MAINTENANCE RESPIRATION IN CROP LEGUMES

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To my Mother,

my late Father,

my Sister and my Brother.

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SUMMARY

Maintenance respiration refers to energy production for processes other than the synthesis of new cell materials, and it follows therefore that as the energy requirements of maintenance processes increase, the quantity of carbon allocated to growth is likely to be proportionately decreased. Whilst the quantitative relationship between respiration (dark CO_2 efflux) and growth has been extensively studied, considerably fewer studies have attempted to quantify the energy requirements of the maintenance processes and experiments reported in this thesis were intended to obtain further information on the nature and intensity of the maintenance processes in some crop legumes (field bean, chickpea, lucerne, pea and kidney bean).

Experiments were conducted using whole plants (grown in a naturally light glasshouse at 20°C) and mature leaves (plants were grown for 20-40 days in a controlled environment room at 20°C). Whole plants were used to compare maintenance coefficients calculated using three different methods, and also to investigate the relationship between the respiration rate during starvation and plant dry weight. The dark respiration of mature field bean leaves was studied to determine: (i) the pattern of respiration of attached and detached leaves during a prolonged dark treatment, since this treatment has been used to estimate maintenance respiration, (ii) the source of carbohydrate for respiration during the dark treatment, and (iii) whether leaf senescence occurs during the treatment.

The three methods of measuring the maintenance component were: (i) to allow the CO_2 efflux to decay in prolonged darkness to an asymptotic value which is then taken to be the maintenance value; (ii) to plot the dark CO_2 efflux as a function of net CO_2 uptake over a range of irradiances and take maintenance as the dark efflux when the net CO_2 uptake is zero; (iii) to plot total CO_2 uptake as a function of the growth rate and take maintenance as the CO_2 efflux when the growth rate is zero. The maintenance coefficients so calculated for field bean, chickpea and lucerne using methods (i) and (ii) were similar; however coefficients calculated using method (iii) were significantly higher ($P < 0.05$). Method (i) was considered to provide the best estimate for maintenance because there was less opportunity for growth processes to contribute to CO_2 efflux. Method (ii) resulted in estimates for maintenance respiration which contained CO_2 efflux due to phloem loading of assimilate in the leaves and therefore there was greater opportunity for structural growth. These latter processes (phloem loading and structural growth) were considered to contribute to a greater extent to CO_2 efflux when the maintenance coefficient was determined using method (iii).

It is concluded that method (i) gives minimal estimates of the maintenance requirements, but has the advantage of being simple and amenable for the mass screening of plants if those with a 'low' maintenance respiration are to be identified.

Maintenance respiration consumed the equivalent of 2% of the dry weight per day in field bean, chickpea and lucerne. The growth efficiency (Y_G) was the same for all species (0.69 ± 0.01) and was unaffected by the method of calculation.

There was a linear relationship between the respiration rate of whole plants after 48 hours in the dark, and: (i) the dry weight, (ii) the total nitrogen content, and (iii) the organic nitrogen content. These relationships are interpreted to mean that protein content was being maintained during starvation.

The respiration rate of whole field bean plants was essentially constant during a 12 hour night. Defoliation and removal of leaves from the assimilation chamber at the beginning of the night removed 20-30% of the dry weight yet hardly affected the respiration rate of the remaining plant parts immediately following defoliation. The respiration rate subsequently declined linearly with time. This result is interpreted to mean that phloem loading of sucrose in the leaves maintains a constant flow of assimilate to the 'sinks' of the rest of the plant.

The rate of dark respiration of mature field bean leaves was dependent upon the irradiance level during the previous photoperiod. Studies on the pattern of change in dark respiration in prolonged darkness revealed the following:

(i) Dark respiration was essentially constant during the normal 12 hour night and there was a rapid loss in leaf dry weight during this period.

(ii) After 12 hours, the respiration rate decayed to an asymptotic value at about 24 hours.

(iii) The gas exchange quotient ($\mu l\ CO_2/\mu l\ O_2$) remained above 0.90 for 60 hours when leaves were detached at the end of the photoperiod. When attached leaves were measured, the quotient was above 0.90 during a normal (12h), but subsequently declined in a complex way, which indicated that protein was being oxidised.

The energy cost for phloem loading and sucrose synthesis were calculated from the literature to be equivalent to 0.0527gC respired/gC translocated. The experimental value, based on the difference in the respiration rate of mature leaves during the normal night and after 60 hours darkness was 0.0560gC/gC. The maintenance respiration rate determined on leaves detached at the beginning of the night was 52.2 mg glucose (g dry weight)$^{-1}$ 24h^{-1} whilst the rate for attached

leaves after 60 hours of darkness was 18.2 mg glucose (g dry weight)$^{-1}$ 24h^{-1}. The latter figure is concluded to represent energy requirements for the maintenance of cell ion concentrations and for protein turnover. The former also includes the cost of sucrose synthesis and phloem loading.

Most of the starch accumulated in mature field bean leaves during the photoperiod was mobilised during the night. Soluble sugar loss was rapid during the normal night, but the equivalent of between 30 and 50% of the sugar present in the leaf at the end of the photoperiod was retained within the leaf vacuoles and was able to provide carbon for leaf maintenance during the prolonged dark treatment.

Measurements of chlorophyll and protein were made on mature field bean leaves during 60 hours darkness. There was no measurable decline in leaf chlorophyll (on an area basis) and there was a gradual increase in the concentration of insoluble and total protein. The concentration of the soluble protein and RuBPC remained steady with time in the dark, suggesting a small loss in RuBPC which may have been respired. Photosynthetic CO_2 assimilation resumed when leaves were re-illuminated with 200 µmol m^{-2} s^{-1} at intervals of 12 hours during the 60 hour dark treatment. However the rate of assimilation at this irradiance declined after leaves had been in the dark for 12 hours. There was no evidence of senescence in mature field bean leaves until after at least 48 hours darkness.

STATEMENT

This thesis contains no material which has been accepted for the award of another degree or diploma at any University, and to the best of my knowledge and belief, the thesis contains no material previously published or written by another person except when due reference is made in the text of the thesis.

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D.E. IRVING

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CHAPTER I GENERAL INTRODUCTION

Since James (1953) published his book 'Plant Respiration', a considerable amount of new information has been gained about respiratory metabolism. Although dark respiration was, at one time, largely viewed as a 'black box' which lead to an inevitable dissipation of organic molecules previously gained during photosynthesis, it is now recognised as a process which contributes to crop growth in a very positive sense, for the reason that respiratory metabolism produces two different kinds of product, ATP (the energy currency of the plant), and carbon skeletons (which form the units from which more permanent cell constituents are assembled), and each are essential to plant growth. A by-product of respiration is CO_2. The main biochemical pathways of carbon and energy metabolism are well established, yet the role of respiration in the growth physiology of crop plants is still imperfectly understood. It is now the responsibility of the crop physiologist to quantify the relationships between rates of respiration in cells of a variety of plant organs, and levels of crop productivity.

Evolution of current thinking on the relationship between respiration and growth, appears to have been influenced in three main ways. Firstly, Beevers (1961) emphasized the connection between respiration and energy production, not only for growth, but also for maintenance of existing structure. Secondly, Pirt (1965) developed an analysis which enabled the energy requirements for growth and maintenance to be quantified in bacterial cultures. Thirdly, the concept of basal metabolism, which was well established in the field of animal physiology, was appreciated as being relevant to the concept of maintenance in plant physiology (McCree 1970).

'Maintenance respiration' refers to energy production for processes other than the synthesis of new cell materials and it follows therefore, that as the energy requirements of maintenance processes increase, the quantity of carbon allocated to growth is proportionately decreased. In higher plants, the maintenance processes are thought to involve mainly: (i) the maintenance of cell protein which is subject to continual turnover; and (ii) the maintenance of ion concentrations across cell membranes (Penning de Vries 1975a).

Animal physiologists (e.g. Blaxter 1962) and microbiologists (e.g. Stouthamer 1979) are more cognizant of the functional relationship between respiration for growth, and respiration for maintenance. In fact, recent developments in the concept of growth and maintenance respiration in higher plant physiology can be credited largely to the researches of microbiologists. It was Pirt (1965) who analysed the relationship between the specific growth rate (μ) and the yield of

biomass (Y) when bacterial cultures were grown in the presence of a limiting supply of energy substrate. The plot $^{1}/Y$ against $^{1}/\mu$ invariably gave a straight line of slope 'm' (a maintenance coefficient) and intercept $^{1}/Y_G$ (a growth coefficient). Pirt was the first to provide a unified conceptual approach to growth and maintenance.

In the wake of Pirt's analysis, McCree (1970) quantified CO_2 influx and efflux from growing plants with an empirical equation, in which respiration was expressed as a function of carbon assimilation and plant mass. Thornley (1970) explained this equation in terms of growth and maintenance coefficients, and for the first time, higher plant respiration was put on a quantitative footing. Penning de Vries *et al.* (1974) and Penning de Vries (1975a) calculated growth and maintenance requirements from a knowledge of biochemical pathways and the results they derived were consistent with results obtained using gas analysis. This served to give the concept of growth and maintenance theoretical credence.

As a result of these, and more recent studies, we now have a greater awareness of the role of respiration in higher plant growth. Our knowledge of how the intensity of maintenance processes affects growth, is however, still lacking. Maintenance requirements can be determined experimentally when growth is considered to have ceased. However while microbiologists have recourse to the technique of continuous culture, in which the concentration of energy substrate can be strictly controlled, such methodology is not appropriate for the culture of higher plants. As a consequence, it is extremely difficult to supply substrate to a plant at such a rate that growth does not occur. Moreover, plants often have considerable carbohydrate reserves from which to draw in times of substrate limitation.

Animal physiologists starve their subjects in order to stop growth and so obtain an estimate of basal metabolism. This technique was tried, and used to estimate maintenance in higher plants by McCree (1970, 1974). Calculations of the growth efficiency employing this technique to quantify maintenance (Silsbury 1977) yielded numbers consistent with those computed from proximate analysis following the method of Penning de Vries *et al.* (1974). This result gives credence to the belief that the rate of CO_2 efflux during starvation quantitatively estimates the intensity of maintenance processes. For this reason, the starvation method was extensively used in the present study, which was initiated as a contribution toward understanding the quantitative relationship between respiration and maintenance processes.

Following the introductory chapters (Literature Review - Chapter II, and Materials and Methods - Chapter III), the thesis is arranged to deal with maintenance respiration on two levels of organisation. The first level, that of

the whole plant, is examined in (Chapters IV and V), whilst the second level, that of the mature leaf, is covered in (Chapters VI, VII and VIII).

In Chapter IV, energy expenditure for protein turnover and for maintenance of ionic concentrations, are discussed as causative factors in the relationship between the starved rate of dark respiration and dry weight which was established by McCree (1970). In addition to confirming this relationship, it was found that respiration was also a function of the organic nitrogen (protein) content of whole plants, which suggested that protein turnover was the major energy consuming process of maintenance. Results of experiments on roots and shoots, showed that rates of protein turnover in roots and shoots separately, may be quite different.

In Chapter V, a comparison of methods of determining the maintenance coefficient, is made since the starvation method, which has been widely used, has been criticised on the grounds that such a treatment is quite dissimilar to 'normal' growing conditions. Three methods were used:

(i) the starvation (steady state) method, in which the rate of CO_2 evolution following about 48 hours in continuous darkness is assumed to represent maintenance respiration;
(ii) the dynamic method, in which maintenance respiration is determined as the rate of CO_2 efflux when net carbon assimilation in the light is zero; and
(iii) the gross uptake method, in which the rate of carbon consumption when the growth rate is zero, is taken to represent maintenance respiration.

The latter method (which is based on Thornley's (1970) balance equation) yielded specific rates of maintenance respiration which were significantly higher than rates determined by the other methods. These rates were also higher than values for total maintenance requirements calculated by Penning de Vries (1975a) which were based on theoretical maintenance energy requirements. The other two methods gave essentially equivalent results which were comparable to Penning de Vries' estimates.

The methods are assessed with a view to determining which coefficient best reflects the magnitude of the maintenance processes. It is concluded that the assumptions made, regarding the processes which contribute to growth and maintenance, largely determine the choice of the 'true' maintenance coefficient and in the present study, the starvation method was favoured. It was also found that the maintenance coefficient and the growth efficiency were similar for all of the three nitrate fed legume species tested (field bean, lucerne and chickpea) regardless of the method used. A debudding treatment, which caused thickening of stems and

petioles resulted in a lower organic nitrogen content of kidney bean and tobacco plants and a significant reduction in the rate of maintenance respiration.

The first set of experiments conducted on mature leaves, reported in Chapter VI, sought estimates of maintenance respiration from direct measurement of the rate of CO_2 efflux according to Penning de Vries (1975a). Preliminary experiments in which CO_2 efflux was monitored over a 60 hour period of darkness revealed first, that the rate of respiration varied with the level of incident irradiance, and second, after 12 hours dark (the normal night), a rapid decay in the respiration rate, which stopped after a further 12 to 24 hours. In contrast, leaves which had been detached immediately following the photosynthetic period, exhibited a constant rate of CO_2 efflux for 36 hours. Other differences between attached and detached leaves are detailed within the chapter, but it is concluded that following the photosynthetic period leaves possess a respiratory component additional to the maintenance component arising from the energy requirements for protein turnover and maintenance of ion concentrations, which is hypothesised to support sucrose synthesis and phloem loading.

Aspects of carbon metabolism in starved mature leaves are investigated in Chapter VII. The topic addressed was the source of carbon for respiration during starvation. McCree (1970) suggested that the decay in whole plant respiration, which occurred 13-14 hours into the dark period, was due to the depletion of a pool of assimilate, and the switching from one pool to another. The data showed that during the 12 hour night following irradiation, starch and soluble carbohydrate were exported from the mature leaves. After 12 hours in one experiment, and 24 hours in another, the rapid decline in soluble carbohydrate had stopped, and the subsequence disappearance of soluble carbohydrate was at a much lower rate. The biphasic pattern of consumption suggested that respiratory substrate was derived initially from a metabolic pool and subsequently from a storage (possibly vacuolar) pool. This interpretation supports the hypothesis enunciated by McCree, however there was also evidence that amino acids derived from protein breakdown also served as a respiratory substrate during the prolonged dark treatment.

The maintenance of protein content during the prolonged dark treatment is the subject of investigation in Chapter VIII. Studies on wheat and barley have revealed large losses of soluble protein within 2-3 days of a dark treatment (Wittenbach 1978, Peterson *et al.* 1973). In view of the conclusions drawn earlier, regarding the role of respiration in maintaining the protein content of plant tissue, it was thought prudent to assay for leaf protein in order to verify that protein was indeed, being maintained. No significant decline in the concentration

of soluble protein (including RuBPC) or insoluble protein was detected within 48 hours of a dark treatment. However, the fact that the RuBPC concentration remained constant during this time i.e. RuBPC and the dry weight both declined to the same extent, suggested that RuBPC served as part of the respiratory substrate in the leaf. Moreover, the ability of the leaf to assimilate CO_2 became impaired during 60 hours of darkness. It is concluded that whilst maintenance of non-RuBPC protein occurred, RuBPC itself was apparently not maintained.

Chapter IX consists of a general discussion of the processes contributing to the maintenance requirement, methods used for the measurement of the maintenance requirement, and the consequences of maintenance for the growth of the plant.

CHAPTER II LITERATURE REVIEW

INTRODUCTION

Green plants absorb radiant energy by the process of photosynthesis and store it in the chemical bonds of organic molecules (principally carbohydrate). The subsequent conversion of these molecules into plant cell protoplasm is termed biosynthesis, and involves respiratory metabolism, in which some molecules serve as the substrate and others serve as the energy source. Thus, respiration has a dual function: (i) to provide carbon skeletons for the whole range of units from which cell constituents are assembled, and (ii) to provide energy for the synthesis of these units.

This literature review is arranged in three parts. The first deals with the pathways leading to CO_2 evolution; the second, with mitochondrial electron transport pathways; and the third, with the growth and maintenance concept of respiration.

PATHWAYS OF CO_2 EVOLUTION

Pathways of Hexose Oxidation

The principle pathways of hexose-phosphate oxidation are shown in Figure 2.1.

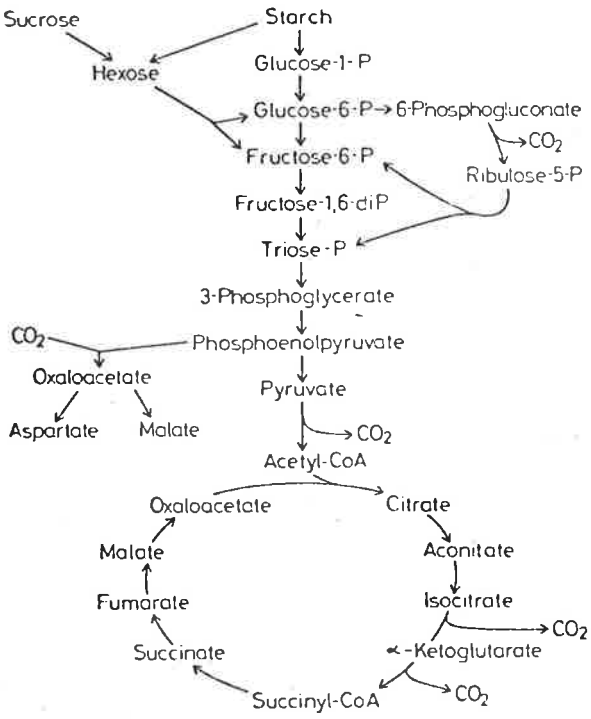

FIGURE 2.1. Principal reactions responsible for the respiration of carbohydrate (from ap Rees 1980).

glycolysis

During glycolysis, one molecule of glucose is oxidised to pyruvate with the net synthesis of two molecules of ATP (adenosine triphosphate). Two molecules of carbon dioxide are evolved by the pyruvate dehydrogenase-catalysed conversion of pyruvate to acetyl-CoA, if this step is operable, and two molecules (net) of $NADH_2$ are produced, which if oxidised in the cytochrome pathway, are equivalent to 6 molecules of ATP (assuming the P/O ratio to be three). Glycolytic enzymes are located mainly in the cytoplasm, but they also occur in the chloroplast, since starch degradation to triose-phosphate is usually *via* glycolysis.

the oxidative pentose phosphate pathway

This pathway branches from glycolysis and is an alternative pathway for glucose-6-phosphate oxidation. For every six molecules of glucose-6-phosphate entering, five may be recycled with the production of six molecules of carbon dioxide and 12 molecules of $NADPH_2$.

The pathway is not usually closed (i.e. it does not normally recycle) except possibly in chloroplasts when starch mobolisation is inhibited (Stitt and Heldt 1981) or when there is a high demand for intermediates for biosynthesis, and instead, the triose-phosphate formed as an intermediate is shuttled back into the glycolytic pathway (ap Rees 1980). Ap Rees (1980) considers there to be no convincing evidence that the $NADPH_2$ generated in the oxidative pentose phosphate pathway is oxidised in the respiratory electron transport chain, although it may be oxidised by soluble oxidases e.g. ascorbic oxidase, and phenol oxidase (Beevers 1961). Thus within the cell, there is a potential for considerable carbon dioxide evolution which is not associated with ATP synthesis.

the tricarboxylic acid cycle

Pyruvate may be converted to acetyl-CoA which is then fed into the tricarboxylic acid cycle. Alternatively, glycolytic phosphoenolpyruvate can be carboxylated by phosphenolpyruvate carboxylase to yield oxaloacetate, or, pyruvate can be converted to malate by malic enzyme. Both oxaloacetate and malate can enter the mitochondria by means of exchange transporters located in the mitochondrial membranes (Wiskich 1980), and then serve as substrates for the tricarboxylic acid cycle. During each revolution of the cycle, two molecules of carbon dioxide are evolved in addition to the production of three molecules of $NADH_2$, one molecule of $FADH_2$ and one molecule of ATP from a substrate level phosphorylation. When one molecule of hexose is oxidised, the cycle rotates through two revolutions in which six pairs of electrons are conserved in six molecules of $NADH_2$ (equivalent to 18 molecules of ATP) and four pairs in two molecules of $FADH_2$ (equivalent to four

molecules of ATP). In addition, two substrate level phosphorylations occur for a final total of 24 molecules of ATP synthesised, with four molecules of carbon dioxide being released.

In summary, for every molecule of glucose completely oxidised, the potential exists for the synthesis of 38 molecules of ATP and six molecules of CO_2 if the glycolytic pathway is used, but additional CO_2 evolution can occur if the branch to the oxidative pentose phosphate pathway is employed.

Other Pathways Involved in Carbon Dioxide Evolution

Davies (1973) proposed a 'pH stat' in which phosphoenolpyruvate was carboxylated by phosphoenolpyruvate carboxylase to produce oxaloacetate which was then converted to malate. These reactions consume hydroxide ions when the cytoplasmic pH increased above about pH 7.2. At lower pH values (below about pH 7.0) malic enzyme was considered to decarboxylate malate to pyruvate with the production of both carbon dioxide and hydroxide ions. Raven and Smith (1976) envisaged this 'stat' as being involved in nitrate assimilation in species which reduce nitrate in their shoots. Malate, synthesised in the leaf in response to hydroxide ion production during nitrate reduction, is transported to the root *via* the phloem. The malate is decarboxylated to pyruvate, the hydroxide ion released reacts with carbon dioxide to form the bicarbonate ion, and the latter is exchanged at the root membrane for further nitrate, thus maintaining electrical neutrality and raising the pH of the external solution. This scheme has been criticised recently by Deane-Drummond (1982), who did not find the expected alkalinisation of the medium during nitrate uptake by barley seedlings. She has suggested instead, that hydroxide ion release is associated with nitrate reduction rather than with nitrate uptake.

If in fact a mechanism involving spatial separation of CO_2-assimilation and CO_2-release not involving carbohydrate metabolism does exist in some plants, then both the amount of carbon dioxide fixed by photosynthesis in the leaves and the amount evolved in root respiration will be over-estimated when root and shoot gas exchange are measured separately. When gas exchange is measured on whole plants there will be no net carbon dioxide uptake in those plants which transport and decarboxylate all malate derived from shoot nitrate assimilation. However, not all plants transport and decarboxylate malate arising from nitrate assimilation.

There is also evidence for carbon dioxide uptake by root nodules associated with carboxylation of phosphoenolpyruvate to provide oxaloacetate for tricarboxylic acid cycle activity during intense nitrogen fixation in soybean (Coker and Shubert 1981). Such carbon dioxide uptake would result in lowered net carbon dioxide evolution and thus the underestimation of dark respiration.

Considerable potential for carbon dioxide release associated with biosynthetic reactions arises in the pathway leading to pentan synthesis for producing hemicellulose and pectin in wheat leaves. This pathway was found to release 40% of the total ^{14}C labelled glucose applied, and was due to the decarboxylation reaction converting UDP glucuronic acid to UDP xylose (see ap Rees 1980).

MITOCHONDRIAL ELECTRON TRANSPORT PATHWAYS

During conventional respiration reducing equivalents in the form of $NADH_2$ and $FADH_2$ generated in the tricarboxylic acid cycle are oxidised in the cytochrome pathway and ATP is synthesised (Figure 2.2). Addition of cyanide, which blocks the

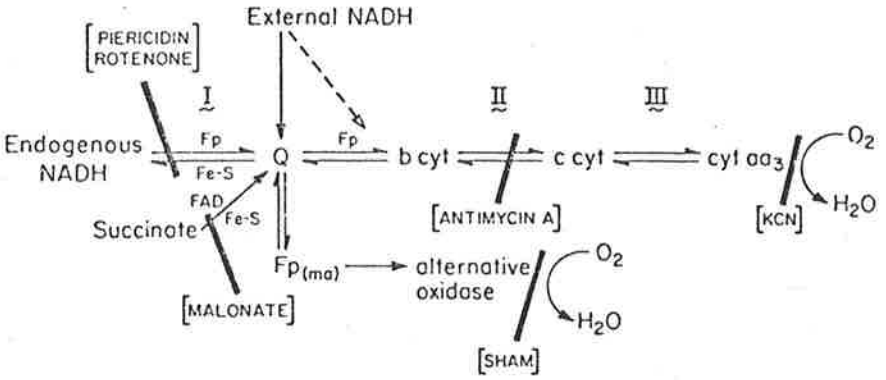

FIGURE 2.2. Probable respiratory pathways of plant mitochondria (from Day *et al.* 1980).

terminal cytochrome oxidase, severely depresses oxygen uptake. However, in many tissues (the most extensively studied are the spadix of *Arum maculatum* and aged potato slices), the mitochondria show oxygen uptake which is resistant to cyanide, and in this circumstance oxygen uptake can be accelerated in much the same way as when the electron transport chain becomes uncoupled. Indeed, it is generally accepted that the cyanide resistant pathway is non-phosphorylating (Day *et al.* 1980). Wilson (1980) disagreed, as he could detect ATP synthesis in the presence of cyanide. He has also shown that calcium uptake could be maintained by mung bean mitochrondia in the presence of 660 μM potassium cyanide.

The model of electron transport devised by Bahr and Bonner (1973) envisages the cyanide resistant pathway (also called the alternative pathway) as branching from the conventional cytochrome pathway at ubiquinone ('Q' in Figure 2.2). These authors also consider that the branch pathway becomes operative only when the electron transport capacity of the cytochrome chain is exceeded. Although the identity of the alternative oxidase is unknown, some inhibitors, such as the substituted hydroxamic acids (e.g. SHAM), have been identified, and when applied together with cyanide, often completely inhibit oxygen uptake. The application of SHAM by itself may inhibit respiration, but although this may show that the alternative pathway is present, it does not prove *in vivo* operation.

To date, a role for the alternative pathway is purely speculative. Palmer (1976) suggested that the alternative pathway functions to enable operation of the tricarboxylic acid cycle when the energy charge is high, (presumably during periods of high carbon inflow and when carbon skeletons, and not energy, are required). Day *et al.* (1980) suggested that carbon input to the cycle is maintained by the acceleration of glycolysis, possibly by way of a 'Pasteur Effect', by lowering the cytoplasmic energy charge. However, the alternative pathway is expected to engage when the cytoplasmic energy charge is high, and since the energy charge has not been observed to change very much despite marked changes in metabolism or metabolic conditions (Wiskich 1980), the means by which glycolysis is deregulated, is unknown. Lambers (1979) has proposed that the operation of the alternative pathway in roots occurs when the roots are supplied with more carbohydrate than is required for storage, growth and maintenance. Although it has attractions this model does not propose a mechanism for the switch from the conventional to the alternative pathway. Control of this switch is important since the pathway is hypothesised to be an energy "overflow" mechanism, and whilst the alternative pathway is operating, the cytochrome pathway is saturated (i.e. producing ATP) and the tricarboxylic acid is also fully operational, both features being characteristic of a cell with a high demand for energy and carbon skeletons. A better model for energy dissipation would involve the deletion of phosphorylation sites on the cytochrome pathway.

THE CONCEPT OF GROWTH AND MAINTENANCE RESPIRATION

Historial Introduction

Two major collections of work serve as the historical roots for the development of the maintenance concept; the work of Blackman and Parija (1928) and Kidd and West (1930) on fruit storage, and that of F.G. Gregory and colleagues on respiration and protein metabolism.

The classic studies of mature fruit respiration conducted by Blackman and Parija, and Kidd and West were aimed at investigating factors which led to premature ripening and the consequent decay of stored apples. These workers were, in effect, seeking to regulate 'basal' or 'maintenance' respiration. Temperature was found to be the major regulator, and when combined with reduced oxygen concentration and elevated carbon dioxide concentration, respiration could be held at a low rate, fruit carbohydrate was conserved, and tissue damage was minimal.

Another interesting phenomenon which arose during these studies was the 'climacteric', a phase of rapid CO_2 evolution which persisted for about one week, and was associated with the ripening of fruits (such as apples, pears and bananas) which had been stored at temperatures above $10^{o}C$ (Kidd and West 1930).

The agent responsible for the climacteric was subsequently identified as ethylene (Gane 1937). Potato tubers were also found to exhibit a respiratory climacteric upon treatment with ethylene (Solomos and Laties 1975). Interestingly, cyanide also triggered the climacteric in potato tubers and the ethylene-induced climacteric was found to be cyanide insensitive. Solomos and Laties (1976) suggested that for ethylene to stimulate respiration, the cyanide resistant pathway must be present. Nevertheless, Theologis and Laties (1978a, b) found that the pathway was inactive at the height of the climacteric in fruit slices and in aged cyanide resistant slices of bulky storage organs. The implication is, that although the potential for cyanide resistant respiration exists during fruit ripening, climacteric respiration represents fully coupled respiration.

Gregory and Sen (1937), studied protein and carbon metabolism in acutely potassium starved barley plants and observed that a high respiratory rate was associated with high levels of amino acids in the tissue, and suggested that a protein cycle was in operation. In this cycle, amino acids derived from degraded protein were deaminated and the carbon residue respired, and in this way it was envisaged that the rate of CO_2 release should reflect the rate of turnover of the protein cycle.

This work was completed by Richards (1938) who showed that a quantitative relationship existed between dark respiration and protein content, and suggested that a given rate of CO_2 evolution could maintain a given quantity of protein. Such a statement implies that respiration supplies energy for protein synthesis to compensate for continuous degradation, a belief common today (Huffaker and Peterson 1974), although the notion of a protein cycle is no longer strongly supported, at least not during normal growth.

This brief historical summary outlines important facits of the concept of maintenance and growth respiration which have recurred in more recent times, namely the role of temperature, the role of the cyanide resistant pathway, and the relationship between respiration and the maintenance of protein content.

Recent Developments in the Concept of Growth and Maintenance Respiration

More recent developments, which have addressed the same problems as outlined above, have derived from investigations on whole plants. Many studies on crop growth (particularly that of clover) show that the crop growth rate (determined by dry matter harvesting) increases until an 'optimal' leaf area index is achieved (usually at canopy closure when the leaf area index is three to four), and that at higher leaf area indices, the growth rate declines (e.g. Davidson and Donald 1958 and Fukai and Silsbury 1976). Furthermore, Stern and Donald (1962) observed that the 'optimum' leaf area index increased with irradiance level. Other studies

using gas analysis, show no such 'optimum', but rather, a 'critical' leaf area index (McCree and Troughton 1966 and King and Evans 1967), in which gross carbon dioxide uptake is maximal at canopy closure, and thereafter, changes very little with increasing leaf area index. Dark respiration shows a similar variation. This observation lead McCree and Troughton (1966) to conclude that plants adapt their respiration such that the rate of respiration remains a constant proportion of the gross carbon dioxide uptake. In addition to this constant relationship McCree (1970) found (in graphical plots of respiration against gross carbon assimilation) that when the gross assimilation rate was extrapolated to zero, larger plants had a higher (basal) respiration rate. When he combined these observed relationships, McCree derived a two component model of respiration:

$$R = k\,Pg + cW$$

where: R = total respiration ($gCO_2\ m^{-2}\ 24h^{-1}$)
Pg = gross carbon dioxide assimilation ($gCO_2\ m^{-2}\ 24h^{-1}$)
W = dry weight ($gCO_2\ m^{-2}$)

k is an efficiency (dimensionless), c is a rate constant (dimension = $time^{-1}$)

The best fit to this data was obtained when $k = 0.25$ and $c = 0.015$. Thornley (1970) pointed out that this two component model was of the same form as that used by Pirt (1965), who measured growth efficiency and maintenance in bacterial cultures. Pirt measured the yield of bacteria per unit of substrate at different specific growth rates (i.e. substrate limited growth) and obtained the relationship:

$$\frac{1}{Y} = \frac{m}{\mu} + \frac{1}{Y_G}$$

where: Y = yield of bacteria (g dry weight (g substrate)$^{-1}$)
μ = specific growth rate (g g^{-1} h^{-1})
m = maintenance coefficient (g substrate (g dry weight)$^{-1}$ h^{-1})
Y_G = growth efficiency (g dry weight (g substrate)$^{-1}$)

Thornley (1970) extended Pirt's analysis to derive the equation:

$$R = (1 - Y_G)\,Pg + mY_G\,W$$

where: R = total respiration (g CO_2 $24h^{-1}$)
Pg = gross carbon dioxide assimilation (g CO_2 $24h^{-1}$)
W = dry weight (g CO_2)
Y_G = growth efficiency (g CO_2 (g CO_2)$^{-1}$)
m = maintenance coefficient (g CO_2 (g CO_2)$^{-1}$ $24h^{-1}$)

which is in the same form as the McCree equation. McCree's 'k' was recognised as

being a function of the growth efficiency, whilst 'c' was a maintenance coefficient. Conversion of the constants 'k' and 'c' into the more theoretically meaningful terms 'Y_G' and 'm' respectively,is easily achieved (e.g. Hansen and Jensen 1977, McCree and Silsbury 1978).

More recently, Thornley (1977) analysed growth and respiration in a slightly different way. In his model (Figure 2.3), Thornley put forward the more realistic view that respiration draws on a supply of stored substrate to provide energy and carbon for growth only (growth of degradable and non-degradable structures). A

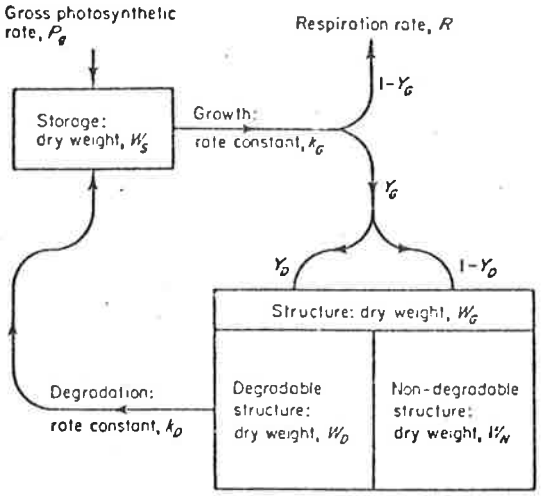

FIGURE 2.3. Model for respiration, growth and maintenance (from Thornley 1977).

decay constant is assigned to the degradable structure, and the degradation products are converted back into available substrate in the storage pool. Although maintenance is not included in the model the processes which result in degradation can be interpreted as giving rise to a maintenance requirement. The present model has no provision for wastage respiration (Thornley 1971). Senescence could in part be accounted for by the breakdown of degradable structures and should therefore be included in maintenance. McCree (1982) modified the model slightly by including in the storage component a provision for both starch accumulation and degradation which resulted in a better correlation between observed and calculated rates of carbon exchange by stands of white clover.

Barnes and Hole (1978) concluded that the Thornley model was mathematically compatible with the earlier two compartment model of McCree (1970) if the degradable structure was proportional to protein content, rather than being dependent on gross carbon assimilation as assumed by Thornley(1977). The analysis of Barnes and Hole also confirms the idea that a maintenance requirement is necessitated by the breakdown of the degradable structure. In addition to confirming the traditional two component model of respiration, Barnes and Hole consider the Thornley model to provide a formal theoretical basis for that view.

The Underlying Significance of the Growth Efficiency and Maintenance Coefficient

The concepts of growth and maintenance were given a biochemical basis in the work described by Penning de Vries *et al.* (1974, growth efficiency) and Penning de Vries (1975a, maintenance).

growth efficiency

In the first publication a method for calculating the theoretical efficiency of biosynthesis using quantitative biochemistry was described. From a knowledge of the composition of the biomass, the amount of growth substrate (glucose in this instance) conserved in that biomass, and the amount respired in providing energy, the growth efficiency could be calculated using equations describing the formation of each compound synthesised by the plant. Factors characterising the conversion of substrate to end product are: the 'production value'; the 'oxygen requiring factor'; and the 'carbon dioxide production factor'. These represent: the weight of end product; the weight of oxygen consumed; and the weight of carbon dioxide produced; each divided by the weight of substrate required for carbon skeletons and energy production. The magnitude of each factor varies with the end product formed. Thus for carbohydrate, the value is 0.87, for organic nitrogen compounds, 0.48, and for lipid 0.36, (Penning de Vries 1972). Therefore, as the level of reduction increases from carbohydrate to lipid the weight of substrate respired to provide the energy required for synthesis increases.

Substrate requirements for energy production are largely determined by the P/O ratio which, for the purpose of the calculations, was set at three. Any value between two and three hardly affects calculation of the 'production value' in rapidly growing plants, although the ratio becomes more critical with declining relative growth rate, when there is a greater energy demand for maintenance.

There is a good agreement between values for the growth efficency calculated using this quantitative biochemical approach (0.7 - 0.8) and estimates obtained from gas exchange analysis (Penning de Vries 1972). This result is considered to provide a formal biochemical basis for the growth efficiency. More recently, McDermitt and Loomis (1981) have proposed a method of calculating the growth efficiency from an elemental analysis of the products. This method 'short-cuts' that of Penning de Vries *et al.* (1974) and gives highly comparable results.

