'AN INVESTIGATION INTO THE EFFECT OF ENVIRONMENTAL FACTORS INFLUENCING PHOTORESPIRATION AND CARBON DIOXIDE COMPENSATION POINT (T) IN HORDEUM VULGARE L.'

Patrick Fair.

A Thesis Submitted to the Faculty of Science,
University of the Witwatersrand, Johannesburg
for the Degree of 'Doctor of Philosophy.'

Witwatersrand University

1972.

This work is dedicated to my wife, Jean for her love and patient understanding during the production of this work and to my Mother and Father who have helped to support me during my university career.

DECLARATION.

I hereby declare that the whole of the work now submitted as a thesis for the degree of Doctor of Philosophy is the result of my own investigation, except where acknowledgement to other work is made.

The substance or part of this thesis has not been submitted in the past, nor is being nor is to be submitted for a degree in any other university.

Patrick Fair

ACKNOWLEDGEMENTS.

I wish to express my gratitude to Professor Cresswell for his invaluable advice, assistance and understanding and to Mr. John Tew with whom I worked while producing a major part of this thesis.

I would like to extend my thanks to Mr. Kaufman for his technical assistance and to Mr. Bunning for preparing the photographs.

Thanks are also due to Mrs. Phillips for her willing assistance in typing this manuscript.

I wish to acknowledge the Senior Bursarship granted to me by the University of the Witwatersrand and the C.S.I.R. bursary, which enabled this work to be undertaken. The Hartmann and Braun infra-red gas analyser was bought with a C.S.I.R. grant.

ABBREVIATIONS

A.A.	amino acid
ATP	adenosine triphosphate
C_3- plants	plants possessing the photosynthetic Calvin Cycle.
C_4- plants	plants possessing the C_4 - dicarboxylic acid pathway of photosynthesis.
mM	millimolar
NAD	nicotinamide adenosine dinucleotide
3-PGA	3- phosphoglyceric acid
p.p.m.	parts per million/milligrams per litre
RuDP C'ase	ribulose 1-5, diphosphate carboxylase.

ABSTRACT

Initial work indicated that the CO_2 compensation point (T) varied for any given set of conditions in a single plant species. This was in direct contradiction to the widely held view that the T was of a fixed value for any given plant species. Further-more it was found that high light intensities (above limiting values) resulted in a rise in the T, a point again at variance with general belief. These two points were investigated further and led to a possible explanation for the function of the peroxisome being presented.

Hordeum vulgare L. (Barley) was used exclusively for all experimental work. Experimental work was related to varying ages of the barley plant and specific leaves. Plants were grown in either nutrient liquid culture or soil supplemented with a nutrient solution. The plants were grown under closely controlled conditions in a phytotron. Experimental procedure entailed the use of an infra-red gas analyser and a paramagnetic/oxygen analyser for the determination of the T and the monitoring of oxygen levels respectively. Enzyme activity determinations were made on nitrate reductase and by Mr. Tew on ribulose 1-5, diphosphate carboxylase and glycollate oxidase. The reasons for the choice of these enzymes are presented in the introduction. Carbon 14 labelling experiments were also conducted to determine the distribution of C^{14} in the basic (amino acids), acidic (organic acids), and neutral (sugars) fractions. A 'lost fraction' was also determined and it has since been established that this fraction is composed mainly of sugar-phosphates.

Experimental conditions were varied with regard to nutrient status, light intensity, and oxygen-carbon dioxide gas concentrations. The effect of these variations in experimental conditions were then determined on T, nitrate reductase, ribulose 1-5, diphosphate and glycollate oxidase, as well as C^{14} distribution into the acidic, basic, neutral and 'lost' fractions of the plant.

The conclusions of this investigation are listed below:-

(1) T was shown to increase with increasing light intensity up to 100,000 lux, above limiting light intensities.

(2) The T response at a given light intensity was shown to vary with leaf age, high light intensities (30,000 lux) giving a lower T than at low light intensities (10,000 lux) in a young expanding leaf, and the reverse of this pattern in fully expanded leaves.

(3) T is shown to be affected by varying nutrient conditions, when nitrogen is supplied as NH_3 a higher T point is recorded when compared to nitrogen supplied as nitrate.

(4) From the results it is suggested that one possible major function of the glycollate cycle is the supply of carbon skeletons for amino acid metabolism in the light.

(5) The ecological implications of points 1 and 2 are discussed.

CONTENTS

FIGURES AND DIAGRAMS

PAGE

DIAGRAM NO.

PLATES AND TABLES

TABLE NO. PAGE

IN JUCTION

Preliminary work on the T of C_3 plants suggested that this point was not a fixed point in any one plant but varied with time. Most workers (Jackson and Volk 1970) had implied that for a given set of conditions a plant had a fixed T. This observation in conjunction with the observation that light intensities above limiting values did in fact affect T, led to two closely parallel lines of research being conducted. One, to elucidate the effect of high light intensities on T of Hordeum vulgare and two, to study the fluctuation in T under a given set of conditions and elucidate the reason for and implications of this fluctuation.

