.01$	$P<0.001$	$P<0.01$	$P<0.01$
	s.e.m.	0.04	0.04	0.22	0.24

Prob. Are probabilities based on the F-test from ANOVA.
s.e.m. is the standard error of the mean from ANOVA.

Leaf emergence and expansion
The value of the phyllochron was significantly increased by P deficiency. In P1 plants, PHY was 32% higher than in the control plants (P4) (Table 3.2). The final area of individual leaves was also significantly affected by the treatments (Table 3.3). In P1, the final leaf area of individual leaves was reduced by about 20 to 64%, compared to P4 plants. The reduction in LER accounted for most of the variation observed in the final leaf area of individual leaves. In P1 plants, LER of the different tested leaves was reduced about 18-55%, compared to control plants.

Table 3.2 Values of the phyllocron (PHY), and the number of tillers per plant at the final harvest (average of 5 plants per treatment per block), for the different levels of P supply. Different letters among treatments indicate differences statistically significant by a Tuckey test ($P<0.05$) after ANOVA. For the tillering rate and tillering duration the values between brackets are the standard error of the mean of each treatment ($n=3$).

		P1	P2	P3	P4
Phyllochron [dd leaf^{-1}]		124.1 a	108.4 ab	110.2 ab	93.6 b
Total number of tillers per plant		1.13 b	1.6 b	2.83 a	3.17 a
Tillering rate [tillers m^{-2} d^{-1}]					
	T1	29.5 (2.6)	48.9 (12.8)	49.9 (3.4)	68.1 (9.2)
	T2	11.7 (4.9)	31.0 (19.1)	58.7 (11.2)	62.7 (5.3)
	T3	-	-	25.4 (7.9)	37.7 (5.0)
Tillering duration [d]					
	T1	4.5 (0.15)	3.4 (0.7)	3.5 (0.2)	2.5 (0.2)
	T2	8.9 (2.1)	7.1 (2.3)	3.1 (0.6)	2.7 (0.3)
	T3	-	-	6.1 (0.9)	4.8 (0.6)

Table 3.3 Final leaf area, leaf expansion rate during quasi-linear phase (LER) and duration of the quasi-linear phase of leaf expansion (DUR). Different letters among treatments indicate differences statistically significant by a Tuckey test ($P<0.5$) after ANOVA.

	Leaf 4	Leaf 5	Leaf 6	Leaf 7
Leaf area [cm^2]				
P1	4.4 c	13.2 ab	15.2 b	15.6 b
P2	7.3 bc	11.0 b	18.2 b	20.8 a
P3	8.8 ab	15.6 a	18.3 b	19.2 ab
P4	12.4 a	17.2 a	26.1 a	22.0 a
s.e.m.	0.9	0.8	1.2	1.0
LER [cm^2 d^{-1}]				
P1	1.4 b	2.3 a	2.7 b	3.6 a
P2	2.2 ab	3.1 a	3.1 ab	3.9 a
P3	2.8 a	3.3 a	3.1 ab	4.6 a
P4	3.2 a	3.4 a	3.4 a	4.7 a
s.e.m.	0.26	0.33	0.12	0.42
DUR [d]				
P1	4.7 a	5.4 a	5.4 a	5.44 a
P2	4.2 a	5.2 a	7.0 a	6.86 a
P3	4.9 a	5.4 a	8.1 a	5.84 a
P4	4.3 a	5.6 a	7.5 a	5.50 a
s.e.m.	0.38	0.54	0.67	0.56

The duration of the *quasi*-linear phase of leaf expansion did not differ among the treatments, and was on average 4.5 (s.e.m.=0.4), 5.4 (s.e.m.=0.5), 7.0 (s.e.m.=0.7) and 5.8 days (s.e.m.=0.6), for leaves 4, 5, 6, and 7, respectively. Despite of the significant effects of the P deficiency on the values of LER, a correlation analysis (Table 3.4), did not show any significant relationship between LER and different indices of P status of the plants, i.e. Leaf P%, Leaves P% or Shoot P%.

Table 3.4 Correlation coefficients between different plant characteristics.

	AMAX[1]	LER[2]	SLA$_{nl}$[3]	Leaves P%	Leaf P%	Leaves SLP
Shoot P%	0.5	0.13	-0.14	-0.9	0.9	0.9
Prob.[4]	<0.01	0.3	0.3	<0.0001	<0.0001	<0.0001
N.[5]	48	48	48	48	48	48
Leaf SLP	0.43	0.17	-0.2	0.94	0.97	0.91
Prob.	<0.01	0.2	0.07	<0.0001	<0.00001	<0.0001
N.	48	48	48	48	48	48
Leaves SLP	0.474	0.1	0.037	0.96	0.93	1
Prob.	<0.001	0.4	0.8	<0.0001	<0.0001	
N.	48	48	48	48		
Leaf P%	0.44	0.16	-0.2	0.98	1	
Prob.	<0.01	0.2	0.1	<0.00001		
N.	48	48	48	48		
Leaves P%	0.49	0.14	-0.14	1		
Prob.	0.001	0.3	0.3			
N.	48	48	48			
SLA$_{nl}$	0.03	-0.04	1			
Prob.	0.8	0.7				
N.	48	48				
LER	0.3	1				
Prob.	<0.5					
N.	48					

[1] AMAX = Photosynthesis rate at light saturation
[2] LER = Leaf expansion rate during the *quasi*-linear phase
[3] SLA$_{nl}$ = Specific leaf area of recently expanded leaves
[4] Prob = Level of probability
[5] N. = Number of data points.

Leaf and canopy phosphorus
Total plant P content increased by seven fold between P1 and P4 treatments, and low P plants partitioned more P to the roots than high P plants (Fig. 3.2a). The profiles of Leaf P% at the last harvest are presented in Fig. 3.2b. Differences among treatments within the same leaf number were statistically significant. At the fourth harvest, older leaves within the canopy had a lower P concentration than more recently expanded ones. Interestingly, the slopes of the decay of Leaf P% within the canopy did not differ among P treatments.
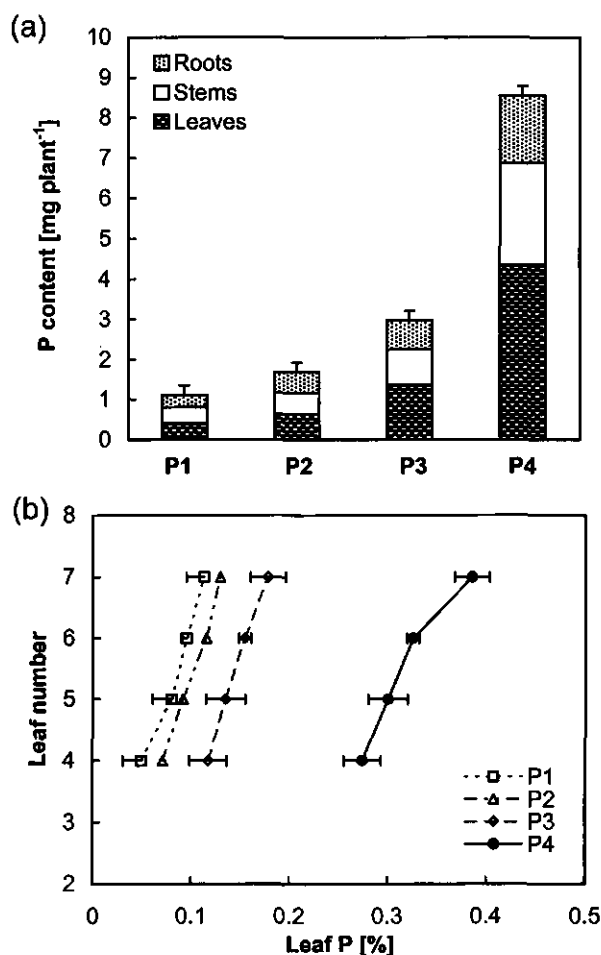
Figure 3.2 (a) Phosphorus content in roots, stems and leaves at harvest four. Bars are standard errors for the total P content. (b) Profile of Leaf P% within the canopy at the time of the fourth harvest for the different levels of P supply. Bars are standard errors.

Leaf assimilate production

The response of leaf photosynthesis (A) to irradiance at different levels of P supply when leaves 4 and 5 reached maximum size, is presented in Fig. 3.3. eq. 3.1, presented before, satisfactorily described all the data sets with R^2 higher than 0.9. Table 3.5 summarizes the values of the different parameters used in eq. 3.1 for the different levels of P supply. Figure 3.3 shows that in P1 plants, P deficiency decreased AMAX in recently expanded leaves, by 43 and 57% relative to treatment P4 for leaves 4 and 5, respectively. Dark respiration (DR) and the apparent quantum yield (QY) were not significantly affected by the P treatments. The correlation analysis presented in Table 3.4 indicates a strong positive relationship between the value of AMAX and the P status of individual leaves both, on weight (Leaf P%) and area basis (Leaf SLP).
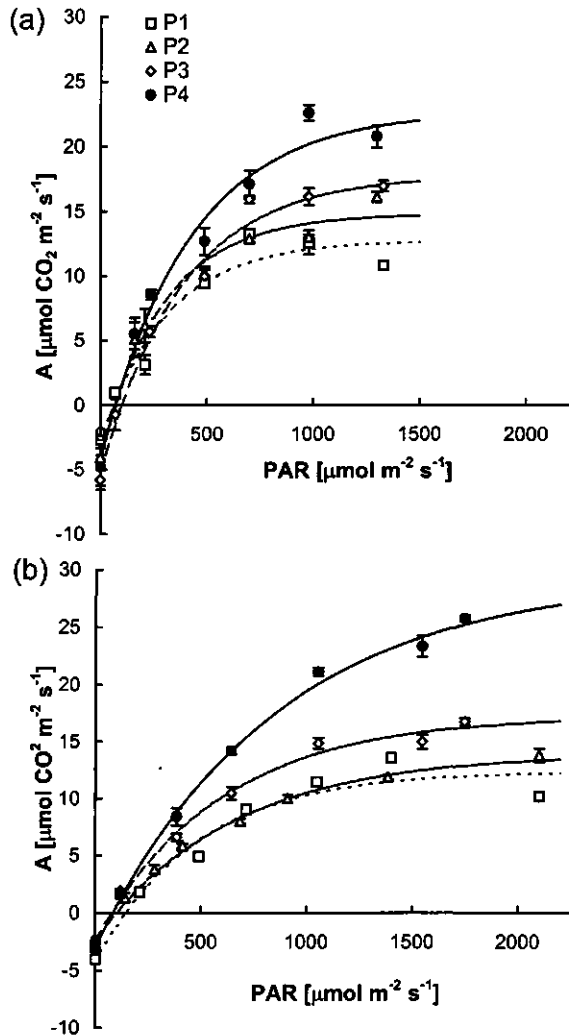
Figure 3.3 Light response curves of photosynthesis (A) for leaf 4 (a) and leaf 5 (b), at the different levels of P supply. Every dot is the average of three replications and bars represent the standard errors. Lines were fitted by eq. 3.2.

Combining the data from recently expanded and older leaves a non linear relationship ($R^2=0.79$, $n=40$, $P<0.01$) between Leaf P% in individual leaves and their relative AMAX, i.e. relative to the AMAX of leaves from the treatment P4 (Fig. 3.4) was obtained. From the fitted function AMAX was zero when the threshold value of Leaf P was 0.067%P, and the critical Leaf P% for AMAX, i.e. the Leaf P% where AMAX is 95% of the maximum was 0.27%P. The specific leaf nitrogen (SLN) of leaf 4 varied from 0.4 to 0.1 mg cm^{-2}, and did not show any relationship with AMAX (not shown).
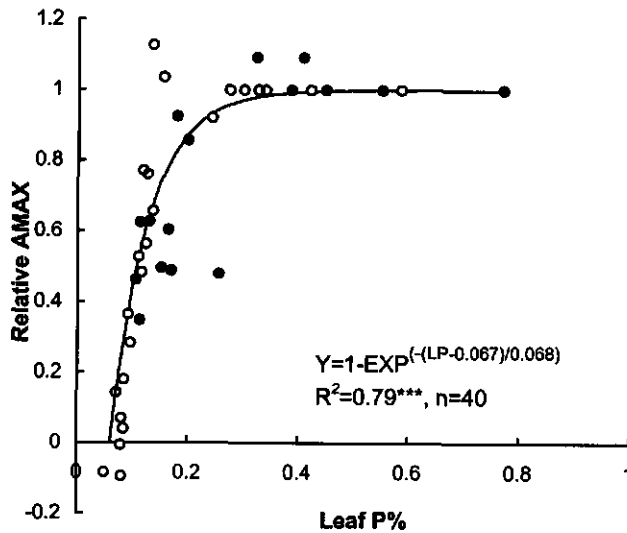
Figure 3.4 Relative photosynthesis at high radiation calculated with respect to the value of AMAX of P4 plants for each harvest. Closed symbols are from recently expanded leaves and open symbols from older leaves.

Tillering

The final number of tillers per plant was significantly affected by the treatments (Table 3.2). At the time of the final harvest 100% of the tested P4 plants presented three tillers (T1, T2 and T3), the coleoptile tiller (T0) did not emerge. At treatment P4 tiller T4 was present only in 17% of the tested plants. Plants of treatment P3 presented T1, and T2, while only 80% of the plants had T3. With P2 plants, T1 was present in all the tested plants and T2 only in 60%. At treatment P1, all the tested plants presented T1, and only 13% T2. In high-P plants (P4), the rate of tiller emergence (TRATE, tillers $m^{-2}d^{-1}$) was inversely related to the time it took every tiller order to appear in 100% of the tested plants (TDUR, days) (Table 3.2 and Fig. 3.5a). Higher tiller orders presented a slower TRATE and a higher TDUR. Since P deficiency not only increased the value of PHY, but also strongly reduced TRATE and slightly increased TDUR (Table 3.2), P deficiency increased the delay and dispersion on the emergence of tillers. The relative value of TRATE for every tiller order, relative to the corresponding order in P4 plants, was related to the concentration of P in shoots (Shoot P%) (Fig. 3.5b). The relative tillering duration was closely, but inversely related to the relative tillering rate (Fig. 3.5c). From the data in Fig. 3.5b it was derived that the value of Shoot P% leading to a relative tiller rate of 0.95, the critical threshold for the relative values of TRATE, was 0.38%.

Simulation results

In order to check the performance of the morphogenetic model, the rate of plant leaf area expansion (cm^2 $plant^{-1}$ d^{-1}) was calculated and daily integrated, by giving the observed values of LER for the individual leaves of each treatment as an input, i.e. data from Table 3.3.

Table 3.5 Net photosynthesis at light saturation (AMAX), dark respiration (DR) and apparent quantum yield (QY) of leaf 4 and 5, for different levels of phosphorus fertilization. Between brackets is the standard error of the fitted parameter.

	P1	P2	P3	P4
Leaf 4				
AMAX [μmol CO_2 m^{-2} s^{-1}]	12.75 (1.49)	14.8 (1.01)	17.8 (1.31)	22.76 (2.06)
DR [μmol CO_2 m^{-2} s^{-1}]	2.78 (1.45)	3.54 (1.18)	5.34 (1.04)	4.19 (1.64)
QY [μmol CO_2 μmol^{-1} PAR]	0.049 (0.017)	0.06 (0.012)	0.06 (0.01)	0.065 (0.013)
Leaf 5				
AMAX [μmol CO_2 m^{-2} s^{-1}]	12.44 (1.51)	14.01 (0.54)	17.3 (0.94)	29.4 (1.5)
DR [μmol CO_2 m^{-2} s^{-1}]	4.23 (1.59)	2.33 (0.39)	2.39 (0.68)	2.99 (0.61)
QY [μmol CO_2 μmol^{-1} PAR]	0.034 (0.01)	0.025 (0.002)	0.033 (0.004)	0.038 (0.003)

Using the observed LER as an input in the model (thin lines in Fig. 3.6), the model described well the observed values of leaf area for all the treatments. When calculating plant leaf area as a function of the potential leaf expansion rate, and the supply of assimilates from photosynthesis (thick lines in Fig. 3.6a), results simulated by the model agreed well with observed results at P4 and P3. The simulation model also satisfactorily simulated production and partitioning of dry matter of treatment P4 (Fig. 3.6b). However the model overestimated the final leaf area by about 29 and 14% for the treatments P1 and P2, respectively, if compared with the results of the morphogenetic model.

The simulation model was used to estimate the proportion of the reduction in total leaf area in treatment P3 that could be explained by a lack of assimilates and by direct effects of P limitation on leaf expansion. Table 3.6 presents the observed total leaf area at P4 and P3, the simulated total leaf area in P4 (P4s), and the simulated total leaf area in P3 plants calculated as a function of the effects of P deficiency on AMAX, PHY and tillering (P3s), and as a function of the effects of P deficiency only on AMAX (P3s[b]). Taken into account the effects of P on AMAX, PHY and tillering, 68% of the observed reduction in total leaf area between P4 and P3 plants was explained. It was also calculated that the effects of P on AMAX alone were very small, and that direct effects of P on leaf expansion accounted by a 32% of the observed reduction in total leaf area between P4 and P3 plants.

An analysis of sensitivity of the four calculated variables: total leaf area (TLA, cm^2 plant^{-1}), total dry weight (TDW, g plant^{-1}), specific leaf area (SLA, m^2 g^{-1}), and radiation use efficiency (RUE, g (total biomass) MJ^{-1} (intercepted PAR)), to $\pm$10% changes in the value of the parameters: AMAX at the top of the canopy, the apparent quantum yield (QY), and the phyllochron (PHY), was performed for treatments P4 and P3 (Table 3.7).

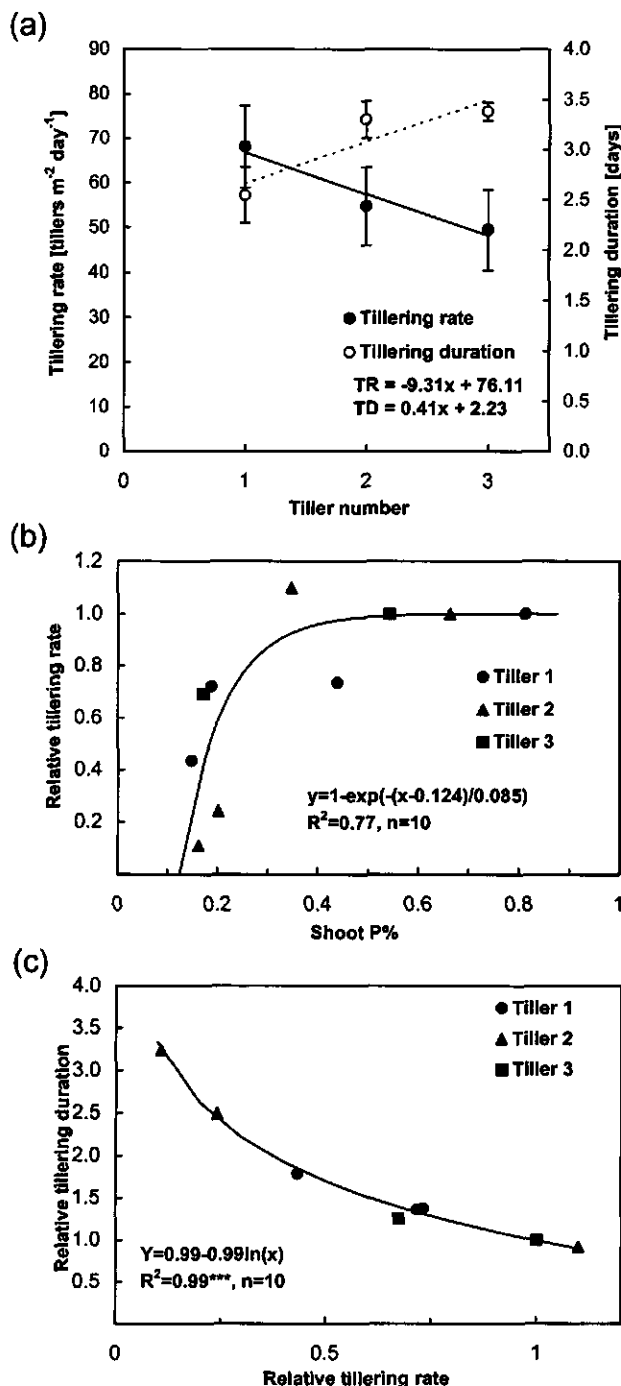Figure 3.5 (a) Rate of tillering calculated on a m^2 basis for P4 plants and duration of tiller emergence, for each tiller order. Every point is the average of three replications. (b) Relative tillering rate with respect to the control (P4) for each tiller order, versus the mean P concentration in the shoot. (c) Relative tillering duration versus the relative tillering rate. Bars represent standard errors.

The analysis was not performed for treatments P2 and P1 because the model did not satisfactorily simulate those treatments. A sensitivity coefficient was calculated as $SC=(\Delta V/V) / (\Delta p/p)$, where V and p are the model variables and model parameters, respectively. Values of SC higher than 1 or lower than -1 indicate a high sensitivity, while a SC between 0.5 and -0.5 indicates a low sensitivity of the variable to a change in the parameter (Thornley and Johnson, 1990). From Table 3.7, the values of PHY and QY seemed to be most critical independently of the level of P supply, while AMAX showed to have little impact on the studied variables. Total leaf area was most sensitive to changes in PHY, while total dry weight, specific leaf area and radiation use efficiency were most sensitive to changes in QY.

Table 3.6 Observed total leaf area in treatments P4 and P3, % of change in total leaf area between P4 and P3, simulated total leaf area of treatment P4 (P4s) and treatment P3 (P3s) taking into account the effects of P deficiency on AMAX, PHY and tillering, and taking into account only the effects of P deficiency on AMAX (P3s[b]), and the estimated direct effects of P deficiency on leaf expansion.

Observed total leaf area			Simulated total leaf area			
P4	P3	% Change	P4s	P3s	P3s[b]	Direct effects
244.1	142.3	-41	265.8	173.9	263.2	32%

Discussion

In high P plants, the values of the photosynthesis rate per unit of leaf area at high radiation, the apparent quantum yield and the dark respiration, agree with those reported by other authors (Evans, 1983; Marshall and Biscoe, 1980; Penning de Vries et al., 1989). Phosphorus limitation consistently decreased the value of AMAX in recently expanded leaves, i.e. 21 to 57% relative to the value of AMAX of P4 plants (Fig. 3.3 and Table 3.5). Phosphorus deficiency limits photosynthesis through a reduced regeneration of ribulose 1,5-biphosphate (RuBP), while the activity of RuBP is usually not affected (Brooks, 1986; Brooks et al., 1988; Rao and Terry, 1995). RuBP regeneration capacity of leaves can be reduced if the availability of fixed carbon, the initial activity of the Calvin cycle enzymes, and/or the supply of ATP and NADPH are limited. Under moderate P deficient conditions Rao and Terry (1995) proposed that RuBP regeneration is most probably limited by the supply of Ru5P and /or the initial activity of the Ru5P kinase.

