A Physiological Explanation for the Canopy Nitrogen Requirement of Winter Wheat
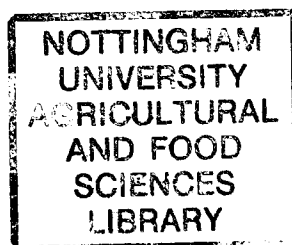
by

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ACKNOWLEDGEMENTS

Dedicated to

my father,

Peter James Critchley,

whose memory has remained with me throughout.

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ABSTRACT

Nitrogen (N) fertiliser is one of the most important agronomic inputs and yet the application recommendations for winter wheat (*Triticum aestivum* L.) still remain imprecise. This increases costs both to the wheat grower and to the environment. An understanding of the canopy nitrogen requirement (CNR) is required before any improvements in fertiliser recommendations can be made. The CNR is defined here as the minimum amount of N required to produce and maintain a canopy and be efficient in light capture and conversion. This thesis aimed to provide a physiological explanation for this requirement in winter wheat, based on canopy structure and radiation geometry, to test the hypothesis that CNR can be predicted from canopy architecture.

Variation in CNR was predicted from canopy architecture using data from the literature and a series of principles developed here. The prediction for the photosynthetic N requirement of the laminae and leaf sheath was based on the light distribution, modelled by a form of Beer's Law, and maximising N use efficiency (NUE). The structural N in the true stem was predicted from stem dry weight, stem area and the assumption that 0.3% of stem dry weight is structural N. Field experiments in 1997/8 and 1998/9 were designed to test these predictions by creating a wide range of canopy architectures through three seed rates (20, 320 and 640 seeds m^{-2}) and two varieties (Soissons and Spark). In the 1998/9 experiment there was also a low fertiliser N treatment to reduce the amount of luxury N uptake.

The CNR of any particular treatment was stable with canopy development and increased canopy size through depth and was an average of 2.2 g m^{-2}; lower than the original proposed CNR of 3.0 g m^{-2}. The CNR of the low seed rate canopy was also greater than the conventional seed rate and Soissons unexpectedly had a greater CNR than Spark.

The results confirmed that the green area, light extinction coefficient (*k*) and incident light could explain the light flux and its distribution through the canopy. However, in the low seed rate and early growth, Beer's Law appeared not to hold because full

ground cover was not achieved. Leaf N requirement decreased linearly with increased canopy size through depth and most canopies distributed N to maximise NUE. The results suggested that of the 50% of total N in the stem, 35% was photosynthetic N in the leaf sheath, 25% was structural in the true stem and the remaining 40% was transport, metabolic, storage and luxury N. The photosynthetic N requirement was overestimated in the prediction indicating that the leaf sheath had a lower N requirement than the lamina. Direct measurements of structural N requirement in the stem could not be made but there was supporting evidence for the relationship with canopy architecture.

It is suggested that these principles could be used by growers to predict the CNR from canopy characteristics and by breeders to identify traits that could improve yield. Further detailed analysis of photosynthetic requirements and function of N in the stem would allow the development of a more quantitative prediction scheme, which would be the next step to greater precision in fertiliser recommendations.

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1 INTRODUCTION

Current nitrogen (N) fertiliser recommendations for wheat are based on empirical relationships between fertiliser N applications and crop yield (MAFF, 1994), for a range of previous crops and soil types. These schemes have improved the precision of N applications but have limitations because a prediction of potential yield is required for the calculation of fertiliser N. It has been suggested that precision in fertiliser application recommendations can be increased by considering the canopy N requirement (CNR) (Sylvester-Bradley *et al.*, 1997a). The 'requirement' of the canopy for N, is defined in this thesis as the minimum amount of N per unit green area that will accumulate dry matter at the maximum rate. This not only includes photosynthetic N but also 'support' N. Here, support N is that which is contained in compounds for structure, transport and metabolism. This thesis aims to provide a physiological basis for a new approach to assessing CNR from canopy architecture. This chapter begins by describing the background to the thesis and then describes the basis on which it is proposed that CNR can be assessed.

1.1 GENERAL INTRODUCTION

The current UK recommendations (MAFF, 1994) for N fertiliser applications are based on the economic optima derived from the diminishing response of yield to fertiliser N (Sylvester-Bradley *et al.*, 1997a). These recommendations are imprecise for many agricultural situations. In relation to about 200 $kg\,ha^{-1}\,N$ that is conventionally applied, the average error in prediction of application is 40 $kg\,ha^{-1}\,N$ and it can be up to 100 $kg\,ha^{-1}\,N$ (Sylvester-Bradley *et al.*, 1987). Growers are more inclined to over apply N, since the price of fertiliser is relatively cheap compared to the potential return in yield and there is no economic benefit from maximising efficiency of N use. In this way, the grower increases the likelihood of not limiting crop growth or yield due to N deficiency.

Over-applications often result in increased nitrate leaching, disease spread, lodging and higher costs (Jenkinson, 1986). Increased concern over health and environmental

risks of N over-application has also resulted in fertiliser restrictions. Therefore there is a need to increase the precision of fertiliser N use to meet the canopy N requirement. The requirement for N may be determined through understanding the physiological roles of N in the formation, support and maintenance of the green canopy (Sylvester-Bradley *et al.*, 1990b; Barraclough, 1997) as well as the response of photosynthesis to N content per unit green area. It is therefore appropriate to consider the N requirement expressed on a green area basis.

Green area index (GAI) is a measure of the canopy size. It describes the ratio between the total projected area of green material and the ground area it covers, and is therefore dimensionless (Sylvester-Bradley *et al.*, 1997a). Work carried out by the University of Nottingham and the Agricultural Development and Advisory Service (ADAS) (Sylvester-Bradley *et al.*, 1990b) revealed a relatively consistent relationship between projected green tissue area and N content per unit ground area (Figure 1.1). The coefficient describing the slope of this relationship was termed the 'canopy nitrogen requirement' (CNR) (Sylvester-Bradley, Stokes & Scott, 1990a). A similar relationship was also observed in lucerne with accumulated GAI through depth (Lemaire & Gastal, 1997), in sugar beet with a range of canopy sizes (Scott, Jaggard & Sylvester-Bradley, 1994) and in oilseed rape with increased GAI through crop development (Stokes *et al.*, 1998). The CNR of wheat proved to be more stable than the N concentration of dry matter with the increase in canopy size (Grindlay, Sylvester-Bradley & Scott, 1993; Grindlay, Sylvester-Bradley & Scott, 1997). It has therefore been claimed to have greater potential in diagnosing canopy N requirement. The suggested explanation for stability of CNR through the crop's growth was based on the decreasing leaf N content per unit shoot green area, but the increasing stem N content per unit shoot green area (Grindlay *et al.*, 1997). As canopy size increases, a greater proportion of the leaves are shaded, thus reducing the leaf N needed for photosynthesis. However, this is compensated by the increase in stem N to support the leaves in the more illuminated layers of the canopy (Lemaire *et al.*, 1991; Grindlay *et al.*, 1997; Lemaire & Gastal, 1997). This implies that the decrease in leaf N is balanced by the increase in stem N for the CNR to remain stable. However, this idea has not been thoroughly tested. There is a need for a more detailed analysis of the distribution, function and control of the N in wheat leaves and particularly in the

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stems, as well as the allometric relationships between these two tissues. This should provide a robust physiological basis for the prediction of CNR.
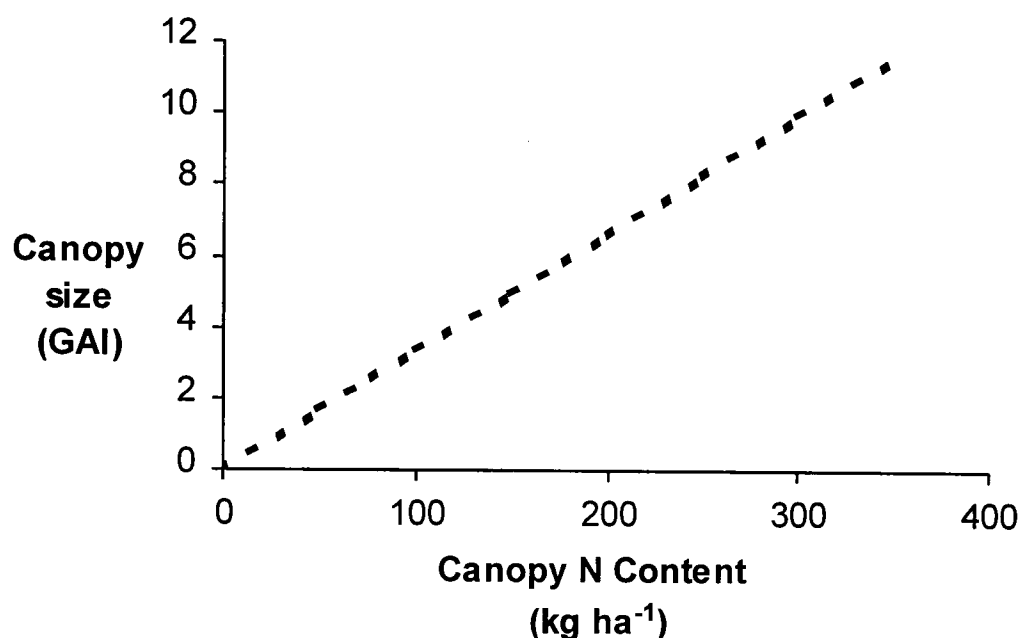
Figure 1.1 The relationship between canopy N content per unit ground area and green area index (GAI) during crop development (adapted from Sylvester-Bradley *et al.*, 1990b).

The CNR of UK grown winter wheat appears to be 30 kg of N to build each hectare of green tissue, as canopy size increases and then decreases throughout the stages of development from early March through to senescence (Sylvester-Bradley *et al.*, 1990b). Stages of development or 'growth stages' (GS) refer to the important phases of the wheat life cycle and are defined by the Decimal Code (Tottman, 1987). The N requirement of the canopy is for the whole shoot, including green leaves, stems, the dead leaves but excluding the ears which are storage organs for the N translocated from the canopy plus any taken up during grain fill. The N required by the shoot is subsequently redistributed to the ears for storage. A CNR of 30 kg ha^{-1} has been assumed to apply to all canopies. However, significant variation in winter wheat CNR from 11 to 51 kg ha^{-1} was found for a range of varieties, sites-seasons and fertiliser N in the UK (Stokes *et al.*, 1997; Foulkes *et al.*, 1998). There has been no explanation of this variation, although field trials have revealed that unfertilised crops have a lower

CNR than conventionally fertilised crops (Grindlay *et al.,* 1997; Stokes *et al.,* 1997). There is no evidence that N reserves are not present in these crops and so the measured N may have been a 'content' rather than 'requirement'. The increased measured canopy N 'content' with fertiliser may have been because of extra N taken up above that needed for requirement. The general aim of this thesis is to understand CNR in terms of the physiological functions of N, not only in the leaves but also in the stem. It should then prove possible to assess the CNR for each set of growing conditions from known canopy characteristics which can be related to these functions. The remainder of this chapter reviews the literature concerning the functions of N in wheat from which a hypothesis is developed about how CNR may be predicted. This begins with the effect of canopy architecture on radiation geometry in the next section.

1.2 CANOPY ARCHITECTURE AND RADIATION GEOMETRY

Photosynthesis at whole canopy level depends on the amount and distribution of the light within the whole canopy and the response of the leaf N content to that distribution. The architecture or size, structure and geometry of the canopy affects light interception and the light profile within the canopy (Nilson, 1971). The distribution of the leaf N content within the canopy is adjusted to the light environment to maximise carbon gain (Field, 1983; Hirose & Werger, 1987b; Anten & Werger, 1996), which is the basis for the leaf N requirement here. The relationship between canopy architecture and light distribution is now discussed in detail.

1.2.1 Light distribution

The amount and distribution of light penetrating a crop canopy, depends on the incident radiation, canopy size and geometrical arrangement of the leaves. It is widely assumed that the light available at depths within the canopy can be described by a form of Beer's Law developed by Saeki, described by Monteith (1965):

$$I = I_o \, e^{-k\,L} \qquad\qquad \textbf{Equation 1.1}$$

where, I_o is the incident solar radiation and I is the radiation received at a level within the canopy (above which there is green area index of L). Light interception therefore depends on the size of the canopy green area (L) and the foliage geometry of the crop, which is represented by a light extinction coefficient (k) described in more detail in section 1.2.2. Beer's Law assumes light is extinguished in a homogenous medium and therefore the Monsi-Saeki equation assumes random leaf distribution. As the size of the canopy increases, there is a diminishing increase in fractional interception (f) that can be described by a negative exponential relationship (Hipps, Asrar & Kanemasu, 1983). The fraction of light intercepted can be calculated from L, if the light extinction coefficient is known:

$$f = 1 - e^{-k\,L}$$

Equation 1.2

1.2.2 Light extinction coefficient

The extinction coefficient, k, is analogous to the absorption coefficient in Beer's Law (Baret, Andrieu & Steven, 1993). This describes the plant and canopy characteristics that affect light interception, mainly leaf angle, but also including leaf shape, leaf thickness and vertical stratification of leaf area, which affect the transmission (τ), reflection and absorption of light (Monteith, 1965). The light itself (whether direct or diffuse) and the angle at which it penetrates the canopy is also important in measuring the light interception. As solar elevation increases, k decreases (Hipps et al., 1983).

The angle of leaves can be an important factor affecting photosynthetic rate, although the optimum angle depends upon the angle of the incident light and the canopy size (Trenbath & Angus, 1975). As leaves expand, the proportion of support structure to the whole length of the leaf decreases causing the leaf to curve downwards. Conditions which allow the growth and lengthening of the leaf such as increased N and water supply, will also increase the chances for the leaf to droop. Differences between species and cultivars show that these are genetically controlled (Table 1.1).

Table 1.1 Light extinction coefficient (k) for a range of canopies (Azam-Ali, Crout & Bradley, 1994; Monteith, 1990)

Canopy species	Light extinction coefficient (k)
Kale	0.94
Rice	0.43-0.86
Maize	0.56-0.78
Barley	0.48-0.69
Wheat	0.40-0.70
Groundnut	0.40-0.66
Ryegrass	0.29-0.43
Gladiolus	0.20

The angle of leaf inclination may also change with development stage, during which the leaf length, spacing, orientation and stem length may change. As plant density increases, space becomes limited and there may be mutual support so that the leaves become more erect. An increase in leaf angle (leaves more erect) was observed with increased population density in maize (Williams *et al.*, 1968). At similar GAI, this will allow more light to penetrate to the deeper parts of the canopy. Fukai *et al.*, (1990) showed that at reduced shoot density barley had a reduced k (more erect leaves). This was more likely to be the result of the non-uniform leaf distribution due to wide spacing.

The inclination of leaves has also been related to yield but the most advantageous angle depends on other crop aspects. Generally, canopies with erect leaves produce a higher yield than prostrate leaves (Trenbath & Angus, 1975). This is due to more light penetration to the lower leaves allowing a more even light distribution and reducing light saturation. Winter & Ohlrogge (1973) showed that at low densities or a low GAI, the crop with the prostrate leaves yielded more through greater light interception, than the same crop with erect leaves. At high densities or a large GAI, the crop with the erect leaves yielded more through greater photosynthetic efficiency. This is due to the

increase in photosynthate produced per quantum with a decrease in light flux (Winter & Ohlrogge, 1973). Generally, leaf angle is related to yield in maize, rice and temperate small-grained crops (Trenbath & Angus, 1975).

The angle of the sun will affect the distribution of photosynthesis within a canopy. In maize, the lower leaves contribute more and the flag leaf less, to total photosynthesis with increasing solar elevation (Puckridge, 1972). A canopy with erect leaves will respond to changes in light flux and light quality, more than a canopy with prostrate leaves, due to the penetration and more even distribution of the light. The optimum leaf angle for maximising photosynthetic efficiency over a range of solar elevations, depends on the size of the canopy. For canopies with a high GAI experiencing high solar elevation, photosynthetic efficiency would be greater if the leaves were more erect, distributing the light within the canopy. Under low solar elevation, it would be more efficient for the leaves to be more prostrate to capture as much light as possible at the top of the canopy, as it does not penetrate as deep. For canopies of low GAI, and at all solar elevations, prostrate leaves would be required to maximise photosynthesis (Trenbath & Angus, 1975). Other conditions that may affect the growth and photosynthesis of the crop, through variations in leaf inclination include lodging, drought stress, pests and diseases (Trenbath & Angus, 1975).

A canopy that is ideal for allowing maximum photosynthetic efficiency would have erect leaves at the top and prostrate leaves at the bottom (Nobel & Long, 1982). This would allow light to penetrate to lower layers, due to little mutual shading. This would also reduce the flux of the light in the upper layers, such that leaves would not be above light saturation and no light would be wasted. The leaves in the lower layers would be more prostrate and therefore able to capture all of the available light penetrating down through the upper layers. The light flux reaching the lower parts would be above the light compensation point, which is the light flux at which there is no net change in photosynthesis (Hall & Rao, 1999). Therefore these leaves remain a source of photosynthetic assimilates adding to the photosynthetic efficiency of the whole crop.

A more sophisticated description of k includes the components of sunfleck (s) and transmission coefficient of a leaf (τ) (Szeicz, 1974). Sunfleck is the fraction of

radiation transmitted by a unit leaf layer without interception and so describes the arrangement and orientation of the leaves (Monteith, 1965). The fraction intercepted by a unit leaf layer is therefore (1 - s). It is assumed that there is a uniform distribution of leaves and also that s is constant throughout the day. However, the latter is not the case in practice due to changes in cloud cover, solar angle and windspeed. Sunfleck has been found to decrease exponentially with canopy depth, in kale and barley (Monteith, 1965) such that

$$s = 0.6 \, e^{-0.15L} \hspace{4cm} \textbf{Equation 1.5}$$

In sugar beet with a GAI of less than 3, photosynthesis increases with decreasing s, as the leaves become more prostrate. With a GAI of more than 5, photosynthesis increases as s increases and the leaves become more erect.

The transmission coefficient (τ) for light used in photosynthesis has been taken as 0.1 (Monteith, 1965; 1972). As the elevation of the sun increases, the amount transmitted through an individual leaf, also increases. In a canopy with randomly oriented leaves, τ is independent of solar angle, although will be less than the maximum at normal incidence and with horizontal leaves (Monteith, 1965). As N content decreases with leaf depth, it might be expected that τ increases.

The fraction of light to be transmitted through the leaf is therefore (1 - s) τ. Incorporating s and τ, the irradiance at a point within the canopy above which there is a green area index of L, can also be described by Equation 1.6 (Monteith, 1965).

$$I = I_o \, [\, s + (1 - s) \, \tau \,]^{\,L} \hspace{3cm} \textbf{Equation 1.6}$$

Through understanding the variation in k, there will be a greater understanding of the variation in light distribution within the canopy. Photosynthetically active radiation (PAR) is the light used for photosynthesis and is therefore important for the determination of leaf N requirement.

1.2.3 Photosynthetically active radiation

The radiation intercepted and used by the canopy for photosynthesis or photosynthetically active radiation, is shortwave electromagnetic radiation with wavelengths between 0.4 and 0.7 μm (Hall & Rao, 1999). A direct beam of light contains between 44 and 45 % PAR. Diffuse light, where scattering has occurred, can contain up to 60% PAR. Combining these two components, brings the ratio of PAR to total incoming solar radiation, to 50% (Monteith, 1972). The smallest indivisible quantity of radiant energy, is called a quantum and a photon is a quantum of PAR. Photochemical reactions depend on the number of photons on a unit of surface area in unit of time. This is the photosynthetic photon flux density (PPFD) and is the most suitable unit for the PAR (Daudet & Tchamitchian, 1993). As photons are so small, they are usually expressed in units of moles converted by the Avogadro constant. It is therefore usual to measure PPFD in $\mu mol\ m^{-2}\ s^{-1}$ (Bonhomme, 1993). Irradiance is the amount of light energy (E) in joules per unit area per unit time ($J\ m^{-2}\ s^{-1}$) and is proportional to the frequency of light (McLaren, 1980; Monteith, 1984).

The distribution of light is important in understanding the leaf N requirement, as the main function of N in the canopy is for photosynthesis.

1.3   PHOTOSYNTHETIC N IN THE CANOPY

More than three-quarters of the total N in leaves is in photosynthetic proteins, both in soluble form and in thylakoids (Field & Mooney, 1986; Evans, 1989c). These are mainly confined to the chloroplast, which is approximately 16% of the total mesophyll cell volume (Heldt, 1997). The structure of the chloroplast is a double membrane, enclosing a stroma that contains a system of thylakoid membranes. These membranes are the site for the light reaction of photosynthesis. They enclose the thylakoid lumen.

Thylakoid proteins within the chloroplast consist of chlorophyll-binding proteins, electron-transport chain proteins and adenosine 5'-triphosphate (ATP) -synthesising proteins. Chlorophyll is a light harvesting pigment and can account for 60-85% of the thylakoid protein (Heldt, 1997). Each chlorophyll molecule contains four atoms of N

(Heldt, 1997) and is always bound to a protein. The chlorophyll-protein complex can be between 20 and 60% of photosynthetic N, depending on irradiance (Hikosaka & Terashima, 1995).

The most abundant soluble protein in plant tissue is Ribulose 1,5-Bisphosphate carboxylase/oxygenase (RUBISCO) located in the chloroplast stroma. It is a large molecule (560 kDa) made up of eight units each comprised of one large and one small subunit (Ramshaw, 1982). RUBISCO has two enzymic activities. It catalyses the carboxylation of Ribulose 1,5-Bisphosphate (RUBP), fixing CO_2 to form two molecules of 3-phosphoglycerate (PGA) within the Calvin cycle (Figure 1.2). It also catalyses the oxygenation of RUBP by O_2, to form phosphoglycollate and PGA (Heldt, 1997). PGA is reduced to form triose phosphate (Triose-P). RUBISCO has a low catalytic rate and a poor affinity for CO_2 (Evans, 1989c) and so is required in large amounts to maintain adequate carboxylation rates for growth.
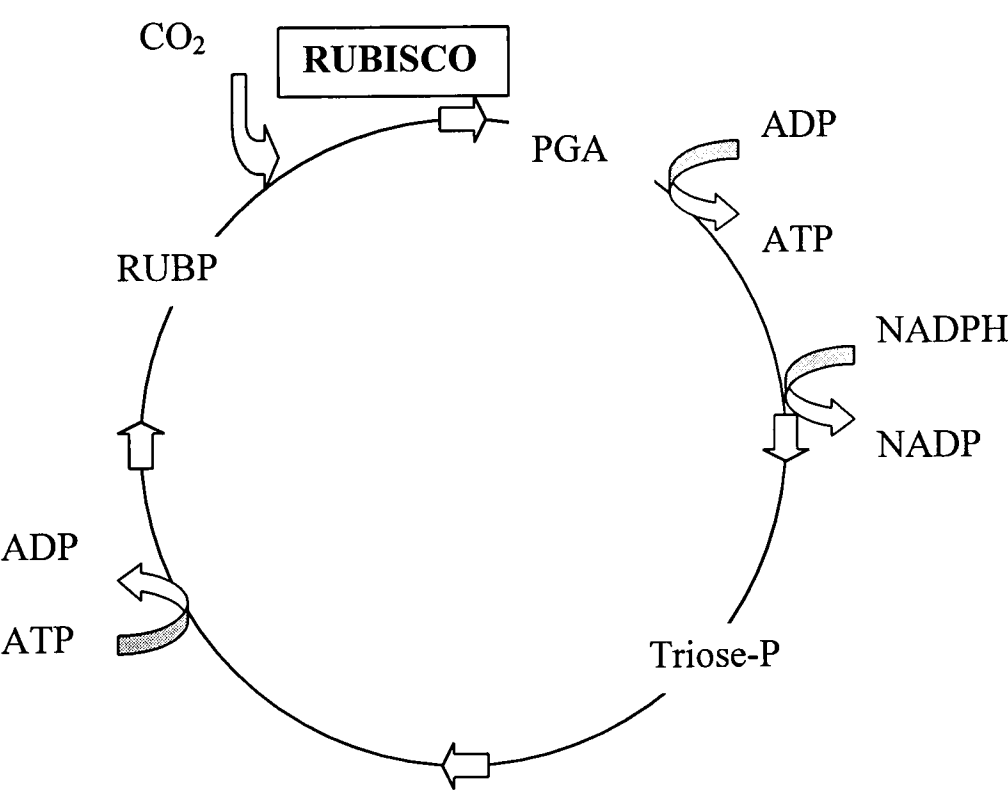
Figure 1.2 The basic reactions of the Calvin cycle (from Heldt, 1997).

The following sub-sections begin by describing the relationships between photosynthesis, leaf N content and light flux on which principles for predicting leaf N requirement are based (Chapter 2). In many of the relationships, it is difficult to determine which is the cause and which is the effect. Here, it is considered that photosynthesis responds to leaf N content and to light flux, but also that there is a response of leaf N content to light flux. These relationships are now discussed. However, it is also possible that the cause and effect may be reversed between leaf N content and photosynthesis, and between light interception and leaf N content.

1.3.1   Response of photosynthesis to leaf N content

The rate of photosynthesis is determined by the quantity of radiation that is absorbed by the receptor pigment, chlorophyll, and can be expressed in terms of the amount of carbon dioxide (CO_2) fixed per unit area per unit time ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$). The rate at which there is no further response to increased PPFD, is the light saturated rate of photosynthesis (P_{max}). The PPFD at which this occurs is the light saturation point.

Leaf N content per unit leaf area can vary according to N supply (Muchow & Sinclair, 1994), leaf age (Hikosaka, Terashima & Katoh, 1994), light flux or PPFD (Evans 1993b), canopy position (Lemaire $et\ al.$, 1991) and time of year (Grindlay, 1997). A relationship between leaf N content per unit leaf area and P_{max} has been observed (Evans, 1989b; Field and Mooney, 1986).

RUBISCO accounts for 40 – 70% of soluble leaf protein in wheat and this proportion appears not to be affected by leaf age or temperature (Lawlor $et\ al.$, 1987a; Evans 1989b). The RUBISCO content per unit leaf area in wheat, measured by Lawlor $et\ al.$, (1987a), ranged between 2.8 and 6.0 $g\ m^{-2}$. RUBISCO content increases with leaf N content, such that there is increased partitioning of total leaf N to RUBISCO with increased leaf N content, under the same irradiance and at the expense of other non-thylakoid N components (Yamashita, 1986; Evans, 1989c). RUBISCO activity also increases linearly with the leaf N content of wheat (Evans, 1983), except at very high N contents (Lawlor $et\ al.$, 1987b). There is an increase in P_{max} with RUBISCO content described by a rectangular hyperbola (Hikosaka & Terashima, 1995). It is suggested that the relationship is not linear due to the increased resistances to CO_2

diffusion (Evans, 1983). The ratio of RUBISCO to chlorophyll is constant, suggesting a close relationship between the light harvesting complexes and the process of CO_2 fixation. The amount of RUBISCO per unit of chlorophyll in wheat leaves ranged between 9 and 13 g g^{-1} (Lawlor *et al.*, 1987a).

Chlorophyll content and activity increases proportionately to leaf N content in wheat leaves, under the same light conditions (Evans, 1989b). The amount of chlorophyll to total N has been measured as 3.7 mmol Chl mol^{-1} N (Evans, 1983). The ratio between total N and chlorophyll also remains constant throughout senescence (Evans, 1983; Lawlor *et al.*, 1987a). There is an increase in P_{max} with chlorophyll content and therefore N. Nitrogen deficient wheat plants have been shown to have a small total chlorophyll content (Shangguan, Shao & Dyckmans, 2000). With increasing the N content through irradiance, however, the proportion of total leaf N allocated to thylakoid protein decreases (Evans, 1989c), such that the chlorophyll content per unit leaf area remains the same with increasing leaf N content (Terashima and Evans, 1988).

The electron transport capacity increases with leaf N content in proportion to the increase in thylakoid protein (Evans, 1989c). There is a linear increase in P_{max} with cytochrome-*f* (Hikosaka & Terashima, 1995). This also contributes to the increased P_{max} with leaf N content.

It is therefore not surprising that an increase in the N supply to wheat results in the increase in photosynthetic rate (Shangguan *et al.*, 2000). Net photosynthesis increases linearly with leaf N content as a percentage of dry matter (Mooney & Gulman, 1979). Expressing both photosynthesis and leaf N content per unit of leaf area, allows any variation in the photosynthetic capacity per unit leaf N to be seen, and reflects the differences in N partitioning, electron transport capacity and RUBISCO activity (Field & Mooney, 1986; Evans, 1989a). A linear relationship between P_{max} and leaf N content was observed in *Lysimachia vulgaris*, but only for leaf N contents of up to 1.12 g m^{-2} (Pons *et al.*, 1989). When leaves from various depths in a closed canopy were measured, the response in the herb *Amaranthus dubius* L. was also linear with leaf N contents of up to 2.10 g m^{-2} (Anten & Werger, 1996). A linear response of photosynthesis to leaf N content was also observed in the mature oak and maple

leaves (Reich, Walters & Ellisworth, 1991). In this case, variation in leaf N content was due to phenological and seasonal changes. Hirose & Werger (1987b) modelled an increase in photosynthesis at light saturation with leaf N content, showing slight curvature such that the response decreased for the perennial herb *Solidago altissima*. Increasing the leaf N content not only increases the amount of gross photosynthesis, but also the 'cost of maintenance,' or respiration. Therefore net photosynthesis does not always continue to increase linearly with N content. A non-linear increase in rate of photosynthesis with leaf N content was also observed in wheat above 1.50 g m^{-2} (Evans, 1983) (Figure 1.3), in *Xanthium canadense* (Hikosaka, Sudoh & Hirose, 1999) and in the chaparral shrub, *Diplacus aurantiacus* (Gulman & Chu, 1981). Gulman & Chu (1981) have shown both a linear and a curved response of photosynthesis rate to leaf N content under a high and low light flux, respectively. There is therefore an interaction with light flux. The activity of RUBISCO continues to increase proportionately even with high leaf N contents. It has been suggested that the relative decrease in photosynthetic rate is due to a decrease in CO_2 diffusion and not inactive RUBISCO enzymes (Evans, 1983; Evans & Terashima, 1988) or the cost of maintenance. It is also probable that in closed canopies at high leaf N contents, photosynthesis is not at a maximum, due to a limitation by light flux. Chlorophyll content and electron transport capacity both increase with leaf N content, and will therefore require greater irradiance to reach maximum photosynthesis for that N content (Evans, 1989c) or have a greater light saturation point. Different photosynthetic responses to leaf N content at high light flux have been compared for a range of species (Field & Mooney, 1986; Sinclair & Horie, 1989; Evans, 1989c). These showed that the C_4 crop, maize, has a greater photosynthetic rate per unit leaf N than the C_3 species. Of the C_3 species studied, wheat and rice have the greatest photosynthetic rate for leaf N contents of up to 1.68 g m^{-2} and evergreens and sclerophylls have the lowest.

Nitrogen use efficiency (NUE) is the photosynthetic rate per unit leaf N (Hirose & Werger, 1987b). The slope and the intercept of the N-photosynthesis response curve determine this. The intercept may be taken to indicate how much N is required by the leaf for non-photosynthetic compounds such as nucleic acids, proteins and amino acids involved in primary metabolism. Sinclair & Horie (1989) reported this to range from 0.2 g N m^{-2} in maize, to 1.0 g N m^{-2} in soybean. They reported wheat to be

similar to rice with an intercept of 0.3 g N m^{-2}, and Evans (1983) observed wheat to have an intercept of 0.2 g N m^{-2} (Figure 1.3). The linearity of the response indicates the use of most N, thereafter, in photosynthesis. Nitrogen use efficiency increases with increasing PPFD (Hirose & Bazzaz, 1998). Maximising NUE throughout the canopy, based on light flux provides the physiological basis for CNR.
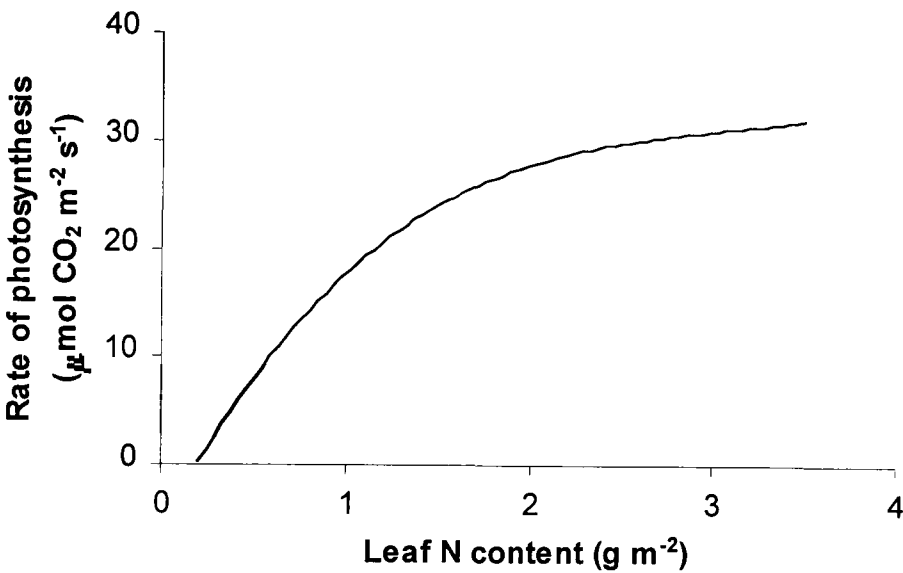
Figure 1.3 Response of photosynthetic rate to leaf N content in wheat under a PPFD of 1800 μmol m^{-2} s^{-1} (adapted from Evans, 1983).

1.3.2   Response of photosynthesis to light flux

Photosynthetic rate in wheat increases with PPFD (Lawlor *et al.*, 1987b; Shangguan *et al.*, 2000), as shown in Figure 1.4. This response curve is well studied for many species (*Solidago altissima* Hirose & Werger, 1987a & b; *Lysimachia vulgaris* Pons *et al.*, 1989). Several models have been developed, describing the hyperbolic relationship (Field, 1983; Hirose & Werger, 1987b; Sinclair & Horie, 1989; Anten *et al.*, 1995a). It can be explained in three sections (Hirose & Werger, 1987b; Evans, 1989b). The initial slope or quantum yield (1), indicates the efficiency in the utilisation of incident irradiance and is dependent on the chlorophyll and therefore leaf N content (Pons *et al.*, 1989). The intercept has a negative value indicating dark respiration, which is linearly correlated to leaf N content (Hirose & Werger, 1987b;

Pons *et al.*, 1989). The linear response of photosynthesis to low light flux is due to the rate of CO_2 assimilation increasing in proportion to the amount of light intercepted. Light, and the electron transport rate, therefore limit CO_2 assimilation here (Hirose & Werger, 1987b). The second section (2) where the response is reduced, is where carboxylation by RUBISCO starts to become more limiting than the electron transport. Photosynthesis eventually reaches a maximum where RUBISCO and therefore N content per unit leaf area, becomes the limiting factor. This is the point of light saturation. The plateau region of the response is where RUBISCO content is fully saturated (3).

In summary, the intercept, the initial slope and the asymptote all increase with increasing N per unit leaf area.
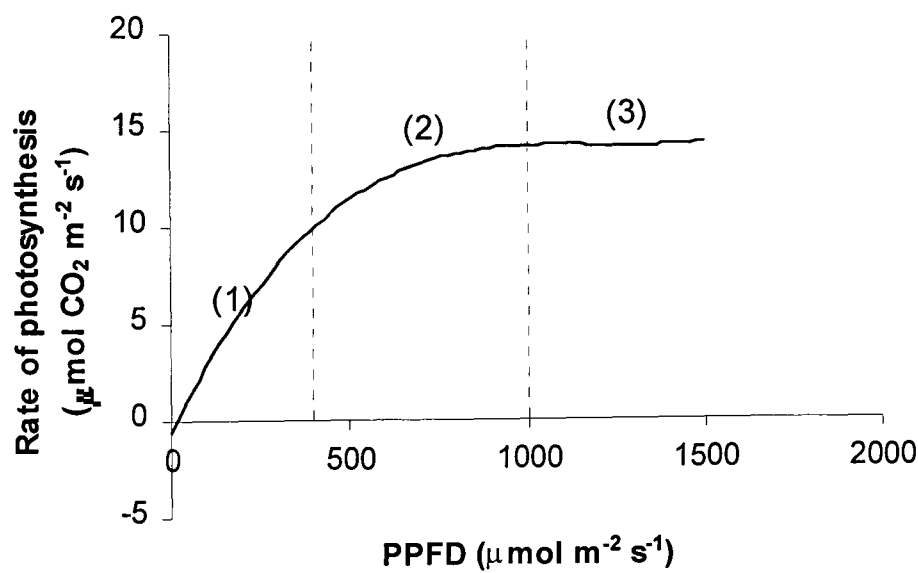
Figure 1.4 Response of photosynthetic rate to increased photosynthetic photon flux density (PPFD) in wheat with 15 mmol l^{-1} nitrate (adapted from Shangguan *et al.*, 2000).

1.3.3 Response of leaf N content to light flux

Photosynthetic rate is greater where light fluxes are higher but also where the amount of RUBSICO is greater (Mooney & Gulman, 1979). This suggests a relationship between leaf N and light and has been observed in many species. There was no correlation between %N in dry weight and daily PPFD in the walnut (*Juglans regia*)

leaves (Klein *et al.,* 1991) or in peach (*Prunus persicsa*) leaves (DeJong, 1986). However, there was an increase in the N content per unit area of leaf with daily PPFD in both tree species. The response of leaf N content to light appears to vary. Peach leaf N content was linearly correlated to number of hours of daily leaf exposure to PPFD of more than 100 μmol m^{-2} s^{-1} (DeJong & Doyle, 1985; DeJong, 1986). They found a strong correlation between leaf N content and light exposure but as the season progressed, leaves with the same exposure to light contained a greater N content. This may relate to the seasonal changes in the canopy of deciduous trees. The relationship between leaf N content and PPFD was also linear in guar (*Cyamopsis tetragonoloba*) (Charles-Edwards *et al.,* 1987) and *Solidago altissima* (Hirose & Werger, 1987b). In other studies the relationship was not linear for all levels of irradiance especially at the higher levels. In the sunflower, there was a linear increase in leaf N content with PPFD, reaching a maximum of 2.3 g m^{-2} at around 1000 μmols m^{-2} s^{-1} with no further increase in leaf N content (Rousseaux, Hall & Sànchez, 1999). A curvilinear response of leaf N content to daily irradiance was also observed in lucerne (Evans 1993b).

Simulated response curves suggest that with increased N content the leaves have higher light saturation points, higher P_{max} and higher light compensation points (Field, 1983, Hirose & Werger, 1987b) (Figure 1.5). Increasing the light available to those leaves with a lower N content has little effect on increasing photosynthetic capacity and therefore carbon gain.
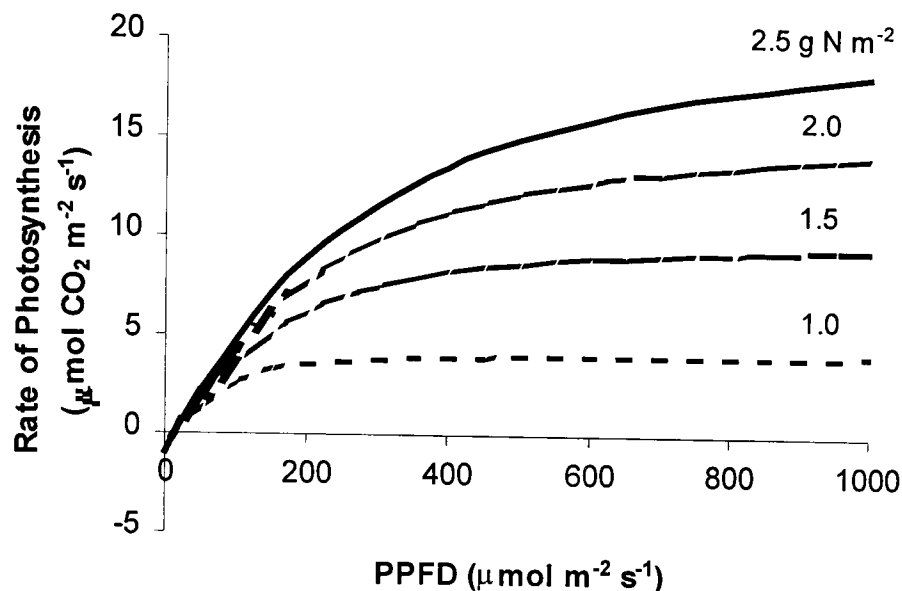
Figure 1.5 The response of photosynthetic activity to photosynthetic photon flux density (PPFD) under various leaf N contents (g m^{-2} leaf area) – indicated by numbers. Adapted from Hirose & Werger (1987b).

1.3.4 Response of leaf N content to light quality

Most of the literature describing the leaf N content in relation to light has focussed on the quantity of photosynthetic light (Hirose & Werger, 1987b; Lemaire *et al.*, 1991). The quality of light, or the ratio of red to far-red light (R:FR), and leaf N content has been less frequently studied (Rousseaux *et al.*, 1999).

The phytochrome photosystem monitors the quality of light, although it may also be involved in detecting the quantity (Frankland, 1986). There are two isomers of phytochrome which are interconvertible by light energy in the red and far-red regions of the spectrum (Presti & Delbrück, 1978). Red light converts phytochrome red (Pr) to phytochrome far red (Pfr) while far red light does the reverse. The R:FR ratio is therefore sensed by the proportions of Pr and Pfr present (Smith & Whitelam, 1990).

As light penetrates the canopy, and red light is absorbed, the R:FR ratio decreases from 1.1 to about 0.1 (for a GAI of >5) on reaching the soil (Ballaré & Casal, 2000).

This occurs due to the strong absorption of red light but insignificant absorption of far-red light by chlorophyll (Smith, 1982). In shade avoiding plants, such as wheat, the R:FR ratio is detected and stimulates appropriate responses such as enhanced petiole and stem extension, increased partitioning to shoot at the expense of the root and reduction in tillering (Holmes & Smith, 1977; McLaren & Smith, 1978; Morgan & Smith, 1981; Deregibus *et al.*, 1985; Smith & Whitelam, 1990, Ballaré & Casal, 2000). It has been suggested that plants are not only able to perceive the presence of neighbouring vegetation but also its proximity, through the detection of light quality (Smith, Casal & Jackson, 1990). Although it appears that there is no direct evidence from the literature, it seems possible that the response of leaf N content could be triggered by the change in R:FR ratio.

1.3.5 Acclimation of photosynthetic apparatus to light flux

Leaves are also able to acclimatise to light through the change in the partitioning of N to photosynthetic apparatus. The proportion allocated to thylakoids increases from 20 to 40% of total leaf N, under low light at the expense of the enzymes in the Calvin cycle (mainly RUBISCO) (Evans 1989a & c; Hikosaka & Terashima, 1995). Therefore, where leaf N content is reduced through the response to low light, the chlorophyll content per unit leaf area remains stable. In photosynthetic acclimation to low light, there is therefore greater proportional investment of leaf N in the light-harvesting complex at the expense of the electron transport and Calvin cycle capacity. There is also a decrease in the cytochrome f content and an increase the amount of chlorophyll content, so that there is a decrease in the electron capacity per unit of chlorophyll with low irradiance (Evans, 1987). Conversely, there is an increase in electron capacity per unit of chlorophyll at high irradiance, due to the increase in cytochrome f content (Evans, 1989c). The electron capacity per unit of total N is unaffected by reduced light because of the increased partitioning of N to cytochrome f (Evans, 1989c; Hikosaka & Terashima, 1995). Despite the increased partitioning of N to chlorophyll, the photosynthetic capacity per unit leaf N is decreased with the reduction in light levels (Gulman & Chu, 1981; Evans, 1989a; 1993a).

1.3.6 Leaf age

Leaf age has been recognised as a confounding factor in the relationship between leaf N content and PPFD, with respect to canopy depth (Friedrich & Huffaker, 1980; Field & Mooney, 1986). Usually the leaves at the top of the canopy are the most illuminated but are also the youngest. An experiment was carried out by Hikosaka *et al.,* (1994), in which a vine was grown horizontally to avoid mutual shading and a range of N treatments was applied. A decrease in leaf N content per unit leaf area and chlorophyll content was observed with an increase in leaf age, but only when N was limiting. With canopy-type shading imposed, there was a gradient in leaf N content along the gradient in PPFD. This also occurred when inverse canopy-type shading was imposed. Therefore, with a low N treatment both PPFD and leaf age significantly contribute to the gradient in leaf N content. This may also be expected in a dense stand where increased N competition per plant results in N deficiency. With a high N supply, the PPFD is the stronger driving force of the gradient in leaf N content. A similar observation was made on Italian ryegrass (*Lolium multiflorum*) leaf, in which the older part of the leaf (tip) experienced the greatest PPFD and the greatest N content per unit area (Prioul, Brangeon & Reyss, 1980). In the wheat crop, the effect of leaf N content on the light saturated photosynthetic rate, was increased slightly by leaf age only when there was N deficiency (Dreccer *et al.,* 2000). Therefore, it may be concluded that unless the plant is N stressed, leaf age is not a major factor in determining the gradient of leaf N content with canopy depth.

1.3.7 Leaf N content and canopy position

The canopy of a crop intercepts light, such that a gradient in PPFD down the canopy develops. As the canopy expands, upper layers intercept more light so that less is able to penetrate to lower layers. The light gradient therefore increases and photosynthetic acclimation occurs, involving the redistribution of N from lower to upper leaves. This non-uniformity of N over the whole canopy is reflected in the canopy's photosynthetic capacity (DeJong & Doyle, 1985).

There is a strong relationship between leaf N content per unit leaf area and depth down the canopy (Hirose and Werger, 1987a; Grindlay *et al.,* 1997). Typical values of leaf N content at the top and bottom of the canopy in Lucerne are 2.6 g m^{-2} and

0.5 g m^{-2} respectively (Lemaire *et al.*, 1991). This is similar to soybean with 2.2 g m^{-2} and 0.5 g m^{-2}, top and bottom, respectively (Shiraiwa & Sinclair, 1993). In comparison with soybean, the leaves at the top of the wheat canopy have a smaller N content (1.9 g m^{-2}), but slightly greater at the bottom (0.7 g m^{-2}) (Grindlay *et al.*, 1997). The N content of peach tree leaves were greater both at the top and bottom, ranging from about 3.3 to 1.2 g N m^{-2}, respectively (DeJong & Doyle, 1985; DeJong, 1986). There is also a diminishing decrease in leaf N content with depth of the annual herb *Xanthium canadense* canopy (Hikosaka *et al.*, 1999).

There is a linear decrease in leaf N content per unit leaf area with cumulative leaf area from the top of the canopy in lucerne (Evans, 1993b) and soybean (Shiraiwa & Sinclair, 1993). The decrease in wheat leaf N content with cumulative leaf area also appears to be linear, but in the unfertilised crop the decrease is exponential due to relatively more N in the upper leaves (Grindlay *et al.*, 1997).

The ratio or N content per unit leaf area, decreased with depth down the canopy of a guar crop (Charles-Edwards *et al.*, 1987). Results suggested that the degradable, metabolically active N and in turn, the photosynthetic capacity within the leaf, declines with increasing shading. Remobilisation of the photosynthetic N occurs within the canopy to maximise use of the available light. Once the canopy has reached a certain size, virtually all the incident light will be intercepted. The remobilisation of N means that any additional N taken up by the roots, will be used to maintain the structure of new shoots and other non-photosynthetic components. This will essentially replace the structural N lost in those leaves which abscise. With the increase in canopy size and subsequent shading, the chlorophyll-proteins and the carboxylating enzymes are degraded in low PPFD. The N released from this will be translocated to the actively growing parts (Evans, 1993a).

Gradients in leaf N content are not just the result of vertical gradients in PPFD. Drouet & Bonhomme (1999) observed a relationship between horizontal local light and leaf N content gradients along leaves in maize.

1.3.8   Radiation Use Efficiency

Radiation use efficiency (RUE) is the conversion of light interception into total crop biomass, expressed in g MJ^{-1}. It therefore reflects the balance between the photosynthetic production and respiratory losses. The theoretical minimum energy required to reduce one molecule of CO_2 is 9.5 quanta of PAR (Penning de Vries et al., 1989 - see Azam-Ali et al., 1994). RUE is greater in pre-anthesis canopies with a lower light extinction coefficient as the light is distributed over a greater leaf area and therefore results in less light saturation (Green, 1989). The RUE increases with leaf N content, although the response is hyperbolic (Muchow & Sinclair, 1994). However, RUE decreases with increasing PPFD (Dreccer et al., 1998). RUE is expected to increase with the optimal N distribution, such that more N is allocated to the more illuminated leaves and reducing light saturation (Dreccer et al., 1998). Sinclair & Muchow (1999) reported the range of maximum values for RUE in wheat to be 1.46 - 2.93 g MJ^{-1} PAR.

1.3.9   Optimal leaf nitrogen distribution

Maximisation of daily carbon (C) gain will occur with N redistribution, such that, carbon gain in the receiving leaf will be greater than the reduction in C gain in the leaf exporting N. This is the requirement for optimisation of N distribution. It is expected that light will drive this. In any one leaf position, there is a diminishing increase in photosynthesis with increasing N content (Field, 1983).

The relationships between N and photosynthesis, PPFD and photosynthesis and PPFD and N have been modelled to simulate optimum leaf N distribution for maximum canopy photosynthesis (Field, 1983; Hirose & Werger, 1987a; Sands, 1995). The optimal and observed leaf N contents increased with PPFD and total N availability (Field, 1983; Hikosaka & Terashima, 1995). Combined simulations showed that the youngest leaves, at the top of the canopy, receive greater PPFD and therefore have the highest optimal N contents. Field (1983) predicted the daily carbon gain with optimal and actual N distributions in *Lepechina calycina* Benth. Epl., (Field, 1983). There was a small increase of 1% in carbon gain with the optimum compared to the actual leaf N distribution in *Lepechina calycina* Benth. Epl., (Field, 1983). The increase in carbon

gain due to optimal N distribution in the more dense stand of *Solidago altissima* was 4.7% (Hirose & Werger, 1987a). The optimal total N content was 2.2 g N m^{-2}. Pons *et al.*, (1989) observed a slightly greater increase of 5% in *Lysimachia vulgaris*. Similar observations were made by Anten *et al.* (1995a) in sorghum, rice and soybean. Bindraban (1999) modelled the effect of a uniform leaf N distribution on canopy growth. He found no increase in canopy growth even with a leaf N concentration of 4 mg g^{-1}, as lower leaves would be limited by light and not N.

There are several possibilities for the discrepancies between observed and optimal leaf N distribution. There will be extra costs involved in redistributing N, such as the degradation and resynthesis of photosynthetic enzymes and amino acid transport. However, the cost of redistributing the N in terms of amino acid transport are slightly less than the benefits it provides in extra carbon gain (Field, 1983). The actual distribution is more uniform than the optimal N distribution with the actual N content being lower at the top and higher at the bottom of the canopy than the optimal. It was suggested that the lower leaves were not able to reduce their N content to levels as low as the optimum, as the optimum was below that required for maintenance (Hirose & Werger, 1987a). It was also suggested that there may be a lag period in the response of N to fluctuations in PPFD (Pons *et al.*, 1989). The lower leaves would also be able to take advantage of occasional sunflecks, by having more than the minimum required for maintenance.

In summary, N in the wheat canopy is used mainly for photosynthesis, but there is evidence that it is also in compounds used for structural support, transport and storage which will be described in the next section. Nitrogen is distributed between the leaves to maximise carbon gain by responding to the profile of PPFD, which is a function of canopy architecture.

1.4   OTHER FUNCTIONS OF N IN THE CANOPY

The common notion is that canopy N is used predominantly in photosynthesis. As a result, most of the literature has concentrated on laminae, as these are the main photosynthetic organs. However, the stem also contributes significantly to the N

content of the whole plant, yet its main function is seldom seen as being for light capture and photosynthesis.

In many studies of cereals and grasses the stem is not separated from the leaf sheath. The sheath could be expected to be similar to the laminae which it supports, so in many studies on cereals, 'stem' N may include a reasonable amount of photosynthetic N. However, the true stem is relatively large compared to the sheath and is almost totally enclosed. Thus N in structural components are also likely to be an important constituent of true stem N content. A considerable amount of N may also be reserved in the stem for later redistribution. Puckridge and Donald, (1967) reviewed a paper which suggested that in barley, the stem, including the leaf sheaths, contained the largest proportion of reserve N. Nitrogen in soluble, transportable forms may also be present in the xylem and phloem within the true stem. The amounts however, are likely to be small and may depend on stage of development.

To summarise, the main function of N in the leaves has been identified as photosynthetic. The main role of N in the stem is less well understood; this N may be involved in several supportive roles including, structure, reserve, metabolism and transport (Grindlay et al., 1997). These roles may also exist to a minor extent within leaves. It is difficult to distinguish, either physically or chemically, between metabolic, structural and storage proteins. For instance structural proteins are usually fibrous, but the non-fibrous storage protein, glutelin, may also be considered as a structural protein (Miège, 1982). Thus proteins with different functions are best distinguished according to evidence of that function rather than through direct measurements. Each function will now be considered in turn.

1.4.1   Structural function of N

The wheat stem extends from the nodes situated along its length. In mature wheat, there are usually six fully extended internodes (Percival, 1921; Hector, 1936; Knapp, Harms & Volenec, 1987). The upper portion of the internode is ten times stronger than the lower portion (Juniper, 1979). The nodes are solid, while generally, the internodes are hollow (Hector, 1936). A study of the cross section of an internode reveals four main tissues: epidermal, hypodermal, vascular and parenchyma.

The epidermis is a thick outer layer of the stem, with rows of stomata similar to those in the leaf (Hector, 1936), penetrating to the parenchyma. A cuticle is present where the leaf sheath is absent (Percival, 1921). The hypoderm is a continuous layer beneath the epidermis. This consists of strengthening, lignified sclerenchymous tissue fibres (Percival, 1921; Knapp *et al.,* 1987). There are two types of parenchyma tissue, identified and depicted by their colour: green and colourless. The green assimilating parenchyma tissue embedded in the hypoderm runs in narrow parallel bands up the length of the stem and contains chlorophyllous tissue (Hector, 1936). These become closer with height, but are almost absent at the base of the stem (Percival, 1921). The soft colourless ground parenchyma tissue extending from the hypoderm into the middle of the stem, breaks down at later stages of development to leave a hollow stem (Percival, 1921; Juniper, 1979). At the base of the stem, the parenchyma becomes lignified providing the required strength (Percival, 1921). Small vascular bundles are located in the hypoderm, whilst larger bundles are located more centrally, in the parenchyma. The xylem is located nearer the centre of the stem, while the phloem is towards the periphery. Strengthening sclerenchyma surrounds the vascular bundles. Thin-walled cells containing chloroplasts extend down the length of the internode within the hypoderm (Percival, 1921; Juniper, 1979). Leaf sheaths surround the whole of the lower internodes and most of the upper internode length. They serve to protect and support the stem (Percival, 1921; Hector, 1936).

The diameter of the stem increases with height up the canopy (3.43 - 4.18 mm), but the upper internode (peduncle) has a smaller diameter than the lower (3.03 mm) according to Percival (1921). More recently, Easson, Pickles & White (1992) reported stem diameter to decrease slightly with height up the canopy, after the initial increase for the bottom two internodes. The thickness of the stem wall decreases with height (Percival, 1921) and so it would be expected that the structural N requirement per unit stem area would increase with canopy depth.

Plant cell walls consist of fibrous crystalline cellulose fibres and non-crystalline sugars packed between the fibres, which provide a reinforced matrix. In early stages of development there are specific proteins embedded in the cell wall, possibly to assist with the plasticity of the wall during the expansion phase (Juniper, 1979). These will possibly be broken down afterwards. At later stages of development, lignin is formed.

Lignin contains very little N. Not only is it rigid and resistant to decay but it is an enzyme inhibitor and forms chemical bonds with other cell wall components (Juniper, 1979). Other substances of the cell wall include the waterproofing polyesters: cutin and suberin (Juniper, 1979). However, these also contain little N.

Juniper (1979) performed a chemical analysis of barley straw at harvest, identifying cellulose (41.3% DM), hemicellulose (31.3%), lignin (7.1%), ash (mineral element) (3.0%) and cell contents (16.9%). Most of the N in the stem is likely to be located in the cell contents. Similar results were obtained for spring wheat stems at harvest, with 9% dry weight identified as crude protein (Osaki, Shinano & Tadano, 1991) and therefore 1.4% N in dry weight. This suggests that stem N is not mainly structural but provides for photosynthetic compounds, nucleic acids or reserves.

There are few clear identifications of structural proteins reported in the literature. This may be because they are difficult to identify or because the actual amounts are relatively small. Structural proteins have been identified at the cellular level in the cell membrane and cell wall. Extensin is a cell wall glycoprotein, forming a strengthening link within a protein – glucan network (Miege, 1982; Boulter & Derbyshire, 1977).

Plants allocate little N to their cell walls for structure (Bacon, 1988). Extensin constitutes only 1 - 2 % of the cell wall (Lamport, 1977). Structural compounds in plants such as polysaccharides, cellulose and lignin, are composed of carbohydrates and contain very little N.

Wilman & Altimimi (1982) measured the digestibility of Italian and perennial ryegrass plant parts, with respect to lignin and N content (% DM). The internodes (with sheaths) were found to be low in N and high in lignin, compared to the nodes. The N was 0.49% and 0.86% respectively. Digestibility was found to correlate positively with %N and negatively with lignin content of the cell wall. Upper parts of the stem were more digestible, with a lower lignin content and higher %N. At pollen shedding stage, %N of successive internodes down the stem was 0.95, 0.43, 0.37 and 0.32% DM. %N also decreased with shoot maturation (Wilman, Oujouederie & Asare, 1976). Prior to pollen shedding stage, at head emergence, the lower two internodes were 0.8 and 0.5%N (Wilman & Altimimi, 1982). Also at this time, lignin

was 5.9 and 6.7 % in DM. This appears to indicate the structural function of lignin, and that N in the stem does not play an important role in this.

In summary, the evidence from the literature suggests that it seems unlikely that the N contained in the stem is mainly used for structure. However, certain canopy architecture characters are able to provide the basis for a crude prediction of the stem structural N content, in Chapter 2. There must also be other functions of N in this tissue. Most plant tissues, even those not predominantly photosynthetic (i.e. not leaves) contain a large number of enzymes in small amounts, which collectively will account for some of the N content (Bacon, 1988) and it is also possible that the stem contains reserve N. The constant redistribution of N between plant parts means that some N will also be in transport.

1.4.2 Transport of N

Transport of N between plant parts occurs via the phloem or the xylem. Because N is taken up in the plant through the roots it must be transported to the growing parts of the shoot. As the canopy increases in size, there will be progressive shading of the lower leaves and plant parts by the upper newly formed parts. As a result, it is necessary to redistribute the N from the lower to the upper leaves, where the light flux and therefore the need for N in photosynthesis, is greater. This is physiologically important during senescence, so no N is lost. As grain filling takes place in wheat and reproductive organs develop, it is important for N to be redistributed to these sinks. This is agronomically important for protein crops and animal feed. Nitrogen that is reserved in various forms is also redistributed during canopy expansion or reproductive growth. The xylem of wheat contains N in the form of nitrate and amino acids where movement of N occurs with the transpiration stream from the roots to the shoot (Simpson & Dalling, 1981; Frommer *et al.*, 1994).

As a major part of the total soluble leaf protein is contained within RUBISCO, large quantities of amino acids are derived from its degradation. Studies by Simpson, Lambers & Dalling (1983) have shown that a large amount of N is transported into the glumes via the xylem, reaching a maximum just after anthesis. They also showed the concentration of amino N in xylem sap to increase from 57 μg ml^{-1} at the stem

base, to 104 µg ml^{-1} at the top node. The N is then loaded into the phloem and transported to the grain. A little N in the grain is transported directly from the xylem.

N is transported mainly as amino acids but there are also small amounts of organic acids, nucleotides, proteins and phytohormones (Heldt, 1997, see also Frommer *et al.*, 1994). Large amounts of potassium and sucrose are also transported in the phloem. Hayashi & Chino (1986) carried out chemical analysis of wheat phloem sap. Sucrose, amino acids, and potassium were the main compounds found (each was approximately 30% of the total chemical composition of the sap). The total concentration of amino acid compounds in the phloem was 262 mM and the concentration of nitrate was 8 mM. Of the total amino acids detected in the sap, glutamic acid constituted the highest concentration (30%), followed by aspartic acid at 19%. Eighteen other amino acids were detected by Hayashi and Chino (1986), including serine (7%), threonine (5%), asparagine (4%), glutamine (4%) and arginine (2%).

Amino acid composition was also determined in spring wheat (Osaki *et al.*, 1991). The main free amino acids at the mid-maturing stage in leaves were alanine, glutamic acid, aspartic acid, glutamine and γ-amino butyric acid. In the stem, the main free amino acids were aspartic acid, aspragine, glutamic acid and glutamine.

Transport fluids also carry N-containing molecules in trace amounts: protein-amino acids, non-protein amino acids, purines and pyrimidines, plant growth regulators, peptides and proteins.

The amount of N in transport appears to be very small and so the requirement of the canopy for this is not considered to be very significant. For this reason, the requirement for transport N is not predicted in Chapter 2.

1.4.3 Reserve N

N reserves in plant tissue have been considered to arise through two different mechanisms categorised by Millard (1988) as:

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1. Accumulation or 'luxury uptake' – deposited only when the supply of nutrients exceeds the demand for growth.

2. Storage – deposited solely for later redistribution, even in competition with new growth for nutrients.

1.4.3.1 Luxury uptake

Surplus N may be accumulated in various organs of the plant, including the stem. It may be in the form of nitrate, free amino acids or organic N. This is also referred to as 'luxury consumption' (Grindlay, 1997). Leaves can accumulate more N than they require, at the beginning of the season when low temperatures and light levels limit growth rates, but there is plenty of N available from mineralisation. This has been seen in Italian Ryegrass, in which the positive response of leaf %N to N supply is linear (Wilman et al., 1976). The concentrations of nitrate are greater in the stems and petioles than in the leaves of sugar beet (Armstrong et al., 1986), or of potato (Millard & Marshall, 1986). Of the total nitrate in leaves, most is accumulated within vacuoles. The same applies to the free amino acids. The majority of accumulated organic N is in the form of soluble protein, the most important of which is RUBISCO. Increasing the N supply increases the leaf concentration of RUBISCO. The ratio of RUBISCO to total soluble leaf protein can increase in plants such as mulberry (Yamashita, 1986). In wheat, this was also observed by Evans, (1989c) but not by Lawlor et al., (1987a) where leaf N contents were increased through irradiance. However, at very high leaf protein contents the percentage of RUBISCO protein that remained active decreased. It has been suggested therefore, that RUBISCO may have an additional role of forming a reserve of N (Lawlor et al., 1987b). It is expected that N accumulated in proteins due to excess would be more readily available for degradation at a faster rate than other proteins. However, during senescence in wheat RUBISCO is degraded at the same rate as other soluble proteins (Peoples et al., 1980). More evidence is required to conclude whether or not N is accumulated in the form of RUBISCO.

Puckridge and Donald (1967) found low plant population density wheat crops to have a consistently higher %N in the stems, except for the earliest stage and maturity. However, stems in less dense stands are generally thicker and so would be expected to have a higher proportion of low %N structural compounds, than denser stands. Thus

these results suggest a considerable reserve of N was accumulated in crop stems at low plant densities. However, more photosynthetic N was also expected in the low density crop, due to the greater light, and the results were confounded by age differences between crops, so it is difficult to be conclusive from this.

1.4.3.2 Storage

Storage of N occurs independently of growth rate and N status. This N will therefore be stored even in N deficient plants. Stored N can be remobilised and reused later during reproductive growth (Nair, Grover & Abrol, 1978; Millard, 1988). Nitrogen is stored in the form of nitrate, amino acids or proteins. When the growth rate of plants declines due to N deficiency, the stored N in the leaves may be translocated via the phloem to the roots. This shoot to root cycling allows exploratory root growth within the soil for new N supplies. The presence of nitrate in the root also encourages nitrate uptake through a positive feedback mechanism (Schrader & Thomas 1981). Nitrogen is then translocated back up to the shoot via the xylem. It is also remobilised and used in other parts of the plant during senescence. RUBISCO is thought to be the main source of storage N (Wittenbach, 1979; Peoples *et al.*, 1980) as well as 'luxury uptake'. It appears that the difference between luxury uptake and storage of N is not in the compounds formed as a reserve but in the conditions under which they are formed.

Millard (1988) has reviewed the costs and benefits for each of the different N reserves. For nitrate in vacuoles, there will be energy expenditure in the form of ATP, for transport across the membrane. When the nitrate is remobilised out of the vacuole, it will require a replacement by solutes to maintain leaf turgor. The N reserved as protein is more costly due to the energy consuming processes involved in synthesising RUBISCO (Millard, 1988). There will also be extra energy costs in RUBISCO proteolysis and conversion to amides for export from the leaf. However, the additional Calvin cycle enzymes allow an increase in carbon assimilation, in some plants, though it has not been observed in wheat. It would also reduce the problem of increased osmotic potential which is associated with nitrate. Nitrate reductase activity is stimulated by light and so occurs in the young upper leaves that are more illuminated. N must be remobilised from the lower shaded leaves. The release of nitrate would

require light stimulation so release of nitrate from lower leaves might be constrained. This is not the case if N is stored as protein. Thus protein, particularly in the form of RUBISCO, appears to be the most advantageous and likely method of reserving N (Millard, 1988).