In an attempt to study the fluctuation of the T further, the level of CO_2 uptake (photosynthesis) and CO_2 output (photorespiration) was studied, by observing the enzyme activities associated with these two processes, together with the T. The activity of the enzyme ribulose 1-5, diphosphate carboxylase (4.1.1.f.) was used to assess the POTENTIAL level of carbon dioxide uptake AT T as it is accepted as the main photosynthetic carboxylating enzyme in Calvin Cycle (C_3) plants. The selection of marker enzyme for the decarboxylation process was more complex. Glycollate oxidase (1.1.3.1.) is thought to be one of the main terminal oxidases associated with photorespiration (Tolbert and Yamazaki, 1969) however, the measurement of its activity need not necessarily give a measure of the level of decarboxylation due to the 'glyoxylate glycollate shunt' proposed by Tolbert and Yamazaki (1969) or alternatively the formation of glycine from glyoxylate and its subsequent removal from the peroxisome in this form. In the scheme suggested by Tolbert and Yamazaki (1969) for peroxisomal metabolism, associated with photorespiration, glutamic acid plays a major role. Further as glutamic acid is considered to be the prime product of reductive amination, it was decided to investigate the possibility of using the enzyme nitrate reductase (1.6.6.2.) as a measure of peroxisomal activity in plants grown with nitrate as the sole nitrogen source.

The response of T to increasing light intensities above limiting values may have important ecological consequences - this aspect is also discussed.

REVIEW OF LITERATURE

A. BACKGROUND TO PHOTORESPIRATION.

Photorespiration has only been reliably demonstrated within the last twenty years and received its major impetus with the work of Decker (1955, 1957). Before this, most work had been directed towards establishing whether or not dark respiration continued in the light (Weight, Warrington and Calvin 1951; Steemann Nielson 1953, 1955). In 1940, Myers and Burr showed that under high light intensity, Chlorella produced rates of O_2 uptake 2 to 4 times as great as those of dark respiration.

Decker (1957) showed that a variety of plant species produced carbon dioxide in the light at rates far in excess of those in the dark. He further demonstrated a dark carbon dioxide burst when a plant was moved from light into the dark indicating that photorespiration continued for a short while after the cessation of photosynthesis (Decker 1955). Decker (1957) also established that in a closed system certain plants reduced th[illegible] on dioxide concentration to a level of 50 p.p.m. wh[illegible] remained stable. This point at which photosynthe[illegible] exactly balances respiration is termed the carbon dioxide compensation point (Decker 1957).

B. MEASUREMENT OF PHOTORESPIRATION.

A number of workers (Moss 1962, Lake 1967, Zelitch 1968, Samish and Koller 1968) have shown that photorespiration is a major component of a plants light meta~~t~~bolism and as much as fifty percent of newly fixed carbon dioxide may pass through this pathway. Canvin suggests that upto one hundred percent of newly fixed carbon may pass through the glycollate cycle (pers. com).

The determination of the magnitude of photorespiration has been attempted using numerous methods, a brief outline of which is presented below. All the methods however suffer from the fundamental problem of not measuring refixation of released carbon dioxide by photosynthesis, thus all methods give minimum values of carbon dioxide output.

A number of workers (Decker and Wien 1958, Tregunna Krotkov and Nelson 1961, 1964, Fock and Egle 1966) have utilized the magnitude of the carbon dioxide burst to estimate the size of photorespiration. Krotkov, Runeckles and Zlimar (1958) estimated the magnitude of photorespiration by determining the rate of dilution of specific activity of CO_2 in a closed system by normal CO_2 from the plant material. El-Sharkawy and Hesketh (1965), Goldsworthy (1966) and Zelitch (1968) calculated the level of photorespiration by determining the rate of CO_2 released into CO_2 - free air by the ant material. Ludwig and Canvin (1971) using an open gas-exchange system determined the level of photorespiration by the simultaneous measurement of $C^{12}O_2$ and $C^{14}O_2$ fluxes from plant leaves.

C. CARBON DIOXIDE COMPENSATION POINT (Γ)

As Γ is an equilibrium between CO_2 uptake and CO_2 output, it is apparent that any change in either net CO_2 uptake or net CO_2 output would be reflected in the rise or fall of the Γ. This equilibrium could thus be highly significant in studying the relationship between photosynthesis and photorespiration. Decker (1957, 1959) used the Γ to estimate the level of photorespiration. He assessed photorespiration by extrapolating the curve of net photosynthesis at varying CO_2 concentrations from the Γ to zero CO_2 concentration. The intercept on the x axis giving a measure of photorespiration. Bravdo (1968) and Samish and Koller (1968) showed however that the intercept was a function of stomatal and internal diffusive resistances as well as photorespiration. This would again give an underestimation of photorespiration.

While determining Γ, the possibility of stomatal closure may be discounted as the low CO_2 concentrations pertaining at Γ, would result in the stomata being wide open (Levitt, 1967).

Numerous studies in different laboratories utilizing T as a measure of photorespiration and photosynthesis have been conducted und' r varying enviromental conditions and more recently under varying nutrient conditions. These factors are discussed under the appropriate headings below:-

(a) VARYING OXYGEN CONCENTRATIONS.

Oxygen concentrations above and below atmospheric levels have been shown to markedly affect T. Forrester, Krotkov and Nelson (1966) were the first to study O_2 effects in detail. Using Soybean (Glycine max) they demonstrated that apparent photosynthesis (net CO_2 uptake) was inhibited by increasing levels of O_2 above atmospheric levels. A number of other workers have made similar findings as well as showing that photorespiration was enhanced with increasing O_2 concentration (Tregunna, Krotkov and Nelson 1966, Bjorkman 1966, Zelitch 1968, Hew and Krotkov 1968, Jolliffe and Tregunna 1968). This increase in photorespiration is marked by an increase in the T. Ellyard and Gibbs (1969) using isolated chloroplasts have also demonstrated a decrease in net CO_2 fixation (Warburg effect) and point to its rapid reversability when the tissue is flushed with nitrogen.