In this work dark respiration did not show any particular response to low P supply. This substantiates the observations made by Fredeen et al., (1989), Abadia et al. (1987) and Rao and Terry (1989). The apparent quantum yield presented a tendency to decrease with P shortage in leaf 4, while in leaf 5 no clear pattern was observed. Abadia et al., (1987) found that low P reduced photosynthesis but only at high radiation. They did not find effects of P shortage on the apparent quantum yield. Jacob and Lawlor (1993), however, observed that not only AMAX but also the quantum yield was reduced by P deficiency, and similar results were observed before by Brooks

(1986) in spinach. The particular effects of P limitation on the photosynthetic characteristics of leaves observed in different experiments, and even among different leaves within the same experiment, might seem contradictory if the intensity and timing of the stress is not taken into account. The results from Jacob and Lawlor (1993) as well as those from Brooks (1986) were obtained from water culture experiments where the plants were exposed to extreme P limitations. In those experiments, leaf area and shoot dry weight are usually decreased by more than 90% respect to high P control plants. In this experiment P stress reduced total dry weight in the range of 26 to 60%, depending on the level of P supply. Furthermore, in this work the intensity of the stress seemed to decrease with time, i.e. in low P plants the reduction in LER of leaf 4 was more important than the for leaves 5, 6 and 7 (Table 3.3).

Table 3.7 Sensitivity coefficients for different calculated variables.

Parameter Level of P	**Positive change**				**Negative change**			
	TLA[5]	**TDW[6]**	**SLA[7]**	**RUE[8]**	**TLA[5]**	**TDW[6]**	**SLA[7]**	**RUE[8]**
AMAX[1]								
P4	0.06	0.22	-0.01	0.21	0.08	0.26	-0.05	0.24
P3	0.01	0.23	0.06	0.22	0.01	0.27	0.46	0.26
QY[2]								
P4	0.49	0.88	-0.16	0.78	0.52	0.92	-0.18	0.81
P3	0.08	0.78	-0.16	0.74	0.21	0.85	0.85	0.80
PHY[3]								
P4	-1.11	-0.61	-0.46	0	-1.56	-0.82	-0.37	-0.01
P3	-1.61	-0.81	-1.88	-0.06	-1.77	-0.78	1.07	-0.02

Above the columns spanning header: **Sensitivity coefficients of different calculated variables**

[1] Light saturated photosynthesis (AMAX). The value of AMAX at the top of the canopy was 40 and 35 μmol CO_2 m^{-2} s^{-1} for P3 and P2, respectively.
[2] Apparent quantum yield (QY). The value of QY was 0.06 μmol CO_2 μmol PAR^{-1}, for both P3 and P2.
[3] Value of the phylochron after leaf 5 emergence (PHY2). The value of PHY2 was 38 and 47 dd leaf[-1] for P3 and P2, respectively.
[4] Number of tillers per plant
[5] Calculated total leaf area (TLA).
[6] Calculated total dry weight (TDW).
[7] Calculated specific leaf area for the whole plant.
[8] Calculated radiation use efficiency (RUE).

Photosynthesis in leaves of different age and position showed a significant non linear relationship with their Leaf P% in the range of 0.05 to 0.8 %P (Fig. 3.4). It is well known that only 5-15% of leaf P is intimately involved in leaf photosynthesis (Bieleski, 1973). Accordingly, total P concentrations as reported here would comprise both inorganic-P (largely vacuolar) plus a variety of different organic forms, of which only some will directly be involved in CO_2 assimilation. Since phosphorus in the vacuole is not directly involved in photosynthetic reactions, increases in the internal phosphorus concentration above a certain threshold value would not be expected to
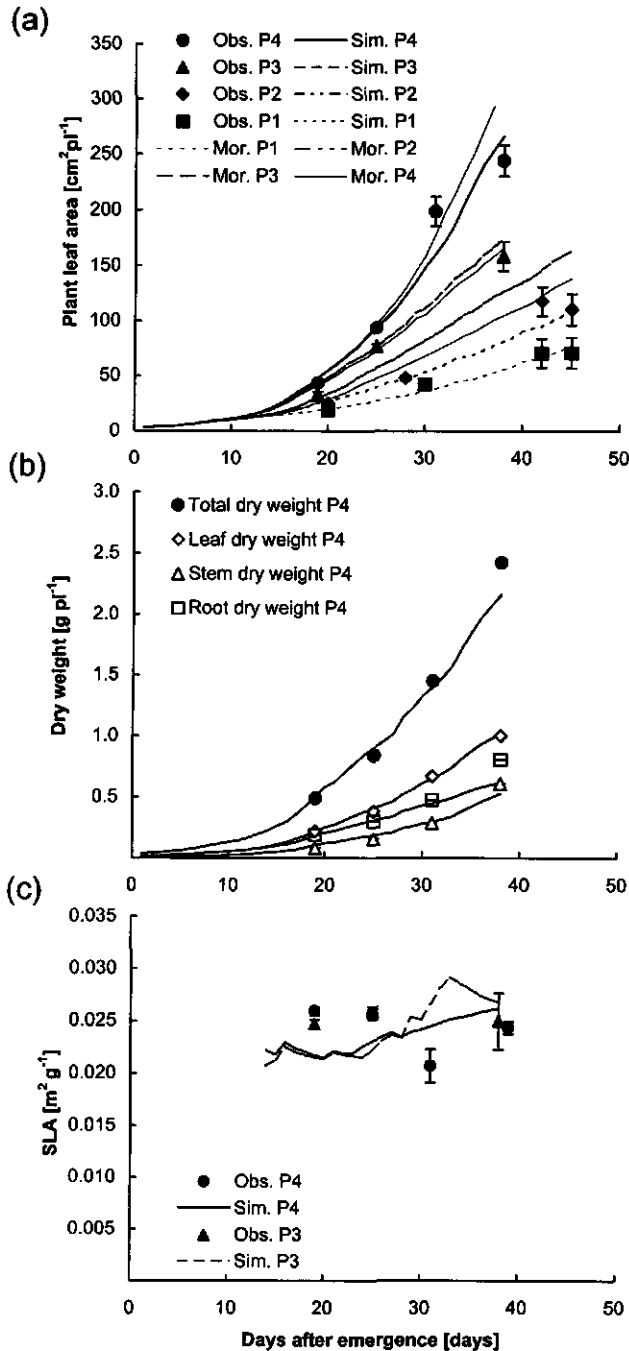
Figure 3.6. (a) Simulated (lines) and observed (symbols) of plant leaf area, (b) dry weight, and (c) specific leaf area, for the different P treatments versus days after emergence. Thick lines in (a) represent the results from the simulation model if calculated as a function of the potential leaf expansion and the supply of assimilates. Thin lines in (a) are the results of the simulation model when the observed LER for individual leaves of each treatment were used as an input in the model. Bars represent standard errors.

result in a further increase in assimilation rate. Here when the leaf P concentration was 0.27%, AMAX reached 95% of its maximum. At lower leaf P concentrations AMAX declined sharply to zero and became negative at a leaf P lower than 0.067%. Hyperbolic relationships between AMAX and Leaf P% and similar critical thresholds values of Leaf P% (0.3%) for AMAX have also been observed by other authors in gamelia (Cromer et al., 1993) and eucalyptus (Kirschbaum and Tompkins, 1990).

The effects of P limitation on the rate of leaf emergence are in line with previous results (Rodríguez et al., 1994), and with results obtained under nitrogen limitation (Longnecker et al., 1993). It is still not clear however why P has such an important effect on the value of the phyllochron. The value of the phyllochron depends on the timing of leaf initiation at the stem apex, and the duration of leaf tip elongation through the whorl of mature sheaths. The duration of leaf tip elongation depends on the exponential leaf expansion rate, i.e. expansion during the lag phase of leaf expansion, and the height of the whorl of the previous leaf (Skinner and Nelson, 1995). Despite it was observed that P deficiency reduced the value of the *quasi*-linear LER the leaf expansion during the lag phase of leaf expansion nor the heights of the whorl were determined. Furthermore even when a low LER might affect the value of PHY, I do not discard the possibility that phosphorus deficiency changes the rate of leaf primordia initiation in the stem apex as previously observed by Rahman and Wilson (1977).

In high P plants, lower order tillers emerged faster, and during a shorter period of time than higher order tillers. Hence homogeneity in the plant population, with respect to the number of plants bearing a certain tiller (e.g. T1, T2 or T3), decreased as the plants developed higher order tillers. Phosphorus deficiency further increased this heterogeneity by decreasing the rate of tiller emergence and increasing the duration of tiller emergence (Table 3.2, Fig. 3.5b and c). This resulted in less tillers per plant in low P plants since the increase in the period of tiller emergence did not compensate for the reductions in the rate of tiller emergence. Rickman et al. (1983) also found that under increasingly unfavourable environmental conditions, e.g. nitrogen deficiency and drought stress, the tiller population decreased as the rate of tiller emergence slowed down, and the tillering duration increased. In this work the relative tillering rate, calculated with respect to P4 plants for each tiller order, was non linearly related to the concentration of P in the shoot (Fig. 3.5b). The critical value of Shoot P% for the relative tillering rate was 0.38. Sato et al. (1996) mentioned a critical Shoot P% of 0.5 for the rate of emergence of T1. However, they restricted their analysis to T1 and mentioned that this critical value differed among wheat cultivars.

The observation that P limitation reduced the individual leaf size through reduced LER while the duration of leaf expansion remained unaffected agrees with most existing data of leaf area expansion under stress conditions (Ong and Baker, 1985; Porter, 1984).

Using a simulation model, it was possible to explain most of the observed reduction (68%) in leaf area of wheat plants grown under a mild P stress conditions (Table 3.6, Fig. 3.6). In P3 plants the effects of P deficiency on leaf emergence and tillering were critical in determining plant leaf area expansion, while assimilate production had a minor effect (Tables 3.6 and 3.7). At P1 and P2 treatments the simulation model overestimated the plant leaf area by 29 and 14%, respectively (Fig. 3.6). This and the

fact that LER was not related to any index of the P status of the plants (Table 3.4) suggests the existence of direct effects of P on leaf expansion.

Phosphorus deficiency can directly limit the size of individual leaves by, producing fewer cells per leaf primordia, and/or limiting cell elongation. Despite there is not direct evidence of the effects of P deficiency on cell division in the vegetative apex, Jacob and Lawlor (1991) observed that in low P wheat plants the cell number in leaves was reduced by 10 fold. Radin and Eidenbock (1984) proposed that direct effects of P deficiency on leaf expansion could be mediated through a limited hydraulic conductivity in roots and consequently a lack of turgor for cell expansion. However recent evidences indicate that the expansion properties of the cell wall rather than a lack of turgor for cell expansion are more likely to limit leaf expansion (Pritchard et al., 1990), particularly since nutrients have been observed to have direct effects on these properties (Palmer et al., 1996).

Conclusions

Phosphorus limitation in wheat reduced both leaf expansion and the light-saturated photosynthesis per unit of leaf area. However tillering was the most sensitive process to P deficiency presenting a critical P% in shoots of 0.38. In this work by means of experimental and simulation techniques the existence of direct effects of P deficiency on the individual leaf area expansion were identified. The main variables that determined the leaf area of the plants under low P were the number of tillers per plant and the rate of leaf emergence per stem. Despite plant metabolism was also impaired at low P supply, its effects on leaf area expansion appear to be minor. In order to clearly quantify direct effects of P deficiency on leaf expansion more detailed research in conjunction with the use of simulation techniques would be required. Particularly more information is needed regarding the effects of P on cell division and cell expansion.

Chapter 4

Leaf primordia initiation, leaf emergence and tillering in wheat (*Triticum aestivum* L.) grown under low phosphorus conditions

Abstract: In two simultaneous experiments the effects of phosphorus (P) supply on leaf area development in wheat (*Triticum aestivum* L.) grown in sand with nutrient solutions were studied. In Exp. 1, I studied leaf emergence, leaf elongation, tiller emergence, shoot growth, and P uptake under four levels of P supply (mM) 0.025 (P1), 0.05 (P2), 0.1 (P3), and 0.5 (P4). In Exp. 2, there were two levels of P supply, P1 and P4, and I examined the effects of P on leaf primordia differentiation and leaf emergence. The phyllochron was calculated as the inverse of the rate of leaf emergence calculated from the regression of number of leaf tips (PHY-Ltip), Haun index (PHY-Haun), and as the cumulated thermal time between the emergence of two consecutive leaves (PHYtt). The plastochron was calculated from the inverse of the rate of leaf primordia initiation in the apex. P deficiency delayed the emergence of leaves on the main stem and on the tiller 1. Phosphorus deficiency increased the time from emergence to double ridge and anthesis. The final number of leaves was not affected by P. The effects of P on the value of the phyllochron were attributed to both a reduced rate of leaf primordia initiation, and to a reduced leaf elongation rate. P deficiency delayed or even suppressed the emergence of certain tillers. In this work a phosphorus deficiency that reduced shoots growth by 25% at 44 days after emergence significantly modified the structure of the plants by increasing the value of the phyllochron and delaying tillering. These results suggest that any attempt to simulate leaf area development and growth of wheat plants for P-limited conditions should include the effects of the deficiency on leaf emergence.

Abbreviations: DAE - days after emergence, DUR - duration of leaf length expansion, dd - degree days, LLER - leaf length expansion rate, P - Phosphorus, PHY-phyllochron, PHY-Haun - phyllochron as a function of the Haun index, PHY-Ltip - phyllochron as a function of leaf tip emergence, PHYtt - phyllochron as a function of cumulated thermal time, T0, T1,.., Tn - order of main stem tillers

Introduction

The response of leaf area to phosphorus (P) is an important factor determining the yield in wheat because leaf area determines the amount of solar radiation intercepted, and consequently the amount of dry matter accumulated by the crop. In wheat leaf area depends on: leaf emergence, individual leaf expansion, and tillering. In addition, leaf emergence has a particular role in determining plant leaf area since leaf and tiller emergence are closely synchronized processes (Klepper et al., 1982; Kirby et al., 1985). After Bunting and Drennen (1966), the effect of different environmental factors on leaf emergence has usually been characterized by the value of the phyllochron (PHY). PHY is also an important parameter in many simulation models of wheat growth and production (Ritchie and Otter, 1985; McMaster et al., 1992; Rickman et al., 1996). The value of PHY can be calculated in different ways, (i) as the inverse of the slope of the relationship between the number of emerged leaf tips and cumulated thermal time, (ii) using the Haun scale (Haun, 1973) instead of the number of leaf tips, or (iii) from the thermal time between the emergence of two consecutive leaf tips (Wilhelm and McMaster, 1995). Major factors determining the rate of leaf emergence in wheat are temperature and daylength (Slafer and Rawson, 1994). Other factors may also affect the phasic development of the plants and the value of PHY, e.g. soil strength (Masle and Passioura, 1987), and water stress (Angus and Moncur, 1977). Nitrogen availability, at non extreme levels, has little effects on the PHY (Frank and Bauer, 1984), while under P-deficient conditions leaf emergence and plant development are usually delayed (Elliot et al., 1997; Rodríguez et al., 1994). The value of PHY depends on the rate of leaf initiation at the stem apex (plastochron, dd), and the duration of the leaf tip elongation through the whorl of mature sheaths. The duration of the leaf tip elongation depends on the rate of leaf elongation and the height of the sheaths (Skinner and Nelson, 1995). Even though P deficiency is known to reduce the rate of leaf elongation (Radin and Eidenbock, 1984), it is not know whether the value of the plastochron might also change under P-deficient conditions. The importance of the rate of leaf elongation altering the phyllochron can be obscured when the phyllochron is calculated following the Haun index, or when is calculated out of the number of emerged leaf tips regressed versus the cumulated thermal time (Skinner and Nelson, 1995). However, calculating the PHY of each leaf from the thermal time accumulated between the emergence of two consecutive leaves, allow us to better capture important environmental and genetic effects on the development of the plants.

In a previous paper it was shown that small increases in the value of PHY as a consequence of P deficiency, were important in determining plant leaf area in wheat (Rodríguez et al., 1998a). However, it is still not known why the value of PHY changes as a function of the P level of the plants. In this work I will study whether the effect of P on the value of PHY is mediated throughout an increased plastochron, a reduced leaf elongation rate or whether both processes are active at the same time. This question will be answered by calculating the leaf elongation rates of each leaf, the rate of leaf primordia initiation, and the value of PHY using the three methods mentioned above, in wheat plants grown under different levels of P supply.

Material and methods

Experimental set-up

Pre-germinated seeds of winter wheat (*Triticum aestivum* L., cv Buck Poncho) were sown in containers filled with washed sand, and grown in the open air at the Facultad de Agronomía, Universidad de Buenos Aires, Argentina (34°35' S, 58°29' W).

In order to have plants of different P nutritional status, I supplied nutrient solutions containing four levels of P (mM): P1 0.025; P2 0.05; P3 0.1 and P4 0.5, as $NH_4H_2PO_4$. The solutions also contained (µM): 1500 KNO_3; 1000 $Ca(NO_3)_2$; 250 Mg SO_4; 125 KCl; 6.25 H_3BO_3; 0.5 $MnSO_4.H_2O$; 0.5 $ZnSO_4.7H_2O$; 0.125 $CuSO_4.5H_2O$; 0.125 $(NH_4)_6 Mo_7O_{24}.4H_2O$; 10 Fe-EDTA. NH_4Cl was added as needed so that the total amount of NH_4 was constant in the different P treatments. Two litres of the nutrient solution was supplied per container every two days and after every rain. Once a week the containers were flushed with deionized water to avoid accumulation of nutrients. There were eighteen containers of 100 L capacity (0.57m diameter and 0.44m depth), with 80 equidistant plants. All determinations were made on plants of the central part of the containers to avoid border effects. The eighteen containers were arranged in two simultaneous experiments, experiment 1 consisted of treatments P1, P2, P3 and P4, and experiment 2 of treatments P1 and P4. Within each experiment treatments were distributed following a completely randomized block design with three replications. The accumulated thermal time over a base temperature of 0°C, was calculated from the average of the maximum and minimum air temperature, measured at the experimental site. In both experiments leaves and tillers were identified as by Klepper et al. (1982).

Experiment 1

The number of leaves and tillers was recorded every two days in 5 homogeneous tagged plants. The leaf number on the main stem and on tiller 1 were determined as the number of leaf tips longer than 1 cm, and as the number of completely expanded leaves plus the fraction of the length of the emerging leaf with respect to the length of the previous one (Haun, 1973). The value of the PHY for each treatment was calculated as: (i) the inverse of the slope of the relationship between leaf number (leaf tip >1 cm) and cumulated thermal time (PHY-Ltip), (ii) the inverse of the slope of the relationship between Haun index and cumulated thermal time (PHY-Haun), and (iii) the thermal time between the emergence (leaf tip >1 cm) of two consecutive leaves (PHYtt).

The length of every emerged leaf on the main stem and on tiller 1, was measured every two days on two tagged plants per experimental unit, until the leaves reached their final length. The rate (LLER, cm dd^{-1}) and duration (DUR, dd) of leaf elongation during the linear expansion phase were calculated using an optimization model (eq. 4.1) that fitted the experimental data iteratively by means of curve-fitting software (Jandel Scientific, Erkrath, Germany),

$$y = a + b \bullet x \qquad \text{if } x \leq c \qquad (4.1a)$$
$$y = a + b \bullet c \qquad \text{if } x > c \qquad (4.1b)$$

where y is total leaf length [cm] , a the y-axis intercept [cm], b the value of LLER [cm dd^{-1}], x is the thermal time since leaf emergence [dd], and c the time when leaf expansion stopped [dd]. The duration of linear leaf expansion was calculated as:

$$DUR = c + a/b \qquad\qquad (4.2)$$

At the stage of double ridge and at anthesis a group of 5 plants per container were harvested and dry weight and P content of shoots were determined. Total P concentration in the shoots was determined after wet digestion in a H_2SO_4-Se-salicylic acid mixture with addition of H_2O_2, by the colorimetric molybdenum-blue method.

Experiment 2
At intervals varying from 2 to 7 days and starting before seedling emergence, three homogeneous plants per container were harvested for dissection and leaf primordia counting under a magnifying glass. In these plants leaf emergence (leaf tip >1 cm) and the Haun index were also determined. The value of the plastochron (dd), defined as the interval in thermal units between the initiation of two successive leaf primordia in the apex, was calculated from the inverse of the slope of the relationship between number of leaf primordia and cumulated thermal time.

Statistical methods
Differences among values of PHY and plastochron were tested by a *t*-test ($P<0.05$). Differences among means of shoot dry weight, shoot P%, LLER, final leaf length and DUR, were tested by Tukey ($P<0.05$) after ANOVA using Sigma Stat (Jandel Scientific, Erkrath, Germany).

Results

Plant growth and development
Plants of treatment P4 (the control treatment) reached anthesis 101 days after emergence (DAE) (Table 4.1). Phosphorus deficiency increased the time to anthesis by 4 to 14% for the different levels of P supply, with respect to P4 plants. After 44 days from emergence, the shoot dry weight for P1 was significantly lower than for P2, P3 or P4. At anthesis, shoot dry weights for P4 and P3 were higher than for P2, and for P2 higher than for P1 plants (Table 4.1). The concentration of P in shoots significantly increased with the level of P supply. When compared with the treatment P1, the concentration of P in P4 plants increased by a factor of 8.6, while dry matter increased by a factor of 2.6 at anthesis.

Leaf differentiation and emergence
The level of P supply did not affect the final number of leaves on the main stem (Table 4.2). The values of PHY-Ltip or PHY-Haun did not differ from each other (*t*-test $P=0.05$, $n=8$). Irrespective of the method of calculation, PHY increased with P deficiency from about 7 to about 35% depending on the level of P supply (Table 4.2 and Figs. 4.1a and 4.1b). When the value of PHY was directly calculated from the thermal time between the emergence of two consecutive leaves (PHYtt) a fluctuation

in its value for the different leaves was observed (Fig. 4.1c). However no particular trend to increase or decrease with the leaf number was present, and as observed with PHY-Ltip and PHY-Haun, P deficiency increased the value of PHYtt. In Figure 4.1c, the highest differences on the value of PHYtt among P levels were observed for leaf 4 and onwards. A *t*-test (*P*=0.01, *n*=8) indicated that the value of PHY-Ltip for tiller 1 did not differ from that on the main stem. However, the effects of P deficiency on the value of PHY-Ltip of T1 leaves were more evident (Table 4.2), and started at a lower leaf (L1) (Fig. 4.2) than those observed on main stem leaves (L4). Under P deficiency PHY-Ltip of T1 leaves was increased from about 70 to 100% depending on the level of P supply.

Table 4.1 Days to anthesis and to double ridge, and shoot dry weight and shoot P concentration at 44 days after emergence (DAE) and at anthesis. Different letters for each variable indicate significant differences, Tukey (*P*<0.05).