The numerical value of the growth efficiency is independent of temperature over the 'normal' range likely to be experienced by a field crop and the magnitude of the variation in the efficiency is quite small for vegetative plants (Table 2.1). Thus although higher temperatures increase the *rate* of respiration, the *amount* of carbon respired to produce *unit* biomass remains unchanged unless the composition of

TABLE 2.1. Literature estimates of growth efficiency (Y_G, g g^{-1}) at different temperatures (°C).

Species	Temperature	Y_G	Reference
Subterranean clover	20	0.69	Silsbury (1979)
Subterranean clover	30	0.75	McCree and Silsbury (1978)
White clover	20	0.75	McCree (1970)
White clover	30	0.74	McCree and Silsbury (1978)
Dwarf beans	20-25	0.75	Moldau and Karolin (1977)
Lolium multiflorum	20	0.78	Hansen (1978)
Lolium multiflorum	20	0.77	Hansen and Jansen (1977)
Grain sorghum	30	0.71	Wilson *et al.* (1980)
Sunflower	10	0.76	Szaniawski and Keilkiewicz (1982)
Sunflower	20	0.75	Szaniawski and Keilkiewicz (1982)
Sunflower	30	0.75	Szaniawski and Keilkiewicz (1982)
Maize		0.65	Penning de Vries *et al.* (1974)
Pinus taeda		0.64	Chung and Barnes (1977)

the new biomass is altered. A high rate of respiration is therefore not necessarily negative with respect to plant growth, but may instead, reflect a high rate of biosynthesis.

One problem with the interpretation of the growth efficiency which is still unresolved, arises from the observation that the shoot converts substrate into biomass with greater efficiency than does the root (Hansen and Jensen 1977; Hansen 1978, 1979; Lambers *et al.* 1979; Szaniawski 1981; Szaniawski and Keilkiewicz 1982). Lambers *et al.* (1979) have suggested that energy derived directly from photosynthesis may result in a high growth efficiency in shoots, and that the possession of wasteful respiration (the alternative, cyanide resistant pathway) contributes to a low efficiency in roots.

the maintenance coefficient

In his second publication, Penning de Vries (1975a) surveyed the literature to calculate the energy and substrate requirements for maintenance in leaves. He found that in the main, energy would be expended in protein turnover at (7 - 13 mg glucose (g dry weight)$^{-1}$ 24h^{-1}) and in maintaining ion concentrations at (6 - 10 mg glucose (g dry weight)$^{-1}$ 24h^{-1}) However, in contrast to the calculations made for the growth efficiency those made for the energy requirements for maintenance are of necessity crude, since accurate estimates of protein turnover and rates of ionic fluxes in higher plants were not available. The appreciable variation in the estimates could be attributed in part, to differences in the biochemical composition of the material from which the information was drawn. In addition, the calculations for maintenance of ionic fluxes apply during darkness and may underestimate the overall energy requirements, since there is clear evidence from Graham and Bowling (1977) that during shoot irradiation additional energy is expended to increase the electrical potential difference across root membranes. In contrast to the energy requirements for protein turnover and the maintenance of ionic fluxes energy expenditure for membrane maintenance was not considered to be high (ca. 1.7 mg glucose (g dry weight)$^{-1}$ 24h^{-1}).

Despite the rather arbitrary assumptions and omissions these calculations nevertheless provide numbers in reasonable agreement to those obtained from measurements made using a variety of techniques and suggest that protein turnover and ion fluxes do indeed contribute to the bulk of the energy expended in maintenance.

Experimental Determination of Growth and Maintenance Coefficients

The recent confirmation of the utility of the two component models of respiration recommends their continued application for the purpose of determining growth and maintenance coefficients. In addition to the Thornley (1970) model, two further models, as presented by McCree and Silsbury (1978), are readily applicable

to whole plant carbon exchange. Carbon exchange is continuously recorded and integrated over 12 hour periods to obtain daytime and nighttime totals (D and N respectively).

Thornley's balance equation may be written:

$$\Delta S = {}^{1}/_{Y_G}\,\Delta W + \Delta Sm$$

which is the equation of a straight line of slope ${}^{1}/_{Y_G}$ (the reciprocal of the growth efficiency) and slope ΔSm. The maintenance coefficient "m" is calculated:

$m = \frac{\Delta Sm}{W}$, where W is the dry weight in CO_2 units. The dependent variable is ΔW, and the independent variable, ΔS. Using McCree's (1982) notation, where N is a negative number, ΔS is in the input of subtrate carbon, calculated as D-N, and the daily accumulation of biomass carbon is calculated as D+N. It is important to note that this analysis is based on 24 hour totals of carbon exchange and refers to growth at constant temperature (20°C) and 12 hour day length.

The dynamic equation of McCree and Silsbury (1978) is written:

$$N = gD + h,$$

in which 'g' is a function of the growth efficiency ($Y_G = \frac{1 - g}{1 + g}$) and 'h' is the maintenance efflux. The maintenance coefficient 'b' is calculated $b = {}^{h}/_{W}$.

The steady state equation (McCree and Silsbury 1978) is written:

$$N = kD + (1 + k)\,cW$$

where 'k' is equal to 'g' above and the term (1 + k) cW, is the maintenance efflux. If Nm = cW, where Nm is the 12 hour total CO_2 efflux after about 48 hours darkness when the rate of CO_2 efflux is constant, then the maintenance coefficient 'c' is calculated $c = \frac{Nm}{W}$.

The relationship between the various maintenance coefficients is represented in Figure 2.4, which is a plot of the 12 hour nighttime CO_2 efflux against the corresponding daytime uptake. The maintenance efflux is determined by extrapolation from the desired x- and y-axis coordinates, to the ordinate. The coordinates are determined as follows: in the Thornley equation, maintenance is determined when the daily (24 hour) accumulation of biomass carbon is zero (i.e. D + N = 0), and the co-ordinates are (D ($\Delta Sm/2$), N ($\Delta Sm/2$)). In the dynamic equation, maintenance is estimated as the CO_2 efflux when the daytime carbon accumulation is zero (i.e. when D = 0), and the coordinates are (0, N). In the steady state method, maintenance respiration is determined in prolonged darkness, but is represented in Figure 2.4 as the efflux (Nm) when the assimilation (Dm) is equal (but opposite in sign) to the rate of efflux after approximately 48 hours dark (i.e. when Dm = Nm) and the co-

ordinates are (-Dm, Nm) .

Ordinate intercept	Derived coefficient	24h maintenance coefficient
Y_1	c	2c
Y_2	b	2b
Y_3	0.5m	m

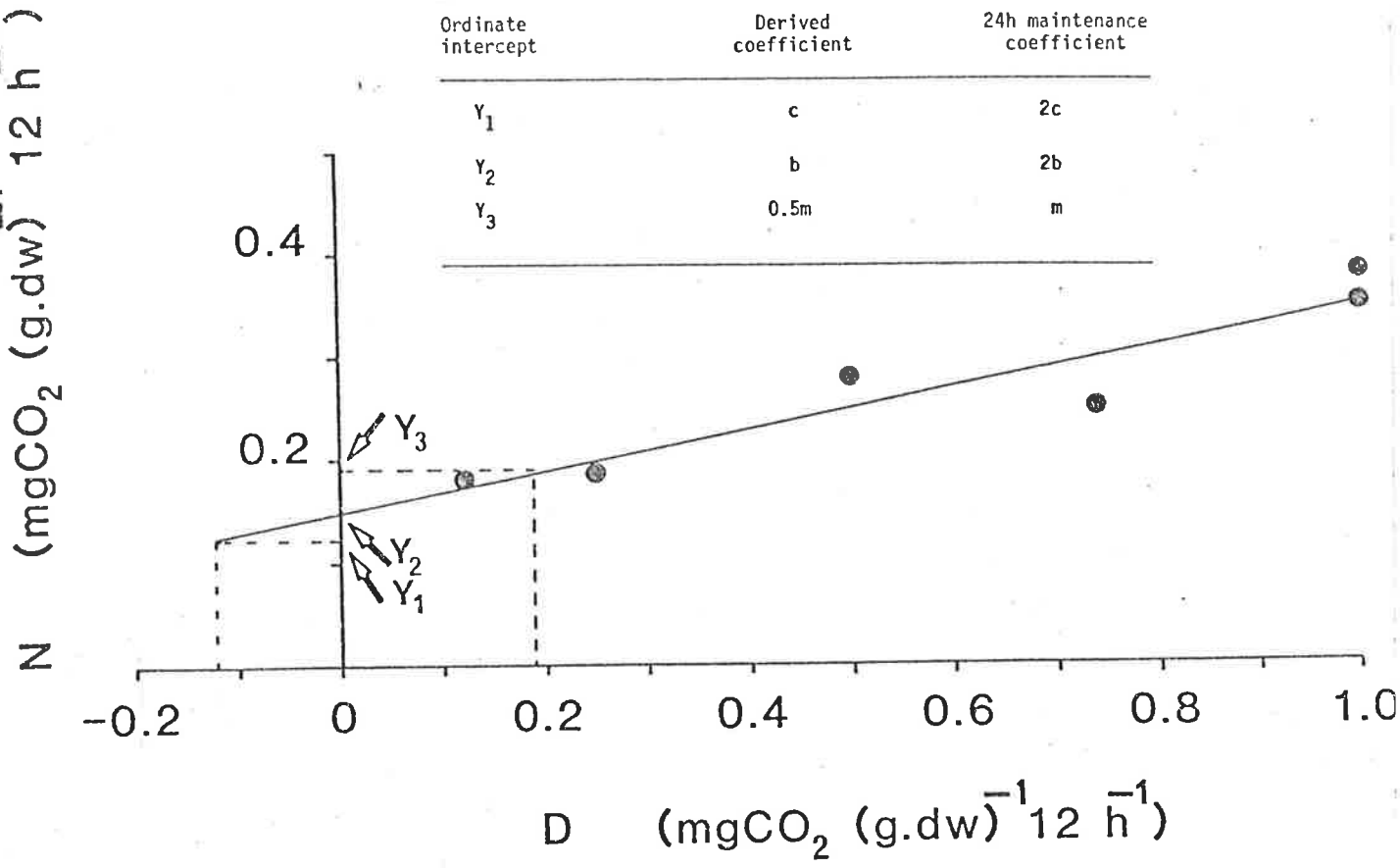

FIGURE 2.4. Relationship between nighttime CO_2 efflux (N) and daytime CO_2 influx (D) for a single field bean plant to show the derivation of maintenance coefficients.

The magnitude of the coefficients (which are represented in the table accompanying Figure 2.4) can therefore be ranked thus, m > 2b > 2c, and poses the question, which is the 'true' maintenance coefficient?

Assumption Made in Calculating Growth and Maintenance Coefficients

(1) It is implicit in the McCree-type analysis that over a 24 hour period, respiratory substrate production and consumption are in equilibrium, since the carbon balance equation does not provide for storage. Evidence in support of this assumption arises from the observation (McCree 1970) that shortly after a 12 hour night, if the plant is not irradiated, respiration begins to decay rapidly. This decay implies that substrate has become limiting for growth. At low temperature (e.g. 10°C) nighttime assimilate consumption may be reduced, leading to carbohydrate accumulation over the 24 hour period (McCree and Silsbury 1978) which can cause an uncoupling of the relationship between daytime carbon uptake and nighttime carbon efflux (Moldau and Karolin 1977).

(2) In the dynamic and steady state methods, nighttime carbon loss is assumed also to represent daytime carbon loss. This subject has been debated for some time, and some authors are adamant that dark respiration is inoperative (Mangat *et al.*

1974) or partially inhibited (Lambers *et al.* 1979) during the light, whilst others conclude that dark respiration does continue in the light (Chapman and Graham 1974).

It is important that the two aspects of respiration (carbon skeleton production and energy synthesis) be separated since whilst oxidative phosphorylation may be blocked during photosynthesis (adenine nucleotides shuttled from the chloroplast, are supposed to inhibit electron transport through the mitochondrial cytochrome pathway by establishing a high cytoplasmic ATP/ADP + Pi ratio), the mitochrondria are the source of *keto* acids which cannot be synthesised in the chloroplast. The tricarboxylic acid cycle should therefore be operational during photosynthesis.

However, the inhibition of mitochondrial ATP synthesis during photosynthesis cannot be now assumed in view of the findings from the two recent studies by Dry and Wiskich (1982), and Stitt *et al.* (1982), which severely test the view that the ratio ATP/ADP regulates oxidative phosphorylation. It is still conceivable however that other factors regulate oxidative phosphorylation during photosynthesis in photosynthetic cells, but the situation is now far from clear.

Another viewpoint in this argument arises from an analysis at a higher level of organisation, the organ (leaf) level. Two points need to be discussed, firstly leaf structure, and secondly, sites of growth. The most important conclusion from the study of Jellings and Leech (1982) was that a large proportion of the cells within a leaf are non-photosynthetic. They calculated that in wheat, barley and oats, the vascular tissue comprised between 30-40% of the cells, the epidermis 10% and the mesophyll 50-60% of the cells. These numbers, certainly infer that dark respiration occurs in cells of the leaf, during the light even if only in non-photosynthetic cells. In fact, since translocation of assimilate from the leaf is usually faster during the light than the dark (Geiger and Batey 1967, Pearson 1974, Ho and Thornley 1978, Chatterton and Silvius 1979), the phloem companion cells (or transfer cells where present), which provide the "push" for long distance translocation (see Giaquinta 1980), must also be operating faster in the light than the dark. It is unlikely that energy for phloem loading is supplied directly from photosynthesis (*via* triose phosphates) since sucrose is synthesised in the cytoplasm and this is the usual form of carbon which is exported from the mesophyll to the companion cells.

The second point to consider is that assimilate production and consumption usually occur in separate parts of the plant. Young developing shoot buds and roots rely on assimilate imported for growth and these tissues respire during the light and the dark, although root respiration may be higher during the light (e.g.Farrar 1981), but not necessarily (e.g. Challa 1976, Veen 1980). This apparent effect of

light can sometimes be explained in part, by diurnal temperature variation. Whilst assimilate export from the leaf may be higher in the light than the dark, the finding of Fondy and Geiger (1982), that import into developing sink leaves at night was similar to that during the day, is particularly interesting and supportive of the basic assumption that non-photosynthetic 'sinks' respire at the same rate during the light and the dark.

Consideration of these two points, lead to the conclusion that dark respiration during the light can be higher than during the dark (on a whole plant basis), and that methods which resort to calculation of growth and maintenance coefficients during the dark are likely to give minimal estimates of the intensity of the maintenance processes.

(3) Since the instantaneous rate of CO_2 efflux in prolonged dark (in the steady state method) is integrated over 24 hours, it is assumed that maintenance is constant during a 24 hour period. This may be a simplification, because Ryle *et al.* (1976) determined from an analysis of $^{14}CO_2$ efflux, that maintenance varied between 2 and 10% of the total efflux over the first 24 hours of measurement. It should be pointed out, however, that there is little evidence that an analysis based on the kinetics of $^{14}CO_2$ release does in fact, reflect the underlying processes of maintenance. It has already been cited, that root transmembrane potential is higher during the light than the dark (Graham and Bowling 1977), although it could be argued that this energy expenditure be debited to growth rather than maintenance, since the higher transmembrane potential during shoot irradiation is expected to reflect ion uptake for growth. This latter example also points to the difficulty in deciding whether energy expenditure for ion uptake should be attributed to the growth or maintenance processes.

(4) Finally, it should be noted that photorespiration does not affect the analysis, since *net* carbon assimilation is measured and photorespiration is therefore taken into account in the overall carbon balance.

Factors Affecting the Maintenance Coefficient

Increase in temperature increases the rate of maintenance respiration. McCree and Silsbury (1978) calculate a Q_{10} value of 1.85 for subterranean clover whilst a value of 2.2 was obtained for radish (Lopes 1979) and for both white clover and sorghum (McCree 1974).

Changes in daylength produce inconsistent results. Hansen and Jensen (1977) found no consistent relationship between the maintenance coefficient and daylength (8 to 16 hour day), whilst Lopes (1979) and McCree and Kresovich (1978) found a

small, but positive relationship. McCree and Kresovich suggest the correlation is due to the higher growth rate obtained when the daylength is longer and is to be expected, since maintenance costs and metabolic activity are probably linked.

Conflicting results are obtained when water stress is imposed. Wilson *et al.* (1980) observed a decline in the maintenance coefficient with increasing soil water deficit, whilst Moldau *et al.* (1980) found the maintenance coefficient to increase, and suggested this to be an adaptive response.

Schwarz and Gale (1981) found that the rate of maintenance respiration of plants which were tolerant of mild salt stress (*Xanthium strumarium*, *Atriplex halimus* and *Phaseolus vulgaris*) to increase when the nutrient solution contained a higher than 'normal' salt concentration. There was no increase in the respiration rate when salt was added to the nutrient solution of *Zea mays*, a salt intolerant species. Schwarz and Gale concluded that the salt stimulated increase in the maintenance respiration rate enabled the former plants to tolerate salt stress.

The wide variety of environmental conditions experienced during growth do not permit any general conclusions to be drawn on possible differences in maintenance requirements arising from species characteristics. However, McCree (1974) found the specific maintenance rate of white clover to be higher than that of grain sorghum when both were supplied with nitrate.

Inconsistencies in the Concept of Maintenance

(1) Some difficulty in the interpretation of maintenance arises because a strict definition is lacking. Penning de Vries (1972) defined maintenance as 'processes to compensate for the degradation of existing structures and organisation. Re-synthesis of hydrolysed proteins is likely to be a part of maintenance processes, but also dry matter accumulation on one level of organisation may sometimes be seen as a part of maintenance on a higher level, e.g. the formation of a new leaf on a plant when it replaces a lost one'. In another publication, Penning de Vries (1975a) considered that maintenance respiration 'refers to the carbon dioxide that results from protein breakdown, plus the carbon dioxide produced in respiratory processes that provide energy for the maintenance processes'.

From the first definition, the level of organisation to be considered is left to the experimenter. Thus when comparing maintenance respiration rates from single leaves with rates from whole plants, swards or crops, it is not surprising that quite large variations (Penning de Vries 1975a) are observed. If, for example, shoot maintenance includes leaf senescence and leaf development, emergence and elongation (i.e. growth), the value for maintenance is expected to

be higher than is the maintenance of fully expanded leaves only, since the specific respiration rate of growing tissue is very much higher than that of mature tissue (Robson and Parsons 1981). In addition, this first definition cannot be accommodated in the model of Thornley (1977), since this model does not consider any loss of the non-degradable fraction, as for instance, when a leaf senesces and abscises. Clearly, the model needs modification to allow for this aspect of senescence, and very recently McCree (1982) had to incorporate death of the non-degradable fraction into his computer simulations when attempting to apply this model to carbon exchange of white clover. Should the model incorporate senescence, it would be more consistent with Penning de Vries' definition. However the connection between maintenance and senescence (in terms of the growth and maintenance concept) seems obscure, and points to one of the major difficulties in applying the concept *viz.* which processes are associated with synthesis, and which with maintenance.

The second definition is also difficult to reconcile with the Thornley model, since it concerns carbon dioxide release only, i.e. complete oxidation of carbohydrate is assumed for energy production, with no provision for carbon skeleton synthesis. For example, when amino acids from degraded protein cannot be recycled because they are transported from an organ or are further oxidised, additional substrate must be consumed for carbon skeleton production if the level of protein is to be maintained.

The uncertainty surrounding the view that CO_2 evolution is solely due to respiratory processes leading to energy production, has already been highlighted in the first section of this literature review.

(2) The bases of expression of maintenance rates are inconsistent. Expression per unit dry weight can lead to variations in the rate due to differences in chemical composition of the material, and expression on a carbon basis may be more appropriate. Some authors suggest that since a large proportion of maintenance arises from protein turnover, respiration would be more accurately explained by relating maintenance to protein content (e.g. Barnes and Hole 1978). This belief is held by many authors although there is, as yet, no evidence to support the view, for the reason that Penning de Vries (1975a) divided maintenance costs more or less evenly between energy requirements for protein and ionic gradient maintenance. The substitution of protein content for dry weight in the McCree (1970) equation (Barnes and Hole 1978) was mainly for mathematical reasons, and relating maintenance to protein content could be misleading if part of the protein is in a storage form which turns over only very slowly (Madison *et al.* 1981). It is interesting to note that in the microbiology literature, maintenance is still presented on a dry weight basis.

These inconsistencies spell out the obvious weaknesses in our knowledge of the underlying causes of the maintenance requirement and point to the need for more data on maintenance respiration and a greater concentration on those underlying processes and their control. Whilst there has certainly been an increase in information on the former point since the publications of Penning de Vries, very little attention has been given to the latter.

Practical Applications of the Growth and Maintenance Concept in Plant Science

(1) Crop growth models. The idea of an 'optimal' leaf area index created problems for crop modellers, since if respiration was taken to be a function of leaf area and was therefore relatively constant after canopy closure, the lower shaded leaves appeared to be parasitic upon the rest of the plant. With the knowledge that respiration is a linear function of carbon assimilation and mass, there was a great improvement between the crop yields observed, and those calculated by model simulations. Many of the more recent crop models now include growth and maintenance components of respiration (e.g. Wann *et al.* 1978; Hodges *et al.* 1978; Sheehy *et al.* 1979, 1980). In addition, the concept has been used successfully by McCree (1970, 1982) to quantify carbon dioxide exchange by white clover plants.

(2) Improving crop yield. The general agreement between calculated values of the growth efficiency and those arising from observation (see Table 2.1) suggests that plants usually synthesise biomass with maximum efficiency, and therefore there appears little scope for improving crop yield by breeding for improved efficiency. In addition, as discussed previously, a higher efficiency may indicate the synthesis of biomass of relatively low energy content.

In contrast, crop model simulations consistently show that final yield is sensitive to the maintenance component of respiration (e.g. Sheehy *et al.* 1979, 1980, Hunt and Loomis 1979) and this suggests that improvement in yield may be accomplished by breeding plants with lower maintenance requirements, as long as the competitive ability of the plants is not impaired. Recent studies by Wilson (1975, 1982, Wilson and Jones 1982) and Robson (1982a, b) show that selection lines of *Lolium perenne* possessing 'low' rates of mature leaf respiration (which is equated with low maintenance) do indeed produce greater dry matter yields after simulated grazing, than do lines in which mature leaf respiration is 'fast'. Moreover, no major differences in chemical composition between the two lines are apparent. So far however, neither the competitive ability of the 'slow' respiring line nor the biochemical reasons for the dry matter yield difference, have been ascertained. Nevertheless, such initially promising results again point to the need for a greater understanding of the maintenance processes and their control, and also point to the real prospect of increasing dry matter production of a pasture grass through practical application of the concept of growth and maintenance.

CONCLUSION

It is clear that the biochemical pathways of carbohydrate oxidation are well established. Hexose phosphate, the usual carbohydrate substrate for dark respiration, is oxidised in the sequence of reactions encompassing glycolysis and the tricarboxylic acid cycle. Reducing equivalents derived from the tricarboxylic acid cycle, are, in turn, oxidised in the mitochondrial electron transport pathway with concomitant ATP synthesis. As a result, CO_2 is released during the oxidation of hexose phosphate, and O_2 is consumed in the terminal oxidation of $NADH_2$ in the electron transport pathway. Although this describes the essence of dark respiration, CO_2 evolution also arises from hexose phosphate oxidation *via* the oxidative pentose phosphate pathway, and oxygen consumption is also dependent on the activity of the alternative electron transport pathway. Unfortunately, the extent to which these two latter pathways contribute to respiration *in vivo* are, as yet, impossible to assess. Nevertheless considerable evidence indicates that respiration measurements can be greatly influenced by the activity of these, and other pathways which involve CO_2 and O_2 exchange.

Although CO_2 evolution may reflect the participation of pathways not linked to energy production, it is usual to assume that CO_2 exchange is representative of the intensity of energy producing reactions, and on this basis, the rate of dark CO_2 efflux can be partitioned into growth and maintenance components. Maintenance respiration refers to energy production for processes other than synthesis, and is expected to include such activities as maintenance of protein content (since proteins are subject to continual turnover) and maintenance of ion concentrations across cell membranes.

Although the individual chemical reactions, and their regulation are important determinants of growth, it is the ability of the plant to integrate these reactions under a wide range of environmental conditions, which determines the adaptability and competitiveness at the whole plant or crop level of organisation. The growth and maintenance concept seems to be one way in which to investigate the integration of biochemical reactions. Incorporation of growth and maintenance coefficients into models of plant and crop growth, have improved the predictive ability of these models, and in the case of the Thornley (1977) model, suggest physiological reasons for the variation in maintenance energy requirements.

The review therefore points to the need to estimate growth and maintenance coefficients (which are the factors which quantify the growth and maintenance processes), and to analyse the extent to which they represent the underlying reactions. It seems that a greater knowledge of the partitioning of carbon between growth and maintenance processes may lead to an understanding of the reasons for

yield increase, not only in terms of seed or fruit, but also of vegetative leaf matter, which is so essential for the improvement of crop and animal production.

CHAPTER III MATERIALS AND METHODS

PLANT CULTURE

Seed of the following legumes, field bean (*Vicia faba* cv. Fiord); chickpea (*Cicer arientinum*, an unnamed line originating from Greece); field pea (*Pisum sativum* cv. White Brunswick) and Lucerne (*Medicago sativa* cv. Hunter River), were obtained from collections at the Waite Agricultural Research Institute. Seed of kidney bean (*Phaseolus vulgaris* cv. Hawkesbury Wonder), and tobacco (*Nicotiana tobaccum* cv. White Burley), were obtained commercially.

Seeds of uniform size were surface sterilised in 50% (v/v) ethanol, washed overnight in water, and sown singly in 15 cm diameter, or 15 x 15 cm square plastic pots. Lucerne communities were established in similar pots by sowing in a 7 x 7 matrix with two seeds at each site (i.e. 98 seeds per pot). The rooting medium was 'Oil Dry', which is normally used for removing spilt oil from garage floors. It is a fritted clay of high porosity and water holding capacity (van Bavel *et al.* 1978) which has proved ideal as a rooting medium for plant growth. Following emergence, seedlings were selected for uniformity and placed in either a temperature controlled glasshouse (20°C) and experienced natural irradiance and daylength, or in a growth room set at 20°C with a 12 hour day provided by high pressure sodium vapour lamps giving selected levels of photosynthetically active radiation ranging from 1500 μmol m^{-2} s^{-1} to 160 μmol m^{-2} s^{-1}.

Except where specified, each pot was irrigated daily with 500 ml of dilute nutrient solution (Table 3.1) containing nitrate (*ca* 7.5 mM), and at no stage were the legumes supplied with nodulating bacteria. All plants grew with no symptoms of necrosis or nutrient deficiency.

TABLE 3.1. Elemental composition of the nutrient solution used for plant growth.

Nutrient	Concentration (mM)	Nutrient	Concentration (μM)
KNO_3	2.52	$FeSO_4.7H_2O$	72
$Ca(NO_3)_2.4H_2O$	2.50	EDTA Na_2	64
$MgSO_4.7H_2O$	1.00	H_3BO_3	46.2
KH_2PO_4	0.50	$MnCl_2.4H_2O$	9.1
		$ZnSO_4.7H_2O$	0.76
		$CuSO_4.5H_2O$	0.32
		$Na_2MoO_4.2H_2O$	0.50

WHOLE PLANT GAS EXCHANGE

Carbon exchange was measured by placing a plant in a 30 litre perspex assimilation chamber irradiated from above by a 400 W 'Metalarc' lamp. The photon flux density incident on the upper inside face of the chamber was 700 $\mu mol\ m^{-2}\ s^{-1}$ (400-700 nm) as measured by LI-COR 172 quantum meter and sensor (Lambda Instruments Corporation, Lincoln, Nebraska). The incident irradiance could be altered by placing nylon mesh screens over the chamber.

Air for the chamber was provided by a small compressor which pumped outside air through a 200 litre drum, over a humidifier, and to the chamber *via* a mass flow controller (Tylan model FC-202 with model RO·14-200 readout box, Tylan Corporation, Torrance, California) at a maximum flowrate of 25 $l\ min^{-1}$. The air entered the chamber under slight pressure, through a line of inlet ports, and was forced past a heat exchanger and over the shoot by a small fan. Exhaust air was vented to waste. Two air streams were drawn for gas analysis; one sample for reference was taken from the air line before the mass flow controller, the other for analysis, came from inside the chamber. These streams were led to an ADC Series 225 infra red gas analyser (Analytical Development Company, Hoddesdon, England), using PVC ('Tygon') tubing at a maximum flow rate of 500 $ml\ min^{-1}$. The analyser was operated in differential mode and was calibrated daily using gas from a compressed air cylinder. Air from the cylinder was, in turn, calibrated by comparison with concentrations of CO_2 in N_2 generated by two cascading Wösthoff gas mixing pumps (Wösthoff, Bochum, Hagenstasse).

Continuous recording of the analyser output by a chart recorder (Omniscribe, Houston Instruments, Austin, Texas) permitted calculation of gas exchange rates. Air flow rates through the chamber were adjusted to allow a maximum depletion of 20 $\mu l\ CO_2\ l^{-1}$. Air temperature within the chamber was monitored continuously with copper-constantan thermocouples placed under the upper and lower leaves, and was maintained by circulating water from a thermostatically controlled water bath, through the heat exchanger. Dewpoint temperature measurements taken during the light period were close to 15°C. The air temperature was 20°C.

LEAF GAS EXCHANGE - INFRA RED GAS ANALYSIS

A small brass chamber (10 cm x 5 cm x 1.5 cm) with a glass lid was used for leaf carbon exchange measurement.

Air was supplied from a compressed cylinder and was humidified by bubbling through a tube containing 0.5 M HCl which was secured in a thermostatically controlled water bath set at 20°C. The air was then divided into two streams, one passed to the chamber *via* a flow meter and then to the analysis tube of the

ADC gas analyser, whilst the other went to the reference tube of the analyser, also *via* a flow meter. The flow rate to the analyser was adjusted to 500 ml min^{-1}. The temperature inside the chamber was continuously monitored using copper-constantan thermocouples and was maintained by conditioning the incoming air, and by circulating water from a water bath, through the base of the chamber. Attached leaves were accommodated in the chamber by removing a small section of the rubber seal from between the lid and chamber top, inserting the leaf, and sealing the petiole with plasticine.

When this equipment was used to measure net carbon assimilation, additional temperature control was achieved by a small flowing water screen placed between the chamber and the light source. Irradiance was supplied by a 150W tungsten bulb which permitted 200 µmol m^{-2} s^{-1} incident on the upper inside face of the chamber. Air flow through the chamber was increased to one litre per minute.

LEAF GAS EXCHANGE - BY MANOMETRY

Gas exchange quotients (µl CO_2/µl O_2) were determined by conventional manometry (Umbreit *et al.* 1957). Paired leaflets were placed in separate flasks with KOH in the centre well (for O_2 uptake) or without KOH (for total gas exchange). Carbon efflux was calculated assuming O_2 uptake per unit fresh weight to be the same in the two vessels. Each measurement was replicated five times. Gas exchange was linear for at least 70 min, and rates of CO_2 exchange were similar to those measured using infra-red gas analysis. The bath temperature was 20°C.

PLANT ANALYSIS

Dry Weight

Dry weight was determined after drying material at 85°C in a force-draught oven, or after freeze-drying (-40°C, 0.013 kPA) for 48 hours.

Leaf Area

Leaf area was determined gravimetrically.

Chlorophyll

Chlorophyll *a* concentration was determined from the absorbtion of 80% (v/v) acetone extracts at 750, 663 and 645 nm according to Arnon (1949).

Carbon Content

Carbon content of dry plant tissue was estimated on ground material (1mm screen) using the wet oxidation technique of Walkley and Black (1958). This technique, adopted because of the simplicity of the measurement, has a tendency to under-

estimate the carbon content (Ho 1976). Ho suggested a correction factor of 1.04 - 1.07 for tomato, and the average value, 1.055, was adopted in the present study. The utility of the Walkley-Black method with correction, was proven by constructing a carbon balance using three small field bean plants. Of four comparably sized seedlings taken from the 20°C glasshouse, three were dried and weighed to estimate the initial dry weight of the fourth, which was placed in an assimilation chamber and irradiated for 12 hours per day. For 20 days, the daily carbon increment was calculated and accumulated to give an estimate of the final dry weight in terms of CO_2 units. The plant was then dried, weighed and the carbon content determined using the corrected Walkley-Black wet oxidation method. The experiment was repeated three times in total. The final dry weight, when converted to CO_2 units, agreed with the final weight determined by gas exchange to within 5%.

Nitrogen Content

Total nitrogen content and organic nitrogen were determined in whole plant studies (Chapters IV and V). Total nitrogen was determined by a micro kjeldahl method after pre-treating a powdered sample overnight with 30% (w/v) salicylic acid - sulphuric acid mixture (Eastin 1978). Recovery of added nitrate was better than 98%. Hippuric acid was used as the standard.

Ammonia in the digest was measured using either an automatic analysing system (Technicon Autoanalyser, Technicon, New York) which employed a colourimetric reaction (the formation of a blue indophenol complex following reaction of ammonia with sodium phenate and sodium hypochlorite), or by steam distillation into boric acid, and titration against potassium hydrogen di-iodate.

Nitrate, in ground plant tissue, was estimated potentiometrically after extraction into aluminium sulphate solution (Baker and Smith 1969). The method employed a nitrate electrode (Orion model 93-07) and double junction reference electrode (Orion model 90-02) which were coupled to an Orion Research model 701A digital meter (Orion Research, Massachusetts).

Organic nitrogen was calculated from the difference between total nitrogen and nitrate nitrogen, and was converted to crude protein by the factor 6.25.

Protein Assay

The Lowry assay (Lowry *et al.* 1951) was employed for protein determination in the two single leaf experiments (referred to as experiment one and experiment two in the text). However, different attributes of leaf protein were measured in each experiment.

methods used in experiment one

All protein extractions were carried out on ice and at temperatures less than 4°C during centrifugation.

soluble protein

Powdered freeze-dried leaf material (10 mg) was ground with sand in a mortar containing 2 ml of grinding buffer. The grinding buffer consisted of 0.2M Tris-HCl, 2.5 mM Na EDTA, 5.0 mM mercaptoethanol and 6.0 mM $MgCl_2$, pH 8.0. The suspension was filtered through nylon cloth and the filtrate centrifuged for 20 minutes at 25,000 x g. Following centrifugation, 0.1 ml of the upper phase was added to 3.9 ml water and 1.0 ml 60% (w/v) trichloroacetic acid (TCA). The sample was allowed to stand for 30 min before centrifugation as above. The supernatant was decanted, the centrifuge tube inverted upon absorbent paper to remove all solution and the precipitate dissolved in 0.4 ml 0.5M NaOH. From this solution, 0.2 ml was assayed for soluble protein (in duplicate) according to Lowry *et al.* (1951) with Bovine Serum Albumen (B.S.A. Sigma A4378) dissolved in 0.5M NaOH as the standard.

insoluble protein

Following the first centrifugation the upper liquid phase was decanted, and the solid residue was resuspended in 1.0M NaOH and heated in a boiling water bath for 90 min. The tubes were then removed from the water bath, cooled, and 0.2 ml assayed for insoluble protein (in duplicate) as before except that the standard was BSA dissolved in 1.0M NaOH.

total protein

Total protein was calculated as the sum of soluble and insoluble protein.

RuBPC

Ribulose 1, 5-bisphosphate carboxylase/oxygenase (RuBPC) was purified according to the method of Thomas and Huffaker (1981) and quantified using the procedure of rocket immunoelectrophoresis (Weeke 1973).

purification

Fresh field bean leaves were ground with sand using mortar and pestle in grinding buffer (described under 'Soluble Protein' measurement) in the ratio 10 ml per gram fresh weight. The buffer also contained 0.5% BSA and 1% polyvinylpyrrolidone. The homogenate was filtered through nylon cloth, centrifuged (25,000 x g for 15 min), the upper phase brought to 35% saturation with ammonium sulphate and then

centrifuged again. The upper phase was then brought to 60% saturation with ammonium sulphate. The precipitate, which was recovered by centrifugation, was dissolved in 10 ml 0.05M HEPES buffer pH 7.8 containing 2.5 mM EDTA, 5.0 mM mercaptoethanol and 6.0 mM $MgCl_2$, and loaded onto a 50 cm x 3 cm column of Sepharose 6B which had previously been equilibrated with column buffer (0.02M HEPES, 1.0 mM EDTA, 1.0 mM mercaptoethanol and 3.0 mM $MgCl_2$, pH 7.8). Fractions containing the enzyme were qualitatively determined by rocket immunoelectrophoresis, pooled, and applied to a 30 cm x 2 cm column of DEAE cellulose (Whatman DE 32) equilibrated with column buffer. The column was eluted with a 400 ml gradient of 2.5 - 200 mM ammonium sulphate in column buffer. Fractions containing the enzyme were pooled, brought to 60% saturation with ammonium sulphate and frozen at -15°C or dipped into liquid nitrogen and stored frozen in vials at -60°C.