Furthermore they found that high levels of bicarbonate (13.0mM) prevented the high O_2 inhibition of apparent photosynthesis and that low levels of bicarbonate (1.0mM) enhanced this inhibition. Dark respiration has been shown to be unaffected by high oxygen concentrations (Forrester et.al. 1966, Tregunna et.al. 1966, Goldsworthy 1966). Tregunna and co-workers (1966) found a linear relationship between O_2 concentration and T. This increase in T with O_2 concentration could be the result of either an increase in photorespiration or a decrease in photosynthesis or both.

Low oxygen levels (below 21%) directly suppress photorespiration with a corresponding drop in T. Fock, Becker and Egle (1970) and Goldsworthy (1968) suppressed CO_2 output decreasing O_2 concentrations from twenty-one percent O_2 to one percent, obtaining T values close to zero. Hesketh (1967) compared net photosynthesis in air (21%, O_2) and in O_2 free air and showed enhancement of net photosynthesis in tobacco, cotton, and bean.

(b) VARYING CARBON DIOXIDE CONCENTRATIONS.

T cannot be determined under a given environment of fixed levels of CO_2 as the two concepts are mutually exclusive. Some evidence does exist however of high levels of CO_2 (300 p.p.m.) inhibiting photorespiration. Tamiya and Huzisige (cited by Jackson and Volk 1970), Ellyard and Gibbs (1969) and Chollet and Ogren (1972) have demonstrated that the inhibition of apparent photosynthesis by high O_2 levels is reduced by increases in CO_2 concentrations above 300 p.p.m. Jolliffe and Tregunna (1968) found that at 21% O_2 the inhibition of photosynthesis was removed by high levels of CO_2 (500 p.p.m.). Egle and Fock (1967) could not obtain a postillumination CO_2 burst at 1200 p.p.m. CO_2 at normal O_2 levels, *suggesting a supression of photorespiration.* Photorespiration however appears to be unaffected by concentrations below 300 p.p.m. (Decker 1959,Hew and Gibbs 1969, Ludwig and Canvin 1971a).Bidwell, Levin and Shephard (1969) report however that photorespiration in bean leaves was lower at 300 p.p.m. than at 30 to 40 p.p.m.

Further evidence for the effect of CO_2 on photorespiration is provided by the work of Zelitch and Walker (1964) and Zelitch (1965) who showed that the synthesis of glycollate was severely inhibited at CO_2 concentrations of more than o.2%. It is well established that glycollate is the substrate for peroxisomal metabolism (Zelitch 1959, Tolbert 1963, Zelitch 1966a and b , Tolbert 1971). Heath and

Orchard (1967) have shown the light compensation point to be reduced at high CO_2 concentrations. Another aspect of the CO_2 concentration is that shown by Hanson and Edelman (1972) who, using carrot tissue cultures have reported that chlorophyll levels declined in 1% carbon dioxide.

(c) EFFECT OF LIGHT INTENSITY.

Work on this aspect, particularly at high light intensities (20,000 lux), is scanty and at best contradictory. It has been reasonably well established that at low light intensities (5000 lux) Γ drops rapidly and hyperbolically with an increase in light intensity (Meidner and Glinka - unpub. res., Heath 1969, Jackson and Volk 1970) reaching constant values at relatively low intensities (Heath and Orchard 1967, Whiteman and Koller 1967). At light intensities above these limiting values the general view, is that held by Jackson and Volk (1970) that Γ does not increase with increasing light intensities. This view is supported by the work of Poskuta (1968). However Samish and Koller (1968) suggest that Γ does infact increase with increasing light intensities. This work is not based on experimental evidence but on a mathematical model. Increasing light intensities have been demonstrated to increase photorespiration (Hoch, Owens and Kock 1963, Goldney and Van Steveninck 1971) and the CO_2 burst (Decker 1959, Tregunna et.al. 1964). Although Wolf (1970) states that Moss (1962) has shown Γ to increase with light intensity Moss in his paper gives no experimental evidence for this statement.

(d) VARYING NUTRIENT SOLUTIONS.

Tregunna et.al. (1964) using "postillumination burst" techniques showed that mineral deficient leaves (yellow leaves showing extreme nitrogen deficiency) have no photorespiration and Ryle and Hesketh (1969) reported that photorespiration is little affected by nitrogen deficiency.

Herath and Ormrod (1972) working with barley, peas, and rape showed clearly that sulphur nutritional status will affect the T values of high T plants and that the magnitude of the effect could be modified by the temperature at which the plants are grown. A higher temperature giving rise to a smaller T difference between the control and the treated plants.

D. VARIABILITY OF T.

It has been generally assumed that the T of a plant is constant under constant environmental conditions (Jackson and Volk 1970, Wolf 1970, Heath 1969). Recent work indicates that at least in certain plants, this is not entirely correct. Jones and Mansfield (1970) have established that a 24 hour rhythm in T of plants, other than crassulacean type plants, exists. Using Coffea arabica they showed that the 'peak' compensation point values could be as much as 40 p.p.m. greater than the 'trough' values. Meidner (1970) working with Xanthium pennsylvanicum demonstrated that debudding and photoinduction (short days) resulted in an increase in stomatal conductance and a marked drop in T after eight days.

E. POSSIBLE MECHANISMS OF CO_2 - O_2 EFFECT.

Attempts to explain the competing effects of CO_2 - O_2 concentrations on the rate of photorespiration have led to two possible mechanisms being proposed. One by Zvi Plant and Gibbs (1970) is based on a Mehler type reaction involving a two carbon fragment produced by a transketolase reaction in the Calvin cycle.