	P1	P2	P3	P4
Days to double ridge**	51 b			41a
Days to anthesis*	115 c	108 b	105.3 b	101 a
44 DAE*				
Shoot dry weight [g pl^{-1}]	0.03 b	0.06 a	0.06 a	0.08 a
Shoot P%	0.07 b	0.08 b	0.08 b	0.21 a
Anthesis*				
Shoot dry weight [g pl^{-1}]	1.62 c	2.79 b	4.62 a	4.14 a
Shoot P%	0.05 c	0.09 c	0.16 b	0.43 a

* data from Experiment 1.
** data from Experiment 2.

In the control plants (P4) the value of the plastochron was a 30% smaller than the value of PHY-Ltip (Table 4.2, and Fig. 4.3a). Extrapolation from the fitted lines in Fig. 4.3a, shows that there were already 4 leaf primordia differentiated in the embryo of the seeds. Data from experiment 2 showed that leaf primordia differentiation proceeded until 41 and 51 days after emergence (double ridge) at P4 and P1 treatments (*P*<0.05) (Table 4.1), respectively. The difference between the plastochron in P4 and P1 plants was not statistically significant. Although its value was 18% higher in P1 than in P4 plants (Table 4.2 and Fig. 4.3a). In Fig. 4.3b, the number of leaf primordia in P1 and P4 plants was plotted versus their respective Haun index. The curvilinear relationships in Fig. 4.3b show that leaf primordia accumulated in the apex of both, P1 and P4 plants. Figure 4.3b, also shows that at any Haun index the number of leaf primordia in P1 plants was always slightly smaller than in P4 plants.

Tillering
Figure 4.4, shows the number of phyllochrons elapsed at the time each main stem tiller, e.g. T1, T2,... T6, first emerged in any of the tested plants. A *t*-test (*P*=0.05)
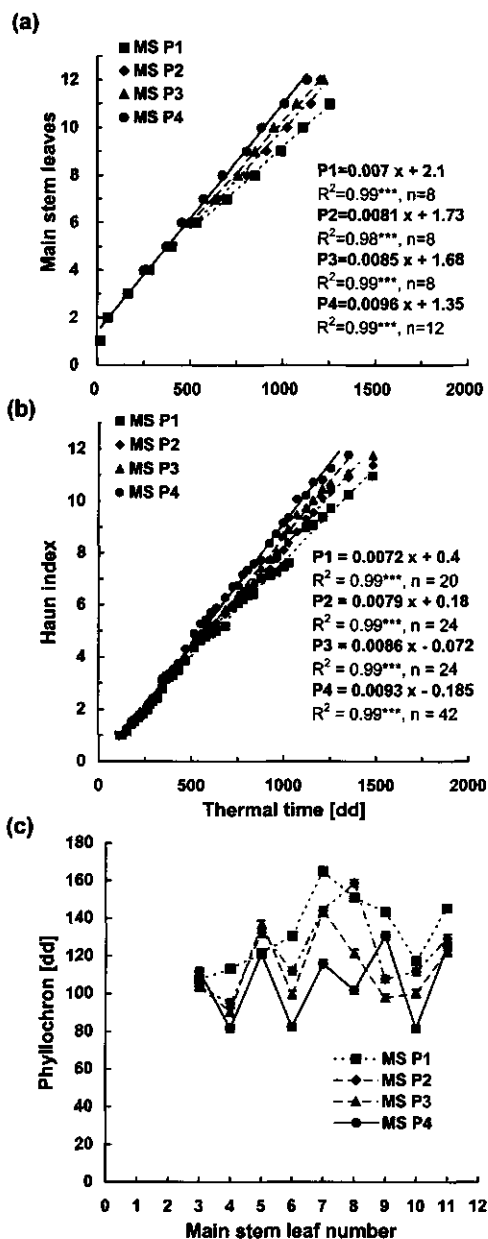
Figure 4.1 Number of main stem leaves (a) and Haun index (b) versus cumulated thermal time (base temperature = 0°C), and value of the phyllochron (c) calculated as the cumulated thermal time between the emergence of two consecutive leaf tips for the different leaves at different levels of P supplied at Exp. 1. Bars are the standard error of the means.

indicated that the slope of this relationship, the synchrony, did not vary with the level of P supply (Table 4.2).
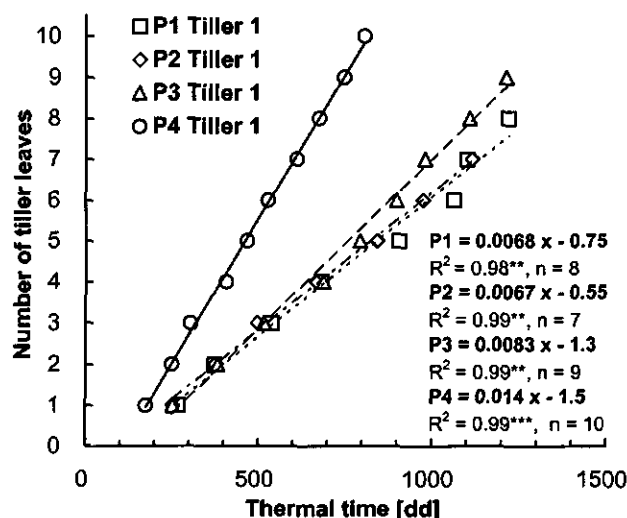
Figure 4.2 Number of leaves on tiller 1 for different levels of P supply, versus thermal time.

Table 4.2 Final number of leaves, value of the phyllochron (dd) of leaves on the main stem and tiller 1 for four levels of phosphorus supply. PHY was calculated as the inverse of the slope of the relationship between leaf number, leaf tip >1 cm (PHY-Ltip), or the Haun index (PHY-Haun), versus the cumulated thermal time. Value of the plastochron (dd) of main stem leaves for treatments P1 and P4. Synchrony (phyllochrons tiller[-1]) between the emergence of main stem leaves and main stem tillers. Different letters for each variable indicate significant differences among the P treatments by a *t*-test (*P*<0.05).

	P1	P2	P3	P4
Main stem leaves				
Final leaf number	11.0 a	11.7 a	12.0 a	12.3 a
PHY-Ltip[1]	141.8 c	123.4 b	117.6 b	104.1 a
PHY-Haun[1]	138.8 c	124.3 c	119.0 b	111.1 a
Plastochron	91.7 a	-	-	77.5 a
Tiller 1 leaves				
PHY-Ltip	147.0 b	149.2 b	120.4 b	70.9 a
Synchrony	-	1.32 a	1.39 a	1.48 a

[1] In the calculus of PHY of main stem leaves, for the treatments P1, P2 and P3 the first four main stem leaves were not taken into acccount.
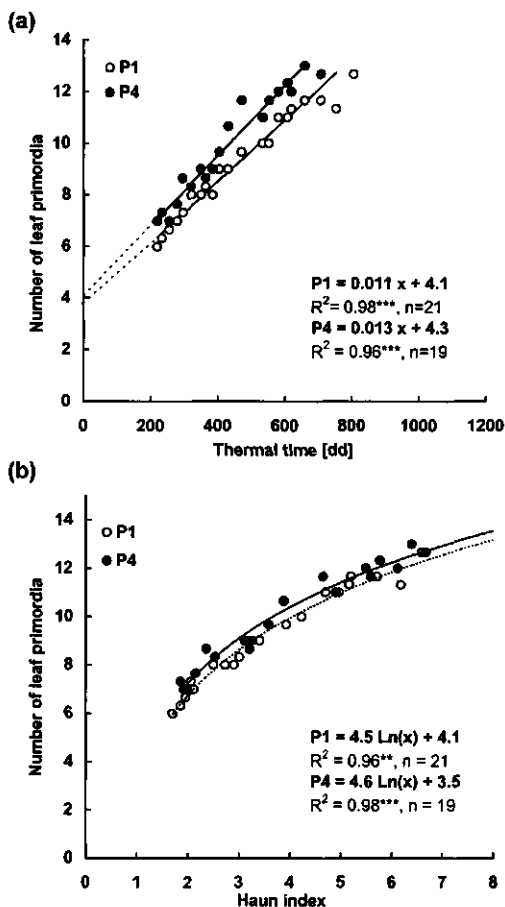
Figure 4.3 Number of leaf primordia in the apex of the main stem of wheat plants growing at two levels of P supply versus cumulated thermal time (a), and (b) versus the Haun index, at Exp. 2.

In average, every tiller number took 1.39 phyllochrons to start emergence in the tested plant population. The value of the synchrony for P1 plants was not calculated since only two points (T1 and T2) were available. However, Fig. 4.4 shows that T1 and T2 in P1 plants started emergence following the same synchrony as P2, P3 and P4 plants. Table 4.3, presents the number of main stem leaves at the time each main stem tiller completed its emergence in more than 50% of the tested plants. Control plants formed T1, T2, T4, T5 and T6. T3 and T0 (the coleoptile tiller) did not emerge in any treatment. Low P supply delayed and suppressed the emergence of certain cohorts of tillers, i.e. in P1 only T1 and T2 emerged, in P2 not only T3 but also T4 failed to emerge, while T5 and T6 were delayed. Tiller emergence in P3 did not differ much from the control plants.

Table 4.3 Main stem tillers present in more than 50% of the tested plant population at the time each main stem leaf emerged, in wheat plants grown under four levels of P supply.

	P1	P2	P3	P4
Main stem leaf number				
3			T1	T1
4	T1	T1		T1, T2
5			T1, T2	
6	T1, T2	T1, T2		
7				
8			T1, T2, T4	
9			T1, T2, T4, T5	T1, T2, T4
10		T1, T2, T5	T1, T2, T4, T5, T6	T1, T2, T4,T5
11		T1, T2, T5, T6		T1, T2, T4, T5, T6
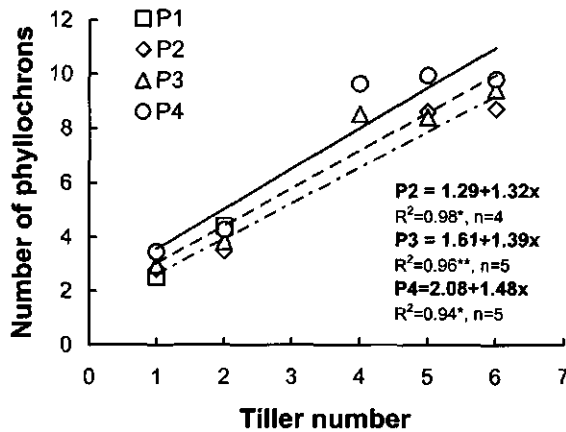
Figure 4.4 Number of phyllochrons elapsed after tiller 1, 2 and 3 start emergence, in wheat plants grown at different levels of P supply.

Leaf elongation rate and duration of leaf elongation
Phosphorus deficiency significantly decreased the final leaf length of leaves 7, 10 and 11 on the main stem (Table 4.3). Differences among P treatments on the final length of tiller 1 leaves were not statistically significant. Due to the change in environmental conditions during the experimental period i.e. the mean air temperature increased from 10 to 18 °C from emergence till anthesis, it was decided to express the values of LLER in cm per unit of thermal time (base temperature = 0°C). Under P deficiency LLER of leaves 5, 7, 10 and 11 were significantly reduced, up to about 50% with respect to P4 plants, depending on the level of P supply and leaf number. Phosphorus deficiency significantly increased the duration of the leaf elongation period only at leaf 7. The effects of P on DUR were clearly smaller than the effects of P observed on the value of LLER. To test whether the effect of P deficiency on LLER affected the value of PHY, in Fig. 4.5a I plotted the value of PHYtt (Fig. 4.1c) versus their respective LLER (Table 4.4).
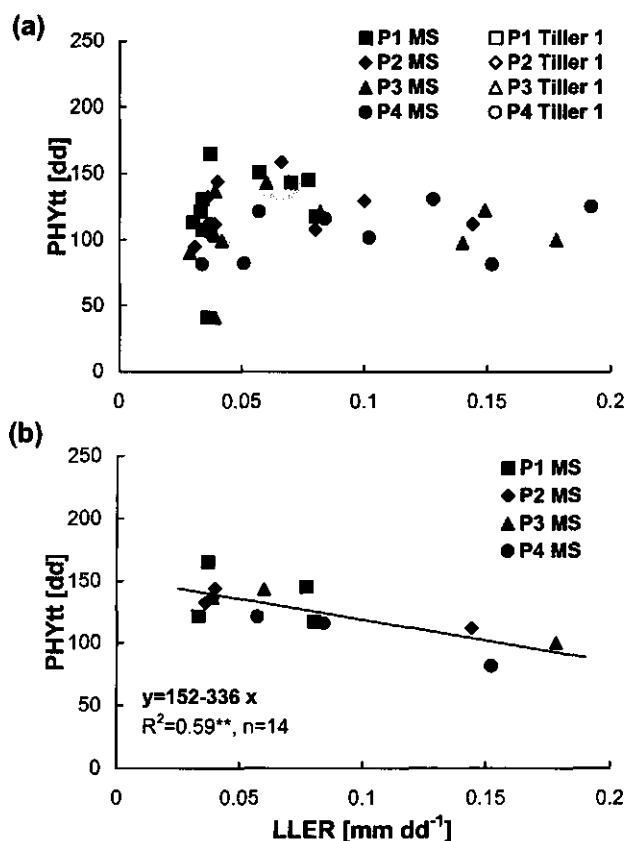
Figure 4.5. Value of the phyllochron for the individual leaves (PHYtt) versus the leaf elongation rate for all the leaves (a), and (b) for those leaves where the rate of leaf elongation was significantly affected by the P treatments.

In Fig. 4.5a, no clear relationship was observed between PHYtt and LLER, however when I eliminated the points corresponding to leaves where the effect of the treatments on LLER were not statistically significant, a negative linear relationship between PHYtt and LLER was detected (Fig. 4.5b).

Discussion

In these experiments the supply of P to wheat plants grown in sand culture was varied to study the effects of P on leaf emergence and its relationship with leaf primordia initiation and the leaf elongation rate. Here, P deficiency reduced the shoot dry weight around double ridge and at anthesis up to a 60%, depending on the level of P supply with respect to the control plants. In control plants (Fig. 4.1a) the random distribution of the data around the regression line indicated that the value of PHY-Ltip was constant during the growing period. In control plants even when the value of PHY was calculated as the cumulated thermal time between the emergence of two consecutive leaves, its value did not consistently increase or decrease with the leaf number.

Table 4.4 Final leaf length (FL, cm), linear leaf length expansion rate (LLER, cm dd^{-1}), and duration of the expansion rate (DUR, dd) of leaves on the main stem and on tiller 1, at four levels of phosphorus supply. Different letter for each leaf number and variable indicate significant differences among P treatments, Tukey ($P<0.05$).

	FL				LER				DUR			
	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4
MSLN												
1	4.9	4.5	4.5	4.5	0.06	0.08	0.05	0.07	94.7	58.3	86.3	63.3
2	5.1	5.5	5.1	5.0	0.03	0.04	0.04	0.03	129.7	147.9	143.7	135.4
3	4.1	4.4	4.3	4.4	0.03	0.04	0.03	0.04	124.7	119.6	119.8	116.3
4	4.6	4.6	4.4	5.0	0.03	0.03	0.03	0.04	153.8	150.1	156.5	144.8
5	5.8	5.9	5.7	6.6	0.03b	0.04ab	0.04ab	0.06a	159.0	165.2	174.1	117.2
6	7.0	6.5	6.9	8.5	0.03	0.04	0.04	0.05	166.0	173.0	207.0	165.0
7	7.1b	7.2b	8.2b	11.0a	0.04c	0.04c	0.06b	0.08a	138.2b	176.8a	193.5a	129.2b
8	7.6	8.5	11.1	11.0	0.06b	0.07b	0.08ab	0.10a	135.6	131.5	139.9	108.2
9	8.2	12.9	15.0	15.6	0.07	0.08	0.14	0.13	108.0	152.8	111.2	123.5
10	14.9c	18.7b	22.6a	19.4ab	0.08b	0.14ab	0.18	0.15a	126.0	130.0	185.0	127.0
11	15.2b	22.3a	26.0a	25.0a	0.08	0.10	0.15	0.19	204.1	258.9	180.4	136.2
12		21.9	22.7	23.7		0.20	0.25	0.30	200.0	292.0		87.0
T1LN												
1	3.1	2.8	3.0	3.0	0.02	0.02	0.02	0.03	123.4	142.2	126.4	108.6
2	4.6	3.7	4.1	5.3	0.03	0.02	0.02	0.05	154.9	162.4	171.1	111.2
3	5.7	4.6	5.3	6.4	0.03	0.02	0.03	0.04	194.7	209.1	196.1	145.8
4	6.3	4.8	6.9	9.3	0.03	0.03	0.05	0.06	218.7	188.3	130.2	141.3
5	7.4	8.2	10.7	11.6	0.05	0.06	0.07	0.10	145.5	137.1	153.1	118.1

MSLN = Main Stem Leaf Number
T1LN = Tiller 1 Leaf Number

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Skinner and Nelson (1994a) observed that an increase of the plastochron and a decrease on the rate of leaf expansion accounted for the increases in the value of PHY observed after the emergence of the leaf number six. Miglietta (1991) found a constant increase in the phyllochron with leaf number in field-grown wheat, and suggested that it could be due to the increased time needed for leaf extension from the apex through an increasingly longer whorl to the point of emergence. In contrast to the results of Skinner and Nelson (1994a), in this study LLER did not decrease but increased with the leaf number, and the rate of production of leaf primordia in the apex was constant. These two factors probably compensated for the greater distance each new leaf had to expand before emerging, and might explain the apparent inconsistency between these results and those of Miglietta (1991). The observation that the values of PHY-Ltip for main stem and tiller leaves were similar, agrees with several reports (Kirby and Riggs, 1978; Klepper et al., 1982), but disagrees with others (Fletcher and Dale, 1974; Kirby et al., 1985). Consequently, it seems that the statement that leaves on the main stem and on the tillers do not always emerge at the same rate would be correct. However, by assuming a unique PHY-Ltip for main stem leaves and tiller leaves Rodríguez et al. (1998a) were able to correctly simulate total leaf area of vegetative wheat plants growing under different levels of P supply using a morphogenetic model. Consequently, whether the values of PHY on the main stem and on the tillers are the same or not seems to have little relevance to the calculation of leaf emergence and plant leaf area.

In P deficient plants, PHY started to be affected after the emergence of the leaf 4 on the main stem, which coincided with the time tiller 1 emerged. At this time probably the seed reserves of P would probably have been exhausted, and the plants started to depend mainly on the P supplied with the nutrient solution.

In this work the value of the plastochron in control plants was about 70% of the value of PHY, and consequently leaf primordia accumulated in the apex and pseudo-stem. Although the effects of P on the value of the plastochron were not statistically significant, in P1 the leaf primordia differentiated at a rate 18% slower than in P4. Primordia formation is primarily a function of the cell division rate and the properties of extensibility of any limiting surfaces acting as constraints on growth (Lyndon, 1994). A promoting effect of P on spikelet primordia initiation was previously observed by Rahman and Wilson (1977). They attributed this effect to an increased rate of cell division in the apex. It is not clear whether P deficiency reduces cell division. Radin and Eidenbock (1984) did not find significant effects of P on cell division of cotton plants, while Jacob and Lawlor (1991) determined that low P reduced the number of cells per plant in wheat and maize leaves by a factor of 10, and in sunflower by a factor of 16. Cell wall extensibility was also proposed to be reduced by P deficiency (Radin and Eidenbock, 1984). The intervention of P in these processes seems to be via alterations in the balance between auxins and cytokinins (Horgan and Wareing, 1980), auxins have been mentioned to control cell wall extensibility (Lyndon, 1994).

A slower rate of leaf primordia differentiation might cause the value of PHY to increase if the value of the plastochron was greater than the value of PHY, and/or the accumulation of developing leaves within the pseudo-stem is reduced. In this work, despite the value of the plastochron in both P1 and P4 plants, were smaller than the

value of PHY, Fig. 4.3b shows that at any value of the Haun index, less leaves were simultaneously elongating in P1 than in P4 plants. I did not measured the elongation rate of the leaves within the pseudo-stem. However, the value of LLER was significantly affected by the P treatments after leaf 4 had emerged, at about the same time that leaf emergence slowed down. Furthermore, the fact that the value of PHYtt was inversely related to LLER, indicates that the leaf elongation rate might have limited leaf emergence. I believe that a smaller number of expanding leaves together with a reduced rate of leaf elongation, were responsible by the increased the value of the phyllochron in P1 plants.

Under potential conditions of crop growth the timing of leaf and tiller emergence are closely related (Davies and Thomas, 1983). However the mechanisms controlling such a synchrony are still unclear (Skinner and Nelson, 1994b; Skinner and Nelson, 1995). Results from this work indicate that phosphorus deficiency not only delayed but also suppressed the emergence of certain tillers without altering the synchrony between main stem leaves and first order tillers. Providing the synchrony does not change, an increased phyllochron together with a decreased rate of emergence of each tiller cohort with in the plant population (Rodríguez et al., 1998a), can delay and even stop tiller emergence (Skinner and Nelson, 1995; Rickman et al., 1983). In these experiments, when the intensity of the P stress was highest, e.g. when the plants had 5-7 main stem leaves, tillering stopped entirely. At less stress (P2) two tillers were skipped, and at P3 and P4 one tiller was skipped. I do not know why treatment P4 skipped T3, most probably at that time the amount of P supplied to P4 plants was too low to support potential growth. The coleoptile tiller (T0) is known to fail when any nutritional or environmental factor is out of the optimum range (Longnecker et al., 1993).

In this work different levels of P supply changed the growth and developmental characteristics of the plants. It was observed that a phosphorus deficiency that reduced growth by a 25% (P3) at 44 days after emergence can significantly modify the structure of the plants by increasing the value of the phyllochron and delaying tillering. P deficiency increased the value of the phyllochron of main stem leaves probably through both a reduced rate of leaf primordia initiation in the apex and a reduced rated of leaf elongation. These results indicate that any attempt to simulate leaf area development and growth of wheat plants under P-limited conditions should take into account the effects of the deficiency on the emergence of main stem leaves. I believe that a better understanding of the effects of P on primordia initiation and leaf expansion could be achieved by studying the effects of P on cell division and on cell expansion.

Chapter 5

Tillering regulation in wheat grown under low phosphorus conditions

Abstract   Phosphorus (P) deficiency limits the yield of wheat particularly by reducing the number of ears per unit of area because of poor tiller emergence. The objectives of this work were to (i) determine whether tiller production under low phosphorus availability is a function of the availability of assimilates for growth or a direct result of low P availability, (ii) attempt to establish a quantitative relation between an index of the availability of P in the plant and the effects of P deficiency on tiller emergence, and (iii) to provide a better understanding of the mechanisms involved in tiller emergence in field grown wheat. Wheat (*Triticum aestivum* L.), cv. INTA Oasis, was grown in the field under drip irrigation on a low-P soil (5.5 mg P kg^{-1} soil Bray and Kurtz (1945)) in Balcarce Argentina. Treatments consisted of the combination of three levels of P fertilization 0 (P1), 60 (P2) and 200 (P3) kg P_2O_5 ha^{-1}, and two levels of radiation reduction, a control (non-shaded) and 65% of reduction in incident radiation from seedling emergence until the end of tillering (shaded). P treatments significantly modified the pattern of growth and development of the plants. Shading reduced the growth and concentration of water soluble carbohydrates in leaves and stems. Leaf photosynthesis at high radiation was reduced by P deficiency but it was not affected by shading. At concentrations of P in shoots lower than 0.42% the heterogeneity in the plant population with respect to the number of plants bearing a certain tiller increased. At a concentration of P in shoots of 0.17% tillering completely ceased. P deficiency directly altered the normal pattern of tiller emergence by slowing down the emergence of leaves on the main stem (e.g. increasing the phyllochron), and by reducing the maximum rate of tiller emergence for each tiller.