There is much literature identifying the seed storage proteins and the amino acids which are predominantly alanine and glutamic acid, (Kaczkowski, Kos & Piór, 1988; Osaki *et al.*, 1991; Weiser & Seilmeier, 1998). Proteins are also present in the meristem (Newcomb, 1967 see Boulter & Derbyshire, 1977). However there appears to be no literature to identify the N reserves in the stem.

Nitrogen can be reserved in some species as compounds identified as Vegetative Storage Proteins (VSPs; Mackown, Van Sanford & Zhang, 1992). They have been defined as having at least two functions: to supply reduced N in the case of defoliation, and to support spring growth when a rapid rate of N supply is required (Louahlia *et al.*, 1999). It would be difficult to distinguish the latter from luxury consumption. Staswick (1994) has reviewed the identification and function of VSPs in soybean. VSPs have been identified in woody species and forage legumes. In perennial ryegrass, three polypeptides were identified (55, 37 and 24 kDa) that accumulated in the leaf sheaths during autumn and winter, and were reduced under low N supply (Louahlia *et al.*, 1999). However, it is not clear if these should be classified as VSPs. Klauer *et al.*, (1996) identified similar glycoproteins with molecular mass of 27 and 29 kDa and lipoxygenase (94 kDa) in legume paraveinal mespohyll (PVM). This is non-photosynthetic, located between the spongy and palisade mesophyll of the leaf, but is associated with vascular bundles in the stem (Staswick, 1994). Its functions are the storage and transport of photoassimilates and N rich proteins (Klauer *et al.,* 1996). It is therefore possible that VSPs are present in the stems of wheat. Nitrogenous storage compounds in the root of biennial Chicory are VSP and arginine (Ameziane *et al.,* 1997). At the end of the season, the amount of total N in the root is unchanged but the percentage of VSP increases, suggesting a role of storage over the winter. No significant amounts of RUBISCO have been detected as VSPs (Klauer *et al.,* 1996) and there appears to be little evidence for VSPs in wheat, similar to those in soybean. However, they may be of different molecular weight and therefore not yet identified as VSPs.

In this thesis, it is thought that there is no requirement for luxury uptake, but that there may be a requirement for storage N. However, it is difficult to predict the requirement for storage N and also to distinguish the storage compounds from luxury uptake or photosynthetic compounds so that the prediction may be tested. Therefore, the storage N requirement has not been predicted in Chapter 2.

1.4.4 Metabolic function of N

In addition to their major role in photosynthesis, proteins also function as electron carriers in photorespiration. The proteins concerned include cytochrome-c (inner membrane of mitochondria, in all eukaryotes), ferredoxin (6-11 kDa) and plastocyanin (11 kDa) (Ramshaw, 1982). The other main metabolic proteins in the leaf include proteinase inhibitors, nitrate reductase, nitrite reductase and phenylalanine ammonia-lyase (Huffaker, 1982).

Primary metabolites include amino acids, carbohydrates, fatty acids, cytochromes, chlorophylls and metabolic intermediates of the anabolic and catabolic pathways (Heldt, 1997). Secondary metabolites also occur with no direct metabolic function. However they may have the function of protection from pathogens, micro-organisms and animals. One group of N rich secondary metabolites formed from amino acids is the alkaloids, which are mainly stored in vacuoles (Heldt, 1997).

Other groups of enzymes identified in plant tissues apart from RUBISCO are, proteases, peroxidase, ATP synthase, phosphorylase (catalyses the reversible phosphorysis of a-glucan) and glycolate oxidase (oxidation of glycolate to glyoxylate in photorespiration, 37kDa) (Ramshaw, 1982). The ratio of ATP synthase to chlorophyll is 1-2:500 (Heldt, 1997).

Other functions of N in the stem may have further supporting roles, including enzyme inhibition (e.g. trypsin and chymotrypsin) and recognition or regulatory proteins. The latter include histones, which regulate DNA transcription and replication (Boulter & Derbyshire, 1977).

There are also large numbers of non-protein nitrogenous compounds such as amino acids, polymers and nucleic acids (Bacon, 1988). Up to 15 % of total organic N in a leaf may be free amino acids (Field & Mooney, 1986), although this is very uncommon. N is involved in cell division through the requirement for nucleic acids such as DNA and RNA, for nucleotides (the building blocks for nucleic acids, phosphorylating agents, energy donor carriers etc.) and for purine and pyrimidine bases, involved in biochemical pathways and operate genetic control over biological processes (Lewis, 1986). Ten per cent of organic N in a leaf is in nucleic acids (Field & Mooney, 1986). Proportions in the stem are not reported in the literature.

The amounts of N required for each of these functions in winter wheat is not clear from reading the literature but crude predictions for some of these, based on the physiological processes and structural aspects of the canopy, are developed in Chapter 2.

1.5 THESIS STRUCTURE

The literature describes the physiological processes that determine the N content of leaves and to some extent of stems. The hypothesis to be tested is that:

The canopy N requirement of winter wheat can be predicted from canopy architecture.

This is the general objective of the thesis but there is also a series of specific objectives as follows:

- To develop a series of principles for the prediction of the N requirement of winter wheat grown in the UK, based on architectural characteristics.
- To test the stability of the CNR with increased canopy size through depth and development, and between husbandry treatments.
- To test the principles for the prediction of the canopy light distribution and to explain the variation in light extinction coefficient (k).

- To test the principles for the prediction of leaf N requirement based on maximising the NUE.

- To test the principles for the prediction of stem N requirement and to further the current understanding of the function of N in stem tissue.

- To suggest improvements for increasing precision in future predictions of canopy N requirement.

The structure of the thesis is therefore based on the physiological relationships between canopy architecture and CNR. Variation in canopy architecture characteristics can arise through crop husbandry practises such as variety, seed rate and N fertiliser (Figure 1.6). Architecture also changes with space through canopy depth and with time through crop development. It is proposed here that the variation in architecture can be related to the variation in CNR. This thesis aims to test a series of principles that might be used to predict the leaf N requirement and the stem N requirement from canopy architecture. The leaf N requirement is based on the minimum amount of N that maximises carbon gain and so is related to the light distribution within the canopy as a result of canopy architecture. The stem N requirement is also based, to some extent, on the same principles as the leaf N requirement but also on the need for mechanical support of the leaf area. Transport and metabolic N are also considered to be part of the requirement for 'support' N, but are more difficult to assess. These links are also illustrated in Figure 1.6. Other factors that would affect the relationship between CNR and canopy architecture include pests and diseases, lodging, water availability and rooting characteristics. However, to provide a useful and simple basis for a series of principles, these factors were not considered. The principles linking canopy architecture to CNR (Chapter 2) are based on the physiological relationships and functions of N described in the previous sections of this chapter.

Two field experiments were also designed to create canopies having a wide range of architectures and to test the hypothesis practically and compare with the predictions. The materials and methods used for these experiments are detailed in Chapter 3. General crop growth measurements taken throughout each field season and at harvest

are described in the first chapter of results (Chapter 4). Chapter 5 describes the description of total N content with the increase in canopy size through depth and time and the variation in this between husbandry treatments. The explanation for the CNR is addressed in the remaining three results chapters. The differences in canopy architecture and the effect on the light distribution within the canopy are presented and discussed in Chapter 6. The effect of the variations in architecture and light on the N content leaf and stem is described is the next two chapters. The chapter describing the N in stems (Chapter 8) in particular provides new information and ideas in understanding stem N function. The general discussion draws together all the main points concerning the prediction of the CNR, and the importance and application of this work within agriculture.
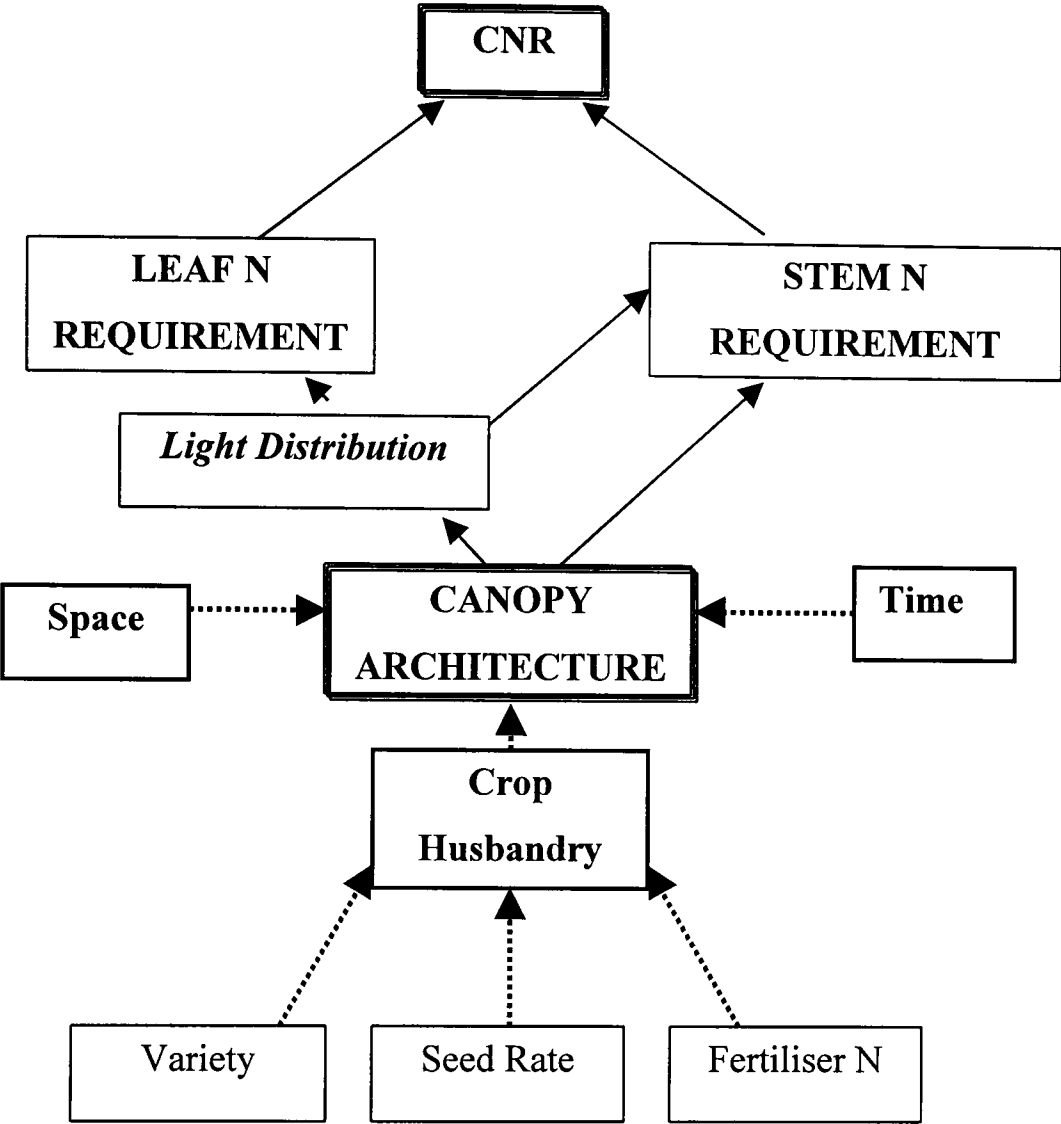
Figure 1.6 Diagram illustrating the ways in which variation in canopy architecture can arise (·······) and the proposed links between canopy architecture and canopy nitrogen requirement (CNR) (——).

2 PREDICTING CNR

2.1 INTRODUCTION

The literature shows that the CNR of wheat is variable (Foulkes *et al.*, 1994; Stokes *et al.*, 1997; Foulkes *et al.*, 1998) but does not provide any physiological explanations for this. It is hypothesised that this variation is due to differences in canopy architecture which are known to change with canopy depth, husbandry and crop development. These are considered in the following sections of the current chapter, resulting in quantitative predictions of CNR with canopy depth for a theoretical canopy and qualitative predictions of the less well-researched effects of husbandry and development.

2.2 DEPTH

The leaf and stem N requirements are predicted separately and then combined for the total N requirement with increased canopy depth. The leaf N requirement can be predicted from accumulated GAI. However, the stem N requirement cannot be predicted directly from accumulated GAI due to the limitations of the literature concerning structural N requirement. Two relevant sources of information are combined to predict the stem N requirement for each layer in a theoretical canopy with a GAI of 6.8. This method of prediction is applied in later chapters where it will be tested for a canopy in the experiment.

2.2.1 Leaf N requirement and depth

A simulated curvilinear relationship between N content per unit leaf area and photosynthetic activity (CO_2 exchange rate (CER)) has been developed at high light flux (Hirose & Werger, 1987b). This work was carried out on *Solidago altissima*, which is a clonal, herbaceous perennial that forms unbranched single stems from short over-wintering rhizomes. At low leaf N contents, photosynthesis is limited by the amount of RUBISCO and at higher leaf N contents it is limited by light. The authors also showed that the simulated response of photosynthetic activity to leaf N was

increased with higher light fluxes (Figure 2.1). The photosynthetic rate per unit leaf N or the nitrogen use efficiency (NUE) therefore increased with leaf N content, after an initial decrease below the light compensation point, until an optimum leaf N content was reached (Figure 2.2). NUE decreased above this point due to disproportionate increases in maintenance costs of large N contents. They also concluded that the nitrogen content that maximised NUE increased with PPFD. However, the simulated relationship between leaf N content and PPFD that maximised NUE was not modelled by Hirose and Werger, so further development of their simulations is now carried out. The leaf N contents for a range of light fluxes where NUE was maximal, are calculated using the following relationships as described by Hirose & Werger (1987b);

$$CER = P - R$$

$$R = 0.120 + 0.346 \, N$$

$$P = [\, \varphi I + P_{max} - \{ \, (\, \varphi I + P_{max} \,)^2 - 4 \, \varphi \, \theta \, I \, P_{max} \, \}^{1/2} \,] / 2 \, \theta$$

$$P_{max} = -7.86 + 12.5 \, N$$

$$\varphi = 0.0211 + 0.0188 \, N$$

$$\theta = 1.10 - 0.251 \, N$$

$$I = PPFD$$

$$NUE = CER / N$$

Where:

N_L = Leaf nitrogen content per unit leaf area (g N m^{-2})

P = Gross photosynthetic rate (net photosynthesis + dark respiration) (μmol CO_2 m^{-2} s^{-1})

I = Photosynthetic Photon Flux Density PPFD (μmol m^{-2} s^{-1})

P_{max} = Gross photosynthetic rate under saturating PPFD (μmol CO_2 m^{-2} s^{-1})

φ = Quantum yield (initial slope of curve) (Relates number of molecules affected in a photochemical reaction (M) to number of photons absorbed (P)

φ=M/P)(μmol CO_2 μmol^{-1})

θ = Convexity (diffusion and carboxylation resistance)(dimensionless)

CER = CO_2 exchange rate (net photosynthetic rate) (μmol CO_2 m^{-2} s^{-1})

R = Dark respiration (μmol CO_2 m^{-2} s^{-1})

The leaf N content required to maximise NUE increases with increasing light flux (Figure 2.3). A curve is fitted through the points and this relationship is used to provide a prediction for the individual leaf N requirement according to light flux experienced. Equation 2.1 describes this relationship.

$$N_L = 0.1828 \, PPFD^{\,0.39}$$ **Equation 2.1**

Where:

$N_L = g \, m^{-2}$ (leaf area) N

$PPFD = \mu mol \, m^{-2}$ (ground area) s^{-1}

The prediction is based on an assumption that plants distribute N between leaves to maximise NUE. Evidence in the literature suggests that plants have an N distribution that is closer to the optimum (based on maximising NUE) than a uniform distribution (Field, 1983; Pons et al., 1989). The optimum N distribution therefore is considered to be a reasonable basis for which a quantitative prediction scheme could be developed and tested.
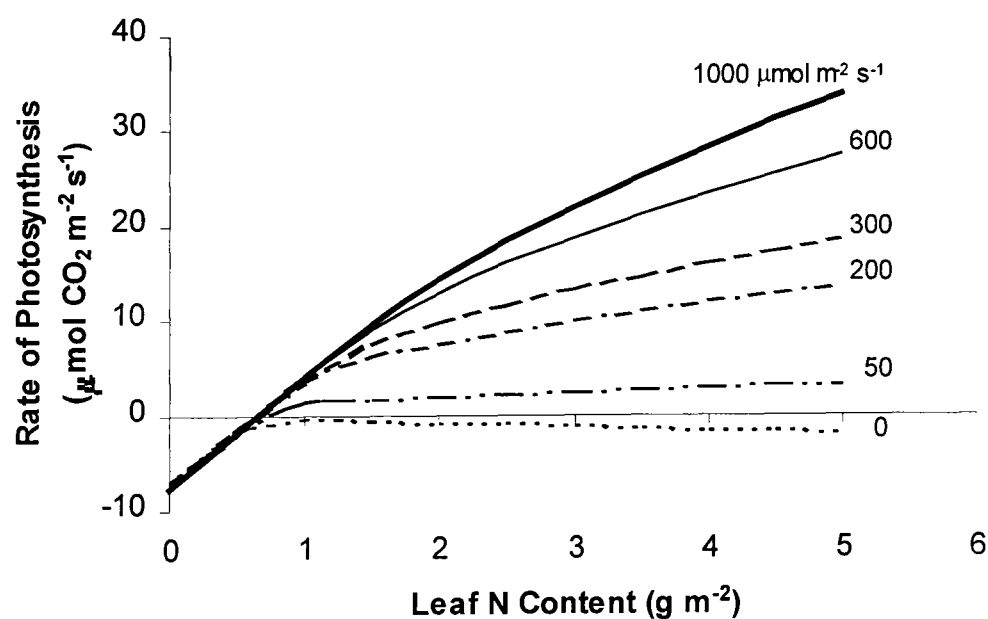
Figure 2.1 The response of rate of photosynthesis to leaf N content per unit leaf area under various light fluxes ($\mu mol \, m^{-2} \, s^{-1}$) – indicated by numbers. Adapted from Hirose & Werger (1987b).
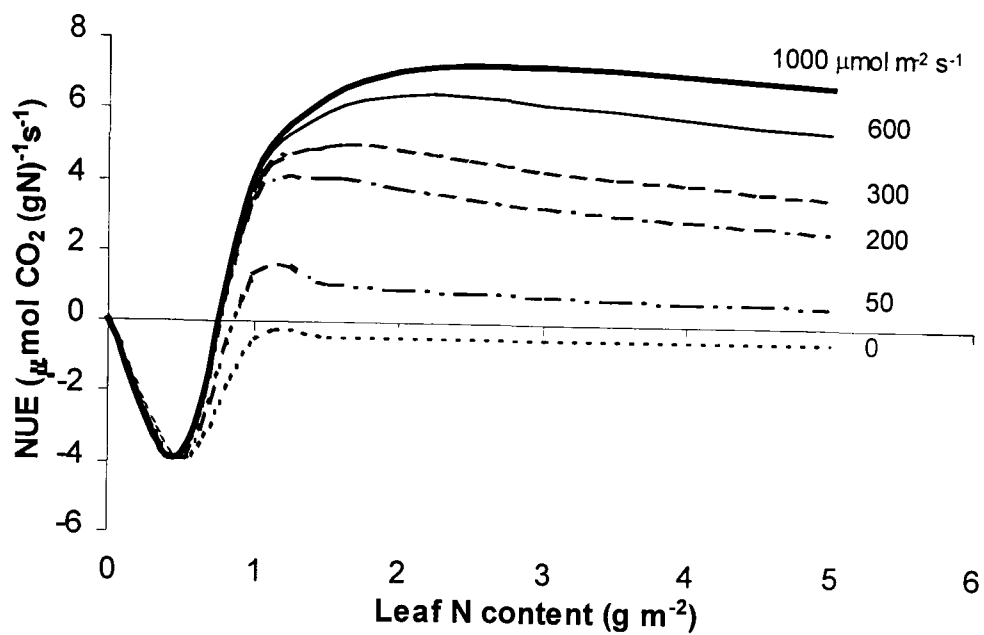
Figure 2.2 The response of nitrogen use efficiency (NUE) to leaf N content per unit leaf area under various light fluxes (μmol m^{-2} s^{-1}) – indicated by numbers. Adapted from Hirose & Werger (1987b).
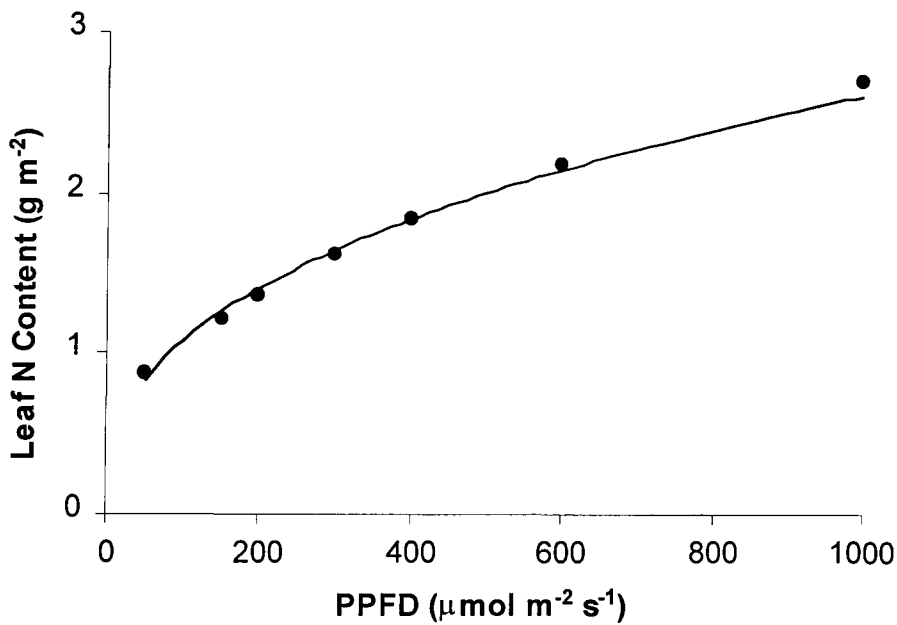
Figure 2.3 The predicted leaf N content per unit leaf area (in this study) required to maximise nitrogen use efficiency (NUE) with increasing photosynthetic photon flux density (PPFD). Fitted curve: $y = 0.1828x^{0.386}$, $R^2 = 0.99$. Based on Hirose & Werger (1987b).

To optimise N content for the whole canopy the PPFD with canopy depth is predicted by assuming an exponential decrease in PPFD with increased GAI, according to the Monsi and Saeki equation based on Beer's Law.

$$I = I_o \, e^{-kL}$$ **Equation 2.2**

Where:

I = PPFD at canopy depth

I_o = Incident radiation

k = Light extinction coefficient

L = Accumulated GAI with depth

The leaf N requirement with increased canopy depth is predicted from accumulated GAI, k and incident radiation, by combining Equations 2.1 and 2.2 (Equation 2.3):

$$N_L = 0.1828 \, (I_o \, e^{-kGAI})^{0.39}$$ **Equation 2.3**

A decrease in leaf N requirement per unit leaf area is therefore predicted with the increase in canopy size through depth (Figure 2.4). This was predicted for a canopy with a k value of 0.5 in June. The incident radiation is based on the long-term mean for June at Sutton Bonington, national grid ref. SK 267 502, 52° 50 N, 1° 15'W, altitude of 50m (573 μmol m^{-2} s^{-1} (average over 16.5 h of daylight)).
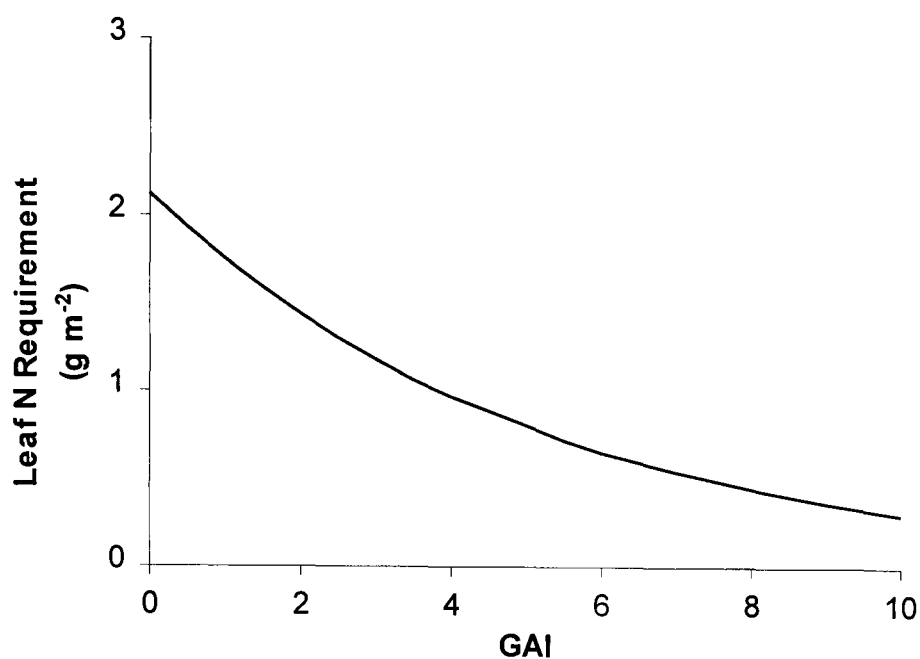
Figure 2.4 The predicted decrease in leaf N requirement per unit leaf area (in this study) with accumulated green area index (GAI) through canopy depth. $Y = 0.1828 \, (573 \, e^{-0.5x})^{0.39}$.

2.2.1.1 General assumptions

The general assumptions used to predict the leaf N requirement are:

- Photosynthetic characteristics of wheat are equivalent to *Solidago altissima.*

- Detached leaves used for the simulation were representative of leaves still attached to the plant.

- NUE and photosynthetic rate are not affected by the difference in conditions between Tokyo and the UK.

- PPFD at the leaf surface in the Hirose and Werger simulations is equivalent to the PPFD per unit ground area.

- Plants distribute N between leaves to maximise NUE.

- The decrease in PPFD with increased GAI followed Beer's Law.

- There is no storage or luxury uptake of N in leaves.

2.2.2 Stem N requirement and depth

Here, the predicted stem N requirement is based on structural and photosynthetic N and is expressed on a stem area basis. The principles for leaf N requirement are applied to predict the photosynthetic stem N requirement from a direct relationship with GAI. Structural N requirement in stems is predicted for a specific canopy size with a GAI of 6.8 and a k of 0.5, that was measured by carrying out stratified clips (Everett, 1995) as a direct relationship with GAI cannot be predicted from data available in the literature. Details of this crop are provided in Table 2.1. Photosynthetic N requirement is also predicted for this specific canopy size to enable stem total N requirement to be predicted. Nitrogen content was not measured by Everett and so the predictions could not be directly tested.

Table 2.1 Green area index (GAI) of stratified clips, for Mercia on 8 June and with 170 kg ha^{-1} N applied (Everett, 1995).

Layer (From canopy top)	Canopy Depth (cm)	Accumulated GAI	Leaf GAI A_L	Stem GAI A_S
1	0-10	0.035	0.04	0
2	10-20	0.487	0.45	0
3	20-30	1.374	0.71	0.17
4	30-40	3.151	1.43	0.35
5	40-50	4.84	1.44	0.25
6	50-60	6.043	0.92	0.28
7	60-70	6.793	0.42	0.33

2.2.2.1 Photosynthetic N of stems

The predicted relationship between photosynthetic N and PPFD is based on Hirose & Werger (1987b), to maximise NUE in the leaf. The response of photosynthetic stem N to PPFD is assumed to be the same as the response of leaf N. Photosynthetic N in the stem is thought to be mainly in the leaf sheath, and so the area of the photosynthetic

surface is taken to be cylindrical. This is then comparable with the leaf N. The photosynthetic N in the true stem, which would experience greatly reduced PPFD due to leaf sheath interception, is not considered as it is assumed to be small. The amount of photosynthetic N in the stem can then be calculated using the same method as calculating leaf N and then multiplying by π, as the projected area of the stem is always measured rather than the cylindrical area. The relationship between photosynthetic stem N (Ns_p) and PPFD is therefore similar to Equation 2.1:

$$N_{Sp} = \pi \, (0.1828 \; PPFD^{\,0.39})$$

Equation 2.4

Where:

N_{Sp} = g m^{-2} (projected stem area) N

PPFD = μmol m^{-2} s^{-1}

The distribution of PPFD within the canopy is already assumed to follow Beer's Law for the prediction of leaf N requirement. Equation 2.2 can therefore be applied to the photosynthetic N requirement of the stem (Equation 2.5):

$$N_{Sp} = \pi \, (0.1828 \, (I_o \, e^{\,-kGAI})^{\,0.39})$$

Equation 2.5

The photosynthetic stem N per unit projected stem area is therefore predicted to decrease with a similar pattern as leaf N requirement, with accumulated GAI through canopy depth (Figure 2.4).

A prediction of stem total N requirement (Section 2.2.2.3) can only be made if both structural and photosynthetic N is predicted for the real canopy measured by Everett (1995). Equation 2.5 is used to predict the change in photosynthetic stem N requirement with canopy depth for each 10cm layer in the canopy (Figure 2.5). The weighted mean stem photosynthetic N per unit stem green area is 3.31g m^{-2}.
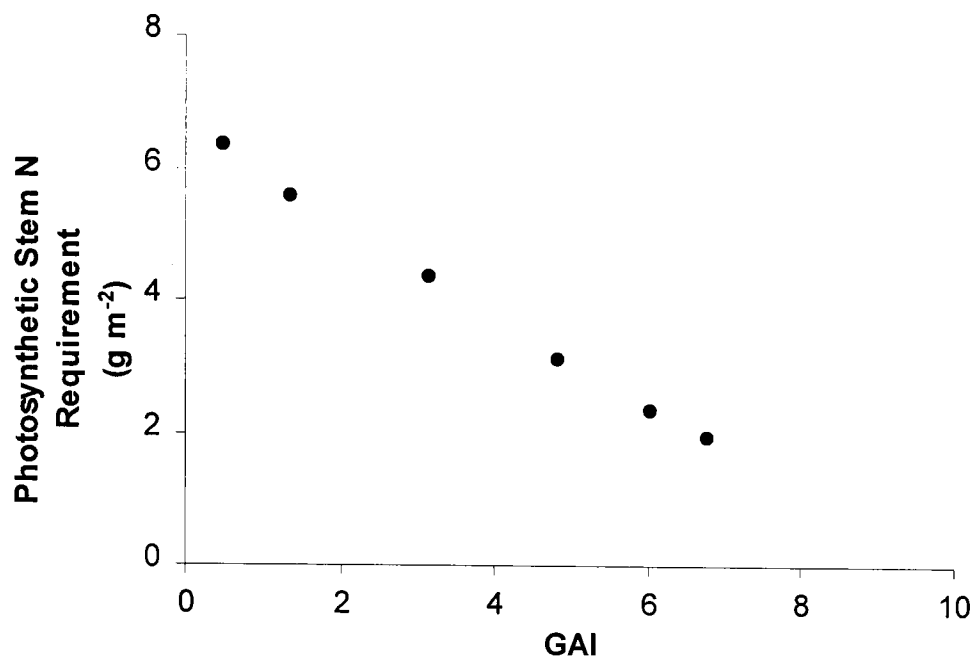
Figure 2.5 The predicted decrease in photosynthetic stem N per unit projected stem area (in this study) with accumulated green area index (GAI) through canopy depth. Data points represent each 10 cm layer.

2.2.2.1.1   General assumptions

The general assumptions used to predict the photosynthetic stem N requirement are:

- All photosynthetic N in the stem is located in the leaf sheath.

- The leaf sheath is of similar structure to the lamina and the relationship between photosynthetic N and PPFD of the leaf sheath is equivalent to that of the lamina.

2.2.2.2   Structural N of stems

The stem N content was measured in spring wheat at around GS 69 (end of flowering) and at harvest by Osaki *et al.,* (1991). They concluded that 25% of the N in stems at GS 69 was not redistributed by harvest and that, at GS 69, 1.33% of stem dry weight (DW) was found to be N. If it is assumed that the N not redistributed by harvest is part of structural tissue (White, 1995), then 0.3% of stem dry weight is structural N. It is assumed that structural N compounds are located in the true stem.

The prediction for structural stem N per unit projected stem area for a specific depth in the canopy (N_{Sst}) is based on 0.3% DW. This ratio is assumed to be constant with canopy depth. The stem internode length, dry weight and diameter are taken from Easson *et al.,* (1992) (Table 2.2) to predict the stem dry weight per unit length (DW^L) (Figure 2.6) and the projected stem area per unit length at a specific canopy depth (A_S^L) (Figure 2.7). The data are for a wheat crop sampled on 26 June and fertilised with 150 kg ha^{-1} N. Accumulated GAI with canopy depth is taken from Everett (1995) for Mercia (Table 2.1). Predictions of stem N are based on a canopy with a GAI of 6.8, *k* of 0.5 and with the same GAI distribution as measured by Everett.

Table 2.2 Stem internode length, dry weight and diameter. Mean of four wheat varieties sampled on 23 June and with 150 kg ha^{-1} N (Easson *et al.,* 1992)

Internode	Internode length (cm)	Internode dry weight (g)	Internode diameter (cm)
Peduncle	29	0.424	0.38
2	20	0.485	0.44
3	10	0.303	0.45
4	7.3	0.242	0.44
5	5.5	0.182	0.35
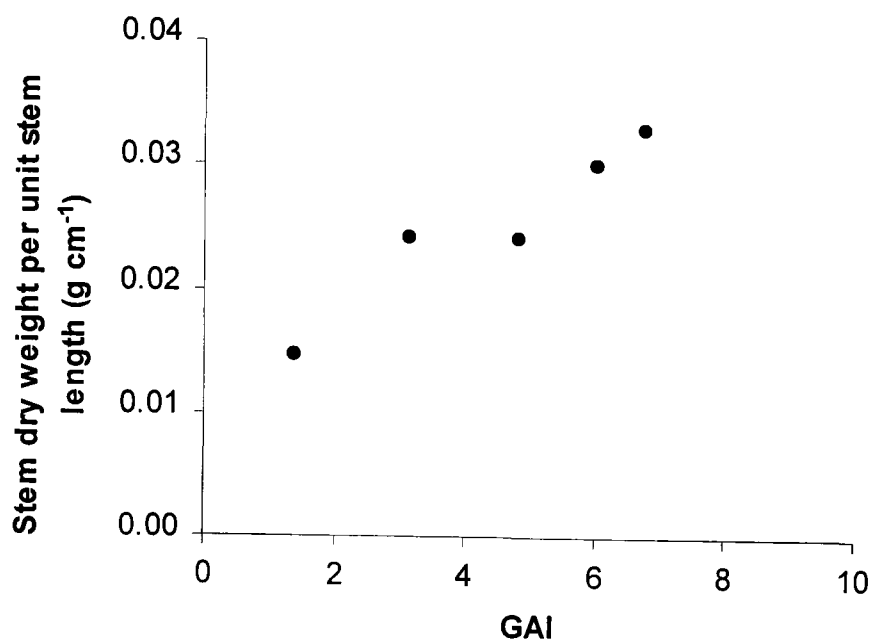
Figure 2.6 The increase in stem dry weight per unit stem length, and corresponding accumulated green area index (GAI) with canopy depth. Data points represent each layer (Easson *et al.*, 1992; Everett, 1995).
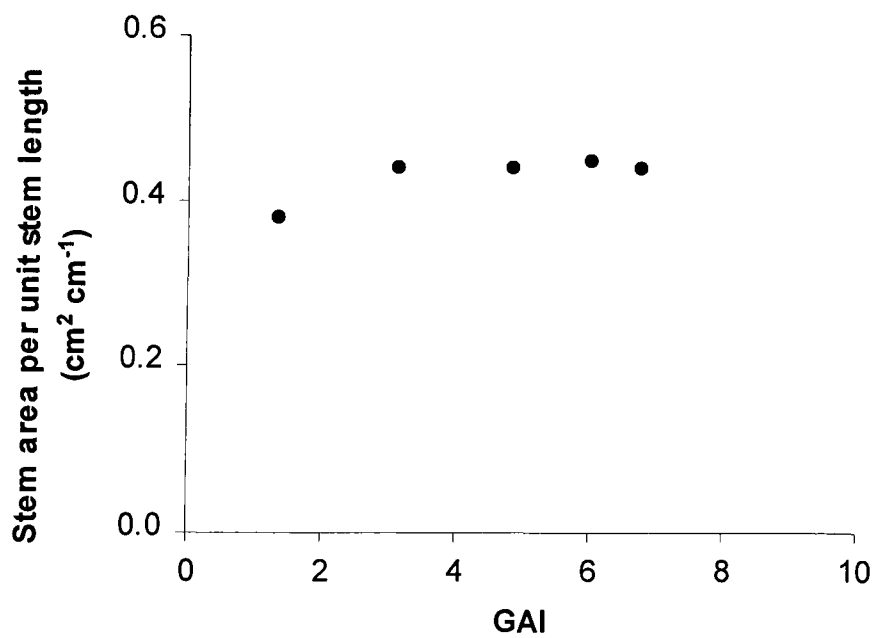
Figure 2.7 The projected stem area per unit stem length, and corresponding accumulated green area index (GAI) with canopy depth. Data points represent each layer (Easson *et al.*, 1992; Everett, 1995).

Structural N per unit stem length for each layer (N_{Sst}^{L}) is calculated from stem dry weight per unit length (DW^{L}) and the % structural N of stem dry weight, for each layer (Equation 2.6).

$$N_{Sst}^{L} = DW^{L} \times 0.003$$

Equation 2.6

Where:

N_{Sst}^{L} = g N m^{-1} for a layer

DW^{L} = g m^{-1} for a layer

Structural N per unit stem area (N_{Sst}) is then calculated for each layer with the projected stem area per unit length of each depth (A_{S}^{L}) (Equation 2.7):

$$N_{Sst} = N_{Sst}^{L} / A_{S}^{L}$$

Equation 2.7

Where:

A_{S}^{L} = m^{2} m^{-1} for a layer

N_{Sst}^{L} = g m^{-2} for a layer

Equations 2.6 and 2.7 could be re-expressed as:

$$N_{Sst} = DW^{L} \times 0.003 / A_{S}^{L}$$

Equation 2.8

Structural stem N content per unit stem area is therefore predicted to increase with canopy size through depth (Figure 2.8). The weighted mean stem structural N requirement per unit projected stem green area, is 1.81 g m^{-2} for a canopy such as that described by Everett (1995).
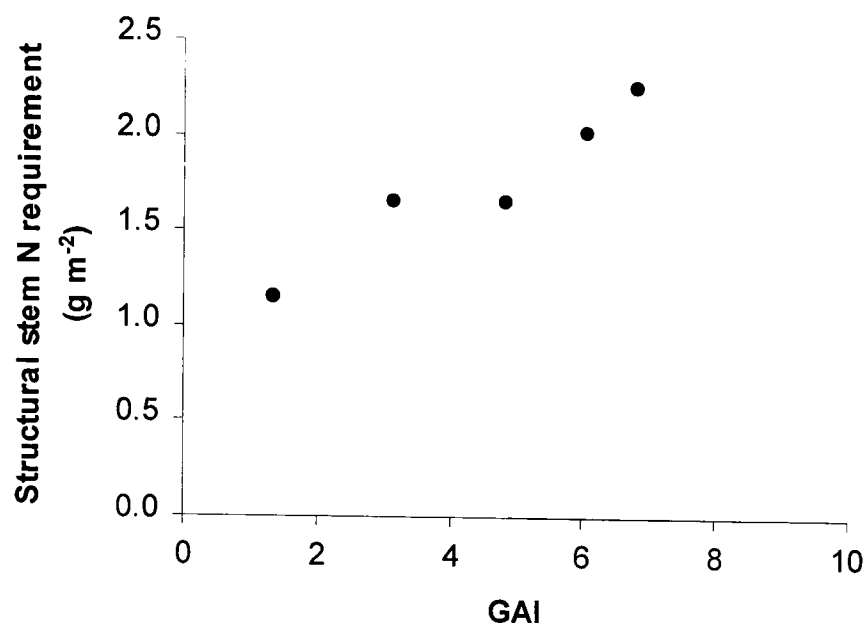
Figure 2.8 The predicted increase in structural stem N per unit stem area required (in this study) with accumulated green area index (GAI) through canopy depth.

2.2.2.2.1   General assumptions

The general assumptions used to predict the structural stem N requirement are:

- N in the stem at harvest is structural N and is optimal for this purpose.
- 0.3% of stem dry weight is structural for all canopy depths.
- All N in the true stem is structural N.

2.2.2.3   Total Stem N

Structural stem N is predicted with canopy depth through stem dry weight, stem area and the % structural N. This can be related to the accumulated GAI with canopy depth. Photosynthetic stem N is predicted through Beer's Law, and the principle of maximising NUE for photosynthesis by the leaf sheath. This can therefore be related directly to accumulated GAI.

Total stem N required per unit stem area (N_S) with accumulated GAI through canopy depth, can be predicted by combining photosynthetic and structural N requirements of

the stem (Figure 2.9). This model assumes that there is no reserve N. The weighted mean stem N requirement per unit stem green area was 1.63 g m^{-2}, for the canopy measured by Everett (1995).

Whilst there is an increase in structural N with canopy depth (Figure 2.8) the decrease in N associated with photosynthesis is much greater (Figure 2.5). The amount of N in structural support compounds appears to be less than expected and so the predicted total N in the stem decreases with canopy depth due to the response of photosynthetic compounds to the light gradient.
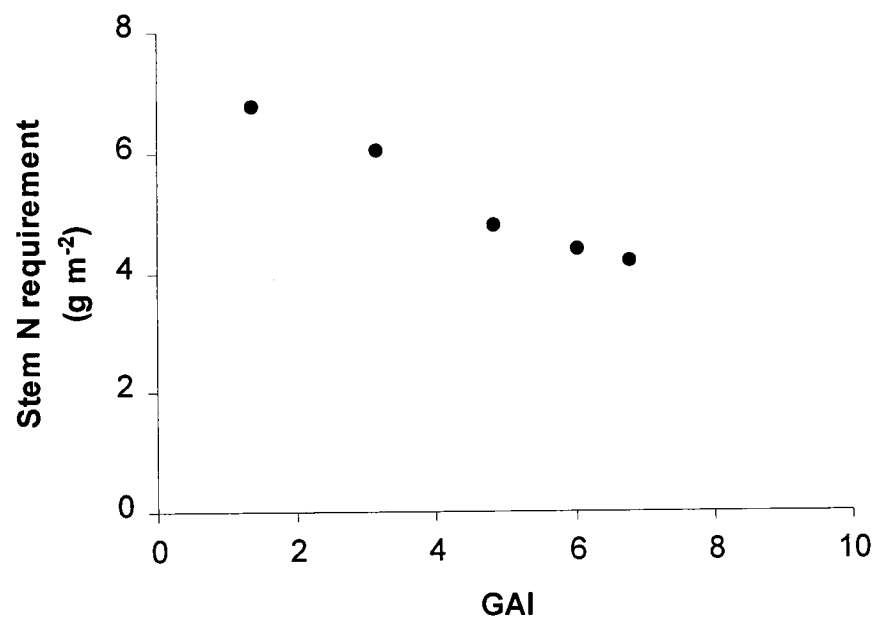
Figure 2.9 The predicted total stem N requirement per unit stem area (in this study) with accumulated green area index (GAI) through canopy depth. Data points represent each depth.

2.2.2.3.1   General assumptions

The general assumptions used to predict the total stem N requirement are:

- There is no reserve N in the stem.
- There is no transport N.

2.2.3 Total N requirement and depth

The total N per unit total green area (N_T), for the whole canopy is predicted from the leaf N requirement per unit leaf area (N_L), the stem N requirement per unit stem area (N_S) and the ratio of both stem and leaf area to the total green area (A_L / A_{LS} and A_S / A_{LS}, respectively):

$$N_T = (N_L (A_L / A_{LS})) + (N_S (A_S / A_{LS}))$$

Equation 2.9

The ratios of leaf and stem green area to total green area are taken from Everett, (1995) (Table 2.1). This canopy had a GAI of 6.8, which is a conventional canopy size. The predictions are based on both the cylindrical and projected stem green area, separately (using π as the correction factor). Both sets of variables are presented in Table 2.3.

Figure 2.10 presents the predicted total N requirement with increased GAI through canopy depth, using a projected stem area. At the top of the canopy where there is much light, this is mainly photosynthetic leaf N. The stem is not present until the third layer (GAI of 0.5). Below this there is a predicted requirement for structural N and some photosynthetic N in the stem which maintains the total N requirement at above 2.00 g m^{-2} despite less leaf N in response to reduced PPFD. A decrease in total N requirement is predicted below a GAI of 3 in response to the decrease in light. Stability is maintained between a GAI of 5 and 6, when the increase in structural N is balanced by the decrease in photosynthetic N per unit green area. At the bottom of the canopy there is an increase in total N requirement, similar to the N requirement at the top (2.20 g m^{-2}). This is mainly structural N in the true stem and a reduced total green area. The mean total N requirement is predicted to be 2.26 g m^{-2} (Table 2.3).

Figure 2.11 illustrates the predicted total N requirement with increased GAI through canopy depth, using a cylindrical stem area. The difference between this and N requirement based on projected stem area is the expression of N content on a greater stem area. The contribution of the leaf N is greater than the stem N to the total N requirement. There is a general decrease in total N when predictions are based on the cylindrical area, except below an accumulated GAI of 5. It is suggested here that the

main reason for this, is the decreasing light within the canopy and the response in decreasing photosynthetic N both in the lamina and in the leaf sheath. Most of the light is extinguished above the bottom layer of the canopy, and so structural N is predicted to stabilise the N requirement to around $1.00 \, \text{g m}^{-2}$. The mean total N requirement is predicted to be $1.56 \, \text{g m}^{-2}$ (Table 2.3).
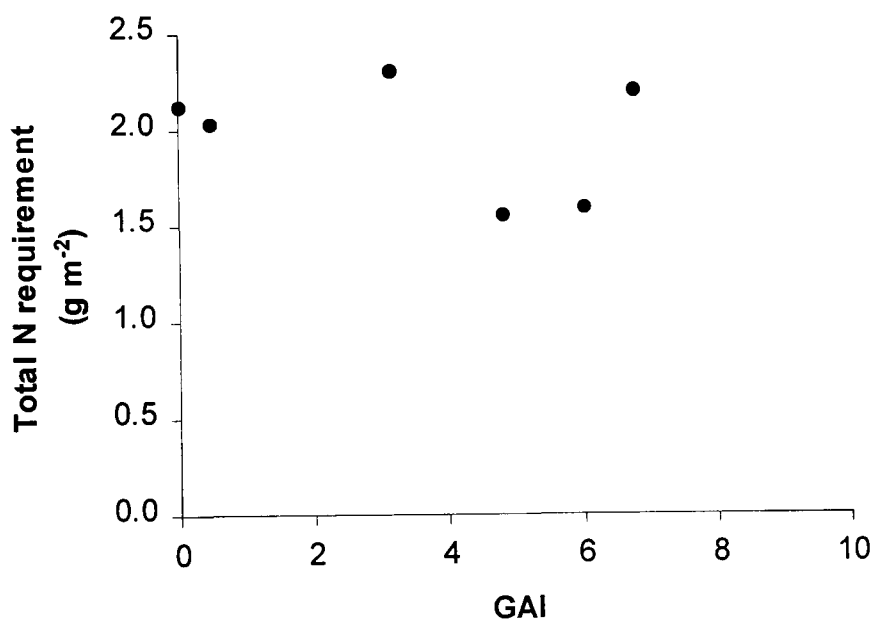
Figure 2.10 The predicted total N requirement per unit total green area (projected stem area) (in this study) with accumulated green area index (GAI) through canopy depth.
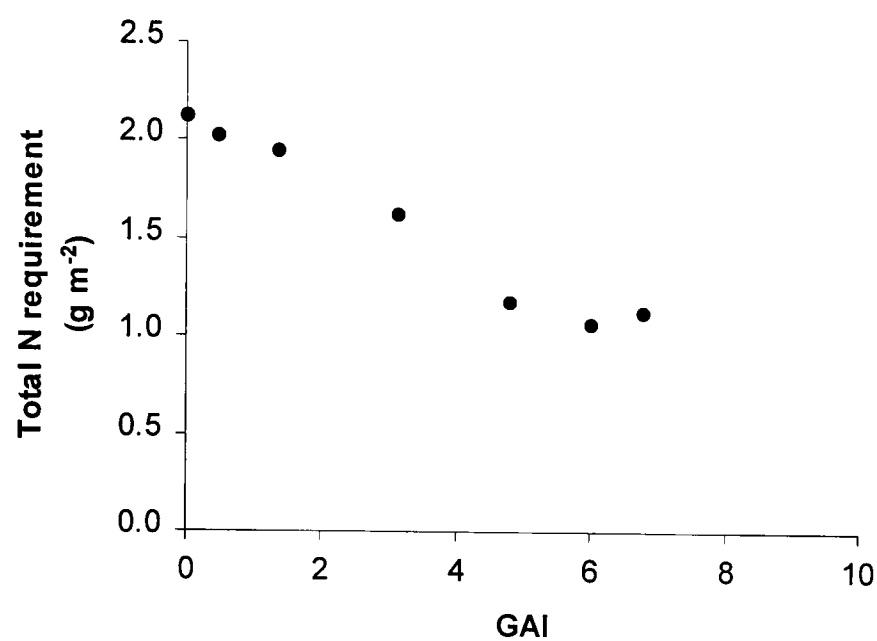
Figure 2.11 The predicted total N requirement per unit total green area (cylindrical stem area) (in this study) with accumulated green area index (GAI) through canopy depth.

Table 2.3 Predicted N requirement (in this study) for the whole canopy with a projected green area index (GAI) of 6.8.

VARIABLE	UNIT OF MEASUREMENT	Projected Stem area	Cylindrical Stem area
A_L	Leaf GAI	5.41	5.41
A_S	Stem GAI	1.39	4.35
N_L	g leaf N m^{-2} green leaf area	1.22	1.22
N_S	g stem N m^{-2} green stem area	5.12	1.63
A_L / A_{LS}	m^{-2} green leaf area m^{-2} total green area	0.80	0.55
A_S / A_{LS}	m^{-2} green stem area m^{-2} total green area	0.20	0.45
N_T	g N m^{-2} total green area	2.26	1.56

2.3 HUSBANDRY

Based on the general principles outlined above, qualitative differences in canopy nitrogen requirement (CNR) between husbandry treatments (variety and seed rate), were predicted. These are summarised at the end of this chapter in Table 2.4.

2.3.1   The principles behind the predictions

Leaf N is assumed to relate to the average light flux within the crop. The leaf number per main stem (culm) and leaf size are used to predict leaf area per shoot. Leaf area per shoot and shoot density are used to predict the GAI which is then combined with the light extinction coefficient (k) to predict average light flux within the canopy. It is assumed that a higher shoot density would have a greater effect on GAI than a higher leaf area per shoot, and that a larger GAI would have a greater effect on light flux than a higher k. Final leaf N per unit GAI is based on the average light flux as leaf N content per unit of light is expected to remain constant.

Nitrogen in the stem is assumed to relate to the requirement for support and photosynthetic apparatus. The prediction of difference in stem N per unit GAI between treatments is based on two main parameters of equal weighting. These are, the ratio of stem length to leaf area per shoot and stem N content per unit stem length. These are predicted by considering the architecture of individual shoots. Leaf size and leaf number per culm are used to predict the shoot leaf area. The height of the stem and the leaf area per shoot are then used to predict the ratio of stem length to leaf area per shoot. Stem dry weight per unit stem length or thickness and photosynthetic N per unit stem length in the stem (assumed similar to leaf N) are used to predict stem N content per unit stem length. Leaf area is assumed to contribute to a large proportion of the total green area, and therefore stem N per unit leaf area is assumed to be similar to stem N per unit GAI.

CNR is predicted from the combination of leaf N and stem N per unit GAI. It is assumed that stem N and leaf N contribute equally to CNR. It is also assumed that

there is no dead material, which would increase the CNR through adding structural N but no green area.

2.3.1.1 General assumptions

The assumptions for the qualitative predictions are therefore:

- Shoot density has more weight than leaf area per shoot in considering the effect on GAI.

- GAI has more weight than k when considering the effect on average light flux within the canopy.

- Leaf N content per unit of light is consistent between treatments.

- N in the stem is related to the requirement for support, and is determined by the equal weighting of the ratio of stem length to leaf area per shoot, and stem N per unit length.

- Predicted ratio of stem length to leaf area per shoot assumes equal weighting of height and leaf area.

- Predicted leaf area assumes equal weighing of leaf number and leaf size

- Predicted stem N per unit stem length assumes equal weighting of stem dry weight per unit length and stem photosynthetic N.

- Green leaf area can be used to estimate total stem area.

- Leaf N and stem N have equal weighting in CNR.

- There is no non-green tissue.

- There is no transport N

- The % structural N in stem dry weight is the same for all treatments.

2.3.2 Variety

The following is summarised in Table 2.4. The two varieties compared are the bread wheat Soissons and Spark. Soissons is an early developing, short stemmed variety compared to Spark. Soissons generally has larger leaves and so it is predicted that this variety has the greater leaf area per shoot. Soissons is photoperiod-insensitive (Foulkes et al., 1998) and so the time to reach GS 31 is shortened, resulting in a reduction in tiller production. Spark therefore has a higher shoot number than Soissons (Foulkes et al., 1998). The greater shoot number in Spark is expected to

overcompensate for the larger leaf area per shoot in Soissons. The GAI is therefore predicted to be greater in Spark. Soissons is a variety that generally has a high k due to its more prostrate leaves (Foulkes *et al.*, 1998), but the difference in green area is expected to have a greater influence on light interception than the difference in light extinction coefficient. Soissons is predicted to intercept less light, thus allowing more light to penetrate to the lower layers. Therefore, it is predicted that Soissons will experience greater light flux within the canopy although the difference will be small. The leaf N content per unit GAI is therefore expected to be slightly greater in Soissons due to the smaller shoot number allowing better illumination of the leaves throughout the canopy.

Soissons is predicted to have a greater green leaf area per shoot and Spark to have a taller stem. Stem length per unit leaf area is therefore predicted to be greater in Spark. Soissons is expected to have a greater stem dry weight per unit stem length due to the thicker stem and also to have a greater amount of photosynthetic N per unit stem length similar to the leaf. The amount of N per unit stem length is therefore predicted to be greater in Soissons. The greater amount of N per unit length in Soissons is not expected to compensate for the greater stem length per unit GAI in Spark and so the stem N per unit GAI is predicted to be slightly greater in Spark.

The combination of the greater leaf N content in Soissons and the greater stem N content in Spark, leads to the prediction that there will be no difference in total N requirement between varieties.

2.3.3   Seed rate

The following is also summarised in Table 2.4. The predicted effect of seed rate on CNR is based on the comparison of a very low seed rate and a very high seed rate (20 and 640 seeds m^{-2}, respectively). The wider leaves at the low seed rate (Kirby & Faris, 1970) are expected to over compensate for the longer leaves at the high seed rate (Kasperbauer & Karlen, 1986), such that leaf size is greater in the low seed rate. The leaf number per culm is also expected to be greater in the low seed rate (Kirby & Faris, 1970). Leaf area per shoot is therefore predicted to be greater in the low seed rate but this is not expected to compensate for the lower shoot number. The GAI of

the high seed rate is therefore predicted to be greater. Less dense canopies are expected to have heavier more 'drooped' leaves (Trenbath & Angus, 1975), greater space and less mutual support. Despite the greater k value expected with low seed rate, the greater GAI of the high seed rate is predicted to allow less light to penetrate through each layer of the canopy. The leaf N content per unit GAI is therefore predicted to be greater in the low seed rate due to the smaller shoot number allowing greater light penetration.

Stem extension is greater in a more dense crop (Puckridge & Donald, 1967) in response to decreasing light quality (Holmes and Smith, 1977) and so the high seed rate crop is expected to have a taller stem. The leaf area per shoot was previously predicted to be greater at the low seed rate and so the ratio of stem length to leaf area is predicted to be greater in the high seed rate. The taller high seed rate crop is expected to have a thinner stem (Percival, 1921) and therefore lower stem dry weight per unit stem length. A lower amount of photosynthetic N per unit stem length in the stem is also expected at the high seed rate, in response to the reduced light flux within the canopy. Stem N per unit stem length, is therefore predicted to be greater in the low seed rate crop. The increase in stem N per unit length with reduced seed rate is expected to be greater than the decrease in stem length per unit leaf area. It is therefore predicted that stem N per unit GAI will be greater at the low seed rate.

Both leaf and stem N content are predicted to increase with the decrease in seed rate, and so the CNR is subsequently predicted to increase with reduced seed rate.

2.4   CROP DEVELOPMENT

The change in CNR with time is predicted by comparing a wheat crop before stem extension (GS30) with a crop at flowering (GS61). The method of prediction and principles are the same as in the previous section.

Leaf number per shoot and leaf size are expected to be greater at GS61 and so green leaf area per shoot is predicted to be greater at GS61. The number of shoots is expected to decrease between GS30 and GS61 (Foulkes et al., 1998) due to tiller

death but the GAI is predicted to be greater at GS61 because of the greater leaf area per shoot. There will also be the presence of the stem at GS61. The larger leaves at GS61 are also expected to be more prostrate (Trenbath & Angus, 1975; Everett, 1995) and so increase the light extinction coefficient (higher k value), due to their weight. The incident radiation increases from 9.5 to 20 MJ m^{-2} s^{-1} between March and June (Sylvester-Bradley et al., 1997b), but the average light flux within the canopy is predicted to be greater at GS30 because of less mutual shading by the smaller canopy. Leaf N content per unit GAI is therefore predicted to be greater at GS30, due to less mutual shading allowing more light within the canopy.

At GS30, the stem has not yet extended, and so no stem N will be required. The stem N content per unit GAI is predicted to be greater at this growth stage, due to the actual presence of the stem.

The leaf N content is expected to decrease, whilst the stem N content is expected to increase with development. The CNR is therefore predicted to remain the same between GS30 and GS61 (Table 2.4).

Table 2.4 Qualitative predictions (in this study) of the effect of variety, seed rate and crop development on canopy characteristics including green area index (GAI), light extinction coefficient (k) and their predicted effect on canopy N requirement (CNR). Indentations indicate parameter hierarchy for predictions.

CANOPY CHARACTER	VARIETY	SEED RATE	CROP DEVELOPMENT
	Soissons / Spark	20 / 640 seeds m^{-2}	GS30 / GS61
Leaf size	So > Sp	20 > 640	61 > 30
Leaf number / culm	So = Sp	20 > 640	61 > 30
Leaf area / shoot	So > Sp	20 > 640	61 > 30
Shoot density	Sp > So	640 > 20	30 > 61
GAI	Sp > So	640 > 20	61 > 30
k	So > Sp	20 > 640	61 > 30
Light flux	So > Sp	20 > 640	30 > 61
LEAF N / GAI	So > Sp	20 > 640	30 > 61
Stem height	Sp > So	640 > 20	61 > 30
Stem length / leaf area	Sp > So	640 > 20	61 > 30
Stem dry weight / length	So > Sp	20 > 640	N/A
Photosynthetic N / length	So > Sp	20 > 640	N/A
Stem N content / stem length	So > Sp	20 > 640	N/A
STEM N / GAI	Sp > So	20 > 640	61 > 30
CNR	So = Sp	20 > 640	30 = 61

3 MATERIALS AND METHODS

An experiment to investigate the distribution of N within the wheat canopy was carried out, during the 1997/8 and 1998/9 winter wheat growing seasons at University of Nottingham farm, Sutton Bonington (National Grid ref. SK 267 502). The site is 52° 50' N, 1° 15'W and at an altitude of 50 m.

3.1 SITE DESCRIPTIONS

The 1997/8 trial took place in field 2. The soil N content of the field was measured at 108 kg ha^{-1} N in a 90cm soil profile, on 17 February 1998. The crop at this time had a GAI of approximately 0.2 and was assumed to contain 6 kg ha^{-1} N (based on 30 kg ha^{-1} N). The plots used were part of the Home-Grown Cereals Authority (HGCA) Seed Rate Project.

The 1998/9 trial was carried out in field 9. The soil N content measured in February was 49 kg ha^{-1} N in a 90cm soil profile. Again, 6 kg ha^{-1} N, was assumed to be in the crop.

Details of the sites are given in Table 3.1

Table 3.1 Details of sites used for trials (National Grid ref. SK 267 502, 52° 50' N, 1° 15'W and altitude of 50 m.

		1997/8	1998/9
Site		Field 2	Field 9
Sowing Date		3 October 1997	12 October 1998
Soil Series		Dunnington Heath	Dunnington Heath
Soil texture		Medium stoney loam to 80cm over clay (Keuper Marl)	Medium stoney loam to 80cm over clay (Keuper Marl)
pH		6.9	6.3
P. K. Mg (ADAS index)		5, 3, 4	4, 3, 4
Organic matter		4.0	not measured
Soil mineral nitrogen in February (GAI = 0.2) (kgNha^{-1})			
0-30 cm		51	20
30-60 cm		30	17
60-90 cm		27	12
Previous cropping	1996/7	Oats	Oats
	1995/6	Sugar beet	Sugar beet

3.2 EXPERIMENTAL TREATMENTS AND DESIGN

Cultivar and husbandry factors were chosen to practically test the hypothesis that variation in CNR may be explained by variation in canopy architecture. Soissons generally has a smaller canopy size, shorter stem, larger and flatter leaves, and a lower shoot density than Spark (Foukes *et al.*, 1998). The conventional farm seed rate of 320 seeds m^{-2} was chosen to represent a conventional crop. Extreme seed rates of 20 and 640 seeds m^{-2} were also selected, to create a significant contrast in canopy architecture. Canopies sown at low seed rates generally have a smaller canopy size, shorter stems, larger and flatter leaves, and a lower shoot density than at higher seed rates. An additional low N treatment was chosen in the second season's experiment to

create a canopy with no luxury uptake of N, as limiting N would restrict canopy growth. This would provide a better comparison to the theoretical CNR.

In 1997/8, the experiment was a randomised factorial block design, with three blocks, two varieties and three seed rates. The same design was held for the following year with the addition of split plot design for the two nitrogen applications superimposed. The split plots were not randomised however, for practical reasons concerning machinery involved in fertiliser application and combining. The 1997/8 trial plots were 1.61 m by 26.41 m, with a row width of 13.2 cm. Destructive samples for this project were taken from the eastern 8 m within each plot, allowing space for other work and for combine yield. The 1998/9 trial was designed to allow plenty of space for more treatments. The actual plot size was 4.83 m wide by 24 m. Each plot consisted of three strips, each of which were Ojard drill widths of 1.61 m. Both trials were drilled with an Ojard drill. The experimental plans are in Appendix I. The wheat varieties Soissons and Spark together with the 20, 320 and 640 seeds m^{-2} seed rates were chosen for their contrasting growth characteristics and CNRs. The variety characteristics are shown in Table 3.2. In 1998/9, the soil mineral nitrogen content was used to calculate the fertiliser rate required to supply the crop with the same amount of N as in the previous experiment. This was applied to the northern half of the plots. The fertiliser applications were reduced by 50% for the southern half of each plot to create the 'low N' treatment. Details of fertiliser applications are provided in Table 3.3 and Table 3.4

Table 3.2 Cultivar characteristics from NIAB (1998). High value shows character to a high degree. (* Represents information taken from Foulkes *et al.,* 1998).

	Soissons	Spark
Parentage	Jena x HN 35	Moulin x Tonic
Breeder	Desprez, France	Nickerson Seeds, Lincs.
Recommendations for use	Fully Recommended for Special Use within the Central, South-east and South-west regions	Fully Recommended for General Use
Year Released*	1988	1990
Shortness of Straw	7	5
Earliness of Ripening	9	7
Grain Bread-Making Quality	7	6
Rht*	Rht1	No
Photoperiod sensitivity*	Insensitive	Sensitive
CNR (g N m^{-2})*	2.72	3.35

Table 3.3 Fertiliser applications for 1997/8 trial

Fertiliser Applications	Rate (kg ha^{-1})	Date
Nitrogen	40	9/3/98
Nitrogen	140	8/4/98

Table 3.4 Fertiliser treatment applications for 1998/9 trial

Fertiliser Applications	Rate (kg ha^{-1})	Date
Nitrogen (all plots)	44	16/3/99
Nitrogen (low N plots)	80	22/4/99
Nitrogen (high N plots)	100	22/4/99
Nitrogen (high N plots)	100	19/5/99

3.3 CROP HUSBANDRY

The split nitrogen fertiliser application ('Nitram', 34.5%) in 1997/8 of 180 kg ha^{-1} N in total in accordance with MAFF fertiliser recommendation for a Soil Index of 0 (MAFF, 1994). Within each season all plots had identical applications of fungicide, herbicide and plant growth regulators (PGRs), as appropriate for that year (see appendix II).

3.4 METEOROLOGICAL DATA

The daily global incoming solar radiation, sunhours, rainfall and temperature data was obtained from the Sutton Bonington weather station, from August 1997 to June 1999.

3.5 COLLECTION OF CROP SAMPLES FOR ASSESSMENT

Destructive samples were taken throughout the growth of the crop, from November until senescence and then a final pre-harvest sample. The dates are shown in Table 3.5.

Table 3.5 Dates of sampling.