A more recent paper by Ogren and Bowes (1971) offers an alternative scheme which adequately explains the extreme sensitivity of photorespiration to CO_2 and O_2 concentrations. They state that, 'The competitive nature of O_2 inhibition suggests, the possibility that O_2 substitutes for CO_2 in the carboxylase reaction, so that ribulose diphosphate is oxidized to 3PGA, the first product of CO_2 fixation, and phosphoglycollate. Increasing O_2 increases the rate of phosphoglycollate production thereby stimulating photorespiration and further reducing the concentration of Calvin cycle intermediates'. This scheme is presented in fig. 21. Recent papers by Bowes and Ogren (1972) and Hall (197') give additional support to this scheme. In this scheme the level of the T would be a direct indication of the balance between the glycollate pathway and carboxylation.

F. SUGGESTED FUNCTIONS OF PHOTORESPIRATION.

Considerable speculation exists as to the role of photorespiration and the question remains largely unanswered.

It has been reliably established that photorespiration is the result of the glycollate pathway, which involves both the peroxisome and the mitochondrion (Tolbert and Yamazaki 1969, Tolbert 1971). This pathway is presented in fig. 21.

Large numbers of peroxisomes are found to occur in chloroplast - containing cells of all C_3 plants studied, and it has been shown that at least 50% of newly fixed carbon passes through the glycollate cycle (Tolbert 1971, Zelitch 1966). Electron micrographs underline its importance and demonstrate the close spatial relationship between the chloroplast, peroxisome and mitochondrion (Frederick and Newcomb 1969, 1971, Laetch 1969, Trelease Becker, Gruber and Newcomb 1971). Tolbert (1971) in his recent review lists a number of possible functions of the peroxisome and states that no one function alone is adequate to account for leaf peroxisomes. He does not consider the synthesis of glycine and serine to be important aspect of peroxisomal function. Contrary to Tolbert's suggestion is the work of Ruis and Kindl (197') which points to a possible connection between amino acid synthesis and peroxisomal metabolism. They suggest from their results that leaf peroxisomes may have more importance in amino acid metabolism than was previously assumed.

Bird, Cornelius, Keys and Whittingham (1972) have recently demonstrated that ATP is produced in the mitochondrion during the conversion of glycine to serine (fig. 21). They state "Our results suggest that one of the main functions of the photorespiratory pathway is to allow the synthesis of ATP in the light in the mitochondrion". This they suggest would provide a biolog..al significance for the process of photo-respiration. In a follow-up paper B.. et.al. (1972b) showw that at least two ATP's are for.. by the conversion of glycine to serine.

G. NITRATE ASSIMILATION AND PHOTORESPIRATION.

Virtually no evidence exists on a possible link, either direct or indirect, between nitrate assimilation and photorespiration. Ruis and Kindl (1971) mentioned above, have implied the possibility of nitrogen metabolism being associated with the peroxisome.

It is well established that nitrate reductase is an inducible enzyme with a short response time and half life, both in vitro and in vivo (Nason and Hageman 1969). Numerous workers have shown that nitrate reductase is susceptable to suppression by ammonia and a number of amino acids and amides (Afridi and Hewitt 1965, Filmer 1966, Stewart 1972). Stewart working with Lemma minor has shown nitrate reductase to be suppressed by asparagine, glycine and ammonia as well as partial suppression by aspartate, glutamate and glutamine. Glycine, glutamate and aspartate are amino acids directly involved in the glycollate cycle.

H. INFLUENCE OF AMINO ACIDS AND AMIDES ON PHOTORESPIRATION AND NITRATE REDUCTASE ACTIVITY.

Numerous workers have demonstrated that excess ammonia in the roots of plants is incorporated into glutamate, aspartate, glutamine and asparagine (Pate 1966, Weissman 1972, Ivanko and Ingoerson 1971, Ingoerson and Ivanko 1971) This suggests the possibility that these compounds rather than ammonia may be responsible for the suppression of nitrate reductase activity in plants. It is well known that nitrogen supplied as ammonia suppresses nitrate reductase activity.

Carr and Pate (1967) working with "field pea" a nodulated plant, point out that Pate (1966) has shown that nitrogen transported from the root is almost entirely transported in the form of glutamate, aspartate, glutamine or asparagine. They further state that these solutes contribute nitrogen and carbon to protein synthesis and that during the latter process amides are consumed and from their amido nitrogen a range of amino acids are formed during photosynthesis, namely serine, glycine, alamine and the aromatic amino acids. Serine and glycine are directly implicated in photorespiration (fig. 21). Weissman (1972) has demonstrated that in sunflower and to a lesser extent in soybean a large amount of the plants nitrogen is incorporated in the root and transported to the leaf as an amide. It is implicit however that if nitrate reductase activity occurs in the leaf of a plant then nitrate must also be transported to the leaf.

I. PATTERNS OF NITRATE REDUCTASE ACTIVITY IN PLANTS.

Carr and Pate (1967) show that maximum nitrate reductase activity decreases with each succeeding leaf and reaches a maximum activity at full expansion of the leaf, thereafter decreasing with age. Harper and Hageman (1972) working with soybean have established patterns of nitrate reductase activity that rise with each succeeding new leaf formed, reaching a peak of activity at full leaf expansion. Cresswell (1961) has shown that nitrate reductase distribution is related to plant age and leaf position.