Purity of the preparation was verified by the observation of the two bands characteristic of the large and small subunits following sodium dodecylsulphate-polyacrylamide gel electrophoresis. Enzyme concentration was determined by the Lowry method following precipitation in 12% TCA.

antibody production

Purified enzyme solution (1.5 ml) was added to 1.5 ml of Freunds' complete adjuvant, and 0.5 ml of the mixture was injected intramuscularly into the hind legs of each of three white rabbits (supplied by the Central Animal House of the Waite Agricultural Research Institute). After two and four weeks the rabbits were injected again, except that the enzyme was mixed with vegetable oil and the injection was subcutaneous. Five weeks after the initial innoculation, the rabbits were bled from an incision in a major vein of the ear. The blood was allowed to congeal, the serum decanted and allowed to congeal again. Following the second decantation, the serum was centrifuged, the upper (serum) phase removed and dispensed into vials, and stored at -15°C.

rocket immunoelectrophoresis

Slab gels (Agarose A, Pharmacia) were made by heating aliquats of stock gel ((1% v/v) in Tris-barbitone buffer) until dissolved, cooling to 50°C and adding 100 µl of rabbit serum. The solution was carefully mixed and poured into a mould (which measured 70 mm x 70 mm x 1 mm) and allowed to solidify. After removal from the mould twelve 3 mm diameter wells were punched from the gel, five wells were used for standards whilst seven were used for samples. (The Tris-barbitone buffer consisted of 0.1M Tris-HCl, 0.1M sodium barbitone and 0.02% (w/v) sodium azide, pH 8.6, which was diluted in the proportion 30 ml buffer to 70 ml water for use). The wells were loaded with 2 µl of purified enzyme diluted in Tris-barbitone buffer or 2 µl of sample taken from the supernatant (previously assayed for soluble protein),and immediately subjected to electrophoresis.

Electrophoresis was accomplished by placing two slab gel plates inside a two compartment perspex box containing Tris -barbitone buffer. Filter paper wicks connected the gel to the buffer at each end, and the complete unit was run within a refrigerator at 133V for 10 hours. After electrophoresis the gels were soaked in 0.9% (w/v) NaCl for four hours, rinsed with water, covered with filter paper, soaked in absolute ethanol and dried in an oven at 70°C for one hour. The gels were then stained with Coomassie Blue (1.25 g Coomassie Brilliant Blue R-250 (Sigma), 454 ml 50% (v/v) ethanol, 46 ml glacial acetic acid, filtered through Whatman No.1 filter paper before use) for one hour, destained (75 ml glacial acetic acid, 925 ml 5.4% (v/v) ethanol) for 30 min and dried.

The concentration of RuBPC in the samples were obtained by reference to the peak heights of the standards.

methods used in experiment two

Total protein only was determined from 10 mg of powdered, freeze-dried leaf material which was first clarified of chlorophyll by extraction overnight in 6 ml 80% (v/v) acetone at 4°C. (Eze and Dumbroff 1982). The sample was then centrifuged, the acetone-chlorophyll mixture decanted, and the colourless residue was suspended in 10 ml 0.1M NaOH containing 1% (w/v) SDS (BDH Chemicals, Dungey and Davies 1982). Protein was solubilised overnight (12h) at 80°C in pyrex tubes capped with glass marbles. The solution was then cooled, thoroughly mixed, allowed to settle (in a 30°C water bath) and 0.1 ml was taken for protein assay (in triplicate) according to Lowry *et al.* (1951). The standard was BSA dissolved in 0.1M NaOH and 1% (w/v) SDS.

Starch Measurement

This method was adapted from Haslemore and Roughan (1976), Haissig and Dickson (1979), and Clegg (1956).

sample preparation

Ground freeze-dried tissue (10 mg) was freed of protein and soluble sugars by three separate extractions in 5 ml 80% (v/v) ethanol for one hour at 80°C. Between extractions, samples were centrifuged and the ethanol supernatant decanted and pooled for analysis of soluble carbohydrate at a later time. The decolourised residue was oven dried overnight, wetted with 1 ml 80% (v/v) ethanol, then 2 ml water was added, the tubes capped with glass marbles and placed in a boiling water bath for one hour to gelatinise the starch. The tubes were then cooled and 1 ml 0.2M acetate buffer (pH 4.5) added, followed by 0.1 ml purified amyloglucosidase, the tubes capped with glass marbles and incubated for 24 hours at 55°C. Samples were then diluted to 10 ml with water, mixed, and 0.5 ml taken for glucose assay (in duplicate).

standard preparation

Soluble starch standards were run with the samples. Standard (2 mg ml^{-1}) was prepared by ethanol extraction of soluble starch (BDH Chemicals) as above. The starch was dried at 60°C, ground to a powder with a mortar and pestle, and 100 mg was dissolved in 20 ml water and heated in a boiling water bath until translucent. The solution was then cooled and diluted to 50 ml with water. Standards (containing acetate buffer) were prepared to give 0 - 40 µg starch in a final volume of 0.5 ml. Preliminary tests revealed 100% starch recovery from standards after six hours incubation with amyloglucosidase.

amyloglucosidase preparation

Amyloglucosidase from *Aspergillus niger* (BDH Chemicals) was freed of reducing sugars and coloureds by diluting 20 ml with an equal volume of water and subjecting the solution to a series of acetone precipitations at -15°C until the final precipitate (collected by centrifugation) was colourless. The precipitate was dissolved in minimal water and freeze-dried (-40°C, 0.013 kPa) for 24 hours. The power was stored dessicated at -15°C. Active preparation was obtained by dissolving 15 mg in 25 mM sodium citrate (pH 6.0) and storing at 4°C.

glucose assay

Glucose concentrations were estimated by mixing 0.5 ml sample, or 0.5 ml of glucose standard containing 0 - 40 µg glucose in dilute acetate buffer, with 4 ml Sigma enzyme-colour reagent and incubating the mixture in tubes capped with glass marbles at 37°C for 30 min. The enzyme-colour reagent contained glucose oxidase, peroxidase and o-dianisidine dihydrochloride (Sigma Chemical Co., St. Louis, MO, Technical Bulletin 510 (1-78). The absorbance at 450 nm was read within an additional 30 min and the glucose concentration in the samples was calculated from the linear regression derived from the standard curve. The calculated glucose concentration was corrected by the factor 0.95 which arises because 0.95 g starch yields 1.0 g glucose upon hydrolysis.

Soluble Carbohydrate Measurement

Pooled ethanol extracts from the starch analysis were reduced to a final volume of 4.5 ml by evaporation in a 90°C water bath. Samples were then cooled, 0.5 ml 10% (w/v) neutral lead acetate added, and stored overnight at 4°C. Next day the samples were centrifuged and 0.1 ml of the upper phase was taken (in triplicate) for analysis by the phenol sulphuric acid technique (Dubois *et al.* 1956). A sucrose standard (80 mg ml^{-1}) was diluted in 80% (v/v) ethanol and lead acetate to give 0 - 40 µg sucrose in a final volume of 0.1 ml. Sucrose concentrations in samples were calculated from the regression equation fitted to the standards.

CHAPTER IV INTERRELATIONSHIPS BETWEEN RESPIRATION IN PROLONGED DARKNESS, DRY WEIGHT AND NITROGEN CONTENT

INTRODUCTION

As described in Chapter II, McCree (1970) found the rate of CO_2 efflux from white clover plants after about 48 hours in the dark, to be proportional to the plant dry weight. This rate of efflux, when incorporated into a two component equation for dark respiration, was subsequently interpreted as describing the intensity of maintenance processes (Thornley 1970).

The maintenance requirement can be considered to arise because of the occurrence of protein degradation and the necessity to maintain ion concentrations across cell membranes at specified levels (Penning de Vries 1975a). However, it is difficult to reconcile this view with the relationship between respiration and dry weight noted above. It is more likely therefore, that respiration is related to some attribute of the dry weight, such as the protein content of the tissue, but may be so for two different reasons. The first of these may be the catalytic activity of the enzymatic proteins. The second is that respiration provides energy to maintain the level of protein. This latter hypothesis is examined in the present chapter by placing plants in the dark for 48 hours and measuring the degree of association between the rate of CO_2 efflux at this time and the nitrogen content (total nitrogen, nitrate nitrogen and organic nitrogen) of the plant tissue.

When respiration measurement on whole plants had been completed, the roots were separated from the shoots and the specific rates of root and shoot respiration determined separately using several plants. This treatment was prompted by the finding that the specific root respiration rate was higher than the shoot respiration rate in several species of *Senecio* (Lambers 1979). Lambers explained this phenomenon as due to the presence of wasteful (alternative pathway) respiration in the roots. However, Lambers (1980) showed that the alternative pathway became progressively less active over a 16 hour night, which implies that after 48 hours of darkness, the pathway should be inactive. Therefore, if the alternative pathway is inactive the specific rates of root and shoot respiration should be similar after 48 hours of darkness. The objective of this part of the study was to test this hypothesis.

METHODS

Plants were grown in the 20°C glasshouse and subsequently transferred to the assimilation chamber in the laboratory (for details refer to Chapter III) and exposed to two days of high irradiance (*ca* 700 μmol m^{-2} s^{-1}) before being left in the dark until the CO_2 exchange rate declined to an asymptotic value (about 48 hours).

This steady state rate of efflux was taken to be the maintenance respiration rate for whole plants. This rate was noted and then the roots were severed from the shoots of several plants and the CO_2 exchange rate measured separately on each part in order to determine the relative contribution of the root and shoot to the total efflux.

When these measurements of carbon exchange had been completed root material was washed free of rooting medium and together with the shoot material was oven dried, weighed, ground through a 1 mm sieve, and analysed for total nitrogen, organic nitrogen and nitrate as described in Chapter III. Rates of carbon exchange were calculated on a daily (24 hour) basis.

RESULTS

Whole Plant Studies

A relationship between starvation respiration rate and dry weight held for each of the species tested (field bean, pea, chickpea, lucerne and tobacco), and furthermore, the relationship was common to all the species (figure 4.1). In addition, when plant respiration rate was plotted against the organic nitrogen content of the tissue,a common, direct relationship was found (figure 4.2). A strong relationship was also apparent when total nitrogen content was taken as the dependent variable, and in this instance, the regression was:

$$Y = 132.3\ X - 1.8 \qquad r^2 = 0.78, \quad n = 50$$

where: Y = rate of carbon efflux after about 48 hours dark (mgC $24h^{-1}$ $plant^{-1}$)

X = total nitrogen content (mg N $plant^{-1}$)

Repiration was also related to nitrate content but the variance explained was comparatively low ($r^2 = 0.23$, $n = 48$).

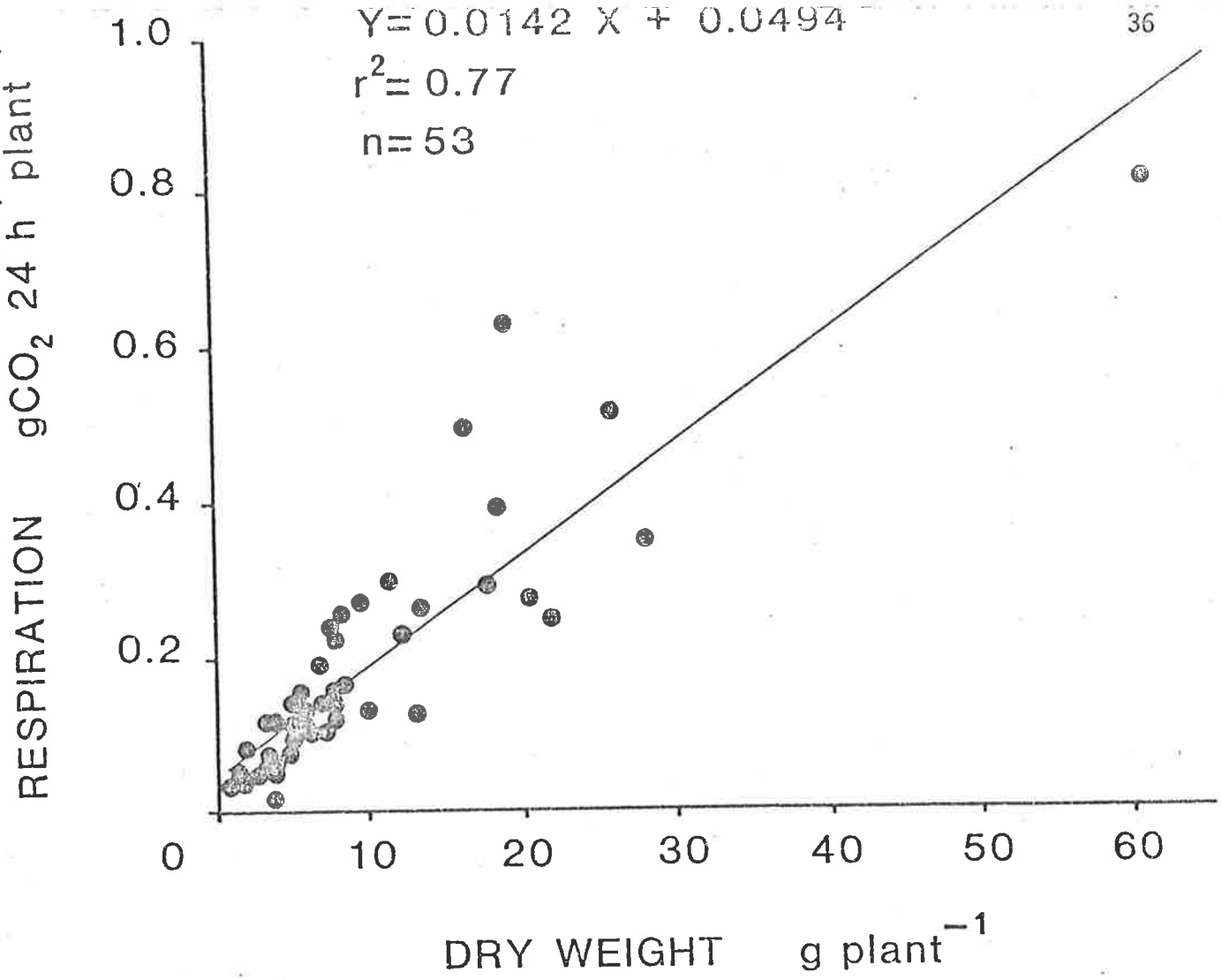

FIGURE 4.1. The relationship between the respiration rate after 48 hours darkness and plant dry weight. Data for field bean, pea, chickpea, lucerne and tobacco are fitted to a common regression line.

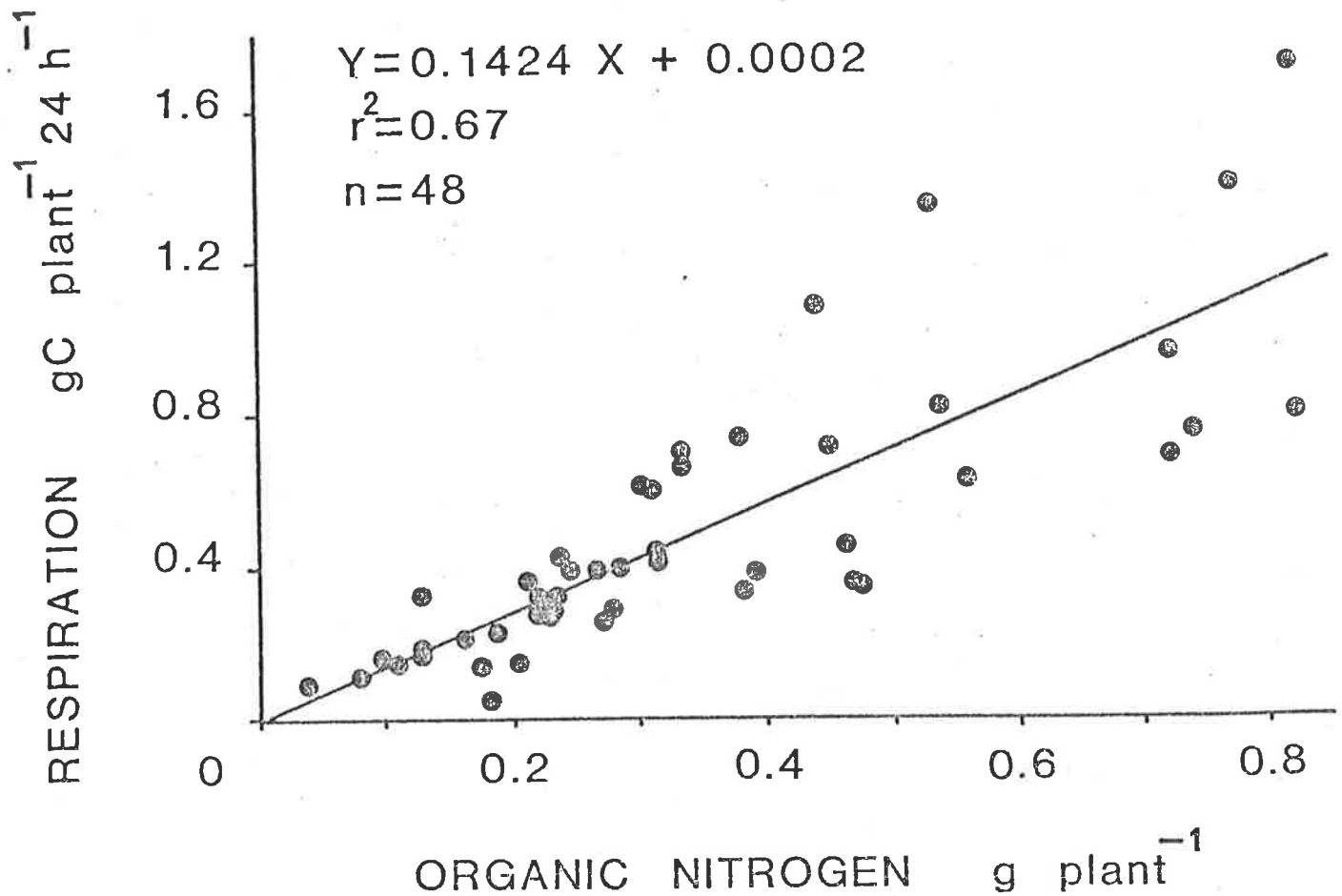

FIGURE 4.2. The relationship between the respiration rate after 48 hours darkness and organic nitrogen content of whole plants. Plant species used are indicated in the legend to figure 4.1.

root and shoot respiration in prolonged darkness

Of 15 observations made, the specific rate of respiration was greater in the root than the shoot on 10 occasions (table 4.1). The pot and rooting medium (following root removal) were replaced in the gas exchange chamber to assess the contribution of microorganisms within the rooting medium to root respiration, but no CO_2 exchange was detected at the flow rates used in these experiments.

TABLE 4.1. Comparison between specific rates of root and shoot maintenance respiration estimated for fieldbean, tobacco, pea and chickpea. Results were obtained after plants had been kept in the dark for between 48 and 60 hours. Units are $mgCO_2$ (g dry weight)$^{-1}$ $24h^{-1}$. † indicates data where shoot maintenance is greater than root maintenance respiration.

Species	Root maintenance		Shoot maintenance	
Field bean	37.08		18.14	
	26.01		8.04	
	16.04		16.69†	
	38.30		27.43	
	43.34		28.63	
	33.73		20.97	
	13.71	mean : 29.74	15.34†	mean : 19.32
Tobacco	47.96		6.48	
	44.46		21.24	
	31.53		14.98	
	19.16	mean : 35.78	22.79†	mean : 18.25
Pea	18.19		19.17†	
	65.97	mean : 42.08	26.91	mean : 23.04
Chickpea	42.45		45.22†	
	48.25	mean : 45.35	32.47	mean : 38.85

nitrogen concentration in roots and shoots

Concentrations of nitrogen components within the root and shoot fractions are shown in table 4.2. Total nitrogen concentrations were similar in root and shoot compartments of the legumes, but in the roots a large proportion of the total nitrogen was in the form of nitrate. Nitrate concentration was very much lower in the shoots. In contrast, shoot total nitrogen was higher than root total nitrogen in tobacco plants, and nitrate concentration was higher in the shoot than the root. The distribution of nitrate ions within the plants probably reflects the major sites of nitrate reduction.

TABLE 4.2. Concentrations of total nitrogen, organic nitrogen and nitrate nitrogen (% dry weight) in roots and shoots of legume species and tobacco plants kept in darkness for between 48 and 60 hours. Values are means together with the standard error.

Species	Total N		Organic N		Nitrate N	
	root	shoot	root	shoot	root	shoot
Legumes	4.18 ± 0.19	4.50 ± 0.17	3.04 ± 0.22	4.03 ± 0.14	1.14 ± 0.10	0.48 ± 0.10
Tobacco	2.91 ± 0.12	3.72 ± 0.24	2.62 ± 0.11	3.05 ± 0.19	0.29 ± 0.07	0.67 ± 0.13

DISCUSSION

The respiration rate of whole field bean, chickpea, pea, lucerne and tobacco plants, measured between 48 and 60 hours of darkness, was highly correlated with the dry weight of tissue, as found by McCree (1970) for white clover.

If the strength of this relationship (as indicated by the value of the coefficient of determination) is the sole criterion on which to base conclusions, then the dry weight determines the rate of starved (maintenance) respiration. However, correlations do not establish cause and effect, and it is difficult to comprehend why respiration should be related to the dry weight, since the bulk of the tissue is in the form of carbon compounds (e.g. carbohydrates, cellulose, lignin) which require little, if any maintenance.

As suggested in the Introduction, it is probable that maintenance respiration is related to the protein content of the tissue. The close correlation between respiration and crude protein content (organic N x 6.25) is evidence for this conclusion, particularly since the ordinate intercept (figure 4.2) is close to zero. However, in order to answer the question posed in the Introduction concerning the cause of the relationship between respiration and protein, two further points need attention. First, a proportion of the total leaf protein is in the form of RuBPC (Huffaker 1982) which is a photosynthetic - and not a respiratory, enzyme. Second, the shoot constitutes the greater part of the total dry matter (around 75% of the total for all species in these experiments). This means that a large proportion of total shoot protein comprises RuBPC. Collectively, all of the above evidence strongly support the view that respiration provides energy for protein maintenance rather than the view that respiration was dependent on the protein content because of the catalytic activity of the protein. However, the fact that the slope in figure 4.2 is not unity, and that respiration is highly correlated with the total nitrogen content and is also correlated with nitrate-nitrogen content, suggests that respiration for the maintenance of ion concentrations has a confounding influence on the relationship between the maintenance respiration rate and the organic nitrogen content. During the dark, when nitrate reduction in the shoot is unlikely to be substantial (Aslam *et al.* 1979), nitrate may have an important influence on respiration because the ions are usually concentrated within a storage (vacuole) compartment (Aslam *et al.* 1976; Shaner and Boyer 1976) and are likely to require energy expenditure to maintain that concentration gradient, since appreciable leakage can occur (Jackson *et al.* 1976). When the magnitude of the regression coefficient for the relationships between respiration and organic nitrogen (142.4 $mgC_{\Lambda}^{24h^{-1}}(mg\ N)^{-1}$) and total nitrogen (132.3 $mgC_{\Lambda}^{24h^{-1}}(mg\ N)^{-1}$) are compared, it is clear that the maintenance of protein is more expensive than is the maintenance of a mixture of protein and nitrate containing the equivalent nitrogen concentration. This calculation suggests, but does not prove, that during starvation

the major component of maintenance is concerned with protein turnover. The element of doubt arises because these results apply to whole plants, and it is likely that the relative intensity of maintenance processes in roots and shoots differ. For example, since roots are continuously exposed to nutrient solution, maintenance of ion concentrations may be higher in the roots than in the shoots.

Upon examination of the root and shoot nitrogen data for legumes (table 4.2), it was tempting to speculate (with the assumption that the alternative pathway is inactive), that energy expenditure to maintain ion concentions in roots was the cause of the high specific rate of root respiration (table 4.1). However, the specific rate of root respiration was also generally higher than the shoot respiration rate in tobacco, despite the fact that the nitrate concentration was lower in the roots. This information, together with the observation that organic nitrogen (i.e. protein) concentration was also lower in roots than in shoots of tobacco, suggests that the high rate of root respiration may be explained by a faster turnover of protein in the roots. Therefore, a comparison was made of calculated protein turnover rates for whole plants (data from the regression in figure 4.2) and for mature field bean leaves (data from Chapter VI). The turnover rates (which are the maximum potential turnover rates) were calculated as follows: assuming that the total CO_2 efflux after about 48 hours of darkness represents carbon derived solely from protein, the average respiration rate calculated from the regression in figure 4.2 (142.4 mgC g N^{-1} $24h^{-1}$), and the protein-specific respiration rate in Chapter VI, can be computed to a loss of protein, with the knowledge that one gram of protein contains 0.544 g carbon (Challa 1976) and that one gram of nitrogen is equivalent to 6.25 g protein. The turnover rates so calculated were 4.1% $24h^{-1}$ for whole plants, and 5.0% $24h^{-1}$ for mature leaves. These data do not support the hypothesis, but the idea should not be totally disregarded, since whilst this calculation may enable an estimate of the maximum turnover rate, it does not impart any information on the *in vivo* turnover rate.

Using data from tables 4.1 and 4.2, the rate of shoot protein turnover was calculated to be 3.8% d^{-1}, whilst the rate for root protein turnover was 7.8% d^{-1}. These data support the hypothesis.

An alternative explanation which may account for the high specific root respiration rates arises from processes associated with root nitrate reduction. Nitrate reduction is very slow (Aslam *et al.* 1979) or absent (Naik *et al.* 1982) in the shoot during the dark. There is little reason to doubt that this also holds when a prolonged dark treatment is imposed, since transpiration and therefore nitrate transport to the shoot, would be negligible. Therefore, nitrate reduction-linked malate synthesis in the shoot, and decarboxylation in the root (Raven and Smith 1976) is unlikely to contribute significantly to the CO_2 efflux. However, it is possible for root nitrate reduction to proceed, at the expense of stored carbohydrate, because nitrate is continually supplied in the nutrient solution. If root nitrate reduction occurs, CO_2 efflux could arise, in part, from the operation of the oxidative pentose phosphate pathway, which is the probable source

of reducing equivalents for the enzyme nitrite reductase, located in the root plastids (Emes and Flower 1979). Measurement of the root gas exchange quotient could be instructive, since if the value of the quotient was about unity, the oxidative pentose phosphate pathway would be implicated, but in addition, organic nitrogen accretion would also have to be proven. Unfortunately, such measurements were not made in the present study.

One further reason for the lower specific shoot respiration rate under these experimental conditions which has been previously overlooked here and elsewhere may be attributable to the amount of stem tissue. Stem tissue can constitute a considerable proportion of the total shoot biomass, but is likely to respire at a very lower specific rate than is the leaf tissue. The effect of the stem tissue may therefore be to contribute to an overall reduction in the specific rate of shoot respiration.

CONCLUSIONS

The rate of CO_2 efflux after a prolonged dark treatment is related to the protein content of the tissue, but this efflux is likely to contain a component of respiration associated with the maintenance of ionic concentrations in the plant, in addition to a component which supplies energy to maintain the tissue protein content.

The alternative respiratory pathway is assumed not to contribute to respiration after 48 hours dark, but the specific root respiration rate is still generally higher than the shoot specific rate, and this could be due to the operation of the oxidative pentose phosphate pathway in the roots, possibly for nitrate reduction or to protein turnover being faster in roots than in shoots.

Both of these points highlight the necessity to quantify the intensity of the individual maintenance processes.

CHAPTER V APPLICATION OF THE CONCEPT OF GROWTH AND MAINTENANCE TO CROP PLANTS

INTRODUCTION

In the present chapter, three methods of apportioning CO_2 exchange into growth and maintenance components are compared. The three methods are: (i) allowing the CO_2 efflux to decay in prolonged darkness to an asymptotic value which is taken to be the maintenance value; (ii) using the 'y'-axis intercept from a plot of dark CO_2 efflux as a function of net CO_2 uptake determined over a range of irradiances i.e. when the net CO_2 uptake is zero; (iii) using the 'y'-axis intercept of a plot of total CO_2 uptake as a function of the growth rate i.e. when the growth rate is zero.

Due to the simplicity of measurement, the starvation method (method (i)), has been extensively employed to estimate maintenance respiration (e.g. McCree 1970, 1974; McCree and Silsbury 1978; Jones *et al.* 1978; Silsbury 1979, Chapter IV of this thesis). McCree and Silsbury (1978) have previously shown that the starvation method and the dynamic method (method (ii)), give essentially equivalent results for the maintenance coefficient in subterranean clover. However, the starvation method can be criticised on the grounds that a prolonged dark treatment could result in CO_2 fluxes which are not a normal feature of plant growth under average growing conditions (Thornley 1977, Robson and Parsons 1981). For this reason, it may be more satisfying to estimate the intensity of maintenance processes whilst the plant experiences 'normal' light and dark cycles.

The objectives therefore were to examine the variability both between and within each method, and to assess the extent to which the values from each technique represents the 'true' maintenance value.

METHODS

Growth and maintenance coefficients were determined from the relationship between daytime carbon assimilation and nighttime carbon efflux. Whole plants (field bean, lucerne and chickpea) 10 - 50 days after emergence were individually removed from the temperature controlled glasshouse and placed in the assimilation chamber (see Chapter III for details) and subjected to an irradiance level of 700 μmol m^{-2} s^{-1} and temperature of 20°C for three days to establish steady state carbon production and consumption. The irradiance was then progressively reduced each day for four days by placement of a series of nylon mesh screens over the chamber. The lowest irradiance level experienced by the plant was about 100 μmol m^{-2} s^{-1}. Subsequently, the irradiance was gradually increased to the original level by daily removal of the nylon screens. The plant was kept for two days at

the highest irradiance (700 μmol m^{-2} s^{-1}) before the chamber was darkened with light-proof cloth and the gas exchange was traced until the rate of efflux became constant, which was usually between 48 and 60 hours of darkness.

Additional plant species (pea, kidney bean and tobacco) were used to determine maintenance requirements according to the steady state method only (described below) and these plants did not experience the variable light regime. Instead, following removal from the glasshouse, individual plants were placed in the assimilation chamber (under the same conditions as described above) for three days to establish steady state carbon production and consumption, and then the chamber was darkened until the rate of CO_2 efflux became constant.

The growth efficiency (Y_G) was calculated from the growth coefficients ('g' and 'k') which were determined from the dynamic and steady state analyses (described below) for comparative purposes, and also for the reason that Y_G has a biochemical interpretation. Rates of maintenance respiration derived from the dynamic and steady state analyses were converted into daily (24 hour) rates in order to give them the same units as the maintenance coefficient 'm' derived from the gross uptake method.

Calculation of Growth and Maintenance Coefficients by the Dynamic Method

Values for net carbon uptake during the light (D) and carbon efflux the following night (N) obtained by varying the daily irradiance level (as above), were used to calculate the growth and maintenance coefficients by linear regression according to the dynamic method of McCree and Silsbury (1978):

$$N = gD + h$$

where: N = integrated nighttime carbon efflux (g CO_2 $12h^{-1}$ $plant^{-1}$)
D = integrated daytime carbon influx (g CO_2 $12h^{-1}$ $plant^{-1}$)
g,h are constants

Following the method of McCree and Silsbury (1978) 'g' is a function of the growth efficiency (Y_G) where:

$$Y_G = \frac{1 - g}{1 + g} \text{ g } CO_2 \text{ (g } CO_2)^{-1}$$

and 'h' is the maintenance efflux. The maintenance coefficient 'b' was calculated:

$$b = \frac{2h}{W} \text{ mg } CO_2 \text{ (g } CO_2)^{-1} \ 24h^{-1}$$

where 'W' is the dry weight in CO_2 units.

Carbon exchange was represented on a whole plant basis rather than as specific rates, since leaf expansion was generally slow under the experimental conditions and the relationship between D and N essentially linear (the degree of association between D and N that was required before subjecting the data to analysis, was arbitrarily set such that $r^2 > 0.70$). The dry weight was required in order to calculate the specific rate of maintenance respiration, and was estimated when D was approximately zero, by back-calculation from the final biomass and the sum of the daily rates of synthesis.

Calculation of Growth and Maintenance Coefficients by the Steady State Method.

The Steady State method required that measurement of net CO_2 uptake (D) and CO_2 efflux (N) was made at two time periods, during and immediately following a day of high irradiance (700 μmol m^{-2} s^{-1}), and after 48 hours in continuous darkness when the efflux had decayed to a constant value. At this latter time period carbon uptake was taken to be equal but opposite in sign to the efflux (i.e. -Dm). This efflux is denoted as Nm.

The growth and maintenance coefficients were calculated by the method of McCree and Silsbury (1978):

$$k = \frac{N - Nm}{D - (-D_m)} \text{ g } CO_2 \text{ (g } CO_2)^{-1}$$

Since Dm = Nm, k was calculated:

$$k = \frac{N - Nm}{D + Nm}$$

where: N = integrated nighttime efflux (g CO_2 $12h^{-1}$ $plant^{-1}$)
D = integrated daytime influx (g CO_2 $12h^{-1}$ $plant^{-1}$)
Nm = 12 hour rate of CO_2 efflux after about 48 hours dark (g CO_2 $12h^{-1}$ $plant^{-1}$)
k = constant

The growth efficiency (Y_G) was calculated:

$$Y_G = \frac{1 - k}{1 + k} \text{ g } CO_2 \text{ (g } CO_2)^{-1}$$

The maintenance respiration rate was taken to be the value of the term (1 + k)cW, where Nm = cW, and the maintenance coefficient 'c' was calculated:

$$c = \frac{2\ Nm}{W} \text{ mg } CO_2 \text{ (g } CO_2)^{-1}\ 24h^{-1}$$

where 'W' is the dry weight in CO_2 units.

Calculation of Growth and Maintenance Coefficients by the Gross Uptake Method

If the input of substrate carbon (ΔS) is calculated as the sum of D and N and the growth rate as the difference between the two (ΔW), then the coefficients can be determined by linear regression using the Thornley balance equation (Thornley 1976), so-called, the 'gross uptake method'. The balance equation is written:

$$\Delta S = {}^{1}/_{Y_G} \Delta W + \Delta Sm$$

where:

ΔS	=	daily input of substrate carbon	(g CO_2 $24h^{-1}$ $plant^{-1}$)
ΔW	=	daily growth rate	(g CO_2 $24h^{-1}$ $plant^{-1}$)
Y_G	=	growth efficiency	(g CO_2 (g CO_2)$^{-1}$)
ΔSm	=	maintenance respiration rate	(g CO_2 $24h^{-1}$ $plant^{-1}$)

The value for the growth efficiency (Y_G) was determined directly from the slope of the regression line, whilst the maintenance coefficient 'm' was calculated:

$$m = \frac{\Delta Sm}{W} \quad \text{mg } CO_2 \text{ (g } CO_2)^{-1} \ 24h^{-1}$$

where 'W' is the dry weight in CO_2 units, and was determined when $\Delta W = 0$, by back-calculation as described for the dynamic method.

RESULTS

The Maintenance Coefficient

The three methods gave differing estimates of the maintenance coefficient (table 5.1). An analysis of variance on the coefficients expressed on a carbon basis (the coefficients calculated on a dry weight basis are included for comparative purposes) revealed: (i) within the field bean and lucerne data, there was a significant effect (at the 5% and 1% level respectively) of the method of calculation, and in both species the coefficient determined by the gross uptake method ('m') was significantly higher than the other coefficients; (ii) maintenance coefficients for chickpea were not significantly different, although the mean value for 'm' was considerably higher than the mean values of the other coefficients; (iii) species effects on maintenance coefficients were not significant, regardless of the method of calculation; and (iv) within the steady state data, coefficients calculated for kidney bean and tobacco were significantly lower (at the 1% level) than the coefficients determined for the other species.

TABLE 5.1. Estimates of maintenance coefficients for several species obtained using the three methods described in the 'Methods' Section. All values were obtained at 20°C. Tabulated values are means together with the standard error, and coefficients determined from each method (for field bean, lucerne and chickpea) are presented in two columns. Data in column (i) are coefficients expressed per unit CO_2 equivalent of the dry weight (mg CO_2 (g CO_2)$^{-1}$ 24h^{-1}), whilst those in column (ii) are expressed per unit oven dry weight (mg CO_2 (g d.wt)$^{-1}$ 24h^{-1}). Data for pea, kidney bean and tobacco, were derived from the steady state analysis only.

Species	Steady state '2c'		Dynamic '2b'		Gross uptake 'm'	
	(i)	(ii)	(i)	(ii)	(i)	(ii)
Field bean	16.38 ± 1.27	21.35 ± 1.41	18.37 ± 3.59	25.09 ± 4.94	28.66 ± 4.65	38.69 ± 6.38
Lucerne	19.48 ± 1.55	24.11 ± 2.12	21.55 ± 1.80	25.79 ± 2.02	29.14 ± 2.03	35.82 ± 3.09
Chickpea	21.67 ± 1.61	28.65 ± 2.03	19.63 ± 3.87	25.54 ± 4.61	27.84 ± 3.96	36.30 ± 4.62
Pea	20.50 ± 1.99	26.53 ± 2.37				
Kidney bean	13.36 ± 1.30	16.51 ± 1.59				
Tobacco	14.59 ± 1.72	17.89 ± 1.93				

The Growth Efficiency

The data presented in table 5.2, show that all methods gave essentially the same result when efficiencies were compared within and between species. An analysis of variance confirmed this conclusion. The overall mean (± SE) was 0.69 ± 0.01.