Sample Date Number	1997/8	Growth Stage	Days after Sowing	1998/9	Growth Stage	Days after Sowing
1	17 November	Tillering	45	30 November	Tillering	49
2	15 December	Tillering	73	14 December	Tillering	63
3	12 January	Tillering	101	11 January	Tillering	91
4	9 February	Tillering	129	15 February	Tillering	126
5	17 March	Stem extension	165	29 March	Tillering	168
6	28 April	Flag leaf emerged	207	5 June (Soissons)	Flowering	236
7	26 May	Ear emerged	235	18 June (Spark)	Flowering	249
8	15 June	Flowering	255			
9	29 June	Flowering	269			
10	13 July	Grain fill	283			
Pre-harvest	12 August		313	6 August		298
Harvest	20 August		321	18 August		310

A 0.25 m^2 quadrat was placed within the plot at least 2 rows away from the edge of the plot. The second sample in 1997/8 (15 December) was 0.5 m^2 for the lowest seed rate due to small sample size. Samples in 1997/8 were taken in random positions within the allocated plot length, with at least 30 cm between sampling positions or plot edge. In 1998/9, samples were taken again from the southern end but one sample taken sequentially from each strip within the plot before returning to the first. Crop re-growth made it difficult to assess which area of ground had been previously sampled from and so after the sample had been taken, markers were used to identify the sampled area.

In the first four and five samples taken in 1997/8 and 1998/9 respectively, the plants were extracted from the ground with the roots attached. In later samples stratified clips were performed, except sample 8 of 1997/8. Due to persistent rain and low light levels, the shoots were cut at the base and collected intact.

3.5.1 Intact plant sampling

Plants were dug up and placed into polythene bags containing labels, and tied. Within a few hours, they were placed in the cold room at 4 °C to prevent deterioration of the lamina surface area. In the lab, they were washed, dried and the roots were cut off leaving just the green laminae and stem if present.

3.5.2 Stratified clip sampling

Once stem elongation had begun (GS31), stratified clips were taken in the field, every 10 cm down the crop, across a 0.25 m^2 area. By carrying this out in the field, as opposed to bringing back to the laboratory, the canopy structure was maintained.

A frame, constructed from steel, was used for the first two stratified canopy harvest dates (17 March and 28 April 1998). The height of the crop was not always uniform because of a few taller main shoots. The upper layer was therefore often greater than 10 cm as 0 cm was set to start at the top of the majority of the shoots. The frame was made from two 0.25 m^2 quadrats held above one another and attached with adjustable clamps to a vertical metal rod at each corner. At 10 cm intervals down the length of each rod, were alternating blue and red coloured tapes. Once the frame was placed in the correct position within the plot, the upper quadrat was set level with the top of the canopy and to the nearest coloured marker tape. The lower quadrat was set 10 cm below, to the same colour marker. This upper layer (0 – 10 cm) was removed by cutting across at this lower quadrat level with scissors and using two canes to guide the height when cutting into the centre. All the material from this layer was placed into a polythene bag with a suitable label, and tied. Lowering the bottom quadrat to the next appropriate marker enabled the next layer to be cut and collected. This was repeated down the whole canopy to the ground.

Several difficulties arose during the sampling. The frame became unstable at times and was difficult to adjust quickly in the field. As a result of this, a new frame was designed and constructed to be lighter, more sturdy and easier to use. This frame was made from light aluminium, with the four corner vertical poles held in position with a quadrat at the top. A second quadrat was adjusted to the top of the canopy, similar to the design of the original frame. However instead of lowering this quadrat to the bottom of each layer, metal rods were fed through holes drilled at 10 cm intervals in each of the legs of the frame. These legs were adjustable so that they could be aligned with the top of the canopy, such that the bottom layer was no less than 5 cm or no more than 15 cm.

Although the frame allowed stratified clips to be made in the field and therefore with minimum disturbance of the natural canopy structure, there were still a few problems that could not be avoided. On some occasions when the frame was placed over the crop, a few shoots and leaves at the surrounding edge were slightly displaced. Care was taken to place the frame between the rows more accurately. When the leaf material was cut in layers, occasionally some leaves would drop to the ground through the canopy, and these were retrieved as efficiently as possible. This was especially the case where leaves projected up into higher layers and then drooped into lower layers. It was difficult to cut the leaf in two positions along the length and catch the leaf tip as it fell.

a)   b)

Figure 3.1 Photographs of a) aluminium frame in place within the crop and b) stratified clips taken by cutting across frame level with rods positioned in holes of frame leg.

3.5.3   Pre-harvest samples

Grab samples were taken from each plot before harvest. A handful of shoots (with roots attached) were pulled from the ground, from five systematically chosen places, in each plot. Approximately 100 shoots per plot were collected. These were taken from the second of the three strips making up each plot. Most of the soil was carefully shaken off the roots, taking care not to shake the grain. The samples were then placed, grain first to prevent loss of material, into labelled large paper bags. These were then taken back to the labs and stored for later analysis.

3.6   CROP GROWTH ASSESSMENTS

3.6.1   Plant and shoot density

In the first sample of the 1997/8 and first two of the 1998/9 trial, the number of plants within the quadrat was counted in the field and the plant density (plants m^{-2}) calculated from this. On sample dates 6, 7, 8, 9 and 10 from 1997/8, and sample date 6 (of each cultivar) from 1998/9, the shoot density was measured. This was calculated from the number of shoots in the lower layer of each plot. Fertile, dead and dying shoots were not distinguishable, as the growing shoot tip was not attached due to being stratified.

3.6.2   Green area

It was necessary take a sub-sample (ss) of between 15 and 75% for large samples. This was done by randomly taking a sufficient amount of the whole sample, to perform growth analysis on. The remaining sample (rem) was placed into a labelled paper bag and dried alongside the subsample (after partitioning and green area determination), to work out the sub-sample fraction (SS) (%) on a dry weight (DW) basis. There were no sub-samples taken in smaller samples.

$$(SS) = (DW\ ss\ /\ (DW\ ss + DW\ rem)) * 100 \qquad \textbf{Equation 3.1}$$

The sample was partitioned into appropriate components according to development of the crop. There were six possible components: green lamina, green stem, green ear, non-green lamina, non-green stem, green ear and non-green ear. The projected area of the separated green components was determined in cm^2 (GA), using a moving belt leaf area meter (Li-Cor Model 3100, Lincoln, Nebraska, USA). This was calibrated frequently using a circular metal disc of known area and the belt was kept as clean as possible to reduce errors.

Green area index (GAI) was calculated from green area in cm^2, sub-sample size as a fraction of the whole sample and quadrat size in m^2 (Q).

$$GAI = (GA / 10000) * (1/SS) * (1/Q) \qquad \textbf{Equation 3.2}$$

3.6.3 Dry weight

All plant material was dried in a force-ventilated oven at 80°C for 48 h to ensure drying to a constant weight. The components were then weighed (in g) on a two-point balance as soon as they were cool. The dry weight of each component was then calculated per m^2 ground area:

$$DW \ (gm^{-2}) = DWss * (1/SS) * (1/Q) \qquad \textbf{Equation 3.3}$$

3.6.4 Yield components

The fresh weight of the total sample, the ears and the threshed grain were determined from the grab sample. Chaff dry weight was calculated from the grain and total ear fresh weights. The straw and grain were then dried for 48 h at 85 °C and their dry weight determined. The grain %N was determined by N analysis.

Ear number from the 1997/8 experiment was provided by J. Whaley (pers. com.). Ear number in the 1998/9 experiment was determined by counting total number of ears within the $0.25m^2$ quadrat, in each plot.

3.6.5 True ear green area

To determine the true green area of the ears, as opposed to the projected area of one side, the ears were dissected. Five shoots were taken randomly from plot 16 in which Spark was sown at 320 seeds m^{-2} and fertilised with 244 kg ha^{-1} N. The ears from these shoots were removed from the stem and the projected area of the face and the side of each was measured. The number of spikelets was counted before each was carefully cut away from the rachis, using a knife. The area of the face and side of the rachis was then measured. Alternate spikelets along the length of one side of the ear were chosen for dissection, which totalled either five or six from each ear. It was thought that these would give a good representation of all the spikelets. The face and side of the intact spikelet was measured before the glumes and florets were separated.

The area of each component was measured, again on both the face and the side. The surface area of each spikelet was assumed to be twice the face area plus twice the side area. The convex surface area of each the glume and floret was assumed to be twice the side plus the face. It was necessary to use double-sided sticking tape to hold the face to be measured in place on the leaf area machine. The machine did not detect this. This was repeated with another four ears.

3.6.6 Leaf sheath experiment

Detailed work was carried out on shoots to measure the N content of the leaf sheath and stem separately. Ten intact shoots were taken randomly from the northern end (fertilised with 244 kg ha^{-1} N) of plots 16 and 18, in which Spark was sown at 320 and 20 seeds m^{-2} respectively.

The leaves were cut away from the stems and the total green area of leaf material from the ten shoots was measured. The leaves were then placed into a labelled paper bag.

The height of each shoot (with leaves removed), up to the base of the ear, was measured with a metre rule and the ears were cut off. The projected area of the stem was then measured. The leaf sheath was scored just above each node with a knife and then removed by unwrapping from around the stem. The area of the remaining stem was measured once more, before being placed into a labelled paper bag. As the leaf sheath was curled completely it was impossible to flatten for the leaf area machine. Each section of leaf sheath was taped to a sheet of paper and photocopied. The photocopied shapes were then cut out and passed through the leaf area machine. The original leaf sheaths were carefully removed from the paper and placed in a labelled paper bag. All stem, leaf sheath and lamina samples were then dried in an oven for 48 h (80°C) until constant weight, when the dry weight was then measured and recorded. The N content of the leaf sheath and stem from each shoot and the total leaf N content for all ten shoots was determined.

3.7 NITROGEN ANALYSIS

In preparation for the nitrogen content to be determined, each sample was re-dried to remove any re-absorbed moisture. They were then ground separately into labelled small self-sealing polythene bags, to prevent any moisture re-absorption. The grinding mill was cleaned thoroughly with a small brush, between samples, to prevent any cross contamination. The first step was to determine N content as a percentage of dry weight (%N), for each component.

3.7.1 Near infrared reflection (NIR) spectroscopy

As there were many samples to analyse for percentage N, the cheapest and easiest option was to collect near infrared reflectance spectra. This was done by using a NIRSystems (Silver Spring, MD) model 6500 scanning monochromator over the wavelength range from 400 to 2500 nm at 2 nm intervals, to give 1050 data points for each sample spectrum. The software used to examine the reflectance spectra and produce calibrations, was ISI NIRS2 version 3.11 software (Infrasoft International, Port Matilda, PA). This has proved to be a suitable method of N determination in wheat (Borjesson et al., 1999). It is a six-step process to determine the %N of the scanned samples:

1. Scan

The background spectrum was scanned before each sample. Each sample was then allocated an identification code and scanned, showing the logarithmic reflectance at both the visible and near-infra-red spectrum (400-750 nm and 750-2498 nm, respectively). The spectrums for samples of the same plant component were stored in the same file. Each file was then dealt with separately.

2. Centre

The Eigen vector produced the spectrum as a straight line for each sample. This produces a theoretical average vector. The vectors were then ranked around this average vector.

3. Select

Samples were then chosen by the software to analyse for %N content and then to compare with the vectors in the calibration.

4. Manage

The samples selected for the N analysis were printed out, the analysis carried out, and the %N values entered into the appropriate file. These values were then combined with the Eigen vector information.

5. Calibration

A calibration curve was produced for each plant component from the selected samples analysed and the Eigen vector. This produced an R^2 value, standard deviation, standard error of calibration and F value.

6. Predict

All of the information in the calibration file was applied to the selected data groups. The %N of the unanalysed samples was then predicted.

3.7.2 N analyser

In the cases where the samples were too small to be scanned, or to enable the calibration of the NIR, %N was determined directly using the N Analyser NA 2000. Each dried and ground sub-sample, of 70 – 100 mg, was loaded into a small tin capsule. Each sample was combusted at 1800 ^{0}C in pure oxygen producing a mixture of gases and water. The products were then passed over a copper catalyst at 750 ^{0}C, converting carbon oxides to carbon dioxide and N oxide to N. Sulphur oxides were absorbed onto the copper. Carbon dioxide and water were removed by passing over a series of other catalysts. The amount of N in the sample was determined by the change in thermal conductivity when compared to a reference cell. This information was processed to present the N content as % of sample dry weight.

3.8 LIGHT INTERCEPTION

Photosynthetically active radiation (PAR) (0.4 – 0.7 μm) was measured with depth down the canopy. Light interception data and the incident solar radiation obtained from the weather station were used to determine the actual light environment within the crop.

3.8.1 PAR fractional interception

PAR was measured as photosynthetic photon flux density (PPFD) (μmol m^{-2} s^{-1}), using a sunfleck ceptometer (Delta-T Devices, Burwell, Cambridge, UK). Light measurements were taken before the crop was destructively sampled, and in the same position as the quadrat. Light interception measurements were restricted to just those by the canopy area actually being sampled. This was necessary to reduce errors in calculating the light extinction coefficient (k), and was achieved by covering half of the probe with black plastic. Preliminary experimentation showed this material to be effective at blocking out all of the PAR. There was also no differentiation between the two ends of the probe as on repeated occasions the PAR measured by the whole probe was exactly twice that of when half the probe was covered.

In the case where light interception of the whole crop was being measured, the ceptometer was placed above the canopy to measure incident light (I_o) and then below the canopy to measure transmitted light (I). This was repeated five times at random positions within each plot. The fractional interception (f) by the whole canopy was calculated:

$$f = 1 - (I / I_o) \qquad \text{\textbf{Equation 3.4}}$$

Fractional interception for each layer was estimated from light measurements made with the frame in place and before any stratified clips. Incident light was measured above the canopy but within the frame, to remove any effects of shading. Transmitted light was measured below each successive 10 cm layer using the metal rods to rest the ceptometer probe on.

The light extinction coefficient (k) was calculated from fractional interception (f) and accumulated green area index (L) for each 10 cm layer down the canopy, on the main sampling date at around anthesis for each experimental season (Equation 3.5). The average k was also calculated from this equation with total canopy fractional interception and total canopy size. Equation 3.5 is derived from Equation 1.2 in Chapter 1. The average k of the whole canopy was also estimated through the slope of the regression that described the relationship between the natural log (ln) of PPFD and GAI.

$$k = (-\ln(1-f))/L \qquad\qquad \textbf{Equation 3.5}$$

3.8.2 Light flux per unit ground area

The mean PPFD within each layer (I_M) was from the estimated transmitted PPFD above the layer (I_A) and below the layer (I_B). In the first layer $I_A = I_o$. The mean incident radiation of the two weeks prior to the date the light measurements was used to eliminate any instantaneous variation in incident radiation measured on the day of sampling. Fractional interception of the layer was used to estimate I_B from I_A.

$$I_M = (I_A + I_B)/2 \qquad\qquad \textbf{Equation 3.6}$$

$$I_B = (1-f) \times I_A \qquad\qquad \textbf{Equation 3.7}$$

The mean PPFD within the whole canopy was estimated from the sum of the PPFD for each layer, weighted by the proportion of canopy GAI in that layer.

3.9 FINAL HARVEST – YIELD DETERMINATION

Combining took place on 20 August 1998 and on 18 August 1999 for the two experiments, using a Wintersteiger trial plot combine. Combined areas (A) were accurately measured and varied between 13 and 16 m^2. Samples of grain were collected and the fresh weight (FW) recorded. The fresh weight (FWss) and dry weight (DWss) of a sub-sample was determined for each plot to calculated the %

moisture content (%MC) using Equation 3.6. This was then used to calculate the combine yield adjusted to 15% moisture content, using Equation 3.7.

$$\% \text{ MC } = 100 * (DW/FW)$$ **Equation 3.7**

$$\text{Combine yield } = FW / A \times 10 \times (100 - \%MC) / 85$$ **Equation 3.8**

3.10 STATISTICAL ANALYSIS

Statistical analysis of all data was carried out using the Genstat 5 program (version 4.1; Lawes Agricultural Trust Rothamsted Experimental Station). Both analysis of variance and regression were used to analyse data. A probability value of 0.05 or less ($P \leq 0.05$) was taken to be significant.

3.10.1 Analysis of variance

Where the effect of the treatments on the whole canopy was analysed, a separate analysis of variance was carried out for each sample date. The final two samples taken in the 1998/9 experiment were combined, even though they were taken on different dates, as the samples were taken according to growth stage. A two-way ANOVA in randomised blocks was used to compare treatments, except in the final samples taken at anthesis in 1999. A general analysis of variance was able to incorporate the split-plot superimposed onto the randomised block design in 1998/9. Data that did not meet the assumption of random residuals were Log_{10} transformed, and are indicated in the respective results chapter. Standard error of the difference between treatment means (S.E.D.) and the degrees of freedom (d.f) are presented. The degrees of freedom for comparing means in the 1998/9 experiment were not whole numbers, due the design of the experiment. The error between subplots on different main-plot treatments was made up of two errors, which also had degrees of freedom. The way in which these combined depended on the relative size of the two errors.

3.10.2 Linear Regression

To analyse the treatment effect on a variable with increased canopy size through depth, linear regression was carried out. Where a linear model satisfactorily fitted the raw data, linear regression with groups was carried out. Each group was a separate treatment combination. This provided an equation of a model for each group, but was not able to determine any treatment effects. Treatment effects were analysed through the use of a 'Generalised Linear Model'.

3.10.3 Non-linear regression

Where the data fitted an exponential curve, the equations for the fitted models for each group were determined through non-linear regression. In some cases, a model could not be found that fitted the data satisfactorily. The data were then transformed through the application of an appropriate function, so that a linear model could then be fitted and statistical analysis could be performed.

4   GENERAL CROP GROWTH

The first chapter of results describes the overall growth of the crops, which were studied in both seasons. It begins by providing an account of the weather conditions of the two years. Crop establishment, growth and yield components are then analysed from each year with a comparison of the seasons and treatments.

4.1   METEOROLOGICAL DATA

Crop growth is affected by the weather conditions of the growing season and was therefore expected to vary between years. The following section shows the climatic conditions of the 1997/8 and 1998/9 growing seasons. The most important variables in terms of crop growth, development and yield are the incident radiation (MJ m^{-2}), rainfall (mm) and the mean temperature ($^{\circ}$C). Total incident radiation is presented, of which about 50% is photosynthetically active radiation (PAR). These descriptors are compared to the long-term mean of Sutton Bonington from 1960 to 1990 (Figure 4.1 – 4.3).

4.1.1   Incident radiation

Total incident radiation from both growing seasons followed a similar pattern throughout the year (Figure 4.1). As expected, incident radiation decreased during the autumn when the rate of tiller production was high. Both years, however, experienced less radiation than the long-term average. It remained low during the winter months with the lowest radiation of the season being about 55 MJ m^{-2} in December. During the period of stem extension in spring, the incident radiation increased rapidly with above average values in both years. A peak of 476 MJ m^{-2} was reached in May during 1998, but during 1999 continued to increase into the summer months. Summer radiation in both years was below average except during the period of grain fill in 1999 when a peak of 578 MJ m^{-2} was reached in July.

4.1.2 Rainfall

The seasonal distribution of rainfall in both years was extremely variable, unlike the long-term mean (Figure 4.2). In general, there was no pattern in either year nor were there similarities between years. The annual rainfall in the 1998/9 season (800 mm) was much greater than the 1997/8 season (680 mm) and the long-term mean (660 mm). During the autumn, rainfall was higher than the long-term mean in November of 1997 and October of 1998. The latter was the wettest month of that growing season, with 106 mm of rain. This would have implications for leaching and waterlogging, possibly affecting plant establishment. The winter months experienced both higher and lower than average rainfall, illustrating the variability. At the time of anthesis in June of 1998, rainfall reached an impressive 126 mm; more than double the long-term mean. The rains held back during the summer of 1999 and figures were below average in June and July (28 mm).

4.1.3 Mean Temperature

Mean temperatures were similar in both seasons and were generally slightly higher than the long-term mean (Figure 4.3). Temperatures decreased during the autumn as expected. They remained at their lowest in both years (5.5 °C) during the winter, although were greater than the long-term average. Temperatures increased during spring and as canopy expansion began, with February of 1998 being particularly warm. This increase continued throughout the summer, becoming progressively less towards harvest when a peak of 16 °C was reached.
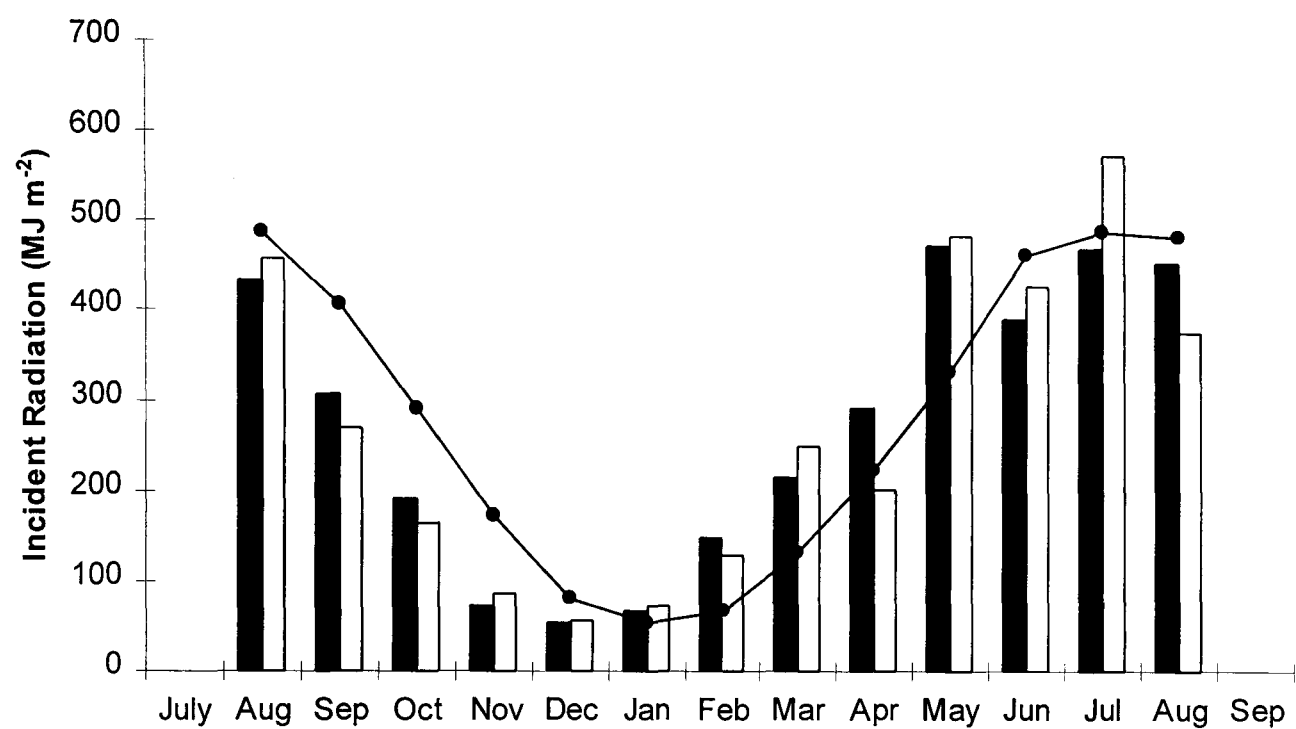
Figure 4.1 Mean daily incident radiation in 1997/8 (■) and 1998/9 (□) compared to the long-term mean (1960 – 1990) (─ ● ─).
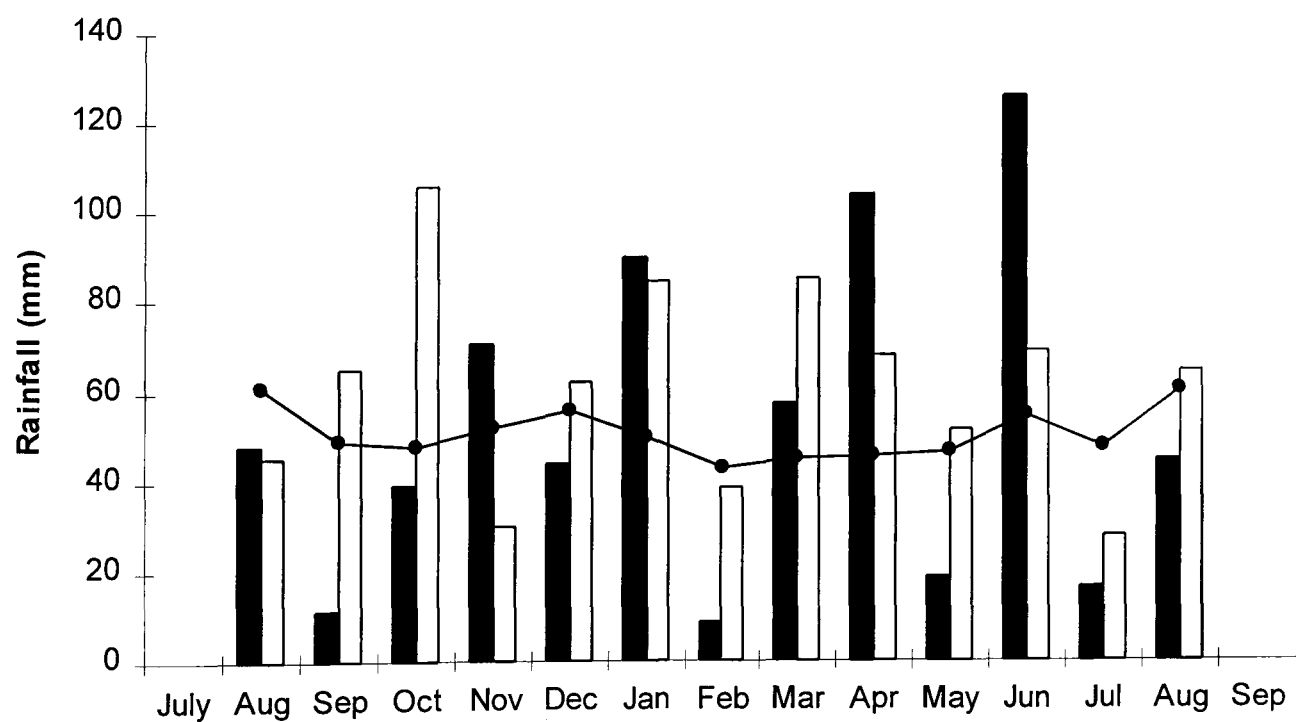
Figure 4.2 Total rainfall in 1997/8 (■) and 1998/9 (□) compared to the long-term mean (1960 – 1990) (─ ● ─).
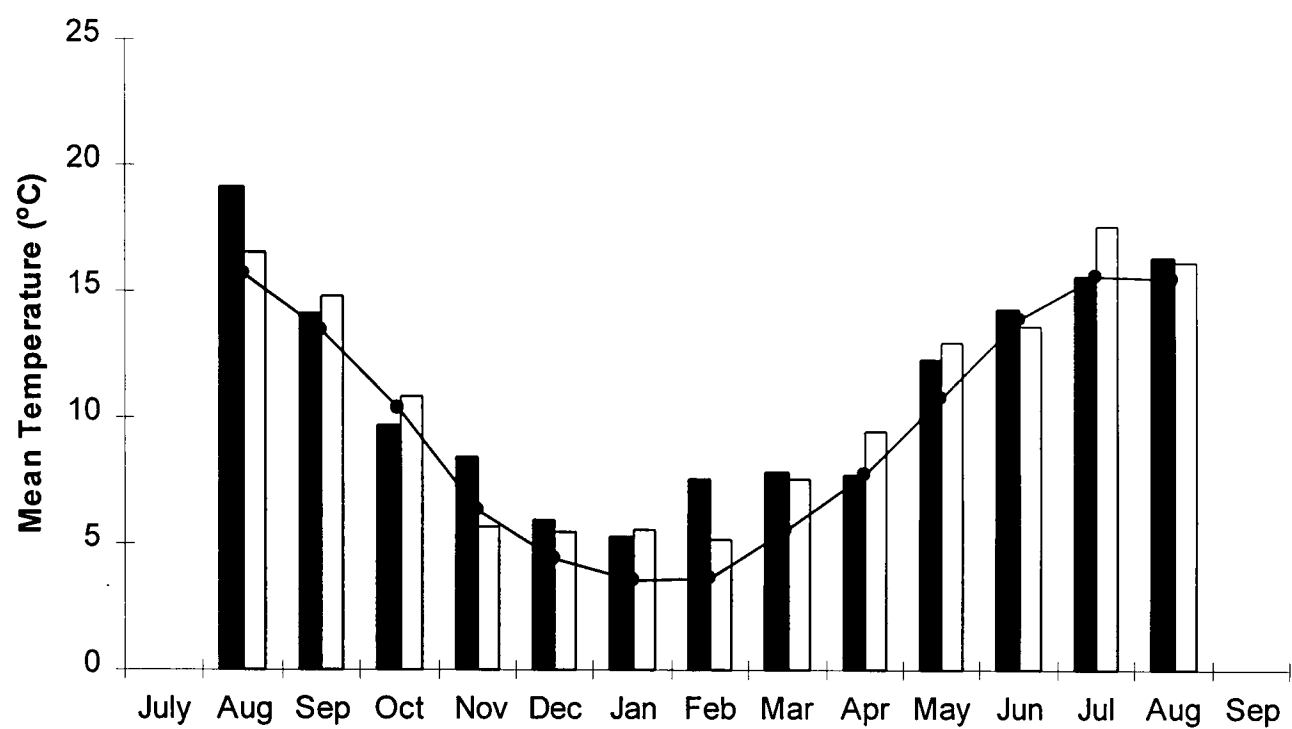
Figure 4.3 Mean temperature in 1997/8 (█) and 1998/9 (☐) compared to the long-term mean (1960 – 1990) (– ● –).

4.2 CROP ESTABLISHMENT

The numbers of plants m^{-2} established by November (GS2) were compared with numbers expected from 100% establishment. A $\log_{10}$ transformation was required to meet the assumptions of the ANOVA. The transformed data, along with S.E.D.s and d.f., is presented in appendix III. Figure 4.5 and 4.6 present the untransformed data.

4.2.1   1997/8 Experiment

4.2.1.1   Plant population

The number of plants established was generally close to one hundred percent (Figure 4.4). This was unexpected at the high seed rate, as there is often reduced establishment with such closely sown seeds. The exception was Spark sown at 640 seeds m^{-2}, in which establishment was considerably lower in all of the replicate plots, averaging 490 plants m^{-2}. There was no significant difference between the varieties, except at 640 seeds m^{-2} (P=0.05). Plant populations increased significantly with seed rate (P<0.001).

4.2.2   1998/9 Experiment

4.2.2.1   Plant population

The plant population for all treatments was close to 100% establishment again (Figure 4.5), even at the higher seed rate. There was a significant increase in plant population with seed rate (P<0.001), with mean populations for 20, 320 and 640 seeds m^{-2} of 21, 319 and 679 plants m^{-2}, respectively. There was no significant difference between Soissons and Spark, which had mean populations of 356 and 324 plants m^{-2}, respectively.
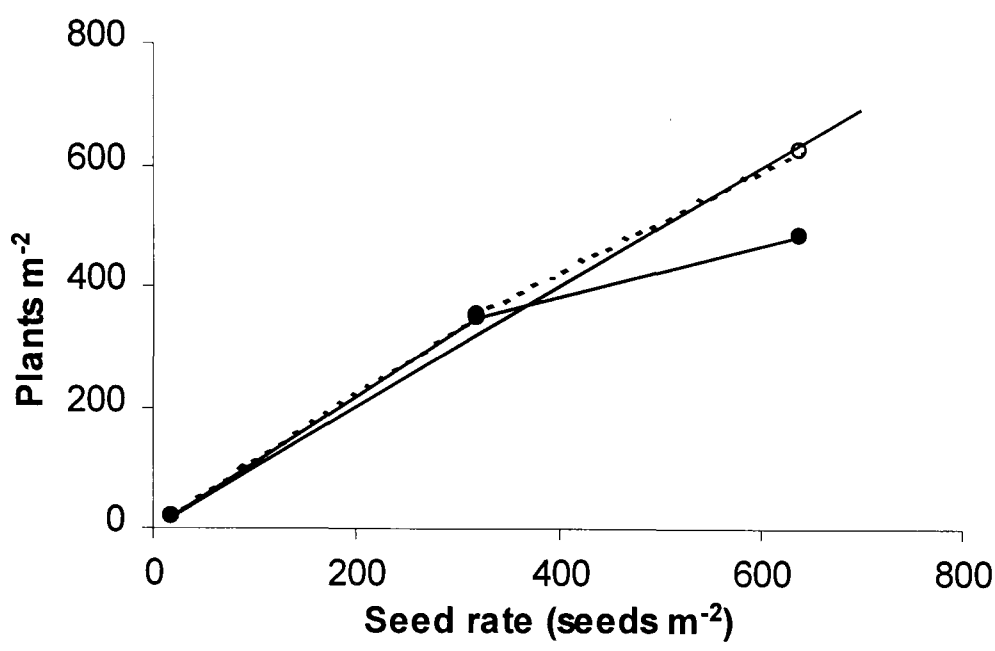
Figure 4.4 Plant population and seed rate of Soissons (····O····) and Spark (—●—) in 1998/9, compared to 100% establishment (——).
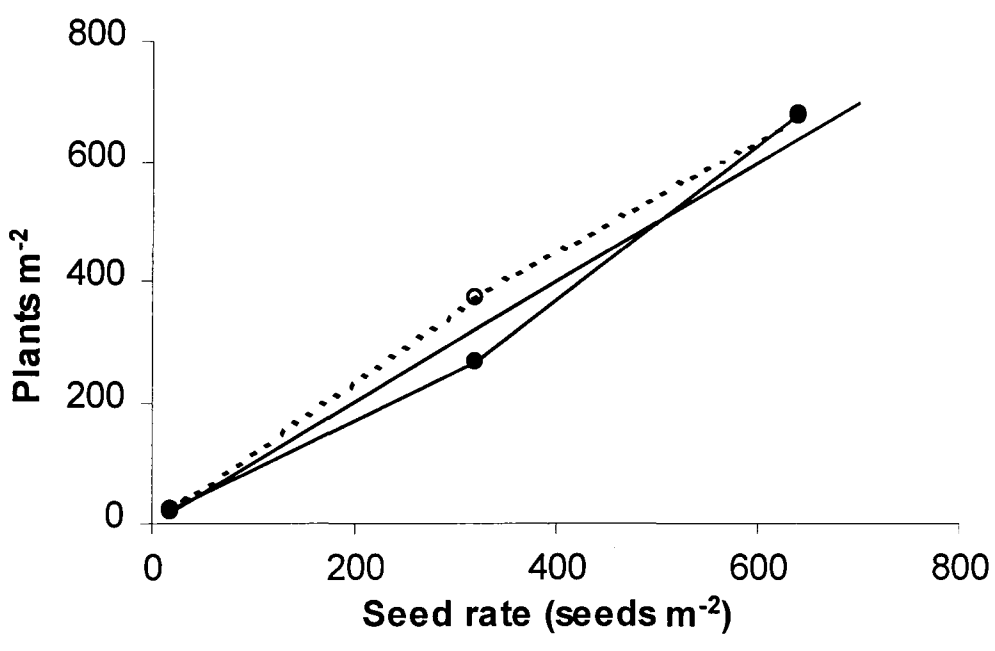
Figure 4.5 Plant population and seed rate of Soissons (····O····) and Spark (—●—) in 1998/9, compared to 100% establishment (——).

4.3 CROP DEVELOPMENT AND GROWTH

The dates of major growth stages (GS) were recorded for each variety. The number of shoots m^{-2}, counted during anthesis, includes main shoots and all tillers that survived that were both fertile and infertile. The projected green area index and N content per unit ground area were measured throughout the season. These gave an indication to the development and growth of the crop.

4.3.1 Development

Table 4.1 shows the developmental differences between the varieties and the growing seasons. A comparison is made with the 'benchmark' crop, as reported in the 'Wheat Growth Guide' (Sylvester-Bradley *et al.*, 1997b). The data from which the 'benchmark' crop is described came from an early sown crop of Mercia grown in 18 site-seasons throughout the UK and sown at 300 seeds m^{-2}. Both experimental growing seasons produced crops that developed earlier than the 'benchmark' crop. This could be the combination of a variety effect with warmer than average winters in both seasons. The development of the crop in the first experiment was faster than in the second, which could have been attributed to the higher winter and spring temperatures, particularly in the February of 1998. The 1997/8 crop was also sown 9 days earlier.

Table 4.1 Comparison of dates of major growth stages in both experimental years and with the 'benchmark' crop (Sylvester-Bradley *et al.*, 1997b).

Growth Stage (GS)	Description	Bench-mark	1997/8 Soissons	Spark	1998/9 Soissons	Spark
	sowing date	7 Oct.	3 Oct.	3 Oct.	12 Oct.	12 Oct.
31	first node detectable	22 April	13 March	17 March	9 April	16 April
39	flag leaf blade all visible	24 May	22 April	28 April	7 May	20 May
59	Ear completely emerged	12 June	26 May	1 June	25 May	7 June
61	start of flowering	16 June	1 June	10 June	2 June	14 June

4.3.2   1997/8 experiment

4.3.2.1   Shoot density

Spark had significantly greater shoot densities than Soissons at anthesis (P<0.001) (Figure 4.6), averaging 830 and 640 shoots m^{-2}, respectively. For each variety, stands sown at 320 and 640 seeds m^{-2} had similar shoot densities and were significantly greater than the stands sown at 20 seeds m^{-2} (P<0.001). Mean shoot densities for the 20, 320 and 640 seeds m^{-2} were 563, 790 and 851 shoots m^{-2}. There was a significant interaction between seed rate and variety (P=0.05) due to the surprisingly high number of shoots in Spark sown at 20 seeds m^{-2}. This was observed in two out of three replicates.

4.3.2.2   N content per unit ground area

The amount of N in the crop, per unit of ground area, increased most rapidly after February (Figure 4.7). The rate of uptake declined after the beginning of June, and appeared to increase again to reach a maximum by early July. This result was unexpected and was not observed in other experiments (Sylvester-Bradley *et al.,* 1997b). Variety generally had little effect on N content throughout the season, although Soissons did have a significantly greater content during March and April (P=0.05). The mean maximum N content in July of Soissons and Spark reached 276 and 266 kg ha^{-1}, respectively. This difference was not significant. Crops sown at higher seed rates had a significantly greater N content, from the beginning of the season until May (P<0.001). There was no seed rate effect after this. The mean maximum N content in July of crops sown at 20, 320 and 640 seeds m^{-2}, was 296, 272 and 245 kg ha^{-1}, respectively. The range at this point of maximum N content, was 240 to 300 kg ha^{-1} in Spark sown at 640 and 20 seeds m^{-2}, respectively. This difference was not significant.

4.3.2.3   Canopy green area index

Variations in canopy expansion and maintenance from the 1997/8 experiment are shown in Figure 4.8. The green area of all crops began to increase rapidly during February, reaching a maximum by the end of June. The rate of decrease in green area

after this varied between treatments. Soissons generally had a significantly greater green area during the spring (P=0.05), but Spark was significantly greater from June onwards (P=0.01). The mean maximum GAI for Soissons and Spark was 8.6 and 9.4, respectively. The maximum canopy size ranged from a GAI of 7.3 to 9.7 in Soissons sown at 20 and 640 seeds m^{-2}, respectively. On the main sampling date (June 29), Spark had a significantly greater total GAI of 8.2 compared to Soissons, which had a GAI of 5.9 (P<0.001). This was expected and can be explained by the greater shoot number of Spark. There was a significant increase in canopy size with increased seed rate (P<0.001) until May. The effect of seed rate was non significant between the crops sown at 320 and 640 seeds m^{-2} during the spring. There was no effect of seed rate after this. The mean GAI in June for crops sown at 20, 320 and 640 seeds m^{-2} was 6.7, 7.8 and 6.7, respectively. The effect of seed rate was significant in Soissons but not in Spark at this time, due to an unusually large canopy in the low seed rate sample. There was therefore a significant interaction between seed rate and cultivar, (P=0.05).
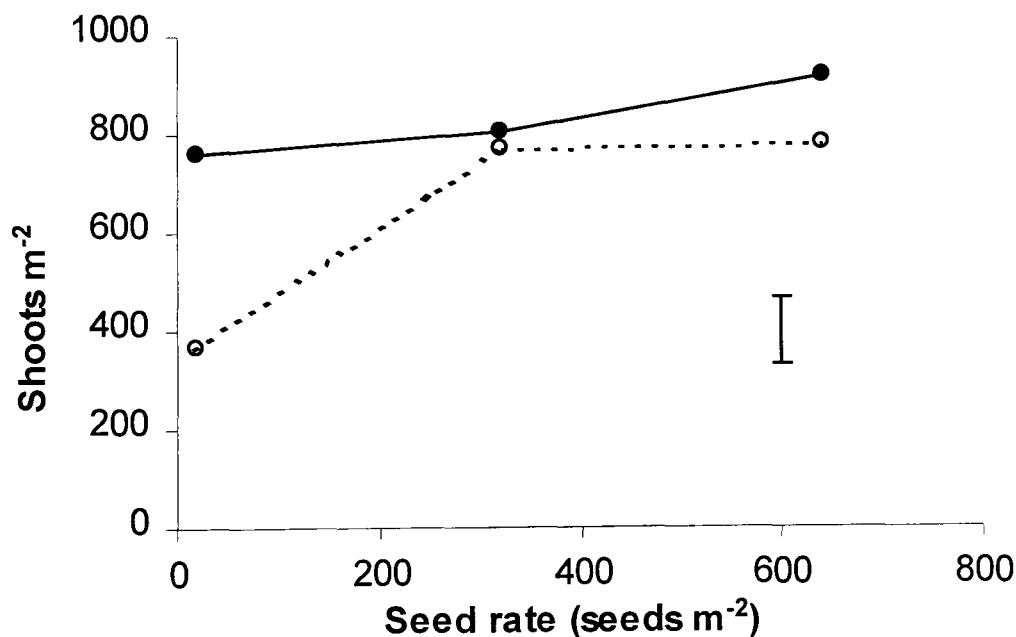
Figure 4.6 Shoot density and seed rate of Soissons ("O") and Spark (—●—) in 1997/8. Error bar represents S.E.D., d.f. = 10.
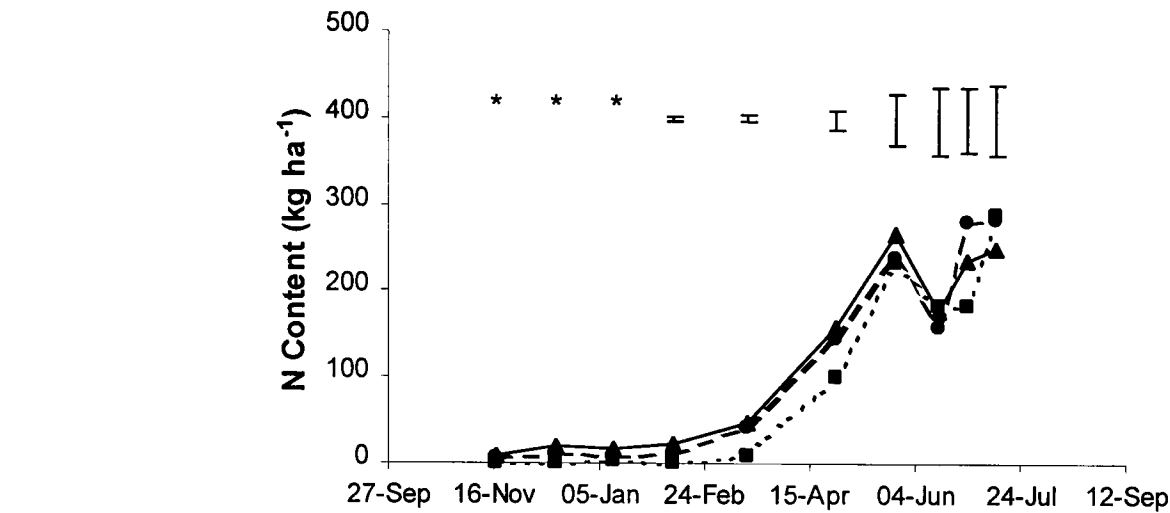
(a)
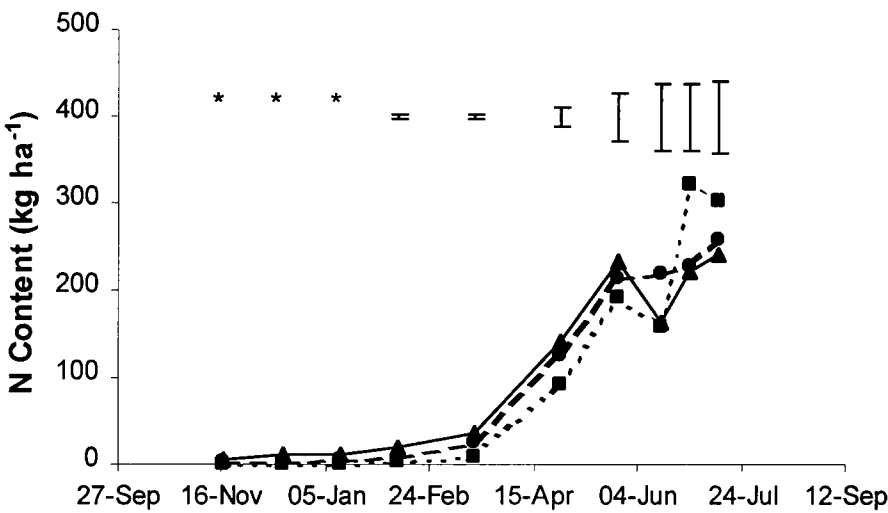
(b)

Figure 4.7 N content per unit ground area of (a) Soissons and (b) Spark sown at 20 (···■···), 320 (--●--) and 640 (—▲—) seeds m^{-2}, in 1997/8. Error bars represent S.E.D., d.f. = 10. Asterisk represents data that required transformation (see Appendix IV).
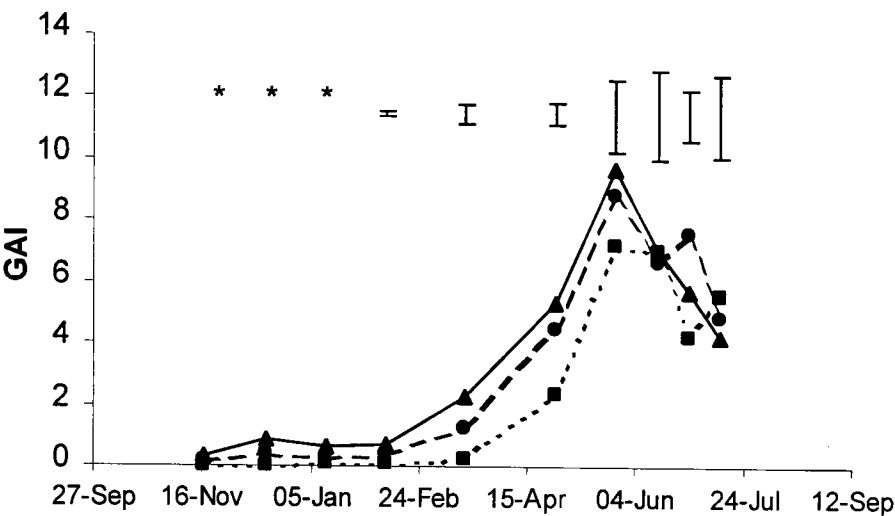
(a)
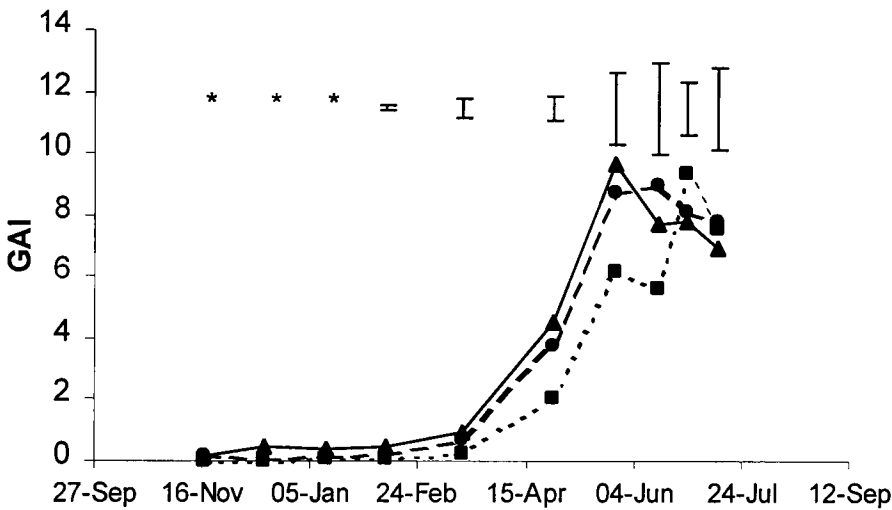
(b)

Figure 4.8 Green area index (GAI) of (a) Soissons and (b) Spark sown at 20 (···■···), 320 (--●--) and 640 (−▲−) seeds m^{-2}, in 1997/8. Error bars represent S.E.D., d.f. = 10. Asterisk represents data that required transformation (see Appendix IV).

4.3.3   1998/9 Experiment

4.3.3.1   Shoot density

Spark had a significantly greater mean shoot density (676 shoots m^{-2}) than Soissons (608 shoots m^{-2}) at anthesis (P=0.05) (Figure 4.9). This effect was smaller with increased seed rate. There was a significant increase in shoot density with seed rate (P<0.001). The means for crops sown at 20, 320 and 640 seeds m^{-2} were 483, 632 and 811 shoots m^{-2}, respectively. Soissons showed a greater increase than Spark. Crops fertilised with 244 kg ha^{-1} N had significantly more shoots m^{-2} (688) than those fertilised with 124 kg ha^{-1} N (595) (P=0.01). This effect increased with seed rate. There was particularly little difference in Spark sown at 20 seeds m^{-2}.

4.3.3.2   N content per unit ground area

The N content per unit ground area increased slightly after February but more rapidly from March onwards (Figure 4.10). Canopies at reduced seed rates contained significantly less N during December and January (P<0.001). This reduction in N content with seed rate was only significant during early spring at the lowest seed rate because crops sown at 320 and 640 seeds m^{-2} contained similar amounts of N at this time. There was no effect of seed rate at anthesis. The mean N content at anthesis for 20, 320 and 640 seeds m^{-2}, was 160, 144 and 147 kg ha^{-1}, respectively. There was generally no significant difference in N content between the varieties, except in February when Soissons had greater N content than Spark (P=0.01). However, by anthesis, Spark had a significantly greater N content of 165 kg ha^{-1} than Soissons with 136 kg ha^{-1} (P<0.001). The crop fertilised with 244 kg ha^{-1} N had a significantly greater N content than the crop fertilised with 124 kg ha^{-1} N (P<0.001). Mean N content was 177 and 123 kg ha^{-1} N, respectively. There was an interaction between seed rate and variety at anthesis (P=0.05). This was due to Spark sown at 20 seeds m^{-2} having a significantly greater N content than Soissons at 20 seeds m^{-2} and Spark at 320 seeds m^{-2}.

4.3.3.3 Canopy green area index

Canopy expansion began during February, with the crops sown at 320 and 640 seeds m^{-2} showing a greater increase than the low seed rate (Figure 4.11). Soissons had a significantly larger canopy than Spark during January and February (P=0.01) but by anthesis, Spark had the larger canopy (P<0.001). The mean maximum canopy size reached by Spark and Soissons was a GAI of 5.9 and 4.8. This difference was due to shoot number and leaf green area per shoot being greater in Spark. Canopy size was also significantly larger with increasing seed rates throughout the season (P=0.01) due to shoot number. The mean maximum canopy size for 20, 320 and 640 seeds m^{-2} was a GAI of 4.8, 5.3 and 5.9, respectively. The effect of the N treatment was measured at anthesis, where crops fertilised with 244 $kg\ ha^{-1}$ N produced significantly greater canopies than those fertilised with 124 $kg\ ha^{-1}$ N (P<0.001), through greater shoot numbers and leaf green area per shoot. The mean GAIs of the canopies at anthesis were 6.1 and 4.6, respectively. There were no significant interactions between any of the treatments.
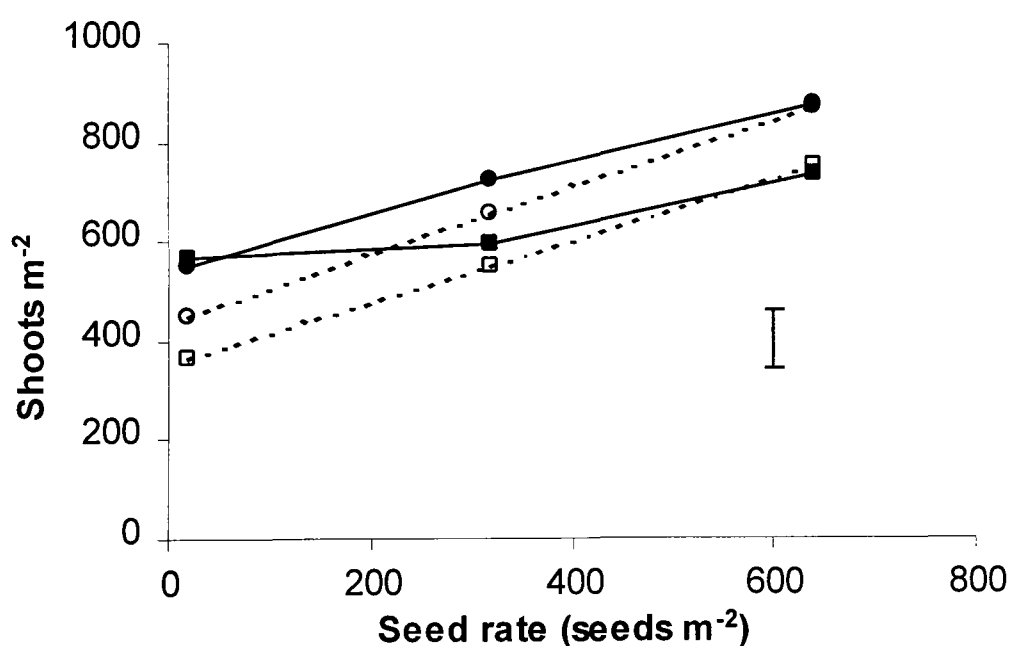
Figure 4.9 Shoot density and seed rate of Soissons (···O···) and Spark (—●—) with 244 kg ha^{-1} N and Soissons (···□···) and Spark (—■—) with 124 kg ha^{-1} N applied, in 1998/9. Error bar represents S.E.D., d.f. = 21.42.
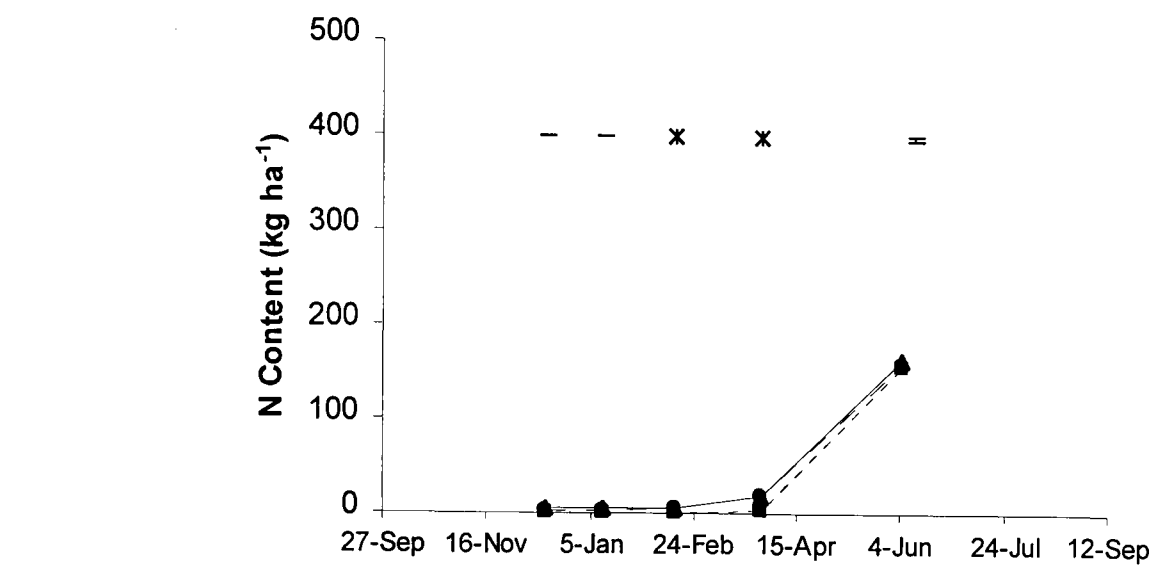
(a)
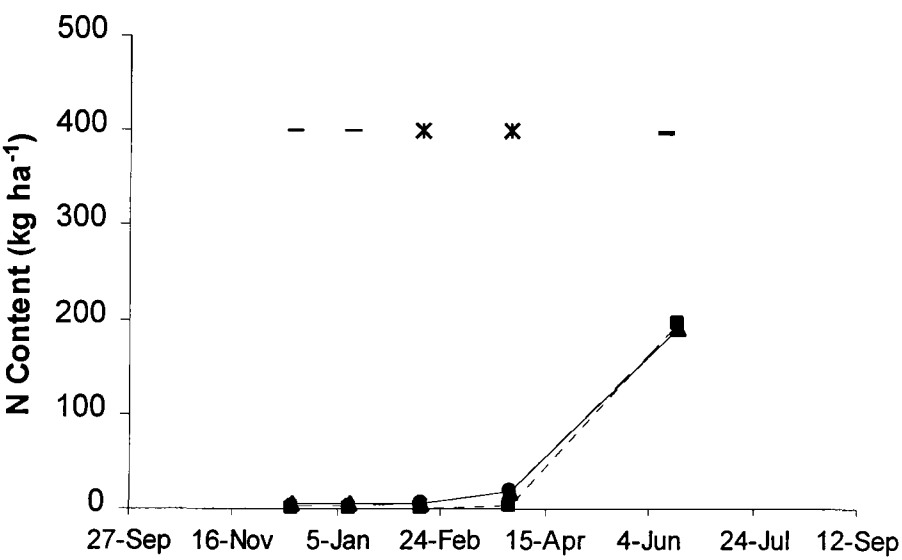
(b)

Figure 4.10 N content per unit ground area of (a) Soissons and (b) Spark sown at 20 ($\cdots\blacksquare\cdots$), 320 (- -$\bullet$- -) and 640 ($-\blacktriangle-$) seeds m^{-2}, in 1998/9. 244 kg ha^{-1} N applied. Error bars represent S.E.D., d.f. = 10. Asterisk represents data that required transformation (see Appendix IV).
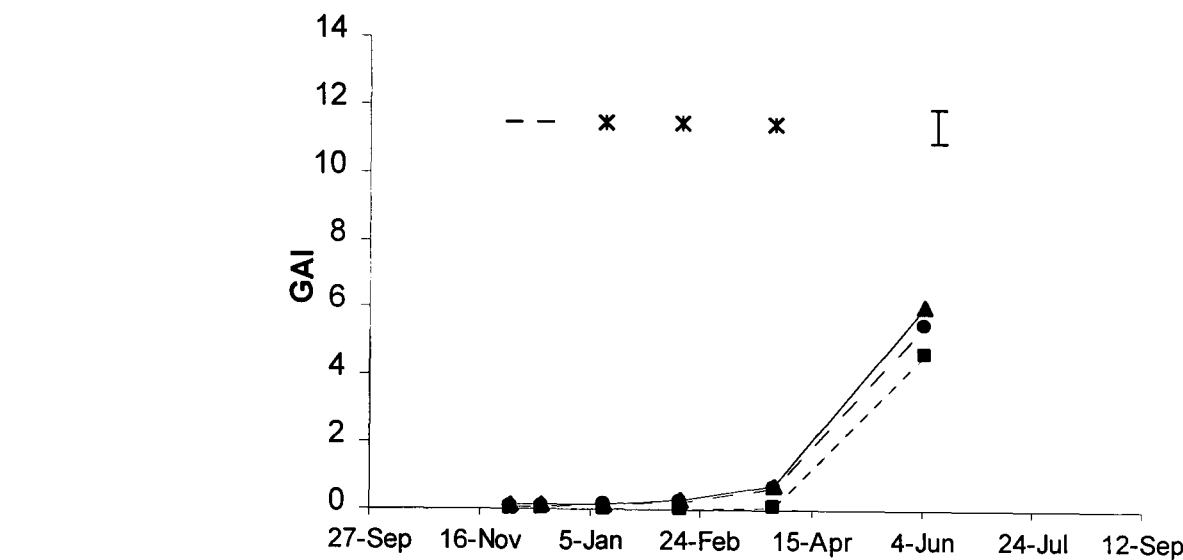
(a)
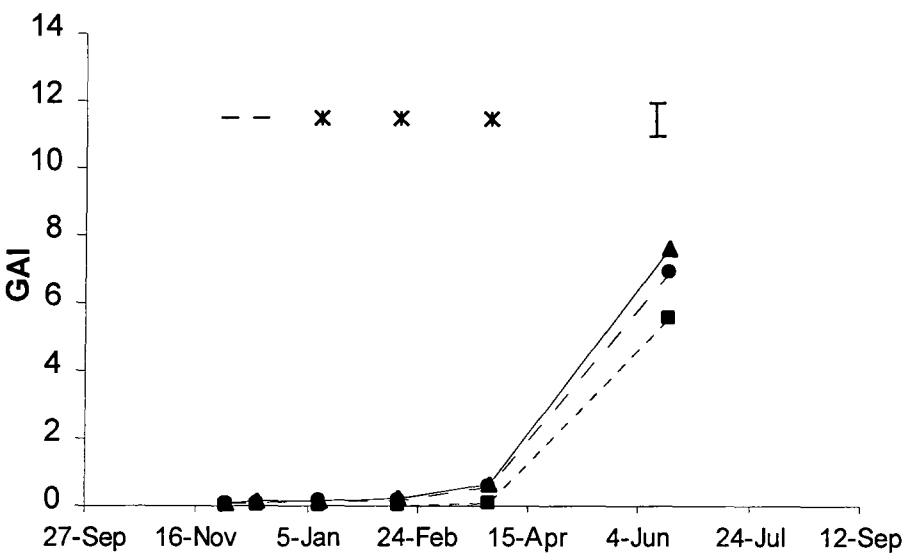
(b)

Figure 4.11 Green area index (GAI) of (a) Soissons and (b) Spark sown at 20 ($\cdots\blacksquare\cdots$), 320 (- -●- -) and 640 (—▲—) seeds m^{-2}, in 1998/9. 244 kg ha^{-1} N applied. Error bars represent S.E.D., d.f. = 10. Asterisk represents data that required transformation (see Appendix IV).

4.4 CROP YIELD COMPONENTS

The results of the combine yield and associated measurements are now presented for each year.

4.4.1 1997/8 Experiment

4.4.1.1 Combine Yield

Soissons had a significantly greater combine yield with a mean of 10.2 t ha^{-1}, than Spark which had a mean of 9.3 t ha^{-1} (P=0.01) (Figure 4.12). The difference was not significant at the lowest seed rate. Crops sown at 320 and 640 seeds m^{-2} produced similar yields within each variety (means of 10.9 t ha^{-1}). These were greater than the crops sown at 20 seeds m^{-2} at 7.4 t ha^{-1} (P<0.001).

4.4.1.2 Ear population

The data were log$_{10}$ transformed to meet the assumptions of the ANOVA. Spark produced a significantly greater mean number of ears (617 ears m^{-2}) than Soissons (559 ears m^{-2}) (P=0.01). This was particularly seen at 320 seeds m^{-2} (Table 4.2). There was also a significant increase in mean ear population with the other seed rates (P<0.001). Mean ear populations for crops sown at 20, 320 and 640 seeds m^{-2} were 300, 701 and 763 ears m^{-2}, respectively. There was no significant difference, however, between ear numbers of Spark at 320 and 640 seeds m^{-2}.

4.4.1.3 Grain N

The amount of N in the grain, as a percentage of grain dry weight, was not significantly affected by variety or by seed rate (Table 4.3). The overall mean of the treatments was 2.14%.

4.4.2   1998/9 Experiment

4.4.2.1   Combine Yield

There was no significant difference between the yield of Soissons and Spark. Mean yields were 10.0 and 10.2 t ha^{-1}, respectively. Yields were significantly increased with increased seed rate (P<0.001) (Figure 4.13). The main effect was between the crops sown at 20 seeds m^{-2} and 320 seeds m^{-2}, with mean yields of 8.0 and 11.2 t ha^{-1}. Crops sown at 640 seeds m^{-2} had a similar yield to the 320 seeds m^{-2} at 11 t ha^{-1}. Crops fertilised with 244 kg ha^{-1} N had significantly greater yields (10.8 t ha^{-1}) than those fertilised with 124 kg ha^{-1} N (9.3 t ha^{-1}) (P<0.001).

4.4.2.2   Ear population

The number of ears in Spark (570 m^{-2}) was significantly greater than in Soissons (469 m^{-2}) (P<0.001). Ear numbers were also significantly increased with seed rate (P<0.001) (Table 4.4). Crops sown at 20, 320 and 640 seeds m^{-2}, had mean ear populations of 392, 567 and 642 ears m^{-2}. The difference between Soissons at 320 and 640 seeds m^{-2} was, however, small. Crops fertilised with more N produced significantly more ears m^{-2} than those with less N (P<0.001), with means of 562 and 504 ears m^{-2}. This difference was greater in Soissons than Spark.