Abbreviations: AMAX - leaf photosynthesis at high radiation, DAE - days after emergence, MTR - maximum tillering rate, PHY - phyllochron, Tn - nth main stem tiller, Td - tillering duration, WSC - water soluble carbohydrates

Introduction

Since the study of Engledow and Wadham (1923) the importance of tillering as a determinant of wheat and cereal yield has long been recognized (Ishag and Taha, 1974; Masle, 1981; Davidson and Chevalier, 1990; Elliott et al., 1997). Mechanisms controlling tiller emergence have also been frequently studied (Klepper et al., 1982; Kirby et al., 1985; Skinner and Nelson, 1994a, 1994b). However, whether under nutrient-limited conditions tiller emergence is directly inhibited by the nutrient or whether it is driven by the supply of assimilates required for growth is still not known (Kirby et al., 1985; Skinner and Nelson, 1994a; Longnecker et al., 1993). Among the major nutrients, phosphorus is the most interesting one because P deficiency limits crop growth and yield in many regions in the world. In addition, P deficiency has been suggested reduce tillering (Woodward and Marshal, 1988; Sato et al., 1996), the rate of individual leaf expansion (Radin and Eidenbock, 1984), and the rate of assimilate production per leaf area (Rao and Terry, 1989; Jacob and Lawlor, 1991).

Models including tiller production under potential growth involves predicting tiller emergence as a function of cumulated thermal time (Ong and Baker, 1985; Porter, 1984; Stapper, 1984). Models of tiller production including water and nitrogen as limiting factors, usually predict tillering as a function of the rate of accumulation of assimilate accumulation as a measure of the availability of resources for growth (Dayan et al., 1981; Charles Edwards, 1984; Porter, 1993; Rickman et al., 1996). Prediction of tiller production in wheat under low phosphorus conditions however, has not been approached yet probably because of the lack of information.

The objectives of this work were to (i) determine whether tiller production under low phosphorus availability is a function of the availability of assimilates for growth or a direct result of low P availability, (ii) attempt to establish a quantitative relation between an index of the availability of P in the plant and the effects of P deficiency on tillering, and (iii) to provide a better understanding of the mechanisms involved in tillers emergence in field grown wheat.

Material and methods

A field experiment was conducted during the 1997 growing season at the INTA Research Station, Balcarce, Buenos Aires, Argentina, (37° 45' S, 58° 18' W; 130 m above sea level) on a typic Argiudol. The soil was low in P (5.5 mg P kg^{-1} soil, Bray & Kurtz I), and had a minimum effective soil depth of 1.5m. Treatments consisted in the combination of three levels of soil P fertilization 0 (P1), 60 (P2) and 200 (P3) kg P$_2$O$_5$ ha^{-1} applied before sowing using commercial ammonium phosphate (P treatments), and two levels of assimilate availability, a control (non-shaded) and 65% reduction in incident radiation from emergence to end of tillering (shaded). Shades were made of a neutral shading cloth stretched over the plots on cane and wire structures. Treatments were arranged in a split plot design, P fertilization treatments were the main plot and shading treatments the subplot. The main plots were disposed in randomized complete blocks with four replications. Rainfall and daily total radiation were obtained from a meteorological station located 500 m from the experimental site. Daily air and soil maximum and minimum temperatures were recorded in the shaded and control

treatments using a data-logger (Li 1100, Li-Cor, Nebraska, USA) and thermocouples. Photosynthetically active radiation intercepted by the shading cloth was measured by placing a line quantum sensor (Delta-T Sun-Scan type SS1, Cambridge, UK) 0.15 m above and below the shading cloth.

On July 20, seeds of wheat cv. Pro-INTA Oasis were sown at a density of 320 seeds m^{-2}. The crop was kept free of weeds, and insect pest were adequately controlled. Soil water content was kept above 60% of field capacity in the first meter of soil depth by drip irrigation. The subplots consisted of four rows, 6 m long and 0.17 m apart with two border rows on each side. A total of 238 kg N ha^{-1} was hand applied as urea, splitting the application in four equal amounts at sowing, emergence, beginning of tillering, and mid-tillering.

Leaf and tiller emergence was monitored every two days on ten tagged plants on each subplot. Total above-ground plant dry weight and its partitioning in stems+sheaths, and leaf blades were monitored by sampling all the subplots every time a main stem tiller emerged on the control plants (non-shaded P3 plants). Sample 1 was taken at 27 days after emergence (DAE) when tiller 1 (T1) was present at least in 50% of P3 non-shaded plants. Following a similar rule for tiller 2 (T2), and tiller 3 (T3), sample 2 was taken at 34 DAE, and sample 3 at 48 DAE. Sample 4 was taken at 61 DAE even though tiller 4 emerged in less than 50% of the tested plants. Samples were taken in homogeneous areas of the subplots by cutting all the plants within a frame of 0.17 m^2. Leaf blade and stem areas were determined using an area meter (Li 3100, LI-Cor, Nebraska, USA). Plant parts were oven-dried 65°C to a constant weight, weighed and ground for P determination. Phosphorus in plant material was determined after digestion with a nitric-perchloric mixture by the molybdovanado-phosphoric acid method. At sampling 3, leaf photosynthesis at high radiation (AMAX), i.e. PAR>1800 μmol m^{-2} s^{-1}, and SPAD readings were taken in the field in the last expanded leaf (collar visible), using a portable photosynthesis system (Li 6200, Li-Cor, Nebraska, USA) and a SPAD meter (Minolta SPAD-502, Minolta Corp., Tokyo, Japan). After the AMAX, and SPAD measurements the leaves were harvested for P determination. The day after AMAX and the SPAD measurements were made, the concentration of chlorophyll a and b and SPAD readings were determined in a separated set of leaves to build a calibration curve for the SPAD-meter. Chlorophyll a and b were determined by the method described by Inskeep and Bloom (1985). Water soluble carbohydrates (WSC) in leaves and stems were determined by the anthrone method described by Yemm and Willis (1954). Samples for WSC were taken between 0800-0900 h to minimize the effects of diurnal variations in carbohydrate concentrations.

During the experimental period three to five plants per plot were harvested at 5 to 7 d intervals and their main stem dissected to determine the developmental stage of the apex, and to count the number of leaf and spikelet primordia. Primordia initiation in the apex was studied from the relationship of total primordia vs. cumulated thermal time over 0°C. The rate of primordia initiation in the apex was calculated by using an optimization methodology that fitted the experimental data iteratively to eq. 5.1 by means of a curve-fitting procedure, Fitnonlinear from Genstat 5 (1987).

$$y = a + b1 \bullet x \qquad \text{if } x < c \qquad (5.1a)$$

$$y = a + b1 \bullet c + b2 \bullet (x - c) \quad \text{if } x \geq c \qquad (5.1b)$$

In eq. 5.1, *a* is the value of the ordinate, *b1* and *b2* are rates of primordia initiation per degree day, *x* is the cumulated degree-days [dd] using a base temperature of 0°C, and *c* is the value of *x* where both lines are crossing.

The maximum rate of tiller emergence and the duration of tiller emergence for each tiller (T1, T2 and T3), were calculated using an optimization model, eq. 5.2, that fitted the experimental data iteratively by means of a curve-fitting software Genstat 5 (1987),

$$y = a + b \bullet x \qquad \text{if } x \leq c \qquad (5.2a)$$

$$y = a + b \bullet c \qquad \text{if } x > c \qquad (5.2b)$$

where *y* [-] is the fraction of the tested plant population having a certain tiller, *a* the y-axis intercept, *b* the value of the maximum rate of tillering (MTR [dd^{-1}]), *x* is the cumulated degree-days [dd] from tiller emergence using a base temperature of 0°C, and *c* is the time when the emergence of each tiller ceased [dd]. The duration of tillering (Td, [dd]) for each tiller (T1, T2 and T3) was calculated by eq. 5.2c.

$$Td = c + \frac{a}{b} \qquad (5.2c)$$

Results

Weather
Mean air temperature was 10.4°C with an absolute maximum of 28.6°C and an absolute minimum of -5.3°C (Figure 5.1). Air temperature under the shade was on average 3°C lower than in the non-shaded plots. Mean daily total radiation during the experimental period was 9.9 MJ m^{-2} d^{-1}. During the experimental period the accumulated amount of rainfall was 133 mm.

Crop growth and development
Leaf area and shoot dry weight were significantly affected by the treatments at all the sampling times (Table 5.1). Interactions between P and S treatments were statistically significant for shoot dry weight at samplings 2 and 4. At 61 DAE leaf area was reduced by 30 and 71% in P2 and P1 treatments compared to P3, while shaded plants had on average 13% less leaf area than non-shaded ones. At 61 DAE, P deficiency reduced the dry weight of shoots slightly more in non-shaded than in shaded plants. However, shading reduced shoot dry weight much more at high levels of P supply (44%) than at intermediate (21%), or at low (27%) P levels (Table 5.1).

The P and shading treatments modified the proportion of leaf area held by tillers. At 61 DAE, about 40% of the total leaf area in non-shaded high-P plants was due to tillers, while this proportion was reduced to a 3.6% in P1-shaded plants (Table 5.2).

In average at 61 DAE, P1 plants had 1.1 leaves less than P3 plants (*P*<0.05) (Table 5.2) and shaded plants had 0.6 leaves less than the non-shaded plants (*P*<0.01).
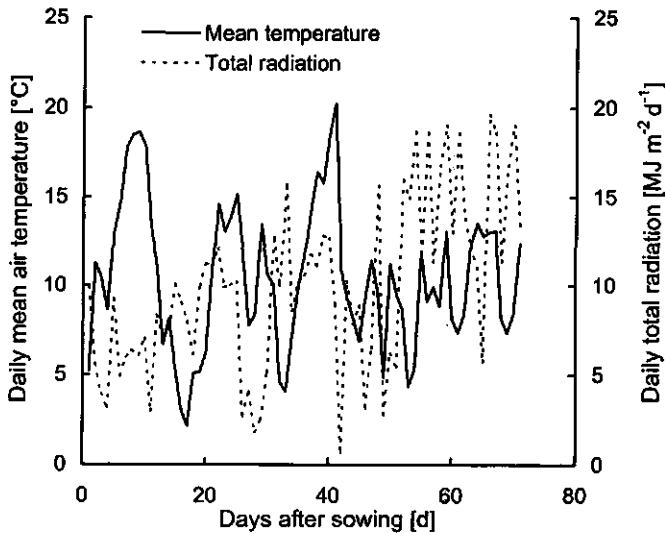
Figure 5.1 Daily mean temperature and mean incoming total radiation during the experimental period as a function of the days after sowing.

Table 5.1 Leaf area and shoot dry weight at 27 (H1), 34 (H2), 48 (H3) and 61 (H4) days after emergence of wheat plants grown at different levels of P nutrition and radiation.

	Non-shaded			Shaded			LSD (0.05)	P	S	PxS
	P1	P2	P3	P1	P2	P3				
Leaf area [cm² m⁻²]										
H1	1425	1731	1982	1481	1328	1777	462	*	NS	NS
H2	2138	3377	4607	2070	2927	3184	1052	*	*	NS
H3	2481	6889	8084	3785	5579	7648	2265	***	NS	NS
H4	8386	19477	31914	7334	19024	23783	6281	***	*	NS
Shoot dry weight [g m⁻²]										
H1	8.8	10.7	11.6	8.0	7.2	8.2	2.4	*	*	NS
H2	15.5	21.6	29.6	11.8	15.8	17.2	4.8	**	***	*
H3	24.6	55.7	69.9	22.8	36.4	46.7	13.5	***	**	NS
H4	66.6	128.5	224.4	48.0	100.3	123.6	23.9	***	***	***

* Significant at $P<0.05$
** Significant at $P<0.01$
*** Significant at $P<0.001$
NS Not significant

Phosphorus deficiency increased the value of the phyllochron (PHY) up to 40%, while the effect of shading was not statistically significant. Regardless of the shading level, at 61 DAE, P1 plants were at Feekes 6 and P2 and P3 plants at Feekes 7 (Table 5.2) (Feekes, 1941). One week later (68 DAE), the terminal spikelet was present in all the treatments.

Table 5.2 Main stem leaf area, tillers leaf area, number of tillers per plant, main stem leaf number (MSLN), phyllochron, and Feekes developmental stage at 61 days after emergence, of wheat plants grown under three levels of P supply and two levels of radiation. Different letters for the values of the phyllochron indicate significant differences (*P*<0.05) by a *t*-test.

	Non-shaded			Shaded			LSD (0.05)	P	S	PxS
	P1	P2	P3	P1	P2	P3				
Main stem leaf area ($cm^2 m^{-2}$)	7375	11951	18339	7066	14875	16412	5085	**	NS	NS
Tillers leaf area ($cm^2 m^{-2}$)	1011	7526	13575	268	4149	7371	2400	***	***	*
Tillers (tillers plant^{-1})	0.77	1.86	2.23	0.07	0.94	1.50	0.59	**	***	NS
MSLN (leaves pl^{-1})	6.2	7.5	7.5	6.0	6.5	7.0	0.7	*	**	NS
Phyllochron [dd leaf^{-1}]	123 c	108 bc	88 a	128 c	103 b	94 ab				
Feekes scale	6	7	7	6	7	7				

*   Significant at *P*<0.05
**   Significant at *P*<0.01
*** Significant at *P*<0.001
NS Not significant.

Table 5.3 Phosphorus concentration in leaf 4, net assimilation at high radiation (AMAX) in leaf 4, water soluble carbohydrates (WSC) in stems and in leaves, and chlorophyll a, b and its ratio in leaf 4, of wheat plants growing at three levels of phosphorus supply and two levels of radiation.

	Non-shaded			Shaded			LSD (0.05)	P	S	PxS
	P1	P2	P3	P1	P2	P3				
Leaf P%	0.15	0.20	0.32	0.13	0.22	0.33	0.04	***	NS	NS
AMAX [$\mu mol\ CO_2\ m^{-2}\ s^{-1}$]	19.4	24.3	27.4	19.7	22.3	27.9	4.3	**	NS	NS
Leaf WSC%	26.2	12.9	9.7	16.0	3.9	5.9	7.2	**	**	NS
Stem WSC%	15.4	11.3	6.3	9.1	3.2	3.2	1.9	**	**	NS
Chl. a [$mg\ m^{-2}$]	313.4	354.4	383.0	329.3	366.9	361.1	64.2	NS	NS	NS
Chl. b [$mg\ m^{-2}$]	64.1	78.6	84.2	75.3	88.3	85.2	16.6	*	NS	NS
a/b	4.9	4.5	4.5	4.4	4.2	4.2	0.37	**	*	NS

*   Significant at *P*<0.05
**   Significant at *P*<0.01
*** Significant at *P*<0.001
NS Not significant.

Assimilate production
Assimilation rate per unit of leaf at high radiation (AMAX), was reduced up to a 30% in P deficient plants (*P*<0.01). Shading did not affect the values of AMAX (Table 5.3). Phosphorus deficiency significantly reduced the concentration of P in recently expanded leaves, and reduced the concentration of chlorophyll b. Phosphorus deficiency increased the concentration of water soluble carbohydrates in leaves and stems, up to 2.8 fold. Shading did not affect the concentration of P in leaves, and
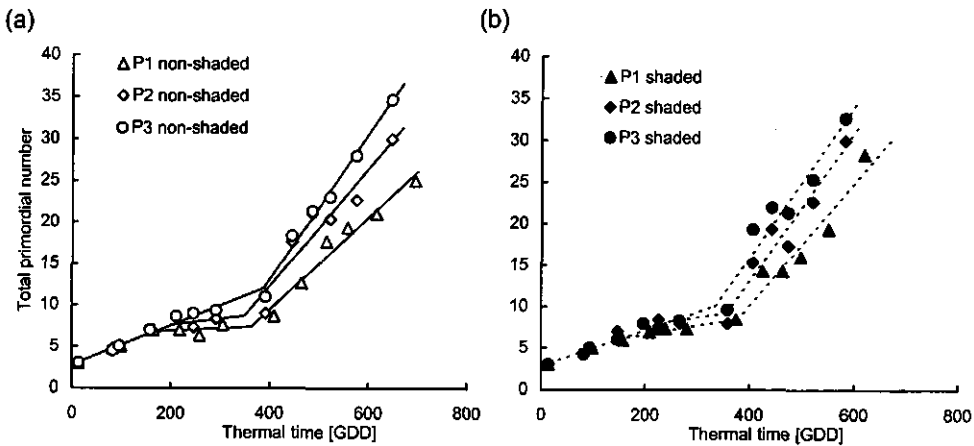
Figure 5.2 Total primordial number versus cumulated thermal time, in non-shaded (a) and shaded (b) treatments for wheat plants grow in the field under different levels of phosphorus supply.

decreased the concentration of water soluble carbohydrates in leaves and stems by about 3.5 fold. Shaded plants had a greater proportion of chlorophyll b than non-shaded plants, consequently their ratio chlorophyll a to chlorophyll b was less.

Primordia initiation and leaf area development
Figure 5.2 shows the total number of primordia on the main stem plotted as a function of thermal time for non-shaded (Fig. 5.2a) and shaded (Fig. 5.2b) plants. The values of the regression parameters obtained by fitting eq. 5.1 to the data of Fig. 5.2, are presented in Table 5.4. At P1 and P2 shaded and non-shaded plants, the data points corresponding to the first three observations were not included since those data points did not differ from the controls (P3 non-shaded and P3 shaded). The value of the parameter a at P3 non-shaded and P3 shaded (Table 5.4) indicates that there were three embryonal leaves in the seed. The rate of primordial initiation showed two distinctive and constant phases. The slower ($b1$), is associated with the initiation of leaf primordia, and the faster ($b2$) with the initiation of spikelets. At high P (P3 non-shaded and P3 shaded), the value of $b2$ was about 4 times higher than the value of $b1$, and under P-deficient conditions this difference increased. Phosphorus deficiency reduced the value of $b1$ by about 87% in non-shaded plants and by 50% in shaded plants. Shading tended to increase the value of $b1$, particularly at low P (P1 shaded and P2 shaded). The value of the abscise at the inflection point (c) did not differ among the treatments and was in average 361.5 dd. According to the regression equations at the inflection point, there were 7.3, 8.6, 12.1, 8.5, 9.2 and 10.04 primordia in the apex for P1, P2, and P3 non-shaded, and P1, P2 and P3 shaded, respectively.
A significant positive relationship was found between the inverse of the phyllochron and the value of $b1$ (Fig. 5.3). At high P (P3 non-shaded and P3 shaded) leaf primordia seems to accumulate in the vegetative apex, data points at the right side of the 1:1 relationship, while at lower levels of P supply (P1 and P2) leaf primordia were initiated at a similar or slower rate than leaf emergence.
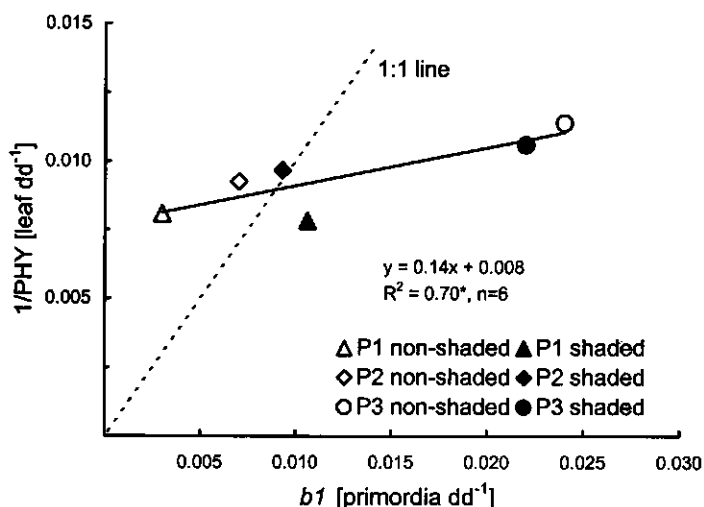
Figure 5.3 Relationship between the value of the inverse of the phyllochron and the rate of leaf primordia initiation in the apex of wheat plants grown in the field under different levels of phosphorus supply and two levels of radiation during tillering.

Table 5.4 Parameters of the regressions between total number of primordia and thermal time, for wheat plants growing under different levels of phosphorus and radiation. Different letters among treatments for each parameter indicate significant differences by a t-test ($P<0.05$).

Parameter	a	b1	b2	c		
	[prim.]	[prim. dd^{-1}]	[prim. dd^{-1}]	[dd]	R^2, d.f.	P
Treatment						
P1 non-shaded	6.3	0.003 b	0.055 b	363 a	0.97, 10	***
P2 non-shaded	6.2	0.007 b	0.070 b	350 a	0.94, 10	***
P3 non-shaded	2.8	0.024 a	0.087 ab	390 a	0.99, 13	***
P1 shaded	4.5	0.011 b	0.073 b	376 a	0.95, 10	***
P2 shaded	5.9	0.009 b	0.088 ab	356 a	0.95, 10	***
P3 shaded	2.7	0.022 a	0.089 a	334 a	0.97, 13	***

a   is the value of y when x=0
b1 is the slope for x<c
b2 is the slope for x≥c
c is the value of x at the inflection point
*** Significant at $P<0.001$.

Figure 5.4 shows the number of phyllochrons elapsed at the time each main stem tiller first emerged in any of the tested plants (1 out of 10 tested plants per experimental unit). In non-shaded plants, this relationship was linear (R^2=0.9, $P<0.001$), while in P3 shaded plants T3 required more phyllochrons to start its emergence. In average in non-shaded plants, a new main stem tiller started to emerge every 1.02 phyllochrons.

In high P non-shaded plants, tillers emerged in the 100% of the tested plant population within a period of 6 to 10 d. Phosphorus deficiency reduced the number of plants presenting a certain main stem tiller, e.g. tiller 1, tiller 2 or tiller 3, and consequently the heterogeneity in the tested plant population, with respect to the number of plants bearing a certain tiller number, increased (Fig. 5.5). Tillers 1, 2 and 3 from treatment P1 shaded, and tiller 3 from treatments P1 non-shaded, P1 shaded and
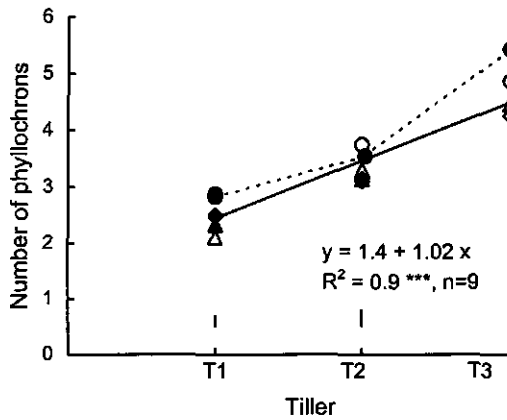
Figure 5.4 Number of phyllochrons required for tiller 1, 2 and 3 to start emergence in wheat plants grown at different levels of P supply and radiation intensity. Bars are standard errors.