TABLE 5.2. Estimates of the growth efficiency (Y_G) for fieldbean, lucerne and chickpea plants obtained using the three methods described in the Methods section. All values were determined at 20°C. Values are means together with the standard error.

Species	Steady state	Dynamic	Gross uptake
Fieldbean	0.70 ± 0.02	0.67 ± 0.02	0.72 ± 0.03
Lucerne	0.72 ± 0.03	0.69 ± 0.04	0.71 ± 0.03
Chickpea	0.68 ± 0.02	0.64 ± 0.01	0.66 ± 0.01

DISCUSSION

The Maintenance Coefficient

On the basis of the discussion of maintenance coefficients presented in Chapter II (figure 2.4), the geometrical relationship between the coefficients should permit a ranking in order of magnitude where m > 2b > 2c. This order was found in the case of field bean and lucerne but not in that of chickpea, because the data for this species were too variable. However, even in the latter, the mean value for 'm' was still considerably higher than the mean value for '2b' and '2c' (table 5.1).

Significant differences between the coefficients '2b' and '2c' were not apparent, although the coefficients should not have the same value since the rate of CO_2 efflux associated with 'b' was determined when D = 0, whereas the efflux associated with 'c' was determined when D < 0. For practical purposes it is concluded that '2b' and '2c' yield coefficients which are essentially equal in magnitude.

Whilst '2b' and '2c' were determined when the daily growth rate is negative, the coefficient 'm' was in contrast, determined when the growth rate was zero, and where D > 0, and this explains why 'm' was higher than the other coefficients. The question which must now be asked is which coefficient (if any) is the 'true' maintenance coefficient? In answering this question, it is logical to reconsider the definitions of maintenance enumerated in Chapter II, and to discuss the extent to which the various coefficients might satisfy the requirements of the definitions.

The coefficient 'm' seems consistent with the first definition which referred to the resynthesis of hydrolysed protein and the replacement of an abscised or dead leaf, as maintenance processes, since the replacement of an abscised leaf results in no net increase in the weight of biomass. McCree (1982) was able to slow the growth rate of white clover plants sufficiently, so that the growth rate was only slightly positive for a period of over 3 weeks. He is of the view that the coefficient 'm' represents the 'true' maintenance coefficient. However, the replacement of a dead leaf could also be construed as synthesis. Although the plant may be maintaining a constant weight of biomass, synthesis processes occur to produce new leaves. Should synthesis processes occur to replace degraded protein within the same leaf, then such synthetic activities should properly be termed maintenance. This interpretation can be extended to consider Thornley's (1977) model (figure 2.3). The model provides for breakdown of degradable structure (e.g. protein in a leaf), and the recycling of the products to storage, which could be a pool within the leaf or in another part of the plant. If the storage material does not supply substrate for resynthesis of the degraded structure in the same leaf, then that leaf will senesce. If substrate is supplied to the same leaf, then the leaf will be maintained. Leaf maintenance in this way, is implied in the model. However, in his application of the model, McCree (1982) included provision for senescence, and this situation opens up the possibility for breakdown products being recycled for the growth of new leaves (or other organs). Put more simply, McCree's analysis refers to the maintenance of a *constant weight* of biomass rather than maintenance of the *existing* biomass.

If carbon balance experiments are conducted using young plants in which senescence is not a complicating factor, then it could be argued that 'm' is an estimate of the true maintenance coefficient. An additional reason why the

magnitude of 'm' is greater than that of '2b' and '2c' could then be attributed to the fact that the latter coefficients were determined on the basis of nighttime CO_2 losses only and may therefore underestimate the true maintenance energy requirement. One could equally argue, perhaps, that 'm' is of greater magnitude because it consists of a component of wasteful (alternative pathway) respiration (Lambers *et al.* 1979). Unfortunately this possibility is largely hypothetical, and it has yet to be conclusively proven that alternative pathway respiration is a normal feature of plant respiration.

The coefficient '2b' may also contain a component of respiration associated with growth. Although '2b' is determined when the net CO_2 uptake in the light is zero, stored carbon could be used for growth during the following night. However, the decreasing irradiance regime of the dynamic test is expected to deplete the level of reserves such that little growth is expected. In determining the coefficient '2c' it is assumed that the prolonged dark period has exhausted the plant of readily accessible carbohydrate and that growth has ceased, in this respect it is not surprising that '2c' and '2b' are of similar magnitude.

It has already been stated that the coefficients '2b' and '2c' are derived when the growth rate is negative, but this need not be an argument to eliminate these coefficients from consideration as maintenance coefficients, since during a normal night the growth rate is also negative. It is therefore unfortunate that carbon biomass is taken as the measure of structure, because in addition to degradable and non-degradable structure, biomass carbon also includes stored carbohydrate which is the substrate for growth and energy production. Carbon loss through respiration, even in prolonged darkness, could come from the carbohydrate rather than the structure pool. Structural material should be the basis used to define the maintenance coefficient but this is a difficult unit to measure. Similar views have been expressed by Penning de Vries *et al.* (1979).

In his second definition (Chapter II), Penning de Vries (1975a) considers that maintenance refers to the CO_2 arising from protein breakdown plus CO_2 produced in respiratory processes that provide energy for the maintenance processes. This definition refers only to CO_2 evolution, which presumably is a result of the complete oxidation of protein (amino acid skeletons) and carbohydrate. The coefficients '2b' and '2c' appear to satisfy this definition, since they represent coefficients of which growth is probably not an important component.

It is clear that the magnitude of the coefficients largely depends on the assumptions made, most particularly in respect to which process are ascribed to maintenance and which to synthesis. There is currently no clearly defined concept of what maintenance really represents. If maintenance respiration is assumed to

represent respiration which provides energy to maintain the protein content and ion concentrations within each individual organ of a plant, then the coefficient '2c' most likely satisfies this assumption, providing that senescence is not a complicating factor in the measurement of the CO_2 exchange rate. Ideally, a maintenance coefficient should represent energy production for the maintenance of existing structure, and not maintenance of a constant weight, since a constant weight can be maintained despite the abscision of a dead leaf, and the growth of a new leaf.

The coefficient '2c' has the advantage of simplicity of measurement, but it should be recognised that the magnitude of this factor depends upon the length of the dark period, for the reason that it usually takes between 36 and 60 hours of darkness before the CO_2 exchange rate becomes constant. As the coefficient is determined in the dark it is likely to represent the minimum maintenance requirement. It is considered unlikely that energy costs for redistribution of materials from mature leaves to growing points would be of significance in the short term. Quite clearly, we are still not in the position to state categorically which is the 'true' maintenance coefficient.

Some of the reasons why the rate of CO_2 efflux in prolonged darkness reflects maintenance energy requirements, were discussed in Chapter IV, but there is some additional evidence which suggests that this rate of CO_2 efflux is related to the maintenance processes. For example, Silsbury (1979) showed that acetylene reduction (a measure of dinitrogen fixation) declined after subterranean clover plants had experienced 13-14 hours of darkness. Within 24 hours of darkness, the CO_2 efflux rate had attained an asymptotic value, and dinitrogen fixation had ceased. This evidence suggests that dinitrogen fixation is a growth process and that the rate of CO_2 efflux following 24 hours in the dark represents maintenance respiration. Recently, Gons and Mur (1980) measured growth and maintenance in the green alga *Scenedesmus protuberans*, and found that specific maintenance rates determined by growth analysis (Pirt 1965) were equivalent to those rates determined by incubation in prolonged darkness. Finally, the 'starvation' method has been widely used for many years to estimate basal metabolism, in the field of Animal Physiology (Blaxter 1964).

Values for the steady state coefficient '2c' (table 5.1) are consistent with the calculated maintenance requirements for leaf tissue published by Penning de Vries (1975a). His values are: 19-34 mg CO_2 (g dry weight)$^{-1}$ 24h^{-1} for the total maintenance requirements, which is partitioned into the requirement for protein turnover (10-19 mg CO_2 (g dry weight)$^{-1}$ 24h^{-1}) and for the maintenance of ion concentrations (9-15 mg CO_2 (g dry weight)$^{-1}$ 24h^{-1}). The whole plant maintenance requirement should be somewhat less than the calculated total requirement, since

the latter refers to leaf tissue containing 4% nitrogen, whereas overall plant protein quality and concentration are likely to be different from these attributes in the leaf.

The Growth Efficiency

The analysis presented in Chapter II provides reasons why the three methods should give equivalent results, and this is borne out experimentally not only in this study, but also in the study by McCree and Silsbury (1978). Since 'k' is the same slope as 'g', it seems that the direct relationship between D and N extends to negative values of D. However the constant 'k' is calculated as the slope between only two points, and one of these points is determined by the coordinates (-D, N) where N = -D, and N, in turn is determined as the rate of CO_2 efflux in prolonged darkness. Thus, it is assumed that -D is an uptake equal to, but opposite in sign to the rate of efflux in prolonged darkness. There is no experimental evidence for this view, but because 'k' and 'g' are equal, this view appears justified.

The mean growth efficiency (0.69) is close to the value (0.65) calculated for maize supplied with nitrate and sulphate (Penning de Vries *et al.* 1974).

Effect of Species

The overall similarity of the growth efficiency for field bean, lucerne and chickpea, implies that the chemical compositions of the plant biomass of these species are similar. This conclusion is supported by results of carbon and nitrogen analyses, but is also indicated by the similarity of the maintenance coefficients. The data for pea (table 5.1) also suggested that the biomass composition of this species was comparable to that of fieldbean, lucerne and chickpea. It appears that the energy costs for growth and maintenance of tissue is dependent on the composition of the biomass, rather than on the species and furthermore, the growth conditions are a more important determinant of biomass composition than is species difference *per se*.

The tobacco and kidney bean plants had been subjected to a debudding treatment, and as a result of the persistent removal of developing buds, the stems of these plants became thickened and fibrous. The organic nitrogen concentration of the tissue was (3.3%), which was much lower than the organic nitrogen content of the other legumes (4.5%), and the maintenance requirement per unit of dry matter was correspondingly lower. Assuming the organic nitrogen content to accurately reflect protein content, these data clearly point out the importance of the protein content in determining the maintenance energy requirement in whole plants.

In all intact, vegetative plants, between 10 and 50 days after emergence, there

was not a significant change in the biomass composition, nor in the specific rate of maintenance respiration. Silsbury (1979) also found little change in the maintenance coefficient of vegetative subterranean clover communities between 48 and 76 days after sowing.

CHAPTER VI DARK RESPIRATION OF MATURE LEAVES DURING PROLONGED DARKNESS

INTRODUCTION

As described in Chapter V, estimation of the maintenance requirements in growing plants, is not a simple task. Penning de Vries (1975a) suggested that maintenance respiration could be measured directly on non-growing attached organs when transport had ceased (between six and 24 hours in the dark for leaves), or on mature non-transporting organs, for example leaves detached from previously irradiated plants. These suggestions have been adopted in the present study. In addition, measurements were extended beyond 24 hours in order to examine respiratory changes which may have arisen during the measurements on whole plants outlined in Chapters IV and V.

Attempts were also made to alter rates of leaf metabolism by exposing the leaves to various treatments e.g. 'high' and 'low' irradiance, debudding and alteration of the nutrient supply. 'High' irradiance treatments (Huffaker 1982) and debudding (Mondal *et al.* 1978; Van Staden and Carmi 1982) were designed to increase leaf protein concentration. Variations in irradiance and nutrient supply were imposed to alter carbohydrate accumulation in the leaves. 'Low' irradiance coupled with three times the normal concentration of nitrate (i.e. 22.5 mM) was intended to deplete the leaf of carbohydrate, whilst a 'high' irradiance in the absence of nitrate, was intended to force the accumulation of carbohydrate within the leaves. The objective of these treatments was to estimate maintenance respiration of mature leaves with nominally different maintenance energy requirements. Mature leaves were used because the CO_2 efflux from these leaves, should not be contaminated with the CO_2 efflux arising from biosynthesis.

METHODS

Growth Treatments

Two experiments using field bean plants were conducted under two irradiance regimes, so called 'high' irradiance and 'low' irradiance, in the growth room at a temperature of 20°C. It is important to note, that where the terms 'low' and 'high' irradiance are used in the present, and subsequent chapters they refer to the contrasting irradiance treatment of separate experiments in which plants were grown and it is not intended that they be interpreted in an absolute sense.

In experiment one, plants were grown for 21 days after emergence and the light flux density on the second mature leaf was 600-750 μmol m^{-2} s^{-1} for the 'high' light, and 300-500 μmol m^{-2} s^{-1} for the 'low' light treatment. In experiment two, plants were grown for 30 days after emergence and the light flux density on the fourth mature leaf was 1100-1300 μmol m^{-2} s^{-1} for the 'high', and 600-800 μmol m^{-2} s^{-1}

for the 'low' light treatment, respectively.

Respiration measurements were also made on leaves from debudded plants (experiment three), in which the stem above the fourth mature leaf was removed. Plants were grown as in experiment two (high irradiance treatment) except that seven days before the measurements, plants were debudded and half the plants placed under a lower irradiance (160 μmol m^{-2} s^{-1}) whilst the other half continued to experience the high irradiance treatment (1100-1300 μmol m^{-2} s^{-1}). In a further experiment (experiment four), plants were grown as in experiment two (high irradiance treatment) but three days before respiratory measurements were made, the plants were divided into two groups, one receiving no nitrate in the nutrient solution (the fourth mature leaf continued to experience the high irradiance 1100-1300 μmol m^{-2} s^{-1}) whilst the other group received three times the normal nitrate concentration of 7.5 mM (and the fourth mature leaf was exposed to a lower irradiance (160 μmol m^{-2} s^{-1}).

Plants providing the source leaves for detached leaf experiments (experiment five) were grown as in experiment one, except that the second mature leaf received higher irradiance (1100-1300 μmol m^{-2} s^{-1}). The same conditions were employed to grow one group of plants to provide attached leaves for gas exchange quotient analysis, whilst another group was grown in the temperature controlled glasshouse (set at 20°C) and received natural radiation levels, which approached about 900 μmol m^{-2} s^{-1} at mid-day. A summary of the treatments is given in table 6.1.

TABLE 6.1. Summary of plant treatments. Irradiance units: μmol $m^{-2}s^{-1}$.

Experiment No.	Days after emergence	Growth irradiance	Treatment irradiance	Special conditions
1	21	600 - 750	600 - 750	-
1	21	300 - 500	300 - 500	-
2	30	1100 - 1300	1100 - 1300	-
2	30	600 - 800	600 - 800	-
3	30	1100 - 1300	1100 - 1300	Debudded
3	30	1100 - 1300	160	Debudded
4	30	1100 - 1300	1100 - 1300	no NO_3^-
4	30	1100 - 1300	160	22.5 mM NO_3^-
5	21	1100 - 1300	1100 - 1300	Detached leaves

Procedures

At the end of the normal day, 30 plants were removed from the growth room and placed in a darkened growth cabinet or a small light-proof incubator, both of which were set at 20°C. If detached leaves were required for measurement they were excised, floated on water and placed in the incubator. Immediately following re-

moval from the growth room, five plants (or five leaves) per treatment were selected for gas exchange measurement (time zero). At subsequent 12 hour intervals, further sets of five plants or leaves were selected. Since only one leaf chamber was available, CO_2 exchange measurements from five leaves, were made sequentially in time although measurements using the Warburg manometers were made simultaneously, since 12 vessels were in use. Detached leaves were blotted dry before being enclosed in the chamber, and for all leaves, approximately 30 min was required for steady state CO_2 exchange to be achieved.

After gas exchange measurement had been completed, a leaf was weighed and its leaf area determined before being frozen in liquid nitrogen and stored at -15°C until freeze-dried (-40°C, 0.013 kPa for 48 hours). After drying, leaves were weighed again, ground to a powder with mortar and pestle, dried under vacuum (-40°C, 0.013 kPa for 12 hours) and stored in screw cap glass vials at -15°C until required for analysis.

RESULTS

The Relationship between Dark Respiration and Previous Irradiance Level

Two experiments were conducted to obtain the data given in table 6.2, which shows the rate of dark respiration of leaves at the beginning of the night, to be closely related to the light flux density (i.e. carbon assimilation) experienced during the previous day.

TABLE 6.2 Relationship between irradiance level (μmol $m^{-2}s^{-1}$) and the mean (± standard error) rate of dark respiration of attached mature leaves, at the beginning of the following night. (mg CO_2 (g dry weight)$^{-1}$ h^{-1}).

Experiment No.	Irradiance	Dark Respiration Rate
1	300 - 500	1.48 ± 0.1
1	600 - 750	2.20 ± 0.3
2	600 - 800	2.59 ± 0.2
2	1100 - 1300	2.73 ± 0.4

Leaf Respiration in Prolonged Darkness

effect of previous irradiance

Figure 6.1 shows typical results of respiration measurements at 12 hourly intervals over a period of 60 hours. Dark respiration (per unit area) remained steady over the first 12 hours (the 'normal' night), but subsequently declined during the following 24 hours until a new, lower, steady rate of gas exchange was attained after 36 hours

of darkness. The overall pattern of the respiratory decay for attached mature leaves (figure 6.1) is very similar to that observed when whole plants are subjected to the same treatment (McCree 1970).

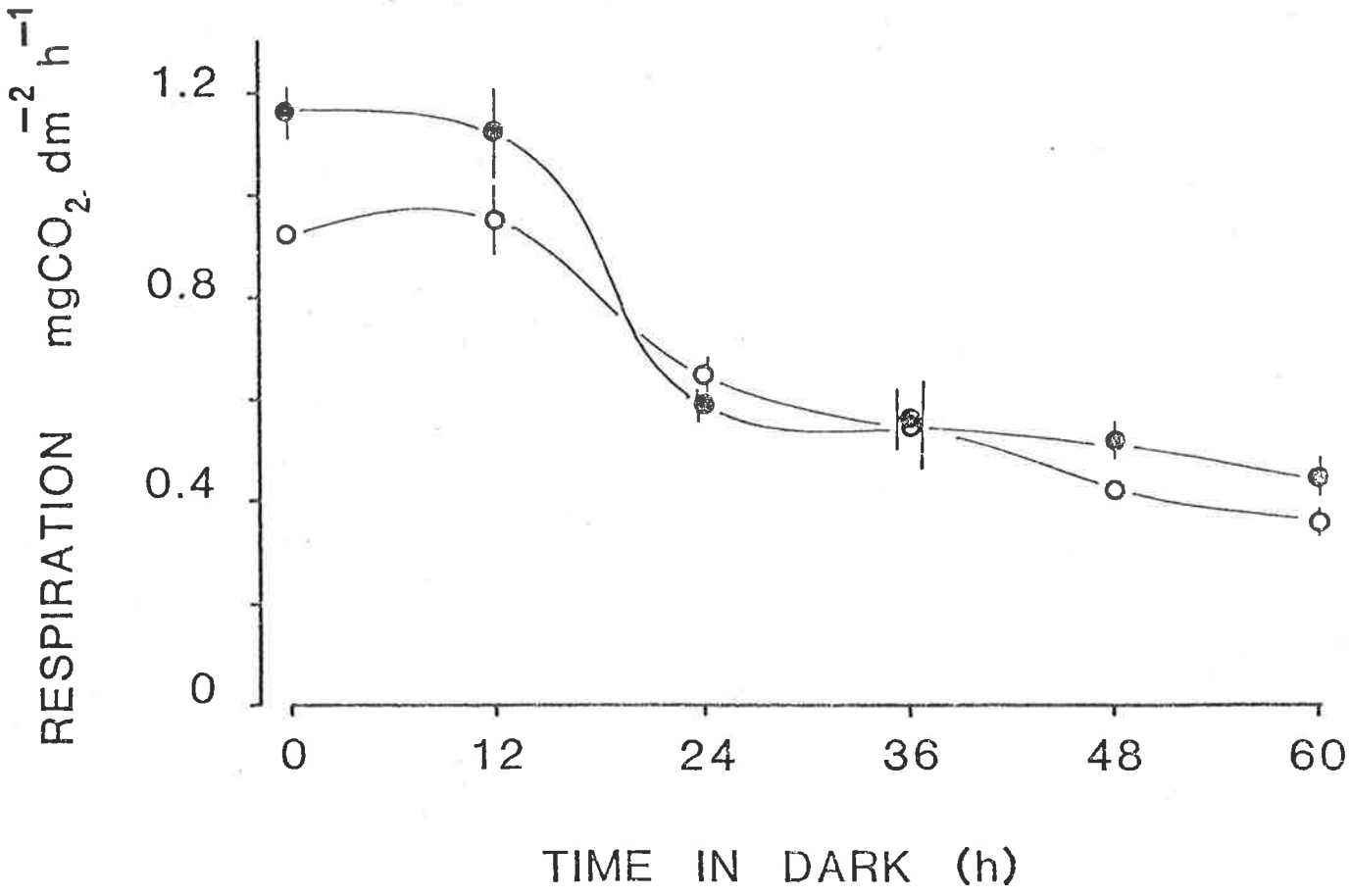

FIGURE 6.1. Time course of dark respiration of the fourth mature, attached field bean leaves, which were previously grown under high irradiance (1100-1300 μmol $m^{-2}s^{-1}$); ●, or low irradiance (600-800 μmol $m^{-2}s^{-1}$); ○. Bars represent standard errors, and where missing are contained within the symbols.

When changes in the dry weight of a leaf were examined, it was found (figure 6.2) that during the normal night, a marked decrease in weight occurred. This was associated (figure 6.1) with a stable rate of gas exchange expressed on an area basis.

effect of leaf detachment

Figure 6.3 shows the pattern of respiration in leaves which had been detached from the parent plant at the end of a 12 hour day and floated on deionised water. There was no decay after 12 hours in the dark, but a small decay occurred after 36 hours in the dark. This was also observed in other detached leaf experiments (data not shown). There was little difference between the respiration rate of attached, and detached leaves, during the normal night.

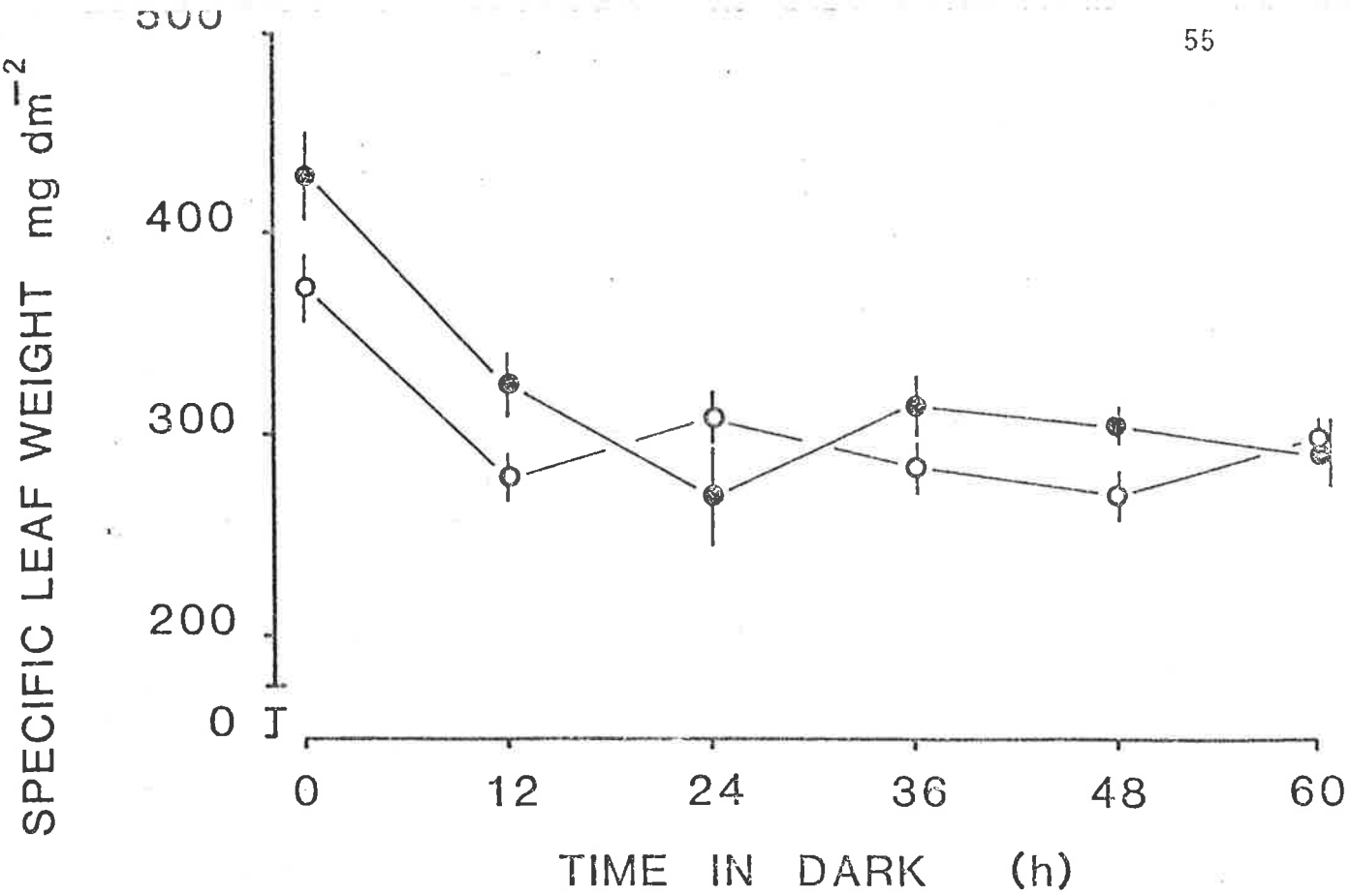

FIGURE 6.2. Changes in the specific leaf weight of the fourth mature, attached field bean leaves, which were previously grown under high irradiance (1100-1300 μmol $m^{-2}s^{-1}$); ●, or low irradiance (600-800 μmol $m^{-2}s^{-1}$); ○. Bars represent standard errors.

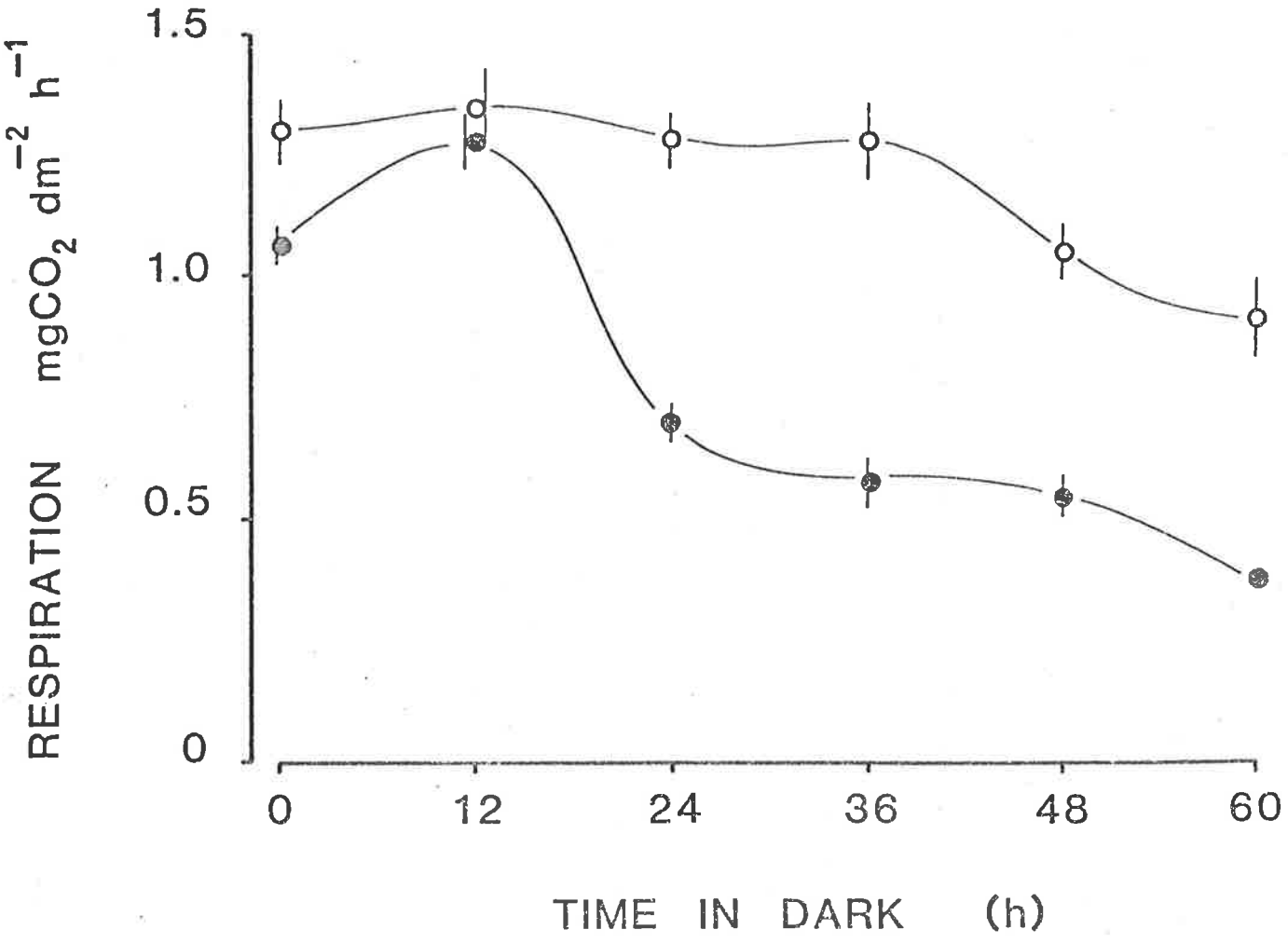

FIGURE 6.3. Time course of dark respiration of the second mature, attached; ●, and detached; ○, field bean leaves, which were previously grown under high irradiance (1100-1300 μmol $m^{-2}s^{-1}$). Bars represent standard errors, and where missing, are contained within the symbols.

To ensure that the detached leaves were not dehydrating, fresh- and dry weights were measured and compared with similar data for attached leaves. The results (table 6.3) show that dehydration was not a factor influencing the respiration of detached leaves.

TABLE 6.3. Test of the effect of detachment on water content (fresh weight : dry weight ratio) of leaves incubated for up to 60 hours in the dark. There were five replicates for the detached leaf treatment and two replicates for the attached leaf treatment. Values are means together with standard errors. Units are mg.

Time in dark (h)	Attached leaves			Detached leaves		
	Fresh weight	Dry weight	Ratio	Fresh weight	Dry weight	Ratio
0	721.5 ± 42.5	103.9 ± 10.1	6.9	555.8 ± 63.8	74.9 ± 7.5	7.4
12	614.5 ± 43.4	72.9 ± 10.0	8.4	526.9 ± 50.1	64.6 ± 7.1	8.2
24	506.4 ± 48.0	51.5 ± 1.4	9.8	690.6 ± 42.0	75.7 ± 5.1	9.1
36	479.2 ± 16.7	54.3 ± 16.0	8.8	553.1 ± 24.0	61.7 ± 2.2	9.0
48	701.7 ± 38.0	77.3 ± 3.5	9.0	535.0 ± 40.0	56.8 ± 4.0	9.4
60	353.4 ± 18.9	36.6 ± 2.3	9.6	399.5 ± 26.0	45.1 ± 2.5	8.9

effect of debudding

The pattern of respiratory decay for attached leaves from debudded plants which were previously exposed to a 'high' irradiance for seven days (figure 6.4) was similar to that observed for leaves grown in experiment two (figure 6.1). There was a constant rate of CO_2 efflux during the 'normal' 12 hour night after which the rate rapidly declined for 36 hours. A contrasting pattern was observed after leaves had been moved to a 'low' irradiance following debudding. The rate of dark respiration was not constant during the normal night, but instead, declined rapidly and attained a lower steady state after about 24 hours of darkness.

It is noticeable that the steady state rate of gas exchange in the 'high' irradiance treatment is higher than that observed in other experiments in which leaves were exposed to a similar photon flux density (e.g. figures 6.1, 6.3 and 6.5), and this could have arisen either because there was a higher protein concentration in the leaves, which necessitated a higher energy demand for maintenance, or because the leaves were thicker and contained a greater mass of respiring material. Although the first possibility was not tested, the second possibility is consistent with the observation that the leaves from the 'high' irradiance treatment were considerably thicker than leaves from the other treatments. For example, the average specific leaf weight during the final 24 hour period of the high irradiance treatment was 865 mg dm^{-2}, compared with 590 mg dm^{-2} for the low irradiance treatment and about 300 mg dm^{-2} for leaves grown in experiment two (figure 6.2).

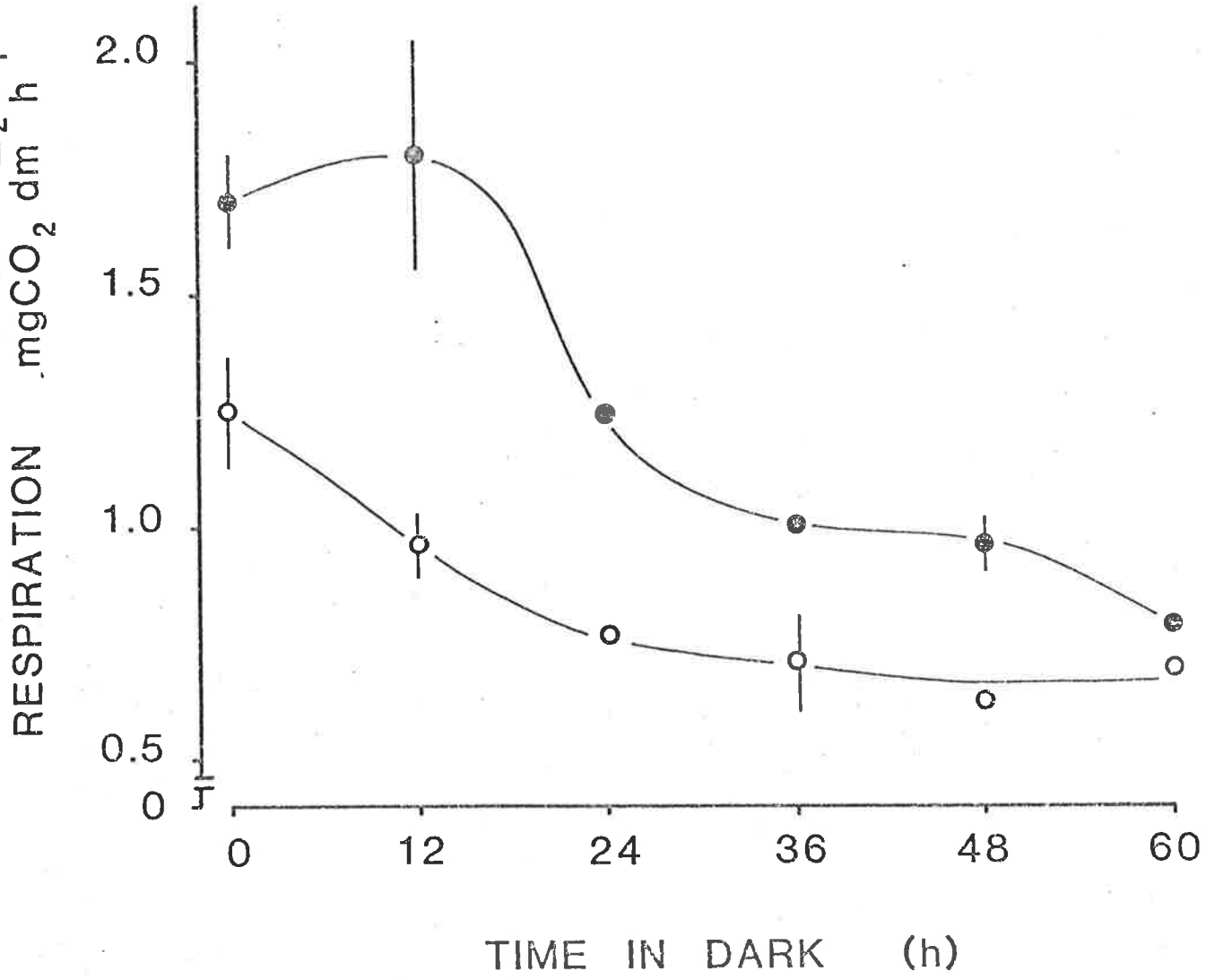

FIGURE 6.4. Time course of dark respiration of the attached fourth mature leaf of field bean plants. Plants were debudded above the fourth mature leaf seven days before measurement and the fourth leaf was then exposed to high irradiance (1100-1300 μmol $m^{-2}s^{-1}$);●, or low irradiance (160 μmol $m^{-2}s^{-1}$);○. Experiment three. Points are means of two replicates, except for the points at 48 hours (low irradiance curve) and at 60 hours (both curves), where points represent measurement on single leaves. On other points, bars represent standard errors and where missing, are contained within the symbol.

effect of altering the nitrogen supply

When plants were exposed to 'high' irradiance, and nitrate was withheld from the nutrient solution, the pattern of dark respiration of the attached leaves (figure 6.5) was similar to that previously observed (figure 6.1). When plants were supplied with 22.5 mM nitrate and exposed to 'low' irradiance, there was a gradual, linear decline in the respiration rate of the mature leaves when the plants were placed in continuous darkness. There was no rapid decay in the respiration rate following the 'normal' night, and during the first 24 hours of darkness, the loss in leaf dry weight was calculated to be only 26.4 mg dm^{-2}. The corresponding dry weight loss from leaves of the high irradiance treatment was 87.1 mg dm^{-2}. There was little difference in the rate of CO_2 efflux between the treatments after 48 hours of darkness, although the rate of efflux was marginally higher in the high light treatment.