4.4.2.3   Grain N

Increasing the amount of N fertiliser from 124 to 244 kg ha^{-1} significantly increased the grain N content from 1.65 to 2.13 % (P<0.001) (Table 4.5). Grain N content was significantly greater in Soissons (1.92%) than in Spark (1.86 %) (P=0.05). There was also a significant increase in grain N with the reduction in seed rate to 20 seeds m^{-2} (P<0.001). The mean grain N contents were 2.00, 1.83 and 1.84 % for crops sown at 20, 320 and 640 seeds m^{-2}.
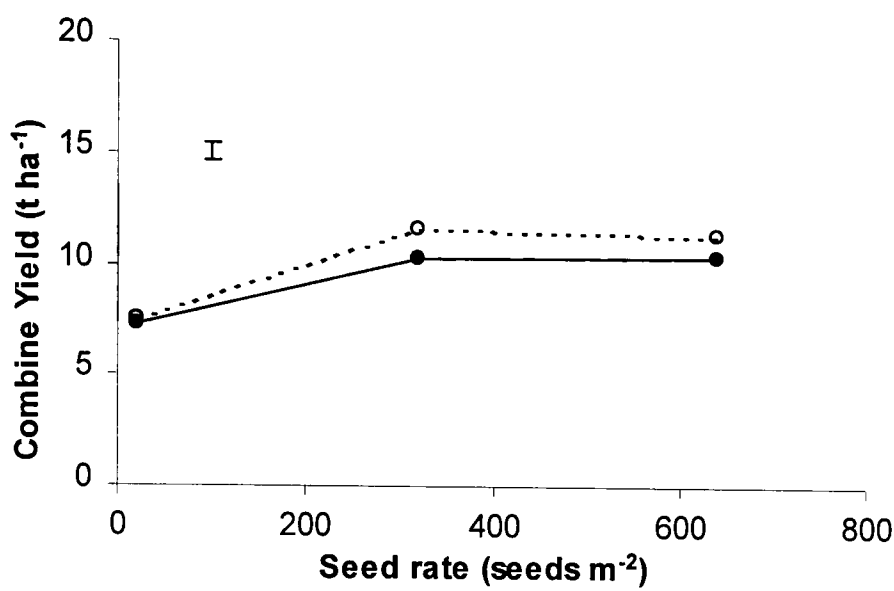
Figure 4.12 Combine yield and seed rate of Soissons (··O···) and Spark (—●—) in 1997/8. Error bar represents S.E.D., d.f. = 10.

Table 4.2 The effect of variety (Var) and seed rate (SR) on ear populations ($\log_{10}$ ears m^{-2}) in 1997/8.

Variety	Seed Rate (seeds m^{-2})			Mean
	20	320	640	
SOISSONS	2.45	2.80	2.88	2.71
SPARK	2.50	2.89	2.88	2.76
Mean	2.48	2.84	2.88	2.73

	P	S.E.D.	d.f.
Var	0.010	0.015	10
SR	<0.001	0.018	10
Var*SR	0.073	0.025	10

Table 4.3 The effect of variety (Var) and seed rate (SR) on grain N (% dry weight) in 1997/8.

Variety	Seed Rate (seeds m^{-2})			Mean
	20	320	640	
SOISSONS	2.14	2.06	2.15	2.12
SPARK	2.19	2.08	2.22	2.16
Mean	2.17	2.07	2.18	2.14
	P	S.E.D.	d.f.	
Var	0.480	0.062	10	
SR	0.344	0.076	10	
Var*SR	0.941	0.107	10	
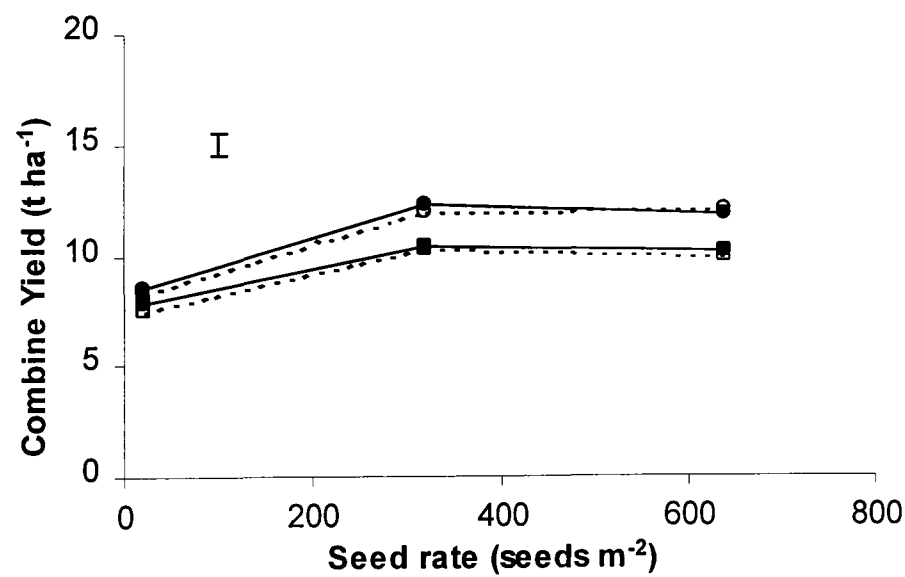
Figure 4.13 Combine yield and seed rate of Soissons (···O···) and Spark (—●—) with 244 kg ha^{-1} N and Soissons (···□···) and Spark (—■—) with 124 kg ha^{-1} N applied, in 1998/9. Error bar represents S.E.D., d.f. = 21.39.

Table 4.4 The effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on ear populations (ears m^{-2}) in 1998/9.

N (kg ha^{-1})	Variety	Seed Rate (seeds m^{-2})			Mean
		20	320	640	
124	SOISSONS	312	494	561	456
	SPARK	395	573	691	553
	Mean	353	534	626	504
244	SOISSONS	407	588	616	537
	SPARK	455	611	699	588
	Mean	431	599	657	562
Mean	SOISSONS	359	541	589	496
	SPARK	425	592	695	570
	Mean	392	567	642	533

	P	S.E.D.	d.f.	[a]S.E.D.	d.f.
Var	<0.001	12.8	10		
SR	<0.001	15.7	10		
N	<0.001	11.5	12		
Var*SR	0.238	22.2	10		
Var*N	0.070	17.2	21.4	0.33	12
SR* N	0.275	21.1	21.4	0.41	12
Var*SR*N	0.931	29.8	21.4	0.57	12

[a]S.E.D. to compare same level of Var, SR or Var*SR.

Table 4.5 The effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on grain N (% dry weight) in 1998/9.

N (kg ha⁻¹)	Variety	Seed Rate (seeds m⁻²)			Mean
		20	320	640	
124	SOISSONS	1.62	1.78	1.61	1.67
	SPARK	1.54	1.78	1.58	1.63
	Mean	1.78	1.60	1.58	1.65
244	SOISSONS	2.24	2.12	2.17	2.17
	SPARK	2.22	2.01	2.02	2.08
	Mean	2.23	2.06	2.09	2.13
Mean	SOISSONS	2.01	1.87	1.90	1.92
	SPARK	2.00	1.79	1.78	1.86
	Mean	2.00	1.83	1.84	1.89

	P	S.E.D.	d.f.	[a]S.E.D.	d.f.
Var	0.032	0.027	10		
SR	<0.001	0.033	10		
N	<0.001	0.023	12		
Var*SR	0.289	0.046	10		
Var*N	0.252	0.035	20.7	0.032	12
SR* N	0.565	0.043	20.7	0.039	12
Var*SR*N	0.888	0.060	20.7	0.055	12

[a]S.E.D. to compare same level of Var, SR or Var*SR.

4.5 CROP GROWTH AND YIELD ASSESSMENT

To evaluate the growth and yield of the crops from the two experiments, each variety sown at 320 seeds m^{-2} at 244 kg ha^{-1} N from each year was compared with the 'benchmark' Mercia crop (Sylvester-Bradley et al., 1997b) (Table 4.6). At GS31, the 'benchmark' crop was generally larger with more N uptake than the crops in either of the two seasons but by GS61, the 'benchmark' crop was smaller. The 'benchmark' crop also had the lowest yield.

Table 4.6 Comparison of crop growth and yield from 1997/8 and 1998/9 experiments with the 'bench mark' Mercia crop.

	Bench mark crop	1997/8		1998/9	
		Soissons	Spark	Soissons	Spark
Sowing date	7 October	3 October		12 October	
Plant population	300	356	352	371	267
GS31 – N Content (kg ha^{-1})	60	41	28	38	58
GS31 – GAI	1.9	1.3	0.7	1.3	2.0
GS31 – Crop biomass (g m^{-2})	160	72	70	167	200
GS61 – N content (kg ha^{-1})	212	240	220	159	194
GS61 – GAI	5.8	8.2	8.6	5.6	7.0
GS61 – Crop biomass (g m^{-2})	1130	1214	1357	1062	1391
GS61 – Shoot number (m^{-2})	600	772	808	655	723
Final crop biomass (g m^{-2})	1740	1933	2080	1887	2086
Yield (t ha^{-1})	9.1	11.6	10.3	12.0	12.3
Ear population (m^{-2})	600	629	773	588	611
Grain N content (% DM)	2.05	2.06	2.08	2.12	2.01

4.6 DISCUSSION

This chapter describes crop development, growth and yield in terms of parameters commonly used by physiologists. This will provide background knowledge of the crop before more detailed results are presented in the following chapters and allow these particular crops to be placed in a wider context. The focus of this discussion is on shoot density and its effect on crop N content per unit ground area and the canopy size, since the CNR is the relationship between these two.

4.6.1 Shoot density

Soissons developed earlier than Spark, as there is an absence of a photoperiod requirement in Soissons for the initiation of the reproductive phase (Foulkes et al., 1998). The vegetative phase of Soissons was therefore shorter and so fewer tillers were produced. Spark was able to compensate for a low plant population better than Soissons, for this reason. Early tiller counts were not made in either year and so it was not possible to comment on tiller production or survival. At the higher seed rate, and particularly in 1998/9, there was no difference in shoot density which suggested that both varieties reached the maximum shoot density possible with the available resources.

At the lowest seed rate, shoot densities were found to be variable. It was observed frequently, that plants at this seed rate were distributed in clusters and with large gaps. Samples were taken from a $0.25m^2$ quadrat which was small enough to fit in some of the gaps. It was therefore possible that one quadrat sample may have just one plant and another may have five plants. This was expected to be a large source of error in many of the measurements of plant growth. Dense canopies have a reduced R:FR ratio, which is known to reduce tillering (Casal, 1988; Gautier, Varlett-Grancher & Hazard, 1999) through the reduction in gibberellins (Kraepiel & Miginiac, 1997). In this way, the crop sown at 320 seeds m^{-2} produced more tillers per plant than the crop sown at 640 seeds m^{-2} and was able to fully compensate for the reduced plant numbers. The crop was not able to fully compensate at the lowest seed rate, despite the very high number of tillers per plant, and still had fewer shoots m^{-2} than the other

seed rates. At the low seed rate, shoot number was limited by the variety's ability to produce and maintain tillers before the end of the vegetative phase. As more tillers are produced at the low seed rate, the space between each tiller on the same plant would be reduced. The spacing between these clumped tillers may be similar or even less than that between shoots from individual plants at the higher seed rate. The resulting decrease in R:FR will cause the reduction in tillering, and so a physiological restriction is induced. However, it may be just the completion of the tillering phase or the limitation of N that curbs the tiller number. Spark sown at 20 seeds m^{-2} in 1997/8, had an unexpectedly high shoot density in two of the three replicates, but this can be explained by the large variation in plant distribution.

The low N treatment was imposed in 1998/9 to remove the possibility of luxury uptake of N occurring. This would reduce the availability of N to the crop so that the amount of luxury N taken up would be limited. Canopies with the low N treatment had fewer shoots, indicating that nitrogen was a limiting resource. This was more evident at the higher seed rates, as there was increased limitation due to the greater number of plants that would decrease the amount of N available per plant. It is suggested here that shoot number at the low seed rate was limited more by the end of the tillering phase, and at the highest seed rate was limited more by resources such as N and light.

4.6.2   Crop N content

The amount of fertiliser N was adjusted in 1999 to supply the crop with the same available N as in the previous year. This was the 'high N' treatment. However, the canopies generally contained more N per unit ground area in 1997/8 than in 1998/9. The greater spring and summer rainfall of 1998 is thought to have increased the recovery of applied N fertiliser, explaining this unexpected difference. N uptake increased throughout the rapid growth period and after maximum GAI was reached.

It was expected that Spark would contain the greatest amount of N because of a greater shoot density. However, there was generally no difference between the varieties in N content per unit ground area despite the greater shoot density in Spark, except at GS61 1998/9 when Spark had a greater N content. Prior to full canopy

expansion, canopies sown at high seed rates had a greater N content than the low seed rate. This was a direct response to shoot density, but also to the rate of development as more dense canopies develop earlier than less dense canopies (Fukai *et al.*, 1990). Canopy expansion and increased growth rate occurs earlier, so that the high seed rate will take up more N per unit ground area than the low seed at the same calendar date. The N uptake was the same for all densities at GS61, when the canopy was close to maximum GAI and most (85 %) of the available N (measured in February) had been taken up.

The apparent greater N per shoot in Soissons and in the low seed rate could have been required to create larger leaves. Therefore, these would have a greater leaf area:stem area. It could also have been required to increase N content of existing leaves and stem for photosynthesis or structure. However, it could have been luxury uptake. It is difficult to determine which of these possibilities it was, without more detailed analysis. This will be addressed in the following chapters.

The nitrogen treatment was applied after GS31 in the 1998/9 experiment, so the effect of N was only observed at GS61 in this year. As expected, N uptake increased with fertiliser N applied. The greater N availability allowed more tillers to be maintained. It is suggested here that if there is more N available than is required to maintain the number of tillers produced, then luxury N would also be taken up. It is thought that this would be the case for the 'high N' crop and that no luxury uptake would occur in the 'low N' crop. It could be hypothesised that the differences in N content per shoot occur because of the differences in shoot number m^{-2} but similar amount of N m^{-2} taken up.

4.6.3   Canopy Size

Rapid canopy expansion began after nitrogen had been applied and coincided with the increase in average temperatures and incident radiation. Green area increased along with N content at this point in the season, suggesting that N drives canopy expansion. This explains the greater maximum canopy sizes of 1998. The mean GAI at GS61 of both varieties sown at 320 seeds m^{-2} (and 244 kg ha^{-1} N in 1999), was 8.4 in 1998 and 6.3 in 1999. This is important for understanding the physiological basis of the CNR

discussed in the followed chapters. Following this period of expansion, the biomass increased in response to increased radiation interception and conversion (Scott *et al.*, 1994).

Soissons had the greater canopy area throughout the spring, following the same patterns as N content. Once again, the variety differences in rate of development could account for this. This also explains why Soissons reached its maximum canopy size generally by the end of May, whereas Spark reached its maximum slightly later, at the beginning of June. At GS61, Spark had a greater GAI than Soissons, in both years. The effect of an increased seed rate on canopy size prior to full canopy expansion was largely due to the increased plant population and shoot density. This was observed throughout the growing season in both years except for June 1998, when there was no seed rate effect. On 29 June 1998, unexpectedly large canopies with a GAI of 9 were sampled at the lowest seed rate. This could only be explained by the clustering of plants which, combined with less senescence than at higher seed rates caused the exceptionally high shoot densities. The observations in the following year were thought to be more usual, when the green area at anthesis was smallest in the low seed rate crops and the difference between the two higher seed rates small.

These results suggested that the greater N content per shoot in Soissons and the low seed rate was not used to increase the leaf area enough to compensate for the reduced shoot density. Although this may have occurred to a certain extent, there is still the possibility that more N is required by these crops for photosynthesis or structure, or that luxury uptake of N is occurring.

As N uptake increased with fertiliser N, the canopy size also increased. Whingwiri & Kemp (1980) made similar observations. The extra N was used to maintain the tillers for longer, but the increase in GAI was not as great as the increase in N uptake. This supports the suggestion that there was luxury uptake occurring in the 'high N' crop and that the 'low N' crop was closer to being N limited. The amount of N in the latter could therefore be taken as the requirement, rather than the content which includes luxury uptake. The following chapters will test this further.

4.6.4 Summary

In summary, shoot density was manipulated through variety choice, seed rate and fertiliser N application. The greater shoot densities in Spark and in high seed rate canopies had no effect on the N uptake per unit ground area at anthesis. The crop fertilised with 124 kg ha^{-1} N in 1999, had both a reduced N content per unit ground area and canopy size, and was thought not to contain any luxury N. The fate of N is determined in the following chapters and will test these ideas and the hypothesis that the CNR can be predicted from canopy architecture. This begins in the next chapter which describes the variation in the relationship between N content per unit ground area and size of the canopy (CNR).

5   CANOPY NITROGEN

This chapter describes the nitrogen (N) content of canopies with different architectures. The observed N contents are compared to the predicted canopy N requirement (CNR) of Chapter 2. The measured N content in the 'low N' crop is thought to be closer to the N requirement through the reduction in available N and therefore opportunity for luxury uptake.

The most comprehensive analysis of the 1997/8 experiment was performed on 29 June, at around GS70 in Soissons and GS67 in Spark. This would have been just before rapid senescence and grain fill, when most of the N was taken up and after the crop had reached its maximum height. The most detailed analysis of the 1998/9 experiment was carried out at a slightly earlier stage of development, at GS65, half way through anthesis. The 1998/9 results also include the effect of the N treatment. A comparison between the treatments was therefore made at these growth stages and is also the main focus of the following three chapters, which attempt to explain the differences in N content described in this chapter.

5.1   HUSBANDRY

Section 5.1.1 describes the accumulated N content per unit ground area with accumulated GAI through depth. The N content per unit green area of the whole canopy is derived from the slope of the regression describing this relationship. The partitioning of N between the leaf and stem is also described. Section 5.1.2 then analyses the change in N content for each successive unit of green area down the canopy.

5.1.1   N content per unit green area

The accumulated N content per unit ground area with depth expressed as accumulated GAI, for canopies of 1998, is shown in Figure 5.1. The results for 1999 are shown in

Figure 5.2 and Figure 5.3. The fitted lines accounted for 96.1 % of the variation in 1998, and 98.6 % of the variation in 1999. Generally, there was no significant intercept and so the slopes have been taken to represent the mean N content per unit of green area. These values are presented in Table 5.1.

In 1998, there was a highly significant effect of seed rate on the N content per unit green area throughout the canopy (P<0.001). At the lowest seed rate the N content was consistently greater than at higher seed rates and across both varieties, despite the larger canopy size of Spark. There was no significant effect of variety in this experimental year.

In 1999, the N content per unit GAI for each canopy supplied with a greater amount of fertiliser N was generally larger than the equivalent of the previous year, but canopies were also smaller. There was a significant effect of seed rate in this experimental year also (P<0.001), where the low seed rate had the greater N content per unit green area. In this year, Soissons had a significantly greater N content per unit green area, than Spark (P=0.01). Canopies with 244 kg ha^{-1} fertiliser N also had a greater N content per unit green area than those with just 124 kg ha^{-1} fertiliser N (P<0.001). The same variety and seed rate effects were also observed at the low N treatment alone.

There was a diminishing increase in leaf N content per unit ground area with canopy size, reaching a total of between 5 and 10 g m^{-2}. The accumulation of N in the stem, however, increased with canopy size. The total N in the stem was also between 5 and 10 g m^{-2}. The N in the non-green tissue at the bottom of the canopy contributed a relatively small amount to the total N content.

An ANOVA was performed on the ratio of N per unit ground area in leaf to that in the stem, to detect any significant treatment effects on partitioning.

In 1998, there was a significant effect of seed rate on the partitioning of N between leaf and stem tissue (P = 0.05) (Table 5.2). In Soissons, this was caused by the greater leaf N to stem N ratio at 320 seeds m^{-2}, compared with other seed rates. In Spark, the effect was due to the increased partitioning of N to the leaf with increased seed rate,

which could indicate a greater leaf N requirement in the low seed rate. There was no effect of variety on the ratio of leaf N to stem N either in this year, although there was an interaction between variety and seed rate (P = 0.05).

In 1999, there was a significant effect of seed rate again (P<0.001)(Table 5.3). However, in this year the effect was inconsistent. The ratio of leaf N to stem N was greater at 20 seeds m^{-2} compared with any other seed rate, but Spark with 244 kg ha^{-1} N was smaller than at any other seed rate, as observed in the previous year. Where the ratio was greater in the low seed rate this meant that more N was partitioned to the leaf in the low seed rate than in other seed rates. This could be explained if there was a greater ratio of leaf to stem green area. Spark had a significantly greater ratio of leaf N to stem N than Soissons in this year (P<0.001), which may be explained if Soissons had a greater ratio of leaf to stem green area. There was no significant effect of fertiliser N on the ratio of leaf N to stem N.

In conclusion, there was no consistent treatment effect on the ratio of leaf N to stem N content per unit ground area.

Fitting regressions to cumulative data reduces the chance of observing differences within individual canopy layers. Therefore, a more detailed look at the N content of successive individual units of green area is presented in section 5.1.2.
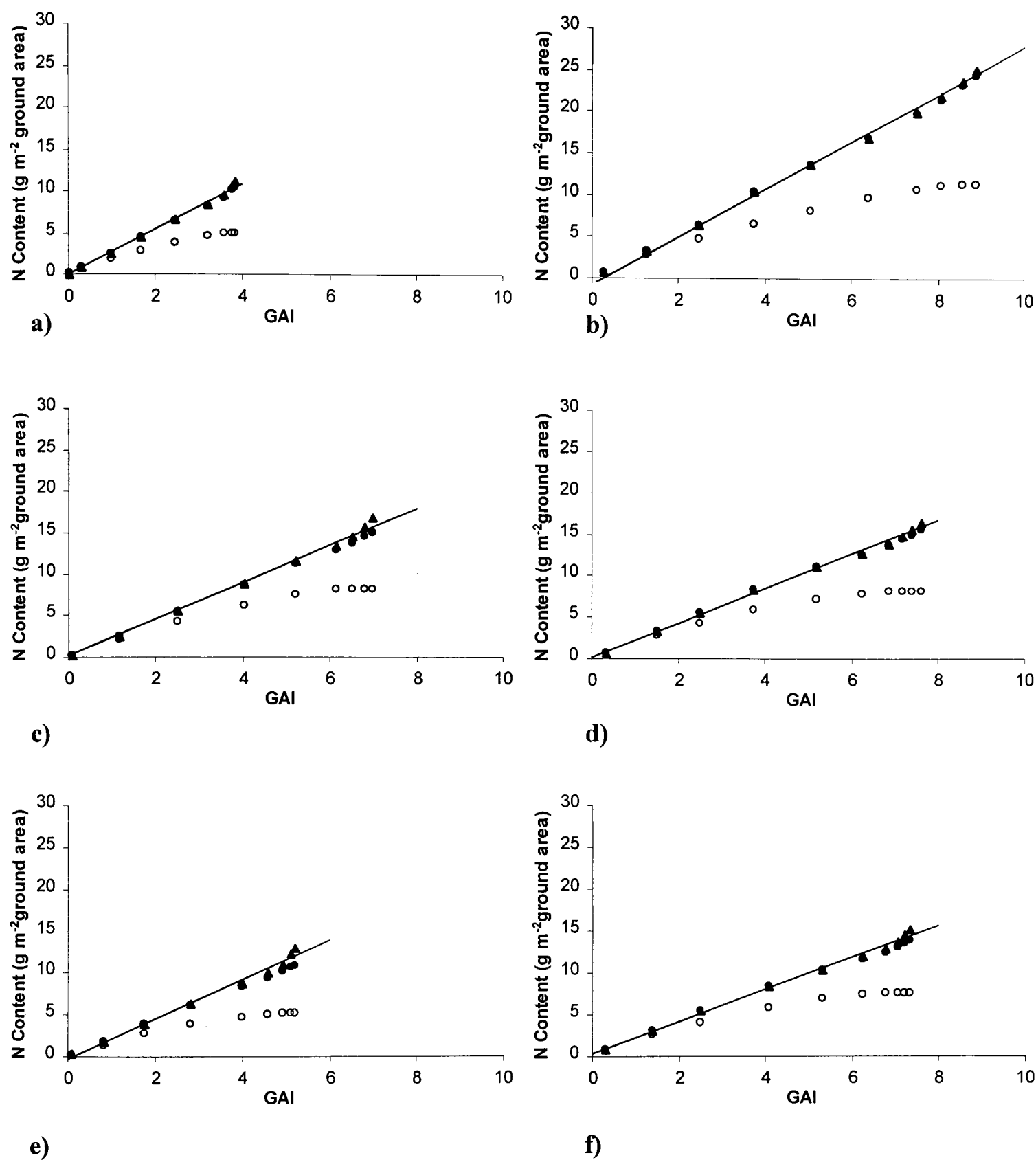
Figure 5.1 The relationship between N accumulation in leaves + stems + non-green tissue (▲), leaves + stems (●) and leaves (○) and depth expressed as accumulated green area index (GAI) (excluding ears), on June 29 1998. a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}. R^2 = 96.1%. Slopes are presented in Table 5.1.
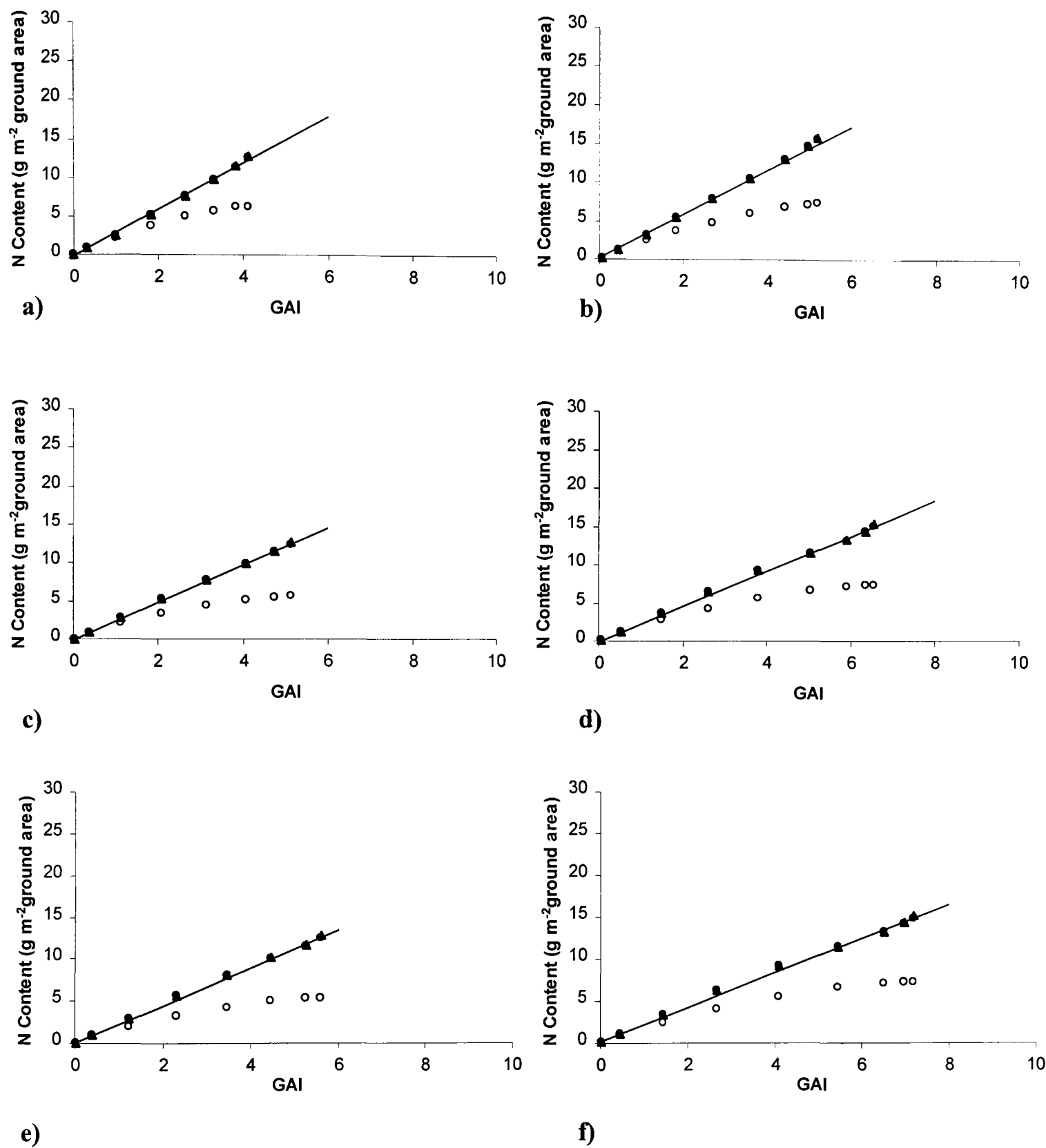
Figure 5.2 The relationship between N accumulation in leaves + stems + non-green tissue (▲), leaves + stems (●) and leaves (○) and depth expressed as accumulated green area index (GAI) (excluding ears) with depth, at anthesis in 1999. 244 kg ha^{-1} fertiliser N. a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}. R^2 = 98.7%. Slopes are presented in Table 5.1.
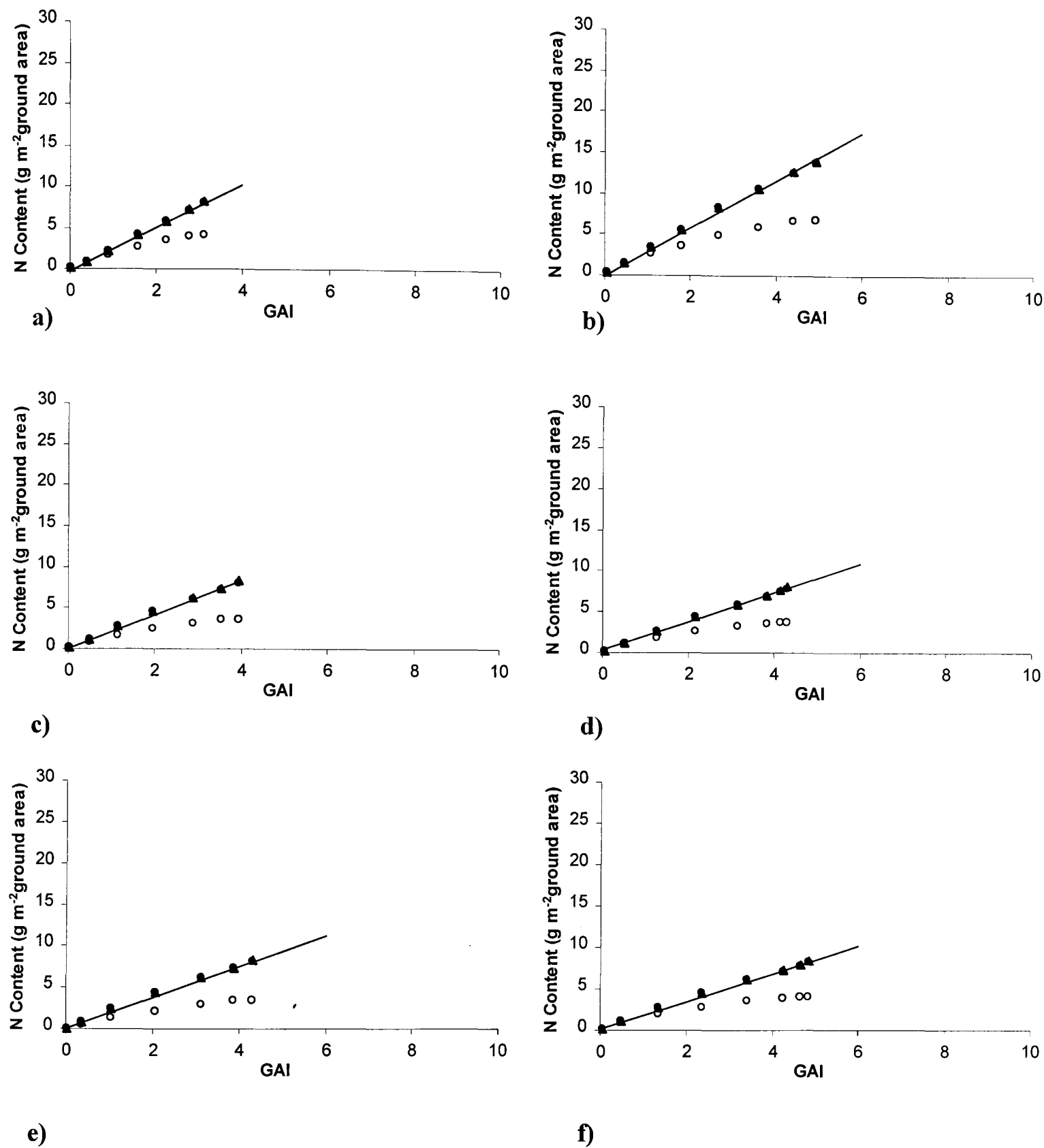
Figure 5.3 The relationship between N accumulation in leaves + stems + non-green tissue (▲), leaves + stems (●) and leaves (○) and depth expressed as accumulated green area index (GAI) (excluding ears), at anthesis in 1999. 124 kg ha^{-1} fertiliser N. a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}. R^2 = 98.7 %. Slopes are presented in Table 5.1.

Table 5.1 Effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on N content per unit green area (g m^{-2}) (total excluding ear) around anthesis, taken from slope of fitted lines in Figures 8.10 – 8.12.

Variety	Seed rate (seeds m^{-2})	1997/8	1998/9 244 kg ha^{-1} N	124 kg ha^{-1} N
SOISSONS	20	2.72	3.01	2.61
	320	2.23	2.43	2.05
	640	2.37	2.25	1.86
SPARK	20	2.89	2.87	2.91
	320	2.07	2.31	1.78
	640	1.92	2.04	1.66
P	**VAR**	0.077	0.01	
	SR	<0.001	<0.001	
	N	n/a	<0.001	
	Var*SR	0.029	0.027	
	Var*N	n/a	0.428	
	SR* N	n/a	<0.001	
	Var*SR*N	n/a	0.013	

Table 5.2 Effect of variety (Var) and seed rate (SR) on the ratio of leaf N content to stem N content per unit ground area on 29 June 1998.

Variety	Seed Rate (seeds m^{-2}) 20	320	640	Mean
SOISSONS	0.89	1.20	0.91	1.00
SPARK	0.92	1.08	1.25	1.08
Mean	0.90	1.14	1.08	1.04

	P	**S.E.D.**	**d.f.**
Var	0.149	0.054	10
SR	0.011	0.066	10
Var*SR	0.016	0.093	10

Table 5.3 Effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on the ratio of leaf N content to stem N content per unit ground area at anthesis in 1999.

N (kg ha^{-1})	Variety	Seed Rate (seeds m^{-2})			Mean
		20	320	640	
124	SOISSONS	1.03	0.82	0.74	0.86
	SPARK	1.03	0.90	0.99	0.97
	Mean	1.03	0.86	0.86	0.92
244	SOISSONS	0.99	0.83	0.75	0.86
	SPARK	0.90	0.96	0.95	0.94
	Mean	0.95	0.90	0.85	0.90
Mean	SOISSONS	1.01	0.82	0.74	0.86
	SPARK	0.97	0.93	0.97	0.96
	Mean	0.99	0.88	0.86	0.91

	P	S.E.D.	d.f.	^{a}S.E.D.	d.f.
Var	<0.001	0.019	10		
SR	<0.001	0.024	10		
N	0.385	0.020	12		
Var*SR	<0.001	0.033	10		
Var*N	0.417	0.028	21.9	0.028	12
SR* N	0.065	0.034	21.9	0.034	12
Var*SR*N	0.270	0.048	21.9	0.048	12

^{a}S.E.D. to compare same level of Var, SR or Var*SR.

5.1.2 N content of individual green area units

The total N content (excluding the ear N) per unit green tissue area of individual green area units with depth expressed as accumulated GAI, on 29 June 1998, is presented in Figure 5.4. The N content changed very little through much of the canopy, except at the bottom where it increased sharply. This was true for all

treatments and was due to the decrease in green area at the bottom. There appeared to be little difference between the varieties in the stable N content of the main part of the canopy. Soissons had a greater N content at the bottom of the canopy where there was slightly more non-green tissue, which caused the increase in the ratio of N content to GAI. In the main part of the canopy, seed rate had the main effect on N content. Canopies sown at the low seed rate had the greatest N content of about 2.6 - 2.7 g m^{-2}, whilst canopies sown at the higher seed rates both had similar N contents of about 2.1 – 2.3 g m^{-2}.

a)   b)
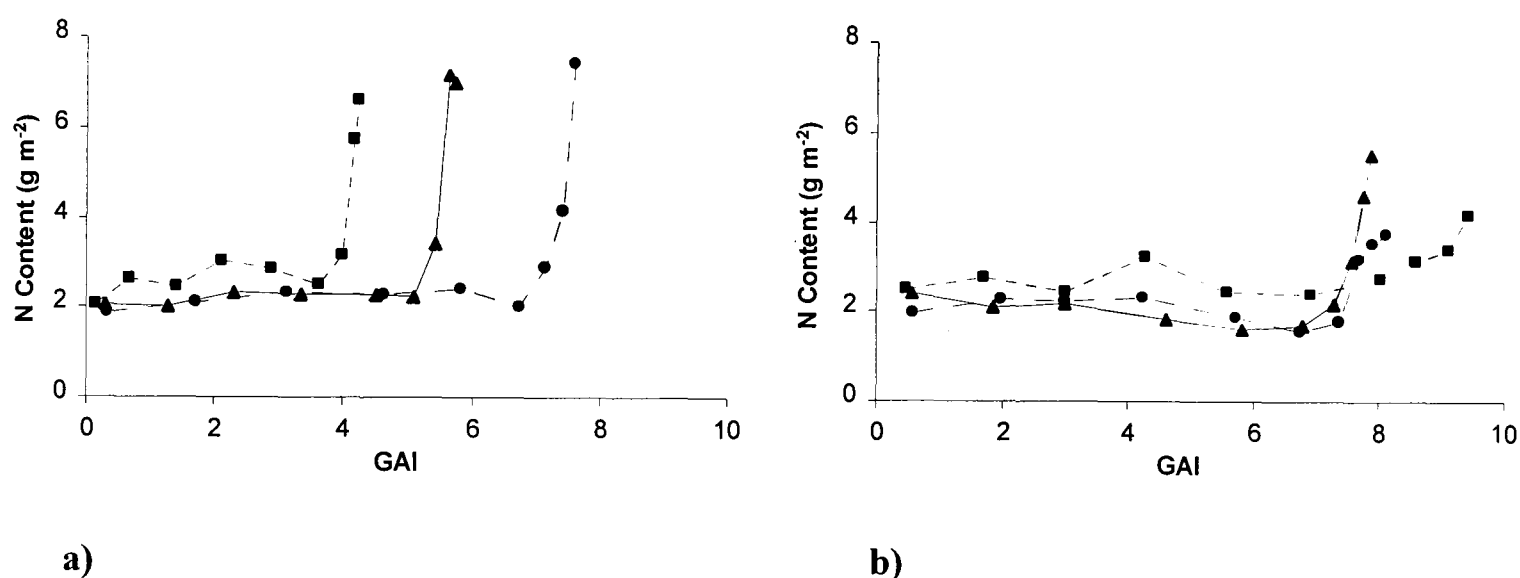
Figure 5.4 Total N (excluding ears) m^{-2} green area (excluding ears) with depth expressed as accumulated green area index (GAI) (including ears), on June 29 1998. a) Soissons and b) Spark sown at 20 (··■···), 320 (- -●- -) and 640 (—▲—) seeds m^{-2}.

The total N content (excluding ears) per unit green area at anthesis in 1999, showed the same general relationship through canopy depth as in the previous year (Figure 5.5). The relatively stable N content observed throughout most of the canopy, was greater than in 1998. There was a smaller increase in N content at the bottom of the canopy compared with 1998. The samples were taken at an earlier stage of development in 1999, with less senescence and N redistribution having occurred. Once again there was little effect of variety on the N content, in the main part of the canopy, but the increase in N content at the low seed rate was clear. Canopies

fertilised with 244 kg ha^{-1} N, and sown at the low seed rate had the greatest N content of around 2.9 g m^{-2}, whilst those at the same N rate but at higher seed rates had an N content of around 2.4 g m^{-2}. As expected, a decrease in fertiliser N application reduced the N content of all canopies. The main part of the canopies sown at 20 seeds m^{-2} had an N content of around 2.5 and 3.0 g m^{-2} in Soissons and Spark, respectively. At 320 and 640 seeds m^{-2}, these decreased to around 2.0 g m^{-2} in both varieties.
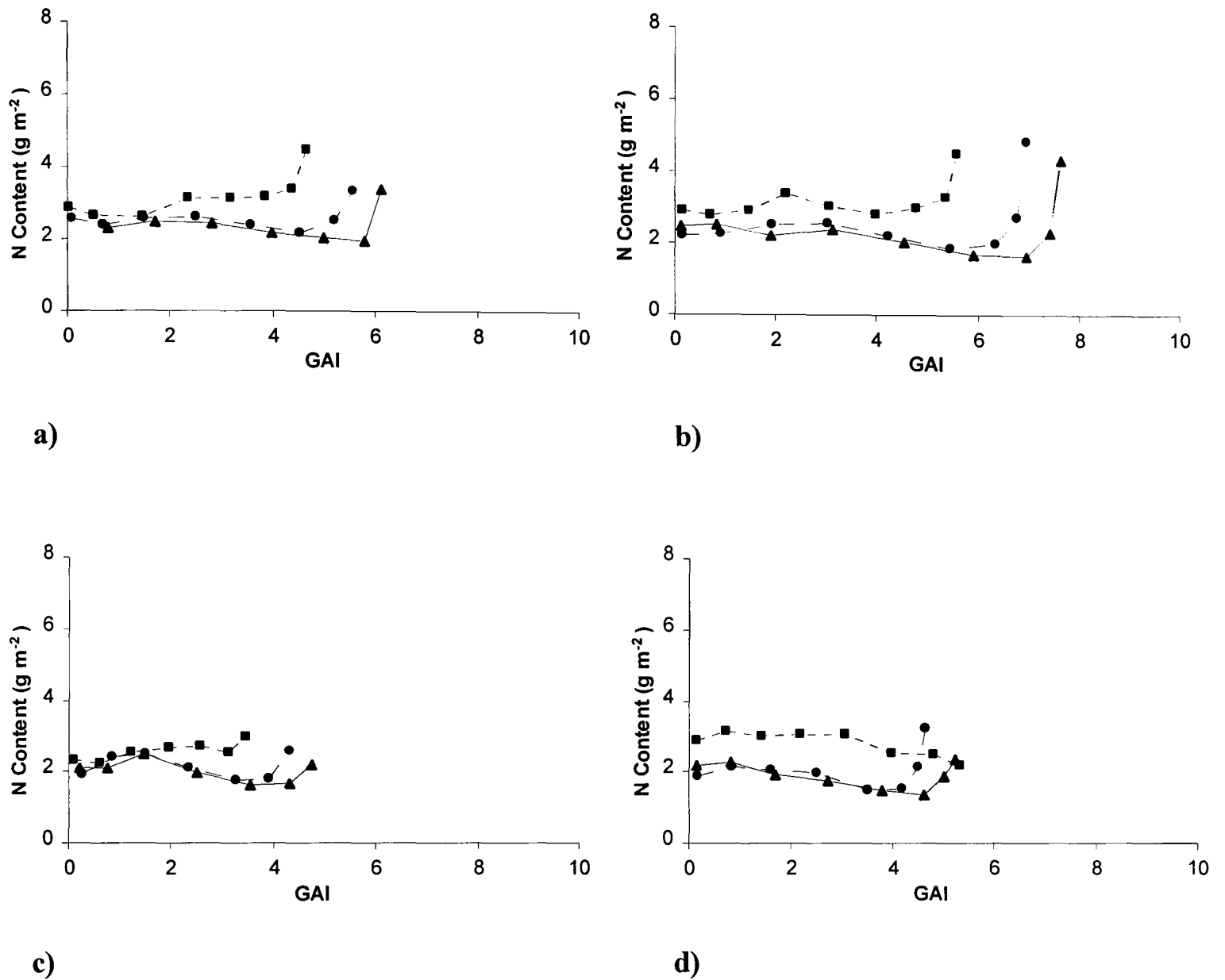
Figure 5.5 Total N (excluding ears) m^{-2} green area (excluding ears) with depth expressed as accumulated green area index (GAI) (including ears), at anthesis 1999 a) Soissons - 244 kg ha^{-1} N, b) Spark - 244 kg ha^{-1} N, c) Soissons - 124 kg ha^{-1} N and d) Spark - 124 kg ha^{-1} N, sown at 20 ("■"), 320 (- -●- -) and 640 (—▲—) seeds m^{-2}.

5.2 CANOPY DEVELOPMENT

Figure 5.6 presents the total canopy N content and GAI measured at several development stages between GS30 and GS71 for Soissons sown at 320 seeds m^{-2} in 1997/8. This shows that the total N content per unit green area and the proportion partitioned to leaves was stable throughout the period of development. This was similar for all canopies.
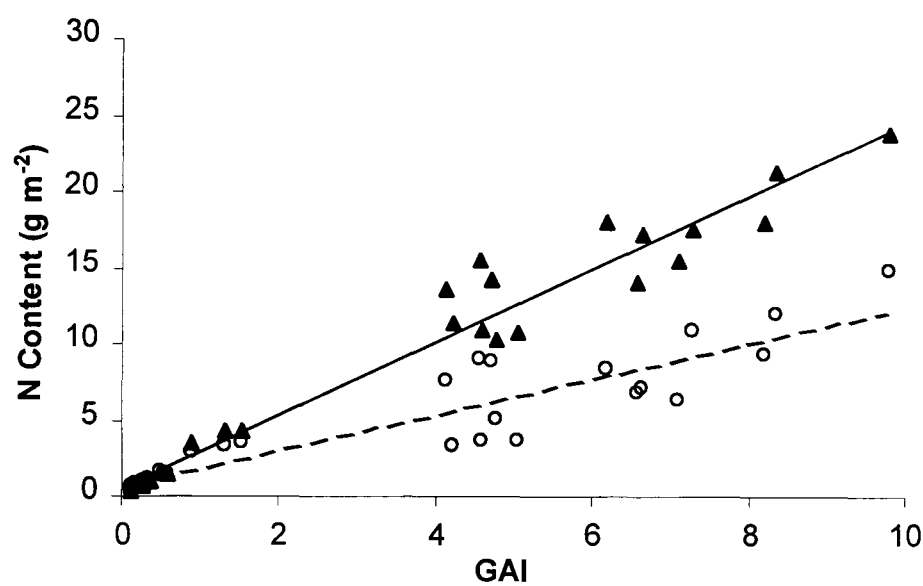
Figure 5.6 The relationship between N accumulation in leaves (O) and in leaves + stems + non-green tissue (▲), and increasing total green area index (GAI) (excluding ears), through development. Soissons - 320 seeds m^{-2}, 1997/8. Fitted lines: y = 0.63 + 1.19x (- - -), R^2 = 85% and y = 0.58 + 2.41x (——), R^2 = 96%, for respective plant components.

Generally, the intercept was not significant and so the slope of the regression has been taken to represent the mean N content per unit GAI. The mean total N content per unit GAI for each treatment is presented in Table 5.4. In both years, the greater N content of the low seed rate observed at anthesis in the previous section was consistent throughout canopy development (P=0.01). Similarly, there was no effect of variety in 1997/8 and Soissons had a greater N content than Spark in 1998/9 (P<0.001). The N

content was also increased with the additional fertiliser N (P<0.001). Also, the actual values for mean N content were similar to those around anthesis.

Table 5.4 Effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on mean N content per unit green area (g m^{-2}) (total excluding ear) throughout crop development.

Variety	Seed rate (seeds m^{-2})	1997/8	1998/9 244 kg ha^{-1} N	124 kg ha^{-1} N
SOISSONS	20	2.47	3.09	2.60
	320	2.41	2.47	2.08
	640	2.46	2.28	1.89
SPARK	20	2.75	3.00	2.89
	320	2.23	2.35	1.82
	640	2.10	2.09	1.68
P VAR		0.267	<0.001	
SR		0.002	<0.001	
N		n/a	<0.001	
Var*SR		0.032	<0.001	
Var*N		n/a	0.402	
SR* N		n/a	0.006	
Var*SR*N		n/a	0.005	

The mean N content per unit green area for each treatment appears to represent the N content measured at each time of sampling throughout the season. However, the N content can be seen in more detail throughout the season in Figure 5.7 and Figure 5.8 where the treatments are compared at each of these sample times.

The time course of the total N content (excluding ears) per unit green area in 1997/8, is presented in Figure 5.7. It appears that there was a large amount of variation throughout the season but the S.E.Ds were also large. Therefore, any change in N

content would not be significant. The N content varied slightly throughout the growing season, beginning at about 2.8 g m^{-2} in November and changed little through the winter. By early spring the N content began to increase, reaching a general maximum of around 3.8 g m^{-2} in March. The N content decreased gradually after May to 2.3 g m^{-2} in July, at the end of the grain filling period. There was generally no effect of variety, except in early spring when Spark had a greater N content than Soissons (P=0.05). The earlier developing Soissons had a larger canopy at this point in the growing season. There was no variety effect at anthesis but an effect on the slope of the regressions has already been shown (Table 5.1), which was thought to be a more sophisticated method of estimating total N content. An effect of seed rate was observed almost constantly throughout the experimental year, although the nature of the effect changed. In the winter, the low seed rate had a significantly reduced N content compared to the two higher seed rates (P<0.001). In December, the N content of the canopies sown at 20, 320 and 640 seeds m^{-2} were 1.99, 2.71 and 2.56 g m^{-2}. After stem extension began in spring, the effect was reversed and the canopies at the low seed rate had a significantly greater N content than the other canopies (P=0.05), through to the end of the sampling period. In July, the N content of the canopies sown at 20, 320 and 640 seeds m^{-2} were 2.48, 2.20 and 2.18 g m^{-2}.

a)   b)
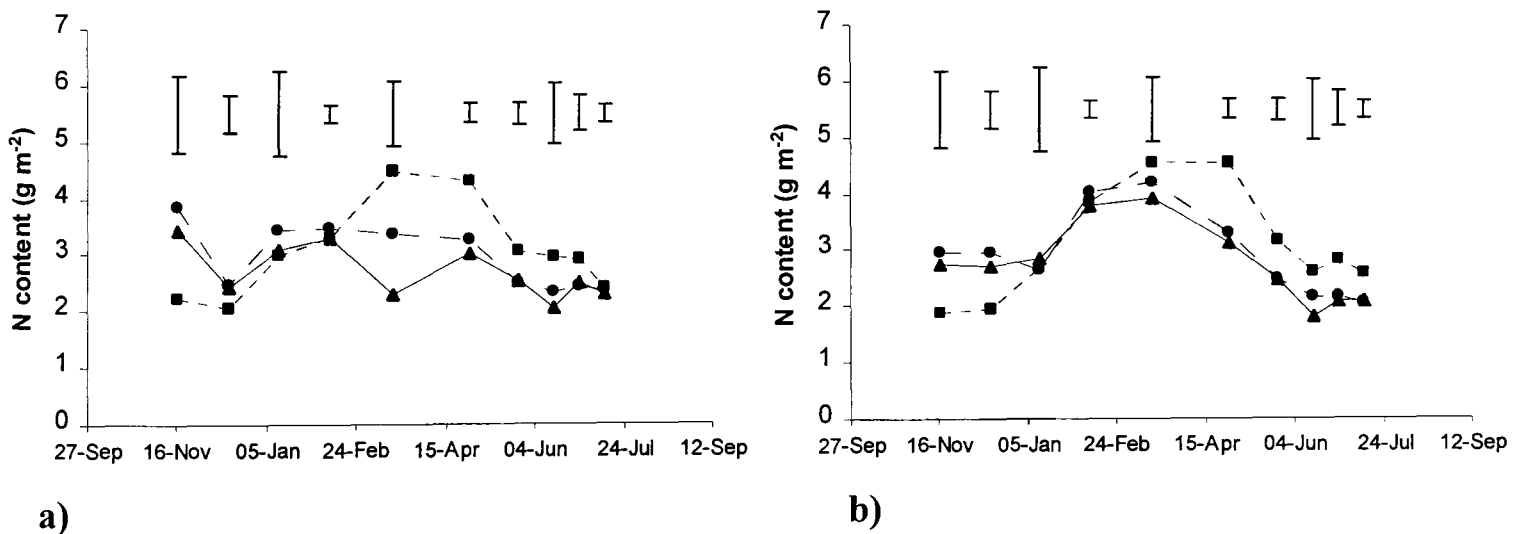
Figure 5.7 The change in total N content (excluding ear) m^{-2} green area (excluding ear) in 1997/8. a) Soissons b) Spark sown at 20 (···■···), 320 (- -●- -) and 640 (—▲—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

The total N content, excluding ears, was more stable through time in the 1998/9 experiment, although there were fewer sample times between March and June than in the previous year (Figure 5.8). It changed very little from around 3.00 g m^{-2} in December through to the early summer. There was a slight decrease by anthesis with a N content of around 2.38 g m^{-2}. These contents were slightly greater than the previous year during the winter, but were lower in the spring. Once again, there was generally no variety effect except in January when Spark had a significantly greater N content (3.31 g m^{-2}) than Soissons (2.66 g m^{-2}) (P=0.05). There was no consistent effect of seed rate on N content in the winter period but from spring onwards, N content was significantly increased with the low seed rate (P=0.01). The effect of decreasing fertiliser N on N content at anthesis has previously been described.

a)   b)
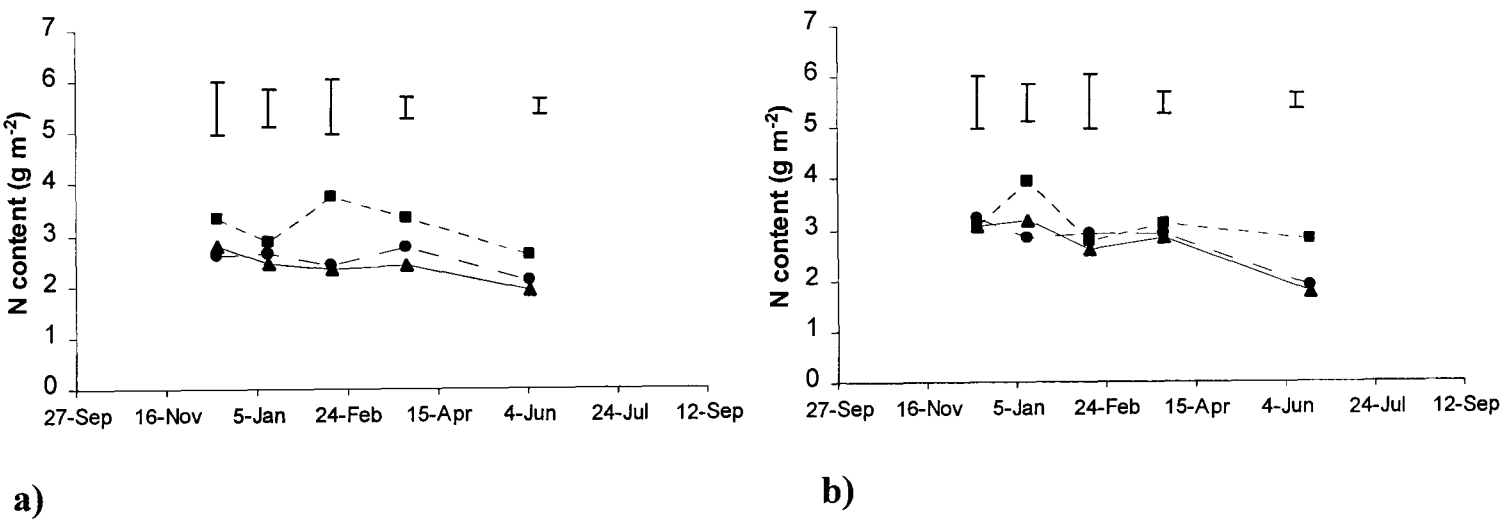
Figure 5.8 The change in total N content (excluding ear) m^{-2} green area (total excluding ears) in 1998/9. 124 kg N ha^{-1} applied. a) Soissons b) Spark sown at 20 ($\cdots\blacksquare\cdots$), 320 (- -●- -) and 640 (—▲—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

5.3 DISCUSSION

This chapter discusses the observations of canopy N content and compares them with the predictions made in Chapter 2. This section aims to test the predictions for variation in CNR within canopies, between canopies and with the development of the canopy.

5.3.1 Testing the prediction for CNR within a canopy

The prediction for variation in N requirement with canopy depth was tested with Spark sown at 320 seeds m^{-2}. It was predicted there would be a relatively stable N requirement throughout most of the canopy but that it would increase markedly at the base. Figure 5.9 shows how this compares to the observed N content with depth in Spark sown at 320 seeds m^{-2} and fertilised with the reduced rate of N. It shows that the actual values and general shape was similar to the prediction but at the base there was not such an increase in N content.
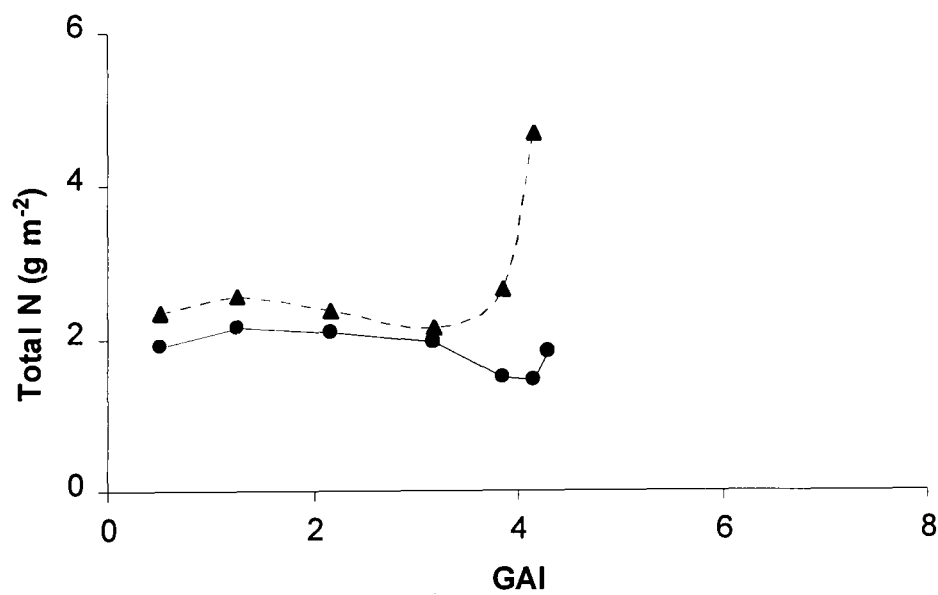
Figure 5.9 A comparison of the predicted N requirement (⋯▲⋯) and the observed N content (—●—) per unit green area with canopy depth (expressed as accumulated green area index; GAI) for Spark sown at 320 seeds m^{-2} and 124 kg ha^{-1} N.

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The N requirement predicted from the canopy characteristics was 2.3 g m^{-2} at the top and 4.7 g m^{-2} at the canopy base with a mean of 2.5 g m^{-2}. Apart from at the base, the observed values were very similar to the predictions. However, the observed mean N requirement of the whole canopy (1.8 g m^{-2}) was slightly overestimated. The comparison shows that the relationship between N requirement and canopy depth could be predicted but that the actual values were overestimated. The relatively constant N requirement throughout most of the canopy suggests that there is some stability in the relationship between N requirement and canopy size. As canopy size increased through depth there was a decreased proportion of total N partitioned to the leaf and increased proportion partitioned to the stem. These results are supported by a similar stable relationship and pattern of partitioning in lucerne, observed by Lemaire & Gastal (1997). The following chapters attempt to explain this stability in CNR throughout the main part of the canopy, as well as the overestimated predicted values of CNR.

5.3.2 Testing the predicted effects of husbandry on CNR

The predictions, made in Chapter 2, for the effect of husbandry on canopy N requirement were tested through the variety and seed rate treatments at the low fertiliser N rate.

The average CNR for all canopies around anthesis was 2.2 g m^{-2}. This is similar to the CNR for Mercia also with a GAI of just above 4 (Stokes *et al.*, 1997) although it is less than the value of 3.0 g m^{-2} previously suggested to be the CNR of winter wheat (Sylvester-Bradley *et al.* 1990b). However, after anthesis, a reduction in N content per unit green area was observed. Therefore, it may be expected that there will be a greater N content per unit green area in the larger canopies measured by Sylvester-Bradley *et al.* (1990b) due to luxury uptake of N or to the earlier growth stage when there was less redistribution of N to the ear and non-green tissue. It is therefore greater than the requirement. The value of 2.2 g m^{-2} observed here is an average value but husbandry effects were detected. These differences are now compared to the predictions.

In Chapter 2, the CNR was predicted to increase with the decrease in seed rate. The results show that the observations agreed with the prediction in both varieties. The CNR increased from 1.9 $g\,m^{-2}$ in canopies sown at 320 seeds m^{-2}, to 2.8 $g\,m^{-2}$ in canopies sown at just 20 seeds m^{-2}. These differences were also consistent throughout the canopy depth. It is proposed that this effect is the result of an increased N requirement for both stem and leaf tissue. There may have also been some luxury uptake of N at the low seed rate but it is thought that this would be minimal and would not explain all of the difference. The same effect of seed rate on N content per unit green area was observed from stem extension and continued through to the end of the season. However, this was not necessarily the CNR. Prior to stem extension, the effect was reversed and there was a greater N content at the higher seed rates. This could have been a result of the difference in canopy size and root development, and therefore the ability to scavenge the available N. It was only once stem extension had begun that the low seed rate took up the same amount of N as the higher seed rates.

Spark was predicted to have the same CNR as Soissons. This was mainly the result of expecting Spark to have a greater stem N requirement but smaller leaf N requirement. Unexpectedly, the results from this project show that Soissons had a greater CNR (2.5 $g\,m^{-2}$) than Spark (2.1 $g\,m^{-2}$). However, this may have been because of the thicker stems of Soissons requiring more structural N, photosynthetic N or the larger ears requiring more mobile N than Spark. In contrast, Foulkes *et al.*, (1998) observed Spark to have a significantly greater CNR than Soissons, over a range of sites and seasons. The mean N contents they measured were greater (2.7 and 3.6 $g\,m^{-2}$ N in Soissons and Spark, respectively) than any of the observed values here, for similar seed rate. This may have been because their samples were taken at earlier development stages when there was less senescence and redistribution of N to the grain, but also because of the likelihood of luxury N. These results suggest that that fertiliser applications may be tailored to the N requirement of the individual variety. The following chapters will attempt to explain the discrepancy between the predicted and observed variety effect.

A more detailed look at the differences in partitioning of N between leaf and stem tissue showed that there was no consistent effect of variety or seed rate or N application. This could indicate that there is a physiological relationship between the

functions of N in the two tissues. The 50% of N partitioned to the stem across all husbandry treatments highlights the importance of this organ as a sink for N. Most of the literature concerning N in plants, has focused on photosynthetic N in the leaves. These results raise the issue of studying N in the stem, if the N requirement of crops is to be fully understood. This is also addressed in the following results chapters.

5.3.3 Testing the predicted effect of canopy development on CNR

The variation in N content throughout the development of the canopy was assessed for each treatment. It was predicted that the CNR of a crop at GS30 and at GS61, would be the same. This was based mainly on a greater leaf N requirement but no stem N requirement at GS30. This was tested by comparing the N content of the canopy before stem extension to the N content of the 'low N' treatment at anthesis. The observations agreed with predictions for all treatments except in Spark at the higher seed rates. Here there was a slight decrease in CNR. Generally, as the canopy expanded and the architecture changed through stem extension and leaf emergence, the N taken up by the canopy was proportional to the green area such that the CNR remained stable. This supports the existence of a physiological relationship between N content and canopy size that could be used in predicting the N required throughout the development of the canopy. Therefore, this indicates that the CNR remains constant throughout the development of the crop.

5.3.4 Summary

This chapter has shown that the average CNR over a range of treatments was 2.2 g m^{-2} but that there was significant variation between treatments. This must be accounted for if the prediction of CNR is to be more precise. As predicted, canopies sown at low seed rates had a greater CNR than higher seed rates and there was generally no effect of canopy development. Unexpectedly, Soissons had a greater CNR than Spark. The observation of a stable CNR through the main part of the canopy and an increase at the base agreed with the prediction, although the value of the CNR was overestimated. The partitioning of N was increased in the stem and decreased in the leaf, with increased canopy size through depth but there was equal partitioning through crop development. The following chapters attempt to explain

these effects and how the principles failed to predict the CNR for some of the treatments. This begins by testing the predictions for the effect of architecture on the light environment within the canopy. Chapter 7 and 8 continue this by testing the predictions for the leaf and stem N requirement.

6 CANOPY ARCHITECTURE AND LIGHT DISTRIBUTION

Predicting the relationship between canopy architecture and light distribution is the first step in predicting canopy N requirement (CNR) from canopy architecture (see Figure 1.6). This chapter provides a detailed description of canopy architecture in terms of crop height and green area. The light distribution within the canopy is used to estimate the light extinction coefficient (k). The mean photosynthetic photon flux density (PPFD) (per unit ground area) within each canopy is estimated from the combination of these characteristics and incident radiation, to help predict the photosynthetic N requirement. The observed effects of canopy depth, husbandry treatment and canopy development on these characteristics are compared to the predicted effects in Chapter 2.

6.1 CROP HEIGHT

The heights of the canopies (including ears) of all treatments are compared in Table 6.1. The ANOVA performed on the data, showed that Spark was 0.08 – 0.12 m taller than Soissons in both years as expected (P<0.001) and also that the low fertiliser N application reduced crop height by 0.06 m (P<0.001). Unexpectedly there was no significant effect of seed rate. The crop was generally taller in the 1997/8 experiment, which may have been because there was also more N uptake per unit ground area. A comparison can also be made to the PGR treated 'bench mark' Mercia crop, which had a height of 0.76 m (including ears) (Sylvester-Bradley et al., 1997b). All canopies were taller than the 'bench mark' crop, except for Soissons with 124 kg ha^{-1} of fertiliser N, applied. The variation in the number of 10cm layers can also be seen from this.

Table 6.1 Comparison of maximum crop height (including ears) of treatment means in the 1997/8 and 1998/9 experiments.