P2 shaded did not emerge at all or were present in less than 50% of the tested plant population. Phosphorus deficiency and shading decreased the value of the maximum rate of tiller emergence (MTR). In Table 5.5 and Fig. 5.6a, it was shown that the value of MTR was positively related to the concentration of P in shoots, and not related to the absolute and relative growth rates of shoots, or to the value of SLA of the leaf from where each tiller originated. The value of MTR of shaded and non-shaded plants, showed a strong curvilinear association with shoot P%, the response being dependent on tiller number (Fig. 5.6a). Tillering duration was inversely associated to the value of MTR, however this association did not always hold for shaded plants, particularly at low levels of P supply (Fig. 5.6b). It was calculated the relative values of MTR (relMTR), and relative Td (relTd), with respect to the controls for P within each shading treatment, for each tiller number. The values of relMTR of both shaded and non-shaded plants presented an asymptotic relationship with shoot P% (Fig. 5.7a, eq. 5.3), and that the relTd was related to relMTR (Fig. 5.7b, eq. 5.4). Given the position of surrounding points for all the treatments in Fig. 5.7a, I choose to exclude from the fitting the value or relMTR for the tiller T3 at treatment P2s. In Figure 5.7b, it was decided to exclude the data point of T3 treatment P1un, because of the small number of observations available for its calculation (Fig. 5.5a). In Fig. 5.7a and b it is also shown that eqs. 5.3 and 5.4 described well results obtained in a previous experiment with the same wheat cultivar at a different plant density (170 plants m^{-2}) (Rodríguez et al., 1998a), small symbols in Figs. 5.7a and b.

$$relMTR = 1 - e^{(-9.5 \bullet (Shoot\ P\% - 0.17))} \tag{5.3}$$
$$R^2 = 0.88, P < 0.001, n = 17$$

$$relTd = 0.89 - 1.2 \bullet \ln(relMTR) \tag{5.4}$$
$$R^2 = 0.82,\ P < 0.01,\ n = 12$$

From eq. 5.3, the value of relMTR became zero at P% 0.17, and relMTR was equal to 0.9 at P% 0.42.
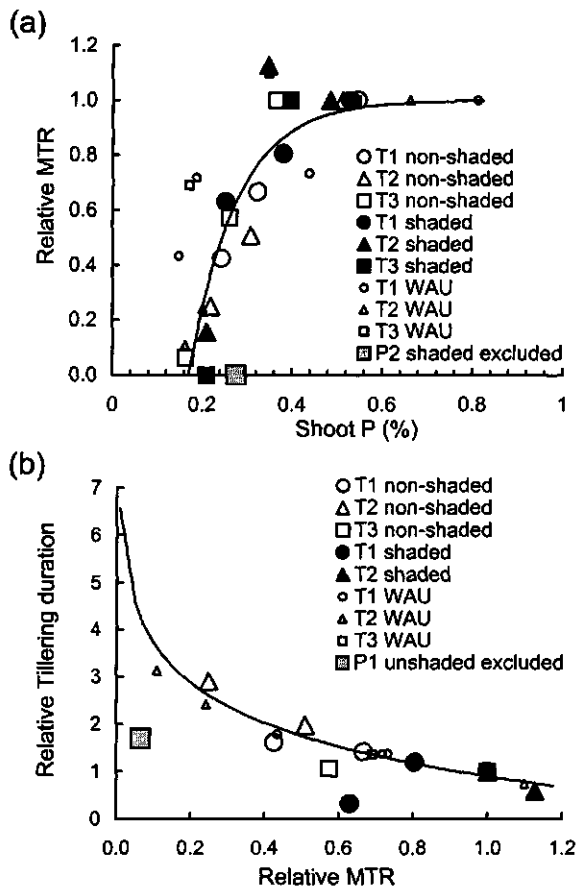
Figure 5.7 Relationship between, (a) relative maximum rate of tillering and the concentration of phosphorus in shoots, (b) the relative tillering duration and the relative maximum rate of tiller emergence, in wheat plants grown in the field under different levels of phosphorus supply and two levels of incident radiation during tillering. Small symbols are the data set taken from Rodríguez et al. (1998a).

Discussion

Two main conditions are known to be required before any primary tiller uniformly emerges in a wheat plant population: (i) a synchrony between main stem leaves and main stem tillers must be satisfied (Klepper et al., 1982; Kirby et al., 1985; Skinner and Nelson, 1994b; Rickman and Klepper, 1995), and (ii) resources should not limit growth (Fletcher and Dale, 1974). However, particularly when growth is limited by nutritional factors (Masle, 1981; Longnecker et al., 1993), the mechanisms involved and the nature of the resources required for the emergence of tillers are not known. Results of this work indicate that tiller emergence in wheat growing under low phosphorus availability was reduced by: (i) delayed emergence of leaves on the main stem, and (ii) direct effects of P on the maximum rate and duration of tiller production.

Table 5.5 Correlation analysis among the maximum tillering rate and plant characteristics.

	Shoot P%	GR	RGR	SLA
Maximum Tillering Rate	0.81	0.31	0.22	0.30
Prob.[1]	<0.001	0.2	0.4	0.2
N.obs.[2]	15	15	15	15
Specific Leaf Area (SLA)[3]	0.19	0.63	-0.34	
Prob.	0.4	<0.05	0.2	
N.obs.	15	15	15	
Relative Growth Rate (RGR)	0.47	-0.15		
Prob.	0.07	0.5		
N.obs.	15	15		
Growth Rate (GR)	0.06			
Prob.	0.8			
N.obs.	15			

[1] Probability
[2] Number of observations
[3] Specific leaf area of the leaf from where each tiller originated from, determined at the time of the emergence of each tiller.
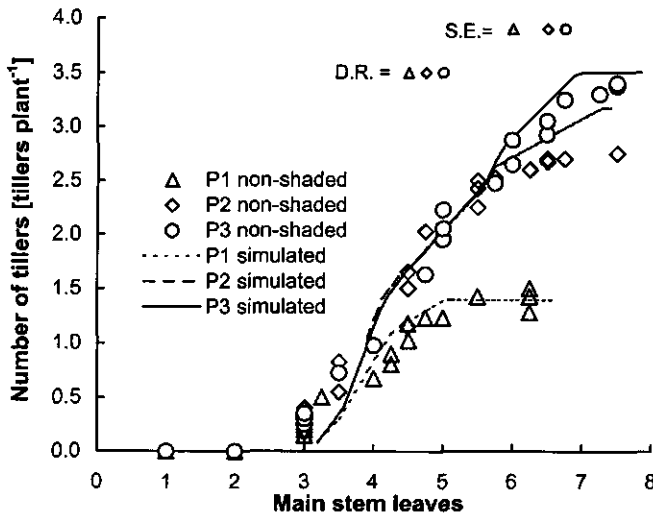
Figure 5.8 Observed and simulated cumulated emergence of main stem tillers and main stem leaves for the non-shaded wheat plants grown under different levels of P supply. D.R. and S.E. indicate double ridge and stem elongation, respectively.

In this experiment, a reduction of 65% in the amount of incident radiation by shading also affected tillering. Shading delayed the emergence of T3 in high P plants (Fig. 5.4), and altered the relationship between Td and MTR of T1 in low P plants (Fig. 5.7b). However, the last observation could be attributed to the small number of observations available for the calculation of these parameters in that treatment (Fig. 5.5a). Using simulation techniques, and assuming that P altered tillering by affecting the values of PHY, MTR and Td it was possible to explain the effects of P deficiency on tillering, in

wheat plants growing in the field. However, further validation of the model remains to be completed.

The value of PHY depends on the rate of leaf primordia initiation in the apex (*b1*), and the duration of the leaf tip elongation through the whorl of mature sheaths. The duration of the leaf tip elongation depends on the rate of leaf expansion and the height of the sheaths (Skinner and Nelson, 1995). It was shown that the rate of leaf primordia initiation was significantly reduced by P deficiency, and that variations in *b1* due to P deficiency explained up to 70% of the variability observed in the rate of leaf emergence. Consequently, I believe that a reduced rate of leaf primordia initiation in the apex caused a first limitation to the emergence of leaves on the main stem of low P plants.

The results indicating that P deficiency reduced the rate of spikelet initiation (*b2*) without affecting the duration of the initiation period, agrees with the observations made by Rahaman and Wilson (1977). In this experiment, all the treatments reached the stage of terminal spikelet at about the same time, indicating that a slower rate of spikelet initiation would reduce the potential number of kernels per ear (Rahaman and Wilson, 1977; Batten and Wardlaw, 1987a, b). The rate of leaf or spikelet primordia initiation depends on the rate of cell division in the apex, a zone that presents a high metabolic rate (Pearen and Nelson, 1989), a high concentration of nutrients (Gastal et al., 1991) and probably a high requirement of P for cell division (Jacob and Lawlor, 1991).

A P deficiency that delays leaf primordia initiation, and delays the emergence of main stem leaves will then also delay the emergence of main stem tillers as the timing of main stem leaf and main stem tiller emergence (Fig. 5.4), are closely synchronized processes (Etter, 1951; Davies, 1974; Davies and Thomas, 1983).

Despite tillers followed the emergence of leaves on the main stem, only in P3 non-shaded plants all the primary tillers, e.g. T1, T2 and T3, completed their emergence in 100% of the tested plant population. In a previous paper (Rodríguez et al., 1998a), found that tillers that were form later, tended to have lower values of MTR and higher values of Td. Gan and McLeod (1997) suggested that an increased tillering duration might be a consequence of the increase on the length of the whorl a tiller has to extend before emerging from a higher positioned leaf on the main stem. In this work, T2 at P3 non-shaded had a higher value of MTR and a lower value of Td than for T1. Furthermore, Rodríguez et al. (1998b) found that the value of PHY did not vary with leaf number while the rate of leaf length expansion systematically increased for twelve successive main stem leaves. This indicates that a possible effect of different heights of the sheaths in higher order leaves on the value of PHY as well as on tiller emergence, would have been nullified by an increase in the rate of leaf expansion. Kirby et al. (1985) indicated that internal factors might be important in regulating tillering. In our opinion the values of MTR and Td with plant development, are more related to sink-source regulations rather than to a position-related mechanism such as suggested by Gan and Mc Leod (1997).

Phosphorus deficiency and shading decreased the number of plants possessing a certain tiller. P deficiency decreased the value of MTR, and increased Td. Consequently, the homogeneity in the tested plant population decreased with respect to the number of plants bearing a certain tiller. This resulted in fewer tillers per plant in

low P plants since the increase in Td did not compensate for the reductions in MTR. Similar results in wheat grown under increasingly unfavourable conditions were obtained by Rickman et al. (1983), and under P deficiency by Rodríguez et al. (1998a).

The critical concentration of P in shoots for tillering, defined at relMTR=0.90, was equal to 0.42% P, and similar to the value (0.38%) previously found with the same cultivar, but at a different plant density (Rodríguez et al., 1998a). Equations 5.3 and 5.4, described well the relative values of MTR and Td found in most of the shaded treatments, and those found by Rodríguez et al. (1998a). This observation, and the fact that the value of MTR was not related to the absolute or relative growth rates of the crop, suggests that MTR in phosphorus-deficient plants primarily responded to P rather than to a limitation in the availability of assimilates.

A shoot P concentration lower than 0.42% systematically increased the heterogeneity in the plant population with respect to the number of plants bearing a certain tiller, particularly as the minimum value of 0.17% P was approached. Greater heterogeneity in the plant population will probably increase the inter- and intra-specific competition in the crop. It is known that plants presenting tillers will intercept radiation and explore the soil profile for resources more efficiently than a poorly tillered plant (O'Donovan et al., 1985). Although Fig. 5.7a clearly indicates a regulating role of P on tillering, the mechanisms involved are still far from understood. Changes in tillering as a response to a low P supply can be interpreted as a response to either an increased competition for P within the plant or to altered levels of endogenous growth regulators induced by low P. Phosphorus deficiency is known to reduce the content of cytokinins in plants of sunflower (Salama and Wareing, 1979). In barley, Woodward and Marshall (1988) found that low phosphorus plants treated with cytokinin-like compounds presented similar tillering rates as high P controls. This suggests that the growth and development of tiller buds in low P plants might not primarily be restricted by nutrients availability but directly by hormonal factors.

Shading might have altered other plant characteristics that affected tillering since the relationhsip between Td and MTR, and the timing of emergence of T3 in P3 shaded plants were altered by the radiation environment. The characteristics of the shaded plants were similar to those reported by others (Fletcher and Dale, 1974; Fisher, 1975; Kemp, 1981). Shaded plants had a lower concentration of WSC in leaves and stems, and decreased ratios of chlorophyll a to chlorophyll b. I believe that treatment P2 shaded failed to produce T3, due to an unbalanced sink:source ratio. Despite P2 shaded plants presented a similar shoot P%, and rate of leaf photosynthesis than P2 non-shaded, P2 shaded plants had the lowest concentration of WSC in leaves and stems. Treatments P1non-shaded and P1 shaded also failed to produce T3, these treatments presented the highest concentrations of WSC in leaves and stems, which indicates that a direct inhibition of P on the formation of sinks was present.

In agreement with the results of Longnecker et al. (1993), it was found that in control plants the relation between the cumulated emergence of main stem tillers and main stem leaves was not linear but tent to decline (departure point) for the latest-formed tillers (see treatment P3 non-shaded in Fig. 5.8). In non-shaded plants P deficiency further decreased the emergence of tillers, and as found by Longnecker et al. (1993), it was observed that the departure point occurred earlier as the intensity of the distress of the plants increased (Fig. 5.8). In this work as in the work of Longnecker et al. (1993),

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the departure point did not correlate with any particular developmental stage of the plants or of the apex. Longnecker et al. (1993), found that after the departure point, tillers continue to emerge at a lesser but similar rate among different nitrogen treatments, and from this concluded that the availability of resources was not determining the reduced rate of tiller emergence among their nitrogen treatments. In this work it was possible to simulate the timing of the departure point, in non-shaded plants, taking into account the effects of shoot P% on the value of the phyllochron, and on the values of MTR and Td. These results indicate that the availability of P was crucial for tillering to proceed at the time of the departure point.

In conclusion, it has been shown that phosphorus directly alters the normal pattern of tiller emergence by slowing the emergence of leaves on the main stem, and by reducing the maximum rate of tiller emergence. In P deficient plants assimilate supply seemed to play a minor role determining the emergence of tillers in wheat.

Significant gains to the understanding of tiller dynamics can still be made, out of studies focused on the regulation of the departure point and tiller survival. Particularly, since increases in yield under a double CO_2 environment, as predicted to happen in about 50 years time, seems primarily to be driven by a higher emergence and survival of tillers (Batts et al., 1996).

Chapter 6

Effects of phosphorus deficiency on the growth of wheat and sunflower plants. A comparative study.

Abstract: Differences among crops with respect to their capacity to produce yield under low phosphorus (P) conditions have been associated to differences in their efficiency to take up soil P and/or to use the plant P to produce growth and yield. The objective of this work was to compare the response of wheat and sunflower plants to different levels of P nutrition in terms of their efficiency to take up and use P for growth. Sunflower (hybrid Paraiso, Nidera S.A) and wheat (Pro INTA Oasis) plants were grown in two simultaneous glasshouse experiments in Wageningen, The Netherlands. In both experiments, the effects of soil P addition on leaf area, dry weight, P uptake, and leaf photosynthesis, were studied in plants grown in pots containing a P-deficient soil. Before sowing the equivalent amounts of 0 to 600 kg of super-phosphate ha^{-1} were added to the pots. In both species, P deficiency reduced the growth of shoots and leaf area expansion. However, in sunflower the effects of P deficiency appeared to be more severe than in wheat. Under a low P supply sunflower plants were particularly more sensitive to P deficiency, first because of a reduced P uptake associated with a smaller rooting capacity, and second because of a higher threshold concentration of P in the leaves with respect to photosynthesis. Once the threshold was exceeded, in sunflower photosynthesis responded faster than wheat to an increase in the concentration of the nutrient in the leaf.

Abbreviations: AMAX - leaf photosynthesis at high radiation; LAI - leaf area index; P - phosphorus, PAR - photosynthetic active radiation; RUE-PAR - radiation use efficiency for PAR

Introduction

Within a global context of increasing food demand, highly variable climatic conditions and increased loss of soil fertility, particularly in countries of non subsidised economies, the efficient use of an increasingly scarce input as phosphate fertilizer has become an essential goal in present agriculture. The fact that there is diversity among species in their ability to take up and to use phosphorus from the environment has been known for decades (Lyness, 1936). Differences among crops with respect to their capacity to produce adequate yields under low P conditions have been associated to differences in their efficiency to take up soil P and/or to use the plant P (Gourley, et al., 1993). Wheat and sunflower crops present several interesting features to be included in a comparative study of the plant response to different levels of soil P availability. Important differences between these two species not only are found with respect to their leaf and root morphology (Osaki, et al., 1994), also with respect to the photosynthetic characteristics of their leaves. Although both species are classified as C3 plants, sunflower presents extremely high rates of net photosynthesis ranging between 25 and 42 μmol CO_2 m^{-2} s^{-1} (Lloyd and Canvin, 1977; Rawson and Constable, 1980), reached at a minimum photon flux density of about 1500 μmol m^{-2} s^{-1} (English et al., 1979). In contrast, wheat represents a typical C3 response to radiation with a maximum photosynthetic rate of 20 to 30 μmol CO_2 m^{-2} s^{-1}, reached at a relatively low irradiance, 900 μmol m^{-2} s^{-1} (Lawlor, 1993). A comparatively high stomatal conductance, high specific rubisco activity and efficient chloroplast electron transport have been cited as factors that contribute to high leaf photosynthesis at high ratidaion (AMAX) in sunflower (Connor and Sadras, 1992). Quantum yield of the two species is very similar, and can be considered to be 0.06 mol CO_2 mol^{-1} (Rawson and Constable, 1980; Lawlor, 1993).

Evidence of a differential response of both species to low P supply is present. Jacob and Lawlor (1991) found that low P supply decreased more strongly leaf area in sunflower than in wheat plants, while net photosynthesis per unit of leaf area was decreased by a similar extent. In a comparative study of C3, C4, monocot and dicot plants, Halsen and Lynch (1996) concluded that monocots had lower leaf P concentrations and better maintenance of leaf production under conditions of P stress than dicots.

A differential response to low P between monocot and dicot species could also be expected after the work of Radin and Eidenbock (1984). These authors found that after transferring cotton plants from an adequate to a low P nutrient solution a decrease in the root hydraulic conductivity preceded any change in the root-shoot ratio, leaf expansion or leaf photosynthesis. In Gramineae species, cell expansion occurs at the base of the leaf blade. This zone is protected from the atmosphere by the sheath of the preceding leaf, and thus there is little transpiration from the zone of elongation. In broadleaf species, cell expansion occurs in leaf blades, which are exposed to the air and therefore transpiring. Therefore, a limited root hydraulic conductivity caused by a low P availability, might be expected to have more severe long-term growth responses in broadleaf plants than in cereals.

In sunflower, Trapani and Hall (1995) found a hyperbolic relationship between both, the rate of leaf expansion and the value of photosynthesis at saturating light intensities,

when plotted versus the content of nitrogen per unit of leaf area. In their work the minimum nitrogen concentration for leaf expansion was higher than the critical value for light saturated photosynthesis. These results would indicate that in sunflower direct effects on expansion could be differentiated from those on the assimilation rate. In cereals, leaf expansion has been observed to be more closely linked to the production of assimilates and their partitioning to roots (Evans, 1972).

The objective of this work was to compare the response of wheat and sunflower plants to different levels of P nutrition with special emphasis on phosphorus uptake and use efficiency.

Material and methods

Plants of sunflower (*Helianthus annuus* L.) Paraiso 5 of Nidera S.A. and wheat (*Triticum aestivum* L) Pro-INTA Oasis, were grown in Wageningen (51° 58' N, 5° 40' E), The Netherlands. In these species, the effects of plant P nutrition on growth, leaf area expansion, and leaf photosynthesis were studied. Using simulation techniques the effects of P deficiency were studied at canopy level by integrating the instantaneous rate of leaf photosynthesis and calculating the value of the radiation use efficiency for photosynthetic radiation (RUE-PAR) as a function of the concentration of P in the leaves. Transmission of radiation trough the glasshouse was 80%.

Cultural techniques and growth conditions
Plants of sunflower and wheat were grown in pots in a glasshouse in two separated sub-experiments. The pots, 5 L and 4 L for sunflower and wheat, respectively, were filled with a sandy soil containing 3 mg P kg^{-1} (Bray and Kurtz, 1945). The pots were arranged to form a canopy of 5 and 173 plants m^{-2} for sunflower and wheat, respectively. Test plants were surrounded by plants of the same treatment as a border. Adequate soil moisture level in the pots was maintained throughout the experimental period. Daily maximum and minimum temperatures were measured inside the glasshouse.

Treatments and experimental set up
In sunflower there were three levels of P supply, that were equivalent to 15 (P1), 60 (P2), and 300 (P3) kg P ha^{-1}, in wheat there were four levels of P supply equivalent to 7 (P1), 15 (P2), 60 (P3), 300 (P4) kg P ha^{-1}. Fertilizer was applied as super-phosphate (ground in mortar). The highest doses of P in each crop was split, half applied and incorporated before sowing and half applied on the soil surface at the 5 leaf stage in sunflower and at 3 leaf stage in wheat. All pots received a basic dressing of macro and micronutrients at the time of sowing. The equivalent of 400 kg N ha^{-1} (as NH_4NO_3), was distributed during the development of the plants. Treatments were randomized within each of three blocks.

Determinations and measurements
In sunflower, the value of AMAX of leaves 7 and 8, 11 and 12, and 15 and 16 were determined at the moment those leaves reached their maximum size. Then the plants were harvested immediately for dry matter and leaf P analysis. At each harvest the leaf

area of each individual leaf was determined using a LI-3100 area meter (Li-Cor, Nebraska, USA). Additionally leaf photosynthesis and leaf P concentration were determined in those leaf insertion numbers where AMAX had been measured at previous harvests, e.g. AMAX and leaf P were measured in leaves 7 and 8 at harvest 1, leaves 7, 8, 11 and 12 at harvest 2, and leaves 7, 8, 11, 12, 15 and 16 at harvest 3. In wheat AMAX and leaf P were measured in leaves 4 at harvest 1, leaves 4 and 5 at harvest 2, leaves 4, 5 and 6 at harvest 3 and leaves 4 to 7 at harvest 4. AMAX was determined using a portable photosynthesis system LCA-2 System ADC (Analytical Development Co. Ltd.), in combination with a lamp (Philips Projection Lamp 6853; 75 W) installed over the leaf chamber resulting in a PAR of 2000 μmol m^{-2} s^{-1}.