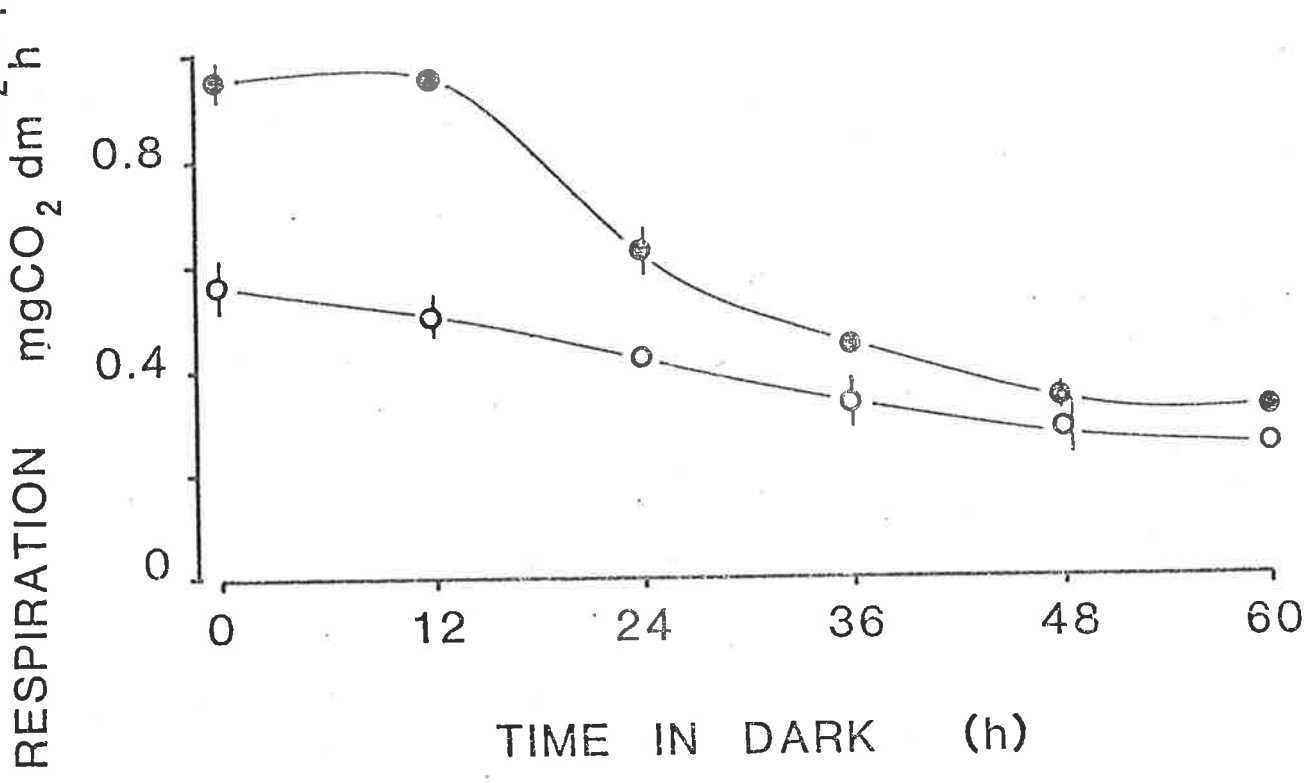

FIGURE 6.5. Time course of dark respiration of the attached fourth mature leaf of field bean plants. Experiment four. Plants were grown under an irradiance of 1100-1300 μmol $m^{-2}s^{-1}$, and 3 days before measurement plants were divided into two groups, one received no nitrate in the nutrient solution and continued to grow at this same 'high' irradiance; ●, whilst the other group received 22.5 mM nitrate, and 'low' irradiance (160 μmol $m^{-2}s^{-1}$); ○. Bars represent standard errors, and where missing are contained within the symbol.

the gas exchange quotient

Figure 6.6 shows results obtained using attached leaves from plants growing in the temperature controlled glasshouse (lower curve), the growth room (centre curve), and using leaves detached from plants which were previously growing in the growth room (upper curve). The gas exchange quotient (μl CO_2/μl O_2) varied in the same manner for both sets of attached leaves, and declined from a value around 0.90 to 0.78 and 0.69 (centre and lower curves, respectively) at 24 hours, increased and then decreased again after 48 hours. No such variation occurred in detached leaves, and the value of the quotient remained above 0.90.

These values reveal possible changes in respiratory substrate from carbohydrate to protein after 12 hours of darkness when leaves remained attached to the plant, but the detached leaves appeared to continue to oxidise carbohydrate for at least 60 h.

Calculation of the Energy Requirement for Sucrose Transport

As noted previously, decay in the respiration rate of mature leaves, when placed in prolonged darkness, was not expected. However, the observation (figure 6.1) that the decay was more pronounced when there was a marked decline in leaf dry weight during the 'normal' night, suggests that leaf respiration provides energy to load

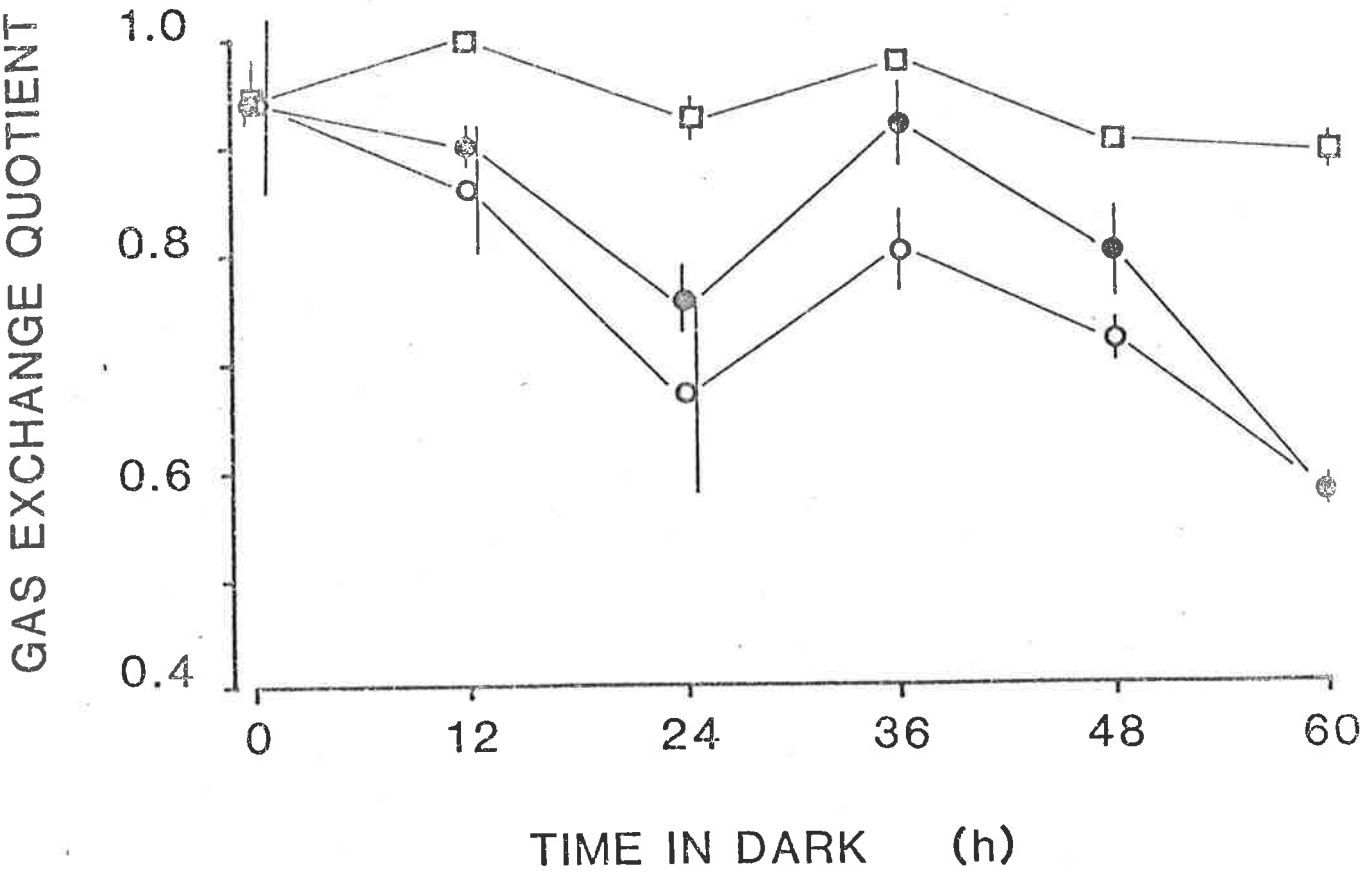

FIGURE 6.6. Time course of the gas exchange quotient (µl CO_2/µl O_2) calculated for mature field bean leaves. Plants were grown in a growth room and leaves were detached at zero hours; □, or leaves remained attached to the parent plants; ●. Other plants were grown in a glasshouse and gas exchange quotients were determined using attached leaves; ○. Bars represent standard errors, and where missing, are contained within the symbols.

sucrose into the phloem. If was therefore decided to calculate the energy requirement for sucrose transport. The calculations (illustrated for one example in appendix I) are made using data from experiments one and two. Respiration measurements were derived from data such as those in figure 6.1 whilst the quantities of carbon translocated were observed from data to be presented in Chapter VII. The energy requirement was calculated in two ways: in the first, the total respiration over the first 12 hours was assumed to contribute to phloem loading whilst in the second, the difference between the respiration rate at 12 hours and 60 hours, was assumed to represent respiration for loading. Thus mature leaf respiration was divided into a loading and a basal (maintenance) component. The results (table 6.4) show that energy costs differ according to the method of calculation, but when the second method is used (column (ii)), the results are less variable.

Calculation of the Maintenance Requirement for Mature Leaves

Data from all five experiments using attached and detached mature field bean leaves are shown in table 6.5. The rates of maintenance respiration of attached leaves (taken after 60 hours of darkness) were hardly affected by the treatments but

TABLE 6.4. Calculated energy requirements for loading the phloem with soluble sugars in the mature leaves of field bean plants. Two experiments were run, each at two levels of irradiance. The calculations were made on the basis of the total respiration rate of the leaves during a 12 hour night (column (i)), or on the basis of the difference between the respiration rate during a 12 hour night, and the rate after 60 hours of darkness (column (ii)). Units are gC respired/gC transported. Summary values are mean, standard error and coefficient of variation (%).

Experiment No.	Irradiance μmol $m^{-2}s^{-1}$		Energy requirement (i)	(ii)
1	600 - 750		0.0503	0.0502
1	300 - 500		0.0503	0.0502
2	1100 - 1300		0.1050	0.0640
2	600 - 800		0.0950	0.0580
		mean	0.0750	0.0560
		s.e.	0.014	0.003
		c.v.	39.7	12.0

were noticeably lower than the rates determined on detached leaves (taken after 12 hours of darkness). The average values for attached leaves on a dry weight and a protein basis correspond to 18.2 mg glucose (g dry weight)$^{-1}$ 24h^{-1} and 68.0 mg glucose (g protein)$^{-1}$ 24h^{-1}, respectively. The corresponding average values for detached leaves are 52.2 mg glucose (g dry weight)$^{-1}$ 24h^{-1} and 200.8 mg glucose (g protein)$^{-1}$ 24h^{-1}.

TABLE 6.5. Maintenance respiration of attached and detached field bean leaves, determined after 60 hours (attached leaves) and zero hours (detached leaves) at 20°C. All treatments (indicated in short-hand) are fully described in the Methods section. Mean values are given together with the standard error for five leaves. Where no error term is apparent, values were calculated from the rate of respiration per unit of dry matter assuming the protein concentration to be 26.4% (the mean value of tissue from experiment 1 and 2. Respiration is on a dry weight basis (mg CO_2 (g dry weight)$^{-1}h^{-1}$) or a protein basis (mg CO_2 (g protein)$^{-1}$ h^{-1}). Experiment 3 was the debudding experiment whilst experiment 4 incorporated changes in the nitrate supply. HL and LL refer to 'high' and 'low' irradiance treatments, respectively.

Attached leaves			Detached leaves		
Treatment	Maintenance respiration per: g dry weight	g protein	Treatment	Maintenance respiration per: g dry weight	g protein
HL Expt 1	1.04 ± 0.38	4.53 ± 0.74	HL	3.76 ± 0.45	14.46
LL Expt 1	0.62 ± 0.14	2.63 ± 0.18	HL	2.70 ± 0.21	10.38
HL Expt 2	1.51 ± 0.14	4.41 ± 0.72	HL	3.11 ± 0.20	11.96
LL Expt 2	1.20 ± 0.08	4.47 ± 0.46			
HL Expt 3	1.16 ± 0.10	4.46			
LL Expt 3	1.16 ± 0.12	4.46			
HL Expt 4	1.25 ± 0.09	4.81			
LL Expt 4	0.94 ± 0.10	3.62			
Mean	1.11	4.17		3.19	12.27

The maximum potential turnover rate of leaf protein can be estimated if it is assumed that the respiration rate represents carbon efflux solely from protein degradation. If the carbon content of protein is 54.4% (Challa 1976), the maintenance rate of attached leaves represents a turnover of 0.05 d^{-1} (50.2 mg protein (g protein)$^{-1}$ 24h^{-1}) whilst for detached leaves the turnover rate is 0.15 d^{-1} (147.6 mg protein (g protein)$^{-1}$ 24h^{-1}).

DISCUSSION

The respiration of mature field bean leaves can be separated into two components in the same manner as can be done for whole plants, i.e. after a 'normal' 12 hour night of more or less stable rate of CO_2 efflux, the dark respiration falls during the next 24 hours, to an asymptotic value. This pattern in mature leaves was unexpected, since such leaves are, by definition, not growing. However, the pattern as described, shows there to be a 'constant' (or basal) component (which can be measured after about 24 hours of darkness) and a 'variable' component of respiration (which is quantified as the difference between the rate of CO_2 efflux at the beginning of the night, and the rate after a further 24 hours in the dark). The initially high rate of respiration associated with the loss in leaf weight, strongly suggests that respiration in mature leaves in addition to providing for leaf maintenance, also supplies energy for assimilate export. Sovonick *et al.* (1974) and Geiger and Sovonick (1975), amongst others, have shown that phloem loading is an energy dependent process, and it is possible to cost this process with a knowledge of both the rate of respiration, and the amount of carbon translocated. As shown in table 6.4, the cost depends on whether the respiration rate during the 'normal' 12 hour night is used, or whether the difference between this rate and the rate after 60 hours of darkness is used for the calculations. It seems logical to exclude the 'constant' component from the calculation because the magnitude of this component is independent of the magnitude of the variable component (table 6.4). This analysis reveals that on average, 53.6 mg of carbon is respired in the export of one gram of carbon in the form of sucrose (i.e. 0.0536 gC/gC). This value is considerably lower than the cost calculated for tomato by Ho and Thornley (1978, 0.22 gC/gC), but is comparable with the value for barley leaves determined by Hitz (1982, 0.08 gC/gC). Much lower rates (0.014 gC/gC) were computed by Sovonick *et al.* (1974) and Komor (cited by Schapendonk and Challa 1980).

The theoretical cost of assimilate loading can be calculated if phloem loading (i.e. movement of sucrose from the free space to the companion cell cytoplasm) requires one molecule of ATP per molecule sucrose transported (Penning de Vries 1975b). One molecule of ATP is equivalent to $^1/_{38}$ molecules of glucose (assuming complete oxidation) and one molecule of sucrose is equivalent to two molecules of glucose (since hydrolysis of sucrose yields a molecule each of glucose and fructose, and the latter are readily interconverted by the enzyme, hexose phosphate isomerase.

The cost of loading one molecule of sucrose, in terms of glucose, is then calculated to be $^{1}/38 /2 = 0.0132$ gC/gC. Furthermore, if the synthesis of one molecule of sucrose requires three molecules of ATP (Penning de Vries 1975b), this cost is calculated to be $^{3}/38 /2 = 0.0395$ gC/gC, and the sum of these costings, which accounts for sucrose synthesis and loading into the phloem, is 0.0527 gC/gC, a value which is very close to that determined in table 6.4, column (ii) (0.0560 gC/gC).

These calculations provide very strong evidence for the separation of mature leaf respiration into two components, in which the energy requirement for sucrose synthesis and loading is costed to the 'variable' component of leaf respiration. The magnitude of the 'constant' component can be determined by prolonging the dark treatment for a further 12 to 24 hours and is likely to largely represent the energy requirements for maintenance of protein content, and the maintenance of ion concentrations within cells.

Such a division of mature leaf respiration was implied by Ho and Thornley (1978). They induced changes in night respiration and translocation by daily changing the photon flux density incident on mature tomato leaves. A linear regression equation incorporating the dark respiration rate and the rate of translocation revealed that respiration accounted for 22% of the carbon translocated, plus 1.1% of the leaf weight. They interpreted this result in terms of a transport dependent respiratory component, and a 'maintenance' component.

The reasons for the wide variation in 'transport' cost is not known, but differences in methodology, and perhaps species, could be responsible for some of it. In regression analysis, the participation of 'extraneous' processes (nitrate reduction or, fluxes of hexoses or ions across cell membranes for example) which may be dependent on one or other of the variables incorporated in the regression, can markedly affect the quantitative relationship between the variables. Although the results presented in this Chapter were not obtained by regression analysis, the latter criticism is still valid. However, the conclusions drawn are consistent with the good agreement between the theoretically determined and the experimental values obtained for the costs of sucrose synthesis and loading.

The treatments applied had only a minor effect on the pattern of dark respiration of mature field bean leaves when conditions during the light period were conducive to the accumulation of high concentration of carbohydrate. For example, the data for experiment one (not shown) and experiment two (figure 6.1) show the respiration rate to be essentially constant during the normal night. Challa (1976) also found the respiration rate of mature attached cucumber leaves to remain constant during the night when leaves had accumulated considerable carbohydrate reserves during the previous day (when plants were grown in 'spring' conditions) and furthermore, he

found the rate to decline during the night when plants were grown in 'winter' conditions, which lead to the complete export of all previously accumulated carbohydrate from the leaf before the end of the night. This latter pattern of leaf respiration was also found in the present study, as shown in figure 6.4 (low irradiance curve). In figure 6.5, there is a very small decay in the leaf respiration rate in the low irradiance treatment, and only a small change in the leaf dry weight during the first 24 hours in the dark. On the basis of Challa's data, these results suggest that the 'available' carbohydrate (chloroplast starch and soluble sugars within the cell cytoplasm) was exported before the end of the normal night in the low irradiance treatments shown in figures 6.4 and 6.5. Chatterton and Silvius (1981) found the programming of chloroplast starch accumulation to be independent of the photon flux density within the range; 320-640 $\mu mol\ m^{-2}\ s^{-1}$, but the present results, together with those of Challa (1976), suggests leaves have a limited ability to adapt their rates of starch accumulation and degradation to the length of the photosynthetic period, when the irradiance level is relatively low. In figure 6.4, the 'low' irradiance is 160 $\mu mol^{-2}\ s^{-1}$, whilst in Challa's study the 'low' irradiance was 30 $W\ m^{-2}$, or approximately 120 $\mu mol\ m^{-2}\ s^{-1}$.

The debudding treatment appeared to increase the thickness of leaves rather than the nitrogen concentration as had been desired. Previous experiments using debudded plants (Chapter V) showed the root and shoot tissue to become more lignified and the concentration of nitrogen fractions (organic nitrogen and nitrate nitrogen) to be lower than in plants which remained intact. Therefore, it is possible that following debudding, the demand for reduced nitrogen is lowered and the roots may actively exclude nitrate and/or there may be an efflux of stored nitrate from the root into the nutrient medium (e.g. Pearson *et al.* 1981). Consequently, the absence of reduced nitrogen (or nitrate) may explain why there was no accumulation of protein within the mature leaves. Alternatively, the protein concentration may have been low because the recently assimilated nitrate was incorporated into the protein of the emerging axillary buds, which were continually removed. The high rate of respiration of the leaves of both irradiance treatments after 36 hours of darkness (figure 6.4) is therefore likely to be due to the greater weight of respiring tissue within unit area (i.e. higher specific leaf area), rather than to a higher protein concentration of the leaf tissue, since high irradiance can stimulate the formation of additional layers of palisade parenchyma in the leaves (Salisbury & Ross 1969).

The removal of nitrate from the nutrient solution, and the exposure of the leaves to 'high' irradiance (figure 6.5) was intended to force the accumulation of non-structural carbohydrate. It was anticipated that decay in the respiration rate in the dark would be delayed until after 12 hours, since the absence of NO_3^- was expected to reduce the 'sink' demand for carbohydrate. The decay occurred at 12 hours however, as in the previous treatments. Thus, it seems that most of the carbohydrate accumulated

during the light, was mobilised from the leaf by the end of the following night despite the imposed treatment. It is possible that nitrate previously stored within the stem and root tissue, may have been mobilised during the period of nitrogen 'starvation' to provide a 'sink' for the exported carbohydrate. Nevertheless, there is a suggestion from the data for the 'high' irradiance treatments in figures 6.4 and 6.5, that decay in the respiration rate was not completed until about 36 hours of darkness. The decay in the respiration rate of both treatments in figure 6.1, was essentially complete in 24 hours. Whether or not the 'high' irradiance curves in figures 6.4 and 6.5 represent a real change in the pattern of leaf respiration in prolonged darkness, can only be elucidated by additional replication and more frequent measurement than used here.

The pattern of change in the gas exchange quotient (figure 6.6) is difficult to interpret. The low value of the quotient for attached leaves after 24 hours in the dark, suggests that protein was oxidised. It is possible that upon exhaustion of the pool of exportable assimilate, the supply of hexose was insufficient to support the respiration rate, which necessitated the oxidation of amino acids until carbohydrate became available either from vacuolar release, or from the translocation of sucrose into the leaf from elsewhere in the plant. The gas exchange quotient should then return to unity but this is approached only by the growthroom-grown plants. After 36 hours the data suggest the continued oxidation of protein and/or lipid.

An alternative explanation for the low value of the gas exchange quotient at 24 hours could be the incomplete oxidation of amino acids which would result in a value of 0.7 (James 1953), and the increase at 36 hours could be due to complete oxidation, to NH_3 and CO_2, which would increase the quotient to a value between 1.0 and 1.3.

It is assumed that cell wall hydrolysis and oxidation of the resulting glucose was not the reason for the high value for the gas exchange quotient at 36 hours. It is also unlikely that assimilate was transported into the mature leaves, since the whole plant was placed in the dark, although the movement of carbohydrate into mature leaves has previously been reported. Quinlan and Weaver (1969) were able to force the import of carbohydrate when they increased the 'sink' strength of a mature vine leaf by shading it. Thrower (1977) showed that import into mature leaves of *Brassica parachinensis* can occur during the rosette stage of growth, but not when the plant becomes elongate. Whatever the cause of the changes in the gas exchange quotient, the data suggest there to be metabolic differences between attached and detached leaves.

Although the detachment of a leaf removes the assimilate sink and eliminates long distance transport, the protracted high initial rate of dark respiration, for up to 36 hours (figure 6.3), suggests that some transport component is present in such

* Respiration rates of attached and detached leaves (26.64 and 76.56 mg CO_2 (g d.wt)$^{-1}$ 24 h^{-1}, respectively) are greater than whole plant respiration rates shown in table 5.1, steady state method (21.35 mg CO_2 (g d.wt)$^{-1}$ 24 h^{-1}). These data show that the assumption made by Wilson (1975), that specific respiratory rates of mature leaves to be equal to the specific maintenance rates of whole plants, is not valid.

leaves. The accumulation of radiocarbon label in veins of leaves, recently fed labelled CO_2 and then detached from the plant, has previously been observed (e.g. Eschrich and Burchardt 1982; Hartt and Kortschak 1964; Leonard and King 1968) and this suggests that the veins may serve as a sink for carbohydrate, and that sugars are accumulated within the veins against a concentration gradient (i.e. loading is active). Such active loading would account for the high respiration rate over the first few hours of detachment, but the reasons for the prolonged high respiration rate is more puzzling. Three suggestions are put forward. First, following detachment, leaf starch probably degrades at a much slower rate than when it is attached and the carbohydrate exported. Under these circumstances, considerable quantities of CO_2 may be evolved from the oxidative pentose phosphate pathway which is functional in the chloroplasts (Stitt and Heldt 1981). As a result of the operation of this pathway, $NADPH_2$ can be generated, and reduce ferridoxin *via* NADP reductase. Ferridoxin can, in turn, reduce O_2, NO_2^- or H+ in the process termed 'chloroplast respiration' (Kow *et al.* 1982). The high value of the gas exchange quotient could arise as a consequence of chloroplast respiration. Second, glucose and fructose may accumulate in the leaf cell cytoplasm, and may require energy for compartmentation, probably within the vacuole (Guy *et al.* 1979). Third, it is feasible that the high carbohydrate availability may stimulate efflux of nitrate from leaf vacuoles by generating a sink for reductant and thus stimulating respiration. However, Aslam *et al.* (1976) found that light was necessary to stimulate nitrate efflux from the (vacuolar) storage pool of etiolated barley leaves, and that exogenously applied glucose could not replace the light effect. Clearly, the isolation of the leaf from the plant has produced a functional change in leaf metabolism and has transformed it from a constitutive organ responsible for the synthesis and transport of sucrose to the rest of the plant.

The differences in metabolism between attached and detached leaves detailed above, therefore imply that caution be applied in interpreting the rate of CO_2 evolution from mature leaves in terms of maintenance respiration.* The effects of these differences can easily be recognised by the degree to which experimental values for maintenance in detached leaves, deviates from the values calculated by Penning de Vries (1975a). Total maintenance requirements of leaves were calculated to range between 13 and 23 mg glucose (g dry weight)$^{-1}$ 24h^{-1}, whilst the experimentally determined values were 18.2 mg glucose (g dry weight)$^{-1}$ 24h^{-1} for the attached leaves (after 60 hours of darkness) and 52.2 mg glucose (g dry weight)$^{-1}$ 24h^{-1}, for detached leaves. Figure 6.3 shows there to be little difference in the respiration rate of attached and detached leaves during the 'normal' night. The total maintenance energy requirements computed by Penning de Vries (1975a) are exceeded by the requirements determined from experimental measurement. The main reason for the discrepency is due to the fact that the energy costs for sucrose synthesis and phloem loading are not included in Penning de Vries' calculations. If leaves are detached from the parent

plant, this does not eliminate the loading component of respiration because sucrose can be accumulated within the veins of the leaf. Moreover, detachment may cause changes in cell metabolism, as outlined earlier.

The maintenance cost attributable to protein turnover calculated by Penning de Vries (1975a) ranged between 7 and 13 mg glucose (g dry weight)$^{-1}$ 24h^{-1} values which are also exceeded by those from experimental measurement of the rate of CO_2 efflux from leaves during a prolonged dark treatment. Two explanations for this discrepancy can be invoked. Firstly, the calculated costs may underestimate the true rate of protein turnover for two reasons: (i) the calculations assume that all proteins turnover at an average rate of 10% per day when it is likely that the rate of turnover will vary with environmental conditions; and (ii), present techniques are not suitable for the derivation of absolute rates of turnover, since an extended incubation is required to obtain sufficiently large changes in protein content, and as a consequence techniques are biased toward measuring turnover of proteins with low turnover rates (Dungey and Davies 1982). Secondly, respiration comprises components concerned with maintenance of ion concentrations and for membrane maintenance, in addition to a component due to protein maintenance. Discrimination between these explanations will depend on more accurate estimates of protein turnover than presently available.

CHAPTER VII. CARBOHYDRATE EXPORT AND SOURCES OF SOLUBLE SUGARS FOR LEAF RESPIRATION DURING PROLONGED DARKNESS

INTRODUCTION

McCree (1970) suggested that the decay in the rate of dark respiration of clover plants which occurred after 12 to 14 hours in continuous darkness was due to the depletion of one pool of photosynthate and the switching to another. It is conceivable that the first of these pools is the leaf starch which has accumulated during the normal day. The evidence for this view arises from the publications of Chatterton and Silvius (1979) and Challa (1976) who investigated leaf starch synthesis in the light and degradation in the dark in soybean and cucumber, respectively.

Chatterton and Silvius found the rate of starch accumulation to be inversely related to the length of the daily photosynthetic period. Furthermore, plants grown in a seven hour photosynthetic period partitioned a higher proportion of their daily foliar accumulation into starch and sugars (90%) than did plants grown in a 14 hour photosynthetic period (60%). Both Chatterton and Silvius (1979) and Challa (1976) found that the starch reserve was essentially depleted by the beginning of the subsequent photosynthetic period and these observations prompted Chatterton and Silvius to propose that leaf starch accumulation is a programmed process and not simply the result of a limitation in translocation. Their data also suggest that the decay in plant respiration noted by McCree may be related to the depletion of the leaf starch.

In Chapter VI, the respiration rate of mature field bean leaves which had been kept in prolonged darkness, was found to decline rapidly once the loss in leaf dry weight had stopped. Challa (1976) also found the respiration rate of cucumber leaves to decline at the end of the night, at the time when starch exhaustion was imminent. The general concurrence in the decline in both mature leaf, and whole plant respiration noted in these previous studies, suggests that the decay in whole plant respiration may be due at least in part, to the decay in the respiration rate of the mature leaves. An alternative interpretation, and one which is more consistent with the growth and maintenance concept of respiration, is that following the depletion of leaf starch, the rate of substrate supply for growth and energy production in the rest of the plant becomes limiting. Experiments contained within the present chapter were designed to allow differentiation between these two hypotheses.

The second pool referred to by McCree (1970) has been suggested to constitute the pool of soluble sugars, which are compartmented in vacuoles within the cell

(Lopes 1979). Lopes made this suggestion after he found that radish leaves contained appreciable levels of soluble carbohydrate at the end of a 60 hour dark treatment. This implies that when maintenance respiration is determined as the rate of CO_2 efflux after about 48 hours darkness, respiration is reliant on the soluble sugar pool within the vacuoles as the sole source of substrate. However, a prerequisite for the acceptance of this postulate is that a pool of carbohydrate of sufficient size to support the observed rate of respiration must be present. This point is also examined in the present study in relation to mature field bean leaves.

METHODS

To elucidate the reason for the decay in plant respiration mature leaves (containing the stored starch) were removed from plants and continuous recordings of the rate of CO_2 exchange from the defoliated plants (stem, petiole, root and leaf buds) were made for up to 60 hours of darkness. Field bean plants were grown at $20^{o}C$ in a growth room, with the upper leaves receiving between 1300 and 1500 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation for 12 hours per day. Forty days after emergence, single plants were placed in a darkened assimilation chamber (see Chapter III for details) at the end of a 12 hour day. Since only one chamber was available, experiments were run sequentially in time. Following placement in the chamber, plants were subjected to a defoliation treatment, in which all the fully expanded leaves were trimmed from the plants and removed from the chamber. This treatment was carried out at one hour or 11 hours into the dark period, whilst the control plants remained intact.

The pattern of starch degradation and the size of the soluble carbohydrate pool in the leaves were determined in two experiments, the details of which are reported in Chapter VI. Briefly, plants for experiment one were taken 21 days after emergence and placed in continuous darkness for 60 hours at $20^{o}C$. Five mature attached leaves were then subjected to respiration measurement every 12 hours from the end of the normal day, frozen in liquid nitrogen and freeze dried. In experiment two, the same procedure was followed, except that plants were taken 30 days after emergence, and the fourth mature leaf used for respiratory measurement. Tissue was weighed, ground to a powder with a mortar and pestle, re-dried and stored in screw cap glass vials until analysed for 80% ethanol-soluble carbohydrate (as sucrose) using the phenol sulphuric acid technique. Starch in the ethanol-insoluble residue was estimated after hydrolysis with amyloglucosidase and determination of the resulting glucose with glucose oxidase-peroxidase-dianisidine dihydrogen chloride reagent. Full details of these analyses are contained in Chapter III.

The Time Course of Whole Plant Carbon Exchange Following Defoliation

The characteristic decay in the respiration rate following the normal 12 hour night is clearly shown by the control treatment in figure 7.1.

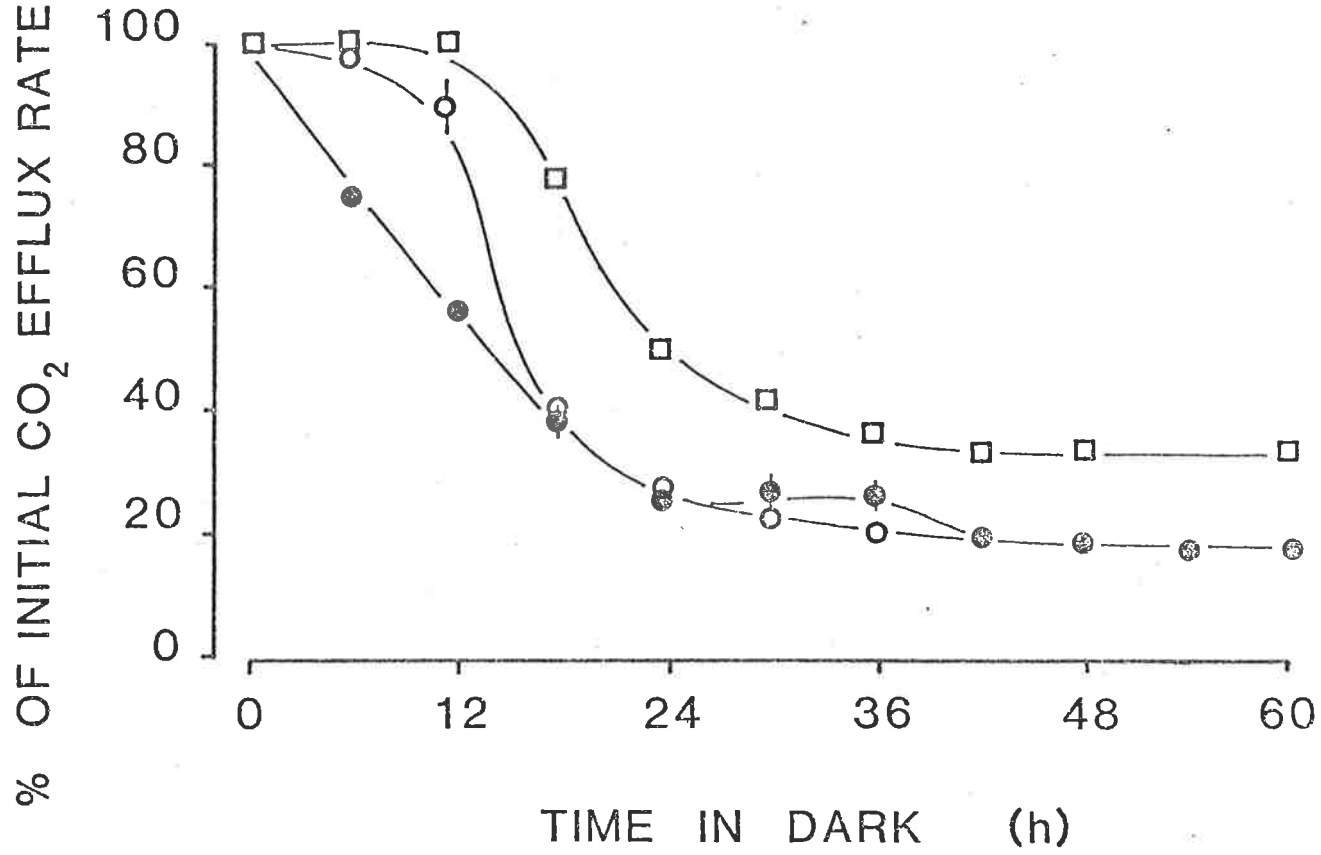

FIGURE 7.1. Decay in the respiration rate of field bean plants which were defoliated one hour into the dark period; ●, 11 hours into the dark period; ○, or remained intact; □. Defoliation treatments were replicated with five plants, the control, with two plants. Bars represent standard errors, and where missing, are contained within the symbols.