Variety	Nitrogen (kg ha^{-1})	1997/8 (m)	1998/9 (m)
SOISSONS		0.88	0.75
SPARK		1.00	0.83
S.E.D.		0.001	0.007
d.f.		10	10
	124		0.76
	244		0.82
S.E.D.			0.006
d.f.			12

6.2 GREEN TISSUE AREA

One of the most descriptive characteristics of canopy architecture is the green tissue area and its distribution. The development of total green area through the growing season has been described in the previous chapter. The area of the green leaf, stem and ear components with depth through the canopy and the total green area, is now presented for each treatment.

6.2.1 The effect of husbandry on green area distribution of green stem, leaf and ear.

6.2.1.1 1997/8 experiment

Spark had more stem than Soissons (GAI of 2.0 and 1.6) and a significantly greater leaf (lamina) green area (P<0.001) (GAI of 5.7 and 3.7). This effect was due to the increased number of shoots in Spark, as there was no significant difference in leaf or stem area per shoot, between the varieties. Surprisingly, the proportion of leaf to stem area was greater in Spark (P=0.05) (Table 6.2). There was no variety effect on green

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area of ears, which may be due to the greater ear number of Spark counteracted by the greater ear size of Soissons (P=0.01).

Generally, there was no consistent effect of seed rate on stem green area but crops sown at 320 seeds m^{-2} had a greater stem area (GAI of 2.0) than either the lower or higher seed rates (GAI of 1.6 and 1.8, respectively) (P=0.01). This effect may have been due to the decreased stem green area per shoot with the increase in seed rate from 320 to 640 seeds m^{-2}, whilst shoot density at the 320 and 640 seeds m^{-2} were similar. The increase between 20 and 320 seeds m^{-2} was explained by the increase in shoot number. This seed rate effect on stem area was found in Soissons but not Spark. This was due to the unexpected but consistently large canopy size of Spark sown at 20 seeds m^{-2} as well as the greater tillering capacity of Spark. This caused a significant interaction between variety and seed rate in this year (P=0.01). Similarly, there was no consistent effect of seed rate on the leaf green area as the crop sown at 320 seeds m^{-2} had a greater leaf area than either of the other seed rates. This was again due to the decrease in leaf green area per shoot with the increase in seed rate and the similar shoot densities at 20 and 320 seeds m^{-2}. A significant interaction between treatments was also found, which again was due to Spark (P=0.05). Unexpectedly there was no effect of seed rate on the proportion of leaf to stem area. In this experimental year, there was no effect of seed rate on ear green area index which was due to the reduced shoot density compensating for greater ear area at the low seed rate.

The total green area was greater in Spark than Soissons due to the larger shoot density of Spark. There was no effect of variety on total green area per shoot. There was a seed rate effect in Soissons but not Spark, due to reasons previously described in terms of stem and leaf green area. Total green area per shoot generally decreased with increasing seed rate (P=0.01).

Figure 6.1 illustrates the differences in green area down the canopy and between the treatments. The stem green tissue area increased down the canopy to a depth of approximately 0.65 m, below which it decreased. Leaf area also generally increased with depth, reaching a maximum at a depth of about 0.45 m and then decreased gradually with depth. The leaf area was maintained more at depth in the low seed rate.

The slightly greater stem and leaf area per layer due to increased shoot number and the extra layer in Spark explains its greater total stem and leaf area. The smaller stem and leaf area at each layer, due to the lower shoot number explains the reduced total areas in the low seed rate. Ears were generally present in the upper 0.20 m of the canopy.

6.2.1.2   1998/9 experiment

In the 1998/9 experiment, Spark had a significantly greater total stem green area than Soissons (P=0.001) with GAI of 1.4 and 1.2. Again, this was due to the increased shoot number of Spark as there was no significant effect of variety on stem green area per shoot. Spark also had a greater leaf green area (4.1) than Soissons (1.2) (P<0.001). This was due to both increased shoot density and increased leaf green area per shoot of Spark (P=0.05). The proportion of leaf to stem area was greater in Spark, in this year as well (P=0.001) (Table 6.3). In this experiment, Soissons had a slightly greater ear GAI (0.44) than Spark (0.39) (P=0.005). The greater ear green area per shoot of Soissons (P<0.001) over compensated for the greater shoot density of Spark.

There was an effect of seed rate on stem green area (P<0.001) but unlike 1997/8, an increase in seed rate produced a canopy with an increased stem area. This was because of the greater shoot number at the higher seed rate. Stem green area per shoot was reduced at the high seed rate (P=0.01). The mean total stem GAI of canopies sown at 20, 320 and 640 seeds m^{-2} were 1.0, 1.3 and 1.5, respectively. The leaf GAI of crops sown at 20, 320 and 640 seeds m^{-2} was 3.3, 3.7 and 4.0, respectively. This was a significant increase (P=0.05) also due to the increased shoot number over compensating for the decrease in leaf green area per shoot at the higher seed rates (P<0.001). As expected, there was a reduced ratio of leaf to stem area with increasing seed rate (P<0.001) (Table 6.3). Surprisingly, there was no seed rate effect on ear GAI, although there was a decrease in ear green area per shoot with increased seed rate (P<0.001).

Increasing N fertiliser application significantly increased the total stem green area (P<0.001) due to the increase in shoot number. Crops fertilised with 244 kg ha^{-1} N and those with 124 kg ha^{-1} N had a mean stem GAI of 1.4 and 1.1 respectively. The

canopies with the greater fertiliser N application had a significantly greater leaf GAI (4.2 compared to 3.1) (P<0.001). The leaf green area per shoot was also increased (P<0.001). The ratio of leaf to stem area decreased at the reduced fertiliser rate (P<0.001) (Table 6.3). The crop with more N fertiliser had a greater ear GAI compared to the crop with less N (0.46 and 0.38, respectively) (P=0.01), again due to more shoots.

The distribution of stem and leaf green area down the canopies in 1998/9, was similar to 1997/8 with the exception that the green area did not decrease as much at the base of the canopy in 1998/9 (Figure 6.2 and Figure 6.3). The canopies with more N fertiliser had an extra layer due to height and slightly more green area per layer due to an increased shoot number and leaf area per shoot. Ears were present mainly in the upper 0.20 m of the canopy, but also in the next layer down particularly in the low seed rate. The green areas of all components were greater in the 1997/8 experiment, possibly due to the larger supply of available N in the soil, as previously described.

Table 6.2 Effect of variety (Var) and seed rate (SR) on leaf and stem green area index (GAI) as a proportion of total GAI (excluding ears) on June 29 in the 1997/8 experiment.

	Variety	**Seed Rate (seeds m^{-2})**			**Mean**
		20	320	640	
Leaf	SOISSONS	70.0	71.7	67.8	69.8
	SPARK	76.2	72.4	73.6	74.07
	Mean	73.1	72.1	70.7	71.95
Stem	SOISSONS	30.0	28.3	32.2	30.2
	SPARK	23.8	27.6	26.4	25.9
	Mean	26.9	28.0	29.3	28.1

	P	**S.E.D.**	**d.f.**
Var	0.042	1.82	10
SR	0.591	2.23	10
Var*SR	0.432	3.16	10

Table 6.3 Effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on leaf and stem green area index (GAI) as a proportion of total GAI (excluding ears) at anthesis in the 1998/9 experiment.

	N (kg ha^{-1})	Variety	Seed Rate (seeds m^{-2})			Mean
			20	320	640	
LEAF	124	SOISSONS	75.1	71.3	70.2	72.2
		SPARK	75.6	73.7	73.4	74.1
		Mean	75.4	72.3	71.8	73.2
	244	SOISSONS	76.9	74.8	72.1	74.6
		SPARK	75.0	76.3	75.7	76.0
		Mean	76.4	75.6	73.9	75.3
	Mean	SOISSONS	76.0	73.1	71.1	73.4
		SPARK	75.8	74.8	74.6	75.1
		Mean	75.9	73.9	72.9	74.2
STEM	124	SOISSONS	27.9	28.7	29.8	27.8
		SPARK	24.4	26.3	26.6	25.9
		Mean	24.7	27.7	28.2	26.9
	244	SOISSONS	23.1	25.2	27.9	25.4
		SPARK	24.0	23.7	24.3	24.0
		Mean	23.6	24.5	26.1	24.7
	Mean	SOISSONS	24.0	26.9	28.9	26.6
		SPARK	24.2	25.2	25.5	24.9
		Mean	24.1	26.1	27.2	25.8

	P	S.E.D.	d.f.	[a]S.E.D.	d.f.
Var	0.002	0.41	10		
SR	<0.001	0.50	10		
N	<0.001	0.42	12		
Var*SR	0.016	0.71	10		
Var*N	0.499	0.58	21.85	0.59	12
SR* N	0.154	0.72	21.85	0.72	12
Var*SR*N	0.681	1.01	21.85	1.02	12

[a]S.E.D. to compare same level of Var, SR or Var*SR.
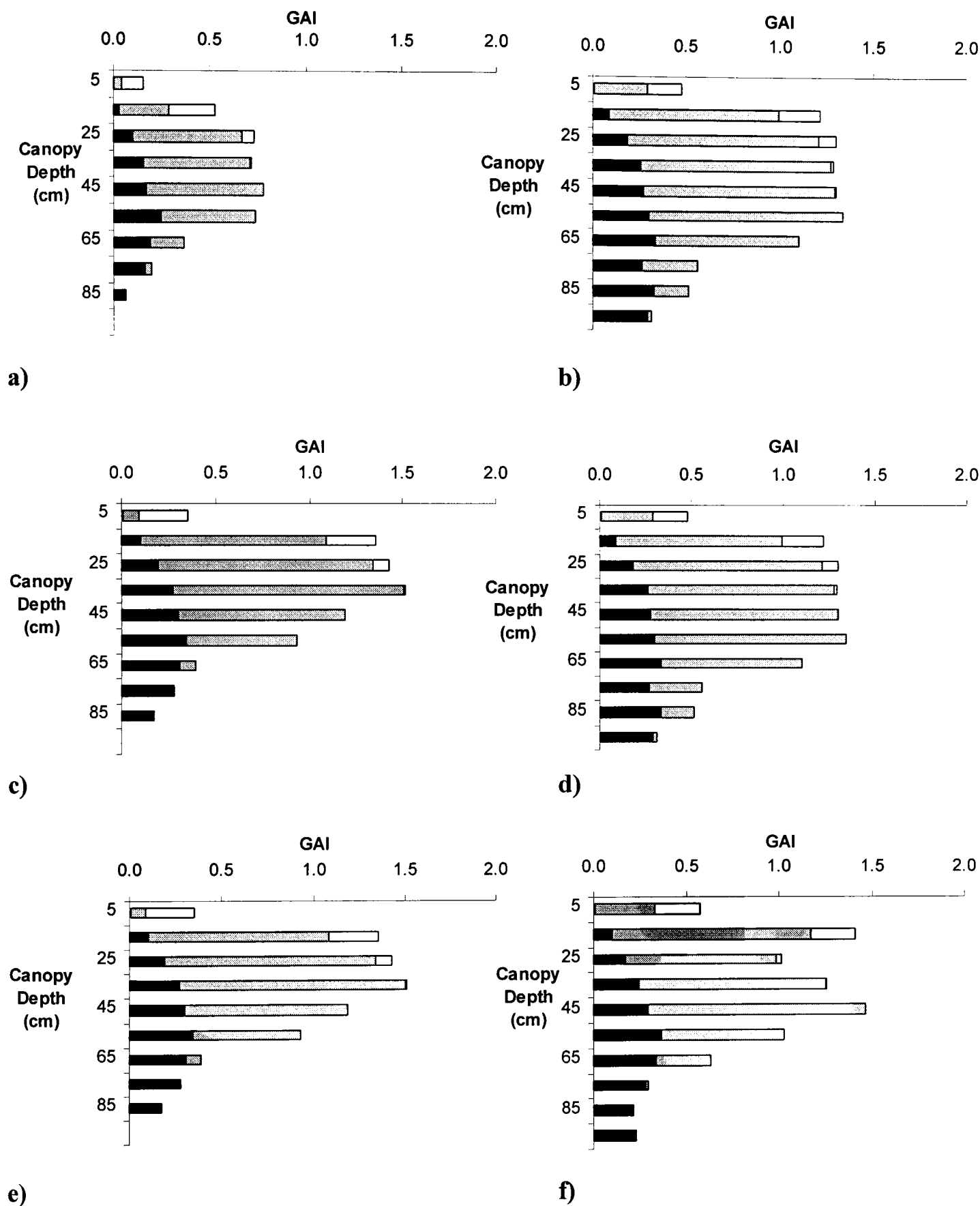
Figure 6.1 The distribution of green tissue area (expressed as green area index; GAI) between the components: stem (■), leaf (▨) and ear (□), down the canopy on June 29 of the 1997/8 experiment; a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}.
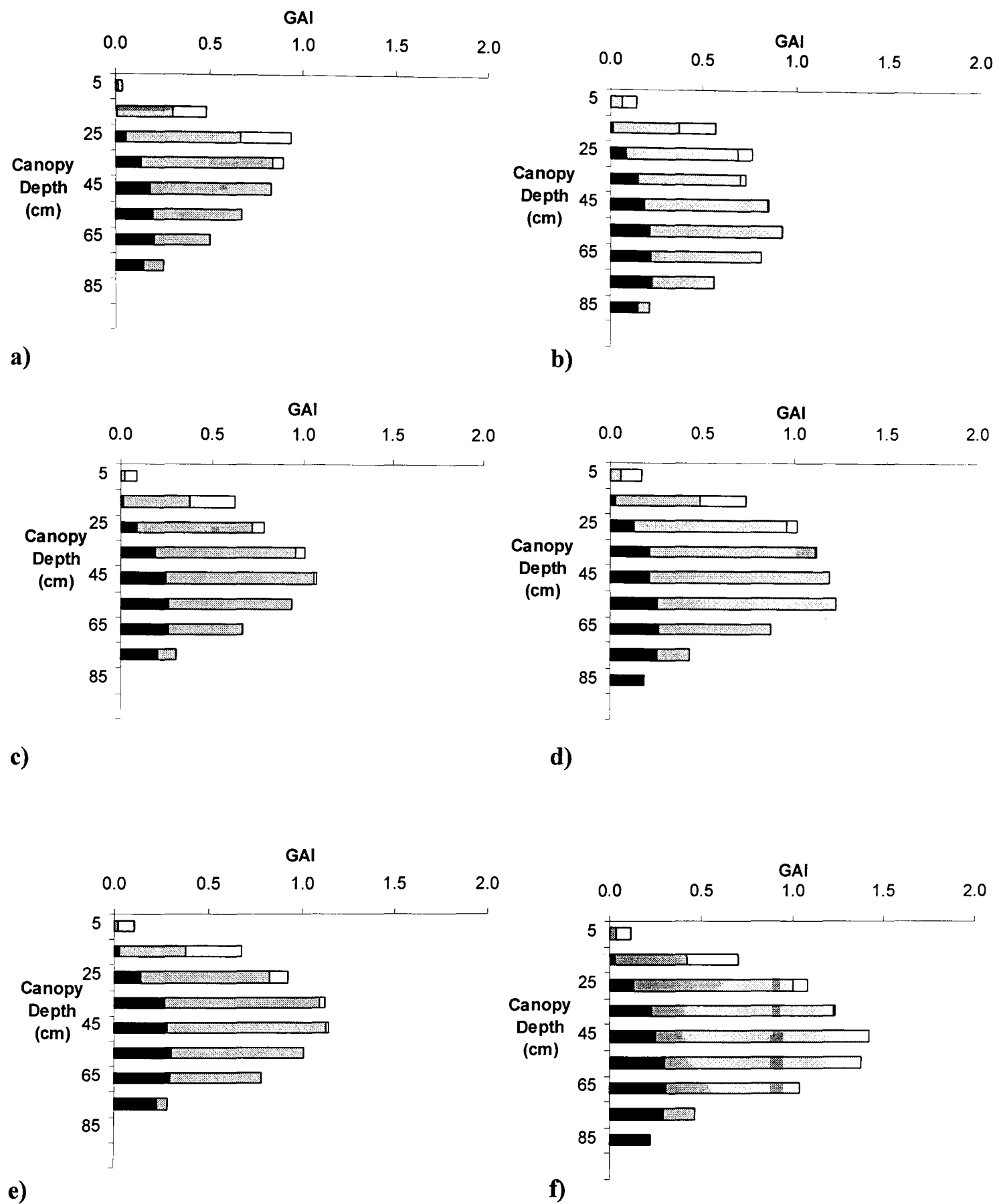
Figure 6.2 The distribution of green tissue area (expressed as green area index; GAI) between the components: stem (■), leaf (▨) and ear (☐) down canopies with 244 kg ha^{-1} fertiliser N, at anthesis in the 1998/9 experiment; a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}.
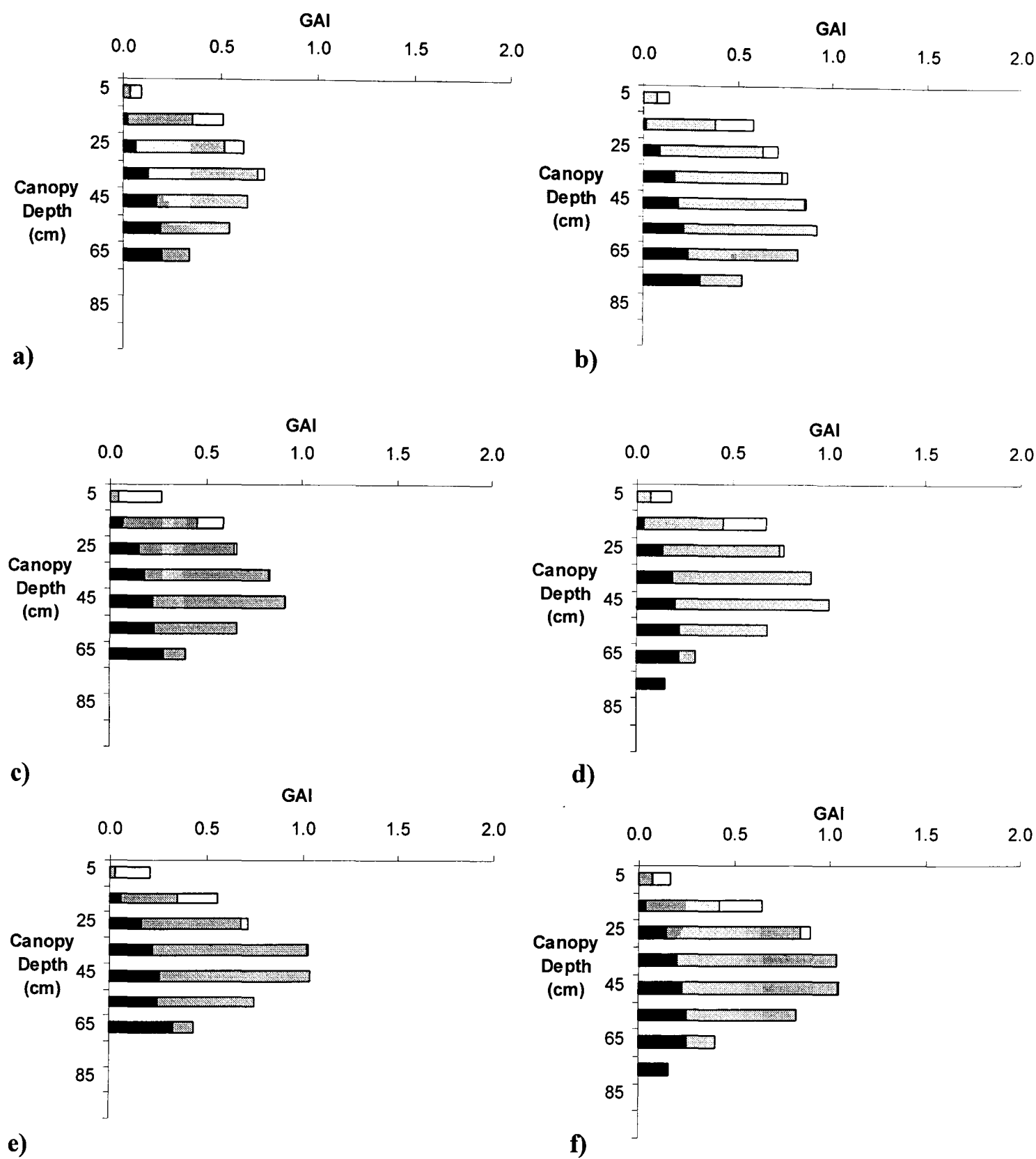
Figure 6.3 The distribution of green tissue area (expressed as green area index; GAI) between the components: stem (■), leaf (▨) and ear (☐) down canopies with 124 kg ha⁻¹ fertiliser N, at anthesis in the 1998/9 experiment; a) Soissons – 20 seeds m⁻², b) Spark – 20 seeds m⁻², c) Soissons – 320 seeds m⁻², d) Spark 320 - seeds m⁻², e) Soissons – 640 seeds m⁻² and f) Spark – 640 seeds m⁻².

6.2.2 Conventional and corrected green area

Green area is conventionally determined by measuring the projected area of the crop components. This is a suitable method for leaf area, as it is generally a thin, two-sided flat organ with the upper side being the main intercepting surface. In most cases, the area of the stem and the ear is determined in the same way, with just the projected area of one side being measured. The stem is cylindrical and the ear is more of a cuboid shape with indentations due to the spikelets. Clearly, to understand the physiological N requirement, the true intercepting surface of the ear and the stem must be considered. Two investigations were carried out to estimate the error in measuring the projected area of the stem and ear and to suggest a correction factor for each component.

6.2.2.1 Stem green area

Table 6.4 presents the stem (attached leaf sheath and true stem) green area (cm^2) of individual shoots determined by three methods (projected area, flattened area of the leaf sheath and cylindrical surface area calculated from the projected area). As expected, there was a significant increase in the projected green area of a single stem with a decrease in seed rate (P=0.01). Surprisingly, there was no significant effect of seed rate on the flattened green area of the attached leaf sheath, suggesting that there may have been more overlap at the higher seed rate. The effect of seed rate on overlap was found to be significant (P=0.05). There was no overlap at the low seed rate, where as at 320 seeds m^{-2}, 10% of the cylindrical stem area was overlapped by leaf sheath. On average, the conventional projected area of stem was only 31% of the true sheath area. This figure significantly decreased with increased seed rate (P=0.05). The calculated total stem surface area based on a cylinder (calculated by multiplying the projected area by π) was not significantly different from the flattened sheath area, despite the overlap at the high seed rate. This suggests that a correction factor of 3.142 may be used to calculate the total cylindrical stem green area from the projected stem and leaf sheath area. It could be used to estimate k more precisely, by representing the true area of the leaf sheath that would be intercepting the light.

The true stem (with the leaf sheath detached) was pale green, indicating that some of the N was photosynthetic. As expected, the projected area of the true stem was greater

at the lower seed rate (P=0.01) (Table 6.5). The total surface area of the true stem was calculated as the surface area of a cylinder. The conventional projected area of stem and leaf sheath was on average 41% of the true stem surface area. Seed rate did not significantly affect this. To calculate the surface green area of the true stem from the conventional projected area of the stem and attached leaf sheath, a correction factor of 2.44 (the reciprocal of 41 %) might be used. Combined with further investigation into relationships concerning stem N, this could be used in predicting the N requirement for structural or transport compounds in the true stem.

Table 6.4 Comparison of methods used to measure individual leaf sheath (LS) green area and relationship to the attached true stem (S) and leaf sheath projected area.

Seed rate (seeds m^{-2})	Projected stem (S+LS) (cm^2)	Leaf sheath (LS) (cm^2)	Cylindrical stem (S+LS) (cm^2)	Projected stem (S+LS) (% LS)
20	28.5	88.5	89.6	32.3
320	24.0	82.9	75.5	29.2
MEAN	26.3	85.7	82.6	30.7
S.E.D	1.22	3.66	3.82	1.20
d.f.	18	18	18	18

Table 6.5 Comparison of methods used to measure individual true stem (S) green area and relationship to attached true stem and leaf sheath (LS) projected area.

Seed rate (seeds m^{-2})	Projected stem (S+LS) (cm^2)	Projected true stem (S) (cm^2)	Cylindrical true stem (S) (cm^2)	Projected stem (S+LS) (% cylinder s)
20	28.5	22.8	71.5	40.0
320	24.0	18.4	57.6	42.9
MEAN	26.3	20.6	64.6	41.4
S.E.D	1.22	1.39	4.36	2.42
d.f.	18	18	18	18

6.2.2.2 *Ear green area*

The mean projected area of the ears examined (Spark sown at 320 seeds m^{-2}) was 7.6 cm^2 (Table 6.6). The total area of the separated but intact spikelets was 43.3 cm^2 and the total area of the separated components of the spikelets (glumes and florets), was 49.3 cm^2. This dissection of the ear showed that the conventional method of projected area measurement was only 18 % of the total area calculated by separating spikelets, and was reduced slightly to 16 % when the total area of all the separated spikelet components were also measured. The method of measuring the area of ear components was generally satisfactory, although there were occasions when the area may have been overestimated. This was due to the component not being held firmly enough to prevent the rolling bar on the green area machine from pushing it over to the side, or due to it being squashed and thus increasing the surface area. The results suggest that a correction factor of 5.6 (reciprocal of 18 %) is required to convert the projected ear area to the true green area. This could be used to increase the precision of estimating *k*.

Table 6.6 Comparison of methods of measuring ear green area.

	Projected area (cm^2)	Spikelet area (cm^2)	Component area (cm^2)	Projected as % Spikelet area	Projected as % component area
MEAN	7.6	43.3	49.3	17.9	16.0
S.E.	1.25	10.92	14.22	2.20	3.05

6.3 LIGHT EXTINCTION COEFFICIENT (*k*)

This section describes the photosynthetic photon flux density (PPFD) within the canopy for each 10cm layer and the effect of husbandry treatment, canopy depth and canopy development on the light extinction coefficient (*k*). The light profile through the canopy is presented for each husbandry treatment around anthesis. The light fluxes were transformed by taking the natural log (ln) to carry out an ANOVA on the fitted lines. The slopes of the fitted lines represent the light extinction coefficient (*k*). These are presented in Table 6.7. The equations used to estimate *k* for each layer and for the whole canopy in examining the effect of canopy development are provided in section 3.8 of Chapter 3.

6.3.1.1 Effect of husbandry on the light extinction coefficient

Figure 6.4 presents the exponential decrease in light flux (per unit ground area) with increased canopy depth expressed as accumulated GAI for the 1997/8 experiment (P<0.001). Variety and seed rate significantly affected the distribution of PPFD within the canopy (P<0.001). The light flux available per unit GAI was greater in Spark than in Soissons (P<0.001) due to the greater light extinction in Soissons (P<0.001), which had more prostrate leaves and therefore less penetration. The mean value of *k* for Spark and Soissons was 0.45 and 0.63 respectively. Canopies at 320 and 640 seeds m^{-2} experienced reduced light flux per unit GAI (P<0.001) and greater light extinction (0.55 and 0.62, respectively) (P<0.001) compared with the 20 seeds m^{-2} (0.43).

a)

b)

c)

d)
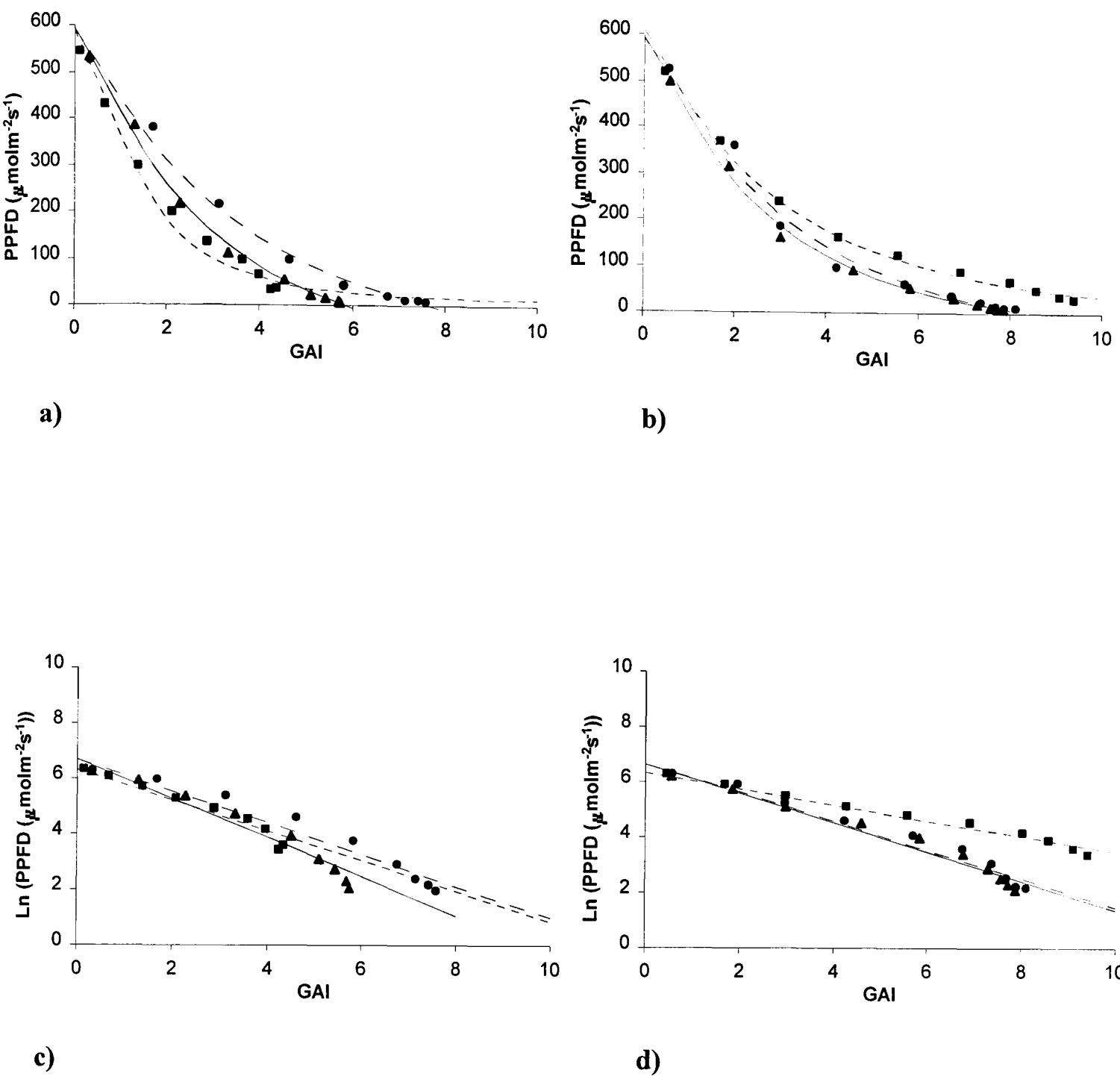
Figure 6.4 The decrease in photosynthetic photon flux density (PPFD) with canopy depth (expressed as accumulated green area index; GAI (including ears)). 29 June 1998. a) Soissons - untransformed b) Spark – untransformed, c) Soissons - ln transformed) and d) Spark - ln transformed). Sown at 20 (■), 320 (●) and 640 (▲) seeds m^{-2}. R^2 = 99% (fitted curves) and 94% (fitted lines) for 20 (....), 320 (-----) and 640 (——) seeds m^{-2}. Slopes presented in Table 6.7.

The exponential decrease in PPFD with canopy depth expressed as accumulated GAI (P<0.001) in 1998/9, is shown in Figure 6.5 and Figure 6.6. In this year, the decrease at the bottom of the canopy was less than in the previous year, possibly due to the larger canopy sizes in 1997/8 causing greater mutual shading. The light flux was generally greater in Spark canopies (P<0.001) and the difference was seen particularly within canopies fertilised with less N. There was a seed rate effect (P<0.001), in which the canopies sown at 20 seeds m^{-2} experienced greater light fluxes. Canopies sown at the two higher seed rates and fertilised with more N had similar light fluxes, except in Spark with low fertiliser N rate, where the canopy sown at 320 seeds m^{-2} had greater light fluxes than expected. This caused the interaction between variety and seed rate (P=0.01). Canopies fertilised with more N experienced significantly lower light fluxes within the canopy (P<0.001). The rate of light extinction was generally greater in Soissons (P<0.001) with a mean k value of 0.45 compared to 0.41 in Spark. The light extinction coefficient was also increased (reduced light penetration) with the increase in seed rate (P<0.001). The mean k values were 0.33, 0.42 and 0.48 in the canopies sown at 20, 320 and 640 seeds m^{-2}. There was significantly reduced light extinction with greater N application (increased light penetration) (P=0.05). The values of k for canopies with 124 and 244 kg ha^{-1} N applied were 0.44 and 0.42. There was also an interaction between variety, seed rate and N (P<0.001) which may have been caused by the greater decrease in light within Spark sown at the high seed rate and fertilised with less N. Generally, there was a greater difference between varieties and seed rates at the lower N application. These findings were generally consistent with the previous year.
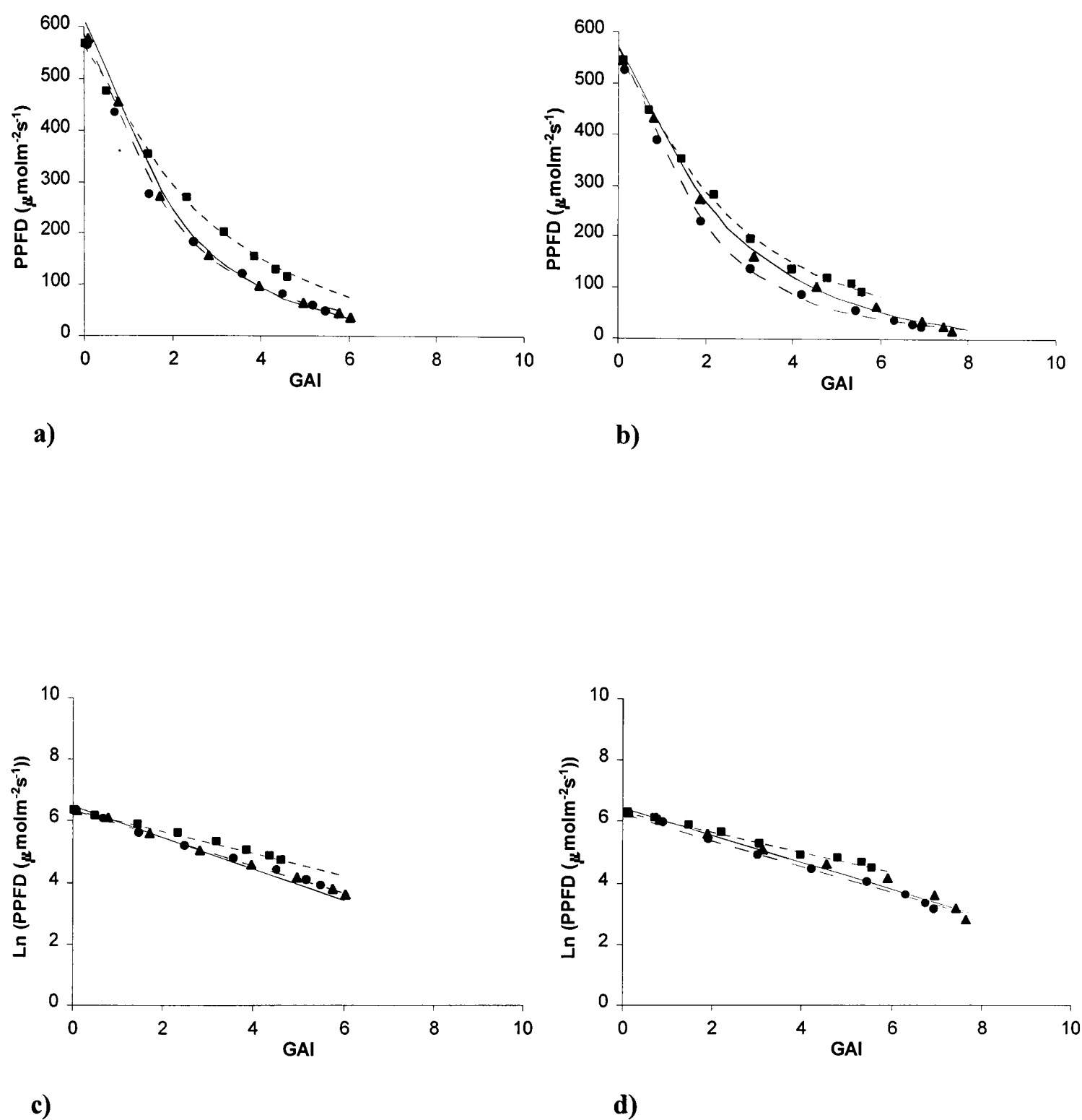
Figure 6.5 The decrease in photosynthetic photon flux density (PPFD) with canopy depth (expressed as accumulated green area index; GAI (including ears)), with 244 kg N ha^{-1}. Anthesis 1999. a) Soissons - untransformed b) Spark - untransformed , c) Soissons - ln transformed) and d) Spark - ln transformed). Sown at 20 (■), 320 (●) and 640 (▲) seeds m^{-2}. R^2 = 96% (fitted curves) and 91% (fitted lines) for 20 (....), 320 (-----) and 640 (——) seeds m^{-2}. Slopes presented in Table 6.7.
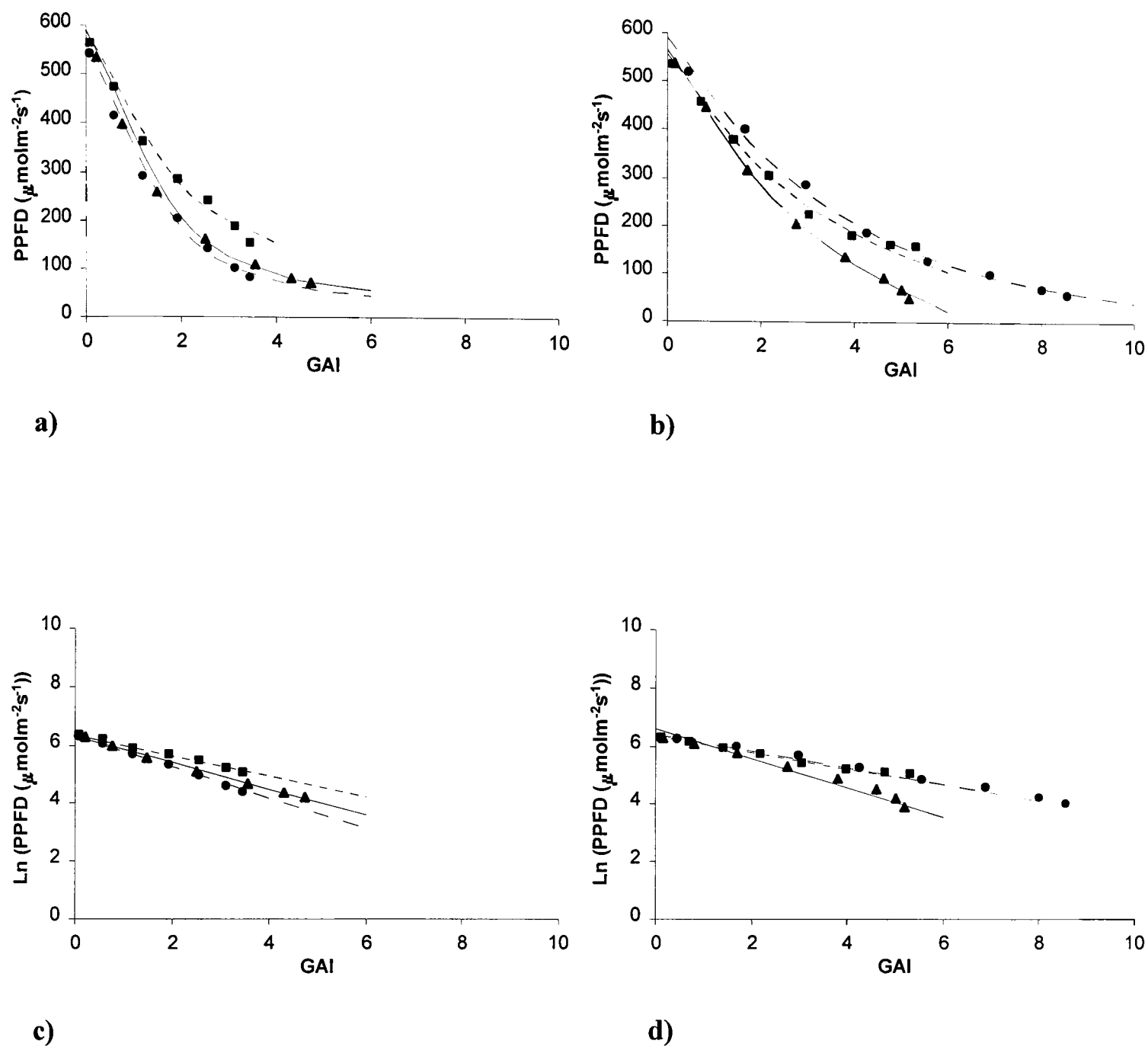
Figure 6.6 The decrease in photosynthetic photon flux density (PPFD) with canopy depth (expressed as accumulated green area index; GAI (including ears)) with 124 kg N ha⁻¹. Anthesis 1999. a) Soissons - untransformed b) Spark - untransformed , c) Soissons - ln transformed) and d) Spark - ln transformed). Sown at 20 (■), 320 (●) and 640 (▲) seeds m⁻², at. R² = 96% (fitted curves) and 91% (fitted lines) for 20 (....), 320 (-----) and 640 (——) seeds m⁻². Slopes presented in Table 6.7.

Table 6.7 Effect of variety (Var), seed rate (SR) and N fertiliser rate (N) on light extinction coefficient (k) around anthesis, taken from the slopes of the fitted lines in Figures 6.4 – 6.6.

Variety	Seed rate (seeds m^{-2})	1997/8	1998/9	
			244 kg ha^{-1} N	124 kg ha^{-1} N
SOISSONS	20	0.56	0.36	0.36
	320	0.58	0.45	0.54
	640	0.73	0.51	0.46
SPARK	20	0.29	0.35	0.28
	320	0.52	0.43	0.46
	640	0.53	0.42	0.52
P	VAR	<0.001	<0.001	
	SR	<0.001	<0.001	
	N	n/a	0.021	
	Var*SR	<0.001	0.121	
	Var*N	n/a	0.401	
	SR* N	n/a	<0.001	
	Var*SR*N	n/a	<0.001	

6.3.1.2 Effect of canopy depth on the light extinction coefficient

The change in light extinction coefficient with canopy depth, expressed as accumulated GAI is presented in Figure 6.7. ANOVAS performed on each 10cm layer showed that Soissons generally had a higher k than Spark (P<0.001). Surprisingly, there was generally no significant effect of seed rate or any interaction between variety and seed rate. The means of the varieties over all seed rates have therefore been presented. The top of the canopy generally had a high k that decreased initially with depth. Soissons generally had a greater k throughout the canopy due to the more prostrate nature of its larger leaves as observed in the field. The greatest difference of 0.4, was around an accumulated GAI of 5. The presence of awns on the ears of

Soissons could also account for *k* being greater at the top of the canopy where there was little leaf tissue. Here, Soissons had a mean *k* of 0.76 and Spark 0.52. At the bottom of the canopy *k* increased slightly, which was possibly due to the increase in non-green tissue intercepting light. There was also greatest variation within each treatment. On average only 6% of the total biomass was non-green tissue and so was considered not to be significant overall.
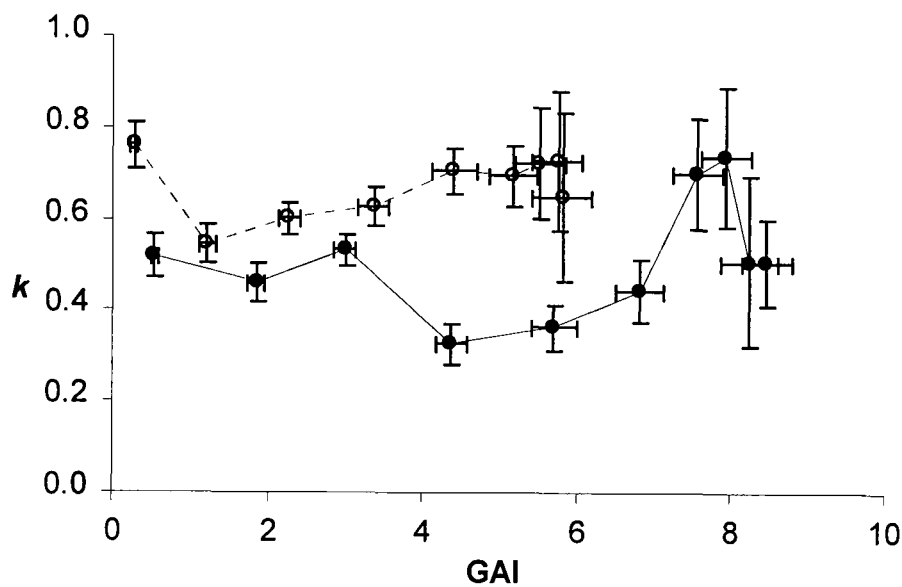
Figure 6.7 The change in *k* with canopy depth (expressed as accumulated green area index; GAI (including ears)) of Soissons ("O") and Spark (—●—), on 29 June in the 1997/8 experiment. Mean of all seed rates. Error bars represent variety S.E. for each layer.

Figure 6.8 shows the change in *k* with canopy depth expressed as accumulated GAI, at anthesis in the 1998/9 experiment. Surprisingly, there was no significant effect of any of the treatments on *k* at each 10cm layer and so the mean of all treatments is presented. The absence of any effect might have been due to the methodology applied causing large variation and inconsistency within treatments and between each layer. The errors appear small as values of *k* greater than 1 were set to the maximum value of 1. For a *k* value greater than 1, the GAI would be less than 1 which would not fully cover the ground. Therefore these values have been limited to the maximum of 1. The varieties were sampled at the same developmental stage in this experimental year,

unlike the previous year. The change in *k* with depth was similar to the previous year in that the top of the canopy had a high value of *k* (0.8) which decreased to 0.4 for the main part of the canopy. The bottom layer increased to a surprisingly high value of 0.75 that was greater than in the previous year although there was actually less non-green tissue (2.5% total biomass).
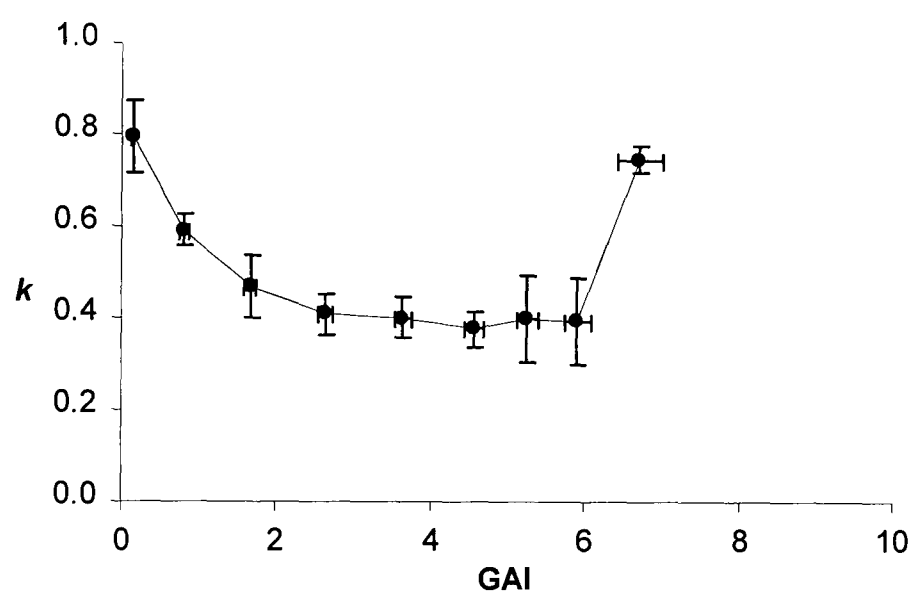
Figure 6.8 The change in *k* with canopy depth (expressed as accumulated green area index; GAI (including ears)) at anthesis in the 1998/9 experiment. Mean of all treatments. Error bars represent variety S.E. for each layer.

6.3.1.3   Effect of canopy development on the light extinction coefficient

The average light extinction coefficients of the canopies throughout the 1997/8 experiment are presented in Figure 6.9. Persistent rain prevented light measurements and therefore the estimation of *k* values, for some of the sample dates. Conditions were dry enough for the light in only one block to be measured on 26 May and so there is no S.E.D. for this date. There was much variation in the values for *k*, and in particular within the canopies sown at 20 seeds m^{-2} and during earlier months in the season when the canopy was small. It was therefore not unexpected that there was much error due to the size of the canopy. In theory canopies smaller than a GAI of 1 can not be evenly distributed, covering the entire surface of the ground. In practice

they would therefore not have met the assumptions of homogeneity in Beer's Law. For this reason, the *k* values of the low density crop in November, January and March, have been omitted. During the autumn and winter early tillering period there were no treatment effects on *k* or any interactions. The value of *k* at this time was about 0.54. In March, once canopy expansion had begun, there was a significant effect of seed rate (P=0.05) The canopies sown at 20 seeds m^{-2} had a smaller *k* value than those sown at 320 and 640 seeds m^{-2} (0.40, 0.62 and 0.60, respectively). However, there were again no significant effects in April, although in this season the mean *k* of all canopies was at its lowest (0.44) in this month. At the final measurement made in July, Soissons had a significantly greater *k* value (0.69) than Spark (0.55) (P=0.05). There was also a significant effect of seed rate at this time (P=0.05), when canopies sown at 20 seeds m^{-2} had a smaller *k* than at 320 and 640 seeds m^{-2} (0.45, 0.69 and 0.71, respectively).

a)

b)
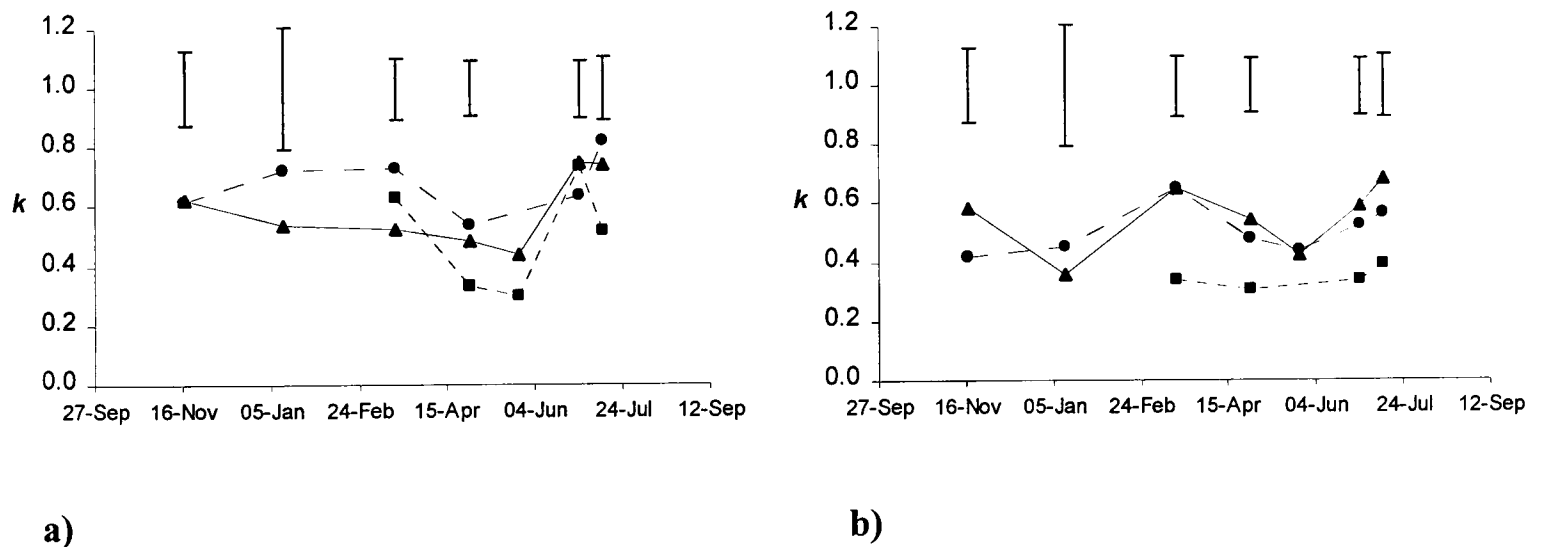
Figure 6.9 The development of the light extinction coefficient (*k*), in 1997/8. a) Soissons and b) Spark sown at 20 (···■···), 320 (--●--) and 640 (─▲─) seeds m^{-2}. Error bars represent S.E.D., d.f. = 5 (Jan), 6 (Nov- March), 8 (June) and 10 (July).

During the autumn winter and early spring period of the 1998/9 experiment, there was an effect of seed rate on *k* (P=0.05) (Figure 6.10). The canopies sown at 320 seeds m^{-2} had a larger mean value of *k* than the other canopies, suggesting that they had more

prostrate leaves. There were no effects other than this throughout the winter and spring, until March. During the winter, the mean value of k decreased from 0.43 to an extremely low 0.19, with much variation. After canopy expansion had begun in March, as expected, Soissons had a significantly larger k than Spark (P=0.01). As in the previous year, there was a significant effect of seed rate on k, in March. However, the nature of the effect was reversed. In 1999, the canopies sown at 20 seeds m^{-2} had a larger k than the other canopies. A comparison of values between the years showed no consistency. There was no N treatment introduced until after these sample dates and so the only sample with which the effect of N could be examined, was anthesis (described in Figure 6.10). However, with this method of estimating k, there was no significant effect of N at anthesis. There were also no effects of variety or seed rate, unlike the method of estimating k through regression analysis. There was a significant interaction between variety, seed rate and nitrogen. The very nature of the variation and inconsistency of these results suggests that either k was extremely variable, that the method of measurement was not satisfactory, or that the theory of Beer's Law was not always obeyed.

a)

b)
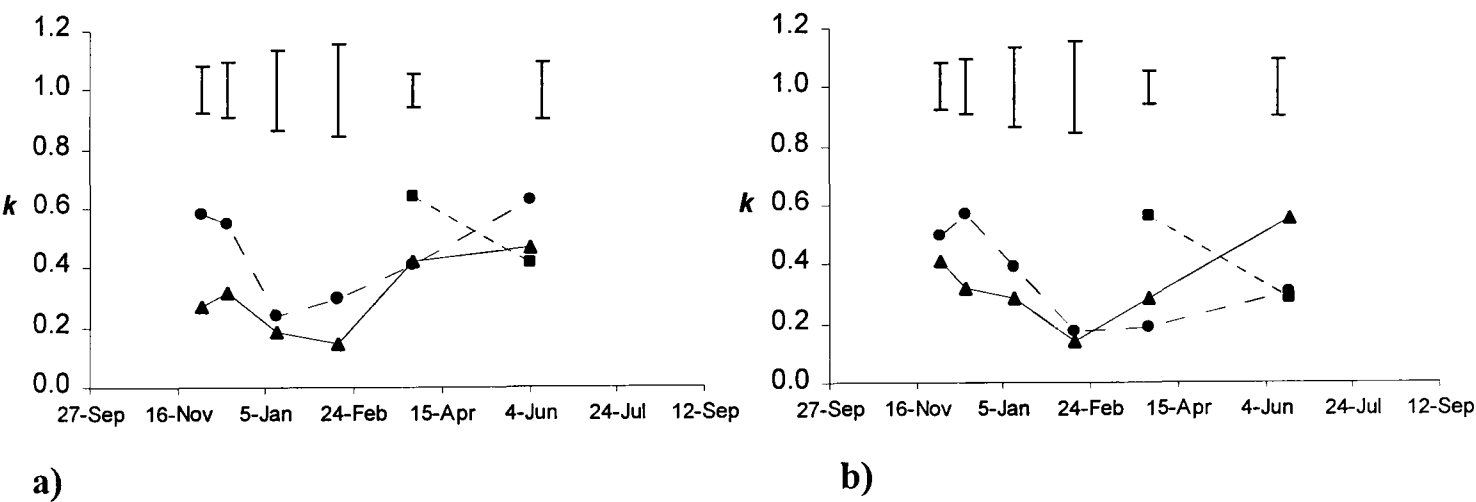
Figure 6.10 The development of the light extinction coefficient (k) in 1998/9. 124 kg N ha^{-1} applied. a) Soissons and b) Spark sown at 20 (···■···), 320 (--●--) and 640 (—▲—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 6 (Nov-Feb), 10 (March-June).

6.4 PHOTOSYNTHETIC PHOTON FLUX DENSITY

The effect of husbandry treatment and canopy development on mean PPFD (per unit ground area) within the whole canopy is described in this section. The mean PPFD was estimated for each of the canopies. This reflects the combination of the light extinction coefficient, canopy size and the incident radiation. The method of estimating this is described in Section 3.8.2. It could be used to predict mean leaf N content and then CNR from canopy architecture.

6.4.1 Effect of husbandry on mean PPFD

Soissons and Spark experienced a similar mean PPFD within the whole canopy (176 and 156 μmol m^{-2} s^{-1}, respectively). The smaller canopy size of Soissons (due to smaller shoot number) was compensated by the more prostrate nature of the leaves, with respect to Spark. Despite the absence of a seed rate effect on total green area index, there was a significant effect on mean light flux (P=0.05) due to the seed rate effect on the value of k. Crops sown at 320 and 640 seeds m^{-2} experienced similar light fluxes of 158 and 155 μmol m^{-2} s^{-1}, respectively but those at 20 seeds m^{-2} experienced a mean of 187 μmol m^{-2} s^{-1}. There was no interaction between treatments. Figure 6.11 shows the mean PPFD within the canopies.
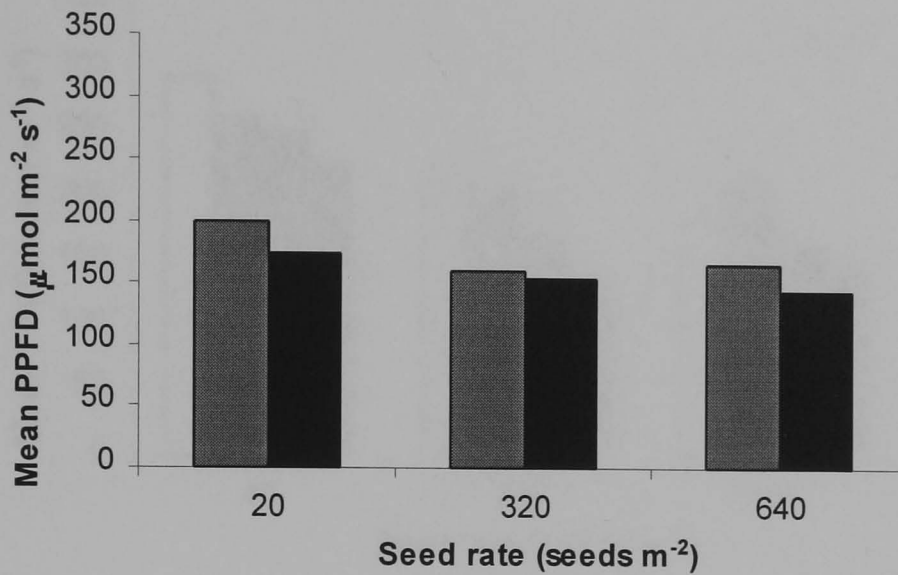
Figure 6.11 Mean photosynthetic photon flux density (PPFD) (per unit ground area) in Soissons (▨) and Spark (■) canopies on 29 June in the 1997/8 experiment. S.E.D = 16.6, d.f. = 10.

Mean light fluxes within the canopies at anthesis of the 1998/9 experiment were greater than the previous year (Figure 6.12). Incident radiation was slightly greater in 1999 at this time of sampling, but it is more likely that this was due to the smaller canopy sizes in general and therefore less shading. The effect of variety and seed rate was consistent with the previous year. Soissons experienced similar mean light fluxes to Spark (216 and 202 μmol m^{-2} s^{-1}, respectively). As expected, the effect of seed rate was highly significant (P<0.001). Canopies sown at 320 and 640 seeds m^{-2} again experienced similar light fluxes of 185 and 180 μmol m^{-2} s^{-1} respectively and those sown at 20 seeds m^{-2} had a mean of 261 μmol m^{-2} s^{-1}. Canopies fertilised with 244 kg ha^{-1} N allowed less light to penetrate due to their greater shoot numbers increasing canopy size and higher k value, and therefore had a smaller mean light flux (187 μmol m^{-2} s^{-1}) than the smaller crops fertilised with 124 kg ha^{-1} N (231 μmol m^{-2} s^{-1}) (P<0.001).
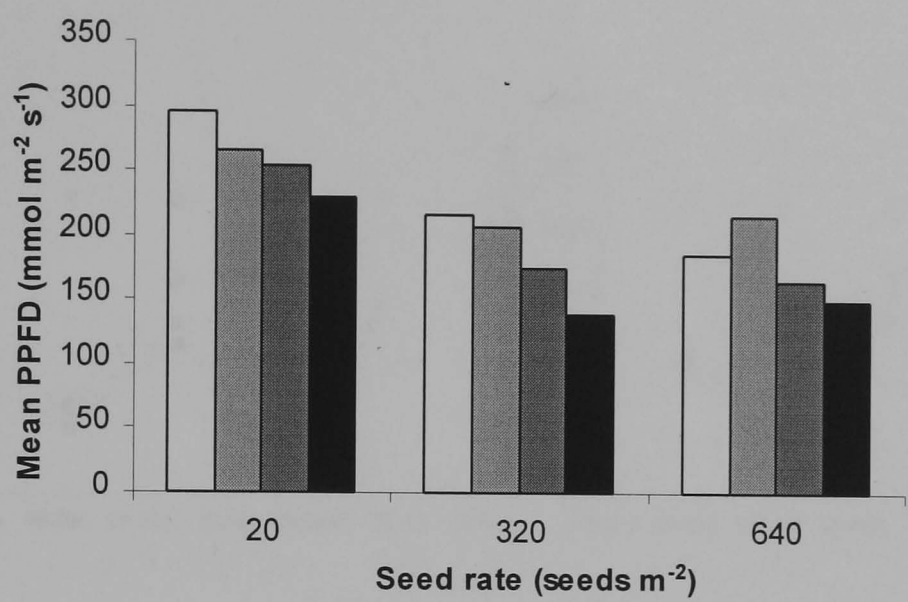
Figure 6.12 Mean photosynthetic photon flux density (PPFD) (per unit ground area) in Soissons - 124 kg ha^{-1} N (▢), Spark - 124 kg ha^{-1} N (▨), Soissons - 244 kg ha^{-1} N (▨) and Spark - 244 kg ha^{-1} N (■) canopies at anthesis in the 1998/9 experiment. S.E.D = 26.09, d.f. = 20.91.

6.4.2 Effect of canopy development on mean PPFD

The mean light flux within the canopy throughout the year followed a similar pattern to incident radiation through the autumn, winter and spring months (Figure 6.13). Therefore there was a decrease in mean PPFD during the autumn and early winter followed by an increase during the spring. Throughout this period, Spark experienced greater light fluxes within the canopy than Soissons (P<0.001), because of the larger canopy resulting from faster rate of development in Soissons. Mean PPFD decreased significantly with increasing seed rates (P<0.001). After April, canopy expansion was rapid and the mean PPFD decreased as mutual shading increased despite the increasing incident radiation. There was no effect of variety, but the seed rate effect remained (P<0.001), with the lowest seed rate experiencing much greater mean light flux. This effect was maintained, although differences reduced, throughout the rest of the season. During July, when there was much senescence and therefore rapid

decrease in canopy area, the mean PPFD in the canopy increased as a result of reduced shading and high incident radiation.

a)   b)
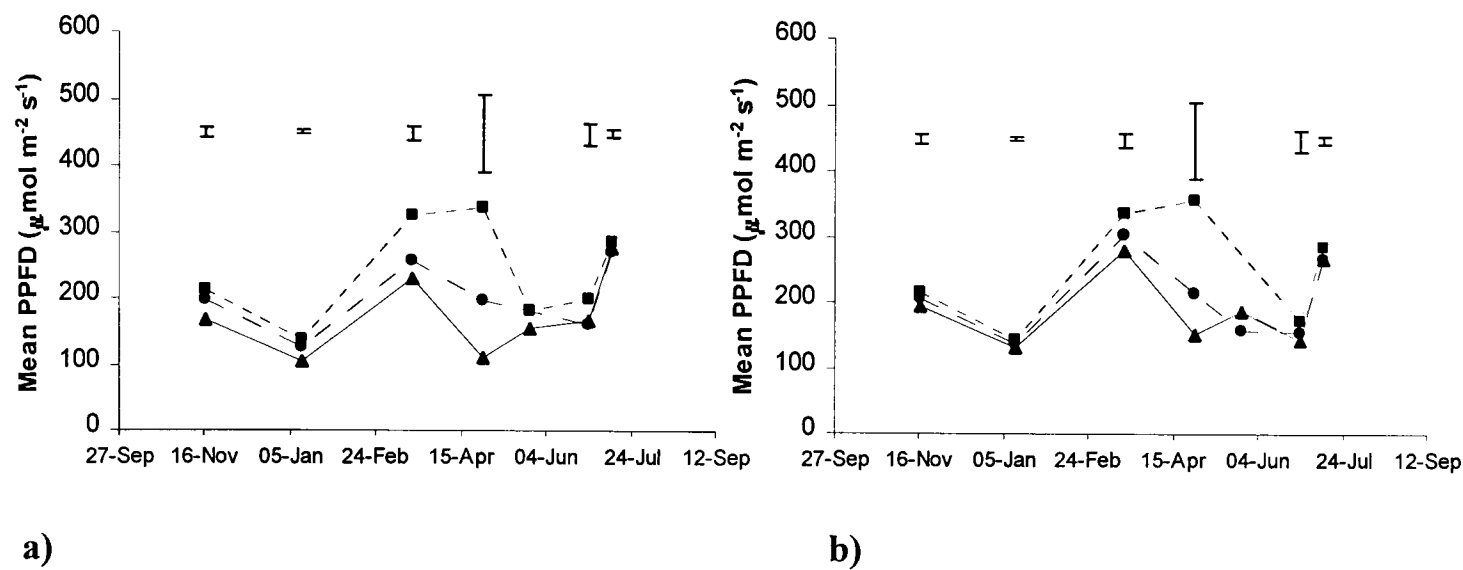
Figure 6.13 Mean photosynthetic photon flux density (PPFD) (per unit ground area) in 1997/8. a) Soissons and b) Spark sown at 20 (·¨■¨·), 320 (--●--) and 640 (—▲—) seeds m⁻². Error bars represent S.E.D., d.f. = 10 (Nov-April, July) and 8 (June).