After harvest, dried plant material was wet digested in a H_2SO_4 / salicylic acid / H_2O_2 / selenium mixture and concentrations of total N and P were colorimetrically measured in the digests using an automated continuous-flow system. For more details of the experimental set up see Rodríguez et al. (1998a; b).

Data on AMAX (μmol CO_2 m^{-2} s^{-1}) versus the concentration of P in the leaf (Leaf P%) were fitted to eq. 6.1 using Sigma Stat (Jandel Scientific, Erkrath, Germany). At each species differences among treatments in growth and leaf area expansion were tested by ANOVA using Sigma Stat (Jandel Scientific, Erkrath, Germany).

$$ \text{AMAX} = a \bullet \left(1 - e^{\left(-b \bullet \left(\text{Leaf P\%} - c \right) \right)} \right) \tag{6.1} $$

Simulation model

Assimilate production at canopy level was calculated using the subroutines of the model SUCROS (Goudriaan and van Laar, 1994), modified to account for the effects of the concentration of P in the leaf on AMAX (eq. 6.1). In the model, the apparent quantum yield (QY, μmol (CO_2) (μmol (PAR))$^{-1}$) was assumed not to be affected by P, and set at 0.06 for both crops. In sunflower, the extinction coefficient varies with LAI as in Villalobos et al. (1996), in wheat extinction coefficient was set to 0.6 (Meinke, 1996). Maintenance respiration was set as 15% of the daily gross assimilation and effects of temperature on photosynthesis were taken from Horie (1977) for sunflower and from Goudriaan and van Laar (1994) for wheat. As I was only interested in calculating radiation use efficiency (RUE) during vegetative stages, the same coefficient for growth respiration was used for both crops (Penning de Vries et al., 1989). The model calculates the intensity of incident radiation, and the proportion of incident radiation that is diffuse at different depths within the canopy. Canopy assimilation rate is calculated by accumulating the instantaneous assimilation rates over three layers and integrating the instantaneous rates over the day (Hammer and Wright, 1994), by using the three-point Gaussian method (Goudriaan, 1986). The models were written using the programming environment denominated Fortran Simulation Translator 2.0 (Rappoldt and van Kraalingen, 1996). I ran the model for the combination of the following conditions: open (LAI = 1) and closed canopies (LAI=4), and high (25 MJ m^{-2} d^{-1}) and low (9 MJ m^{-2} d^{-1}) total radiation intensities, varying the concentration of P in the leaves from 0.01 up to 0.8 %. Air temperature was set to 20°C.

Results and discussion

In both species phosphorus deficiency reduced shoot biomass production and leaf area expansion (Table 6.1). In sunflower however, the effects of P deficiency appeared to be more severe than in wheat. At 42 days after emergence total dry weight and total leaf area were reduced with 82 and 83% in low-P sunflower plants, and with 61 and 67% in low-P wheat plants.

Table 6.1 Total dry weight, and total leaf area in wheat and sunflower plants grown under different levels of phosphorus supply after 42 days from emergence. Different letters for each crop variable indicate significant differences (*P*<0.05) by Tuckey.

Wheat		
	Leaf area	Total dry weight
P level	[m^2 m^{-2}]	[g m^{-2}]
P1	1.35 b	159.9 c
P2	1.87 b	233.9 bc
P3	2.41 b	304.4 b
P4	4.14 a	416.2 a
s.e.m.	0.23**	24.0**
Sunflower		
P1	0.03 c	26.5 c
P2	0.10 b	72.0 b
P3	0.18 a	148.5 a
s.e.m	0.008**	7.0**

*   significant at *P*<0.05
**   significant at *P*<0.01
*** significant at *P*<0.001
s.e.m. is the standard error of the mean after ANOVA.

A differential response to P supply of the two species can be explained in terms of differences in their efficiency to take up P from the soil (Föse et al., 1991), and/or differences in their P use efficiency (Sattelmacher et al., 1994). The efficiency for nutrient uptake of a particular species can be simply characterized by both the extension of its root system, and its uptake capacity per unit of root weight. Available computer simulation models for nutrient uptake clearly show that P uptake responds most sensitively to changes in root extension (Barber, 1984). In these experiments the partitioning of dry weight between roots and shoots changed little among P treatments (Figs. 6.1a and b). In wheat the average root:shoot ratio was 0.4 (c.v.=9%) while in sunflower it was much lower, 0.15 (c.v.=6%). For rather immobile nutrients as P, a higher root:shoot ratio will allow a more extensive and fast soil exploration for resources, particularly under conditions of low P supply (Barber, 1982; Clarkson, 1985). However root morphological characteristics as length, diameter, and duration and length of root hairs, can be more relevant for uptake than root biomass. P uptake not only depends on the extension of the root system but also on its uptake efficiency (Barber, 1984; Cogliatti and Clarkson, 1983).

(a)

(b)
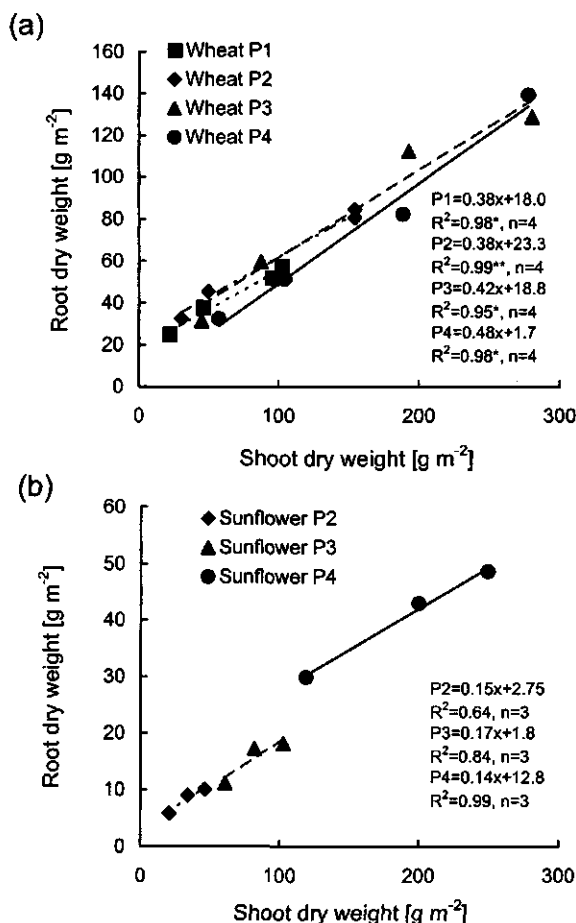
Figure 6.1 Root versus shoot dry weight in wheat (a) and sunflower (b) plants, growing under different levels of phosphorus supply.

In Fig. 6.2, it is shown that at a low and intermediate levels of P supply the averaged P uptake efficiency among the different harvests tend to be higher in sunflower than in wheat. Wheat is known to present a higher specific root area (m^2 g^{-1} root) than sunflower (Osaki et al., 1994). Consequently, a higher uptake rate per unit of root weight in sunflower would indicate a higher uptake efficiency if expressed per unit of root area (Barber, 1984). However, it is not possible to discard that the observed differences in uptake efficiency between wheat and sunflower, were due to a differential extent of the P depletion in the pots. At low P supply the P use efficiency increased by up to 2.6 and 1.6 folds in wheat and sunflower plants, respectively (Fig. 6.3). At high levels of P supply, if P is not longer deficient, the P use efficiency becomes less because part of the P will be accumulated as non metabolic pool in the vacuole (Bieleski, 1973). Phosphorus in the vacuole is not directly involved in photosynthetic reactions, consequently increases in its internal P concentration above a certain threshold would not be expected to result in further increases in growth.
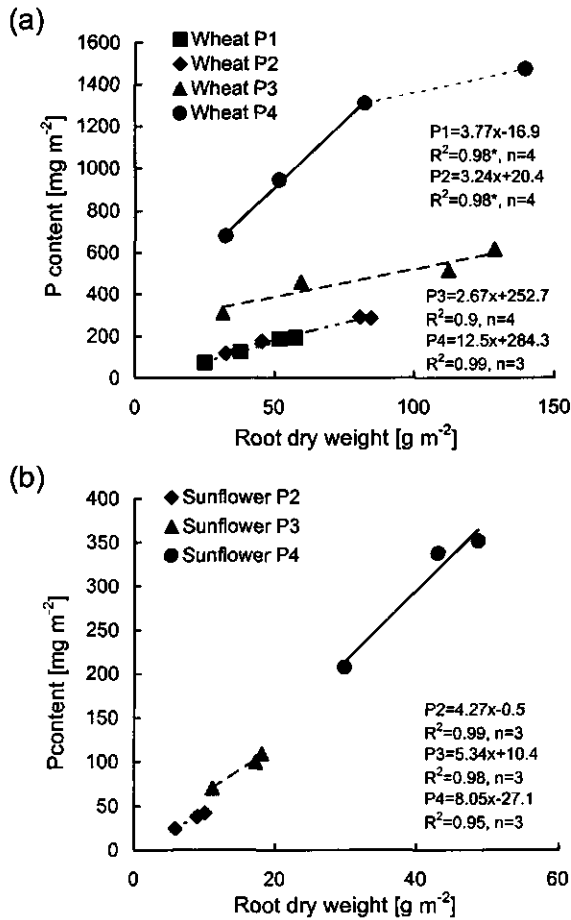
Figure 6.2 Plant phosphorus content versus the root dry weight in wheat (a) and sunflower (b) plants, growing under different levels of phosphorus supply.

Figure 6.3 shows that sunflower presented higher values of P use efficiency than wheat plants. Differences in P use efficiency between wheat and sunflower plants might be due to differences in the rate of photosynthesis and/or differences in the compartimentation of P at the cellular level (Ricardo, 1994). The rates of photosynthesis at high radiation in fully expanded leaves of sunflower plants, were a 73% higher than in wheat plants when grown at non limiting levels of P supply (Fig. 6.4). Figure 6.4 also shows that the values of AMAX in wheat and sunflower plants growing under different levels of P supply were asymptotically related to the concentration of P% in their leaves, and that recently expanded and older leaves responded similarly to leaf P. From the fitted equations, sunflower presented a slightly higher minimum leaf P concentration for AMAX, 0.08 versus 0.066%P, respectively. The sensitivity of AMAX in the two crops to variations in leaf P% can be characterized by the value of coefficient b in eq. 6.1. A high value of b in eq. 6.1
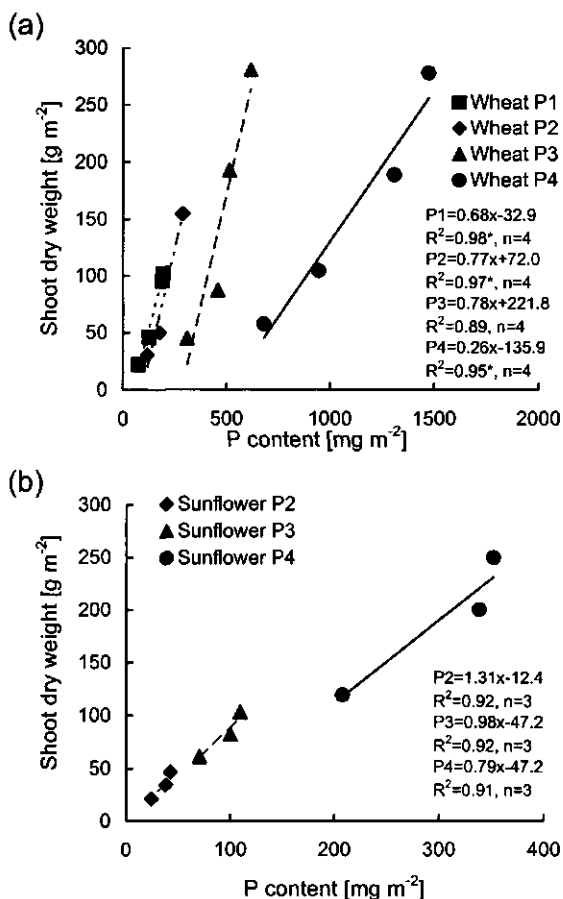
Figure 6.3 Shoot dry weight versus the phosphorus content in wheat (a) and sunflower (b) plants, growing under different levels of phosphorus supply.

indicates a high sensitivity of AMAX to leaf P%. From the fitted equations it is shown that the sensitivity of AMAX to leaf P% in sunflower was a 25% higher than in wheat, indicating that leaf photosynthesis in sunflower would react faster to increases in the internal P status than in wheat.

Using simulation techniques I calculated the values of RUE-PAR for total biomass in wheat and sunflower plants as a function of the leaf P concentration, for different canopy sizes and radiation levels (Figs. 6.5 and 6.6). In Fig. 6.5 the values of RUE, predicted for a closed canopy (LAI=4) and high levels of total radiation (25 MJ m^{-2} d^{-1}), are consistent with those observed experimentally for these two species (Kiniry et al., 1989; Trapani et al., 1992).

Figure 6.5 also shows that RUE is less responsive to changes in AMAX at high levels than at low levels of AMAX, particularly for sunflower. As observed with nitrogen (Sinclair and Horie, 1989), the lower sensitivity of RUE at high values of AMAX caused the steep response of RUE to leaf P% as observed in Fig. 6.6. In Fig. 6.5 it is also shown that despite sunflower has almost twice as high AMAX values than wheat, little difference can be observed on RUE. Furthermore from Fig. 6.5 it seems
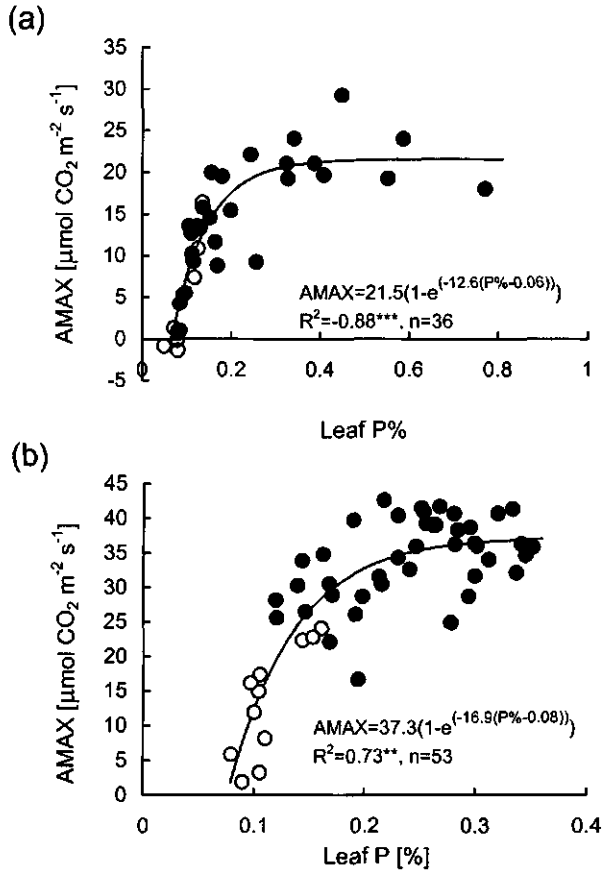
Figure 6.4 Photosynthesis at high radiation (AMAX) as a function of the concentration of P in the leaf of wheat (a) and sunflower (b) plants growing under different levels of phosphorus supply. Closed and open symbols are for recently expanded and older leaves, respectively.
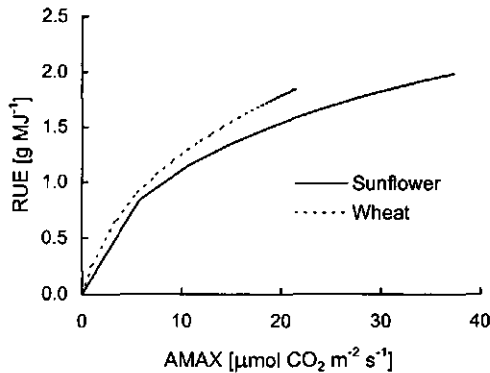
Figure 6.5 Calculated radiation use efficiency (RUE) as a function of photosynthesis at high radiation (AMAX) for sunflower and wheat plants.
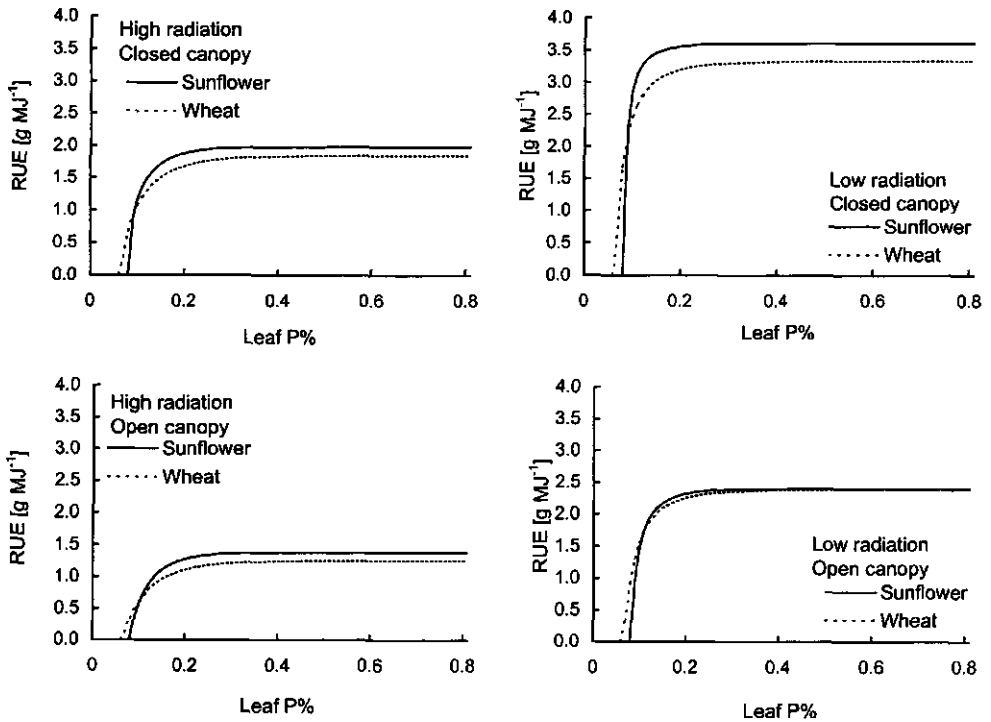
Figure 6.6 Calculated radiation use efficiency (RUE) as a function of the concentration of phosphorus in the leaf of wheat and sunflower plants, for different canopy and radiation conditions.

that in wheat there would be some scope for increasing crop biomass accumulation by further increasing the value of AMAX, while in sunflower the response is flattening.

Figure 6.6 shows that at high levels of P nutrition sunflower always presented higher values of RUE particularly at high levels of radiation, probably due to its higher value of AMAX. However under P-deficient conditions wheat plants presented higher values of RUE than sunflower. At values of leaf P concentrations of 0.08% RUE of sunflower plants was zero, however in wheat the value of RUE was still at a 36% of its maximum. Halsted and Lynch (1996) also found that dicot species were more sensitive than monocot species to low-P conditions. In their work they concluded that carbon allocation and utilization more than carbon assimilation were important in determining the growth of plants under P stress. From the results of this work as well as from those found before (Rodríguez et al., 1998a; b; c), it is most likely that sink size, e.g. leaf initiation in the apex, individual leaf expansion, and tillering, will primarily determine the response of the plants to P deficiency.

In conclusion, this work shows that under conditions of low P supply sunflower plants were particularly more sensitive to P deficiency as a consequence of, (i) a lower P uptake due to a smaller rooting capacity, and (ii) to a higher threshold concentration of P in the leaves with respect to photosynthesis. Once the threshold is exceeded, photosynthesis responded faster in sunflower than in wheat to an increase of the leaf P concentration.

Chapter 7

General discussion

Phosphorus is known to play an important role in leaf expansion (Radin and Eidenbock, 1984), and in the photosynthetic carbon metabolism of leaves (Walker and Sivak, 1986; Terry and Rao, 1991). The following sections in this chapter will discuss the main results of this work in the general context of our present understanding of the effects of P on plant development and growth. Here reference will be made to the implications of these results for the development of comprehensive simulation models of the growth and production of wheat and sunflower growing under limiting conditions of P.

Phosphorus deficiency as a sink and source limitation

Whether P deficiency directly reduces the individual leaf area expansion, causing sink limitation, or whether leaf expansion is reduced by a lack of assimilates for growth as a consequence of impaired photosynthesis (source limitation) was the guiding question of this study.

Sink limitation

Sinks can be defined as regions in the plant which are heterotrophic for photosynthetically fixed carbon. All meristems are sinks, and the organs produced by the action of meristems remain sinks (Pollock and Farrar, 1996), at least till they become autotrophic. Here I considered as a growing sink: vegetative and reproductive apices; tiller buds, and expanding leaves. From this work it can be concluded that P deficiency reduces the activity of the sinks through reductions in, (i) the expansion of individual leaves, (ii) the rate of leaf primordia initiation, (iii) the rate of leaf emergence, and (iv) tiller emergence.

By using experimental and simulation techniques, the existence of direct effects of P deficiency on the rate of expansion of individual leaves was identified in sunflower and wheat. Direct effects of P on leaf expansion accounted for 1/6 and 1/3 of the total observed reduction in the plant leaf area of low-P sunflower (48%) and wheat (41%) plants. The effects of nutrients on leaf expansion have long been recognized (Radin and Eidenbock, 1984; Palmer et al., 1996). However, no previous information was available with respect to the response of AMAX and leaf expansion to P in wheat and sunflower.

The findings in this work indicate that even when P limits the individual leaf expansion in wheat and sunflower plants, there was no direct relationship between the concentration of P in the individual leaves and the rates of individual leaf expansion. Whether under P-deficient conditions leaf expansion is limited through the lack of turgor required for cell enlargement was questioned in this thesis (Chapters 3 and 4). However, as proposed by others (Takami et al., 1982; Gallagher, 1985) leaf expansion could also be limited by an impaired availability of assimilates required for leaf

growth, or by unknown leaf epidermal properties that may change in response to a low plant P status (Freeden et al., 1989; Palmer et al., 1996). Leaf epidermal properties are known to limit cell wall expansion (Pritchard et al., 1991), however it is not known whether nutrient deficiencies can modify these properties (Palmer et al., 1996) or whether growth regulators might also be involved (Salama and Wareing, 1979). In this work, a lack of assimilates as a consequence of P deficiency explained up to a 42% of the observed reduction in total leaf area in sunflower, but no such effect on leaf area expansion was found in wheat. In gamelia, Cromer et al. (1993) found that a limited supply of photoassimilates constituted a stronger restriction for division than for the enlargement of primordial leaf cells. Furthermore, Wenkert et al. (1978) observed that the exclusion of morning sunlight in soybean resulted in zero leaf area growth that same afternoon. In dicots, 90% of the cell population of a mature leaf originates from after unfolding. Consequently rates of individual leaf area expansion as I measure in this work might be affected by limitations in the availability of assimilates for growth., particularly in sunflower a dicot species.