When all the mature leaves were removed from the plants after one or 11 hours of darkness, the rate of CO_2 efflux from the remaining roots, stems and immature leaves rapidly declined. In these young plants defoliation removed between 20 and 30% of the dry weight, yet immediately following defoliation the respiration rate of the remaining plant was hardly affected. The reason for the small effect of leaf removal was due to the fact that the respiration rate of the mature leaves was only a small proportion of the total respiration, and this is illustrated by the following data. It should be noted however, that the respiration rate of the leaves from defoliated plants was not measured, but such measurements were made in experiment one, and the data are presented here. The respiration rate of the whole plant, and subsequently, the respiration of the second mature leaf, was measured at the beginning of the night. The average specific respiration rate for five plants was determined to be 13.5 mg CO_2 (g dry weight)$^{-1}$ h^{-1} whilst the corresponding rate for the leaves was 2.2 mg CO_2 (g dry weight)$^{-1}$ h^{-1}. The average

dry weight of the plants was 0.62 g whilst the average weight of all the mature leaves on each plant was 0.26 g. The contribution of the leaves to the total plant respiration rate can now be calculated if it is assumed that all the mature leaves on each plant respire at the same specific rate. This contribution is calculated thus:

$$\frac{\text{leaf respiration}}{\text{plant respiration}} = \frac{2.2 \text{ mg } CO_2 \text{ (g d.wt)}^{-1} \text{ h}^{-1} \times 0.26 \text{ g d.wt}}{13.5 \text{ mg } CO_2 \text{ (g d.wt)}^{-1} \text{ h}^{-1} \times 0.62 \text{ g d.wt}} \times 100\%$$

$$= 6.8\%$$

Therefore leaf respiration accounted for about 7% of the total plant respiration immediately following the photosynthetic period in this experiment. In another experiment, the proportion was calculated to be 10.5%, and these figures show that respiration is more intense in the growing points, such as the shoot buds and the roots than in the mature leaves, during the early growth of these field bean plants.

The Time Course of Carbohydrate Concentration in the Mature Leaves

In experiment one, starch accumulation was highest in leaves which had previously been exposed to the highest irradiance (figure 7.2). There was a considerable decline in the leaf starch concentration over the normal 12 hour night, but starch was not exhausted until sometime between 12 and 24 hours of darkness. The upper line in figure 7.2 may be misleading in this context, because the leaves were not sampled at the 24 hour time period.

The levels of soluble sugars (figure 7.3) similarly declined very rapidly over the first 12 hours of darkness, but thereafter declined at a steadier rate. There was little difference in sugar concentration between leaves of the two irradiance treatments after 12 hours.

In experiment two, the decline in starch (figure 7.4) was comparable to that in experiment one (figure 7.2) and starch had been completely mobilised within 24 hours of darkness, but the rate of soluble sugar loss (figure 7.5) appeared to continue at a high rate for the first 24 hours. However, it is probable (on the basis of the pattern in figure 7.3) that the soluble sugar concentration fell to about 50 mg (g dry weight)$^{-1}$ in both curves soon after the 12 hour sampling time. There was no measurable loss between 24 and 36 hours, but thereafter the sugar concentration declined in the low irradiance treatment but increased slightly in the high irradiance treatment.

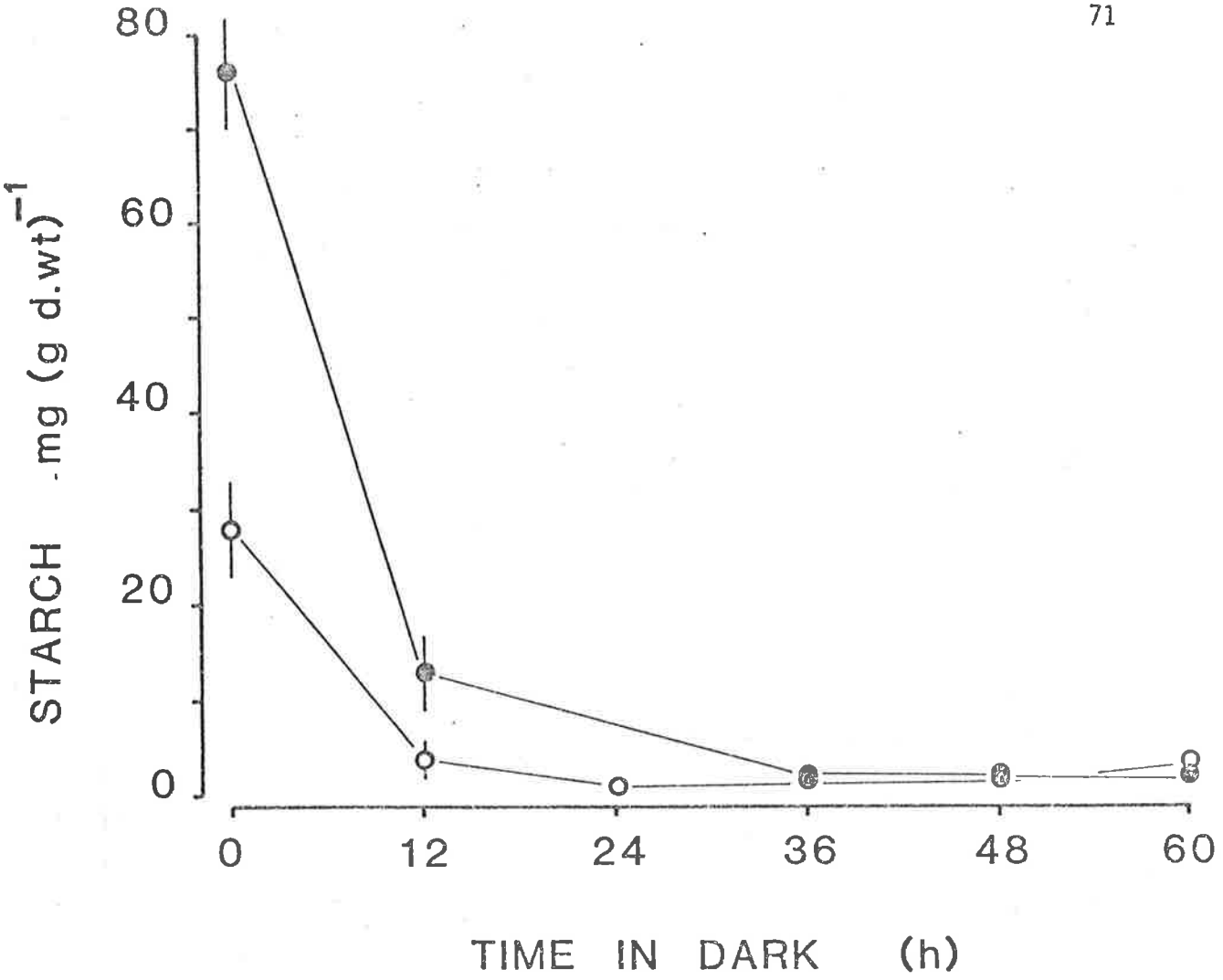

FIGURE 7.2. Time course of starch concentration in the second mature, attached field bean leaves. Experiment one. Leaves were previously exposed to high irradiance (600-750 μmol $m^{-2}s^{-1}$); ●, or low irradiance (300-500 μmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors, and where missing, are contained within the symbols.

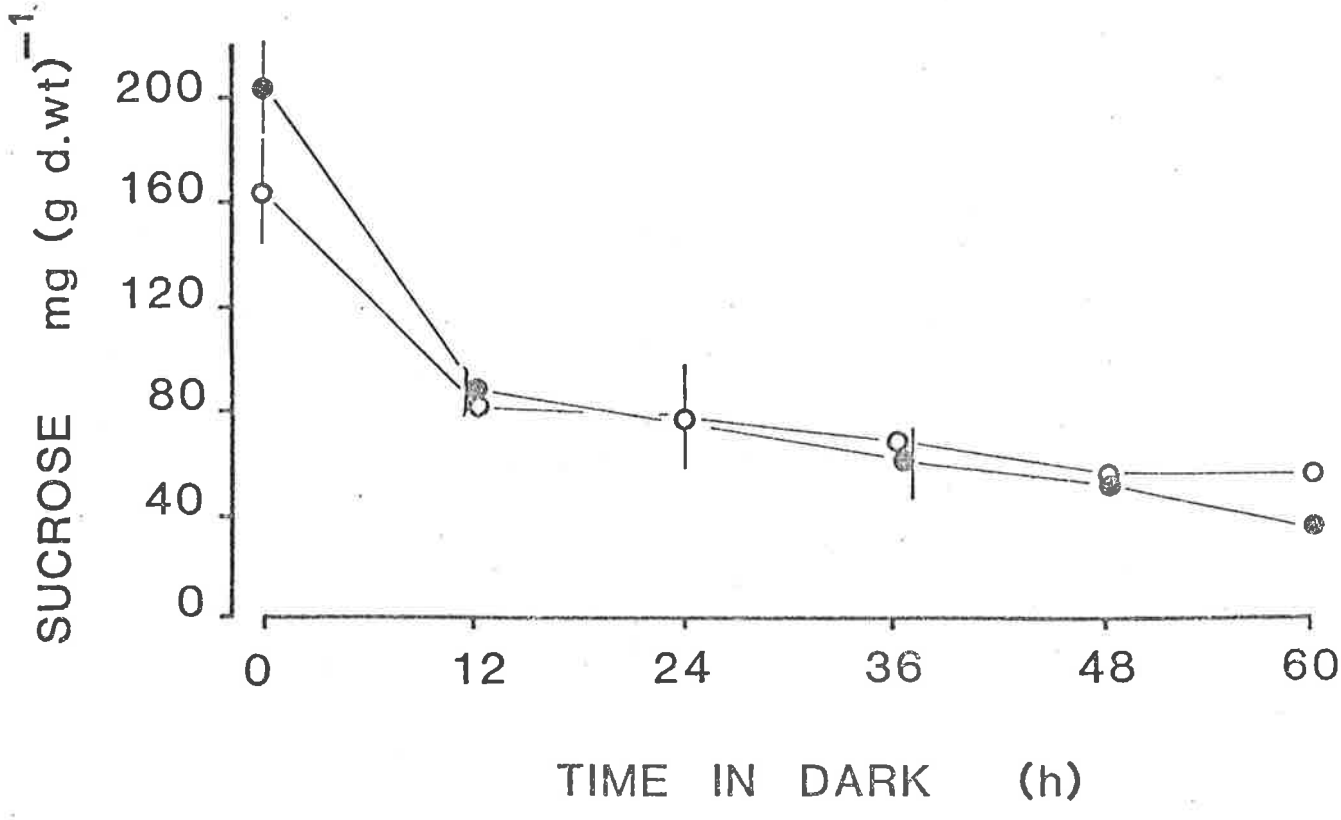

FIGURE 7.3. Time course of soluble carbohydrate (sucrose equivalent) in the second mature, attached field bean leaves. Experiment one. Leaves were previously exposed to high irradiance (600-750 μmol $m^{-2}s^{-1}$); ●, or low irradiance (300-500 μmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors, and where missing, are contained within the symbols.

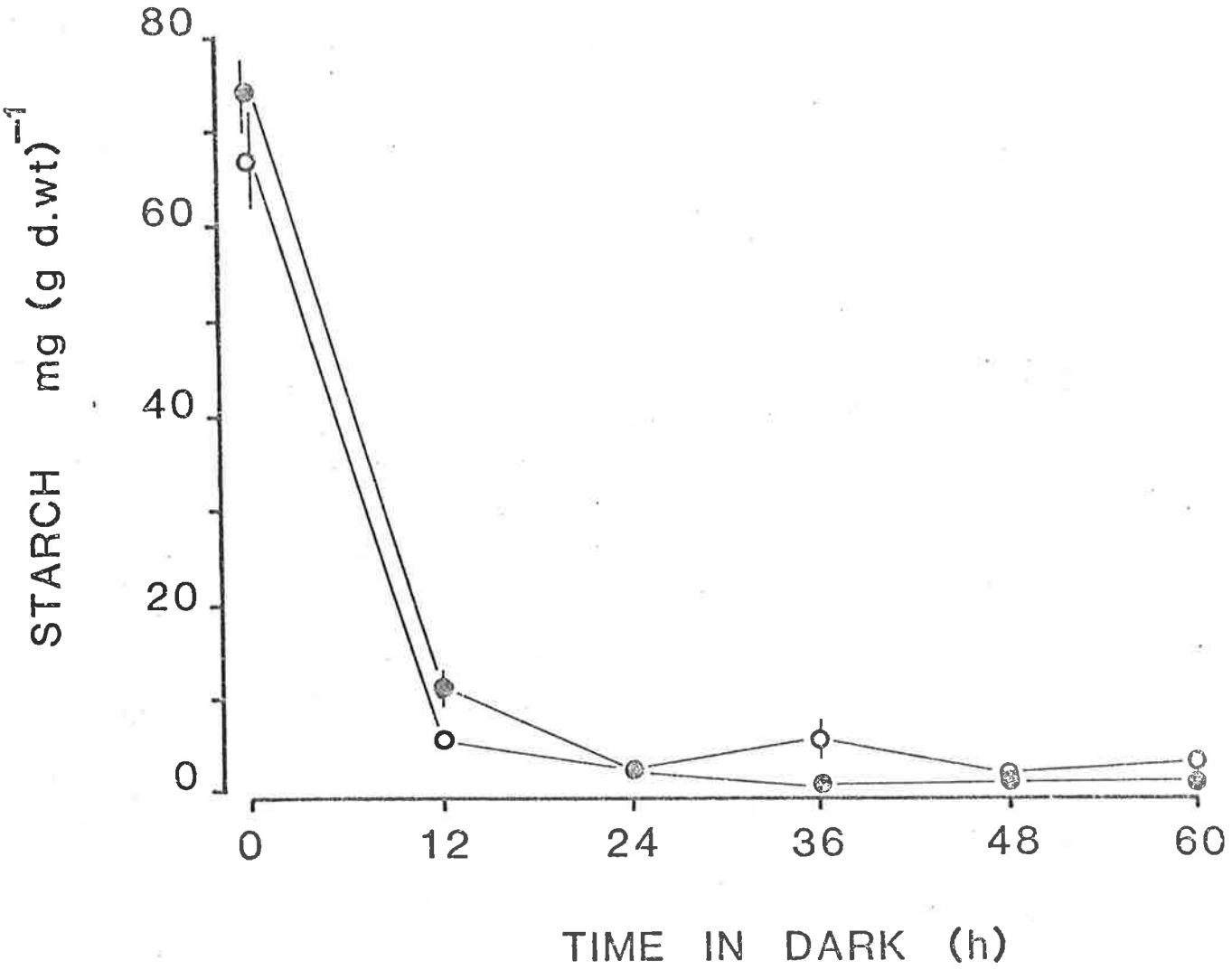

FIGURE 7.4. Time course of starch concentration in the fourth mature, attached field bean leaves. Experiment two. Leaves were previously exposed to high irradiance (1100-1300 µmol $m^{-2}s^{-1}$); ⊛, or low irradiance (600-800 µmol $m^{-2}s^{-1}$); O, during growth. Bars represent standard errors, and where missing, are contained within the symbols.

The Carbohydrate Pool for Leaf Respiration

The size of the pool of soluble carbohydrate potentially available for leaf respiration was calculated, and is shown in table 7.1. The figures are based on the sucrose concentration and the respiration rates of leaves 60 hours after darkening. The data show that the sucrose pool (converted to glucose units) which was present after 60 hours, was sufficient to last a further four to six days, provided that the rate of respiration did not change.

DISCUSSION

Although the dark respiration rate of the mature leaves (Chapter VI) and the whole plant (figure 7.1, control treatment) decay concurrently, this does not mean that the decay in whole plant respiration, which occurs 12-14 hours into a dark period, is caused by the decline in the respiration rate of the mature leaves. The evidence for this, is that immediately following defoliation, the rate of CO_2 efflux from the plant did not fall to the maintenance rate. This did not occur for the reason that only a small proportion of the total respiration was attributable

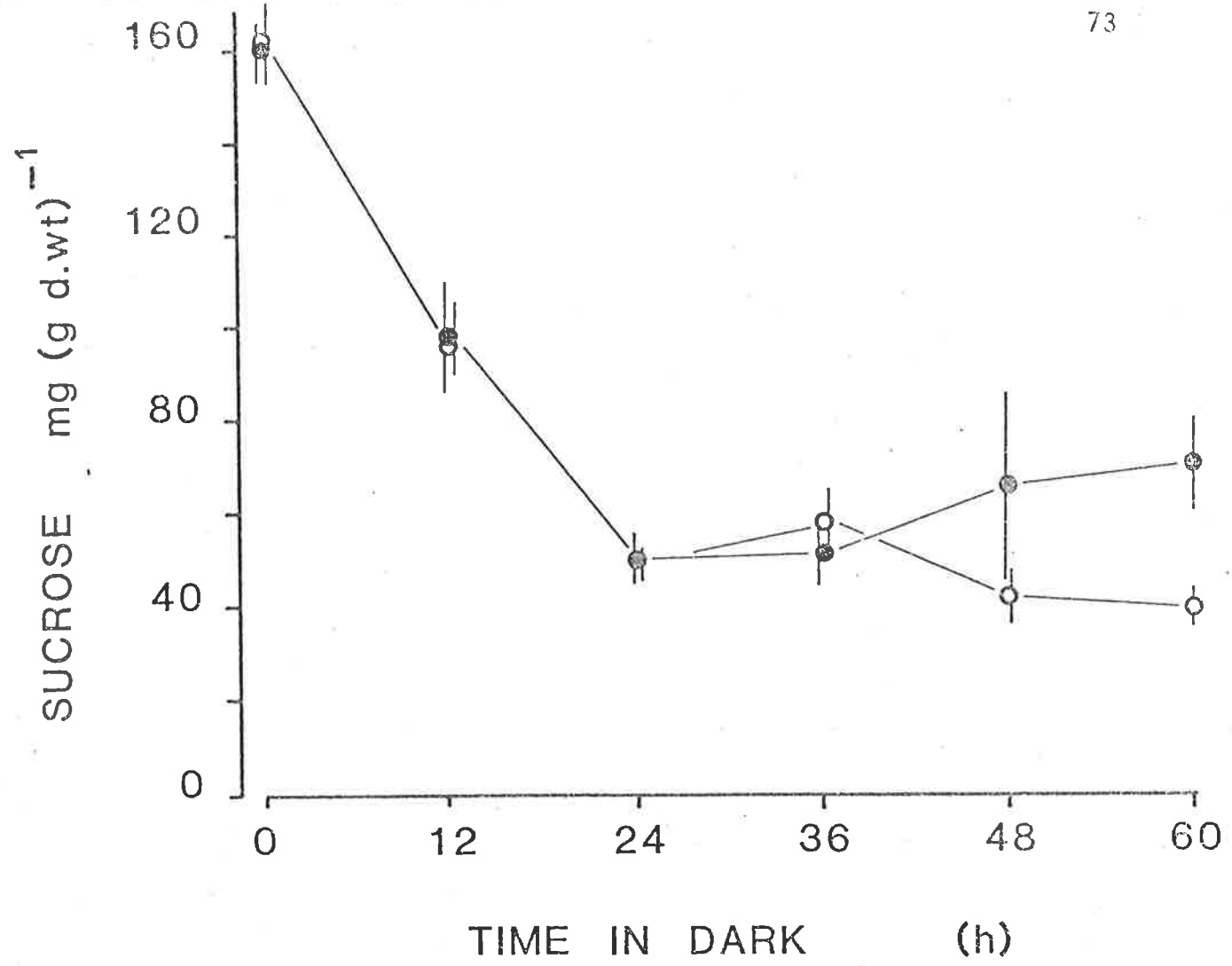

FIGURE 7.5. Time course of soluble carbohydrate (sucrose equivalent) in the fourth mature, attached field bean leaves. Experiment two. Leaves were previously exposed to high irradiance (1100-1300 µmol $m^{-2}s^{-1}$); ●, or low irradiance (600-800 µmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors, and where missing, are contained within the symbols.

TABLE 7.1. Respiration rate after 60 hours of darkness, size of the sucrose pool (in glucose units) and the life of that pool, calculated for mature field bean leaves from two experiments, each run at two irradiance levels. Data are means of five replicates. Units: irradiance, µmol $m^{-2}s^{-1}$; respiration rate mg CO_2 (g dry weight)$^{-1}$ $24h^{-1}$ and mg glucose (g dry weight)$^{-1}$ $24h^{-1}$; pool size, mg glucose (g dry weight)$^{-1}$; pool life, days (24h).

Experiment No:	Irradiance	Respiration rate: mgCO_2	Respiration rate: mg glucose	Pool size	Pool life in days
1	600 - 750	1.04	17.02	72	4.2
1	300 - 500	0.62	10.14	56	5.5
2	1100 - 1300	1.51	24.70	144	5.8
2	600 - 800	1.20	19.64	82	4.2

to the mature leaves. The low specific respiration rate of mature tissue compared to meristematic tissue (Robson and Parsons 1981) and the small proportion of the plant biomass in the form of mature leaves, were both contributing factors. Nevertheless, the leaves accumulated high concentrations of non-structural carbohydrate during the photosynthetic period, and the translocation of this carbohydrate to the growing points in the plant ensured that the rate of biosynthesis in the 'sink' organs (roots and shoot buds), was not limited by substrate during the 12

hour night. This is implied from the constant rate of CO_2 efflux during the normal night.

The linear decline in the rate of CO_2 efflux following leaf removal at the beginning of the night (figure 7.1) implies that this treatment had the effect of inducing a limitation in the supply of substrate for biosynthesis. The reason for this is not immediately obvious, since following the photosynthetic period there should have been considerable soluble carbohydrate within the transport pathways of the plant, as Geiger and Batey (1967), Ho and Thornley (1978) and Pearson (1974) have clearly shown that the rate of carbon export from the leaves in the light is more than double the rate in the dark. Fondy and Geiger (1982) found the rate of carbon import to the sink leaves of sugar beet plants to be similar during both light and dark, and this suggests that the rate of carbon supply to the sink organs is regulated. It was therefore expected, that following defoliation at the beginning of the night, the rate of CO_2 efflux would be buffered by the carbohydrate within the transport pathways and would therefore remain steady for at least a short time (say 1 - 2 hours). However, the rate of CO_2 efflux only remained constant during the 12 hour night, when the mature leaves remained attached to the plant, and this suggests that the mobilised leaf carbohydrate must 'push' the assimilate already in the transport pathways (as a result of carbon assimilation in the light) to the sink organs. This 'push' can be generated during the loading of sucrose into the phloem companion cells of the leaf (or transfer cells if present) and the sucrose can originate either from synthesis during the previous photosynthetic period, or from the degrading starch.

Following depletion of the assimilate contained within the transport pathways, growth and respiration of sink organs is maintained during the night at the expense of sucrose derived from leaf starch degradation. These ideas are consistent with the observations on cucumber plants which were noted by Challa (1976). He found the respiration rate of the roots to be essentially constant during a 24 hour period when plants were grown in 'spring' conditions, whilst a marked diurnal pattern of root respiration was observed when plants were grown in 'winter' conditions, where the respiration rate declined during the last 4 hours of the night. The leaves of 'spring' grown plants were found to respire at a constant rate during the night and the concentration of leaf starch attained a minimum value at the end of the night. In contrast, the respiration rate of leaves of 'winter' plants declined during the night, and leaf starch was exhausted approximately 4 hours before the end of the night.

The general pattern of starch and soluble sugar mobilisation from the mature leaves of field bean plants seems to be established. There is a rapid translocation of starch and soluble sugars during the normal night, but not all of the starch is degraded, and a high proportion of the soluble sugar fraction also remains. The small quantity of starch which survives translocation during the normal night may be exported during the first hour or two of the subsequent photosynthetic period (Pearson 1974). Unfortunately, the leaves were not sampled for starch and sugar analysis at more frequent intervals and the precise pattern of mobilisation of these compounds is not obvious. Nevertheless, the published evidence (Gordon *et al.* 1980; Fondy and Geiger 1982) suggests that at the beginning of the night the sucrose concentration declines first and must fall to a critical concentration within the leaf before the starch is degraded. It may take from one to two hours darkness before starch degradation proceeds, and this time interval is probably dependent upon the concentration of sucrose within, and its rate of translocation from, the leaf.

The mechanism by which sucrose can regulate the degradation of leaf starch is not known for certain, but direct regulation is unlikely because sucrose is present in the cytoplasm and vacuole (Outlaw *et al.* 1975), whilst starch is contained within the chloroplast. The most likely mode of regulation is an indirect one, in which inorganic phosphate has a major role (Herold 1980). In pea chloroplasts, starch breakdown is predominately *via* starch phosphorylase action (Stitt *et al.* 1978), an enzyme which requires inorganic phosphate in order to function. The product of starch degradation is triose phosphate which can be exported to the cytoplasm *via* the phosphate translocator in exchange for further inorganic phosphate. If inorganic phosphate is not available for exchange as, for example, when the level of phosphorylated compounds is high, then starch breakdown is inhibited. When sucrose is synthesised and exported, inorganic phosphate is released, and may then exchange for additional triose phosphate and permit starch degradation.

The data in figures 7.2 to 7.5 inclusive, reveal that the soluble sugars formed the major part of the non-structural carbohydrate pool in the leaves at the end of the daily photosynthetic period. For example, when the concentrations of starch and sucrose, determined at the beginning of the dark treatment, are converted into the equivalent carbon concentrations, the ratios of sucrose to starch in experiment one are: 5.8 for the low irradiance treatment, and 2.8 for the high irradiance treatment. The corresponding ratios for experiment two are: 2.4 and 2.1 for the low and high irradiance treatments respectively. However, since the sucrose concentration within the leaves is relatively constant after 12 hours of darkness (experiment one) or 24 hours of darkness (experiment two), it seems reasonable to expect that the sucrose concentrations at these time periods (in the respective experiments) reflects the concentration of sugars compartmented in the

vacuoles, since cytoplasmic sugars should have been either respired or translocated. If it is further assumed that there was no net sugar accumulation into the leaf cell vacuoles during the previous photosynthetic period, the ratios of sucrose to starch can be recalculated on the basis of cytoplasmic sugars and chloroplastic starch, both of which are the products of photosynthesis during that light period. The ratios reduce to: 2.8 and 1.6, for experiment one (low and high irradiance treatments respectively), and 1.7 and 1.5 for experiment two (low and high irradiance treatments respectively). Huber (1981) found the sucrose to starch ratio to be species dependent. Isolated cells or protoplasts from several crop species were permitted to assimilate $^{14}CO_2$ for 20 minutes and the ratios of sucrose to starch produced were determined to be: 0.8 for peanut, 1.0 for tobacco and soybean, 5.0 for wheat and 5.4 for barley. The present data for field bean is more consistent with the ratios found for the dicotyledonous plants studied by Huber. However, these ratios are calculated on the basis of the daily accumulation of starch, and the instantaneous concentration of sucrose after vacuolar sucrose and translocated sucrose have been taken into account. During the light it is possible, as has been determined in sugar beet leaves (Fondy and Geiger 1982), that the sucrose concentration must increase to a threshold value in the leaf (or specifically within the cytoplasm) before starch accumulation can begin. The sucrose concentration within the leaf may then remain constant and in equilibrium with the rate of translocation. However, to be certain of these events in field bean it is necessary to analyse leaves for soluble carbohydrate and starch concentration during one or two diurnal cycles.

Following the translocation of the sugars from the leaf during the normal 12 hour night, there were still high concentrations of soluble sugars remaining within the leaves. It was estimated (assuming the sugar present in the leaves at the 12 hour time period to be contained in the vacuoles) that the vacuoles contained 50% of the sugar present in the leaf at the end of the photosynthetic period in experiment one (low irradiance treatment). The proportion was about 30% in experiment two. With this, and the foregoing information, consideration can now be given to the source of carbohydrate for leaf respiration during the normal night and the extended dark period.

In experiment one and experiment two leaf respiration during the normal night was calculated to consume between 5 and 9% of the non-structural carbohydrate within the leaf in the form of starch and 'cytoplasmic' sugars. There can be little doubt that soluble carbohydrate was the substrate oxidised during this time, and this conclusion is supported by the high value (greater than 0.9) for the gas exchange quotient which was noted in Chapter VI.

During the 48 hour interval from the 12 hour to the 60 hour sampling period, the pool of soluble carbohydrate within the leaves (which is assumed to be contained within the vacuoles) was of sufficient size to support the measured rate of maintenance respiration of the mature leaves. Furthermore, the carbohydrate pool present at the 60 hour time period was of sufficient capacity to support the measured respiration rate for a further four to six days (table 7.1). This did not mean that sucrose derived from the vacuole was the *only* substrate for respiration, but rather, that the leaf contained a pool of sucrose with the potential to support the observed respiration rate. What is not known, is whether the rate of transfer from the vacuole to the cytoplasm and the mitochondria was sufficient to support the observed rates of respiration during the prolonged dark period. Certainly, the decline in soluble carbohydrate concentration in figure 7.3 (both curves) and in figure 7.5 (low irradiance curve) is consistent with this view. Challa (1976) showed that leaves of cucumber plants grown under 'winter' conditions were completely free of soluble carbohydrate by the end of a 16 hour night. This suggests that if sucrose was stored in the vacuoles of this plant, then transfer across the tonoplast was unrestricted. Also in support of the view that stored sucrose can be mobilised and respired, Farrar (1980) argued that the decay in the respiration of *whole plants* represents a change from a 'mobile' pool to a 'storage' pool of carbohydrate, and not necessarily a change from growth and maintenance respiration, to maintenance respiration only. In relation to switching from growth to maintenance respiration, Farrars' idea is not valid in so far as mature leaves are concerned, since mature leaves are not growing and do not possess 'growth' respiration. In terms of whole plants, Farrars' assertion cannot be challenged for two reasons. Firstly, roots and shoot buds may have continued access to carbon which has been stored within the stem or root, and secondly, meristematic tissues continue to respire at a faster rate than mature tissues, even after 80 hours of darkness (Robson and Parsons 1981). Nevertheless the proportion of tissues which may continue to grow during the prolonged dark period is likely to be very small.

There is one point of evidence which does not support the hypothesis that vacuolar sugars serve as the only substrate for respiration in starved leaves. The data in figure 7.5 (high irradiance curve) shows that a slight increase in soluble sugar concentration occurred after 36 hours of darkness, and this can only be explained as if it is assumed that the loss in dry weight was due to the respiration of a substrate other than the soluble sugar fraction. Furthermore, the gas exchange quotient data presented in Chapter VI (figure 6.4) also suggested that soluble sugars were not the only substrate respired. Carbohydrate is the usual respiratory substrate (ap Rees 1980) and if some other substrate is oxidised, this implies that carbohydrate is either unavailable, or that the rate of supply is insufficient to satisfy the energy requirements of the cell. It seems that during a prolonged dark treatment in some instances (as indicated in figure 7.5, high

irradiance curve) the rate of supply may be insufficient, and,as will be discussed on Chapter VIII RuBPC could be the source of additional respiratory substrate. Clearly the evidence for the view that only vacuolar-derived sugars support respiration in mature leaves during a prolonged dark treatment (Lopes 1979) is inconsistent, however,it is without doubt that a proportion of the sugars which have survived translocation from the leaf can traverse the tonoplast and subsequently undergo oxidation for energy production.

To summarise, the continual export of carbon from the mature leaves serves to maintain the input of carbon to sinks elsewhere in the plant and therefore when whole plants are placed in darkness for longer than about 12 hours, the rate of respiration decays, due to the depletion of starch from the mature leaves. Within the leaves soluble carbohydrate from the cytoplasmic compartments of the leaf serve as the substrate for respiration during the normal night, and stored sugars (located in the vacuoles) can be used as a source of respiratory substrate during an extended dark period. In some instances an additional substrate may need to be oxidised.

CHAPTER VIII VARIATION IN THE PROTEIN CONTENT OF MATURE LEAVES DURING PROLONGED DARKNESS

INTRODUCTION

In Chapters IV to VII, calculations were made in which it was assumed that the rate of CO_2 efflux during a prolonged dark treatment reflected respiration primarily for the maintenance of protein. However, no direct evidence in support of this view has yet been presented, here or elsewhere. Data in Chapter VII indicates that protein may be a source of CO_2 when field bean leaves are kept in prolonged darkness.

Studies using whole wheat plants (Wittenbach 1978) and detached barley leaves (Peterson *et al.* 1973) revealed a large loss in leaf soluble protein within 2 - 3 days of a prolonged dark treatment. Furthermore the photosynthetic enzyme ribulose bisphosphate carboxylase/oxygenase (RuBPC) was the major protein degraded in these studies, accounting for 90% of the soluble protein lost from barley leaves, and 80% of that lost from wheat leaves. RuBPC normally constitutes between 40 and 80% of the total soluble protein in leaves of C-3 species (10 to 20% in C-4 species), depending on the growth conditions.

Results of the time course analyses of RuBPC loss during prolonged darkness by Peterson *et al.* (1973); Peterson and Huffaker (1975) and Wittenbach (1978) showed that the loss of RuBPC paralled in many respects, the decay in whole plant respiration (illustrated in figure 7.1). This observation suggested that during the final stages of the dark decay the leaves may respire amino acids derived from protein breakdown as suggested in Chapter VII, and specifically from RuBPC protein. It is the objective of the present chapter therefore, to determine the extent to which RuBPC protein may be respired in the leaves of field bean plants during a prolonged dark treatment.

METHODS

Two major experiments were conducted as described in Chapter VI. Briefly, field bean plants for experiment one were grown for 21 days after emergence. The light flux densities on the second mature leaf were: 600-750 μmol m^{-2} s^{-1} for a 'high light' treatment, and 300-500 μmol m^{-2} s^{-1} for a 'low light' treatment. The photoperiod was 12 hours, and the temperature was 20°C day and night. At the end of a normal day (12 hours of light) 30 plants were placed in the dark for up to 60 hours, and at 12 hour intervals, five plants were taken for respiration measurement on the attached second mature leaf. Following this measurement, the leaf was detached, the leaf area determined and the leaves frozen in liquid nitrogen and stored at -15°C until freeze-dried. Dried material was weighed, ground to a powder

and re-dried. Soluble protein concentration was determined by the Lowry method (Lowry *et al.* 1951) following extraction in Tris-HCl buffer. RuBPC was quantified by rocket immunoelectrophoresis of an aliquot of the soluble protein extract. Insoluble protein was determined by the Lowry method, following extraction in hot 1M NaOH, and total protein concentration was calculated as the sum of the soluble and the insoluble fractions. The method of analysis are described in detail in Chapter III.

For experiment two, plants were grown for 30 days after emergence. The light flux density on the fourth mature leaf was 1100-1300 μmol m^{-2} s^{-1} for the 'high' and 600-800 μmol m^{-2} s^{-1} for the 'low' light treatments, respectively. The temperature was 20°C day and night, and respiratory measurements were made as for experiment one, except that the fourth mature leaf was used. Dried, ground tissue was subjected to analysis for total protein only, which was extracted in 0.1M NaOH, containing 1% SDS, for 12 hours at 80°C. Protein concentration was determined using the Lowry assay.

Chlorophyll measurements were made on leaves which remained attached or were detached from the parent plant and kept in the dark for up to 60 hours. Two separate batches of plants were grown as in experiment one except that the second mature leaf received higher irradiance (1100-1300 μmol m^{-2} s^{-1}). At the end of a normal day, whole plants from one batch were placed in a darkened incubator set at 20°C, whilst leaves were excised from another batch of plants, floated on water in plastic boxes, and then placed in the incubator. At 12 hour intervals five of the detached leaves were frozen in liquid nitrogen and stored at -15°C until freeze-dried. Dried leaves were then ground with a mortar and pestle and 10 mg was extracted in 80% acetone. The extract was assayed for chlorophyll according to the method of Arnon (1949). Following excision from the parent plants at 12 hour intervals, 'attached' leaves were frozen in liquid nitrogen and freeze-dried. Discs were punched from the dried leaves which were then ground, extracted with 80% acetone and assayed for chlorophyll as above.

In order to determine the effects of prolonged darkness on the photosynthetic apparatus a batch of 30 plants were grown as for experiment one. At the end of the normal day plants were placed in a darkened incubator for up to 60 hours. At 12 hour intervals five plants were removed from the incubator, and the net carbon assimilation rate of the attached second leaf of each plant determined upon exposure to an irradiance of 200 μmol m^{-2} s^{-1}.