The pattern of mean light flux within the canopy during the 1998/9 experiment (Figure 6.14) was different to the previous year, in that there was no decrease during the winter. This was surprising, as it was expected that for this period of slow canopy expansion, mean PPFD per unit ground area would follow incident radiation. In this experiment, there were no treatment effects until February. During the beginning of canopy expansion, Spark experienced greater light fluxes, due to the smaller canopy size at that time (P<0.001). There was also a seed rate effect (P<0.001), in which light flux was reduced with increased seed rates, as expected. However, there was an interaction in February when the samples taken for Soissons sown at 320 seeds m⁻², were particularly low. Mean light within the canopy had decreased greatly by anthesis, due to the size of the canopy being near maximum. At this time, there was no variety effect. The effect of seed rate remained (P<0.001), but the difference between the canopies sown at 320 and 640 seeds m⁻² was reduced, as expected. Canopies fertilised with 244 kg ha⁻¹ N experienced less light within the canopy than those fertilised with 124 kg ha⁻¹ N (P<0.001).
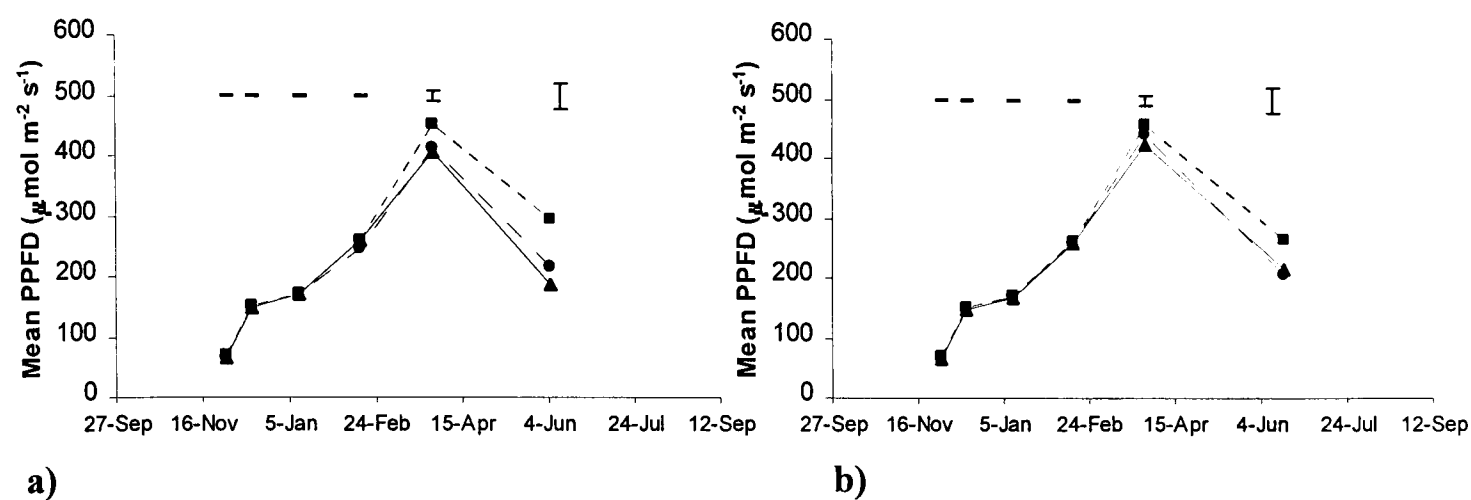
Figure 6.14 Mean photosynthetic photon flux density (PPFD) (per unit ground area) in 1998/9. 124 kg N ha^{-1} applied. a) Soissons and b) Spark sown at 20 ($\cdots\blacksquare\cdots$), 320 (--●--) and 640 (—▲—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

6.5 DISCUSSION

This chapter has described canopy architecture, light distribution and light flux in relation to canopy depth, husbandry treatment and canopy development. The observed effect of these factors is now compared to the predictions made in Chapter 2.

6.5.1 Light Distribution

There is a light gradient within canopies, where leaves at the top of a canopy experience greater light fluxes than the shaded leaves at the bottom of the canopy. The decline of light with canopy depth has been described by an exponential model for many crops including faba bean (Del Pozo & Dennett, 1999), *Solidago altissima* (Hirose & Werger, 1987b) and a range of C_3 and C_4 mono- and dicotyledonous crops (Anten, Shieving & Werger, 1995b). The results from this chapter have shown that the distribution of photosynthetic photon flux density within the wheat canopies can be described by an exponential model following Beer's Law, and supports evidence from Grindlay *et al.* (1997). This study provides evidence for the validity of Beer's Law, however, there are exceptions which will be discussed later in this section. According to Beer's Law, the exponential distribution of PPFD within a canopy is governed by the light extinction coefficient, **k** (Monteith, 1965). Generally this is assumed to be constant with depth (Szeicz, 1974; Anten *et al.*, 1998; Dreccer *et al.*, 2000) and so a value for the whole canopy was estimated through Beer's Law. Generally, the low seed rate experienced greater light fluxes at any one depth. Beer's Law assumes that this was due to the greater transmission through each leaf layer, but fails to take into account the light within the large gaps found at this seed rate. This will be returned to, later. A more detailed analysis for each layer showed that estimated values of **k** were not stable throughout the canopy depth, which will also be discussed later. The predictions for husbandry and crop development effects on mean PPFD within the canopy are now compared to the observations.

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6.5.2 Mean PPFD of the whole canopy

It was predicted that the mean PPFD within the canopy would be greater in Soissons than Spark. Unexpectedly, there was little difference in mean PPFD between the two varieties and therefore the mean leaf N requirement is expected to be similar for both varieties. The same is also expected for the photosynthetic N requirement of the leaf sheath. It was predicted that the greater shoot density of Spark and therefore green area, would overcompensate for the greater k of Soissons, so that Soissons would experience a greater mean light flux per unit ground area. The observations agreed with the qualitative predictions for the difference in GAI and k between the varieties. This suggests that although the principles might be sufficient for predicting the qualitative differences in GAI and k, the next step in predicting the combined effect of these parameters is not precise. It was difficult to predict a value for an extinction coefficient and it is clear that the main factor controlling k is genetic (Angus, Jones & Wilson, 1972; Monteith, 1990). The difference in k was underestimated. For a more precise prediction of the PPFD in the canopy a value for k is required.

As predicted the mean light flux within the low seed rate canopy was greater than in the high seed rates. This can be explained by the smaller size of the canopy but also to the unexpected reduced light extinction coefficient at the low seed rate. A higher leaf (and leaf sheath) N requirement is be expected in the lower seed rate and low N crop where light flux is greatest. An attempt is made, in the next sub-section, to explain the reduced k at the low seed rate.

The mean light flux within the canopy increased through the winter, spring and early summer, with increasing incident radiation. When the canopy had expanded enough to cause significant mutual shading, the mean light flux decreased as expected. This continued until senescence caused a decrease in the canopy size and allowed greater light transmission. This suggests that the change in light flux with increased canopy size through time was not the same as the change with depth, due to the effect of the increase in incident radiation.

The difference in mean PPFD within the canopy between treatments depends on the extent of the treatment effect on k and GAI. As the estimation of k and its effect on

PPFD appears to be the main cause of the discrepancies between the predictions and the observations, this will now be discussed.

6.5.3 Light extinction coefficient (k)

Many of the differences between observations and predictions in canopy architecture and light distribution appear to be because Beer's Law does not apply for all situations and because of the practical errors in estimating k. The results show that k generally increased at the top and the bottom layers of the canopy. There are a number of possible explanations for these observations.

Firstly, the true light intercepting area of the ear is often underestimated when the projected area is measured. The projected area of one side of an ear was found to be approximately 18% of the total surface area of all the components of an ear (glumes and florets). There will also be reflection and transmission between and within the ear components. This underestimation of ear green area might partly explain why the light extinction coefficient is larger in the upper layers of the canopy (Thorne *et al.*, 1988), particularly seen in the 1998/9 experiment. The same might also be true of the green stem, as suggested by Monteith (1990). Detailed experimental work carried out here, suggested a correction factor of x 5.6 for the ear and x 3.142 for the stem (leaf sheath area). The light extinction coefficient was re-calculated for each layer in a range of canopies using these correction factors separately as well as combined to determine the effects (data not shown). Correcting the ear green area reduced the value of k to below 1, but not in Soissons at the low seed rate. The ear green area of Soissons could have been underestimated more than Spark because of the presence of awns. Awns intercept up to 9% of visible radiation (Olugbemi, Austin & Bingham, 1976) but were not detected by the green area machine in this investigation. Therefore, the green area of the awns could not be measured, but may be significant enough to justify an increase in the correction factor for awned varieties. The main effect of the corrected stem green area was to reduce the value of k to less than 1 at the bottom the canopy at both high and low seed rates. The combination of corrected ear and stem green area had a stabilising effect on k throughout the canopy with a value that appeared biologically possible (<1), although there is still some doubt concerning the distribution of foliage at the low seed rate (discussed later). It is also

likely that not all sides of the ear and stem will be in direct sunlight at any one time, which must be considered further before implementing any correction factors. Therefore, further modifications to the correction factors are required. Angus *et al.*, (1972) also obtained *k* values greater than 1 at the top of the barley canopy which they disregarded, attributing them to experimental error.

Secondly, sunfleck is the fraction of radiation transmitted by a unit leaf layer without interception (Monteith, 1965) and is dependent on leaf angle. It is reported to decrease exponentially with depth to an accumulated GAI of 6 (Szeicz, 1974). The range according to this exponential equation is from 0.8 at the top of the canopy and 0.5 at the bottom of a canopy with a GAI of 6. The decrease in sunfleck would result in a larger value of *k* with depth but this was generally not observed, except at the canopy base were *k* increased markedly. However, sunfleck was not measured directly in these experiments and so cannot be accounted for.

Thirdly, the presence of non-green senesced leaf material at the bottom of the canopy (2.5 – 6 % total biomass), might contribute to the increase in *k* at the bottom of the canopy, along with the underestimated stem GAI. The difficulty in inserting the ceptometer probe between the shoots at the bottom meant that it was not always positioned horizontal to the ground. This would cause an overestimation in the amount of light intercepted and therefore an increased *k*. Taking all of these considerations into account, it is difficult to have confidence in some of the estimations of *k* for some of the layers in the canopy. The value obtained through regression analysis is thought to be a more reliable method, as it is a more sophisticated approach using individual data points.

The slopes of the regressions in Figures 6.4 – 6.6 showed that *k* decreased with the reduction in seed rate. However the validity of Beer's Law in estimating the value of *k* at the low seed rate is questionable. A uniform coverage of ground area by green area is assumed for Beer's Law to be used to estimate *k*. At the low seed rate, for individual layers and particularly early in the growing season when GAI was less than 1, there could not be full ground cover and so this assumption could not be made. At low seed rates, wheat maintains more tillers per plant, than at high seed rate, and

leaves could be more erect because of reduced space within any one plant. However, the outer tillers would have more space and therefore leaves would be more prostrate. A balance between these effects may mean that overall, there might not be a seed rate effect on k, as observed in a monocotyledonous perennial herb by Schieving and co-workers (see Del Pozo & Dennett, 1999). At very low seed rates, such as 20 seeds m^{-2}, it was expected there to be more N available per plant and therefore larger leaves containing more N. It might be more reasonable to expect a greater k due to larger heavier leaves becoming more prostrate as well as a reduced transmission coefficient, such as in the faba bean (Del Pozo & Dennett, 1999). The large variation at early growth might also be due to the invalidation of Beer's Law. Early canopies also have a clumped dispersion of foliage, whereas Beer's Law assumes random dispersion (Nilson, 1971). The difference is in the gap frequency or the probability that the incident light reaches the soil (Baret et al., (1993), which will be greater at a lower seed rate and early growth. This could lead to an underestimation of k as the light reaching the ground has not passed through an area where the leaf tissue is present. This could account for the low k at low seed rate, but k was not always smaller at the beginning of the season. Gap frequency can be used to estimate the sunfleck (Daudet & Tchamitchian, 1993). This must be accounted for if the light extinction coefficient is to be estimated more precisely. Nilson (1971) proposed that a negative binomial model and a form of the Markov Chain model were able to describe the gap frequency in clumped foliage. Baret et al., (1993), supported the use of the Markov Chain model in clumped sugar beet and wheat canopies for a range of sowing dates, densities and development stages, using measured parameters that include leaf position (dispersion coefficient), leaf area index, and leaf inclination. However, they concluded that it would not be suitable when the crop is water stressed or windblown, as measurements of leaf angle would not be representative.

Clearly, one of the main sources of variation in measuring k was the experimental error. The light extinction coefficient was estimated by taking measurements of green area and light flux within each layer of the canopy. A number of suggestions for improving the field measurements of light and the GAI are now discussed. Light measurements should always be taken when the sun is overhead and when there is no cloud cover, so that k is not overestimated. However, this is not possible in the UK. The frame used to measure the layers covered only 0.25 m^{-2} ground area, and only

three replicate light measurements were made in each layer. Increasing the area of the frame would provide a better average, reduce the proportion of light intercepted by the frame itself and increase the precision in the measurement of GAI. More care should also be taken to insert the ceptometer probe without disturbing the canopy. The stratified clips should be taken from the same position in the canopy that the light measurements were taken. For practical reasons, this was not always possible. More care should be taken to catch all the clipped material in the layer without allowing it to drop to the ground. This was difficult if the leaf tip drooped into the layer below. However, even if these practical problems have been overcome there is still the problem that crops do not always obey Beer's Law of light capture. Crops with a GAI of less than one at the beginning of the growing season and crops that are widely spaced at low seed rate, do not meet the assumption of homogeneity. Modification of the model for light capture is therefore necessary, possibly through the Markov Chain model. However, extra parameters must also be measured.

6.5.4 Summary

This chapter has shown that the treatments were sufficient to create a wide range of canopy architectures with which to test the hypothesis. Green area index increased with seed rate, N rate and development and with Spark compared to Soissons canopies. The exponential decline in light flux with canopy depth followed Beer's Law. As predicted, light flux within the canopy decreased with increased seed rate and canopy development. Unexpectedly, there was no effect of variety because the difference in k could not be quantified in the prediction. The reduced k in the low seed rate canopy did not agree with the prediction and there was much variation in k with canopy depth and development. Beer's Law did not appear to hold for the canopies at low seed rate or during early growth suggesting that a more sophisticated approach is required. Leaf size, transmission coefficient, gap frequency, dispersion coefficient and leaf angle are required for a more sophisticated estimation of k. Correction factors for the light intercepting leaf sheath and ear area have also been suggested. The differences in the light profiles and mean PPFD between treatments were expected to bring about associated differences in the N requirement of leaves and leaf sheaths, which will be tested in the following chapters.

7   LEAF NITROGEN

The next step in predicting canopy N requirement (CNR) from canopy architecture is to predict the relationship between light flux and leaf N requirement. The leaf N content is compared between husbandry treatments, canopy depths and development stages. These effects are compared to the predictions. The observed relationship between leaf N content and light flux through canopy depth, is compared to the theoretical relationship between leaf N requirement and light flux based on maximising N use efficiency (NUE) (Section 2.2.).

7.1   LEAF N CONTENT PER UNIT GROUND AREA

Leaf N content per unit ground area and canopy depth is described in this section. Leaf N content per unit green area is then estimated from the amount of N in the leaf tissue per unit ground area and the green area of the whole canopy or of the lamina.

7.1.1   1997/8 experiment

The profiles of leaf N content per unit ground area within canopies around anthesis are presented in Figure 7.1. There was a general decrease in leaf N content with canopy depth, except for the first and second layer where there was little leaf tissue. This profile showed treatment effects which were similar to that of the green area of leaf tissue, described in the previous chapter. This was seen particularly in Spark sown at 20 seeds m^{-2} which had an unexpectedly large leaf green area and also leaf N content per unit ground area. However, the decrease in leaf N content was generally more rapid than the decrease in leaf green area at lower layers. Spark sown at 320 seeds m^{-2} illustrated this well, where the leaf green area was unchanged to a depth of 65 cm but there was a decrease in leaf N content from a depth of 15 cm. As expected, the decrease in leaf N content was more rapid with increased seed rate and in the variety Soissons.

7.1.2 1998/9 experiment

The profiles of leaf N content per unit ground area at anthesis in the 1998/9 experiment were similar to the previous year (Figure 7.2). There was a decrease in leaf N content with canopy depth that became progressively more rapid than the decrease in leaf green area. Again, the N content was marginally smaller in Soissons than in Spark at each layer and the decrease with depth more rapid. In canopies fertilised with 244 kg ha^{-1} N, there were only small differences in profiles between the seed rates. In those with 124 kg ha^{-1} N the canopies sown at 20 seeds m^{-2}, particularly Spark, had slightly greater N content with each depth. As expected, all canopies with reduced fertiliser N had a reduced N content at each layer. However, the difference was reduced at the low seed rate. The decrease with depth was also reduced in these canopies.
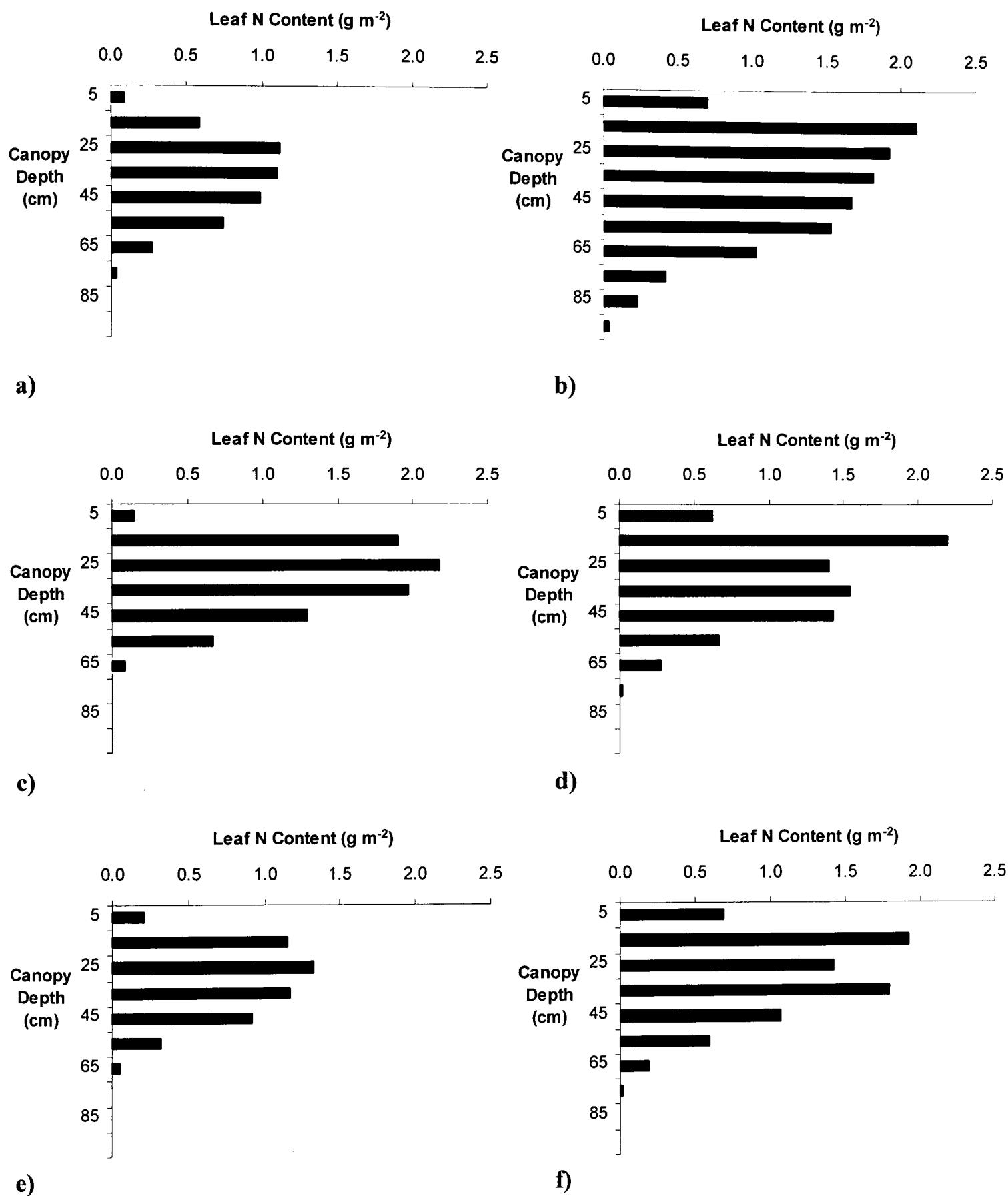
Figure 7.1 The N content of green leaf tissue (g per m^{-2} ground area) down canopies on June 29 of the 1997/8 experiment; a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}.
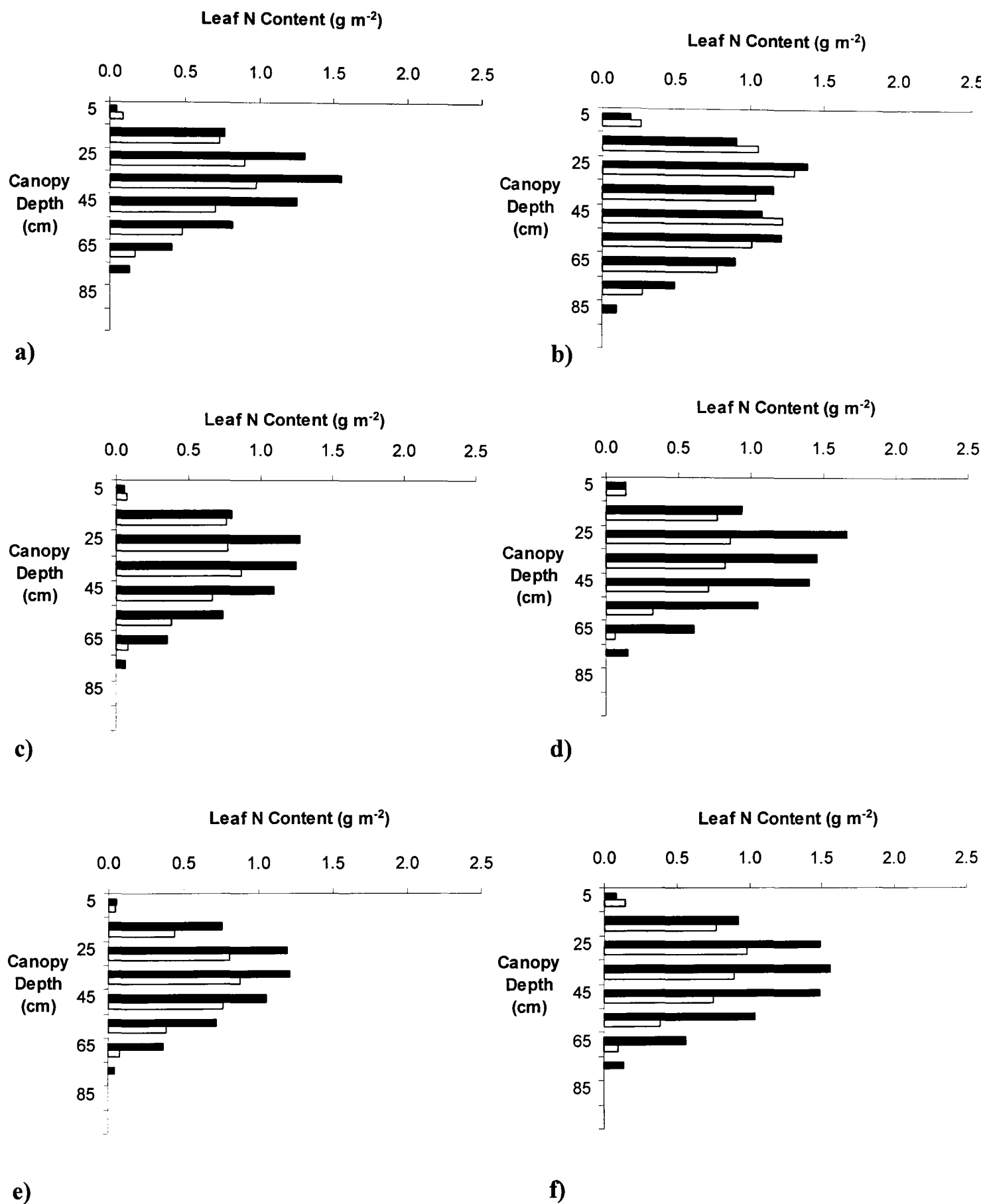
Figure 7.2 The N content of green leaf tissue (g per m⁻² ground area) down canopies, at anthesis in the 1998/9 experiment; a) Soissons – 20 seeds m⁻², b) Spark – 20 seeds m⁻², c) Soissons – 320 seeds m⁻², d) Spark 320 - seeds m⁻², e) Soissons – 640 seeds m⁻² and f) Spark – 640 seeds m⁻². 244 (■) and 124 (□) kg ha⁻¹ fertiliser N.

7.2   LEAF N CONTENT PER UNIT GREEN AREA

The leaf N content per unit green area is determined for each layer in the canopy from the % N in leaf, leaf dry weight and the green area of each layer. Leaf N content can be expressed either on a leaf green area basis, or total green area (leaf and stem) basis. N content per unit green leaf area would provide an indication of the photosynthetic capacity of the leaf, since the two are related (Evans, 1989c). Here, CNR is the total N requirement on a total green area basis (excluding the ears), therefore, expressing leaf N content on the same basis would be more consistent and help with the development of predictions in CNR.

7.2.1   The effect of husbandry on the distribution of leaf N with increasing canopy size through depth

7.2.1.1   1997/8 experiment

Figure 7.3 presents the significant decrease in leaf N content per unit total green area, excluding ears, with increasing canopy depth expressed through accumulated GAI (including ears) (P<0.001). The fitted linear regression models explained 80% of the variation in observed data. Spark had a significantly greater leaf N content for each individual unit of GAI than Soissons (P<0.001) but the slope or rate of decrease in leaf N content was similar. Parallel lines best represented the difference between the varieties. Seed rate had significant effect on the rate of decrease in leaf N content with increased canopy size (P=0.05), however, the effect of seed rate was not consistent between the varieties. Generally, the greatest seed rate had the lowest leaf N content for each individual unit of GAI (P<0.001). Separate lines with different intercepts and slopes best represented the difference between seed rates (P=001).

a)   b)
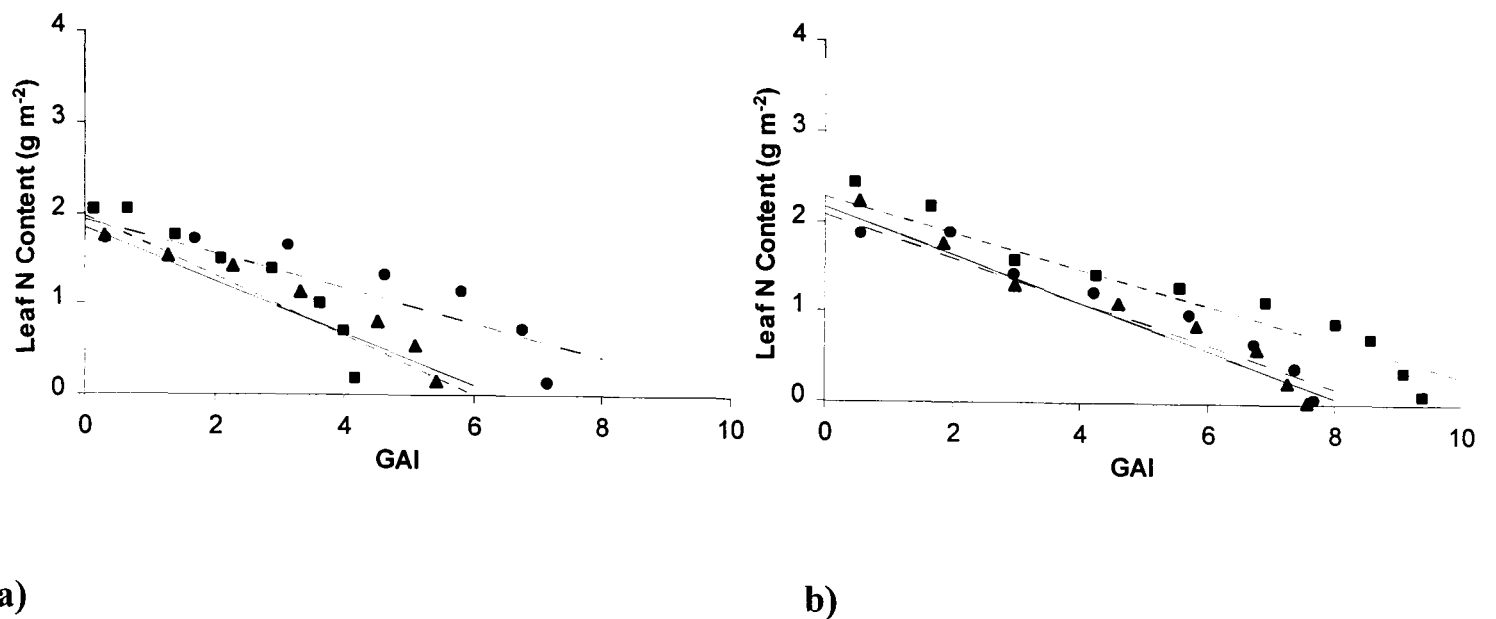
Figure 7.3 The decrease in leaf N content per unit green area (total excluding ears) with canopy depth (expressed as accumulated green area index; GAI (including ears)) in a) Soissons and in b) Spark, sown at 20 (■), 320 (●) and 640 (▲) seeds m^{-2}, on 29 June in the 1997/8 experiment. Fitted lines: R^2 = 80%, for 20 (....), 320 (-----) and 640 (——) seeds m^{-2}.

7.2.1.2   1998/9 experiment

The significant decrease (P<0.001) in leaf N per unit green area (excluding ear), with the increase in canopy size through depth in the 1998/9 experiment, is shown in Figure 7.4. Again, the linear models fitted the data well (R^2 = 89 %). In this year, the rate of decrease in leaf N content was greater in Soissons (P<0.001). As expected, the leaf N content distribution within canopies sown at 320 and 640 seeds m^{-2} were generally similar. The canopies sown at 20 seeds m^{-2} had a greater rate of decrease in leaf N content than either of the other two seed rates (P<0.001) and generally a greater leaf N content for each individual unit of GAI (P<0.001). The leaf N contents at the bottom of the canopy were therefore similar. There were significant interactions with both seed rate (P=0.05) and N supply (P=0.01). Canopies fertilised with less N had a smaller leaf N content throughout the canopy (P<0.001), but particularly in Soissons (P=0.01). The rate of decrease was also slightly greater in the canopies with reduced

N supply (P=0.05). Therefore separate lines with different intercepts and slopes best represented the difference between all of these treatments.

The total canopy leaf N content per unit green area could not be determined from the regressions and so section 7.2.2 presents the mean leaf N content calculated from total N content per unit ground area divided by total GAI (excluding ears).

a)

b)

c)

d)
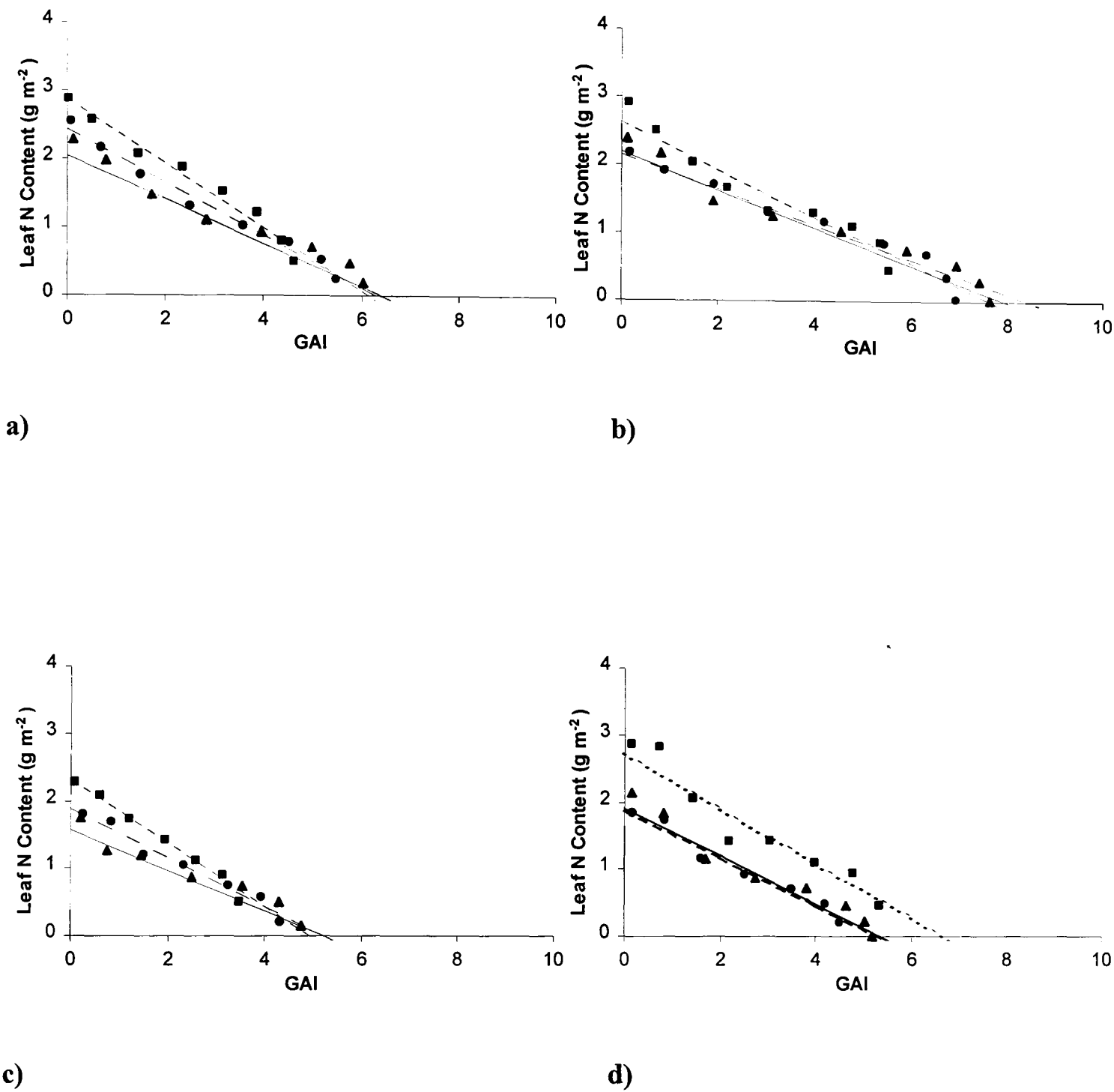
Figure 7.4 The decrease in leaf N content per unit green (total excluding ears) with canopy depth (expressed as accumulated green area index; GAI (including ears)) in a) Soissons - 244 kg N ha^{-1}, b) Spark - 244 kg N ha^{-1}, c) Soissons - 124 kg N ha^{-1} and d) Spark - 124 kg N ha^{-1}, sown at 20 (■), 320 (●) and 640 (▲) seeds m^{-2}, at anthesis in the 1998/9 experiment. Fitted lines; R^2 = 89 %, for 20 (....), 320 (-----) and 640 (——) seeds m^{-2}.

7.2.2 The effect of husbandry on mean leaf N content.

7.2.2.1 1997/8 experiment

A comparison of the mean leaf N content per unit green area (excluding ears) of all treatments is shown in Figure 7.5. The mean of all treatments was 1.14 g m^{-2} and there was no effect of variety. However, there was a decrease in leaf N content with the increase in seed rate (P=0.05). The leaf N content of canopies sown at 20, 320 and 640 seeds m^{-2} was 1.28, 1.12 and 1.01 g m^{-2}.
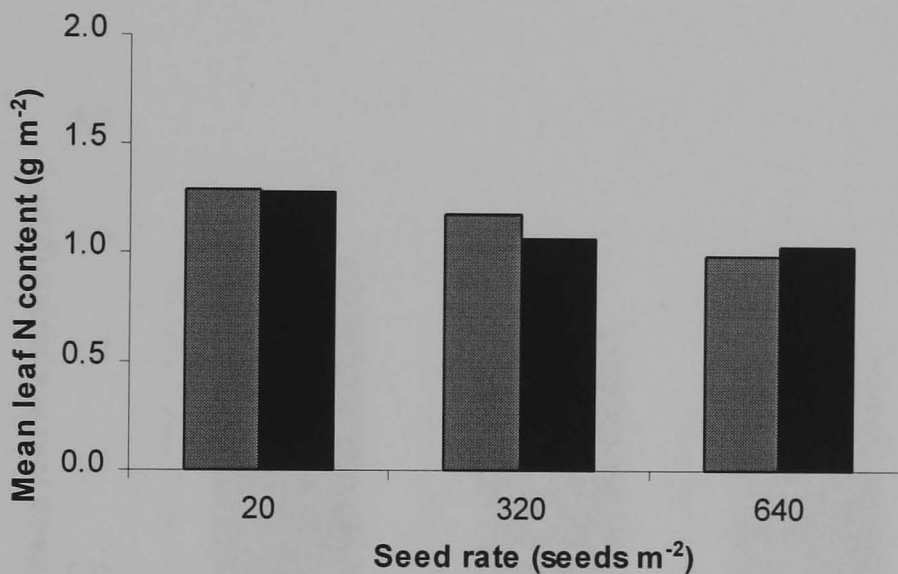
Figure 7.5 Mean leaf N per unit green area (total excluding ears) in Soissons (■) and Spark (■) canopies on June 29 in the 1997/8 experiment. S.E.D = 0.122, d.f. = 10.

7.2.2.2 1998/9 experiment

The mean leaf N contents for canopies from the 1998/9 experiment are presented in Figure 7.6. A comparison between the years showed that there was generally little difference between the N content of canopies, except at the low seed rate which had a greater N content in 1998/9. There was an increase in leaf N content at the low seed rate as in the previous year (P<0.001) but the effect reduced between the two higher

seed rates. The mean leaf N content of canopies sown at 20, 320 and 640 seeds m^{-2}, was 1.42, 1.00 and 0.91 g m^{-2}, respectively. There was also an increase in leaf N content with N application (P<0.001) where leaves of canopies fertilised with 244 and 124 kg ha^{-1} N had a mean N content of 1.20 and 1.02 g m^{-2}. Again, there was no effect of variety on mean leaf N content. Both had a mean leaf N content of 1.11 g m^{-2}.

Section 7.2.3 describes the change in mean leaf N content per unit GAI, through crop development.
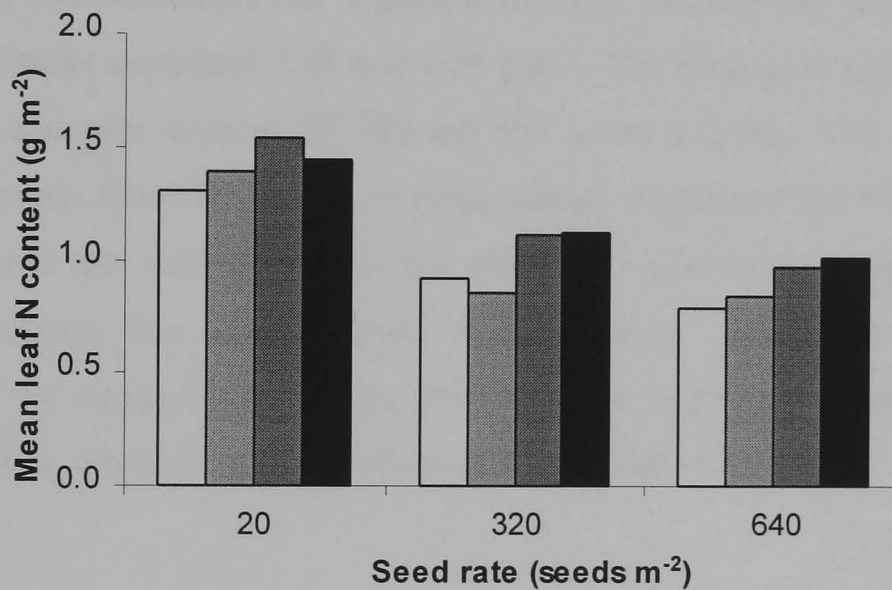
Figure 7.6 Mean leaf N per unit green area (total excluding ears) in Soissons - 124 kg ha^{-1} N (☐), Spark - 124 kg ha^{-1} N (▨), Soissons - 244 kg ha^{-1} N (▨) and Spark - 244 kg ha^{-1} N (■) canopies at anthesis in the 1998/9 experiment. S.E.D = 0.063, d.f. =20.75.

7.2.3 The effect of crop development on mean leaf N content

7.2.3.1 1997/8 experiment

The general change in mean leaf N content per unit green area (excluding ears) is shown in Figure 7.7. Leaf N content increased during the winter and early spring tillering period with the maximum generally reached by around February. The decrease in leaf N content began shortly after this and continued throughout canopy expansion and the grain filling period. During the early tillering period, the canopies sown at 20 seeds m^{-2} had a consistently smaller leaf N content than the canopies at the other seed rates (P=0.05). There was no effect of variety. Following this, at later stages of tillering, there was no seed rate effect but Spark had a greater N content than Soissons (P<0.001). This effect remained throughout the growing season, except in May and June. The maximum leaf N content for both varieties was in February when Spark and Soissons contained 3.88 and 3.26 g m^{-2}. The maximum leaf N content, in February, for canopies sown at 20, 320 and 640 seeds m^{-2}, was 3.57, 3.67 and 3.47 g m^{-2}, respectively. Once the period of rapid canopy expansion had begun there was an effect of seed rate once again, but the effect was reversed, corresponding to the original predictions. The canopies sown at 20 seeds m^{-2} had greater mean leaf N contents than the remaining seed rates (P=0.05). The smallest N content was at the end of the season, when the mean of all canopies was only 0.93 g m^{-2}.
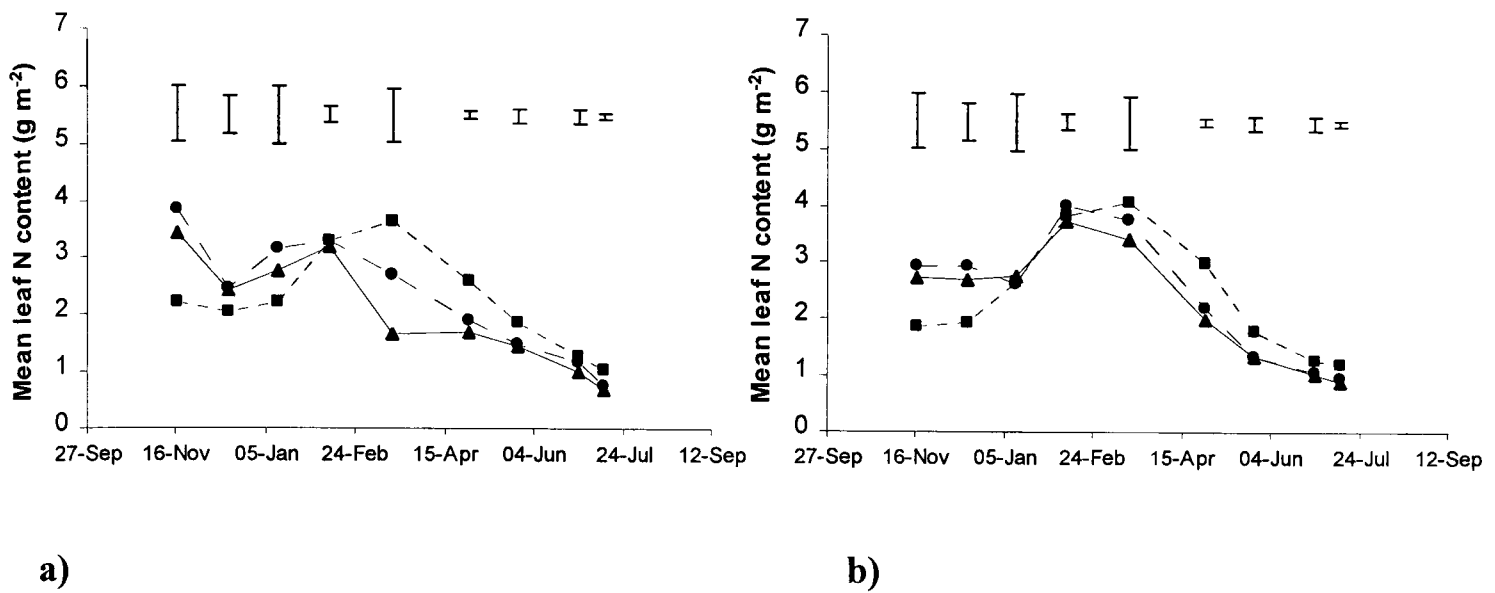
Figure 7.7 The change in mean leaf N per unit green area (total excluding ears) in 1997/8. a) Soissons and b) Spark sown at 20 (···■···), 320 (--●--) and 640 (─▲─) seeds m⁻². Error bars represent S.E.D., d.f. = 10.

7.2.3.2   1998/9 experiment

There were fewer sampling dates in the 1998/9 experiment but there were enough at the beginning of the year to see a slightly different pattern of leaf N content compared to the previous year (Figure 7.8). There was no initial increase in leaf N content during the early tillering period in winter, seen in 1997/8. The maximum leaf contents were in December and not in February. These were 2.91 and 3.11 g N m⁻² in Soissons and Spark and 3.20, 2.91 and 2.92 g N m⁻² in canopies sown at 20, 320 and 640 seeds m⁻², respectively. There were no treatment effects at this time but in January, Spark had a significantly greater leaf N content than Soissons (P=0.05) and canopies sown at 20 seeds m⁻² had a significantly greater content than those at the other seed rates (P=0.05) which was the reverse of the effect observed in the previous year. There were no treatment effects in February, but the effect of seed rate was significant again in March. There was little change in the leaf N content of canopies overall, until stem extension had begun and leaf N decreased. By anthesis, leaf N content was reduced to a mean of 1.11 g m⁻², similar to the previous year.
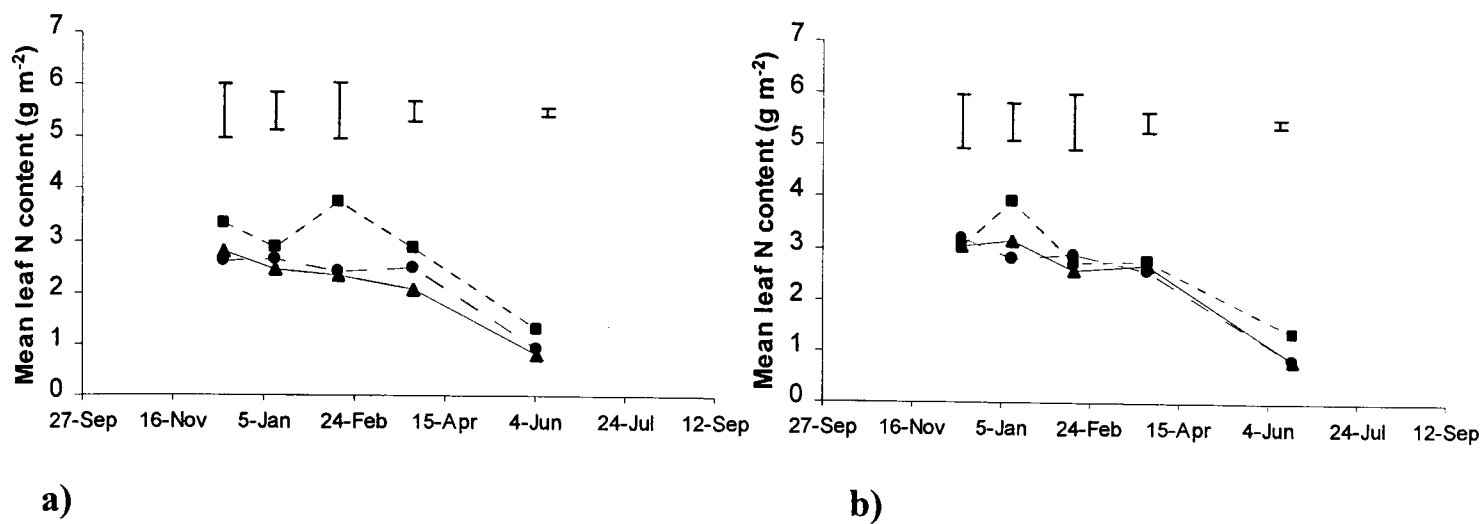
Figure 7.8 The change in mean leaf N per unit green area (total excluding ears) in 1998/9. 124 kg N ha⁻¹ applied. a) Soissons and b) Spark sown at 20 (···■···), 320 (--●--) and 640 (─▲─) seeds m⁻². Error bars represent S.E.D., d.f. = 10.

7.3 THE RELATIONSHIP BETWEEN LEAF N CONTENT AND LIGHT FLUX

The previous chapter described the change in the light flux within the canopy due to canopy size and depth, and the effect of husbandry. Section 7.2.1 has shown that the distribution pattern of leaf N per unit green area within the canopy was not the same as the distribution of light flux. This section begins by investigating the relationship between photosynthetic photon flux density (PPFD) measured within the canopy and leaf N content per unit leaf area. The effect of husbandry and crop development on this relationship is also examined.

7.3.1 The effect of husbandry on the response of leaf N content to decreasing light with increasing canopy size through depth

Asymptotic regression curves were fitted to describe the response of leaf N content per unit leaf area to increased light flux per unit ground area measured with increased canopy height. This is also compared to the predicted relationship based on the N response to maximise nitrogen use efficiency (NUE), in chapter 2. However, treatment comparisons could only be carried out with linear regressions. Therefore, a

log transformation was performed on PPFD to linearise the regression (Sokal & Rohlf, 1995).

7.3.1.1 1997/8 experiment

There was an increase in leaf N with light (P<0.001) until the light flux reached approximately 200 μmol m^{-2} s^{-1} (Figure 7.9). The leaf contained around 1.8 - 2.0 g N m^{-2} at this flux. There was little further increase in leaf N content with light fluxes greater than this, suggesting a maximum leaf N content of 2.0 - 2.2 g N m^{-2}. The minimum leaf N content was about 0.7 g m^{-2}. Figure 7.9 shows the PPFD with a log transformation and fitted linear regressions. There was a significant effect of seed rate on the relationship between leaf N content and light flux within the canopy as the rate of increase of leaf N content with light flux increased at the low seed rate (P=0.01). The low seed rate had a greater leaf N content at high light fluxes but the high seed rate had a greater N content at lower light fluxes. Leaf N contents of canopies at 320 and 640 seeds m^{-2} were similar to one another. There was also a significant effect of variety on the relationship between leaf N content and light flux (P<0.001). The rate of increase in leaf N content with light flux was greater in Spark which had a greater leaf N content at high light fluxes and lower N content at lower light fluxes, than Soissons. Therefore separate lines with different intercepts and slopes best represented these treatment differences. A comparison with the prediction shows a similar shaped response but observed values slightly greater than predicted for all treatments, especially at low PPFD.

a)

b)

c)

d)
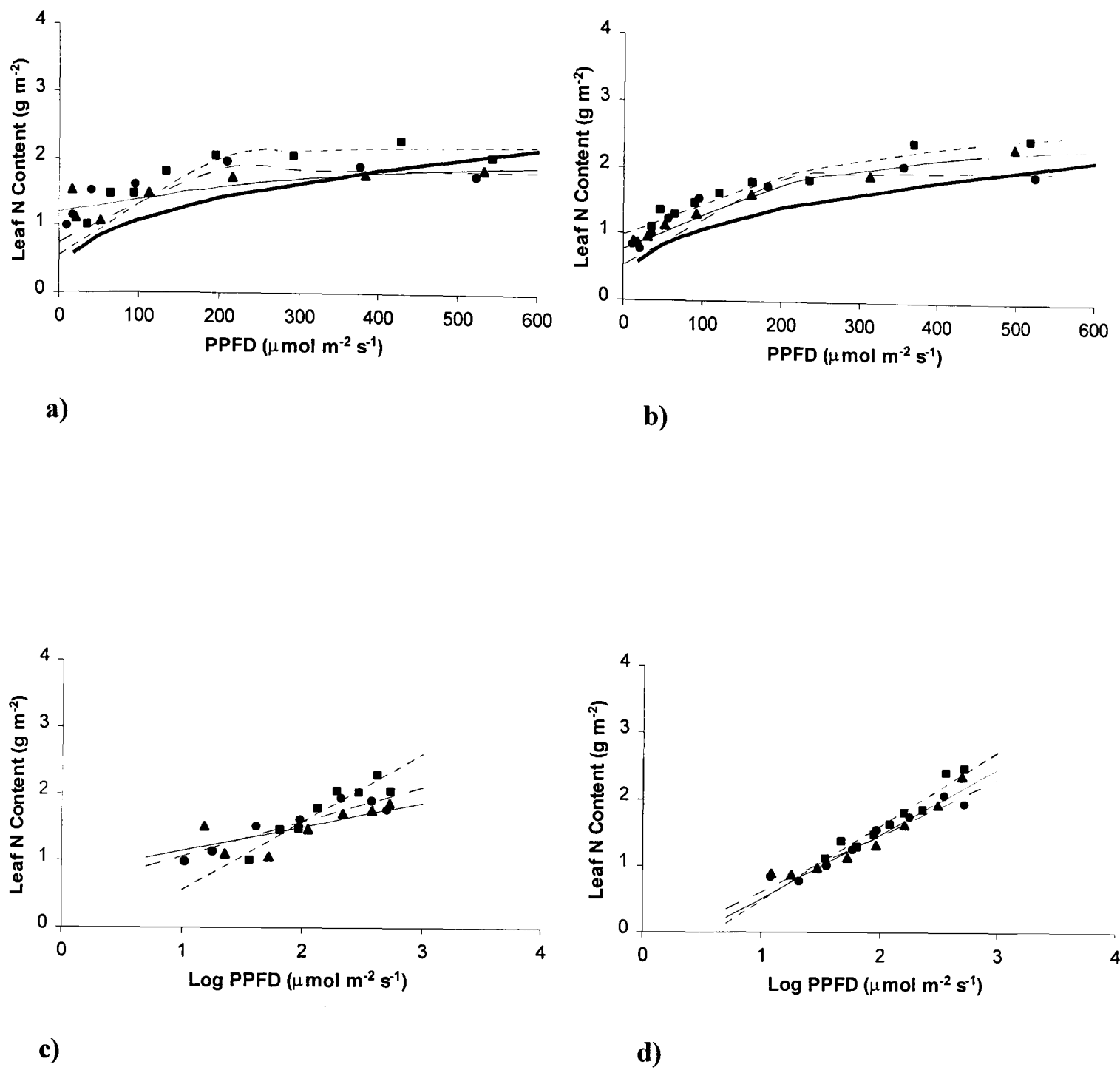
Figure 7.9 The relationship between leaf N per unit green leaf area and photosynthetic photon flux density (PPFD). 29 June 1998. a) and c) Soissons, b) and d) Spark, sown at 20 (■), 320 (●) and 640 (▲) seeds m^{-2}. Fitted curves: R^2 = 76 %, Fitted lines: R^2 = 72 % for 20 (....), 320 (-----) and 640 (——) seeds m^{-2}. Predicted leaf N content at which nitrogen use efficiency (NUE) is maximum (—).

7.3.1.2   1998/9 experiment

Figure 7.10 and Figure 7.11 show the relationship between leaf N content per unit leaf area and light flux per unit ground area at anthesis in the 1998/9 experiment. The response was similar to that in the previous year, with an increase in leaf N with light flux described by an exponential function (P<0.001). The increase was again greater at low light fluxes but the flux at which the rate of increase reduced was not as clear as in the previous year. It appears to have been at around 150 μmol m^{-2} s^{-1}. A comparison between years shows that the minimum leaf N content was again at about 0.7 g m^{-2} and the maximum slightly greater at around 2.0 - 2.5 g m^{-2} (however, this difference may not be significant). There was a significant effect of seed rate on leaf N content per unit of light with canopy depth (P<0.001). Canopies at the lowest seed rate had greater rate of increase in leaf N content with light. Leaf N content was greater in the low seed rate at the top of the canopy where fluxes were greater than 300 μmol m^{-2} s^{-1} but the difference was reduced at low fluxes. This was consistent with the previous year. Again, there was also an effect of variety (P<0.001) in which Spark had a greater rate of increase in leaf N content with light than Soissons. Spark had a greater leaf N content than Soissons at high light fluxes, but lower leaf N content at low light fluxes. Canopies fertilised with 244 kg ha^{-1} N had a greater leaf N content than those fertilised with 124 kg ha^{-1} N for all light fluxes, although the effect was reduced at the high light fluxes (P<0.001). The rate of increase in leaf N content with light flux was therefore greater in the reduced N crop. Leaves exposed to 200 μmol m^{-2} s^{-1} in the 'high N' crop had a N content of 1.8 – 2.0 g m^{-2} (similar to the previous year) and in the 'low N' crop, 1.0 – 1.5 g m^{-2}. There was a significant interaction between seed rate and variety (P=0.05) and between N and variety (P=0.05) in the rate of increase. These may be have been due to different tillering patterns of the varieties, causing different amounts of luxury N uptake. Separate lines with different intercepts and slopes best represented all of these treatment differences. The canopies at the low fertiliser rate had similar leaf N contents to that predicted. The canopies with 244 kg ha^{-1} N had leaf N contents above the values predicted except for the lower layers in the canopy of Soissons which were similar. A reduction in N supply reduced the leaf N content closer to the predicted leaf N requirement. However, the leaf N content at the bottom of the canopy, where light flux was low, was below the predicted requirement. The mean leaf N content per unit leaf area, per

mol photons within the whole canopy was estimated from the mean leaf N content and the mean light flux and a comparison between treatments was made (section 7.3.2).

a)

b)

c)

d)
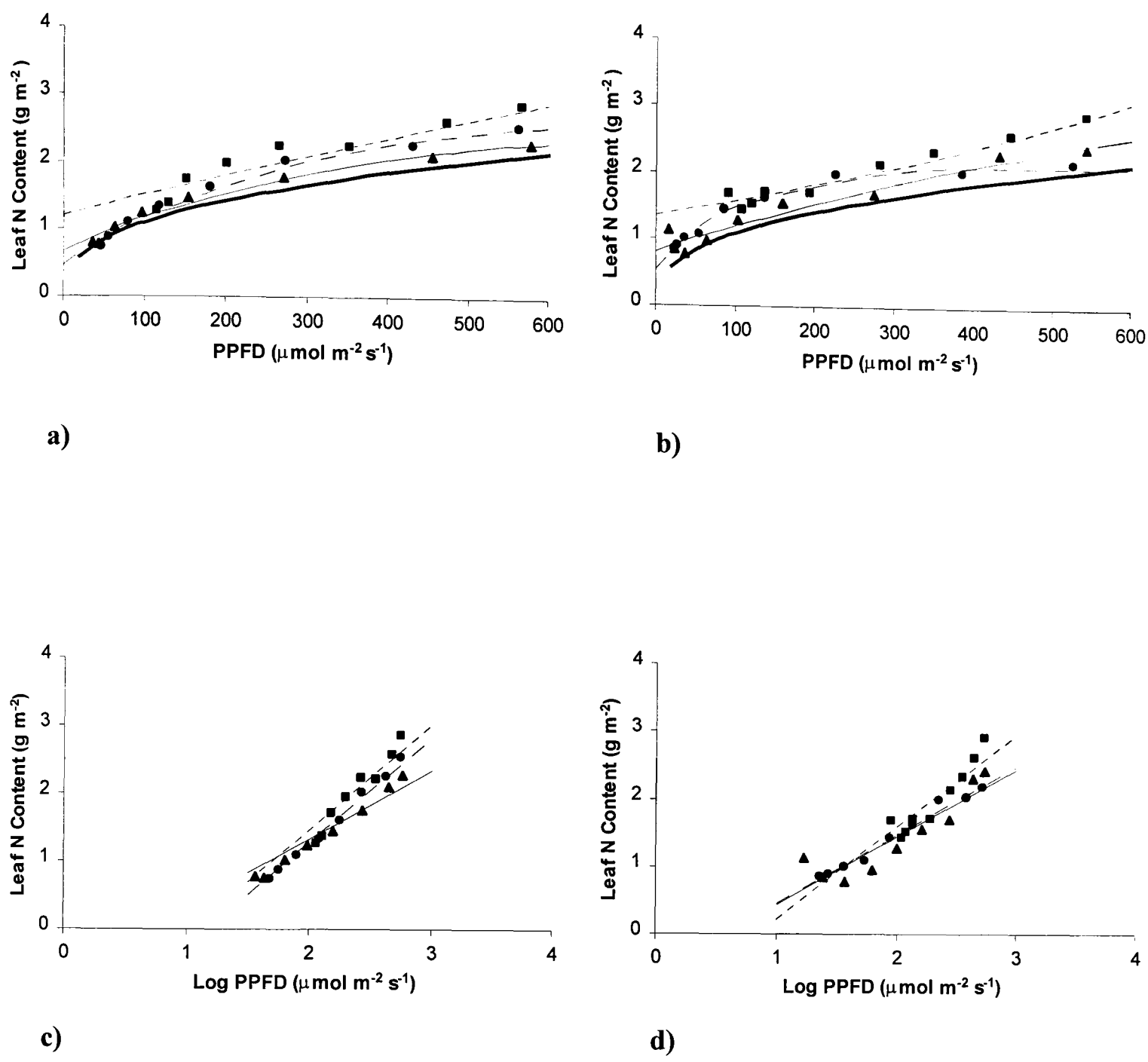
Figure 7.10 The relationship between leaf N per unit green leaf photosynthetic photon flux density (PPFD) with 244 kg N ha^{-1}. Anthesis 1999. a) and c) Soissons, b) and d) Spark, sown at 20 (■), 320 (●) and 640 (▲) seeds m^{-2}. Fitted curves: R^2 = 85 %, Fitted lines: R^2 = 83 % for 20 (....), 320 (-----) and 640 (——) seeds m^{-2}. Predicted leaf N content at which nitrogen use efficiency (NUE) is maximum (—).

a)

b)

c)

d)
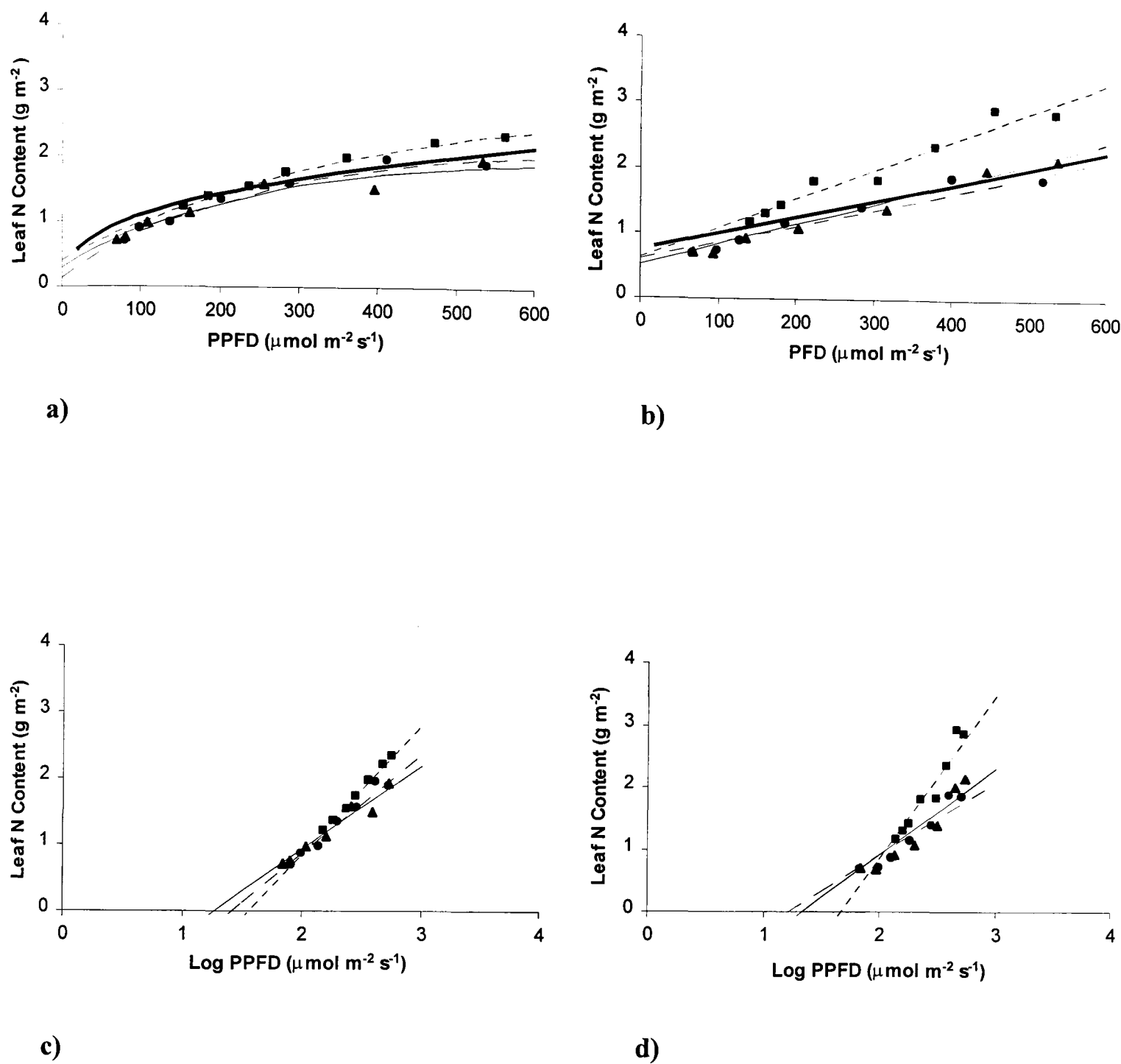
Figure 7.11 The relationship between leaf N per unit green leaf area and photosynthetic photon flux density (PPFD) with 124 kg N ha⁻¹. Anthesis 1999. a) and c) Soissons, b) and d) Spark, sown at 20 (■), 320 (●) and 640 (▲) seeds m⁻². Fitted curves: R^2 = 85 %, Fitted lines: R^2 = 83 % for 20 (....), 320 (-----) and 640 (——) seeds m⁻². Predicted leaf N content at which nitrogen use efficiency (NUE) is maximum (—).