J. LIGHT AND NITRATE REDUCTASE.

Hewitt (1970) mentions that the dependence of nitrate reductase activity on light is not wholly accounted for by its requirements for reducing potential, and is related to the light intensity and duration. Kannagera and Woolhouse (1967) demonstrated that the synthesis of nitrate reductase requires light and photosynthetic activity. Jordan and Huffaker (1972) have shown that photosynthetic CO_2 uptake is required for significant levels of nitrate reduction in leaves.

K. POSSIBLE MECHANISM FOR GENERATION OF $NADH_2$ FOR NITRATE REDUCTASE IN GREEN LEAVES.

Recently Klepper, Flesher and Hageman (1971) have published a paper suggesting a possible mechanism for the generation of $NADH_2$ for nitrate reductase in green leaves. They conclude that sugar-phosphates migrate from the chloroplast to the cytoplasm and are the prime source of energy and that the oxidation of glyceraldehyde 3 - phosphate was ultimately the in vivo source of $NADH_2$ for nitrate reduction.

L. CARBON 14 DIOXIDE LABELLING PATTERNS.

The literature on carbon labelling patterns is voluminous and merely a brief survey of basic labelling trends is presented.

Earliest label in C_3 plants is well established to be in 3 PGA and very soon afterwards is found in glycollate (Calvin and Bassham) (1962). Hess and Tolbert (1966) have shown using time - course labelling experiments that at four seconds 3PGA was a 100 times more labelled than glycollate, however at four to eleven seconds glycollate became uniformly labelled (i.e. both carbons labelled).

The further metabolism of glycollate (fig. 21) is also well established, with the amino acids, glycine and serine playing an important role (Hess and Tolbert 1966, Tolbert 1971). Labelling patterns confirm this finding, the major amino acids labelled being serine, alanine and aspartate (Calvin and Bassham 1962, Tolbert and Cohen 1953, Hess and Tolbert 1966, Carr and Pate 1967).

Wolf (1970) in his review states that there is fairly general agreement that glycollic acid is the source of CO_2 liberated in photorespiration and that it arises from the carboxyl carbon of glycollic acid.

Carr and Pate (1967) state that during the expansion stage of the leaf much of the assimilation of CO_2 is directed towards the manufacture of essential structural material in the developing cells especially that of chloroplasts. In this regard it is interesting that Tolbert (1971) mentions that glycine and serine are the precursors for all C_1 biosynthesis and glycine and succinate are precursors for porphyrin synthesis.

Carr and Pate (1967) further emphasize that CO_2 from photosynthesis is fixed largely into serine, glycine, alanine and other compounds not arriving in quantity from the roots, this pattern remaining constant throughout the photosynthetic life of the leaf, with however the major percentage (90%) of newly fixed CO_2 being incorporated into sugars as the leaf ages.

Ongun and Stocking (1965) using tobacco, studied C^{14} distribution in the light and serine incorporation into the carbohydrates of chloroplasts. They found that the major count in protein derived from serine and glycine. Furthermore serine was metabolised only in the light.

M. CARBON DIOXIDE AND STOMATAL ACTIVITY.

It is a well established fact that CO_2 has an effect on stomatal activity. High intracellular CO_2 levels resulting in stomatal closure and low intracellular CO_2 levels result in stomatal opening (Meidner and Mansfield 1968, Levitt 1967, Sutcliffe 1968) Raschke (1972) and Pallaghy (1972) have demonstrated that stomata start to close at CO_2 concentrations as low as 200 p.p.m. and severe closure occuring at levels above 400 p.p.m. CO_2.

Midday closure of stomata is a well known phenomenon and occurs in C_3 plants, particluarly in hot climates (Meidner and Mansfield 1968). An explanation for this closure was offered by Heath (1952) and Heath and Orchard (1957) who suggested that closure was caused by increasing intracellular levels of CO_2 as a result of increasing photorespiration with increasing temperature. Heath and Orchard (1957) demonstrated this phenomenon using onion.

Boyer (1971) has shown that low leaf water potentials result in a nonstomatal inhibition of photosynthesis in sunflower at high light intensities.

N. ECOLOGY OF C_3 AND C_4 PLANTS.

There is much speculation as to the ecological implications of the two main photosynthetic pathways, the C_3 and C_4 systems (Black 1971, Bjorkman 1971, Hatch and Slack 1970).

Bjorkman (1971) states that the available experimental evidence strongly suggests that the C_4 pathway represents an adaption to habitats with high temperatures, high irradiance, and limited water supply during the active period of growth. Hatch and Slack (1970) and Black (1971) also stress the water conserving aspect of C_4 plants, namely that they produce approximately twice the dry weight as do C_3 plants, for the equivalent loss of water.

It is well established that C_4 plants are widely distributed in tropical and sub-tropical regions, particularly in areas of high temperatures, high light, and markedly seasonal rainfall (Hatch and Slack 1970, Black 1971, and Bjorkman 1971). Hatch and Slack (1970) further emphasize that the C_3 grasses are generally found in regions of more uniformly distributed rainfall, either in elevated habitats, on mountain slopes or in rain forest. They also point out that photorespiration must be considered as one possible factor limiting the growth of Calvin Cycle plants in the tropics, for at temperatures in excess of 30°C the rate of photorespiration can approach half that of photosynthesis. Hatch and Slack (1970) state clearly that C_4 plants primary requirement would be drought tolerance and the ability to grow rapidly following rain. It is thus apparent that C_4 plants are not normally under conditions of water stress during their growth period, especially when one considers that C_4 plants constitute the majority of the vegetation found in tropical swamps, such as: *Phragmites communis*, *Cyperus erectus* and *Miscanthidium teretifolium* taken from the Okavango Swamps in Botswana (Fair and Cresswell - unpub. res.)