RESULTS

The Time Course of Leaf Chlorophyll Content

Changes in chlorophyll *a* content for both attached and detached leaves during

60 hours of darkness are shown in figure 8.1. The induction of senescence is sometimes accompanied by the loss of leaf chlorophyll, however it is clear from figure 8.1 that no loss occurred in these experiments. An analysis of variance indicates that the chlorophyll *a* content in the attached leaf treatment is significantly higher ($P < 0.01$) at the zero and 36 hour time periods than at the other sampling times, but the reason for this difference is not known. There is also a significant difference ($P < 0.001$) between the treatments, and the chlorophyll concentration may be higher in the 'attached' than the 'detached' treatment due to the combined effects of variation between the two batches of plants and the method of analysis. Chlorophyll content in the 'attached' leaves was determined from leaf discs punched from interveinal areas, whilst the analysis on detached leaves was made on a leaf powder containing veins, which could effectively reduce the amount of chlorophyll containing material taken for analysis.

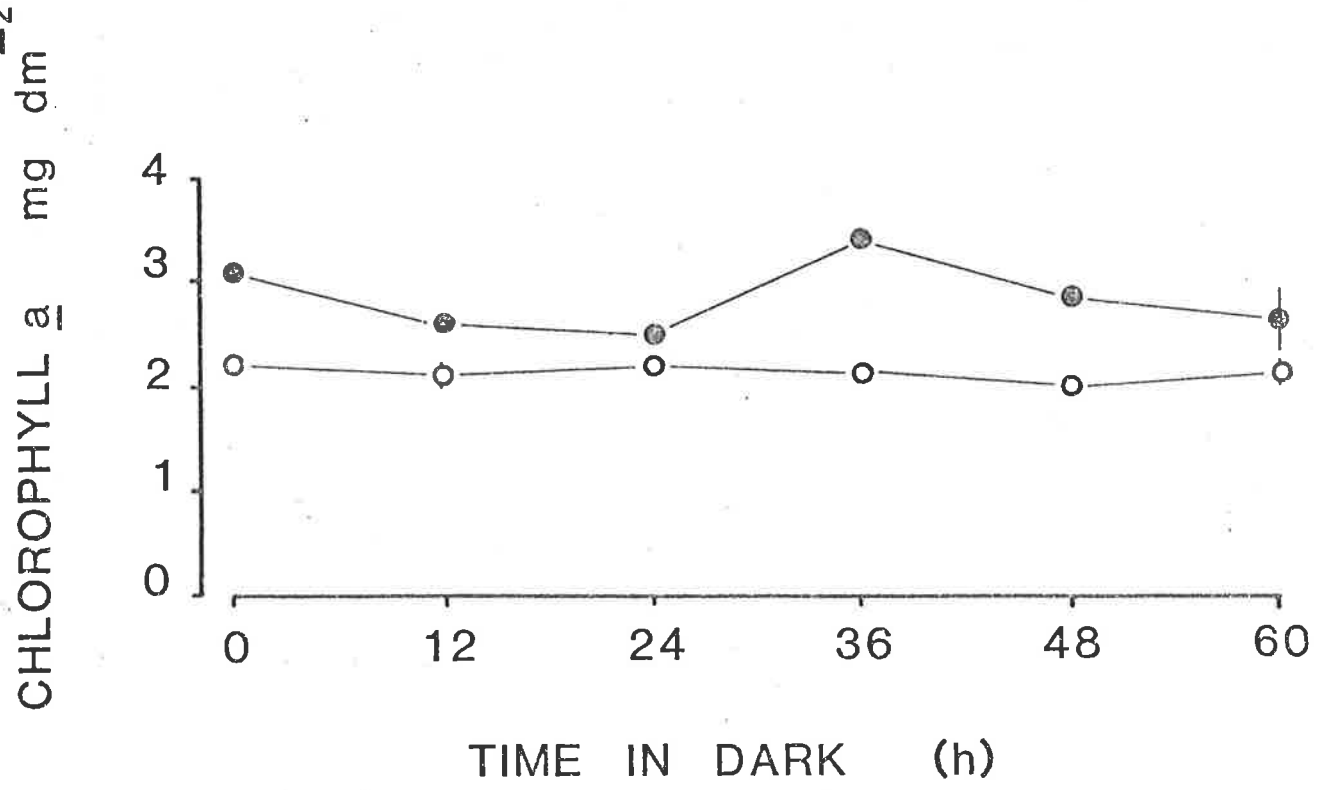

FIGURE 8.1. Time course of the chlorophyll *a* concentration in attached; ●, and detached; O, mature field bean leaves. Bars represent standard errors, and where missing, are contained within the symbols. $LSD_{[.05]} = 0.3$.

The Time Course of Leaf Protein Concentration

soluble protein

The concentration of soluble protein appeared to remain fairly constant throughout the 60 hour dark period (figure 8.2). An analysis of variance confirms this conclusion and furthermore, indicates that there were no differences between the treatments. The protein concentration appeared to increase after 12 hours of

darkness in the high irradiance treatment, however leaves initially had a high starch content (Chapter VII) which effectively 'diluted' the protein concentration at the first period of measurement.

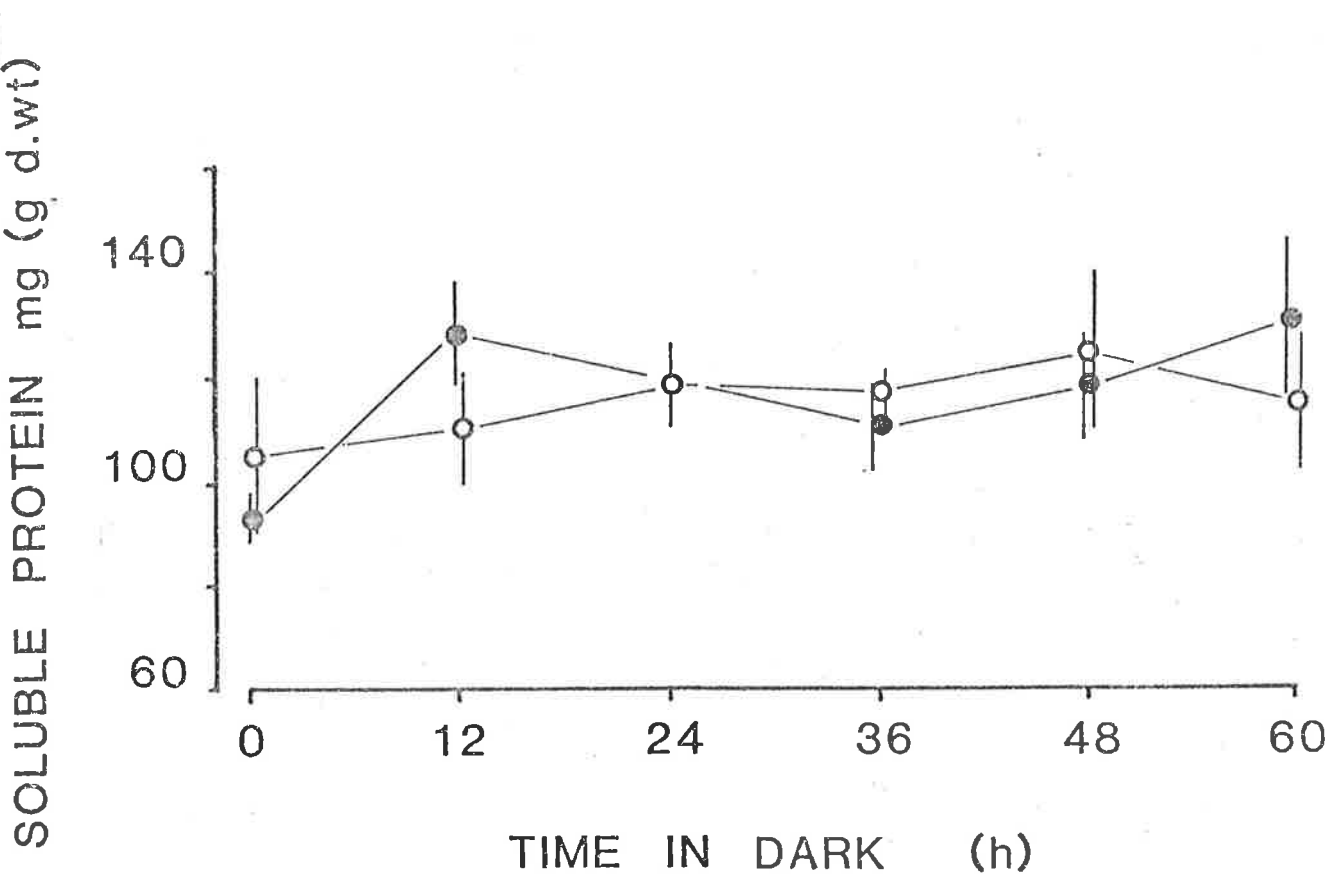

FIGURE 8.2. Time course of soluble protein concentration in the second mature field bean leaves. Experiment one. Leaves were previously exposed to high irradiance (600-750 µmol $m^{-2}s^{-1}$); ●, or low irradiance (300-500 µmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors.

The time course of RuBPC concentration is shown in figure 8.3. Despite the large variation in the data for the low light treatment at 36, 48 and 60 hours, there was no evidence for a decline in the concentration of this protein in prolonged darkness. This view is supported by an analysis of variance, which in addition, revealed that there was a significant difference ($P < 0.01$) between the two treatments. A difference is not surprising, but the fact that the concentration of RuBPC was higher in the leaves of plants grown under low irradiance than in the leaves exposed to the high irradiance was unexpected. Freidrich and Huffaker (1980) reported that the proportion of the soluble protein in the form of RuBPC was about 80% in barley leaves grown under an irradiance of 550 µmol m^{-2} s^{-1} compared to 50% in leaves grown under 400 µmol m^{-2} s^{-1}. As shown in table 8.1, this trend is not apparent in the present results.

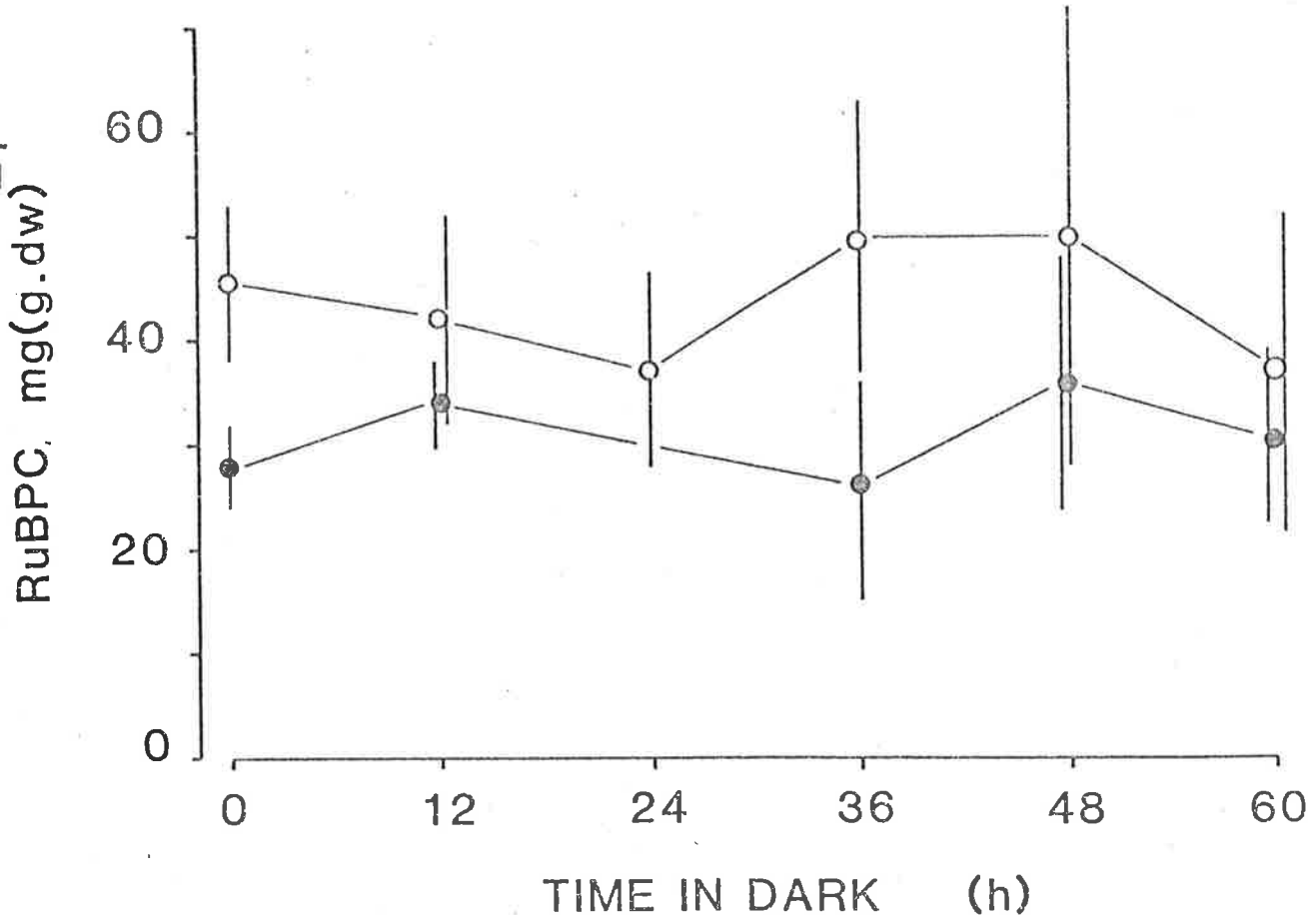

FIGURE 8.3. Time course of ribulose bisphosphate carboxylase/oxygenase (RuBPC) concentration in the second mature field bean leaves. Experiment one. Leaves were previously exposed to high irradiance (600-750 μmol $m^{-2}s^{-1}$); ●, or low irradiance (300-500 μmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors. $LSD_{[.05]}$=15.

The reason for this unexpected result is not known, however it is possible that the long term storage of the high irradiance tissue (which was stored at -15°C for one month after completion of the respiratory measurements, freeze-dried and stored at -15°C for a further eight months) was a contributing factor. During such long term storage, proteases may have degraded the RuBPC protein specifically, or some modification to the active sites may have reduced the immune response of the antibody to the antigen. Since the soluble protein concentration was the same in both treatments (it was expected to be higher in the high irradiance treatment) the degradation of RuBPC to amino acids by proteases may be the best explanation. The low irradiance samples were processed soon after completion of the measurements (two weeks), and the proportions of RuBPC to the soluble protein (table 8.1) are of the expected magnitude. The concentration of RuBPC determined in the 'low' irradiance material should therefore be accurate. This result illustrates the need for rapid processing of plant material following removal from the plant.

insoluble protein

Variation in insoluble protein concentration is shown in figure 8.4. There was a gradual increase in concentration with time in the dark which was significant

TABLE 8.1. RuBPC concentration as a percentage of both the soluble protein, and the total protein content of mature field bean leaves grown under high irradiance (600-750 μmol $m^{-2}s^{-1}$) or low irradiance (300-500 μmol $m^{-2}s^{-1}$). Values are means together with the standard errors.

Time in dark (hours)	High irradiance		Low irradiance	
	soluble	total	soluble	total
0	30.5 ± 6.1	17.9 ± 3.5	46.0 ± 13.3	24.7 ± 5.1
12	29.9 ± 7.7	16.9 ± 1.3	38.1 ± 4.9	21.1 ± 5.6
24	-	-	31.4 ± 5.8	19.3 ± 4.8
36	23.8 ± 8.4	13.0 ± 5.5	46.3 ± 13.1	29.1 ± 13.0
48	30.3 ± 9.4	16.9 ± 5.7	42.5 ± 18.9	23.6 ± 10.2
60	24.3 ± 7.5	13.6 ± 3.7	31.6 ± 7.2	15.6 ± 6.3

($P < 0.01$), but there was no difference between the treatments. The insoluble protein fraction represents mainly membrane bound and other structural protein, and the increasing concentration noted was consistent with the view that dry weight loss was from some alternative carbon source.

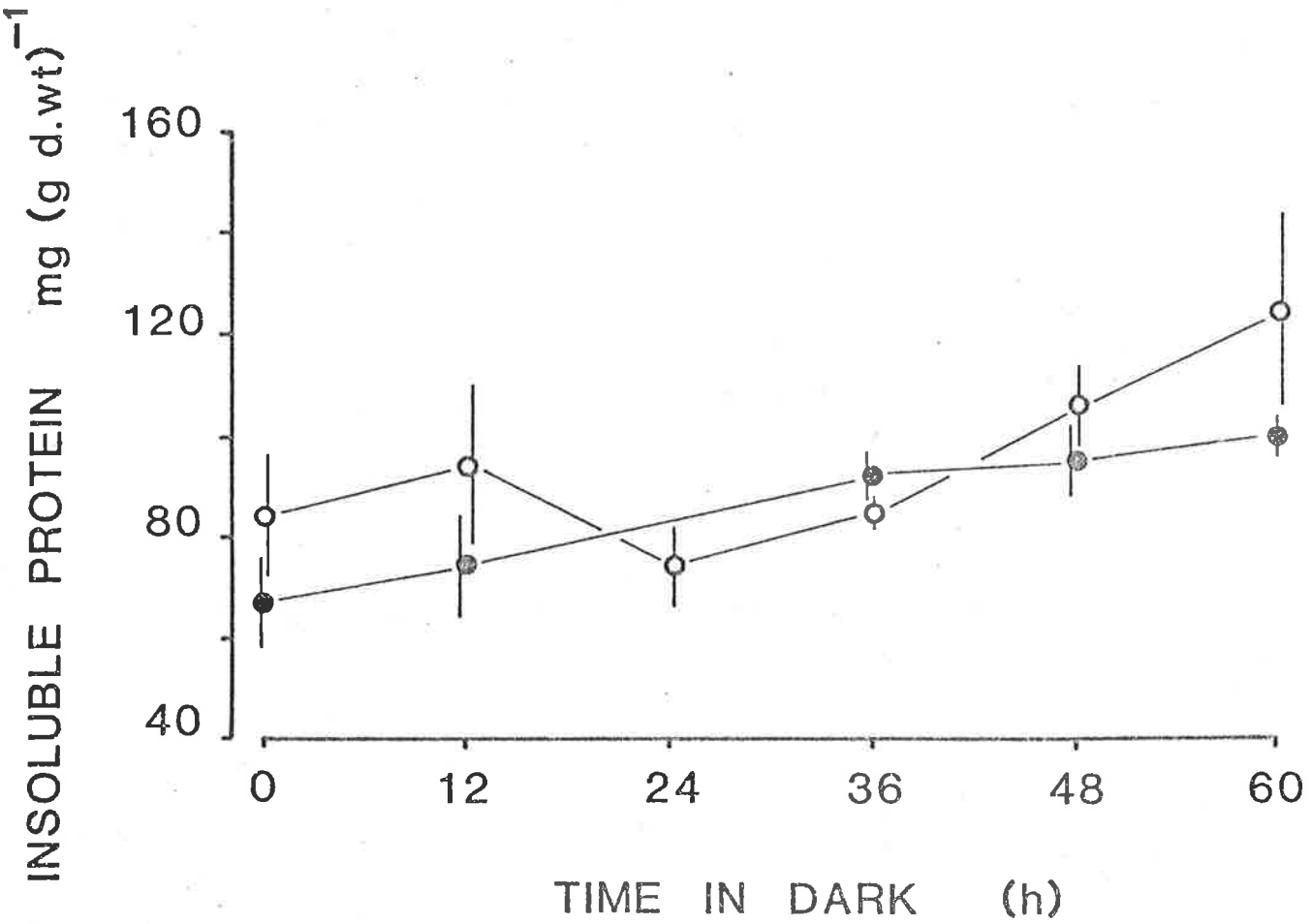

FIGURE 8.4. Time course of insoluble protein concentration in the second mature field bean leaves. Experiment one. Leaves were previously exposed to high irradiance (600-750 μmol $m^{-2}s^{-1}$); ●, or low irradiance (300-500 μmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors. $LSD_{[.05]} = 27$.

total protein

There was also a significant increase ($P < 0.01$) in the total protein concentration with time in the dark (figure 8.5), but surprisingly, there was no effect of irradiance level. Indeed, the light treatments were originally introduced in order to evaluate the effects of altered protein concentration on maintenance respiration, and it is possible that this result arose either because the light treatments were not sufficiently different, or because the second leaf was more dependent for its development on seed nitrogen reserves rather than on currently reduced NO_3^-.

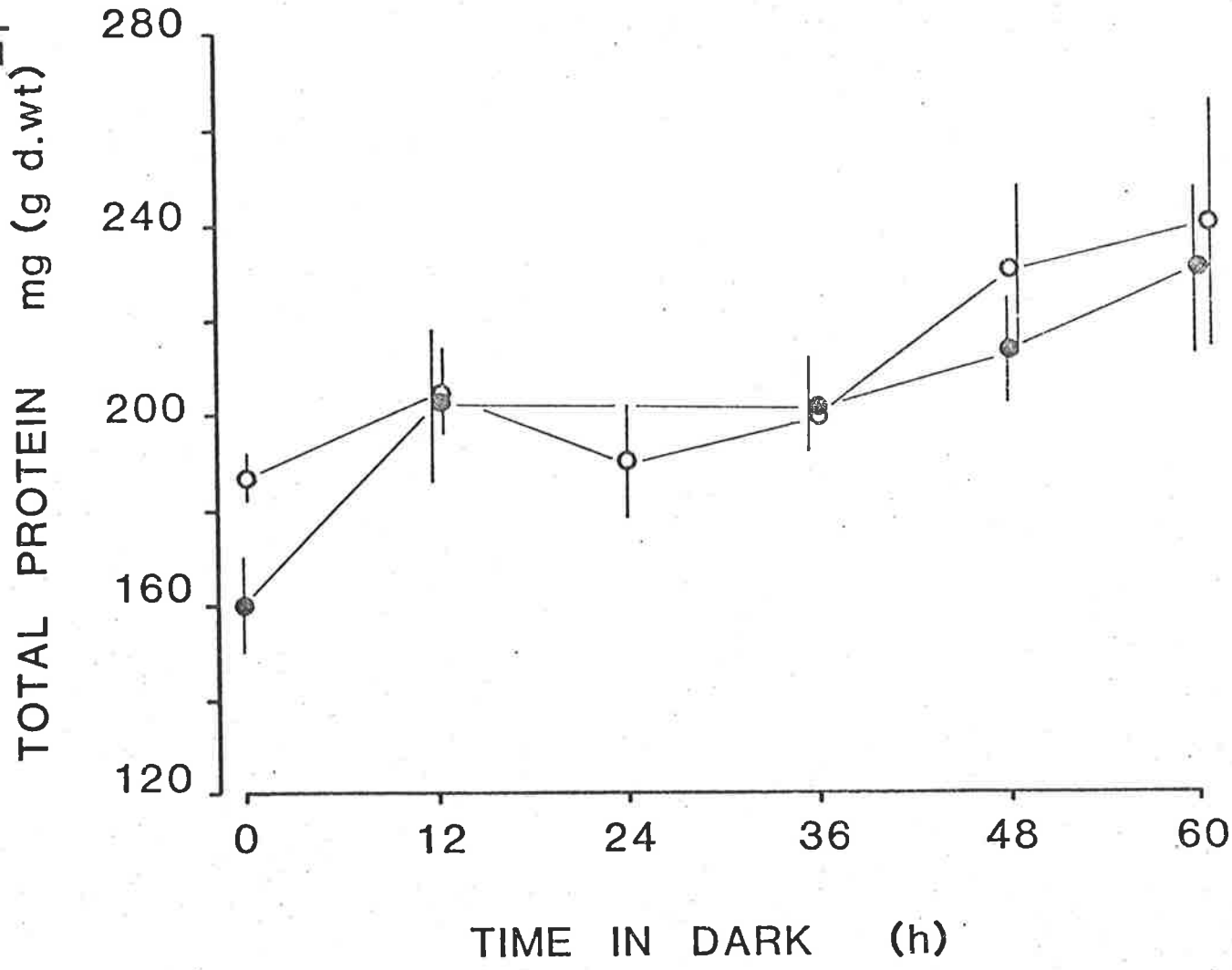

FIGURE 8.5. Time course of total protein concentration in the second mature field bean leaves. Experiment one. Leaves were previously exposed to high irradiance (600-750 μmol $m^{-2}s^{-1}$); ●, or low irradiance (300-500 μmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors. $LSD_{[.05]} = 37$.

A second experiment was therefore designed, which incorporated more contrasting irradiance regimes and older plants. The fourth mature leaf was chosen for study and there was additional replication. Since the data from experiment one showed no decline in protein concentration from the soluble or insoluble fraction only the total protein was assayed in experiment two. The

data (figure 8.6) largely confirm the previous results. There was a significant effect of time in the dark ($P < 0.001$), and in addition, the expected difference in protein concentration between the light treatments was realised ($P < 0.001$). Moreover, in accordance with the higher irradiance regimes used, the concentration of the total protein was considerably higher in experiment two than was the case in experiment one.

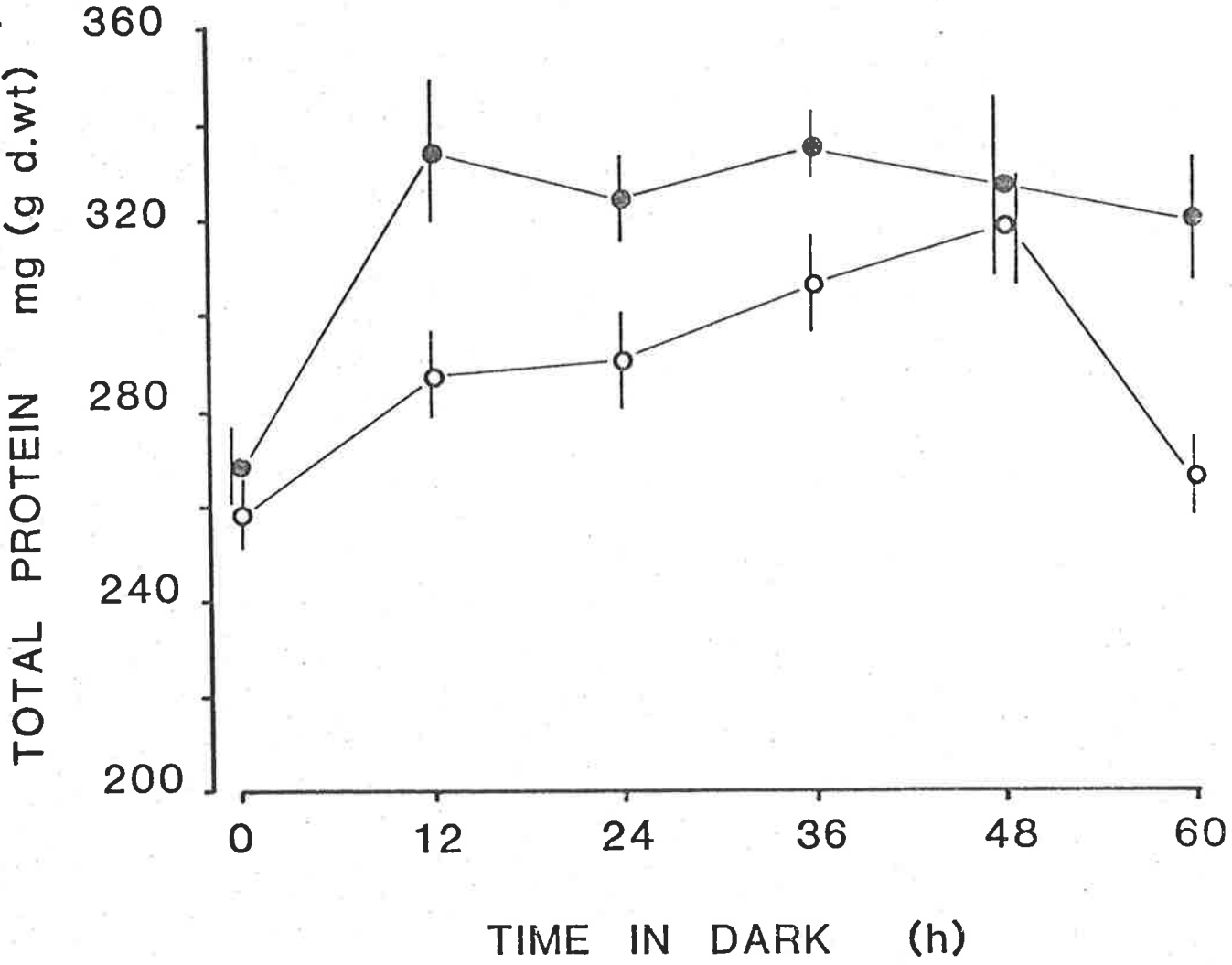

FIGURE 8.6. Time course of total protein concentration in the fourth mature field bean leaves. Experiment two. Leaves were previously exposed to high irradiance (1100-1300 µmol $m^{-2}s^{-1}$), ●, or low irradiance (600-800 µmol $m^{-2}s^{-1}$); ○, during growth. Bars represent standard errors. $LSD_{[.05]} = 31$.

The protein concentration appeared to increase after 12 hours in the dark in the high light treatment (figure 8.6), but thiswas undoubtedly due to protein 'dilution' by the high level of starch in the tissue immediately following the photosynthetic period, as explained previously. Subsequent to the 12 hour time period, there was no change in protein concentration. In the low light treatment, there was a significant, but gradual increase in protein concentration for up to 48 hours in the dark, but contrary to the previous results from experiment one, there was a rapid loss of protein during the ensuing 12 hour period.

Theoretical Protein Degradation Rates

The data for maintenance respiration from table 6.4 were used to calculate a potential rate of protein loss and the theoretical maximum life of leaf protein which could be expected if the respiration rate represents CO_2 loss solely from protein, and that the respiration rate continues unabated until all protein is degraded. The results (table 8.2) showed that the calculated rates of protein loss would account for the loss of between 8.5 and 17.4 (mean : 12.5) mg protein (g dry weight)$^{-1}$ 24h^{-1}. Losses of this magnitude are not apparent upon inspection of figures 8.5 and 8.6, and the decline in protein concentration after 48 hours darkness in the low light treatment (figure 8.6), was far greater than could be expected if carbon residues resulting solely from protein degradation were respired (the observed decline was *ca* 50 mg protein (g dry weight)$^{-1}$ in a 12 hour period).

TABLE 8.2. Average leaf protein content, calculated rate of protein loss and theoretical protein life for mature field bean leaves. The second mature leaf was used for measurements in experimental one, whilst the fourth mature leaf was used in experiment two. Measurements were made after 60 hours of darkness. Plants were grown in two experiments and in each, leaves were exposed to contrasting irradiance regimes. Units are: irradiance, μmol m^{-2}s^{-1}; protein content, mg leaf^{-1}; protein loss, mg leaf^{-1} 24h^{-1} and mg (g dry weight)$^{-1}$ 24^{-1}; protein life, days (24h).

Experiment No.	Irradiance	Protein content	Protein loss per unit: leaf	g d.wt	Protein life in days
1	600 - 750	16.0	0.87	17.4	18
1	300 - 500	13.8	0.44	8.5	31
2	1100 - 1300	19.5	1.03	12.7	20
2	600 - 800	22.1	1.19	12.4	19

Recovery of Photosynthesis After a Prolonged Dark Treatment

At 12 hour intervals after darkening (including the first hour), attached leaves were exposed to an irradiance of 200 μmol m^{-2} s^{-1} and the net carbon assimilation rate was recorded when a steady state had been achieved. The results (figure 8.7) showed there to be no significant decline in the rate of net carbon assimilation with time in the dark, if the rate at zero time is taken as the reference. There was however, a significant effect ($P < 0.01$) of time in the dark. The rate of net CO_2 assimilation increased following exposure to light after the leaves had been kept in the dark for 12 hours, and this rate was maintained at a uniformly high value for between 12 and 36 hours in the dark. After 36 hours darkness, there was a rapid decline in the ability of the leaf to assimilate CO_2 upon re-illumination.

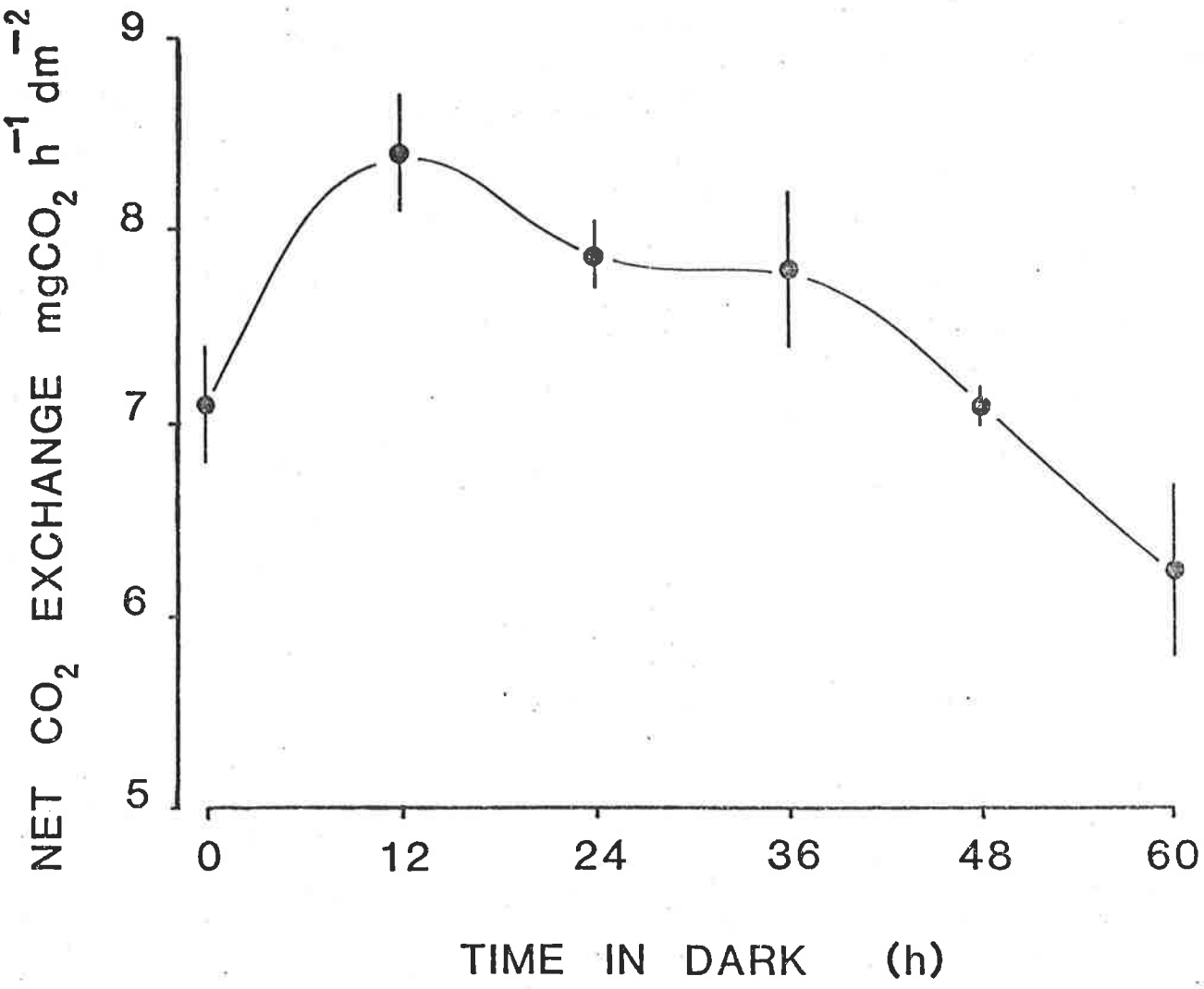

FIGURE 8.7. Net carbon exchange rate of the second mature, attached field bean leaves, when kept in prolonged darkness and periodically exposed to an irradiance of 200 μmol $m^{-2}s^{-1}$. Bars represent standard errors. $LSD_{[.05]}$ = 0.9.

DISCUSSION

Measurements of the total protein concentration in mature field bean leaves during a 60 hour dark treatment, revealed there to be no significant decline in protein concentration for the first 48 hours*. However, for the concentration of both the soluble protein and the RuBPC protein fractions to remain constant with time in the dark,despite there being a gradual loss in dry weight (due to respiration),indicates that both protein fractions declined at the same rate as the decline in the dry weight. This suggests that carbon residues from the degradation of RuBPC could have been utilised in respiration. In addition to serving as a substrate for respiration another possible fate for amino acids derived from RuBPC degradation is their translocation from the leaf to other parts of the plant. Indeed, RuBPC is considered to be a major source of carbon and nitrogen for the growth of developing tissue during senescence of mature leaves. However apart from the data in figure 8.6 (low irradiance curve), where the rate

* There was also no significant difference in the amount of protein per unit leaf throughout this period (data not shown).

of protein loss after 48 hours darkness was far higher than the loss calculated on the basis that the protein was respired, there was no evidence for the translocation of protein from the leaf previous to the 48 hour time period in any experiment.