7.3.2 The effect of husbandry on the response of mean leaf N content to mean PPFD

The effect of husbandry on the relationship between leaf N content and light flux is best described by the mean number of mol of N per unit leaf area, per mol of photons per unit ground area per second, received by the leaf. This cannot be estimated from the regression analysis in the previous sub-section, and so the mean leaf N content (in mols) is divided by the mean light flux (in mols). This results in a mean for the whole canopy.

7.3.2.1 1997/8 experiment

The results presented in Figure 7.12 show that despite the effect of seed rate and variety on the relationship between leaf N and light at various light fluxes within the canopy, there was no effect of either on the mean leaf N content per mol of photons received. This suggested that there was a direct relationship between photosynthetic leaf N and light flux, for the canopy as a whole. The mean of all canopies was 692 mol N m^{-2} (mol photon m^{-2} s^{-1})$^{-1}$.
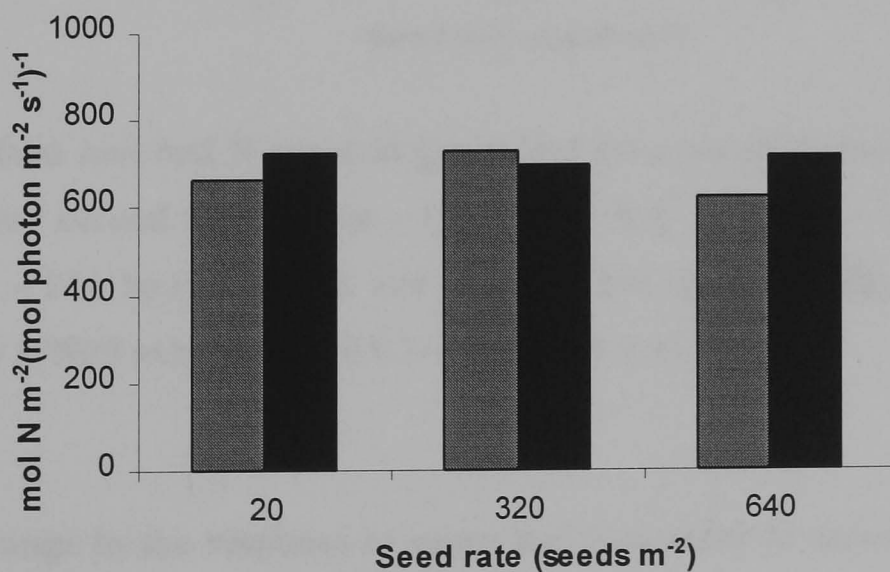
Figure 7.12 Mean mol leaf N per unit leaf green area per mol photons per unit ground area per second in Soissons (▨) and Spark (■) canopies on June 29 in the 1997/8 experiment. S.E.D = 68.1, d.f. = 10.

176

7.3.2.2   1998/9 experiment

The number of mol of leaf N per mol of photons at anthesis in the 1998/9 experiment is shown in Figure 7.13. The mean of all of the treatments was less than in the previous year (532 mol N m^{-2} (mol photon m^{-2} s^{-1})$^{-1}$. This was due to the significant decrease with reduced fertiliser N (P<0.001). The results were also slightly less than in 1998 for the canopies fertilised with 244 kg ha^{-1} N. There was again no effect of variety or seed rate.

Section 7.3.3 describes the change in mean leaf N content per mol of photons throughout crop development.
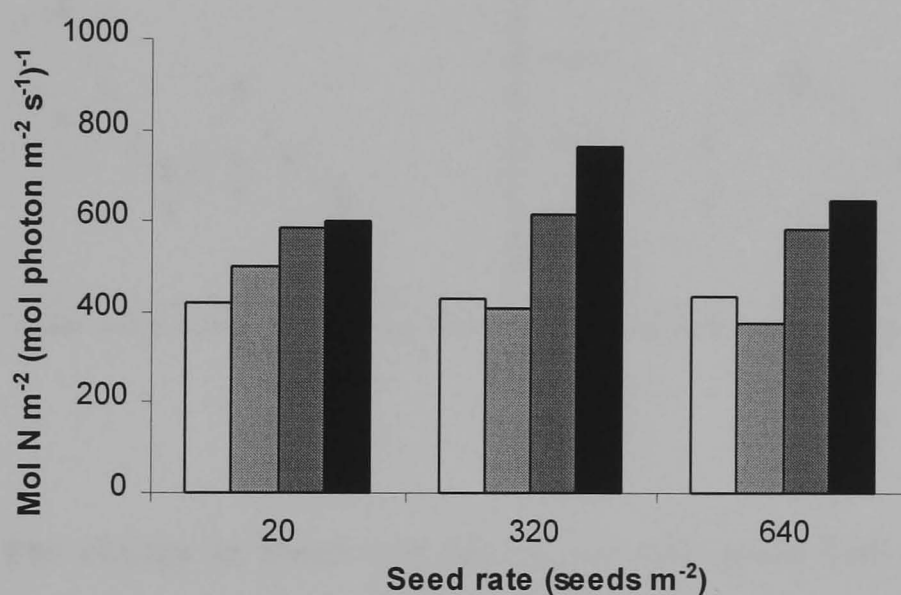
Figure 7.13 Mean mol leaf N per unit green leaf area per mol photons per unit ground area per second in: Soissons - 124 kg ha^{-1} N (☐), Spark - 124 kg ha^{-1} N (▨), Soissons - 244 kg ha^{-1} N (▨) and Spark - 244 kg ha^{-1} N (■) canopies at anthesis in the 1998/9 experiment. S.E.D = 85.0, d.f. = 19.75.

7.3.3   The change in the response of mean leaf N content to mean PPFD with crop development

7.3.3.1   1997/8 experiment

Figure 7.14 shows the change in the mol of leaf N per mol of photons throughout the 1997/8 experiment. Surprisingly, there was a general decrease beginning in winter

when the crop was small and irradiance was low. However, the variation surrounding the data points indicate little significant change throughout the growing season until the decrease in June and July. This was the period of development when N was translocated from the leaves to the grain, and there was also an increase in light flux. There were generally no significant effects of treatment as expected, except for a variety effect in July. Soissons was more developed than Spark and so more of the N would have been redistributed to the grain.
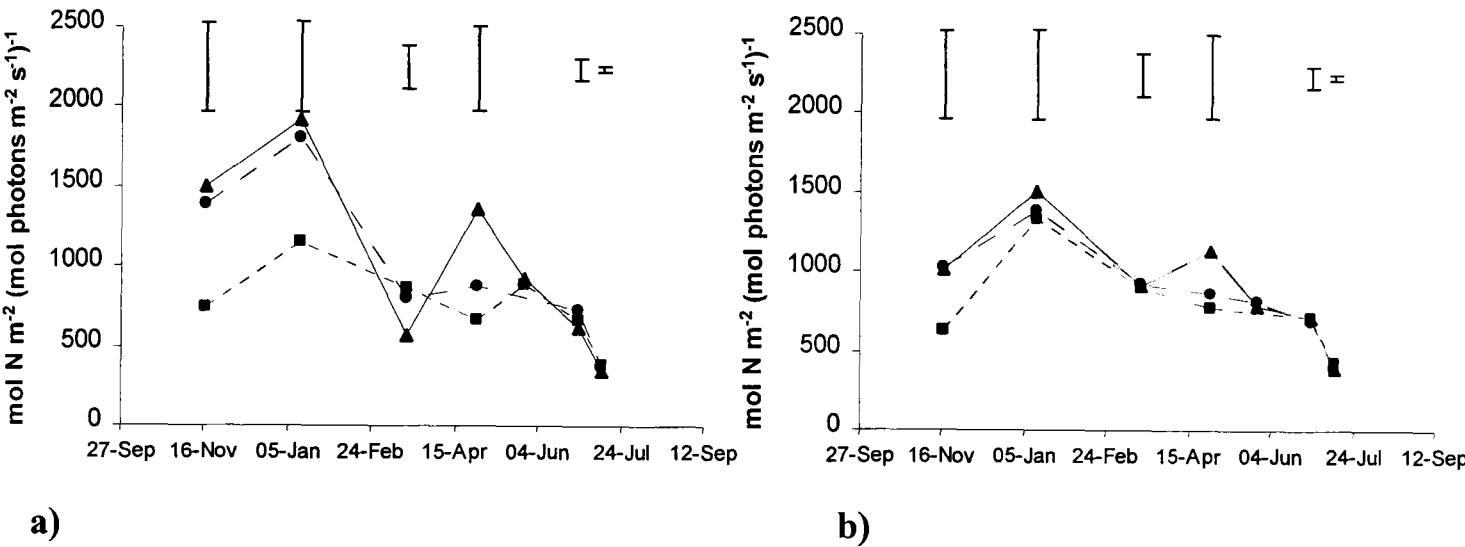
Figure 7.14 The change in mean mol leaf N per unit green leaf area per mol photons per unit ground area per second in 1997/8. a) Soissons and b) Spark sown at 20 (···■···), 320 (--●--) and 640 (—▲—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

7.3.3.2   1998/9 experiment

A similar decrease in leaf N per mol photons with the progression of the growing season, occurred in the 1998/9 experiment, as in the previous year. Figure 7.15 presents the canopies fertilised with 244 kg ha^{-1} N. The decline was more significant in this year, with April having particularly low values. It therefore appears that there was a slight increase at anthesis and values at this time were comparable with the previous year. Again, there were generally no significant effects of variety or seed rate, only the effect of N previously described at anthesis.
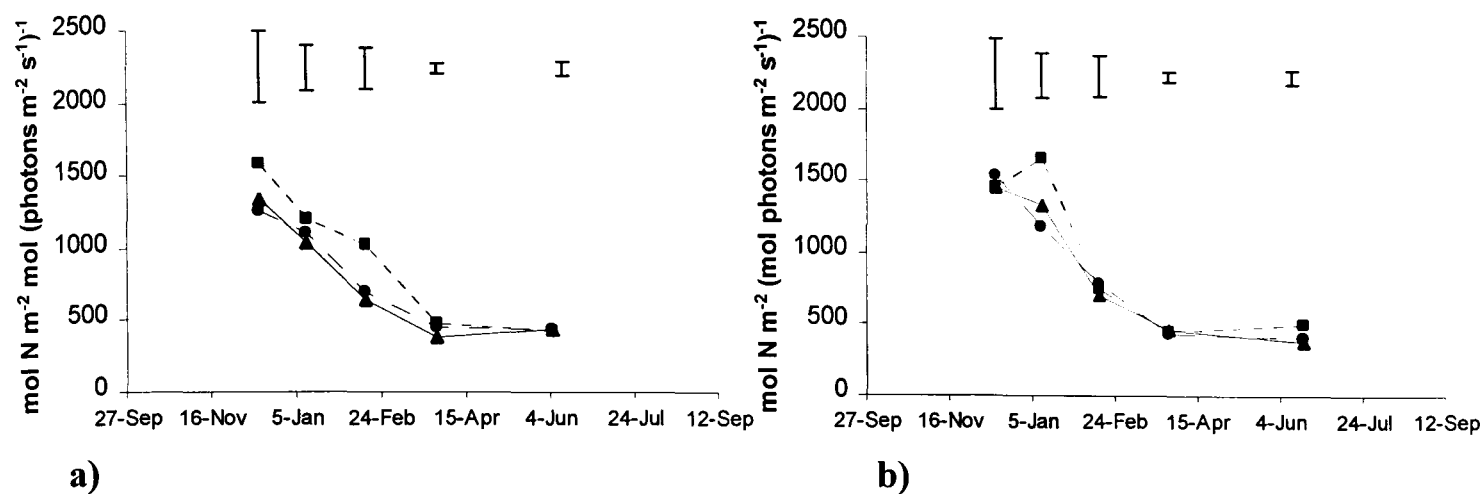
Figure 7.15 The change in mean mol leaf N per unit green leaf area per mol photons per unit ground area per second in 1998/9. 124 kg N ha^{-1} applied. a) Soissons and b) Spark sown at 20 (┄■┄), 320 (--●--) and 640 (─▲─) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

7.4 DISCUSSION

This section tests the predictions of the variation in leaf N requirement within canopies, between canopies and with crop development. An explanation of the variation in leaf N requirement is provided in terms of the relationship with photosynthetic photon flux density (PPFD) that has been previously related to canopy architecture. The physiological mechanism for the response of N to light flux is discussed in terms of light perception and N use efficiency (NUE).

7.4.1 Testing the prediction of leaf N requirement with canopy depth

A decrease in leaf N content per unit green area was observed with canopy depth and a linear model could be fitted to all treatments in both seasons (Figures 7.3 – 7.5). The observed and expected variation in leaf N requirement with accumulated GAI through depth is now compared to test the predictions in Chapter 2. Figure 7.16 presents the comparison for Spark sown at 320 seeds m^{-2} in 1998/9 and with 124 kg ha^{-1} N. The prediction is made from Equation 2.3 (Chapter 2) using the observed k value of 0.46 (Table 6.7) and incident radiation of 568 $\mu mol\ m^{-2}\ s^{-1}$.

The comparison shows that the observed leaf N requirement was very similar to the prediction. The regression analysis showed that they both had the same rate of decrease, although the predicted values were slightly greater than the observed. The mean leaf N requirement was predicted to be 1.4 g m^{-2} but was observed to be 1.2 g m^{-2}. Shiraiwa & Sinclair (1993) also observed a similar linear relationship with canopy size in soybeans, but others observed an exponential decline in N content for other species (Field 1983, Anten *et al*, 1995b). Bindraban (1997) measured the profile of leaf N content per unit leaf area with accumulated GAI through canopy depth in a wheat canopy with a GAI of about 8. The values of 2.5 and 0.3 g m^{-2} N at the top and bottom of the canopy, respectively, show that the range was greater than the observed values presented in Figure 7.16. However, this was a canopy with high N availability and therefore likely to contain luxury N. Wheat canopies with no fertiliser N measured by Grindlay *et al.*, (1997) had upper (1.9 g m^{-2}) and lower leaf N contents (0.7 g m^{-2}) that were exactly the same as the observations in Figure 7.16. This lower

value has also been observed in lucerne (Lemaire *et al.,* 1991). Generally, the principles behind the prediction of leaf N requirement based on maximising NUE seemed appropriate. The response shape of leaf N content per unit leaf area to PPFD, for all treatments, was similar to the predicted response based on maximising NUE (Equation 2.1). This was also similar to the response curve in sunflower (Rousseaux *et al.,* 1999) and lucerne (Evans, 1993b).
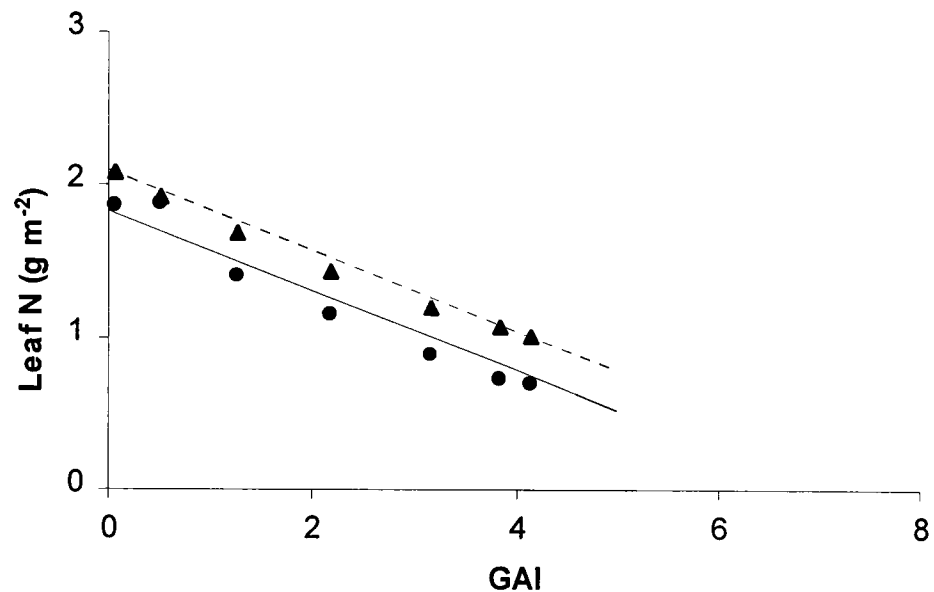
Figure 7.16 A comparison of the predicted (▲) and observed (●) leaf N requirement per unit leaf area with canopy depth (expressed as accumulated green area index; GAI) for Spark sown at 320 seeds m^{-2} and 124 kg ha^{-1} N. Fitted lines; R^2 = 92%, for predicted (.....) and observed (——).

7.4.2 Testing the predictions for the effect of husbandry and development on leaf N requirement

At anthesis, the mean leaf N content per unit green area of all treatments and in both years was 1.1 g m^{-2}. This was about half of the total N content per unit green area; the other half being located in the stem. Measurements in the reduced fertiliser N treatments showed that the mean leaf N requirement per unit green area was 1.0 g m^{-2}.

The mean leaf N requirement per unit green area increased from 0.9 to 1.3 g m^{-2} with the decrease in seed rate from 320 to 20 seeds m^{-2}. This agrees with the qualitative prediction in Chapter 2. There was little difference between the canopies at the two higher seed rates, as their architectures and light fluxes were similar. The N uptake was the same for all seed rates at anthesis and so there would have been more N per plant at the low seed rate. The relationship between the production of leaves and tillers and the development of roots (Klepper, Belford & Rickman, 1984) suggests that low seed rate canopies will have greater root spread per plant. Therefore, the low seed rate would be able to scavenge a sufficient amount of available N to compensate for the reduced plant number. The increase in GAI per plant at the low seed rate, due to increased tillering, could not compensate for low plant density and so there was also more N available per shoot in these canopies to meet the greater photosynthetic requirement.

It was predicted that Soissons would have a slightly greater leaf N requirement than Spark. This was because a greater mean light flux was expected in Soissons. However, there was no difference in either leaf N requirement or mean light flux between the two varieties tested. The mean of all seed rates was 1.0 g N m^{-2} in both. There was no effect of variety on the mean leaf N content per mol photons within the canopy and so the leaf N content can therefore be explained by the light environment created by canopy architecture. This also suggests that the actual N required to increase leaf area and be effective in light conversion was the same.

There was no consistent change in the leaf N content throughout canopy development until stem extension. As predicted, there was a decrease in leaf N requirement per unit green area after the beginning of stem extension and when fertiliser N had been applied. However, the greater leaf N content in spring, prior to stem extension could also include luxury N (Grindlay, 1997). When light levels increased and the canopy expanded, there was an increase in mutual shading (Lemaire & Gastal, 1997). There would be proportionately more leaves at the bottom of the canopy with less N, as more of the N was concentrated at the top of the canopy. This mobilisation of N would also be in response to the increased incident radiation during the late spring and summer.

Although the predicted variation in leaf N requirement was observed in most cases, there were some small differences in the response of leaf N requirement to PPFD. An attempt is now made to explain these differences and to discuss some of the assumptions in the prediction.

7.4.3   Light perception

One of the main assumptions for the prediction is that the PPFD measured at the surface of the leaf in the simulation is perceived in the same way as the PPFD measured within the canopy. There are several possible mechanisms for the perception of light quantity and response of N. First, it is thought that the decrease in production of photosynthetic metabolites and carbon supply increases the expression of genes that stimulate N export from leaves (Anten, Hikosaka & Hirose, 2000). This has been found with leaf senescence due to age, (Hensel *et al.*, 1993), but not with shade induced senescence (Pons & Bergkotte, 1996). A direct relationship between photosynthesis and N export or import has not been found. Second, it has been proposed that certain compounds in xylem sap such as cytokinins might act as a signal to N redistribution (Pons & Bergkotte, 1996). The transpiration rate increases with increased radiation and so the partitioning of xylem sap, along with any compounds it contains, should be partitioned according to the radiation. However, there is no evidence for cytokinins or any compound to be directly related to the light environment (Kraepiel & Miginiac, 1997). Third, photoreceptors may also have a role in perception of light quantity. Blue light absorbing pigments appear to be the most likely of all photoreceptors to have the ability to perceive light quantity, gradients and duration (Frankland, 1986; Jackson & Thomas, 1997). However, the mechanism of how the photoreceptor actually perceives the light quantity is still unclear.

For this thesis, it was decided that PPFD should be expressed per unit of ground area in preference to green area. This is based on the theory that N responds to the light flux rather than to the light actually intercepted. It is also supported by the observation that emerging leaves have already adjusted their N content to local light fluxes before total emergence (Prioul *et al.,* 1980). Data from the experiment has also supported this. The relationship between leaf N content and light flux per unit ground area within the canopy was compared to the relationship between leaf N content and light

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intercepted per unit green area. Figures 7.10 and 7.11 show that when individual curves were fitted to the data set for each treatment in 1998/9, 85% of the variation in leaf N content could be explained by the relationship with PPFD per unit of ground area within the canopy. However, when the leaf N content was plotted against the PPFD intercepted per unit of green area, only 69% of the variation in leaf N content could be explained by the relationship with PPFD intercepted (data not shown). This comparison has shown that leaf N content had a stronger relationship with PPFD per unit of ground area within the canopy than with intercepted PPFD per unit green area. However, the combination of some of these mechanisms cannot be ruled out.

7.4.4 The requirement to maximise NUE

In this thesis, the leaf N requirement is to maximise NUE in each leaf according to the amount of light it experiences. It is then assumed that the distribution of N between leaves will then be optimised. The results have shown that there is great similarity between the predicted leaf N requirement for individual leaves and the observations within a whole canopy. However, at the canopy level the leaves are not independent from one another and may affect the response of N to light in other parts of the canopy. Therefore, the distribution of N in response to light may not always be optimal and may not maximise NUE. Anten *et al.*, (1995b) suggest that stands with a high LAI or *k* value have a steeper N distribution than those with low LAI or *k*. This was not supported by either the seed rate or the N treatment.

A detailed look at the N content per unit green area showed that there was more N in the leaves of the low seed rate compared to higher seed rates, at the top of the canopy under high light flux. This was also indicated by the steeper gradient of the distribution of N between leaves (Figure 7.4). The results here suggest that there was more N than required for maximising NUE, in this low seed rate at the top of the canopy (Figure 7.11). An experiment by Sticksel *et al.*, (1999) found that tillers contained more N in the straw than main stems. As the low seed rate crop had more tillers per plant than the higher seed rates and assuming that some of the extra storage N is located in leaves, this might explain the effect at the top of the canopy. This implies that the low seed rate crop does require this extra N, perhaps to increase the

amount of mobile N per tiller. However, there is still the possibility of luxury N at the low seed rate.

Increasing the N supply can increase leaf N content (Muchow & Sinclair, 1994). It follows that by increasing the fertiliser N, the greater leaf N content would allow the linear response to light to continue at greater light fluxes (Hirose & Werger, 1987b). However, not all of the extra N supplied in the 'high N' treatment was used to increase the N content of the upper leaves but was distributed proportionately more to the lower leaves. No evidence was found in the literature to suggest that wheat leaves can have a N content much above the observed maximum of 2.2 g m^{-2}. At the top of the canopy, the CO_2 diffusion rate may limit carbon gain that may then have the effect of restricting further N import into these leaves. There might even be photoinhibition also limiting carbon gain and N import (Bjorkmann, 1988 see Anten et al., 2000). Leaf N contents greater than this would be not beneficial in terms of photosynthetic efficiency, because of the increased maintenance costs (Hikosaka & Terashima, 1995) particularly if the extra N is luxury. However, extra N in the leaves at the top of the canopy could still be used to increase the light saturated photosynthesis (P_{max}), despite the decrease in NUE due to increased rates of respiration. At low light flux NUE is increased through the investment in chlorophyll, while acclimation to high light flux increases NUE through investment in RUBISCO (Evans, 1989c; Hikosaka & Terashima, 1995). It might be that there is a larger capacity for increasing chlorophyll than RUBISCO content or a greater return from the acclimation to low light compared to high light, that explains the greater allocation of the extra N to lower leaves. Here, the extra N in the leaves at low light flux may have been luxury, as photosynthesis is limited by light. Some of this may be in the form of nitrate in vacuoles or RUBISCO (Millard, 1988), thought to be both a reserve compound as well as functional photosynthetic compound. The work of Pearcy et al., (1994) (see Ryel & Beyschlag, 2000) has shown that when shaded leaves are exposed to sunfleck there is a 10 to 30 minute increase in photosynthesis to a light saturated rate. It is suggested that the leaves in low light will benefit from this extra N when there are sunflecks. The comparison with the predicted leaf N requirement showed that the 'high N' crop had greater leaf N contents than were required to maximise NUE. This supports the notion that reducing the N supply is advantageous in terms of NUE (Hikosaka & Terashima, 1995), and also that there is luxury N in the leaves of the high N canopies.

7.4.5 Summary

The mean leaf N requirement per unit green area was 1.0 g m^{-2} for a range of seed rates and varieties. The observed decrease in leaf N requirement in response to decreased light flux with canopy depth agreed well with the prediction based on maximising NUE. The decreased mean leaf N requirement with increased seed rate and canopy development also agreed with predictions. Unexpectedly, there was no effect of variety but this can be explained by the incorrect prediction of mean light flux. The results also support the assumption that leaf N responds to PPFD perceived on a ground area basis, although it is possible that there is a combination of mechanisms involved in the response of N to light. The response of leaf N to PPFD agreed well with the prediction although not all canopies were found to distribute the N to maximise NUE. Leaf N at the top of the low seed rate canopy was greater than the requirement to maximise NUE. It was suggested that there is an additional requirement for storage of mobile N with the increased tiller number. Canopies with increased fertiliser N had a greater N content than required throughout the canopy and would therefore have had a reduced NUE. The principles developed in Chapter 2 for the prediction of variation in leaf N requirement have been supported by the results. Chapter 8 will now test the predictions for the stem N requirement.

8   STEM NITROGEN

This final chapter of results describes the variation in stem N content with canopy depth, husbandry treatment and crop development. The aim of this chapter is to test the predictions of stem N requirement developed in Chapter 2 based on the requirement for support but also photosynthetic N. Support N is defined here as non-photosynthetic N and may be mechanical or physiological and includes compounds for structure, transport and metabolism. Mainly structural and photosynthetic N is considered here. The role of N in the stem is less well understood compared to in the leaves. Studying the N in the stem at an individual shoot and whole canopy level will provide a better understanding of the stem N function and therefore requirement.

8.1   STEM N CONTENT PER UNIT GROUND AREA

This section describes the distribution of stem N content per unit ground area with canopy depth. In the next section, stem N content per unit green area is calculated from this and the green area of the whole canopy.

8.1.1   1997/8 experiment

Figure 8.1 presents the stem N content per unit ground area with canopy depth. Generally there was an initial rapid increase in stem N content with depth down to about 40 cm, with a gradual decrease in N content below this. This did not reflect the change in stem green area with depth (Chapter 6), which increased gradually and was generally maintained. There appeared to be little difference in stem N content between the two varieties despite the greater shoot number in Spark, indicating a greater stem N content per shoot in Soissons. The effect of seed rate was inconsistent. At the lowest seed rate, Soissons had a slightly smaller maximum N content of 1.2 g m^{-2}, compared to 1.4 g m^{-2} at the higher seed rates. However, Spark had a greater N content at all layers in the low seed rate but also had an unexpectedly large canopy. With increased seed rate, the rate of decrease in N content in the bottom layers of the canopy also increased. This may have been directly related to the larger green area.

8.1.2   1998/9 experiment

The distribution of stem N content per unit ground area with canopy depth, at anthesis in 1999, is illustrated in Figure 8.2. There was an initial increase in stem N content with depth in the upper layers similar to the previous year. Below a depth of 40 cm the decrease in N content was generally more rapid than in the previous year. There were negligible differences between the Spark and Soissons. The effect of seed rate was similar to Soissons in the previous year, in which the decrease in stem N content with depth was reduced at the low seed rate. The maximum stem N content was 1.5 g m^{-2} for all seed rates. The main difference in stem N content was observed between the N treatments. Those canopies with less fertiliser N had less N content throughout the canopy depth. This difference seemed greater than the difference in stem green area.
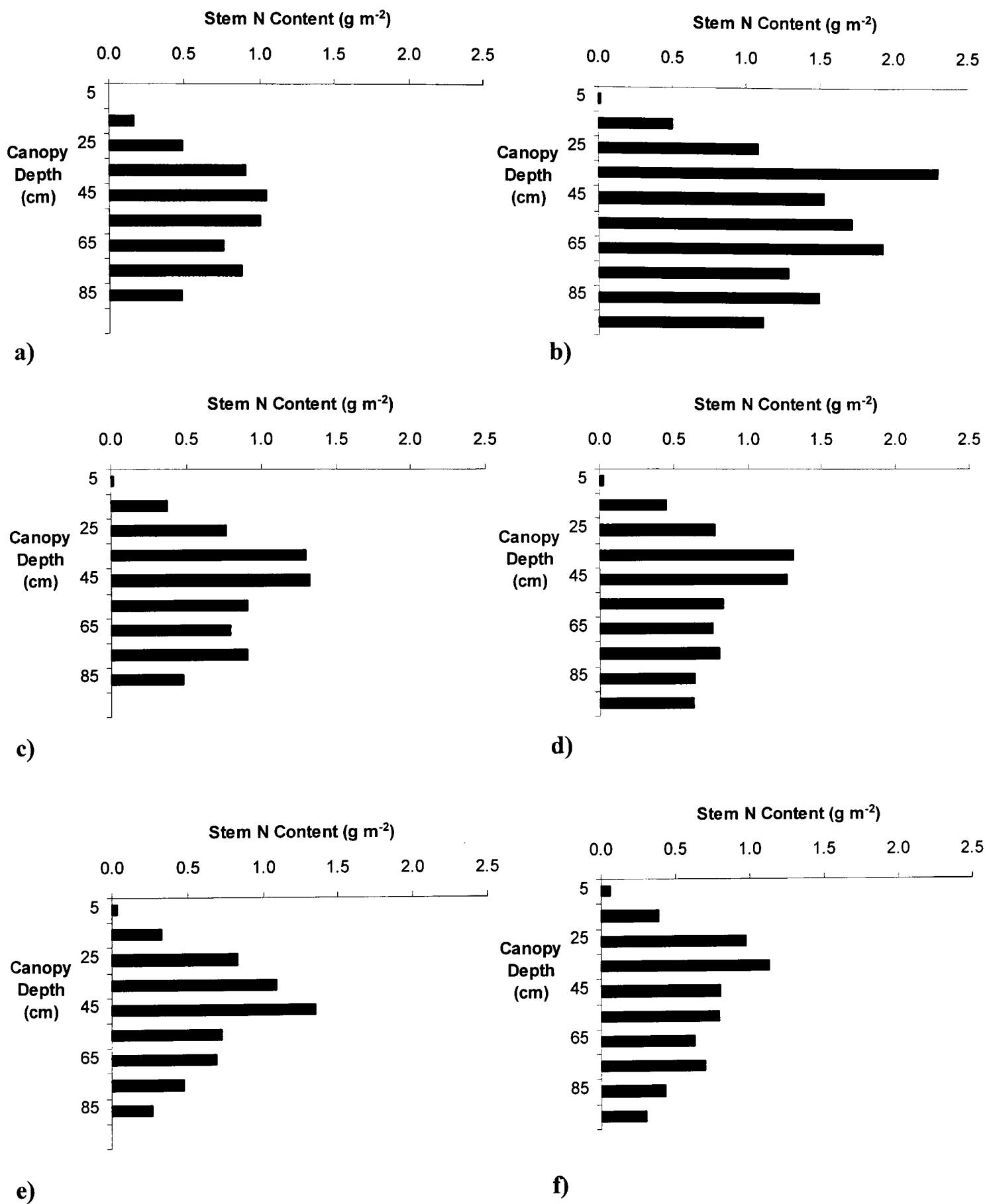
Figure 8.1 The N content of green stem tissue (g m^{-2} ground area) down canopies on June 29 of the 1997/8 experiment; a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}.
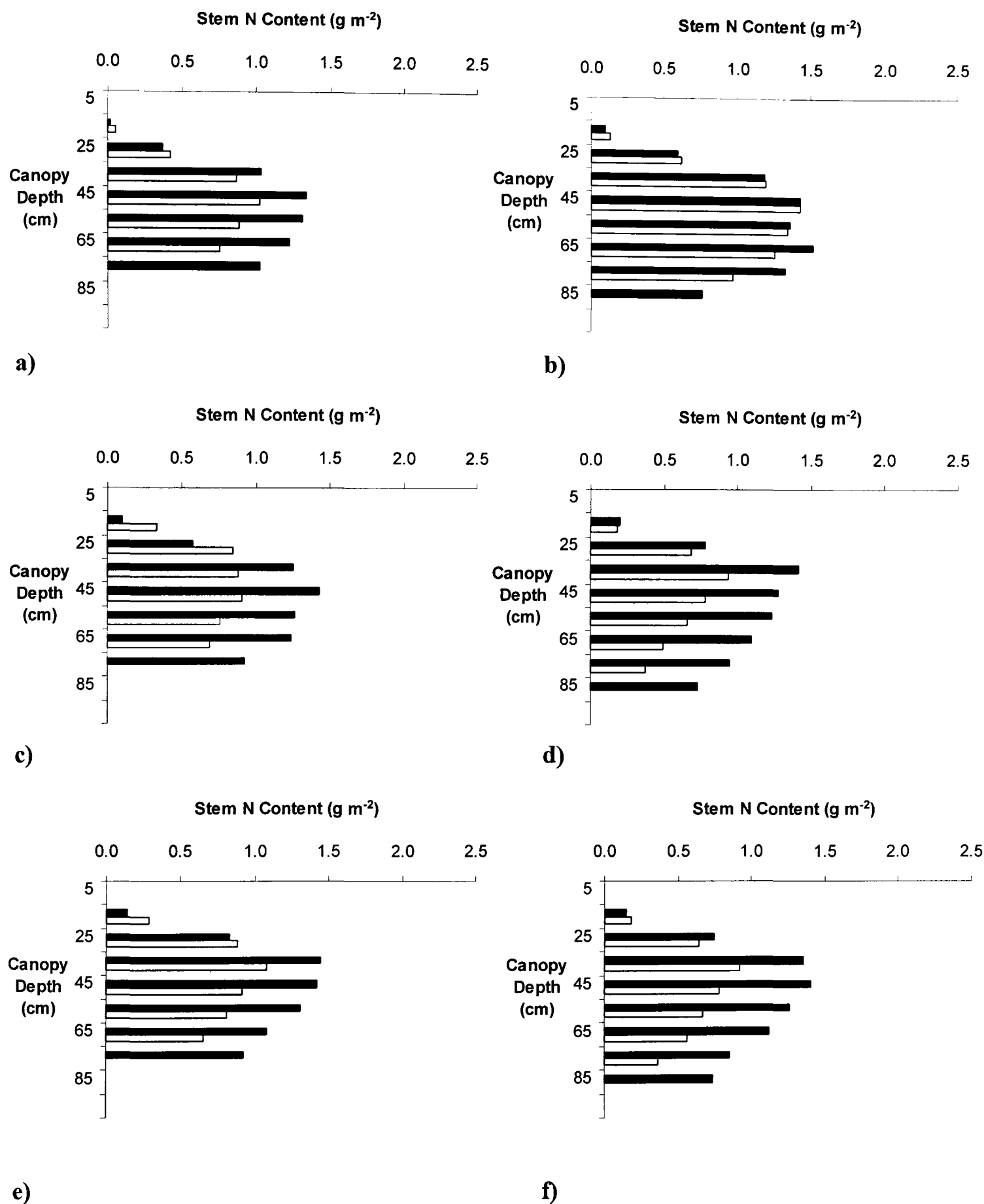
Figure 8.2 The N content of green stem tissue (g m^{-2} ground area) down canopies, at anthesis in the 1998/9 experiment; a) Soissons – 20 seeds m^{-2}, b) Spark – 20 seeds m^{-2}, c) Soissons – 320 seeds m^{-2}, d) Spark 320 - seeds m^{-2}, e) Soissons – 640 seeds m^{-2} and f) Spark – 640 seeds m^{-2}. 244 (■) and 124 (□) kg ha^{-1} fertiliser N.

8.2   STEM N CONTENT PER UNIT GREEN AREA

Nitrogen content of the stem is expressed on a canopy green area basis in this section, to correspond directly with the leaf N content and to understand the partitioning between plant components illustrated in Figure 5.1 - 5.3. Stem N content per unit total green area (excluding the ears) was determined from the % N in stem, stem dry weight and the lamina and stem green area of each layer.

8.2.1   The effect of husbandry on the distribution of stem N with increasing canopy size through depth

8.2.1.1   1997/8 experiment

There was a significant increase in stem N content per unit total green area (excluding ears) with increased canopy size through depth in 1998 as shown in Figure 8.3. Due to the large residual variation, a suitable model could not be fitted to the data. Several transformations were applied to the data, suggested by Sokal & Rohlf (1995), but none of these resulted in a satisfactory linear relationship. A statistical comparison of the treatments therefore cannot be made. There was a general increase in stem N content per unit GAI with increased canopy size through depth, but especially at the bottom of the canopy. Values increased from around 0.2 g m^{-2} N at the top, to around 3 g m^{-2} N at the bottom. The canopy sown at 20 seeds m^{-2}, appeared to have a consistently greater stem N content throughout the canopy compared to higher seed rates. The exception was at the bottom of Spark, which was an unusually large crop. There appeared to be no difference between the varieties.
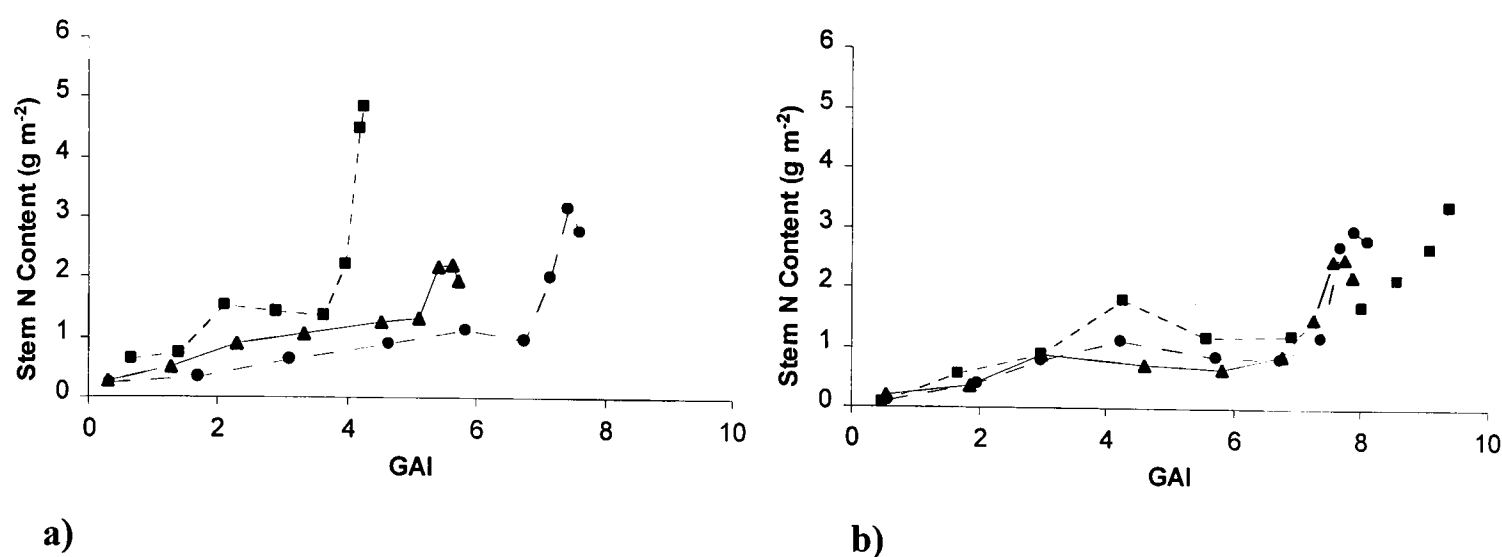
Figure 8.3 The increase in stem N content per unit green area (total excluding ears) with canopy depth (expressed as accumulated green area index; GAI (including ears)) on 29 June 1998. a) Soissons, b) Spark. Sown at 20 (..■..), 320 (---●---) and 640 (─▲─) seeds m^{-2}.

8.2.1.2 1998/9 experiment

Figure 8.4 shows the increase in stem N content per unit green area with increased canopy size through depth, in the 1998/9 experiment. Once again, a satisfactory transformation could not be found to carry out a comparison of linear regressions. The increase in stem N content was greatly increased at the base of the canopy, where the green area was reduced. Values of up to 4 g m^{-2} N were measured. This increase was not as great in the 'low N' crop, in which the maximum stem N content was only 2 g m^{-2}. The crop sown at 20 seeds m^{-2} had a greater stem N content at similar canopy size, particularly at the bottom of the canopy. Once again, there appeared to be no effect of variety.
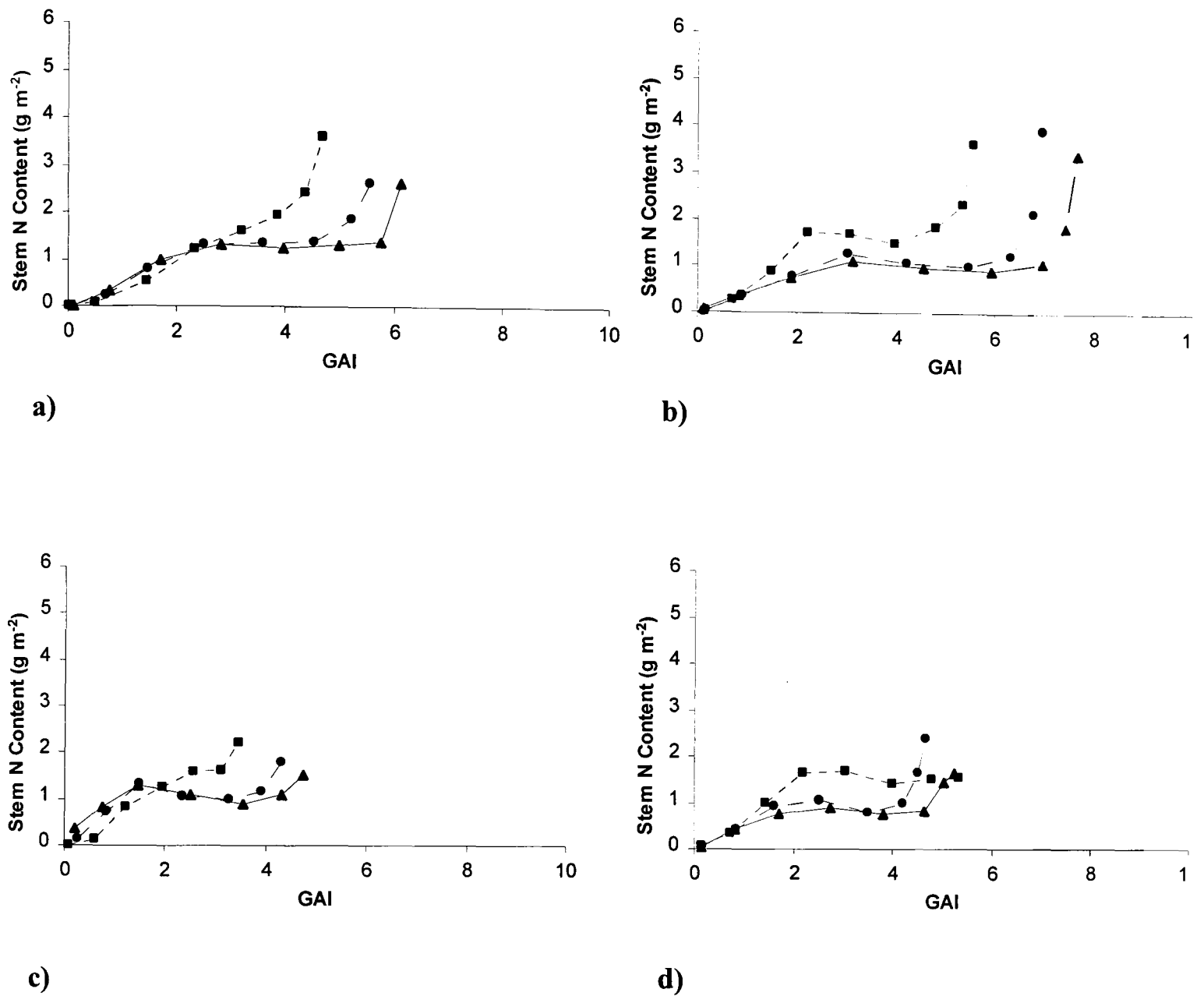
Figure 8.4 The increase in stem N content per unit green area (total excluding ears) with canopy depth (expressed as accumulated green area index; GAI (including ears)). Anthesis 1999. a) Soissons - 244 kg N ha^{-1} b) Spark - 244 kg N ha^{-1}, c) Soissons - 124 kg N ha^{-1} and d) Spark – 124 kg N ha^{-1}. Sown at 20 (..■..), 320 (---●---) and 640 (─▲─) seeds m^{-2}.

8.2.2 The effect of husbandry on mean stem N content.

8.2.2.1 1997/8 experiment

The mean canopy stem N content per unit green area (excluding ears) of all treatments is presented in Figure 8.5. As expected, there was a significant increase in stem N content at the low seed rate, (P<0.001). This effect was not observed at the higher seed rates. The stem N content of canopies sown at 20, 320 and 640 seeds m^{-2}, was 1.44, 0.99 and 0.97 g m^{-2}, respectively. Soissons and Spark had mean stem N contents of 1.18 and 1.09 g m^{-2}, respectively, but this difference was not significant.
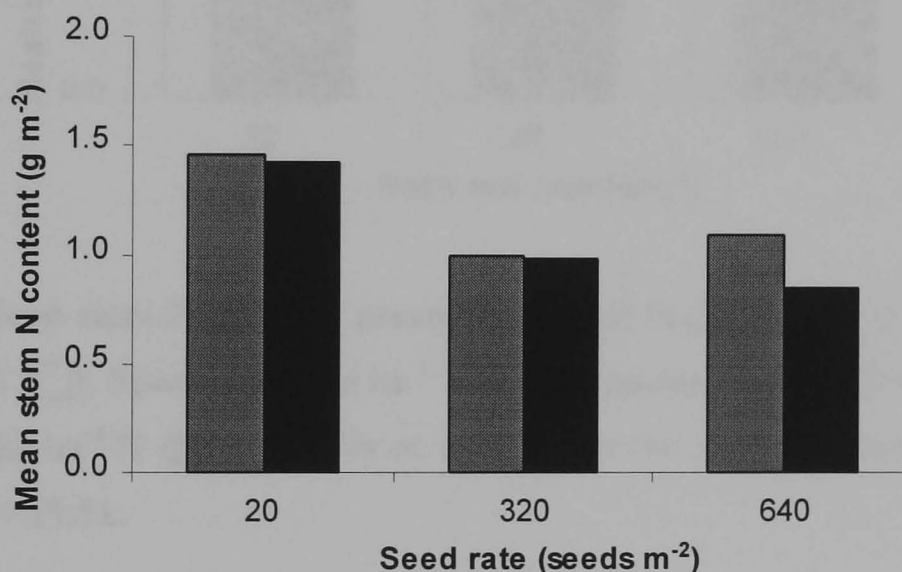
Figure 8.5 Mean stem N per unit green area (total excluding ears) in Soissons (▨) and Spark (■) canopies on June 29 in the 1997/8 experiment. S.E.D = 0.1640, d.f. = 10.

8.2.2.2 1998/9 experiment

Figure 8.6 presents the mean canopy stem N content for each treatment in 1999. Again, there was a significant increase in stem N content with decreased seed rate (P<0.001), but the effect was reduced at the higher seed rates. The stem N content of canopies sown at 20, 320 and 640 seeds m^{-2}, was 1.45, 1.15 and 1.07 g m^{-2},

respectively. This was similar to the previous year. In this year the effect of variety was significant. Soissons had a greater stem N content than Spark (1.28 and 1.17 g m^{-2}, respectively) (P=0.05). Canopies fertilised with 244 kg ha^{-1} N had a greater stem N content than those fertilised with 124 kg ha^{-1} N (P<0.001). The mean stem N contents were 1.34 and 1.11 g m^{-2}, respectively.
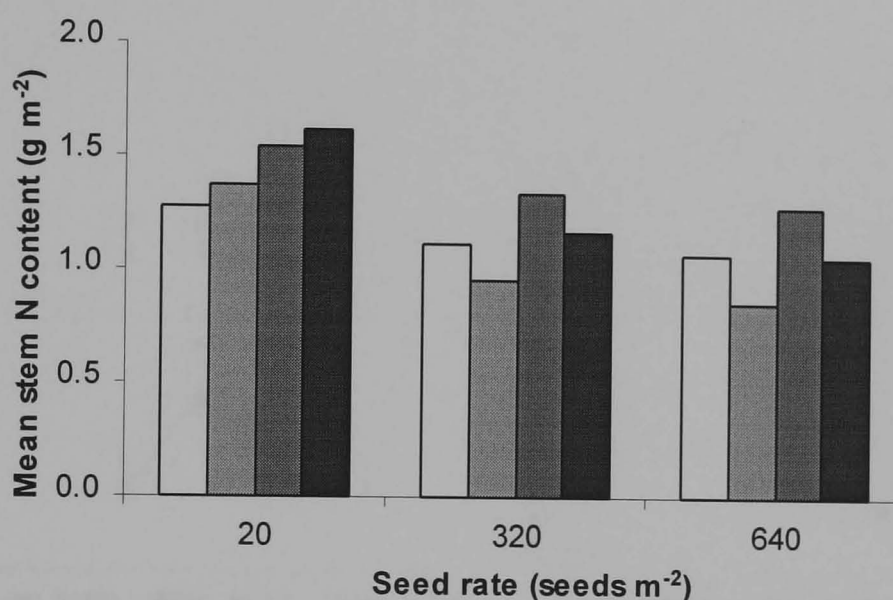
Figure 8.6 Mean stem N per unit green area (total excluding ears) in Soissons - 124 kg ha^{-1} N (☐), Spark - 124 kg ha^{-1} N (▨), Soissons - 244 kg ha^{-1} N (▨) and Spark - 244 kg ha^{-1} N (■) canopies at anthesis in the 1998/9 experiment. S.E.D =0.0951, d.f. = 19.31.

8.2.3 The effect of crop development on mean stem N content

8.2.3.1 1997/8 experiment

The change in mean stem N content per unit green area through time is presented in Figure 8.7. Very low values were observed in March immediately after stem extension had begun (GS31 15 March) and when the ratio of stem to leaf area was small. This may also have been pseudostem (leaf sheath) rather than true stem tissue at this time. Stem N content generally increased rapidly at the beginning of this period, reached a peak in April when fertiliser N was applied and canopy expansion was greatest, and then changed little through to the end of the growing season. At the beginning of stem extension, Soissons had a significantly greater stem N content. The

peak reached in April was 1.61, 1.21 and 1.17 g m^{-2} N for canopies sown at 20, 320 and 640 seeds m^{-2}, respectively. This seed rate effect was significant throughout the majority of the growing season (P=0.05). Soissons and Spark reached maximum stem N contents of 1.43 and 1.24 g m^{-2}, that were significantly different (P=0.05). The variety effect diminished possibly as the difference in development was reduced. When grain fill had begun, in July, there were no treatment effects.

a)   b)
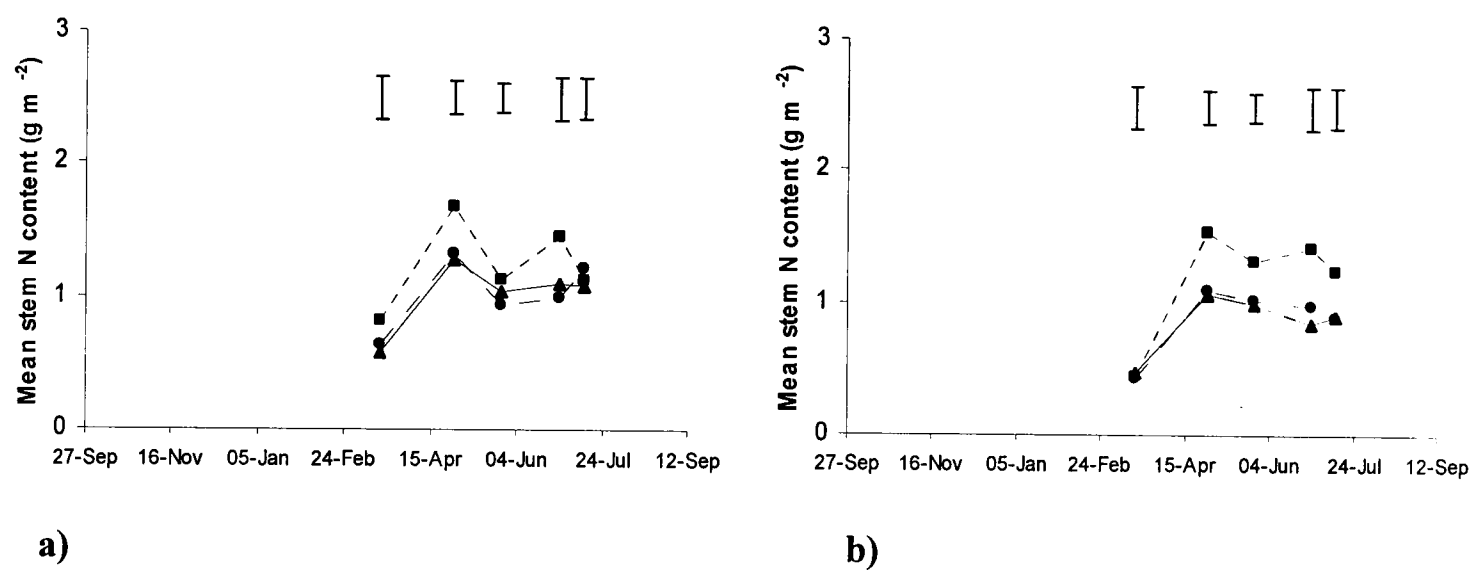
Figure 8.7 The change in mean stem N per unit green area (total excluding ears) in 1997/8. a) Soissons and b) Spark sown at 20 ("···■···"), 320 (--●--) and 640 (—▲—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

8.2.3.2   1998/9 experiment

There were fewer samples taken after the beginning of stem extension in the 1998/9 experiment and consequently the change in mean stem N content through time was seen in less detail than in the previous year (Figure 8.8). The same general increases in stem N content for all treatments were observed between March and anthesis. At the beginning of stem extension stem N content was again very low in all treatments. At this time there was a significant increase in stem N content with the decrease in seed rate but particularly at the lowest seed rate (P=0.05). Unlike in the previous year, there was no variety effect. By anthesis, there were significant effects of all treatments as previously described.
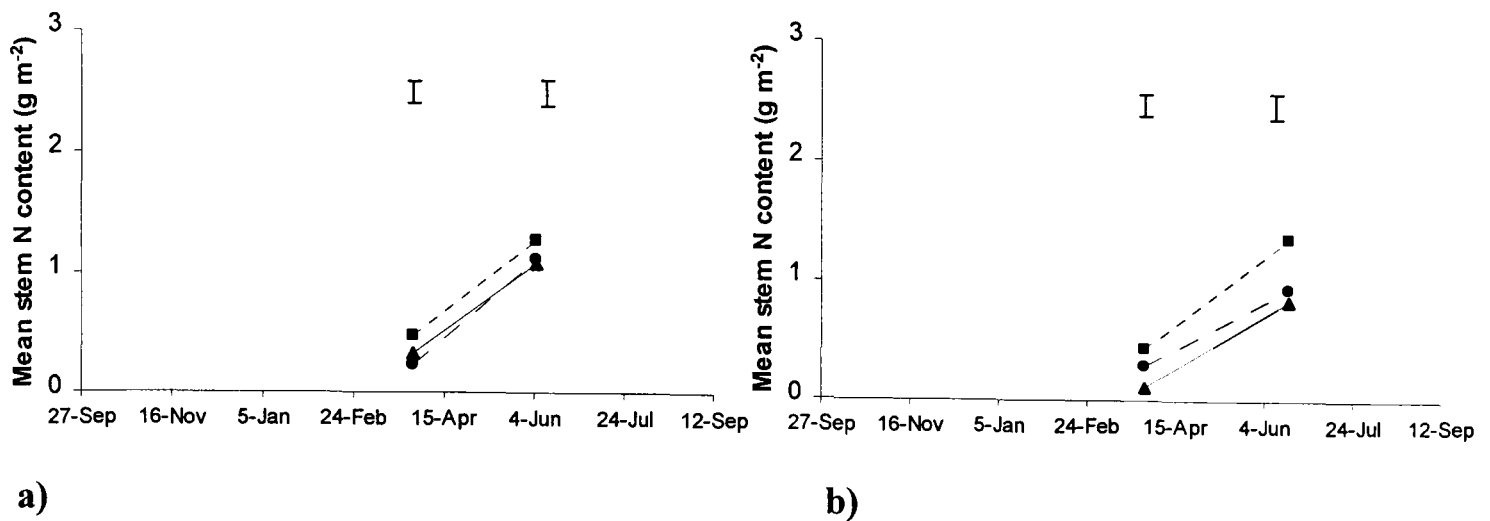
Figure 8.8 The change in mean stem N per unit green area (total excluding ears) in 1998/9. 124 kg N ha^{-1} applied. a) Soissons and b) Spark sown at 20 ($\cdots\blacksquare\cdots$), 320 (--$\bullet$--) and 640 (—$\blacktriangle$—) seeds m^{-2}. Error bars represent S.E.D., d.f. = 10.

8.3 THE RELATIONSHIP BETWEEN STEM ARCHITECTURE AND STEM N REQUIREMENT

In the previous section, stem N content was expressed on a total green area basis and was therefore largely dependent on the leaf area. This does not give an indication of the direct relationship between stem N content and architecture that is proposed in this thesis. Detailed architectural stem characteristics are now considered with the variation in stem N content of individual shoots with canopy depth and between husbandry treatments. The principles used in the prediction for stem N requirement are tested through the comparison with Spark at 320 seeds m^{-2} and fertilised with 124 kg ha^{-1} N.

8.3.1 Variation in architecture and stem N requirement per unit stem area with canopy depth

The prediction of stem N requirement with canopy depth, in Chapter 2, is based the stem dry weight and projected area. This sub-section presents these characteristics

observed in Spark sown at 320 seeds m^{-2}. This is compared to the theoretical canopy from the literature.

The stem dry weight per unit stem length and stem area per unit stem length are considered to be important parameters relating to structural N per unit stem area (Section 2.2.2). Data from the literature was used to predict these for a theoretical canopy with a GAI of about 7. As expected, the stem dry weight per unit stem length increased for each extra unit of green area with canopy depth in Spark (Figure 8.9). The relationship was linear with accumulated GAI. The rate of increase in stem dry weight was greater in Spark, with smaller values at the top and larger values at the bottom compared to the theoretical canopy. The stem area per unit stem length increased with accumulated GAI through canopy depth for half of the canopy (Figure 8.10), which provides an explanation for the increase in stem dry weight in this part of the canopy. The theoretical canopy had a relatively constant stem area, which was only observed in Spark in the lower half of the canopy. However, in the theoretical canopy stem tissue was not measured sufficiently high up where the thinner peduncle would be present. Also, Spark generally has a thinner stem than other varieties which is supported by other ongoing work (P.Berry, pers. comm.). The stem dry weight per unit stem area of Spark increased with GAI through canopy depth at a greater rate than the theoretical canopy (Figure 8.11). The increase was exponential because of the greater stem dry weight but constant area, in the lower half of the canopy. As canopy depth and size increases, the stem becomes heavier with the requirement for more support.