Under P-deficient conditions the strength of the sink is also reduced, as leaf primordia initiation and leaf emergence are delayed, and tillering is restricted. According to the simulation results of Chapters 2 and 3, the effects of P on leaf emergence in sunflower, and leaf and tiller emergence in wheat influenced the size of the leaf area in both species. With wheat, P deficiency modified the proportion of leaf area held by tillers. At 61 days after emergence in high P plants up to about 40% of the plant leaf area was held by tillers while in low P plants this fraction was reduced to about 12%.

Major factors determining the rate of leaf emergence in wheat are temperature and daylength (Rawson and Slafer, 1994). However, nutritional factors also modify the rate of leaf emergence (Longnecker and Robson, 1994; Rodríguez et al., 1994; Sato, 1996; Elliott et al., 1997). In cereals when new tillers emerge from a shoot they depend on that shoot for carbohydrates until at least one blade is fully expanded (Masle, 1981). Consequently tillers are the major sink for carbohydrates and any factor influencing tiller number or blade growth will influence source-sink relations. It has been shown that under low P conditions the emergence of leaves on the main stem was affected by the particular effects of P on the value of the plastochron, and on the rate of expansion in leaf length. Leaf emergence was observed to affect tiller emergence. Phosphorus deficiency did not alter the value of the synchrony. The effects of P on tiller emergence through the values of the maximum tillering rate and the tillering duration had not been described before. Morphogenetic models for the development of the leaf area in wheat were developed using these mathematical relationships. These equations give a simple and clear framework for future modelling of the effects of phosphorus deficiencies on tiller emergence.

Source limitation
In both wheat and sunflower the rate of photosynthesis at high radiation was reduced by P deficiency. The response of AMAX to the mean P concentration of the leaves was non-linear and saturated at concentrations of P in leaves of 0.22 and 0.27%, for sunflower and wheat respectively. Photosynthesis was affected much less by low P at low radiation than at high radiation, and despite stomatal conductance is generally

reduced at low P (Rao and Terry, 1989; Jacob and Lawlor, 1991; Qiu and Israel, 1992), mesophyll factors rather than restricted CO_2 diffusion limits photosynthesis (Jacob and Lawlor, 1991).

Although P reduced photosynthesis at high radiation, water soluble carbohydrates (WSC) accumulated in stems and leaves of low-P wheat plants. Accumulation of WSC under low P conditions has been observed by others (Qiu and Israel, 1992; Rao et al., 1990) and was attributed to a stronger limitation of the sink rather than the source under P-deficient conditions. In this work, WSC were not determined in sunflower. However in sunflower plants grown at low-P, assimilate production seemed to play an important role determining the growth of the plants. Using simulation techniques it was shown that a lack of assimilates due to the effects of P deficiency on AMAX, explained up to a 42% of the observed reduction in plant leaf area of low-P sunflower, while none in wheat plants. This differential response between wheat and sunflower might be attributed to differences in their requirement of assimilates for maintenance and growth. Sunflower is known to present higher maintenance respiration (0.025 g CO_2 g^{-1} d^{-1}, at 20°C; Amthor, 1984) than wheat (0.016 g CO_2 g^{-1} d^{-1}, at 20°C; van Keulen and Seligman, 1987), and also a higher carbohydrate requirement for growth (1.862 and 1.415 g glucose g^{-1} dry matter of storage organs, in sunflower and wheat respectively; Penning de Vries et al., 1983). Furthermore, a differential capacity to balance alterations of the source:sink ratio might also exist between wheat and sunflower. In low-P wheat plants, the total number of growing points, e.g. total number of leaves per plant, was severely reduced as tiller emergence slowed down or stoped. However, the size of the sink in vegetative low-P sunflower plants, is only determined by the effect of P on the rate of leaf emergence. Consequently, in wheat due to the strong limitation to sink growth, the amount of assimilates available for growth was probably less limiting than in sunflower.

Modelling growth and development under P limiting conditions and future needs
A primary aim of crop research is to explain crop behaviour, and crop models are a powerful tool for testing our understanding of crop behaviour (Seligman, 1990).
According to Barber (1982) the simulation of crop response to P should include simulation of the dynamics of P in the soil-root interface, root surface extension, P uptake and plant growth and development as a function of a supply-demand function. Unfortunately, there is still not enough information on these processes to be able to make any significant simulation attempt (Monteith, 1996). In this work the emphasis was on studying whether under P limitation growth is sink and/or source limited. Here direct effects of P on leaf expansion and canopy assimilation were put into a quantitative context by combining experimental and simulation techniques. After this work, major progress was made with respect to modelling crop growth under P-deficient conditions. Useful information is found in: (i) the relationships between the value of AMAX in wheat and sunflower and the P-status of the leaves, (ii) the understanding of leaf emergence as a function of the effect of P on the plastochron and on the rate of leaf elongation, (iii) the description of tillering in a plant population as a function of maximum tillering rate and tillering duration, and (iv) the effects of P on these processes. However further developments are still needed as it is still not known how P directly limits leaf expansion, how the partitioning of assimilates among aerial

organs and roots is regulated. Furthermore studies under increased CO_2 conditions indicated that the critical concentration of P in shoots for dry weight production might increase with the concentration of CO_2 (Rogers et al., 1993; Conroy et al., 1994). This, and the fact that economical sources of P are becoming scarce, indicate that P is going to become a more important limitation to agricultural production.

Summary

Phosphorus (P) deficiency limits the growth and yield of crops in many regions in the world, while in others the use of P fertilizers has become excessive resulting in increased leaching of P to surface waters and in other forms of environmental pollution. Alleviating nutrient deficiencies with a minimum use of fertilizers requires to identify the specific physiological basis of the yield limitation, and if possible to make beneficial modifications of the identified processes. This thesis gives a process oriented study to improve, and further develop our capability for understanding and simulating wheat and sunflower growth for conditions of low phosphorus availability. Here observations at leaf, plant and crop levels were made in wheat and sunflower grown at different levels of P supply. The information obtained at different levels of observation was integrated in simulation models that helped to understand and quantify the importance of each process determining growth under P-deficient conditions.

In Chapters 2 and 3, the effects of P on leaf expansion and canopy assimilation were put into a quantitative context by combining experimental and simulation techniques. The question was whether plant leaf area under P-deficient conditions is directly inhibited by P, or whether leaf expansion is also limited by the lack of assimilates as a consequence of effects of P on the rate of leaf photosynthesis. According to the results of simulation models, reduction of the expansion of individual leaves in P-deficient sunflower plants, was attributed to both direct effects of P shortage, but also to a lack of assimilates for growth. In wheat however, the availability of assimilates did not affect the expansion of individual leaves. A sensitivity analysis of several model parameters showed that both in wheat and sunflower the parameters determining the generation of leaves and tillers, e.g. phyllochron in wheat and sunflower, and tillering in wheat, are crucial in determining plant leaf area expansion.

Chapter 4 focuses on the relationship between leaf emergence, and the rates of (i) leaf primordia initiation, and (ii) individual leaf expansion, in wheat plants deficient in P. Here a P deficiency that reduced shoot growth by 25% at 44 days after emergence significantly modified the structure of the plants by increasing the value of the phyllochron and delaying or even suppressing the emergence of tillers. Under P deficient conditions, the value of the phyllochron responded to both the rate of leaf initiation in the apex (plastochron) and to the rate of leaf elongation.

In Chapter 5, the effects of phosphorus and assimilate supply, on tillering in wheat were described from a field experiment. The objectives of this work were, (i) to determine whether tiller production under low phosphorus availability is a function of the availability of assimilates for growth or whether a direct inhibition by P was involved, (ii) to establish a quantitative relation between an index of the availability of P in the plant and the effects of P deficiency on tillering, and (iii) to provide a better understanding of the mechanisms involved in tiller outgrowth in wheat plants grown in the field. P deficiency directly inhibited tillering by slowing down the emergence of leaves on the main stem, and by reducing the maximum rate of tiller emergence for each primary tiller index. Irrespective of the level of P supply, in general terms the availability of assimilates had little effect on the maximum rate of tiller emergence. A

concentration of P in shoots lower than 0.49%, systematically increased the heterogeneity of the plant population with respect to the number of plants bearing a certain tiller. At a concentration of P in shoots less or equal to 0.17%, tillering completely ceased. The information obtained from this experiment was integrated in a simulation model that explained well the observed results. The main assumptions in this simulation model are, (i) P deficiency increases the value of the phyllochron, (ii) P deficiency reduces the maximum rate of tillering, (iii) P deficiency does not change the relationship between the emergence of main stem leaves and main stem tillers (synchrony).

In Chapter 6, a comparative study between the response of wheat and sunflower to different levels of P supply was made by using data obtained in the experiments described in Chapter 2 and 3. For both species, P deficiency reduced the biomass production and leaf area expansion. However, in sunflower the effects of P deficiency appeared to be more severe than in wheat. Under low P supply conditions sunflower was more sensitive than wheat to a P deficiency, because of a reduced P uptake associated with a smaller rooting capacity, and because of a higher threshold concentration of P in the leaves with respect to photosynthesis.

Chapter 7 presents a general discussion of the main results obtained in this work in the general context of our present understanding of the processes involved in crops grown under P-deficient conditions. Here the effects of P deficiency on crop growth are analysed in terms of source-sink relationships. It is concluded that in wheat shortage of P affects plant growth particularly through limitations in sink size, rather than in the availability of assimilates for growth. For sunflower, however reductions of both the sink and source were important to the response of the plants to low P.

Samenvatting

Tekort aan fosfor (P) is een beperkende factor voor groei en opbrengst van gewassen in vele delen van de wereld, terwijl in andere gebieden het gebruik van fosfaatbemesting excessieve vormen heeft aangenomen, met als gevolg een toenemende uitspoeling van P naar het oppervlaktewater en andere vormen van milieuverontreiniging. Vermindering van fosfaatgebrek bij een zo laag mogelijk gebruik van meststoffen vereist ontrafeling van de specifieke fysiologische basis van opbrengstbeperking, en zo mogelijk verbetering van de geïdentificeerde processen. Dit proefschrift geeft een proces-georiënteerde studie teneinde onze mogelijkheden om de groei van tarwe en zonnebloem onder fosfaat-beperkte omstandigheden te begrijpen en te simuleren en zo mogelijk verder te verbeteren en te ontwikkelen. Er werden waarnemingen gedaan op blad-, plant- en gewasniveau bij tarwe en zonnebloem, opgekweekt bij verschillende niveaus van P voorziening. De informatie die op de verschillende waarnemingsniveaus werd verkregen, is samengebracht in simulatiemodellen die er toe bijdragen om het belang van elk afzonderlijk proces voor de groei onder P-beperkte condities te begrijpen en te kwantificeren.

In de hoofdstukken 2 en 3 zijn de effecten van P op bladstrekking en gewas-assimilatie in kwantitatieve samenhang gebracht door combinatie van experimentele en simulatietechnieken. De vraag was of het totale bladoppervlak onder P-beperkte condities rechtstreeks wordt beperkt door P, of dat bladstrekking ook wordt geremd door een tekort aan assimilaten voor de groei, dit ten gevolge van effecten van P op de bladfotosynthese. Volgens de resultaten van de simulatiemodellen moet een remming van de bladstrekking bij P-deficiënte zonnebloemplanten zowel worden toegeschreven aan directe effecten van P-tekort als aan een tekort van assimilaten voor groei. Bij tarwe echter had de beschikbaarheid van assimilaten geen effect op de bladstrekking. Een gevoeligheidsanalyse van verscheidene modelparameters toonde aan dat die parameters die de ontwikkeling van het aantal bladeren bepalen, dus bij tarwe en zonnebloem de fyllochron, en alleen bij tarwe ook de uitstoeling, essentieel zijn voor de bepaling van het bladoppervlak van de hele plant.

Hoofdstuk 4 is gericht op verklaring van het effect van de snelheden van vorming van bladprimordia en van individuele bladstrekking op de snelheid van bladverschijning bij tarweplanten. Een tekort aan P dat 44 dagen na opkomst de spruitgroei met 25% had gereduceerd, had de plantstructuur aanzienlijk gewijzigd door een toename van de fyllochron en een vertraging of zelfs onderdrukking van de uitstoeling. Onder P-beperkte omstandigheden was de waarde van de fyllochron gevoelig voor zowel de snelheid van bladinitiatie in de apex (plastochron) als voor de snelheid van bladstrekking.

In hoofdstuk 5 worden de effecten van P en assimilatenvoorziening op uitstoeling bij tarwe beschreven uit een veldproef. De doelstellingen van dit werk waren, (i) om vast te stellen of uitstoeling onder lage P-beschikbaarheid een functie is van de beschikbaarheid van assimilaten voor groei of dat er ook een direct effect van P aanwezig is, (ii) om een kwantitatieve relatie vast te stellen tussen een index voor de P-beschikbaarheid in de plant en de effecten van P-tekort op uitstoeling, en (iii) om een

beter begrip te verschaffen van de mechanismen die betrokken zijn bij de uitgroei van de spruiten van de tarweplant in het veld. P-tekort veroorzaakte een rechtstreekse remming of afname van uitstoeling door vertraging van de bladverschijning op de hoofdstengel, en door reductie van de maximum snelheid van spruitverschijning van elke spruitnummergroep. Wat het niveau van P-voorziening ook was, in het algemeen had de beschikbaarheid van assimilaten nauwelijks effect op de uitstoeling. Bij een P-concentratie in de spruiten lager dan 0.49% werd de heterogeniteit van de plantenpopulatie vergroot met betrekking tot het aantal planten met een zeker aantal spruiten. Bij een P-concentratie in de spruiten beneden 0.17% was er helemaal geen spruitvorming meer. De informatie uit deze deelstudie werd bijeengebracht in een simulatiemodel dat de experimentele resultaten goed kon verklaren. De voornaamste aannames in het model zijn: (i) P-tekort vergroot de waarde van de fyllochron, (ii) P-tekort vermindert de maximum snelheid van spruitvorming (iii) P-tekort beïnvloedt niet de relatie tussen de verschijning van bladeren op de hoofdstengel en de zijspruiten van de hoofdstengel (synchronie).

In hoofdstuk 6 is een vergelijkende studie uitgevoerd naar de respons van tarwe en zonnebloem op verschillende niveaus van P voorziening, gebruikmakend van resultaten afkomstig uit de proeven die zijn beschreven in hoofdstuk 2 en 3. Bij beide soorten verlaagdenbeij een tekort aan P de productie van biomassa en de groei van het blad. Echter, bij zonnebloem bleken de effecten van P tekort ernstiger dan bij tarwe. Bij een lage P voorziening was zonnebloem gevoeliger voor P-tekort dan tarwe vanwege een verminderde P opname in samenhang met een kleinere bewortelingscapaciteit, en vanwege een hogere drempelconcentratie van P in de bladeren met betrekking tot de fotosynthesesnelheid.

Hoofdstuk 7 geeft een algemene discussie van de belangrijkste resultaten die in dit werk zijn verkregen, in de algemene context van ons huidige inzicht in de processen die betrokken zijn bij de groei van planten onder P-deficiënte omstandigheden. Hier zijn de effecten van P tekort geanalyseerd in termen van source-sink relaties. Een conclusie was dat bij tarwe een tekort aan P de groei van de plant vooral beïnvloedt door beperkingen in de sink activiteit, en niet in de eerste plaats door beschikbaarheid van de assimilaten voor de groei. Bij zonnebloem echter was reductie van de activiteit van zowel de sink als de source belangrijk voor de uiteindelijke respons van de planten op lage P voorziening.

Resumen

En numerosos agroecosistemas deficiencias de fósforo reducen el crecimiento y rendimiento de los cultivos, mientras que en otros, el uso excesivo de fertilizantes fosforados han devenido en la contaminación de aguas superficiales y polución. Para eliminar deficiencias de P con un uso mínimo de fertilizantes químicos, es indispensable identificar las bases fisiológicas de la reducción de los rendimientos, para luego realizar las modificaciones beneficas necesarias sobre los procesos identificados. En este trabajo se realizó un estudio mecanístico tendiente a mejorar y desarrollar nuestra capacidad para entender y modelar el crecimiento de trigo y girasol bajo condiciones de baja disponibilidad de P en el suelo. En este trabajo se realizaron observaciones a nivel de hoja, planta y cultivo. Finalmente la informacion obtenida a distintos niveles de agregación fue integrada en modelos matemáticos que sirvieron para dar una mejor comprensión de la importancia de cada proceso estudiado sobre el crecimiento de trigo y girasol bajos condiciones limitantes de P.

En los Capítulos 2 y 3 se estudió si ante deficiencias de P, la expansión del area foliar es directamente inhibida por la deficiencia del nutriente, o bien si los efectos del P sobre la tasa de fotosíntesis limitan la expansion foliar a través de un reducido suministro de asimilados. La reducción en la tasa de expansión de hojas de girasol en plantas deficientes en P, fue atribuida tanto a efectos directos del P como a la falta de asimilados para el crecimiento. En trigo sin embargo, la disponibilidad de asimilados no afecto la expansion foliar. Un análisis de sensibilidad de varios parámetros de los modelos desarrollados mostraron que aquellos parámetros relacionados con el desarrollo del área foliar, filocrono (en trigo y girasol) y macollaje (en trigo) fueron importantes para explicar los efectos de deficiencias de P sobre el crecimiento y la expansion foliar en ambas especies estudiadas.

En el Capítulo 4 los efectos de la deficiencia de P sobre el filocrono en trigo, se estudiaron en relación a los efectos del P sobre, la tasa de diferenciación de hojas en el ápice del tallo principal, y de la tasa de expansión de hojas individuales. En este capítulo una deficiencia de P que redujo el crecimiento aéreo en un 25% a los 44 días luego de emergencia, modificó significativamente la estructura de las plantas incrementando el valor del filocrono y retrasando o bien eliminando la emergencia de macollos. Ante condiciones limitantes de P, el valor del filocrono de plantas de trigo dependió de los efectos de la deficiencia de P sobre el valor del plastocrono y sobre la tasa de expansion foliar.

En el Capítulo 5 se estudiaron los efectos del nivel de nutrición fosforado y la suplementación de asimilados sobre el macollaje de plantas de trigo. Los objetivos de este experimento fueron, (i) determinar si la producción de macollos ante una baja disponibilidad de P es función de la disponibilidad de asimilados para el crecimiento o bien si existe una inhibición directa del P sobre el macollaje, (ii) establecer relaciones cuantitativas entre un índice de la nutrición fosforada de las plantas y los efectos del P sobre el macollaje, (iii) ampliar nuestro conocimiento actual con respecto a los mecanismos involucrados en la emergencia de macollos en plantas de trigo creciendo a campo. La deficiencia de P inhibió directamente el macollaje redujendo la tasa de

emergencia de hojas sobre el tallo principal y la tasa máxima de emerencia de los macollos T1, T2, y T3. La disponibilidad de asimilados no afectó la tasa maxima de macollaje. A concentraciones de P en parte aérea menores a 0.49%, la heterogeneidad en la población de plantas, con respecto al número de plantas que presentaba un determinado macollo, se incrementó. A concentraciones de P en parte aérea menores o iguales a 0.17%, el macollaje se detuvo por completo. Teniendo en cuenta los efectos de la deficiencia de P sobre la emergencia foliar y sobre la tasa máxima de macollaje fue posible construir un modelo de simulación que explicó los resultados obtenidos experimentalmente.

En el Capítulo 6, se realizó un estudio comparativo de la respuesta de trigo y girasol ante distintos niveles de fertilización con P, utilizando la información experimental de los Capítulos 2 y 3. Para ambas especies la deficiencia fosforada redujo la producción de biomasa y la expansión del area foliar, sin embargo, en girasol los efectos de la deficiencia de P fueron más severos que en trigo. Ante condiciones de un bajo suministro de P, el girasol fue más sensible que el trigo a deficiencias de P debido particularmente a, (i) una menor absorción del nutriente asociada con una menor capacidad de enraizamiento, y (ii) un mayor umbral de concentración de P en hojas para la fotosíntesis.

En el Capítulo 7 se discuten los principales resultados obtenidos en estos trabajos, en el marco de nuestro conocimiento actual de los procesos involucrados en el crecimiento de cultivos bajo condiciones limitantes de P. Aqui los efectos de deficiencias de P sobre el crecimiento de los cultivos es analizado en función de sus efectos sobre las relaciones fuente-destino. En este capítulo se concluye que para trigo, las deficiencias de P afectan el crecimiento particularmente a traves de una reducción en el tamaño de los destinos mas que a una escasa disponibilidad de asimilados para el crecimiento. Para girasol sin embargo, deficiencias de P limitaron tanto a el tamaño de los destinos como la suplementación de asimilados por la fuente.

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Curriculum Vitae

Daniel Rodríguez was born on 17 October 1962 in Buenos Aires, Argentina. From 1982 till 1989, he studied for his B.Sc. degree at the Facultad de Agronomía Universidad de Buenos Aires (FAUBA). In 1989, he joined the Departamento de Suelos Cátedra de Fertilidad y Fertilizantes at FAUBA. Between 1989 and 1992, he worked as teaching and research assistant at FAUBA. In 1992, he started his M.Sc. at the Sub-department of Theoretical Production Ecology, Wageningen Agricultural University and graduated in 1994. In 1995, he continued his work at FAUBA, and in 1996 he started his PhD programme at the Sub-department of Theoretical Production Ecology. In 1997, he got a research grant from the International Foundation for Science (IFS) of Sweden, and a scholarship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina to support this work.