The data in figure 8.7 clearly show that following 12 hours of darkness the ability of the leaf to assimilate CO_2 was impaired. It is equally clear, that at time zero the rate of net carbon assimilation was depressed. This may have been due to stomatal closure, but unfortunately, measurements of stomatal conductance were not made. Another explanation may be that the accumulated starch and soluble sugars caused feed-back inhibition of photosynthesis (Neales and Incoll 1968). Although the evidence in favour of this hypothesis is still equivocal, there is increasing evidence (Setter *et al.* 1980, and references cited therein), that the reduction in photosynthetic rate observed in source-sink manipulated systems, which may lead to the accumulation of non-structural carbohydrates, is effected by stomatal closure. However, the potential to assimilate CO_2 was increasingly lowered subsequent to the 12 hour time period, and this observation is consistent with the idea that the quantity of RuBPC had declined. A less likely explanation for the progressive reduction in the ability of leaves to assimilate CO_2 with time in the dark is that the chloroplast membranes were degraded, due to a gradual increase in the concentration of the insoluble protein fraction (figure 8.4). In addition, Wittenbach (1977) has shown that membrane permeability was maintained in leaves of wheat seedlings for periods of up to five days of darkness and could be extended to eight days with cytokinin treatment. Lem *et al.* (1980) found that dark incubation of field bean leaf discs for up to 96 hours, had little effect on both the quantity or quality of fatty acids in the major lipid fractions. Interestingly, current opinion is that proteases, which may be specific for RuBPC, are located within the chloroplast (Nettleton, Bhalla and Dalling unpub. 1983), suggesting that RuBPC can be degraded without the breakdown of the chloroplast membrane, and without concomitant loss of chlorophyll.

The loss of RuBPC in these experiments was sufficiently small to have had no measurable effect on the total protein concentration (figure 8.5), and the data in figure 6.2 showed the dry weight loss to be very small. In addition, the data in figure 8.2 show the rate of protein loss to be considerably lower than the loss calculated by assuming RuBPC was the sole source of carbon for respiration. Collectively, these observations support the conclusion drawn in Chapter VII, that a substrate in addition to carbohydrate may contribute to the CO_2 efflux when leaves are kept in prolonged darkness. This additional substrate appears to be RuBPC. Therefore, whilst maintenance of non-RuBPC protein seems to occur during a short dark period, RuBPC seems to degrade. Whilst the maintenance of non-RuBPC is inferred from the present data clear evidence for this viewpoint was presented by Peterson and Huffaker (1975).

The degradation of RuBPC should not be surprising, since if the plant is kept in prolonged dark it becomes heterotrophic. Leaves contain a considerable store of carbon and nitrogen in the form of RuBPC (25% of the total leaf protein content in the low irradiance treatment, table 8.1), which is a photosynthetic enzyme no longer required, and it seems reasonable to expect this protein to be preferentially degraded either to provide substrate for energy production or to enable the translocation of carbon and nitrogen from the leaf to points where these elements are in demand for growth. If it is considered from the viewpoint that the leaf and/or plant is adapting to a changed environment (continual darkness), the loss of RuBPC protein should not be alarming, since it seems that RuBPC can fulfil the role of a true 'storage protein' as suggested by Huffaker (1982). In the absence of amino acid translocation . the oxidation of amino acids is expected to contribute to respiration for cell maintenance.

Several published reports also illustrate the ability of plant material to maintain the protein content within the tissue during brief periods of darkness. For example, Goldthwaite and Laetsch (1968) recorded the retention of chlorophyll and protein in *Rumex* sp. leaf discs for up to two days, and Dungey and Davies (1982) found no measurable loss of protein from barley leaf segments floated on water for 60 hours. Peterson and Huffaker (1975) showed that only a minimal loss of non-RuBPC protein occurred from barley leaves during 72 hours of darkness, whilst there was no loss in RuBPC activity until after 30 hours darkness (Peterson *et al.* 1973). Wittenbach (1978) found that senescence in wheat seedlings was completely reversible during the first two days of a dark treatment, and Vonshak and Richmond (1975) showed that ^{14}C-leucine incorporation into isolated tobacco leaf chloroplasts, extracted from leaves and stored in darkness, was reduced by 40% after two days of dark, but was unimpaired if the leaves had been treated with kinetin. Van Staden and Carmi (1982) showed that leaves of decapitated *Phaseolus vulgaris* retained 75% of their soluble protein over an eight day dark period.

These studies show that a short period of darkness does not inevitably lead to senescence, but rather, that leaves are able to maintain a level of protein; to reverse the train of catabolic events; and to initiate anabolic reactions when light is provided. The ability of attached leaves to recover from a dark period of short duration may be due to the supply of growth factors from the parent plant which stimulates synthesis of degraded protein. The calculations in table 8.2 indicate that considerable protein degradation was possible, but since protein loss was very small, synthesis must have also occurred, i.e. protein was turning over. A growth factor such as a cytokinin produced in the roots (Carmi and Koller 1979) could promote protein synthesis in shoots for a short time after darkening, but the restricted transpiration over a prolonged period of darkness may reduce the rate of cytokinin transport to the leaves leading to net protein degradation as a result of

the synthesis or activation of proteases (Peterson and Huffaker 1975). However, whilst cytokinins may have an important role in maintaining protein synthesis, they may not be the ultimate governing factor since attached mature leaves of *Lolium temulentum* kept in a CO_2-free atmosphere still lost protein and chlorophyll even though they were exposed to light (Lloyd 1980).

The data presented in this chapter illustrate that no major change in protein content occurred in field bean leaves during 48 hours of darkness, and this suggests that the steady state rate of CO_2 efflux from attached leaves is a reasonable reflection of the intensity of the maintenance processes in the absence of the assimilate loading component of respiration. The single instance when a rapid loss of protein occurred cautions against prolonging the dark treatment much beyond 48 hours.

From an ecological point of view, a short period in the dark may not reduce plants to a state far from normality, since this treatment can be compared to the experience of one or two cloudy days in the field. Under this circumstance, the incident irradiance could be lower than the compensation level and require the mobilisation of stored carbohydrates to enable continued growth. Clearly, there can be no advantage to the plant in initiating senescence processes under such conditions. Perhaps one qualitative difference between complete darkness and low irradiance is that the latter treatment may provide the low level energy requirements for phytochrome mediated morphogenic processes, however, the importance of such processes in the short term are unlikely to be important.

CHAPTER IX GENERAL DISCUSSION

The objective of the present study was to obtain experimental data for the maintenance requirements of some crop legumes. The need for these measurements arises for three main reasons. Firstly, the limits on the theoretical maintenance requirements calculated from basic data by Penning de Vries (1975a), are broad and experimental data are needed to verify the calculations. Secondly, synthesis respiration is sometimes calculated as the difference between the total respiration and the maintenance respiration, and it is therefore essential for estimates of the latter to be accurate, although more recently, the growth efficiency has been determined directly by application of the Thornley balance equation (Thornley 1976). Thirdly, there is a need to monitor progress in breeding plants with 'low' maintenance respiration, since selection lines of *Lolium perenne*, possessing 'low' rates of mature leaf respiration, were found to have superior rates of dry matter production than did lines with 'high' mature leaf respiration (Wilson 1975).

This final discussion is organised in four parts. The first is an appraisal of the methods for measuring maintenance respiration and it is argued that although the steady state (starvation) method gives estimates of maintenance which are minimal values, the method is not an abstraction from real conditions. In the second part, it is argued that the respiration of mature leaves should be included as a part of 'maintenance' rather than allocated to synthesis. The third is a discussion of the sugar pools available for growth and maintenance respiration. The fourth is a brief discussion of modifications to plant processes which may improve the quantity and quality of harvestable product.

METHODS FOR THE MEASUREMENT OF MAINTENANCE RESPIRATION

Three methods of measuring the maintenance component of CO_2 exchange were: (i) to allow the CO_2 efflux to decay in prolonged darkness to an asymptotic value which is then taken to be the maintenance value (the steady state method); (ii) to plot the dark CO_2 efflux as a function of net CO_2 uptake over a range of irradiances and take maintenance as the dark efflux when the net CO_2 uptake is zero (the dynamic method); (iii) to plot total CO_2 uptake as a function of the growth rate and take maintenance as the CO_2 efflux when the growth rate is zero (the gross uptake method).

The magnitude of the maintenance coefficient was dependent on the definition of maintenance adopted. Values were generally lowest for the steady state method and highest for the gross uptake method. The dynamic method generally produced

intermediate values, and this order is expected from the geometrical relationships shown in figure 2.4.

It usually took about 48 hours in the dark before the rate of CO_2 efflux became stable, when employing the steady state technique. Clearly, if there were differences in the rate of maintenance respiration during the light and the dark then these differences could not be elucidated using this approach, and evidence in Chapter VI showed that the respiratory component arising from mature leaves and associated with sucrose synthesis and phloem loading, disappeared after 24 hours in the dark.

For the dynamic method, maintenance respiration was assumed to represent the rate of CO_2 efflux when the net daytime assimilation of CO_2 was zero. A further assumption, that the rate of CO_2 efflux was the same during the light as in the dark, was also made. As discussed in Chapter II, this issue remains contentious, although the recent study by Fondy and Geiger (1982) which showed the rate of carbon input into a monitored sink leaf of sugar beet to be similar during the day and the night, and the study by Challa (1976) which showed the root respiration rate to remain constant in cucumber plants grown in 'spring' conditions, is evidence that the latter assumption was reasonable. However, the evidence that the respiration rate of mature leaves is the same during the light and the dark remains equivocal.

The fact that the dynamic method employed 'normal' diurnal fluctuations in irradiance, and that the maintenance coefficients determined by the dynamic and steady state techniques were similar, indicates that the prolonged dark treatment does not reduce the plant to an abnormal condition as suggested by Robson and Parsons (1981). Instead, this result indicates that both methods essentially deplete the pool of mobile carbohydrate which is readily used for growth and maintenance. The magnitude of the phloem loading component in mature leaves should therefore be small when the daily assimilation of CO_2 is zero, and this appears to be the reason that the two methods gave similar results. In an ecological context, it is highly likely that for part of the growing seasons, crops experience extended periods of low irradiance either because of cloud cover or mutual shading. This could effectively confine the daily CO_2 assimilation and therefore, the night-time CO_2 efflux, to considerably lower values of 'D' and 'N' than those which are commonly imposed in assimilation chambers in the laboratory.

The maintenance coefficient determined using the gross uptake method represented the specific rate of CO_2 efflux when the daily accumulation of CO_2 was zero. The assumption was made that the daytime carbon assimilation and the nighttime CO_2 efflux were in equilibrium (this assumption also applied when the dynamic method was used), and therefore, that the 'total' assimilation, calculated

as the sum of the daytime CO_2 uptake and the nighttime CO_2 efflux, was completely utilised for growth and maintenance during a 24 hour period. These assumptions are required in the application of the Thornley balance equation for the determination of growth and maintenance coefficients. The sudden decay in the dark respiration rate when the night is extended beyond 12 hours (figure 7.1) is considered good evidence for the equilibrium between respiratory substrate production and consumption. The 'gross uptake' maintenance coefficient was determined when 'D' was greater than zero in contrast to the value for 'D' utilised in the dynamic method (where D = 0) and the value in the steady state method (where D < 0). Although the maintenance coefficient was determined when the daily growth rate of biomass carbon was zero, growth in structure can occur nevertheless, since carbon which is accumulated in the leaves during the light can be distributed to other parts of the plant during the night. Such growth is also possible when the dynamic method is used, but probably to a lesser degree because although the net CO_2 uptake was zero during the day, there was still a flux of carbon through the plant. Since daily carbon accumulation is possible in leaves, the 'phloem loading' component of leaf respiration can also contribute to the overall CO_2 efflux from the plant. The gross uptake method probably overestimates the 'true' maintenance requirement because growth of new structure is likely to be considerably greater than when the maintenance coefficient is determined by the other methods.

Most of the assumptions made in growth and maintenance analyses are common to all the methods used, and many may not yet be regarded as proven. Especially problematical in the analyses, is the assumption that the rate of CO_2 exchange in the light, is the same as in the dark in spite of the obvious occurrence of processes such as ion uptake and nitrate reduction, which may vary in intensity diurnally, and which may also utilise photosynthetic energy produced in the leaves. It must therefore be stated, that complete confidence in the ability of any particular method to estimate maintenance accurately cannot be given. However, in accordance with the definition of maintenance adopted in this thesis, the steady state and the dynamic methods are judged to give the 'best' estimates for maintenance energy requirements in the crop legumes studied.

Although the steady state and the dynamic methods yield essentially identical estimates of the maintenance coefficient (table 5.1) the former method is favoured for the estimation of maintenance respiration in crop legumes for the reasons that the technique is quick, simple to use and is amenable to the mass screening of plants with 'low' rates of maintenance respiration (McCree 1974). The method does, however, give an estimate of the minimal maintenance requirements because the transport component of mature leaf respiration is no longer present after about 24 hours in the dark (Chapter VI). Further discussion of this is reserved for the next section.

An argument that a prolonged dark treatment is not abnormal needs substantiating. Penning de Vries (1975a) stated that "the term 'maintenance' is confusing in this <<prolonged dark>> situation since the plant does not maintain its structure". The generality of this statement is not attested by evidence of Farrar (1980) who showed that respired carbon originated from the structural material of 13 day old barley plants only after 48 hours of darkening. Evidence from the present study strengthens support for the view that at least some legumes can maintain the integrity of their cells during a prolonged dark treatment.

The data in Chapter IV which reveals a strong dependence of the maintenance respiration rate of whole plants on the organic nitrogen (i.e. protein) content indicates that protein is being maintained in prolonged darkness. Furthermore, measurements of protein content in mature leaves (Chapter VIII) revealed there to be no loss of protein for at least 48 hours of darkness. Barratt and Woolhouse (1981) calculated the half life of the total soluble protein in the primary leaves of *Phaseolus vulgaris* to be about 1.9d and should this value be representative of the half life of the soluble protein in field bean leaves, then the soluble protein concentration should have been halved after 48 hours in the dark if protein maintenance did not occur. Quite clearly, the respiration of starved plants does in part represent energy production for the maintenance of protein content. Additional evidence for the maintenance of leaf structure arises from the observation that field bean leaves were able to assimilate CO_2 immediately upon re-illumination at any time throughout a 60 hour dark treatment. Eschrich and Burchardt (1983) also observed the immediate assimilation of CO_2 upon re-illumination of maize leaves following a 48 hour treatment. Furthermore, there was not a rapid loss in RuBPC from leaves of field bean as was found when young barley plants were placed in prolonged darkness (Peterson and Huffaker 1975).

Mature attached field bean leaves were found to contain sufficient soluble sugars to support respiration throughout 60 hours of darkness, but the data in figure 6.6 suggests that amino acids (probably derived from limited RuBPC breakdown) can be respired. It can not therefore be assumed that soluble carbohydrate was the sole respiratory substrate in leaves kept in prolonged darkness, but the contribution of protein to CO_2 efflux during this period appears to have been small.

It is concluded that the minimal estimates of the maintenance requirement obtained in prolonged darkness were determined for 'viable' plant tissue. This energy requirement approximated to the equivalent of 2% of the dry weight per day in field bean, chickpea and lucerne plants grown at 20^{o}C and this experimental figure is consistent with the value for the total maintenance requirement determined by Penning de Vries (1975a) based on the theoretical energy requirements for protein turnover and for the maintenance of cell ion concentrations.

MATURE LEAF RESPIRATION AS REPRESENTATIVE OF THE INTENSITY OF THE MAINTENANCE PROCESSES

In this thesis, maintenance respiration refers to energy production for processes other than the synthesis of new cell materials. The synthesis which occurs within a particular organ to maintain the *status quo* i.e. to maintain a given level of protein and to maintain ion gradients across cell membranes, can be considered as a maintenance cost. The replacement of a senescent leaf does not satisfy this definition, but the synthesis and loading of sucrose, a transport sugar, is consistent with the definition because new cell materials are not synthesised. It is therefore proposed that the respiration rate of mature leaves be partitioned into a 'variable' component, dependent upon the rate of sucrose synthesis and export, and a 'constant' component, which is likely to be largely dependent on the rate of protein turnover, and the energy requirement for maintaining ion concentrations.

This view of maintenance departs from that proposed by Penning de Vries (1972, 1975b) who considers that phloem loading is a 'synthetic' process, presumably because sucrose is the substrate for growth. The theoretically defined growth coefficient (Y_G) therefore includes the cost of loading the phloem with sufficient sucrose for the synthesis of unit biomass. Although phloem loading is incorporated into the theoretical constant Y_G, *experimental* measurements of the maintenance coefficient (by the dynamic and gross uptake methods) also include a component associated with phloem loading. The loading component contributed a variable amount to the CO_2 efflux depending on the method used, and this influenced the choice of the maintenance coefficient. The data produced in table 6.2 showed the respiration rate of mature leaves to be dependent upon the incident irradiance and it was subsequently found that a 'high' leaf respiration rate was associated which 'high' rates of carbon export from leaves. The close correlation between the expected contribution of respiration associated with phloem loading and the order of magnitude of the various maintenance coefficients ($2c < 2b < m$) is the basis for the above view, that phloem loading contributes to the maintenance coefficient, and should therefore be considered a maintenance process.

Sucrose which is exported from mature leaves can serve as the substrate for growth, but is also utilised in maintenance of cells along the transport pathway and in 'sink' organs. Sucrose may also be stored in roots and stems. Therefore, in order to reconcile the theoretical energy requirements for growth with the experimentally determined growth requirements, the experimentally measured respiration rates of translocating leaves should be separated into components for phloem loading of sucrose for both growth and maintenance processes which occur outside the mature leaves, and into a component for maintenance processes which

occur within the mature leaves. However, if mature leaf respiration is partitioned in this way, the energy expenditure for phloem loading of sucrose destined for maintenance of tissue outside the leaves is not accounted for in either 'growth' or 'maintenance'. This omission is considered sufficient cause in itself to group the components of loading together under the label of 'maintenance' processes.

If the respiration rate of translocating leaves is partitioned as implied by Penning de Vries (1975b) into growth and maintenance components, then the connection between growth respiration in mature leaves and biosynthesis, is difficult to envisage in the detached leaf experiment (figure 6.3). As shown in figure 6.3, the respiration rate of both the attached and the detached leaves was essentially the same after the first 12 hours in the dark, yet because the detached leaves were separated from the growing tissue in the plant, there could be no synthesis, since mature leaves do not grow.

The two component view of mature leaf respiration is similar to that put forward in a new model for energy-limited and energy-sufficient growth recently published by Pirt (1982). In this model, which refers to microbial growth yields, Pirt puts forward the view that maintenance consists of a constant term and a growth rate-dependent term. The latter term could not be fully explained but Pirt suggested it to be important when some factor other than energy supply limits growth. The growth rate-dependent term was supposed to diminish with increase in the specific growth rate, but if such a model is interpreted in terms of higher plants, then the growth-rate dependent term (associated with phloem loading) should increase with growth rate, since a higher growth rate generally demands greater carbon export from the leaves.

Thornley's (1977) model avoids the inclusion of the fixed maintenance requirement (dependent on dry weight or protein content) demanded by previous growth models. The two component model for the respiration of mature leaves introduces a reason for a variable maintenance energy requirement dependent on the growth rate (McCree and Kresovich 1978 and Penning de Vries 1975a) although there is a further reason for a variable maintenance demand, which is that a higher growth rate may stimulate the synthesis of proteins with different rates of turnover. The inclusion of the processes associated with the synthesis and loading of sucrose could also explain the observation that experimentally determined rates of maintenance respiration in leaves exceed the values calculated on the basis that protein turnover and the maintenance of ion concentrations across cell membranes are the only processes contributing to the maintenance requirement (Penning de Vries 1975a) and also the high rates of leaf respiration which were experimentally determined by Penning de Vries (1975a, his table 3). The fact that the respiration rates of mature leaves

determined during prolonged darkness are consistent with the estimates calculated from basic data by Penning de Vries (1975a) is support for the idea that the respiration rate observed during a prolonged dark treatment reflects the energy requirements for protein turnover and the maintenance of ion concentrations. However, whilst the energy costs for sucrose synthesis and phloem loading can be determined experimentally, the present methods do not allow discrimination of energy requirements for each of the other maintenance processes, and this is a topic of concern.

SUGAR POOLS FOR GROWTH AND MAINTENANCE RESPIRATION

Leaves have the capacity to almost completely export the daily accumulation of starch during a 12 hour night. Thus, leaf starch accumulation appears to be programmed (Chatterton and Silvius 1979) so that sufficient starch is present within the leaf by the end of the photosynthetic period to support the continued growth and respiration of 'sink' organs during the following night. The capacity of leaves to adapt their rates of accumulation to that of export is limited however, as indicated by the exhaustion of non-structural carbohydrate from cucumber leaves before the end of the night (Challa 1976), and the pattern of rapid decay in the leaf respiration rate during the normal night in certain low irradiance treatments (figures 6.4 and 6.5). Normally a residual amount of starch remains within the leaf following export during the night, and may be degraded the following day if the irradiance is very low or when plants are placed in extended darkness.

Subsequent to the complete dissolution of starch and the export of cytoplasmic sucrose sometime after 12 hours in darkness, considerable quantities of sucrose remain within the leaves of field bean plants, presumably located within the vacuoles. This storage pool contained between 30 and 50% ~~and~~ of the sugar present in the leaves following a photosynthetic period and it is unlikely that sugar is transported from the leaves to the remainder of the plant after about 24 hours in the dark, since the specific leafweight (figure 6.2) and the concentration of soluble sugars (figures 7.3 and 7.5) remain essentially constant, or decline only very slowly after this time. This means that following starch exhaustion, leaves have a storage pool from which to draw respiratory substrate for maintenance in times of stress (e.g. low irradiance or inhibited CO_2 fixation due to water stress). The roots and other sink organs probably have their own sugar pools for maintenance and the plant can therefore be separated into several compartments on this basis. The ability of leaves (and other organs) to store sugars within vacuoles may be an important attribute which enables them to tolerate short periods in prolonged darkness (or other stresses which inhibit CO_2 assimilation), since no loss in protein occurred from field bean leaves which contained considerable sucrose during 48 hours darkness, whilst leaves of cucumber plants grown in 'winter' conditions

(Challa 1976) lost protein during the last four hours of a 16 hour night when the soluble sugar pool was exhausted. Wittenbach (1977) and Peterson and Huffaker (1975) found considerable loss in chlorophyll and protein from leaves of young wheat and barley plants respectively, when plants were placed in prolonged darkness. It is likely that in such plants, leaves had not developed the capacity to accumulate sucrose, but at the same time, it is unrealistic to expect leaves to maintain a protein complement designed to enable growth during the light, when plants are kept in the dark.

THE ALTERNATIVE PATHWAY HYPOTHESIS AND PROSPECTS FOR BREEDING 'LOW' MAINTENANCE PLANTS

The cause of the difference in the specific respiration rate between roots and shoots (Chapter IV) remains enigmatic and the subject needs more attention. During 'normal' diurnal changes in irradiance, roots may perform functions (e.g. nitrate reduction, ion uptake), products of which are destined for the shoot. For this reason the roots show a higher rate of specific respiration. However, during starvation these additional processes should not be a prominent feature of root metabolism since the translocation of sucrose from the shoot will be substantially reduced and there will be very little flow of water from the root. The alternative pathway hypothesis does not explain the higher rates of root respiration observed during starvation (Chapter IV) and there is still no conclusive evidence that the pathway operates *in vivo*, although the capacity for cyanide resistant oxygen uptake is undoubtedly present. A recent report by Rustin *et al.* (1983) proposes a role for fatty acid peroxy radicals in the cyanide resistant pathway of plant mitochondria. The hypothesis has a great deal of merit because it does not require the presence of the alternate oxidase (which continues to evade identification), yet can account for the effects of the various inhibitors of the alternative pathway. This hypothesis supports the contention of Goldstein *et al.* (1981) that the alternative respiratory pathway is not a 'true' respiratory pathway. Nevertheless, the alternative pathway and its contribution to respiration is unlikely to be disposed of as an untenable hypothesis yet, and considerable interest in the involvement of the pathway in 'high' and 'low' respiring lines of *Lolium perenne* is presently being shown by research groups in the United Kingdom and Europe. If it should be discovered that wasteful respiration is a normal feature of higher plant growth and is due to there being more energy available than can be used in growth, maintenance and storage, then a clear need to increase 'sink strength' by breeding will be demonstrated.

Perhaps the best prospect for improving yield by reducing maintenance energy requirements is by the reduction or elimination of the synthesis of proteins or products with no physiological role. A particularly interesting example of the

synthesis of unnecessary proteins is given by Rees and Bekheet (1982) who found that the algae *Phaeodactylum tricornutum* and *Tetraselmus subcordiformis* accumulated between seven and 30 times the normal amount of urease when cultured in the absence of nickel. Although urease is a normal constituent of the cell protein in these organisms, nickel deficiency induced the accumulation of non-functional urease, and this suggests that similar over-production of proteins may occur when agricultural crops are grown in soils deficient in nutrients.

Adams and Rinnie (1981) discussed the production of unnecessary proteins and referred specifically to the many seed proteins such as urease, β-amylase, lectins, proteinase inhibitors and proteinases, all of which seem to be dispensible i.e. they are proteins which can be eliminated without detriment to the competitive ability of the plants.

Maintenance processes permit adaptation to environmental changes such as salinity (Schwarz and Gale 1981) and water stress (Moldau *et al.* 1980) by maintaining ion gradients across cell membranes or altering the cell enzyme complement. It is therefore necessary to ensure that reduction in the maintenance energy cost does not inhibit the ability of plants to adapt to such changes in environment. For example, Robson *et al.* (unpub. 1982) reported that the superiority of a 'slow' respiring line of *Lolium perenne* was maintained only when nitrogen supply was adequate. Agricultural grasslands are frequently deficient in nitrogen and Robson *et al.* suggest this to be a reason for the widespread presence of 'fast' respiring lines in grass populations.

Breeding for higher growth efficiency (Y_G) would be of benefit in some instances, for example the production of alternative energy sources such as ethanol from grain. For the purposes of food production either for human or animal consumption, lower growth efficiency may be desirable (i.e. higher protein grain), but the problem of yield reduction arises possibly because of the high energetic cost of protein synthesis (Penning de Vries *et al.* 1974) but also because of the corresponding increase in the maintenance energy requirements, particularly when the protein is of a non-storage form with a high turnover rate. Protein quality could also be improved, but such improvement would not affect the growth efficiency (Penning de Vries *et al.* 1974).

APPENDIX 1.

CALCULATION OF THE ENERGY REQUIREMENT FOR PHLOEM LOADING IN MATURE LEAVES

Calculation of the enery requirement for loading the phloem with soluble carbohydrate in the mature leaves of field bean plants was accomplished in two ways. In the first, the loading component of respiration was determined on the basis of the total respiration rate of leaves during a 12 hour night (denoted ^{a}R). In the second, the basis is the difference between the average rate of respiration during the 12h night, and the rate after 60 hours of darkness (denoted ^{b}R). The calculation shown below refers to the high irradiance treatment of experiment two, and is determined on a leaf area basis. The calculations made for experiment one were on the basis of the whole leaf, since leaf areas were not determined. Nevertheless, the method of calculation was the same. The energy requirement is calculated in units of gC respired/gC transported.

	Hours in the dark	
	T = 0 hours	T = 12 hours
1. Sucrose concentration mg $(g.dw)^{-1}$	160	98
2. Specific leaf weight g dm^{-2}	0.4307	0.3255
3. Total sucrose mg dm^{-2} (1 x 2)	68.912	31.899
4. Glucose equivalent sucrose mg dm^{-2} (3) x 1.92	130.02	61.25
5. Starch concentration mg $(g.dw)^{-1}$	74	11
2. Specific leaf weight g dm^{-2}	0.4307	0.3255
6. Total starch mg dm^{-2} (5 x 2)	31.8718	3.5805
7. Glucose equivalent starch mg dm^{-2} (6) x 1.05	33.4654	3.7595
8. Total glucose mg dm^{-2} (4 + 7)	163.485	65.010

Now:
^{a}R = 9.3518 mg glucose dm^{-2} $12h^{-1}$
^{b}R = 5.6646 mg glucose dm^{-2} $12h^{-1}$
$^{c}W_i$ = (8 (T = 0)) = 163.485 mg glucose dm^{-2}
$^{d}W_f$ = (8 (T = 12)) = 65.010 mg glucose dm^{-2}
$^{e}\Delta W$ = 98.475 mg glucose dm^{-2}
^{f} T = 89.123 mg glucose dm^{-2}

The energy requirement using ^{a}R is calculated:

$^{a}R \div T$
= 9.3518 ÷ 89.123 mgC/mgC
= 0.105 gC/ gC

The energy requirement using ^{b}R is calculated:

$^{b}R \div T$
= 5.6646 ÷ 89.123 mgC/mgC
= 0.064 gC/ gC

The carbon balance can be checked.
The dry weight loss over 12 hours (2.[T=0] - 2[T=12]) = 0.4307 - 0.3255
= 0.1052 g dm^{-2}

Assuming the dry weight loss to be in the form of glucose (40%C), the weight loss over 12 hours
= 0.1052 x 0.4 x 1000 x 180/72
= 105.2 mg glucose dm^{-2}

The calculated weight loss (ΔW) = 98.475 mg glucose dm^{-2}
Measured weight loss / calculated weight loss = 105.2 ÷ 98.475
= 1.07

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a The average respiration rate during the 12 hour night.
b The respiration rate calculated as the difference between ^{a}R, and the rate calculated after 60 hours of darkness.
c The initial weight (T=0 hours) of carbohydrate in the leaves calculated as the glucose equivalent.
d The final weight (T=12 hours) of carbohydrate in the leaves calculated as the glucose equivalent.
e The difference between W_i and W_f.
f The quantity of glucose translocated calculated as $T = \Delta W - {}^{a}R$.

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* SILSBURY, J.H. 1977. Energy requirement for symbiotic nitrogen fixation. Nature 267: 149-150.

APPENDIX II - THESIS DEFENCE

This section is included as required by the Examining Committee in order to defend certain philosophical beliefs I possess and which are contained within the Thesis. Criticisms of these beliefs by one of the referees centres largely on parts of Chapters II, V and IX.

Dr. McCree disagrees with the emphasis I have given to determining the intercept values on a plot of the 12h nighttime CO_2 efflux versus the 12h daytime CO_2 input, and my pre-occupation with questioning which of these values represents 'true' maintenance respiration. The referee is of the opinion that the purpose of dividing respiration into growth and maintenance requirements is to model daily carbon balances of whole plants. However, it is my opinion that the growth and maintenance interpretation need not be used to predict daily carbon balances. For example, McCree (1970) simply divided respiration into two components, one proportional to carbon assimilation, the other proportional to the weight of biomass . In this way, McCree was able to model respiration quite well. It was Thornley (1970) who made the interpretation of McCree's equation in terms of growth and maintenance respiration.

In the Thesis, I envisaged the purpose of dividing respiration into two components as being of help in the interpretation of the underlying causes of the maintenance energy requirement, since as pointed out on page 23, there is evidence that low maintenance is associated with greater dry matter yields after simulated grazing (Wilson 1975). I believed this finding to warrant a study of the factors underlying maintenance (refer final paragraph on page 24) and perhaps this was misunderstood by the referee. Before such study can be contemplated, we need an operational definition of maintenance, and as outlined on page 21, the generally accepted authority on the subject, (F.W.T. Penning de Vries),

has published contradictory and confusing definitions. Futhermore, an examination of the methods for calculating maintenance experimentally seemed logical, particularly since the various intercept points represent different rates of carbon exchange, and therefore, different maintenance coefficients. A survey of the literature revealed a lack of coincidence between the theoretical and experimental rates of maintenance respiration. Accordingly, an interpretation of the various coefficients seemed necessary.

I believe that experimental measurements of maintenance need to be tied to a theoretical framework, and since there is a clear argument for measuring maintenance energy requirements of crop plants, I believe the emphasis given to consideration of the 'true' maintenance coefficient to be extremely relevant and that the discussion is not 'misplaced'. For the purpose of modelling daily carbon balances of whole plants, I agree with Dr McCree, a definition of maintenance is not necessary.

A second point of contention arises from my use of D and N. Dr. McCree appears to justify criticism of my use of these characters on the grounds that the constraints required for their use (equal day and night length, and equal day and night temperature) do not apply in 'nature'. These specific conditions applied in the study and therefore criticism of D and N in this instance is, I believe, unjustified. However, I acknowledge that the recording of the temperature during experimental measurements was omitted (see correction required on page 12(a),), and this may have confused Dr. McCree. The argument that such environmental conditions do not exist in nature is trivial. In fact McCree and Amthor (1983) employed a constant day (30°C) and constant night (10°, 20°C or 30°C) temperature in their experiments, and it is a rare event in nature that the temperature is constant throughout the entire day or the entire night. McCree (1982)

also used 12h day length and 25° constant temperature. The majority of laboratory measurements designed to elucidate the underlying causes of metabolism, are conducted in artificial environment under artificial conditions. Mechanisms are usually the same irrespective of enviromental conditions, but the latter can certainly influence the extent to which mechanisms operate. I agree with Dr. McCree that changes in procedure must be introduced when diurnal temperature fluctuations occur, but such fluctuations greatly complicate interpretation of the maintenance coefficient. As pointed out on page 18 *et seq* the equivalence of respiration rates in the light and the dark is not proven, and therefore Dr. McCree's statement that gross P must be determined by switching off the light during the daytime, especially if the temperature is changing diurnally, must not be accepted as definitive.

The referee then questions my use of the 'steady state' method terminology. This terminology was taken directly from McCree and Silsbury (1978) as stated in the Thesis. Although McCree and Silsbury obtained data in a 'closed cycle', they obtained further steady state data when plants were subjected to high irradiance and continuous darkness. This second procedure was also used in the Thesis (as described on page 42). The 'dynamic' method terminology was also adopted from McCree and Silsbury (1978). The terminology used in this Thesis serves as a label, and is not intended to represent the steady state or otherwise of carbon reserves during measurement of the CO_2 exchange rate. We always assume that steady state carbon production and consumption is achieved during a 24 hour period. Moldau and Karolin (1977) noted that net carbon accumulation over 24 hours can cause uncoupling of the relationship between daytime carbon uptake and nighttime carbon efflux. Such uncoupling was not evidenced in the present study. I find Dr. McCree's criticism somewhat confusing because on the one hand he refers to method III as a 'steady state' method, and on the other

(next paragraph) asserts that it is not a true 'steady state' method. I continue to prefer to use the terms 'steady state' and 'dynamic' as labels.

Dr. McCree chooses maintenance to represent carbon exchange when $\Delta W = 0$, and suggests that this method is analogous to constant low level feeding to maintain constant body weight in animal (or human nutrition) studies. I have referred to the use of maintenance when $\Delta W = 0$ in the Thesis (third paragraph, page 46) and my disagreement with this view is presented on page 94. In addition, I strongly disagree with the above analogy drawn, since contrary to the case for plants, animals do not lose limbs or other organs which can be replaced (except of course in the case of animals such as lizards, where a lost tail may regrow). In animals, when $\Delta W = 0$, this is true maintenance, but in plants, as discussed in the Thesis, I believe this includes synthesis as well as maintenance. I suggest this as the reason why experimental values exceed the theoretical maintenance energy requirements.

Maintenance requirements change continuously, particularly when plants grow, since additional maintainable biomass is being produced. However, whether maintenance requirements fall as plant metabolic rate declines, as stated by Dr. McCree is not proven in my opinion. Data of McCree (1982, his figure 5a) showed metabolism (ΔS and ΔW) to be quite steady or to slightly increase over a 28 day period, yet the'maintenance coefficient' (I do not interpret this coefficient to be a true maintenance coefficient) rapidly fell. To me, this data indicates that maintenance is somewhat independent of whole plant metabolism, and supports the view that I have put forward, that the 'steady state' (starvation) method can give a reasonable estimate of the 'true' maintenance requirement. I suggest 'm' declined in McCree's study because some of the maintainable biomass (leaf material) was senescing. As the amount of maintainable biomass falls, so should the energy requirements for maintenance also fall. Furthermore, under low

irradiance conditions, where the growth rate is reduced (McCree 1982), 'm' may have also declined because the newly synthesised tissue had a lower specific maintenance energy requirement than the tissue produced in the high irradiance environment.

In summary, I believe the philosophical arguments concerning the 'true' maintenance coefficient which I have included in the Thesis, are new, and question our present understanding of maintenance, most particularly in respect as to how we are to interpret experimentally measured values. When attempting to estimate maintenance experimentally, we are still taking a 'stab in the dark', hence the comment on page 94 "It must therefore be stated that complete confidence in the ability of any particular method to estimate maintenance accurately cannot be given." This comment was made because although the steady state (starvation) method gave results consistent with the theoretical values, I realise that this method can lead to accelerated senescence, particularly of leaves which are already beginning to senesce. The data in Chapter VII show that healthy field bean leaves did not senesce (i.e. did not lose chlorophyll or protein) until at least 48 hours in the dark,

The starvation method, which was useful when applied to the crop legumes studied here, may not be a universal method for estimating maintenance. Indeed, I cannot forsee a universal method being developed in the near future.