The stem N content per unit stem area in the Spark canopy, decreased with canopy depth and size (Figure 8.12). The N content remained stable at about 5.2 g m^{-2}, down to an accumulated GAI of about 2. It then decreased rapidly to 2.3 g m^{-2} at the bottom of the canopy. It is proposed that there is a relationship between the stem parameters described here and the structural stem N requirement per unit stem area (Chapter 2). The architecture of the canopy is also predicted to relate to photosynthetic N requirement per unit stem area. This is not compared to the theoretical canopy as there were no corresponding N content data in the literature. However, it will be compared to the prediction for Spark, in Section 8.5.
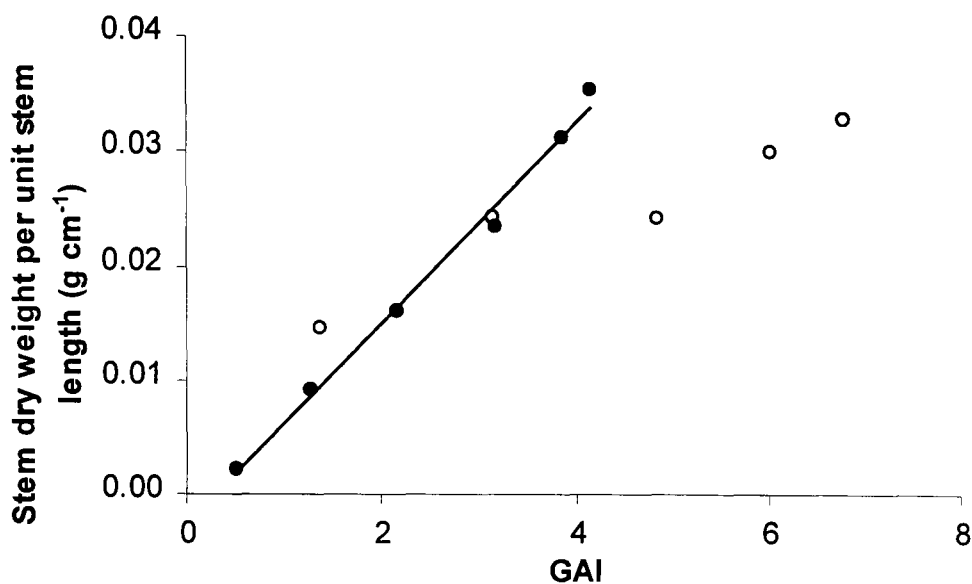
Figure 8.9 A comparison of individual stem dry weight per unit stem length with canopy depth (expressed as accumulated green area index; GAI (including ears)), between the predictions for a theoretical canopy of GAI 7 (O) and observations for Spark 320 seeds m^{-2} 124 kg ha^{-1} N (●). Fitted line for Spark, R^2 = 99%.
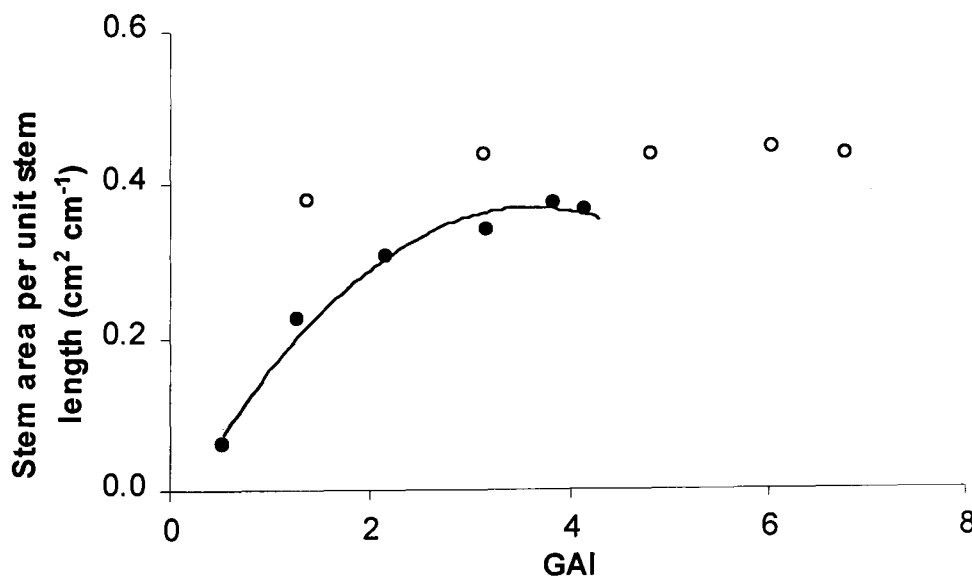
Figure 8.10 A comparison of individual stem area per unit stem length with canopy depth (expressed as accumulated green area index; GAI (including ears)), between the predictions for a theoretical canopy of GAI 7 (O) and observations for Spark 320 seeds m^{-2} 124 kg ha^{-1} N (●). Fitted curve for Spark, R^2 = 98%.
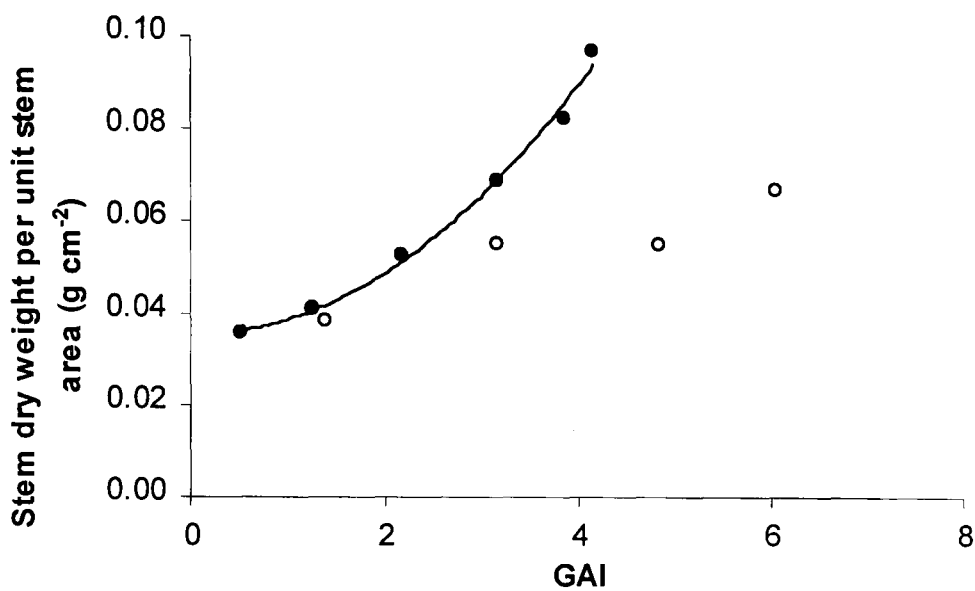
Figure 8.11 A comparison of individual stem dry weight per unit stem area with canopy depth (expressed as accumulated green area index; GAI (including ears)), between the predictions for a theoretical canopy of GAI 7 (O) and observations for Spark 320 seeds m^{-2} 124 kg ha^{-1} N (●). Fitted curve for Spark, R^2 = 99%.
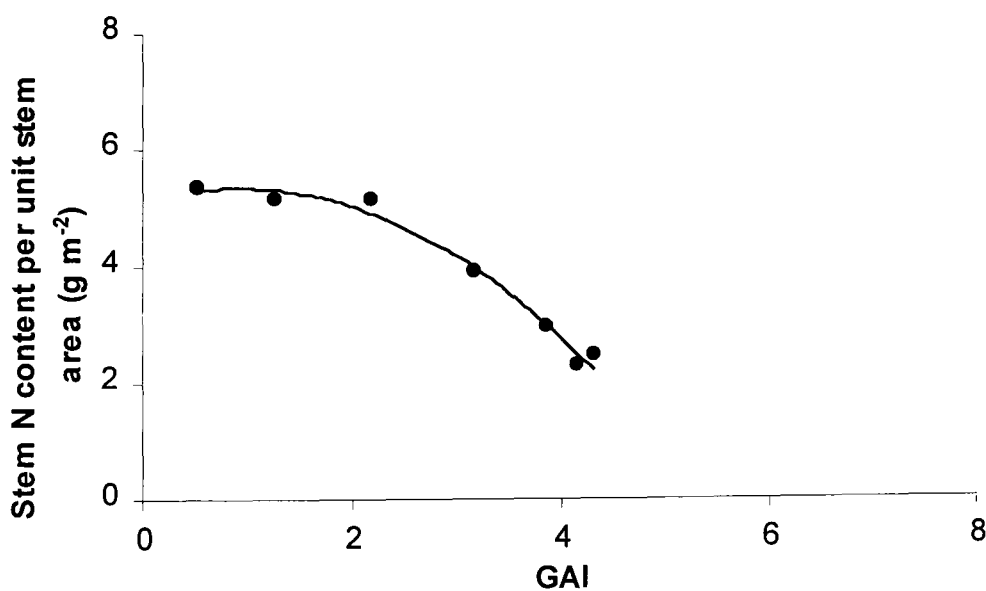
Figure 8.12 Stem N content per unit stem area with canopy depth (expressed as accumulated green area index; GAI (including ears)), for Spark 320 seeds m^{-2} 124 kg ha^{-1} N. Fitted curve, R^2 = 95%.

8.3.2 The effect of husbandry on stem architecture and stem N requirement per unit canopy green area

The predicted effects of husbandry on stem N requirement were based on stem characteristics similar to those considered for the variation with canopy depth (Table 2.4). This included stem length and stem dry weight but also considered the leaf area to be able to compare directly with stem N requirement per unit green area. There was no interaction between N treatment and seed rate or variety for any parameter.

Stem length per unit leaf area increased with the increase in seed rate (P<0.001) which can be directly related to the decrease in leaf area per shoot (Chapter 6). As expected, the stem dry weight per unit stem length decreased with the increase in seed rate (P<0.001). A similar decrease was also observed for the stem N content per unit stem length with increased seed rate (P<0.001). Soissons had a greater stem N content per unit stem length (P=0.05). These results are presented in Table 8.1.

Table 8.1 The effect of variety (Var) and seed rate (SR) on stem parameters in canopies fertilised with 124 kg ha^{-1} N. Values presented are for individual stems. d.f. = 10.

Parameter	Variety	Seed Rate (seeds m^{-2})			Mean
		20	320	640	
Stem	SOISSONS	1.09	1.43	1.80	1.44
Length /	SPARK	1.22	1.50	1.67	1.46
Leaf area	**Mean**	1.18	1.47	1.74	1.45
(cm cm^{-2})		*P*	S.E.D.		
	Var	0.714	0.051		
	SR	<0.001	0.062		
	Var*SR	0.148	0.088		
Stem dry	SOISSONS	19.5	17.4	13.2	16.7
Weight /	SPARK	19.5	18.2	151	17.6
Stem	**Mean**	19.5	17.8	14.2	17.1
length		*P*	S.E.D.		
(mg cm^{-1})	Var	0.225	0.69		
	SR	<0.001	0.85		
	Var*SR	0.532	1.20		
Stem N	SOISSONS	0.155	0.111	0.085	0.117
Content /	SPARK	0.148	0.087	0.071	0.102
Stem	**Mean**	0.152	0.099	0.078	0.109
Length		*P*	S.E.D.		
(mg cm^{-1})	Var	0.026	0.0058		
	SR	<0.001	0.0071		
	Var*SR	0.516	0.1000		

8.4   LEAF SHEATH EXPERIMENT

An investigation into true stem and leaf sheath N was carried out at the beginning of grain fill in 1999 on Spark shoots sown at 20 and 320 seeds m^{-2} and fertilised with 244 kg ha^{-1} N. By separation of the leaf sheath from the true stem, the partitioning of N between these two components was measured and a comparison between the two seed rates made. The N content of the lamina was also measured for comparison with leaf sheath.

The results from this investigation (Table 8.2) showed that there was a greater % N in the leaf sheath compared to the true stem, but less than half that in the lamina. There was more N per unit ground area in the true stem than either the leaf sheath or the lamina because it contained more stem biomass. There was a large proportion of the total N in the stem allocated to the true stem compared to the leaf sheath (63 and 36%, respectively). Surprisingly, the proportion allocated to each component was consistent between the two seed rates suggesting a close relationship between the function of N in leaf sheath and true stem. This appeared to be an important observation that could be used to understand more fully the role of N in the stem. The N content per unit green area was consistently greater in the true stem than the leaf sheath for all expressions of green area. The N content of the leaf sheath was only greater than the laminae when the projected green area was used in the calculation, which is conventionally used. Measuring the N content in terms of its cylindrical area rather than its projected area resulted in the leaf sheath having a lower N content than the laminae. This was also observed in the true stem, although the differences were smaller. These observations were confirmed by the low seed rate which had a heavier true stem, leaf sheath and lamina tissue.

Table 8.2 The % N, dry weight and N uptake and N content of separated true stem and leaf sheath from individual shoot samples of Spark sown at 320 seeds m^{-2} with 244 kg N ha^{-1} in 1998/9. d.f. = 18.

	SEED RATE	TOTAL (stem + leaf sheath)	T.STEM	SHEATH	LAMIN
% N	20	0.97	0.64	1.30	3.3
(% dry matter)	320	0.71	0.53	0.89	2.5
	S.E.D	0.036	0.034	0.047	
Dry matter	20	1423	1111	312	262
(g m^{-2} ground area)	320	1177	894	283	263
	S.E.D.	82.3	63.3	21.6	
N uptake	20	11.16	7.15	4.02	9.43
(g m^{-2} ground area)	320	7.16	4.66	2.50	8.20
	S.E.D.	0.626	0.466	0.216	
N uptake	20		63.8	36.2	
(% total)	320		65.0	35.0	
	S.E.D.		1.41	1.41	
N content	20	7.07	4.52	2.56	1.93
(g m^{-2} total stem	320	4.11	2.67	1.44	1.24
projected area)	S.E.D.	0.197	0.167	0.097	
N content	20	2.25	1.44	0.81	1.93
(g m^{-2} total stem	320	1.31	0.85	0.46	1.24
cylindrical area)	S.E.D.	0.063	0.053	0.031	
N content	20	2.25	1.80	0.82	1.93
(g m^{-2} component	320	1.31	0.85	0.42	1.24
cylindrical area)	S.E.D.	0.063	0.100	0.028	

8.5 DISCUSSION

Nitrogen in the stems of cereals has not been studied in as much detail as in the leaves, despite there being evidence that stems contain a considerable amount of N. The role of N in stems is therefore less understood. This section tests the predictions for the effect of canopy depth and husbandry on stem N requirement. The function of N within this plant organ is also discussed to provide an explanation for these observations.

8.5.1 Testing the prediction of stem N requirement with canopy depth

The stem N requirement per unit stem area of Spark sown at 320 seeds m^{-2} at 124 kg ha^{-1} N was predicted from the stem structural characteristics shown in Figures 8.9 – 8.11 and using the equations in Section 2.2.2. This is now compared to the observed N requirement and presented in Figure 8.13.
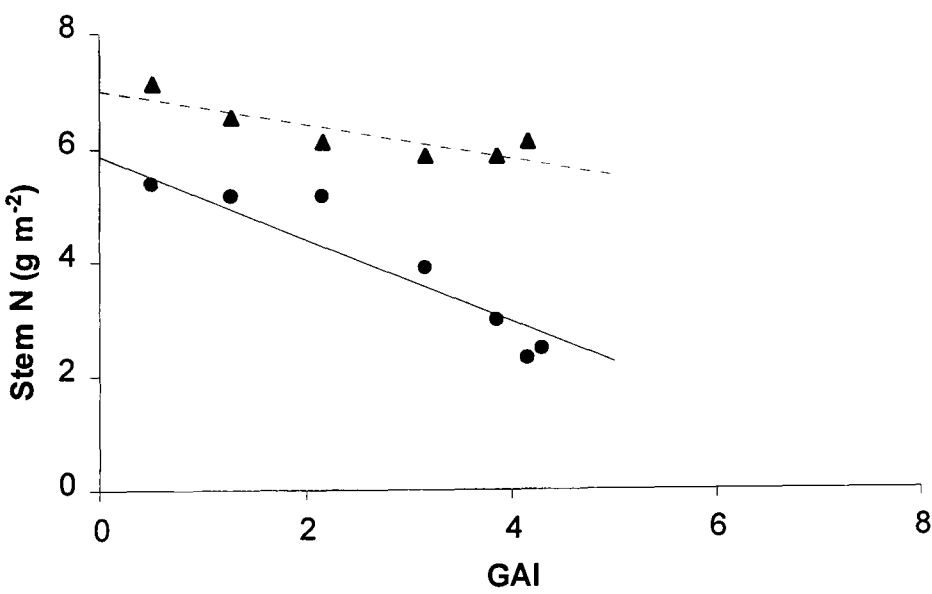
Figure 8.13 A comparison of the predicted (▲) and observed (●) stem N requirement per unit stem area with canopy depth (expressed as accumulated green area index; GAI) for Spark sown at 320 seeds m^{-2} and 124 kg ha^{-1} N. Fitted lines; R^2 = 88%, for predicted (.....) and observed (——).

The observed stem N requirement decreased more than the predicted, which remained relatively constant. The predicted stem N content did not decrease with depth because not only was the photosynthetic N expected to remain high, but also the amount of structural N was predicted to increase greatly. This was based on the large increase in dry weight per unit area with depth. The observed was consistently less than the predicted stem N requirement per unit stem area but the separate fitted lines show that there was a greater difference at the bottom of the canopy. The observed mean stem N requirement was 3.6 g m^{-2} and the predicted was 5.3 g m^{-2}.

The top of the stem contained the greatest N requirement per unit stem area (7.1 g m^{-2}) and it is suggested here that this is due to a high photosynthetic N requirement. Although there was no leaf sheath surrounding the peduncle, it was still green. It is likely there would be little structural N as this had the lowest dry weight per unit area (Figure 8.11).

As expected, there was an increase in stem N content per unit canopy green area with the increase in canopy size through depth, for all treatments. Although this was implied by Grindlay et al., (1997), there were no supporting data. Photosynthetic N in the leaf sheath would decrease in response to the reduced light flux, as seen in the laminae (Chapter 7). Although dry weight increased with depth, there would be proportionately more structural compounds containing low %N and less photosynthetic N, both of which would lower total %N. Lemaire et al., (1991) observed a decrease with canopy depth in lucerne where the %N ranged from 6% to 2% in dry matter. Data from this project show that the %N was lower in wheat, decreasing from 2% to 0.5% with canopy depth (data not shown). The increase in stem N requirement per unit green area was mainly due to the decrease in GAI per layer with canopy depth because, as expected, the stem N content per unit stem area actually decreased with canopy depth.

8.5.2 Testing the predicted effects of husbandry and canopy development on stem N requirement

Contrary to predictions, Soissons had a greater stem N requirement per unit green area than Spark. This was because there was no effect of variety on stem length per unit

leaf area which was expected to be greater in Spark (Table 8.1). It is therefore proposed that the prediction of the variety effect was incorrect, because of the unexpected greater leaf area per shoot in Spark (Chapter 4). Stem N requirement was 1.1 g m^{-2} in Spark and 1.2 g m^{-2} in Soissons.

Stem N requirement per unit green area increased from 1.1 g m^{-2} at 320 seeds m^{-2} to 1.4 g m^{-2} at 20 seeds m^{-2}. As the stem length to leaf area was greater in the high seed rate, this can be explained by the greater stem N content per unit stem length at the low seed rate (Table 8.1). It is proposed that this is because of a greater structural and photosynthetic N requirement at the low seed rate. This seed rate effect agreed with the qualitative predictions (Chapter 2).

The N content of stems increased with canopy size during crop development as expected from predictions (Chapter 2). The N taken up into the shoot expressed on a green area basis was partitioned increasingly to the stem with time. There would have been an increased need for structural compounds as the canopy expanded. Once canopy expansion ended, the stem N content remained relatively stable.

The differences in stem N requirement between each canopy depth and each husbandry treatment can be explained by the function of N in the stem. By increasing an understanding in the function and requirement, some of the differences between the predicted and observed N requirements can now be explained.

8.5.3   Functions of N in the stem

The function and quantity of the N requirement for each of these in the stem has not been found in the literature and so this work provides a new contribution to understanding the role of stem N. Without this understanding, it is difficult to predict the N requirement with precision. Examining the differences between the treatments and where the assumptions were incorrect will improve the predictions.

8.5.3.1 Structural N

It was assumed that structural N per unit stem area is directly related to stem dry weight per unit area and that % structural N in dry weight is constant for all canopy depths and treatments. The decrease in stem N requirement with canopy depth was greater than predicted. Since the previous chapter has shown that the rate of decrease in leaf N requirement was correctly predicted, this suggests that the difference in stem N requirement is not due to an overestimation of photosynthetic N at the bottom of the canopy. One explanation could be that the % structural N in dry weight decreases with canopy depth. This could be because of an increased proportion of structural compounds that do not contain N. However, there is no evidence for this and the difference may be due to a decrease in transport or storage N. Similarly, stem dry weight per unit length was similar in both varieties, but the greater stem N content per unit stem length in Soissons could suggest that Soissons had a greater % structural N in dry weight. However, extra storage for the larger ears in Soissons may also provide an explanation.

It was also assumed that all of the N in the true stem was structural. The predicted structural N requirement per unit stem area (projected) was 1.8 g m^{-2} for Spark sown at 320 seeds m^{-2}. The N content actually measured in the true stem was about one and a half times greater (2.7 g m^{-2}). This implies that there were additional roles of N in the true stem, other than structure, such as transport in the phloem (Simpson *et al.*, 1983) and storage N.

8.5.3.2 Photosynthetic N

It was assumed that the photosynthetic N requirement was directly associated to the leaf N requirement and its relationship with light. Photosynthesis was not measured in these experiments but there is evidence for a significant amount of photosynthesis carried out by the stem (Murthy & Singh, 1979). One explanation for the difference between the observed and predicted stem N requirement measured with canopy depth, is that the photosynthetic N in the leaf sheath has been overestimated. This could be because of a lower maximum N content or light saturation point in the sheath than in the lamina, a difference in the response of N to light flux compared to the lamina, or an overestimation of the light received by the leaf sheath.

It was also assumed that all the N in the leaf sheath was photosynthetic and that the relationship between photosynthetic N and PPFD of the leaf sheath is equivalent to that of the lamina. The lower %N in leaf sheath compared to lamina suggests that the leaf sheath had less photosynthetic N or more structural components than the lamina. The predicted mean photosynthetic N per unit stem area (projected) was 3.9 g m^{-2} N for Spark sown at 320 seeds m^{-2}. This was about three times as much as that actually measured in the leaf sheath where there was the possibility of luxury N (1.4 g m^{-2} N). It was also greater than the leaf sheath N content of the smaller canopy sown at 20 seeds m^{-2} (2.6 g m^{-2}), which had greater light transmission. This indicated that the prediction of photosynthetic N was overestimated. It is suggested that either only one third of the total surface area of the leaf sheath is in direct sunlight (and the N requirement per unit cylindrical area is still similar to lamina), or, the N requirement of the leaf sheath per unit cylindrical area is one third of the lamina. It is assumed that the N is distributed evenly throughout the tissue and so the latter appears more likely. Therefore, the assumption that leaf sheath and lamina tissue are similar is invalid and cannot be used in the prediction for photosynthetic stem N. The comparison between lamina and leaf sheath N content suggests that a correction factor of x 0.34 might be used to estimate the photosynthetic N in the leaf sheath from the lamina.

8.5.3.3 Functions not predicted

It was assumed that there was no transport, luxury or storage N in the stem. However this must be included for the increased precision of the predictions. Nitrogen is mainly transported as amino N and forms 30% of total phloem sap (Hayashi & Chino, 1983; Heldt, 1997). The concentration of amino N transported within the xylem sap was reported to increase with stem height from 57 to 104 μg ml^{-1} (Simpson et al., 1983), which may be because of the accumulation of N exported from the senescing leaves before entering the grain. This could provide an explanation for the increased difference between the prediction and observation with canopy depth (Figure 8.13). Although the N in transport on an area basis in wheat is not provided in the literature, it is thought that these concentrations would be significant. If the N content of the phloem were measured, this would give an indication of the N in transport, at various depths and on an area basis.

The lower seed rate had a greater stem dry weight per unit stem length and was therefore expected to have greater structural N requirement per unit stem length. Therefore, it would also be expected to have lower % N in stem dry weight due to the large amount of low %N structural compounds. The results from the detailed shoot analysis showed that the true stem contained 0.5 %N (of true stem dry weight) at 320 seeds m^{-2} but increased to 0.9 % at 20 seeds m^{-2}. The true stem beneath the leaf sheath was a pale green colour and so would have only contained a small amount of photosynthetic N. The increased % N might be explained by luxury N from the increased N availability per shoot (Puckridge and Donald, 1967) or by the requirement for storage N to increase mobile N in tillers (Sticksel *et al.*, 1999). Storage may be located in the stem as well as in the leaf tissue suggested in Chapter 7.

The distribution of N between the leaf sheath and the true stem was surprisingly consistent between the two seed rates. If there was more luxury or storage N at the low seed rate, then this suggests that it was equally proportioned to the leaf sheath and to the true stem. Another possibility is that the increase in photosynthetic N in the leaf sheath is compensated by the increase in storage in the true stem. N is stored in the form of nitrate, amino acids in vacuoles (Millard, 1988), but also as protein, for example RUBISCO (Lawlor *et al.*, 1987b). So, even with the identification of N compounds, it might have been difficult to distinguish the photosynthetic and the reserve functions.

8.5.4 Summary

This chapter of results has highlighted the importance of considering N in the stem to understand the N requirement of the whole canopy. Approximately 50% of the total canopy N was in the stem, of which 35% was photosynthetic N in the leaf sheath, 25% was structural and the remaining 40% was assumed to be transport, metabolic, storage and luxury. The stem N requirement per unit green area increased with canopy size through depth because of the decreased GAI with depth. Stem N requirement also increased with canopy development because of the increased structural compounds required with crop development. This was despite the decrease in photosynthetic N requirement in both cases. The mean stem N requirement was 1.1 g m^{-2} for a range of seed rates and the two varieties chosen. The stem N requirement increased with the

reduction in seed rate, as there was a greater requirement for structural N, photosynthetic N, and possibly an additional requirement for storage N. Soissons had a greater stem N requirement than Spark due to a greater stem to leaf green area ratio but possibly to a greater structural or storage N requirement also. The results suggest that the principles that the predictions were based on in Chapter 2 overestimated the photosynthetic N requirement of the leaf sheath. Future work would need to include the identification and prediction of transport and storage N in the true stem.

9   GENERAL DISCUSSION

It has become apparent over recent years that there is little understanding of the nitrogen (N) requirement of any crop. Current fertiliser N recommendations based on the requirement of particular crops such as wheat are therefore often imprecise. It is also recognised that the potential for over fertilisation resulting from this, is a problem for the environment (Johnston & Jenkinson, 1989). Advances in machinery technology, breeding, genetics and growth regulation appear to be greater than the understanding of crop nutritional requirements. The links between crop physiology, agronomy and soil science are not sufficient to provide a basis with which to deal with the current problem. This final chapter discusses how this project has attempted to meet the aim of providing a physiological explanation for canopy N requirement (CNR). The implications of the principles and knowledge surrounding the CNR for the researcher, grower and the breeder are also discussed. The limitations of the current work and the work still required to improve predictions of CNR are suggested, and, finally, conclusions are drawn.

9.1   PHYSIOLOGICAL BASIS OF THE CNR

CNR provides an indication of the amount of N a crop requires to maximise crop productivity through the optimal distribution of N. The CNR defined in this thesis is the requirement for the production and efficiency of photosynthetic tissue, its maintenance throughout the period of grain fill and support. It is based on maximising nitrogen use efficiency (NUE) for photosynthesis and the minimum amount required for structural, metabolic, transport and storage compounds to support the canopy in both the leaf and the stem. The gap in knowledge concerning the role of N in the wheat stem and its contribution to total N requirement is newly identified here. Most of the literature has focused on the photosynthetic function of N in the lamina but has largely disregarded the stem. The stem not only has an important role in structural support of the photosynthetic organs but also in photosynthesis and transport. This work has revealed that the stem contains half of the total N in the wheat canopy on

both a ground area basis and total green area basis (excluding ears) indicating that the purpose and fate of the N in this plant organ must be considered more carefully.

Predictions for the CNR of leaves and stem were based on a physiological framework centred on the literature and developed in Chapter 2. This was formulated to test the hypothesis that variation in CNR can be predicted from the variation in canopy architecture. It was tested mainly through experiments set up in the second season. The predictions in CNR were based on the physiological requirement for photosynthetic N in the leaves and stem and structural N in the stem. The amount of N not directly used for photosynthesis or structure was considered to be either luxury uptake, transport N or storage N or a combination of these. Luxury uptake was not considered to be a physiological requirement.

The total N content was measured in all treatments but this often included luxury N for crops grown on high levels of soil mineral N in 1997/8 or treated with high fertiliser N application in 1998/9. The reduction in fertiliser N by 120 kg ha^{-1} decreased combine yield by 16% in the high seed rate and 8% in the low seed rate. By comparison with the yield response to fertiliser N curve (Jenkinson, 1986), the small difference in yield with such a large decrease in fertiliser N indicates that the 'high N' crop had a lot of luxury uptake and the lower N application was closer to the N required for that yield. The N content of crops grown with the lower N application was therefore considered to represent the CNR. The N content of the canopy in which yield increases only a little with extra N supply would be closer to the CNR. This was observed at the low seed rate. However, it is possible that the N content of the 'low N' canopy at the high seed rates could be less than the requirement.

The precision of the prediction for CNR depends on understanding the variation in N requirement and thus determines the value of the application of CNR to each set of growing conditions. The experiments and detailed analysis were sufficient to provide a physiological explanation for CNR. The leaf and stem N requirement can be related to canopy architecture through physiology. There was an increased proportion of shaded leaves in the canopy, which ultimately reduced the requirement for photosynthetic N, on a green area basis. The larger the canopy, the greater the proportion of shading and therefore the greater the decrease in leaf N content. This

generally occurred above an accumulated GAI of 6, whereby 90% of the incident radiation had been intercepted. The N in the leaves responds to the light environment to maximise canopy photosynthesis (Field, 1983) and in such a way that allows mutual compensation of NUE and the photosynthesis per unit of light intercepted or radiation use efficiency (RUE) (Hirose & Bazzaz, 1998). Predictions of leaf N requirement based on maximising NUE were correct. Canopy expansion is dependent on the N in the crop. Where plant parts such as stem and grain demand more N, this will be taken from the leaves through premature senescence. The older lower leaves are the first to senesce because the effect of N limitation is to reduce leaf longevity (Willington et al., 1983, see Jamieson & Semenov (2000). Therefore, as canopy size is increased and where there is little extra N uptake, the amount of N in the extra leaf material is reduced at the bottom of the canopy or with increased canopy development. This study has shown that the decrease in leaf N content is linear and does not follow the same exponential decrease as the light. As total canopy size increases mean leaf N content per unit green area decreases.

CNR was constant with increased canopy size through depth. The decrease in leaf N is accompanied by an increase in stem N per unit green area, with increased canopy size through depth and canopy development. Stem N per unit of stem area decreased indicating that the partitioning of N to the stem, based on the whole canopy size, increased through depth and development. The comparison of predictions and the detailed stem component analysis revealed that the decrease in stem N per unit stem area was predominantly due to the decrease in photosynthetic N in the leaf sheath, as within the laminae. Most of the literature, to date, has concentrated on the importance of the photosynthetic N in the lamina, but this project has highlighted the importance of photosynthetic N in the stem tissue also. This could be an area where breeders could look towards maximising the photosynthesis and contribution toward yield production. It was hypothesised that there would be an increased requirement for structural N but extensive literature research (Wilman & Altimimi, 1982; Bacon, 1988) combined with the evidence from the experiments suggests that the amount of N in structural components is small. There was 30 kg ha^{-1} of N remaining in the crop at harvest which was assumed to be structural N. This was about 15% of the total N uptake. Further detailed work is required to be able to quantify the N in transport and

storage that confound the relationship between stem dry weight and structural N requirement.

CNR was not constant with increased canopy size through variety as Soissons had a greater CNR than Spark. Soissons had a higher light extinction coefficient (k) and Spark produced a greater number of shoots. These canopy characteristics compensated for one another in terms of light flux within the canopy such that, the N requirement for leaf tissue was similar. The genotypic variation in leaf N requirement could be predicted from these architectural characteristics, if they were quantified. The photosynthetic N in the leaf sheath was therefore also expected to have been similar, although this could not be directly tested. The difference in CNR, albeit only 0.1 g m^{-2}, was due to the difference in stem N requirement. The reason for this difference was unclear but there may have been a varietal difference in the proportion of structural N in dry weight, the proportion of stem dry weight to total GAI, or the amount of storage N. Stem N requirement is more difficult to predict than leaf N requirement on the basis of architecture because there are gaps in the knowledge of role of N in the stem and the allometry between stem and leaf tissue.

This work has also shown that the CNR was not constant when canopy size increased through seed rate, although some of the principles still held. The requirement for photosynthetic N was greater in the low seed rate as the light flux was much higher throughout the canopy depth. The amount of N in the lamina (and leaf sheath) per unit of light, was greater in the low seed rate crop at the top of the canopy. This is possibly storage N, but could also be photosynthetic which would reduce the NUE (Hirose & Werger, 1987b), but increase the radiation use efficiency (RUE). Increased RUE has been observed in low-density stands (Whaley *et al.*, 2000), although this could be a direct result of the greater N per shoot available for uptake (Olesen, Jørgensen & Mortensen, 2000). The greater RUE would also explain the maintenance of the yield, despite the lower N application and GAI. The greater photosynthetic requirement, stem weight and the proportion of stem dry weight to GAI in the low seed rate were the cause of the greater total stem N requirement per unit GAI. It has been suggested that tillers are less able to redistribute the N to the grain, than the main stem (Sticksel *et al.*, 1999), but the location of the extra N taken up by tillers to compensate for this, has not been reported. It is suggested here, that extra N is stored in the stem.

Furthermore, the results suggest that this occurs mainly in the true stem, and not in the leaf sheath. The storage of N in the stem of the low seed rate, means that the principles developed for the prediction of the stem N requirement cannot be used alone in this case. It follows that where there is such a high increase in tiller number per plant another principle concerning storage and re-mobilisation, must be considered. However, the low seed rate chosen was lower than any expected to be used by growers and therefore, the proportion of tillers to main stems would not usually be as great as this. In support of this, there appeared to be no increase in storage N in Spark compared to Soissons at the conventional seed rate. In fact Spark, with more tillers per main stem, had a smaller stem N requirement than Soissons.

The treatments selected in the experiments were sufficient to produce variation in N content and in canopy architecture to test the majority of principles developed for the prediction of CNR. The light environment created within the canopy as a response to shoot density, leaf angle and orientation is the principle governor of leaf N requirement and to a certain extent the leaf sheath N requirement. The amount of structural N in the true stem seems to be related more to stem dry weight but on a total canopy area basis is greatly dependent on the canopy size which it supports. This work has demonstrated that there is a physiological basis for CNR to be related to canopy architecture. However, there are still some other considerations and modifications to be made that may improve the precision of the prediction of CNR. These are described in the next section.

9.2   IMPROVING THE PREDICTIONS OF CNR

Ears were not included in the estimation of CNR but they do provide a significant contribution to total photosynthesis, of up to 33% (Puckridge, 1972). The top 10 cm of the canopy intercepted approximately 100 μmol m^{-2} s^{-1} PAR and at least half of the green area measured in this layer was ear. Clearly, the ear does intercept light and has a requirement for photosynthetic N but it has been suggested (Chapter 6) that the GAI of ears is underestimated. For this requirement of the ear to be included in the CNR, a more accurate measurement of the green area must be made. The measured projected surface area of the ear was estimated to be only 18% of the true surface area of all

separated components. A correction factor for this was given to be 5.6 which seemed to be appropriate for estimating k, by reducing it to a value that was biologically possible. Further modification to the correction factor is still required to check accuracy and to account for overlap of each component and treatment effect. The amount of storage N in the ear would interfere with the calculation for CNR, as this would be surplus to the requirement for photosynthetic N. Measurements would therefore have to be taken prior to grain fill.

It was suggested here that the leaf sheath attached to the true stem has an important role in photosynthesis, containing 36% of the total N in the whole stem. The N content of the leaf sheath increased with the greater light available in the low seed rate. As the entire surface of the stem is therefore photosynthetic tissue, the whole stem surface area should also be included, and not just the projected area. The correction factor given for the stem cylindrical area is x π. The N requirement of the leaf sheath can be predicted by the same principles as leaf N requirement, but this work has also shown that the N requirement of the leaf sheath is only 34% of that in the leaf.

Applying these correction factors for ear and stem, the total canopy GAI would almost double a projected GAI of 5.6 to a GAI of 10.4 (for Soissons sown at 320 seeds m^{-2} and fertilised with 244 kg ha^{-1} N). Using this corrected total surface GAI reduces the measured N content from 2.86 to 1.53 g m^{-2} in the same treatment. It is suggested here, that by measuring the total true surface area of the canopy and with further analysis, the explanation of the variation in CNR might be improved. However, the ear and the leaf sheath are both vertical structures which would not intercept light in the same way as the more horizontal laminae. Unlike the lamina, the whole surface of the ear and leaf sheath would not be exposed to direct sunlight throughout the day. This is the case, particularly, when the sun is overhead and the more erect structures absorb less light per unit area than more horizontal leaves (Nobel & Long, 1983). As solar elevation increases, the path length of the light decreases and so there is even less area of ear and stem exposed to the sunlight. On considering the CNR on a daily basis, the actual area exposed to the sunlight at any one point in time might only be half. It seems that the ear and the leaf sheath do not

contain as much photosynthetic N per unit green area as the lamina, possibly because of structural and storage compounds also present. Therefore it is unlikely that these structures have the same photosynthetic capacity per unit area as the lamina.

The N in roots is also not considered to be part of the N requirement, but there is a significant proportion of total N in the root system. The reduction in R:FR ratio, such as for a large canopy or high seed rate, appears to reduce total root biomass (Kasperbauer & Karlen 1986; Knauber & Banowetz 1992). This suggests that the low seed rate canopy will have a greater root biomass and therefore a greater N content. The function of the root system to support the canopy, for example in terms of scavenging for nutrients and better root anchorage, was not considered for the CNR. The N requirement of below-ground biomass should be included in the total CNR.

Both experiments were carried out in the same climate and environment, but it is expected that certain environmental factors will affect the physiology and therefore the CNR. This is now discussed.

9.3   ENVIRONMENTAL FACTORS AFFECTING CNR

The reduction in temperature and the occurrence of water stress reduce shoot to root dry weight ratio (S:R) and this is associated with the decrease in tissue N concentration and specifically RUBISCO (Andrews, Raven & Sprent, 2001). In climates where there is water stress or lower temperatures than experienced in the UK, it can be expected that there is a decreased shoot N requirement as more N is partitioned to root tissue.

Modifications to the prediction of the CNR may be necessary with the continuation of climate change. It is well known that the effects of industrialisation and increased energy use on the climate include the increase in atmospheric carbon dioxide (CO_2) concentration, increase in temperature and increase in ozone (O_3) (IPCC 1996 see Batts et al., 1998). By the year 2050, the current ambient CO_2 concentration of 350 μmol CO_2 mol^{-1} air is predicted to double (Neftel et al., 1985 and Pearman, 1988; see Slafer & Rawson, 1997). The potential impact of this climate change on crop

production is great as there is much evidence for the increase in photosynthesis, growth and crop yield with elevated CO_2 concentrations where there are no other limiting factors (Batts et al., 1997; Drake, Jacob & Gonzàlez-Meler, 1998). In wheat, the increased temperatures reduce the duration of the crop, biomass and grain yield, which in some varieties negates the effect of the elevated CO_2 (Batts et al., 1998). Although the rate of development, leaf number and rate of leaf appearance are not affected by CO_2 concentration (Slafer & Rawson, 1997) canopy architecture is affected through the increased number of tillers and leaf area index (Ewert & Pleijel, 1999). According to the principles developed here, there would be a decrease in the light transmission through the canopy and therefore a lower mean leaf N requirement per unit green area. The increased leaf material is expected to reduce the stem N content per unit green area but the increased tiller number may not be great enough to expect a significant increase in the amount of storage N. This could be controlled through reduced N application. A doubling of current ambient CO_2 has been shown to stimulate photosynthesis in the flag leaf by 50% before anthesis (Mitchell et al., 1999). The increase in photosynthesis occurs despite there being a decrease in RUBSICO (Long & Drake, 1992 see Drake et al., 1998) which suggests that the NUE is increased and therefore the leaf N requirement is reduced. However, elevated CO_2 accelerates chlorophyll degradation which might be explained by the increased N demand by the ear being met by the redistribution of N for the chlorophyll binding proteins (Ommen et al., 1999). Photosynthetic capacity of flag leaves is reduced by elevated O_3 through the decline in content and activity of Ribulose 1, 5-Bisphosphate carboxylase/oxygenase (RUBISCO) (Mitchell et al., 1999). The rate of flag leaf senescence is also increased although this effect is reduced by elevated CO_2 (Ommen et al., 1999). The interactions between elevated CO_2, O_3, and temperature are not consistent amongst the literature. However, the individual effects would suggest a decrease in photosynthetic leaf N requirement but perhaps an increase in the storage N requirement with the predicted climate change. Overall, it can be expected that there will be a decrease in CNR.

9.4 IMPLICATIONS FOR THE WHEAT GROWER

Yield increases can be achieved through improvements in agricultural practices carried out by the grower and through improvements in cultivars by breeding. The increase in physiological knowledge will benefit both these systems of increasing yield. The concept of a CNR could be used by the grower as well as by the breeder. Decisions a wheat grower will take before sowing include variety, sowing date, field and seed rate. The experiments in this project have considered seed rate and variety, for which CNR can be proposed. It would be ideal to be able to quantify the CNR for the grower but this can only be done for this specific experimental situation in the UK climate, where drainage was good and the sowing dates in both years were average. It is the aim of this thesis to be able to produce a series of principles through which the grower can predict the CNR for his cropping situation.

The data show that the CNR for a canopy of Spark sown at a conventional seed rate, was 18 kg ha^{-1} of green area: 9 kg ha^{-1} N was required by the leaf for photosynthesis and 9 kg ha^{-1} N in the stem for support and photosynthesis. Of this, 3 kg ha^{-1} N was required by the leaf sheath for photosynthesis, and 2 kg ha^{-1} N was required by the true stem for structure (assuming that 25 % total N in stems was structural and 35 % was photosynthetic). An extra 4 kg ha^{-1} was also in the stem, which was suggested to be the N in transport, as there was assumed to be no luxury uptake or storage. This is summarised in Table 9.1. For the same seed rate, the CNR of Soissons was proposed to be 21 kg ha^{-1} of green area. The leaf N requirement was similar to Spark (10 kg ha^{1}) as the light environment was similar. The stem CNR was 11 kg ha^{-1}, which was slightly greater than Spark because of the increased ratio of stem to leaf green area in Soissons, but possibly also the increased structural N requirement per unit of stem green area. The leaf sheath photosynthetic N requirement was therefore proposed to be 3 kg ha^{-1} and the structural N requirement of the true stem to be 4 kg ha^{-1}. The transport N was also proposed to be 4 kg ha^{-1}. This is also summarised in Table 9.1. These values are for Spark and Soissons canopies that intercepted 90% and 88% of incident PAR, respectively. This indicates that the canopies reached a suitable size to intercept most of the available PAR and therefore these canopies appear to

have a suitable N supply to reach optimal size. It is also expected that these quantities for the roles of N are close to the requirement.

Table 9.1 Canopy nitrogen requirement (CNR) of two wheat varieties sown at 320 seeds m^{-2} and the N requirement of each shoot component according to function. All values are kg ha^{-1}.

| | VARIETY | |
	Spark	Soissons
CNR	18	21
LEAF N	9	10
STEM N	9	11
Photosynthetic (leaf sheath)	3	3
Structural (true stem)	2	4
Transport (true stem)	4	4

CNR of lower seed rate (20 seeds m^{-2}) was greater than the CNR at the more conventional seed rate. It increased from an average of 20 to an average of 28 kg ha^{-1}, across both varieties. At this low seed rate, Spark had a smaller CNR of 26 kg ha^{-1} compared to Soissons with a requirement of 29 kg ha^{-1}. It is proposed that the variety effect is reversed at the low seed rate because of the magnified effect of variety on tiller number at the low seed rate. There was no effect of variety on stem to leaf area ratio, reducing the dilution effect on Spark stem N requirement. There would also have been a greater requirement for storage of N in Spark, to compensate for the decreased remobilisation of N in tillers. The latter adds a further requirement for storage of N, in the low seed rates. The photosynthetic N requirement in the leaf was 14 kg ha^{-1} in both varieties, as there was no significant difference between light experienced. However, the stem N requirement was 15 kg ha^{-1} in Spark compared to 12 kg ha^{-1} in Soissons. The proposed partitioning of N in the stem according to

function is still based on the assumptions that 25% of stem N is structural, the photosynthetic N requirement is the same in both varieties, according to light environment, and there is an increased requirement for storage N in Spark. It is suggested that Spark had a stem structural N requirement of 4 kg ha^{-1}, a photosynthetic requirement of 5 kg ha^{-1} and a combined transport and storage requirement of 6 kg ha^{-1}. Soissons had a lower stem structural N requirement of 3 kg ha^{-1}, a lower photosynthetic requirement of 4 kg ha^{-1} and a combined transport and storage requirement of 5 kg ha^{-1}. These canopies at the low seed rate only intercepted about 76% of incident radiation, which was limited by the plant population density and consequently the ability to capture radiation. There is still about 20% of the total N in the canopy that is unaccounted for but is likely to be in storage or transport. This must be explained for predictions to be more precise.

The recommended plant density for winter wheat is 300 plants m^{-2} (Sylvester-Bradley *et al.*, 1997b), but recent work carried out between Nottingham University and ADAS suggests that a stand sown at 125 plants m^{-2} can maintain the same yield (Whaley *et al.*, 2000). The grower will need to know how this decrease in optimum plant population will affect the CNR. Decreased seed rates will reduce the plant population density. Relative growth rate (RGR) is the increase in plant material per unit of material per unit of time (Hunt, 1978). The RGR is increased at low plant density (Kirby, 1967). It is suggested that this is the result of increased RUE, through better light distribution in the canopy and more N available per shoot. There was also greater tiller survival (Whaley *et al.*, 2000) which limited the waste of nutrient and assimilates, and a greater leaf area per shoot which increased radiation capture per shoot. An increase in light and crop growth rate has been shown to increase the rate of nitrate uptake through the increase in demand for N based on carbohydrate supply from the shoot (Novoa & Loomis, 1981; Glass 1988; Devienne-Barret *et al.*, 2000). It is suggested from this and from the experimental observations that the stands with the lower plant population have a greater CNR.

Although an increase in CNR with the reduction in seed rate has been established and explained, the seed rate used in this study is too low to maintain yield (Chapter 4) even at higher fertiliser N rates (Ellis, Salahi & Jones, 1999). If the optimum plant population density is 125 plants m^{-2} then analysis of N distribution and content should

be carried out at this density. Until such experiments have been carried out, the CNR for this plant density can only be speculated. The canopy characteristics of a crop at 125 plants m^{-2} are assumed to be closer to the 320 seeds m^{-2} than the 20 seeds m^{-2}, based on a non-linear relationship between seed rate and shoot density. The light environment within the canopy, the structural and the storage requirements were therefore assumed to have greater similarity to the 320 than the 20 seeds m^{-2} canopy. Based on these assumptions, the CNR of an optimum plant population was predicted to be 23 kg ha^{-1}, with 12 kg ha^{-1} in the leaf and 11 kg ha^{-1} in the stem. The component requirements in the stem were predicted to be similar to those in the 320 seed rate. This remains to be tested through experimentation.

The actual N requirement of the crop throughout development needs to be known for correct timing and rate of fertiliser. It is important that the application coincides with the crops' requirement to limit the potential for over fertilisation and loss of excess residual N to the environment. Although growers in the UK and other European countries are now more aware of this, many growers in the United States have still to adopt this strategy described as a 'best nitrogen management plan' (Huang *et al.*, 2001).

9.4.1 Reference book 209 (RB209)

Since beginning this project, the N fertiliser recommendations for UK crops have been revised (MAFF, 2000). There is now a more sophisticated approach to soil N supply assessment with seven indices. It now also considers soil type, soil organic N and winter rainfall. The typical values of nutrient content in organic manure and the calculation of available nutrients from manure have been updated. Crop N recommendations are now also based on the change in grain N concentration over several years, which is thought to be a better indicator than yield to any amendments to the fertiliser N applied. The grain N concentration at the economic optimum rate of feed wheat and bread wheat is reported to be 2.0 and 2.2 % (100% dry weight), respectively (MAFF, 2000). This economic optimum was reached in the 1997/8 crop and the low seed rate of the 1998/9 crop in the 'high N' treatment. In the 'low N' treatment where the N content was considered close to the N requirement, the economic optimum was not reached. This may add an extra dimension to the

estimation of the CNR which would include the storage requirement of the grain or the partitioning of N to the grain, to reach the economic optimum grain N concentration. In the new RB209, there is still no reference to understanding the function of N and therefore the N requirement of the canopy for each cropping situation. The results from this thesis have shown that there is variation in N requirement and that this should be considered when providing fertiliser recommendations.

9.4.2 Canopy management

A new approach in determining the N requirement of a whole crop per unit ground area is based on the optimum size of the canopy, and not the expected yield. The area of the leaves, rather than their dry weight is now proposed to be a more accurate link between the N content per unit ground area and the growth of the crop. The idea of 'canopy management' is to effectively manage the GAI through controlling the amount of N applied (Sylvester-Bradley et al., 1997a). The optimum canopy size is that which is sufficiently large to intercept as much incident radiation as is economically viable (Stokes et al., 1997). This is therefore based on the difference between the cost of N to produce the canopy and the returns in value of extra grain production. The economic optimum GAI for UK winter wheat has been calculated by Sylvester-Bradley et al., (1997a). Since publication, this equation has been revised so that the optimum GAI for the parameter values given is 6.6 but will inevitably vary according to cost of fertiliser N and the value of grain. Once this more sophisticated method of determining CNR has been developed for a wider range of agricultural situations, the values of CNR can be used to estimate the optimum GAI to enable the grower to use the most efficient amount of fertiliser N.

The model assumes CNR is a stable 30 kg ha^{-1}, but the results from this project suggest that CNR varies between seed rates, varieties and site-seasons. The optimum GAI was recalculated replacing the parameter values for the CNR, corresponding k values and incident radiation as measured in the experiment. For Soissons sown at 320 seeds m^{-2} where the CNR was 21 kg ha^{-1}, k was 0.5 and incident radiation (PAR) was 15.3 MJ m^{-2} d^{-1}, the optimum GAI increased to 7.8. This implies that 164 kg ha^{-1} (ground area) of available N is required to produce a canopy of economically

optimum size. However, this Soissons crop actually took up 180 kg ha^{-1} by anthesis, but only achieved a GAI of 5.6. As available N increased, proportionately less was used to increase the GAI which was also reflected in the greater total N content per unit green area. This suggests that as more N is available and canopy expands, other functions of N compete with the role for expansion and maintenance of green area. As there is an increase in mutual shading the R:FR ratio decreases (Ballaré & Casal, 2000). Amongst the typical responses induced by this, the storage of N may be favoured above the production of more green tissue that would encourage more mutual shading and result in decreased RUE. It would also prevent the loss of structural N that might otherwise occur through leaf abscission when light levels become reduced. However, CNR did not increase with development in either experiment here.

A sensitivity analysis was carried out for the parameters in the estimation of optimum GAI. This showed that although the CNR (with a range of 11 to 35 kg ha^{-1}) was sufficient to decrease optimum GAI by almost two, the model was most sensitive to the value of k. An increased value of k from 0.3 to 0.7 observed in the experiment, decreased the optimum GAI by four. The model was least sensitive to the change in incident radiation, which by increasing from 14 to 22 MJ m^{-2} d^{-1} increased the optimum GAI by almost one. The model is sensitive to the price of N fertiliser to the same degree as CNR but is less sensitive to other parameters. Therefore, although CNR does have a strong effect of optimum GAI, the concurrent change in k has a greater effect. It is suggested from this project that there is an interaction between the CNR and N availability, which requires further examination before further recommendations for the economic optimum GAI can be made. However, this work has shown that there is much variation in CNR and that this can significantly affect the economic optimum GAI.

For the grower, it is important to consider the N requirement for the canopy, specific to the agricultural situation. The recommended CNR of 30 kg ha^{-1} of green area is not specific to seed rate, variety or environment, and more importantly does not appear to be precise for most cases. A more precise determination of CNR must be made to improve fertiliser recommendations. The results from this thesis suggest that the grower can consider certain architectural characteristics of the crop to tailor the N

requirement to their own situation. Although limited experimentation prevents quantification, certain guidelines can now be provided:

1. Varieties with more erect leaves have a greater photosynthetic N requirement.
2. Varieties with larger leaves have a smaller photosynthetic N requirement.
3. A reduction in seed rate increases photosynthetic N requirement.
4. Canopies with a greater tiller number have a greater storage N requirement.
5. Thicker stemmed canopies have a greater structural N requirement.
6. Canopies with a larger leaf to stem area ratio have a decreased stem N requirement.
7. Leaf N requirement decreases and stem N requirement increases such that, CNR remains stable throughout the period of development.
8. The grower can calculate when to apply the appropriate N applications, according to the rate of N uptake and size of the canopy aimed for by the major growth stages. This will limit over fertilisation and prevent leaching.
9. The CNR over the range of seed rates and varieties tested is now revised to 22 kg ha^{-1}.
10. The CNR may need adjusting according to the use of the grain, to met the required protein concentration.

9.5   IMPLICATIONS FOR THE BREEDER

The increase in crop yield over the past 50 years is mainly due to the increased duration of the canopy through fertiliser N, irrigation and husbandry, and the increased partitioning of assimilates to the grain through breeding, or harvest index (Richards, 2000). Increased yields are predicted for the future but as the upper limit of the harvest index for intensively bred crops is almost reached (Richards, 2000) breeders must look for other ways to increase maximum yield potential, such as increasing total crop photosynthesis. This could be achieved by selecting traits that increase the size of the canopy, duration of the canopy or photosynthesis per unit canopy area. Some of the physiological relationships discussed in this thesis are able to help identify traits to improve yield potential.

Increases in canopy size would increase the amount of mutual shading, such that a smaller proportion of the canopy would be contributing to carbohydrate production. This is already accepted through Beer's Law, in that there is a diminishing increase in light interception with the increase in canopy size. Nitrogen use efficiency would also be reduced and so this would not be the most economic way of increasing yield potential.

Knowledge of acquisition, distribution and recycling of N within the canopy leads to an understanding in the role of N in canopy senescence. This work has contributed to this at a crop and a physiological level but it could also be extended to a molecular and genetic level to be able to extend canopy duration and grain N accumulation. There is a need to understand the enzymes and mechanisms responsible for the signalling in N remobilisation. In maize, the key enzymes glutamine synthetase (GS) and nitrate reductase (NR) involved in N assimilation have been used as markers to identify genotypes that have greater N remobilisation (Masclaux et al., 2001). In wheat, the cytosolic isoform of GS (GS1) was identified as a regulator for N remobilisation and enhancing the capacity for N accumulation mainly in the grain (Habash et al., 2001). Identification and manipulation of such enzymes would provide the means to enhance canopy duration, NUE and therefore photosynthesis.

Surprisingly, breeders have not needed to select for increased photosynthesis per unit leaf area to improve yield potential (Richards, 2000). This is likely to be because of the responsiveness of the crop and the ease by which yield can be increased through the other means such as harvest index and agronomic practices. However, certain agronomic practises that are intended to extend the canopy duration or size are not always practical. When the lifecycle of the crop is short such as where intercropping is practised or with the continuing rise in global temperatures it would be beneficial to increase photosynthesis per unit leaf area. This could be manipulated by increased light penetration through control of factors affecting k. An erect flag leaf (k value of 0.3) would allow light to penetrate through to layers beneath, such that the flag leaf can avoid light saturation and lower leaves would contribute significantly to canopy photosynthesis. Canopy photosynthesis would increase if lower leaves had a higher k of about 0.6 to maximise light capture and leaf N distribution was optimised such that NUE was maximised for each leaf.

The investigation into light and N content of laminae has shown that there is a maximum N content per unit lamina area of around 2.5 g m^{-2}, despite the increase in light. The response of N to light is reduced generally beyond a PPFD of 300 μmol m^{-2} s^{-1}. This is in the top few layers of the crop where most of the light is intercepted and converted by the upper 2 – 3 leaves. The upper leaves are unable to utilise all the light available, especially during occasions of short duration high light intensity, such as at midday, or sunflecks. If more N were present in the lamina and in form of RUBISCO, then the photosynthetic rate per unit of light (LUE) would increase (Hirose & Bazzaz, 1998). An increase in leaf N content to 4 g m^{-2} in the flag leaf, would increase the photosynthetic capacity by 44%, but the NUE would decrease by 9%. The net amount of carbohydrate produced would be greater, and thus contribute to achieving the greater yield. The extra photosynthetic N would also be available for redistribution to the grain, as more N would be needed to maintain the protein concentration. Breeding could be carried out, by selecting traits that increase leaf N content to around 4 g m^{-2} and thus increase photosynthetic capacity through the increased capacity for RUBISCO. However, a balance must be maintained between LUE and NUE. Nitrogen content and amount of photosynthetic apparatus per unit leaf area may also be increased by reducing specific leaf area (Reynolds, Van Ginkel & Ribaut, 2000; Richards, 2000). It has been suggested that the decrease in light saturated photosynthesis at high light intensity and leaf N content is often the result of an increase in the resistance for CO_2 diffusion (Hirose & Werger, 1987b; Evans & Terashima, 1988), as well as stomatal closure at midday. Maximum canopy photosynthesis could be increased if a lower CO_2 resistance could be selected. The flag leaf must also be able to avoid photoinhibition at such high light intensity that may occur at midday. Breeders could therefore also select for photoprotection of electron transport components. This project has also supported the role of photosynthetic N in the leaf sheath which may be another area for breeders to consider to improve photosynthetic efficiency.

Selecting for varieties that favoured luxury uptake of N, would also be a likely requirement for breeders in the future. This would not only be an advantage in terms of increased mobile N and grain protein concentration, but also for 'mopping up' excess N in the environment.

Many of these suggestions for the application of the work carried out in this project, as well as the explanation of the CNR in terms of canopy architecture and crop physiology, still require further investigation. The next section suggests the nature of the work that should follow on from this project.

9.6 FUTURE WORK

The experiments carried out in the two field seasons covered a wide range of measurements, but more detailed investigation is required to further the understanding of the CNR and its relation to aspects of canopy architecture. Photosynthetic rate in the leaf and in the leaf sheath could be compared to understand response to light and N in each organ. The response of N to changes in both light quality and quantity could also be examined. The chemical analysis of the N compounds in the true stem and leaf sheath is an important next step to improve the understanding of the N use in the stem. Separate measurements of the N compounds in the xylem and phloem, with respect to crop development and crop height, would allow the transport of N to be quantified. Labelled N could be used to trace the movement of N throughout the canopy and the N redistribution from both the leaf and stem tissue, when luxury uptake has occurred. This would indicate whether most luxury uptake was taking place in the stem or in the leaves. Combined with photosynthetic rate measurements this would provide an indication as whether this luxury N was used in photosynthesis. SPAD measurements correlate to total chlorophyll concentrations in wheat (Ommen *et al.*, 1999). This method could be used to indicate chlorophyll concentration. The N content of the roots could also be measured and included in the CNR. More investigation into the true green area of the ear, could also be carried out, for a more precise correction factor. Detailed measurements of the leaf angle, curvature, light transmission and light reflection and sunfleck are required, especially during early growth and with low plant density or low establishment. These measurements can be used to test the limitations of Beer's Law and to estimate the light extinction coefficient more precisely. The amount of structural stem N was taken to be a constant 25 % total stem N and 0.3% stem dry weight. However, this needs to be tested to see whether these values are constant between varieties, seed rates, sowing dates, and canopy height etc. The N compounds in the stem must be identified to

understand their role in this organ. This will involve the separation of chlorophyll, RUBISCO, other soluble proteins, nitrate and amino acids.

A more sophisticated approach to predicting the CNR could be developed through further experiments with other varieties, temperatures, incident light, day length and water stresses to simulate environmental conditions in other countries where crops are grown. Experiments could be carried out in these other environments to examine the effect of these more extreme climates on the CNR and to test the principles thoroughly. The grower could then use these principles based on parameters arising from these to predict the CNR for his own particular situation. It is suggested that the parameters could include; total GAI, stem and leaf green area ratio, stem dry weight, k, stem height and yield. Environmental parameters such as incident radiation, water stress and temperature might also be included once further experiments have been carried out to test this effect on CNR

These suggestions for future research would improve the understanding of N within the canopy and provide some parameters that may be quantifiable and used to predict CNR more precisely from canopy architecture.

9.7 CONCLUSIONS

1. CNR is the minimum amount of N required by the canopy for maximum dry matter production. It does not include luxury N.
2. CNR can be predicted from canopy architecture.
3. N in the leaf tissue is mainly required for photosynthesis.
4. N in the stem tissue is mainly required for photosynthesis and structure.
5. Half of the total N in the canopy is located in the stem. The stem therefore is an important component in determining CNR.
6. CNR is constant when there is a linear increase in N per unit ground area with the increase in GAI.
7. CNR is constant with increasing canopy size through depth and crop development.
8. CNR is greater in Soissons than Spark variety. This would be predicted if the architectural characteristics were quantified.

9. CNR increases at a very low seed rate, due to greater light flux and the requirement for storage.

10. Leaf N content per unit GAI decreases with canopy size due to proportionately more mutual shading.

11. Stem N content per unit GAI increases with canopy size due to the decrease in GAI and increase in non-photosynthetic N.

12. Luxury uptake of N increases at low seed rates and with increasing fertiliser N rates. It may also occur in spring.

13. Of the architectural characteristics studied, GAI, *k*, and the proportion of mainstems to tillers had the greatest effect on CNR.

14. CNR is variable and the previous value of 30 kg ha^{-1} is not representative of all crop situations.

15. More research is required to understand the function of N in the stem and the importance of luxury N.

APPENDICES

Appendix I - Experimental Plans

1997/8 experiment

block 1

variety/seed rate: D 9 2 18 14 5 21 8 13 24 1 23 19 4 10 7 16 3 11 22 20 17 12 6 15 D

1.61 m   0.5 m discard   ▶N   26.41 m

plot no.: D 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 D

4 m

block 2

variety/seed rate: D 3 10 2 17 14 21 11 19 8 22 4 7 6 23 16 5 12 20 9 1 18 24 13 15 D

plot no.: D 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 D

block 3

variety/seed rate: D 5 15 8 2 19 12 6 18 23 4 16 24 9 21 1 13 17 7 14 22 10 20 3 11 D

plot no.: D 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 D

VARIETY/SEED RATE

1	CADENZA - 20 m^{-2}		13	SOISSONS - 20 m^{-2}
2	CADENZA - 40 m^{-2}		14	SOISSONS - 40 m^{-2}
3	CADENZA - 80 m^{-2}		15	SOISSONS - 80 m^{-2}
4	CADENZA - 160 m^{-2}		16	SOISSONS - 160 m^{-2}
5	CADENZA - 320 m^{-2}		17	SOISSONS - 320 m^{-2}
6	CADENZA - 640 m^{-2}		18	SOISSONS - 640 m^{-2}
7	HAVEN - 20 m^{-2}		19	SPARK - 20 m^{-2}
8	HAVEN - 40 m^{-2}		20	SPARK - 40 m^{-2}
9	HAVEN - 80 m^{-2}		21	SPARK - 80 m^{-2}
10	HAVEN - 160 m^{-2}		22	SPARK - 160 m^{-2}
11	HAVEN - 320 m^{-2}		23	SPARK - 320 m^{-2}
12	HAVEN - 640m $^{-2}$		24	SPARK - 640 m^{-2}

1998/9 experiment

N

block 1

6 m

1.6 m discard

plot no.	D	1N	2N	3N	4N	5N	6N	D
variety/ seed rate/N		4	1	6	5	2	3	
plot no.		1S	2S	3S	4S	5S	6S	
variety/ seed rate/N	D	10	7	12	11	8	9	D

24 m

block 2

4 m

plot no.	D	7N	8N	9N	10N	11N	12N	D
variety/ seed rate/N		3	4	6	2	1	5	
plot no.		7S	8S	9S	10S	11S	12S	
variety/ seed rate/N	D	9	10	12	8	7	11	D

block 3

plot no.	D	13N	14N	15N	16N	17N	18N	D
variety/ seed rate/N		3	1	2	5	6	4	
plot no.		13S	14S	15S	16S	17S	18S	
variety/ seed rate/N	D	9	7	8	11	12	10	D

VARIETY / SEED RATE / N RATE

1 Soissons - 20 m^{-2} (240 kgNha^{-1})
2 Soissons - 320 m^{-2} (240 kgNha^{-1})
3 Soissons - 640 m^{-2} (240 kgNha^{-1})
4 Spark - 20 m^{-2} (240 kgNha^{-1})
5 Spark - 320 m^{-2} (240 kgNha^{-1})
6 Spark - 640 m^{-2} (240 kgNha^{-1})

7 Soissons - 20 m^{-2} (120 kgNha^{-1})
8 Soissons - 320 m^{-2} (120 kgNha^{-1})
9 Soissons - 640 m^{-2} (120 kgNha^{-1})
10 Spark - 20 m^{-2} (120 kgNha^{-1})
11 Spark - 320 m^{-2} (120 kgNha^{-1})
12 Spark - 640 m^{-2} (120 kgNha^{-1})

Appendix II – Spray Applications

1997/8 experiment

SPRAY	ACTIVE INGREDIENT (RATE), DATE APPLIED
Plant Growth Regulators	Chlormequat (1610 g ha^{-1}), 21/3/98 2-Chloroethylphosphonic acid (155 g ha^{-1}), Mepiquat chloride (305 g ha^{-1}), 19/5/98
Herbicide Application	Isoproturon (1500 g ha^{-1}), Diflufenican (50 g ha),7/11/97 Metsulforon-methyl (4 g ha^{-1}), Bromoxynil (100 g ha^{-1}), Ioxynil (100 g ha^{-1}) 31/3/98 Fenoxaprop-ethyl (120 g ha^{-1}), Sprayprover (adjuvent: 2 l) 9/5/98
Fungicide Application	Fenpropidin (375 g ha^{-1}) 27/1/98 Prochloraz (405 g ha^{-1}), Fenpropidin (563 g ha^{-1}) 21/3/98 Fenpropidin (563 g ha^{-1}), Cyproconazole (60 g ha^{-1}), Prochloraz (400 g ha^{-1}) Epoxiconazole (126 g ha^{-1}), Fenoprimorph (375 g ha^{-1}) 19/5/98 Fenpropidin (375 g ha^{-1}), Tebuconazole (125g ha^{-1}) 1/6/98
Insecticide Application	N/a
Molluscicide	Methiocarb (600 g ha^{-1}), 11/98

1998/9 experiment

SPRAY	ACTIVE INGREDIENT (RATE), DATE APPLIED
Plant Growth Regulators	Chlormequat (1540 g ha^{-1}), 30/4/99 2-Chloroethylphosphonic acid (116.25 g ha^{-1}), Mepiquat chloride (288.75 g ha^{-1}), 15/5/99
Herbicide Application	Isoproturon (1300 g ha^{-1}) and Diflufenican (20 g ha^{-1}) 2/2/99 Fluroxypyr (100 g ha^{-1}), Metsulfuron-methyl (4 g ha^{-1}), 3/5/99
Fungicide Application	Prochloraz (405 g ha^{-1}), Fenpropidin (562.5 g ha^{-1}), 30/4/99 Epoxiconazole (93.75 g ha^{-1}), Epoxiconazole (125 g ha^{-1}), Fenpropimorph (375 g ha^{-1}), 31/5/99 Fenpropidin (375 g ha^{-1}), 10/6/99 Tebuconazole (250 g ha), Triadimenol (125 g ha), 15/6/99
Insecticide Application	N/a
Molluscicide	Methiocarb (600 g ha^{-1}), 11/99

Appendix III - Plant establishment

(Log$_{10}$ transformed plants m^{-2})

	1997/8		1998/9	
Seed rate	Soissons	Spark	Soissons	Spark
20	1.26	1.27	1.28	1.36
320	2.55	2.54	2.57	2.42
640	2.79	2.68	2.82	2.83
S.E.D	0.043		0.060	
d.f.	10		10	

Appendix IV - Crop Growth

1997/8 experiment

(Log $_{10}$ transformed)

Seed rate	Date	Soissons			Spark			S.E.D.	d.f.
		20	320	640	20	320	640		
N Content	15 Nov	-1.67	-0.30	0.03	-1.93	-0.404	-0.39	0.240	10
(log kg ha^{-1})	15 Dec	-1.35	-0.02	0.29	-1.51	-0.50	0.07	0.118	10
	12 Jan	-0.84	-0.07	0.18	-1.00	-0.32	0.02	0.157	10
GAI	15 Nov	-2.01	-0.87	-0.55	-2.12	-0.87	-0.83	0.120	10
	15 Dec	-1.66	-0.41	-0.09	-1.78	-0.96	-0.36	0.084	10
	12 Jan	-1.28	-0.60	-0.30	-1.41	-0.72	-0.73	0.147	10

1998/9 experiment

(Log $_{10}$ transformed)

Seed rate	Date	Soissons			Spark			S.E.D.	d.f.
		20	320	640	20	320	640		
N Content	15 Feb	1.11	-0.30	-0.23	-1.36	-0.36	-0.31	0.055	10
(log kg ha^{-1})	29 March	-0.50	0.25	0.23	-0.64	0.22	0.27	0.094	10
GAI	11 Jan	-1.83	-0.85	-0.76	-2.16	-0.94	-0.80	0.084	10
	15 Feb	-1.66	-0.68	-0.60	-1.79	-0.82	-0.73	0.059	10
	29 March	-1.02	-0.19	-0.15	-1.13	-0.24	-0.18	0.092	10

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