Steward, Craven, Weerasinghe and Bidwell (1971) state in a recent paper that it is evident that the balance between CO_2 evolved and fixed is so affected by environmental criteria that those responses are important in evaluating the performance of plants in different ecological and climatic situations.

MATERIAL AND METHODS

A. PLANT MATERIAL.

Barley plants, *Hordeum vulgare L.* variety Elses 6 row (C. May Co. Seed Merchants Johannesburg) were used throughout the experiments.

Two growing procedures were utilized depending on the treatment required:-

(a) Plants were grown in soil and supplemented with a Long Ashton nutrient medium (Hewitt 1952). Details of nutrient solution in appendix 14.

(b) Plants were grown in a liquid nutrient culture again employing a Long Ashton nutrient medium. The nutrient medium was changed weekly. The level of the nutrient cultures was topped-up daily with distilled water to maintain a constant level.

All plants throughout the experimental work were grown (except where specified) under constant environmental conditions of fourteen hours light at 27°C and ten hours dark at 22°C. Light intensity was ± 15,000 lux. Only one leaf per plant was utilized. The plant was discarded after a leaf sample was cut.

B. T DETERMINATION

(a) APPARATUS AND CALIBRATION.

A closed circuit gas chamber was constructed, containing an illuminated chamber (plate 1), diaphragm pump, Hartman and Braun I.R.G.A. for T measurement and a Hartman and Braun paramagnetic O_2 analyser (diag. 1). The I.R.G.A. had a relative measuring accuracy of ± 0.25 p.p.m. with a full scale deflection of 100 p.p.m. Calibration of the I.R.G.A. was carried out using standard gas mixtures, supplied by Afrox Ltd. The O_2 analyser was calibrated, using an O_2 free gas and air taken as 21% O_2.
Accuracy was ± 0.25% O_2 and response time 10 sec.

The volume of the leaf chamber was 900 cm^3 and the remainder of the gas circuit a further 900 cm^3.

This gave a total volume of 1,800 cm^3 for the leaf chamber and gas circuit.

The light chamber was illuminated using a Strand Electric, stage spot lamp and the light was supplied with a G.E.C. T6 1000W projector lamp (Plate 1).

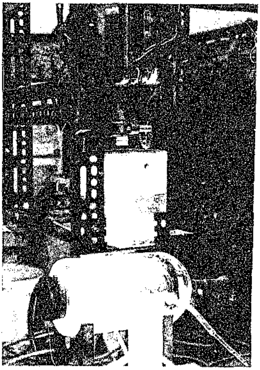
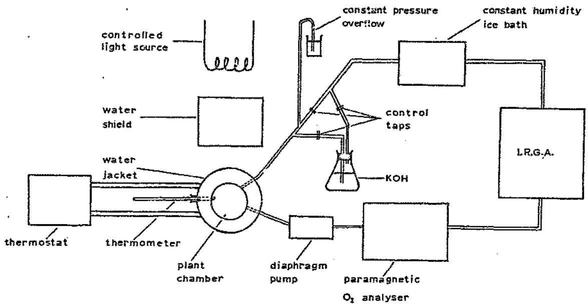


PLATE 1. Showing the illuminated plant chamber for CO_2 compensation point determination.

The light intensity was controlled using a rheostat and measured with a photo-electric cell held in the illuminated leaf chamber, at the level of the leaf surface.

Temperature of the leaf chamber was closely controlled by a water jacket surrounding the chamber. The water temperature was maintained using a Lauda thermostat with an accuracy of .5 of a degree celcius. The light beam was also passed through a 20cm column of water to remove the infra-red spectrum.

Diagram 1. Circuit diagram of system used to determine T.



To maintain a constant water vapour level in the I.R.G.A. an "ice box" was placed in the circuit ahead of the analyser.

A major problem of closed gas circuits is the prevention of CO_2 leaking into the closed circuit, when the CO_2 concentration within the closed circuit is dropped below atmospheric levels existing outside the circuit. In order to reduce this leak 'Tygon' tubing connections were reduced to a minimum and all 'joints' carefully sealed with silicone grease.

The rate of CO_2 leakage was reduced to a constant factor of $\pm$ 7 p.p.m. per hour. This leakage rate was based on the rate of increase in CO_2 concentration in the closed circuit, when the CO_2 concentration was initially reduced to zero by bubbling through concentrated K O H.

As the above leakage rate is small and would in fact be a constant for all T determinations, it was not considered as a factor in assessing the T results.

The constancy of the T determinations was established by demonstrating that a given T value could be maintained by a leaf sample, for periods in excess of 12 hours, at the T originally obtained. The physical measuring apparatus never varying by more than $\pm$ 0.25 p.p.m. from the given T.

All T measured were only recorded after a constant value had been maintained for a period of 15 minutes.

Rates were determined for CO_2 uptake, however no correlation was found between T and rate. Furthermore different rates of CO_2 uptake were obtained from the same material exhibiting the same T. As this investigation was concerned solely with T determinations, rates were not considered.

(b) EXPERIMENTAL CONDITIONS

Temperature of the leaf chamber was maintained between 29° and 30° celcius and a gas flow of 50-60 litres per hour was maintained.

Light intensity was varied according to the experiment being conducted.

Oxygen was maintained at 21%.

(c) TREATMENT OF MATERIAL.