Other publications

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Appendix

FST source code for some of the subroutines of the simulation model presented in Chapter 2. This is not a complete listing, for the subroutines ASTRO, ASSIM and TOTASS from SUCROS see Goudriaan and van Laar (1994).

```
TITLE SUNPHO
****************************************************
* This model calculates leaf area as a function of assimilate
* production considering a minimum and maximum specific leaf
* area for the newly leaves (MINSLA and MAXSLA). Leaf
* expansion rate (LER) is calculated as a function
* of potential leaf expansion rate (LER24) as a function of
* leaf number and temperature. The final LER is defined by
* comparing with the availability of assimilates and MINSLA
* and MAXSLA. This will allow to increase SLA if there is a
* lack of assimilates and to decrease it when there is ample
* supply.
****************************************************

DECLARATIONS
************
DEFINE_CALL TOTASS(INPUT, INPUT, INPUT, INPUT, INPUT,...
                   INPUT, INPUT, INPUT, INPUT, INPUT,...
                   OUTPUT,OUTPUT,OUTPUT)

DEFINE_CALLLEAFEX(INPUT, INPUT, INPUT, INPUT, INPUT,...
                  INPUT, INPUT, INPUT, INPUT, INPUT,...
                  INPUT, INPUT,...
                  OUTPUT, OUTPUT, OUTPUT, OUTPUT,...
                  OUTPUT, OUTPUT, OUTPUT, OUTPUT,...
                  OUTPUT, OUTPUT, OUTPUT)

DEFINE_CALL LEFNUM(INPUT, INPUT, INPUT, INPUT,...
                   OUTPUT,OUTPUT)

MODEL
*****
```

```
WEATHER WTRDIR='C:\SYS\WEATHER\'
WEATHER CNTR='NLDb'; IYEAR=1996; ISTN=1

INITIAL
*******
* Initial parameters and constants
INCON  ILA  = 10.0; WRTI = 1.0; WLVI = 1.5
INCON  ZERO = 0.0 ; WSTI = 0.5; WSOI = 0.0

* Plant density [pl/ha] and phyllochron
PARAMETER  PLANTS = 50000.0; PHY1 = 50.0  ; PHY2 = 38.0
PARAMETER  AMX    = 60.0   ; A    = 1.18  ; B    = 0.55
PARAMETER  EFF    = 0.45
PARAMETER  IRATE  = 2.0      ; DUREX = 230.0 ; LAGD =90.0

* Timing
TIMER STTIME=152.0; FINTIM = 218.0; DELT = 1.0; PRDEL = 1.0
TRANSLATION_GENERAL DRIVER='EUDRIV'

* Printing
PRINT TSUM,DAE,TLACM2,TDW,LDW,SDW,HDW,RDW,TOTSLA

DYNAMIC
*******
FINISH DAE>59.0
* Initial calculations
* Days after emergence = DAE
* 0.8 is the transmisivity of the glasshouse
DTR   = RDD*0.80
DAE   = TIME-151.0

* TDMEAN is the mean temperature
TDMEAN    = (TMN+TMX)/2.0

* DTT is the effective daily thermal time, 4 is the Tb
DTT   = TDMEAN-4.0
TSUM2 = INTGRL(ZERO,TDMEAN)

* TSUM is the integral of DTT
TSUM     = INTGRL(ZERO,DTT)

* Call the calculation of leaf emergence
CALL LEFNUM(TSUM,PHY1,PHY2,DTT,....
            LN,PHY)
```

```
* Call leaf expansion subroutine
CALL LEAFEX(LN,PHY,MINSLA,MAXSLA,GLV,TSUM2,TDMEAN,PLANTS,...
            DTT,IRATE,DUREX,LAGD,...
            PLER,LER24,LER,CUMPH,EXPLEF,LNNLE,EXPR,SLA,...
            PLER1,OBSLER,OBPLER)

* Senescent leaf dry weight ratio (SLWR)
SLWR    = AFGEN(SLWRTB,TSUM)
FUNCTION SLWRTB=0.0,0.00 ,  480.0,0.00,   734.0,0.0.15,...
            853.0,0.061, 979.0,0.04, 1500.0,0.04

* Total Leaf Area in cm2 per plant
TLACM2  = INTGRL(ILA,PLER)

* Green Leaf Area in cm2 per plant
GLACM2  = TLACM2-(LDW*SLWR*MAXSLA*10000.0)

* Calculate the amount of leaf area lost due to the lack of
* assimilates
LOSLAR  = PLER1-PLER
LOSTLA  = INTGRL(ZERO,LOSLAR)

* Green leaf area m2 per plant and green LAI
GLA     = GLACM2/10000.0
LAI     = GLA*PLANTS/10000.0

* SLA is for the newly expanding leaves m2/g
* Min and Max sla is for individual leaves not for the canopy
MINSLA = AMIN1(0.025,0.0103+0.182/((LN+1.0)*(LN+1.0)**0.5))
MAXSLA = MINSLA*1.35
TOTSLA = TLACM2/LDW/10000.0

* Partitioning coefficients, no retranslocation
FUNCTION FLVTB = 0.0,0.57, 793.7,0.31, 916.2,0.070,...
            1000.0,0.0 ,1500.0,0.0
FUNCTION FSTTB = 0.0,0.25, 793.7,0.52, 916.2,0.600,...
            1000.0,0.57,1500.0,0.0
FUNCTION FRTTB = 0.0,0.18, 793.7,0.15, 916.2,0.100,...
            1000.0,0.0 ,1500.0,0.0
FUNCTION FSOTB = 0.0,0.00, 793.7,0.02, 916.2,0.230,...
            1000.0,0.5 ,1500.0,1.0

FLV     = AFGEN(FLVTB,TSUM)


FST     = AFGEN(FSTTB,TSUM)
FRT     = AFGEN(FRTTB,TSUM)
FSO     = AFGEN(FSOTB,TSUM)

* Light extinction
* SCP extinction of visible radiation
PARAMETER  SCP = 0.20

* The extinction coefficient = KDF varies with LAI as in
* Villalobos et al., 1996
QN      = 1.0-EXP(-0.86*LAI)
QD      = ALOG[1.0-1.72*(1.0-EXP(0.863*QN))]
KDF     = QD/LAI

* Daily total CO2 fixation
CALL TOTASS(A,B,DOY,LAT,DTR,SCP,AMAX,EFF,KDF,LAI,...
            DAYL,DTGA,DS0,FAMAX)

* Leaf CO2 assimilation
* Effect of temperature on photosynthesis taken from Horie,
* (1977).
FUNCTION AMTTB=0.0,0.0,  4.0,0.0.0,   10.0,0.5,   20.0,1.0,...
            30.0,1.0,40.0,0.5

AMTMP   = AFGEN(AMTTB, TDMEAN)
AMAX    = AMX*AMTMP

* Carbohydrate production
GPHOT       = DTGA * 30./44.
CRESP       = GPHOT*0.15
*      (gross production of Kg CH2O/ha d)

* Maintenance respiration
MAINT   = MAINTS * TEFF
MAINTS  = MAINLV*WLVG + MAINST*WST + MAINRT*WRT + MAINSO*WSO
TEFF    = Q10**((TDMEAN-TREF)/10.)

PARAMETER Q10 = 2.; TREF = 25.

* Maintenace coefficients according to Horie, 1977
PARAMETER MAINLV = 0.05; MAINST = 0.0075
PARAMETER MAINRT = 0.01; MAINSO = 0.023

* Assimilate CH2O requirement for dry matter production
```

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```
ASRQ   = (ASRQLV*FLV + ASRQST*FST + ASRQSO*FSO) +...
         ASRQRT*FRT
* Growth of organs based on assimilate production
* EXPR is in g/pl
GTW    = (GPHOT - MAINT) / ASRQ
GRT    = (FRT * GTW)
FGRT   = GRT+(EXPR*PLANTS/1000.0*0.5)
GLV    = (FLV * GTW)
FGLV   = GLV-(EXPR*PLANTS/1000.0)
GST    = FST * GTW
FGST   = GST+(EXPR*PLANTS/1000.0*0.5)
GSO    = FSO * GTW

PARAMETER ASRQRT = 1.444; ASROLV = 1.66
PARAMETER ASRQST = 1.513; ASRQSO = 1.862

* Integration of organ growth rates [kg/ha]
* LDW, SDW, RDW, HDW, TDW are in g/pl
* WRT, WLVG, WST, WSO are in Kg/ha

* Daily amount of exported dry weight EXPR is in [g/pl day],
* SUMEXP is in [g/pl], CM2EXP is in [cm2/pl]
SUMEXP=INTGRL(ZERO,EXPR)
CM2EXP=SUMEXP*TOTSLA*10000.0

* Root dry weight
WRT    = INTGRL(WRTI,FGRT)
*        kg/ha
RDW    = WRT/PLANTS*1000.0
*        g/pl
* Leaves dry weight
WLVG   = INTGRL(WLVI,FGLV)
*        kg/ha
LDW    = WLVG/PLANTS*1000.0
*        g/pl
* Stem dry weight
WST    = INTGRL(WSTI,FGST)
*        kg/ha
SDW    = WST/PLANTS*1000.0
*        g/pl
* Storage organs dry weight
WSO    = INTGRL(WSOI,GSO)
*        kg/ha

HDW    = WSO/PLANTS*1000.0
*        g/pl
* Total dry weight [g/pl]
TDW    = RDW+LDW+SDW+HDW
*        g/pl
END
STOP
*---------------------------------------------------------------
* SUBROUTINES
*---------------------------------------------------------------
* SUBROUTINE LEFNUM
*************************************************************
* Purpose: to calculate leaf emergence as a function of PHY
*          and temperatures
*  TSUM   Thermal time                               I
*  PHY1   Phyllochron before leaf 6                  I
*  PHY2   Phyllochron after leaf 6                    I
*  DTT    Daily mean temperature                     I
*  LN     Leaf number                                O
*  PHY    Actual phyllochron                         O
*************************************************************
      SUBROUTINE LEFNUM(TSUM,PHY1,PHY2,DTT,
     & LN,PHY)
      IMPLICIT REAL(A-Z)
      SAVE INILN
* Leaf emergence
      IF(TSUM.LT.PHY1)THEN
         INILN=1.0
         LN=1.0
      ELSE
         IF(TSUM.LE.PHY1*6.0) THEN        !Leaf emergence
            INILN=INILN+DTT/PHY1
            LN=NINT(INILN)
            PHY=PHY1
         ELSEIF(LN.LT.22.0)THEN
            INILN=INILN+DTT/PHY2
            LN=NINT(INILN)
            PHY=PHY2
         ELSE
            LN=22.0
         ENDIF
      ENDIF
      RETURN
      END
```

```
*******************************************
* SUBROUTINE LEAFEX
* Purpose: to calculate the value of LER as a function of
* Leaf position temperature and phosphorus nutrition
* LN     = Leaf number                                    I
* PHY    = Phyllochron                                    I
* MINSLA = Minimum SLA                                    I
* MAXSLA = Maximum SLA                                    I
* GLV    = Amount of dry weight for producing leaves      I
* TSUM2  = Thermal time                                   I
* TDMEAN = Daily mean temperature          [øC]           I
* PLANTS = Plant density                                  I
* DTT    = Daily increase in TSUM tb=4     [dd]           I
* IRATE  = Expansion rate during lag phase                I
* DUREX  = Duration of linear leaf expansion              I
* LAGD   = Duration of lag phase                          O
* PLER   = Potential plant leaf expansion                 O
* LER24  = Potential leaf expansion at 24øC  [cm2/pl d]   O
* LER    = Actual leaf expansion                          O
* CUMPH  = Expanded leaves                                O
* EXPLEF = Expanding leaves                               O
* LNNLE  = Leaves expanding in lag phase                  O
* EXPR   = Export of dw due to sink limitation            O
* SLA    = Specific leaf area                             O
* PLER1  = Actual plant leaf expansion rate               O
* OBSLER = Observed LER for morphogenetic testing         O
* OBPLER = Plant LER from OBSLER for morphogenetic        O
*******************************************
      SUBROUTINE LEAFEX(LN,PHY,MINSLA,MAXSLA,GLV,TSUM2,
     & TDMEAN,PLANTS,DTT,IRATE,DUREX,LAGD,PLER,LER24,LER,
     & CUMPH,EXPLEF,LNNLE,EXPR,SLA, PLER1,OBSLER,OBPLER)

      IMPLICIT REAL(A-Z)
      SAVE EXPAN
      DIMENSION TTSUM(30)
      INTEGER I,COUNT0
      LERC   = 0.0
      OBLERC = 0.0
      COUNT0 = 1
      CUMPH  = 0.0
      EXPL2  = 0.0
      EXPLEF = 0.0
* Temperature effects on leaf expansion rate (taken from
* Villalobos et al., 1996)


      IF (TDMEAN.LT.4.0.OR.TDMEAN.GT.40.0) THEN
        TFAC=0.0
      ELSE
        IF (TDMEAN.LT.24.0) THEN
          TFAC=(TDMEAN-4.0)/20.
        ELSE
          TFAC=1.0-(TDMEAN-24.0)/16.0
        ENDIF
      ENDIF

      TTSUM(1) = 0.0         ! There is 1 leaf at emergence
      IF(AINT(LN)-COUNT0.GT.0)THEN
        TTSUM(AINT(LN))=TSUM2        ! Recording the thermal time
        COUNT0=COUNT0+1.0            ! at each leaf emergence
      ENDIF
      IF (TSUM2.LT.LAGD+DUREX)THEN   ! If L1 didn't finalize
        CUMPH = 0.0                  ! expansion then cumph=0
      ELSEIF(TSUM2.GE.LAGD+DUREX)THEN
        DO 5 I=1,AINT(LN)            ! If it did then
          IF (TSUM2-TTSUM(I).GE.(LAGD+DUREX))THEN
            CUMPH=CUMPH+1.0          ! leaf finalize
          ENDIF                      ! expansion
    5   CONTINUE
      ENDIF

      EXPL=TSUM2/LAGD
      IF (EXPL.LT.1.0)THEN
        EXPLEF=0.0                   ! Expanding leaves
      ELSEIF(TSUM2.GE.LAGD)THEN
        DO 10 I=1,AINT(LN)           ! If it did then
          IF (TSUM2-TTSUM(I).GT.(LAGD))THEN ! leaf is
            EXPL2= EXPL2+1.0         ! expanding
          ENDIF
   10   CONTINUE
        EXPLEF = EXPL2-CUMPH
      ENDIF

      IF (EXPLEF.GE.1.0)THEN
        DO 15 I=CUMPH,CUMPH+EXPLEF
          COUNT=I
          IF (COUNT.LE.12) THEN
            LER24  = MAX(IRATE,-18.3+4.35*REAL(COUNT))
            OBSLER = MAX(IRATE,-14.302+3.403*REAL(COUNT))
          ELSE
```

```fortran
      LER24  = MAX(IRATE,  84.81-4.540*REAL(COUNT))
      OBSLER = MAX(IRATE,  60.9-3.073*REAL(COUNT))
      ENDIF

*  Sum of LER of all expanding  leaves
      LER    = LER24*TFAC
      LERC   = LERC+LER
      OBLERC = OBLERC+OBSLER
15    CONTINUE

*  Adding IRATE cm2 for leaves expanding at low rate due to
*  lost time
      LNNLE  = LN-EXPLEF-CUMPH
      PLER1  = LERC+LNNLE*IRATE
      OBPLER = OBLERC+LNNLE*IRATE
      ELSE
      LNNLE  = LN
      LER24  = IRATE
      LER    = IRATE
      OBSLER = IRATE
      LERC   = IRATE*AMAX1(LNNLE,1.0)
      OBLERC = IRATE*AMAX1(LNNLE,1.0)
      PLER1  = LERC
      OBPLER = OBLERC
      ENDIF

*  Here we make the comparison with the growth rate of leaves
*  MAX and MIN are in cm2/g
*  PLER are cm2/pl

      MAXLGRO=PLER1/(MINSLA*10000.0)
*     g (leaf)/pl
      MINLGRO=PLER1/(MAXSLA*10000.0)
*     g (leaf)/pl
      GLVGPL=GLV/PLANTS*1000.0
*     g (leaf)/pl

      IF (MAXLGRO.LE.GLVGPL) THEN
*  Expansion is less or equal than the supply and produce
*  thick leaves the excess of assimilates is derived to the
*  roots
      FLGR=MAXLGRO
      PLER=MAXLGRO*(MINSLA*10000.0)
      EXPR=GLVGPL-FLGR


      SLA=PLER/FLGR/10000.0
      ELSEIF (MINLGRO.GT.GLVGPL)THEN
*  If expansion is higher than the supply of assimilates,
*  expansion will be reduced depending on assimilates
*  production, and leaves will be thiner.
      EXPR=0.0
      PLER=GLVGPL*MAXSLA*10000.0
      SLA=PLER/GLVGPL/10000.0
      ELSE
      PLER=PLER1
      SLA=PLER/GLVGPL/10000.0
      ENDIF
      RETURN
      END
```

```
        FPLER=MAXLGRO*(MINSLA*10000.0)    !thick leaves
     *  cm2/pl=g/pl *  (m2/g *  cm2/m2)! to the roots
     *  EXPR=GLVGPL-FLGR                 ! and stems.
        g/pl
        SLA=FPLER/FLGR/10000.0
     *  m2/g=(cm2/pl)/(g/pl)/(cm2/m2)

        ELSEIF (MINLGRO.GT.GLVGPL)THEN
          EXPR=0.0
          FPLER=GLVGPL*MAXSLA*10000.0
          SLA=FPLER/GLVGPL/10000.0
        ELSE                             ! will become thiner.
          FPLER=PLER
          SLA=FPLER/GLVGPL/10000.0
        ENDIF                            ! the present supply of
        RETURN                           ! assimilates SLA may still
        END                              ! vary.
```

FST and Fortran source code for some of the subroutines of the simulation model presented in Chapter 5.

```
TITLE TILLERING
DECLARATIONS
**********
ARRAY TRATE(1:TILN), TDUR(1:TILN), TILLER(1:TILN),...
     TILL2(1:TILN)
ARRAY FNTIL(1:TILN), SHOOTP(1:TILN), LOCAL(1:TILN),...
     RATET(1:TILN)

*-------------------------------------------------------------
DEFINE_CALL TILNUM(INPUT, INTEGER_INPUT, INPUT_ARRAY,...
     INPUT,INPUT,INPUT,...
     OUTPUT_ARRAY,OUTPUT_ARRAY,OUTPUT_ARRAY)
*-------------------------------------------------------------
MODEL
*****
     ARRAY_SIZE TILN  = 4
*-------------------------------------------------------------
     TIMER STTIME=211.; FINTIM=275.0; DELT=1.0; PRDEL=1.0
*-------------------------------------------------------------
INITIAL
*******
* Morphogenetics
PARAMETER SYNC   = 0.98; INTCPT = 1.4
INCON     ZERO   = 0.0 ; ONE    = 1.0
PARAMETER BASET  = 0.0
*-------------------------------------------------------------
* Shoot P% to be used in the tillering subroutine
PARAMETER SHOOTP(1:3) = 0.56, 0.54, 0.37; SHOOTP(4:TILN)=0.32
PARAMETER POTTRT = 0.01; POTDUR =90.0
*-------------------------------------------------------------
WEATHER WTRDIR='c:\SYS\WEATHER\'
WEATHER CNTR='balc'; IYEAR=1997; ISTN=1
*************************************************************
DYNAMIC
*******
     FINISH  DAE>150.0
*-------------------------------------------------------------


* WEATHER DATA
**************
     DTMAX    = TMMX
     DTMIN    = TMMN
     DAVTMP   = 0.5 * (DTMAX + DTMIN)
     DTT      = DAVTMP-BASET
     TSUM     = INTGRL(ZERO,DTT)
     DAE      = TIME-(STTIME-1.0)
*-------------------------------------------------------------
* The phyllochron is a function of leaf number according to
* observed data
PHY     = AFGEN(PHYTB,MSLN)
FUNCTION PHYTB=1.0,88.0, 20.0,88.0

* Main stem leaf number (MSLN)
MSLN     = INTGRL(ONE,RLN)

* # leaves/main stem
RLN     = DTT/PHY
* rate of leaf appearance [leaves/day]
*-------------------------------------------------------------
* This calculates the increase in number of tillers
* FNTIL is the final number of tillers/m2 for 270 pl/m2,
* LOCAL is the integral with time of the rate of tillering
* (MTR)
* No more than 4 tillers per plant are allowed

     SWICH    = MSLN-3.0
     TNUM     = AINT(INSW(SWICH,0.0,AMIN1(4.0,TOUT)))
     TOUT     = AMAX1(1.0, (MSLN*SYNC)-INTCPT)
* tillers/pl following sinchrony

     FNTIL    = AMIN1(1.0,TRATE * TDUR)
* #tillers/m2 (final # after delay and dispersion due to P,
* for each tiller cohort, T1, T2, T3 and T4. All considered
* to be primary tillers, there are no secondary tillers. It
* is considered that the tiller appears when it is present in
* 50 or more % of the plants.

     LOCAL    = INTGRL(ZERO,RATET)
     TILLER   = INSW(MIN(LOCAL,FNTIL)-0.5,LOCAL,FNTIL)
     TILL2=   AMIN1(LOCAL,FNTIL)
```

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```
* # tillers/m2 is an array indicating # for the different
* cohort (T1, T2, T3, and T4)

      TOTTN = (ARSUMM(TILL2,1,TILN))
* # tillers / plant. Total number of tilles per plant
* (all cohorts added)

*********************************************************
* Subroutine calls
      CALL TILNUM(TNUM,TILN,SHOOTP,POTDUR,POTTRT,DTT,...
                 TRATE,TDUR,RATET)
*********************************************************
      PRINT DAE,MSLN,TOTTN,TNUM,TDUR,TILLER,LOCAL,FNTIL,...
      TILL2,TSUM,DTT,TRATE

      TRANSLATION_GENERAL DRIVER='EUDRIV'
*********************************************************
      END
*This is for P2
      PARAMETER SHOOTP(1:3) = 0.33, 0.34, 0.29; SHOOTP(4:TILN)=0.22
      FUNCTION PHYTB=1.0,88.0, 2.0,88.0, 3.0,108.0, 20.0,108.0

*********************************************************
* This is for P1
      END
      PARAMETER SHOOTP(1:3) = 0.26, 0.22, 0.15; SHOOTP(4:TILN)=0.17
      FUNCTION PHYTB=1.0,88.0, 2.0,88.0, 3.0,124.0, 20.0,124.0
      WEATHER CNTR='balc'; IYEAR=1997; ISTN=2
      END
      STOP
*SUBROUTINE TILNUM
* Calculates the rate of tiller emergence and the tillering
* interval for each tiller cohort.
*
* TNUM  = Number of tillers according                    |-|
* TILN  = Array size (integer)                            |-|
* SHOOTP = Parameters array of specific leaf P [%P]       |-|
* POTDUR = Potential tillering duration                   |-|
* POTTRT = Potential maximum tillering rate               |-|
* DTT   = Daily mean temperature                          |-|
* TRATE = Maximum relative tillering rate                 |o|
* RATET = Maximum tillering rate                          |o|
* TDUR  = intervals of tillering [days]                   |o|
```

```
*********************************************************
      SUBROUTINE TILNUM(TNUM,TILN,SHOOTP,POTDUR,POTTRT,DTT,
     &                  TRATE,TDUR,RATET)
      IMPLICIT REAL(A-Z)
      INTEGER TILN, I
      DIMENSION SHOOTP(TILN), TRATE(TILN), TDUR(TILN),
     &          RATET(TILN)
      IF (TNUM.LT.1.0)THEN
        DO 10 I=1,TILN
          TRATE(I) = 0.0        ! Here no tillers appeared
          TDUR(I)  = 0.0        ! yet, so rate and duration =0
          RATET(I) = 0.0
10      CONTINUE
      ELSE
        DO 20 I=1,INT(TNUM)
        RELTRT=MAX(0.0,1.0-EXP(-9.5*(SHOOTP(I)-0.178)))
          IF (RELTRT.EQ.0.0)THEN
            RELDUR = 0.0
          ELSE
            RELDUR=0.985-0.99*LOG(RELTRT)
          ENDIF
          TRATE(I) = RELTRT * POTTRT
          RATET(I) = TRATE(I) * DTT
          TDUR(I)  = RELDUR * POTDUR
20      CONTINUE
      ENDIF
      RETURN
      END
```