T was determined using eight leaves of similar age and position. The leaves were cut and immediately placed in 10ml. beakers (4 leaves per beaker) and the water surface coated with wax (plate 2).

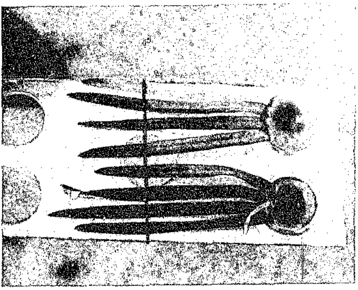


PLATE 2. Showing the eight prepared barley leaves for CO_2 compensation point determination.

Cut leaves were used in prefe nce to attached leaves primarily due to the ease of handling of the plant material in this form. This is particularly important when it is considered that eight leaves are used for each T determination in order to obtain a composite result. The use of cut leaves for T determinations is a commonly adopted technique (Sestak, Z., J. Catsky and P.G. Jarvis 1971).

It is fully realized however that detached leaves may introduce inherent errors into any physiological determinations made. These errors would be due, to the disruption of interchange of metabolites between the leaf and the rest of the plant body. It was felt however that in the short term these effects would be minimal, and that any particular effect that did occur would be common to all determinations that were made. A further effect could be the shock induced in the leaf, when the leaf was removed from the plant. The effect of this shock, if any, remains unknown.

The beakers were held in position by a perspex template, to which the leaves were lightly bound, holding them in a flat plane (plate 2) perpendicular to the light source.

Leaves were numbered in order of appearance, the first leaf to appear being No. 1, the second No. 2 etc.

C. NITRATE REDUCTASE ASSAY

(a) ENZYME EXTRACTS.

500 mg. of freshly cut leaf material was ground in 10ml of cold grinding media, using a chilled mortar and pestle with acid washed sand. The grinding medium was a modified Breidenbach, Kahn and Beevers (1968) medium containing 0.15 M. Tris - HCL buffer (ph 7.5) 0.01M EDTA (ph 7.5), 0.01M KCL, 0.001M $MgCl_2$, and 10mM Dithiotreitol. The extract was squeezed through cheese-cloth, and centrifuged at 1000g for 5 minutes. The supernatant was used as a crude enzyme source. All the above procedures were carried out at $0^{o}C$.

All enzymatic activities have been expressed per gram fresh weight. The method was similar to that of Downton and Slayter (1971). Grinding was tested for its variability against protein and the standard deviation for 20 grinds was less than 1%. Protein was assayed using the method of Lowry, Rosebrough, Farr and Randall (1951).

(b) ASSAY PROCEDURE

The method of Hewitt and Nicholas (1964) was employed. The reaction was as follows:- 1.0ml, 0.1M_2 HPO_4 (pH7.5), 0.05ml, 1.36 x 10^3 M $NADH_2$, 0.2ml, 0.1M K NO_3, 0.2ml extract, made up to 3 ml total volume with water.

Particular care should be taken to use fresh K_2HPO_4 buffer as it was found that buffer of more than a week old, even when kept cold, resulted in significantly lowered activities.

The assay was started by adding the $NADH_2$. Controls omitted the $NADH_2$. The reaction was stopped by the addition of 1 ml. of 1% sulphanilamide in N HCL and the colour developed by the addition of 1 ml. of 0.01% N - (-1- napthyl) - ethylenediamine hydrochloride in water. The colour was read after 30 minutes in an EEl spectrophotometer at 540 n.m.

D. LABELLING: METHOD AND APPARATUS

Labelling was conducted in a perspex box (10 x 20 x 40 cm) (plate 3) from which plant material could be removed at varying intervals. The labelling was conducted in a phytotron chamber held at constant environmental conditions, similar to the plants growth conditions, ie., $\leq$ 500 p.p.m. CO_2 and 21% O_2, unless otherwise specified.

Labelled $C^{14}O_2$ was released in the box by $\pm$ .3ml of 6 N HCl, onto Ba $HC^{14}O_3$, from a dropper sealed into the top (plate 3). Although HCl fumes are known to have harmful effects on biological materials the quantity used ($\pm$ .3ml.) was extremely small.

Given the large volume of the measuring chamber the effect of the HCl fumes were unlikely to have any marked effect on the leaves due to the great dilution of the fumes. This is supported by the linear relationship shown by the figures of total $C^{14}O_2$ uptake for the time-course labelling experiment (Table 2, p.52a).

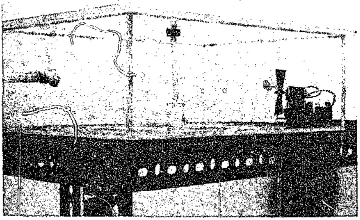


PLATE 3. Showing the perspex 'box' used for the labelling of barley leaves with $C^{14}O_2$.

Additional light was supplied by a 1500 watt tungsten lamp for the experiments requiring a 'high' light intensity.

Plant material for labelling experiments was treated in the same manner as for the T determination (see B (c)).

The $BaHC^{14}O_3$ was obtained from The Radio-chemical Centre, Amersham, England in 1 mci vials. One vial (58.6 mci/m mol) was used for each labelling experiment.

E. SEPARATION PROCEDURE.

(a) EXTRACTION OF MATERIAL.

After exposure to $C^{14}O_2$ the plant material was removed from the labelling box and immediately placed in boiling 80% ethanol.

The tissue was boiled for two minutes. The material remaining, constituted the insoluble residue and was discarded.

The lipids were separated from the alcohol extracts after these were evaporated to dryness using a heated air stream. Chloroform was used to dissolve the lipid fraction, which was then discarded with the chloroform.

After removal of the lipid fraction the water soluble residue was re-suspended in 10ml. of distilled water and a 0.5ml. aliquot of sample removed for radioactive determination - this was used for the determination of the 'total count' of $C^{14}O_2$ taken up by the plant.

(b) SEPARATION OF WATER SOLUBLE COMPONENTS.

The method followed is that presented by Canvin (1960). The water soluble components were fractioned into three fractions by means of ion-exchange resins. The extract was first passed through a 6 x 1cm column of Dowex 50 - x 8 (H+) (Calbiochem purified resin) and then through a 6 X 1cm column of Dowex 1 - x 10 (formate) (Calbiochem purified resin). The formate column was generated from the chloride form with 1M Na formate and 0.1 N formic acid. The Dowex 50 column retains basic compounds and the Dowex 1 column retains the acidic compounds leaving the neutral compounds in the effluent.
The basic compounds were eluted from the column with 50 ml 1N NH_4OH, the acidic compounds were eluted with 40 mls. 4N formic acid followed by 20mls. 8N formic acid.

The four fractions obtained then were:-

(1) Basic - composed mainly of A.A.
(2) Acidic - composed mainly of organic acids but also containing some phosphorylated compounds.

(3) Neutral fraction - composed almost exclusively of sugars.

(4) 'Lost' fraction. - This fraction represented the difference between the 'Total Count' (measured as described in E (a)), and the sum of the above three fractions. This 'Lost' fraction was found to be held on the basic column and has been termed the A_2 fraction by Canvin (pers. com.). This fraction is composed primarily of sugar phosphates. Tew (unpub. data) has eluted this fraction off the basic column and has shown it to contain a high proportion of 3 - phosphoglyceric acid (upto 90%).

After evaporation of the fractions to dryness the precipitate was re-suspended in 10ml. of de-ionized water. Aliquots of 0.5ml were then extracted and the radioactivity determined for each fraction.

F. DETERMINATION OF RADIOACTIVITY.

Aliquots of 0.5ml and the separated fractions were placed in glass scintillation vials. To this was added 15.0 mls. of scintillation fluid (Lips and Beevers 1966a, 1966b) and the number of disintegrations per minute counted on a Packard Tri-Carb Scintillation Spectrometer.

G. VARYING CO_2 AND O_2 CONCENTRATIONS: APPARATUS AND METHODS

Plants were grown in a large perspex box (plate 4) through which a mixture of gasses was passed. The gas mixture was varied by passing gasses from separate N_2, O_2, and CO_2 gas bottles (Afrox) at varying rates, into a mixing bottle and then through the perspex box.

The gas composition was monitored on the papamagnetic O_2 analyser and I.R.G.A.

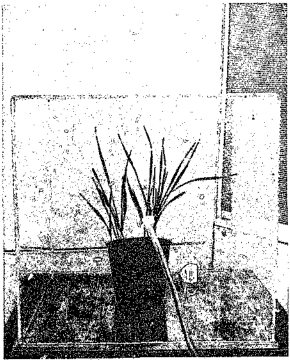


PLATE 4. Showing the perspex 'box' in which barley plants were grown under variable gas concentrations.

Due to the crude nature of the gas control valves, gas values could only be maintained within a given range.

H. INDIVIDUAL EXPERIMENTS.

(a) T AND LIGHT INTENSITY (FIGURE 1)

Eight leaves all of the same age and position (i.e. leaf 2 etc.) were utilized for each set of T determinations. Once T was determined the light intensity was changed using the rheostat. Light intensity range was 10,000 to 100,000 lux. After a stable T was maintained for 15 minutes at the new light intensity, it was recorded. Leaves were sampled at various times during the day.

Reversibility of the T was tested by returning the light intensity to the original level and

The gas composition was monitored on the papamagnetic O_2 analyser and I.R.G.A.

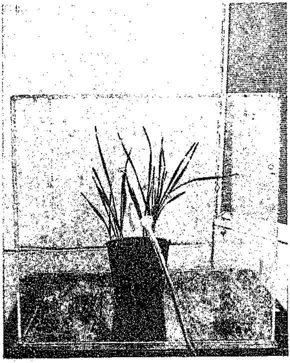


PLATE 4. Showing the perspex 'box' in which barley plants were grown under variable gas concentrations.

Due to the crude nature of the gas control valves, gas values could only be maintained within a given range.

H. INDIVIDUAL EXPERIMENTS.

(a) T AND LIGHT INTENSITY (FIGURE 1)

Eight leaves all of the same age and position (i.e. leaf 2 etc.) were utilized for each set of T determinations. Once T was determined the light intensity was changed using the rheostat. Light intensity range was 10,000 to 100,000 lux. After a stable T was maintained for 15 minutes at the new light intensity, it was recorded. Leaves were sampled at various times during the day.

Reversibility of the T was tested by returning the light intensity to the original level and re-checking the T. If a fluctuation of more than .5 p.p.m. occured, a re-check of the other T levels was carried out.

Plants were grown in soil and supplemented weekly with a Long Ashton Nutrient Medium (appendix 14).

(b) T AND LEAF AGE (FIGURE 2).

The experiments were run over a period of six days. Leaves were sampled once they had reached a length of 8 to 10 centimeters. A new sample of leaves was cut for the T determinations for each succeeding day. Leaves were sampled four hours after the lights switched on in the growth control rooms. T determinations were made at three light intensities namely:- 10,000, 20,000, and 30,000 lux. Determinations at each of these light intensities was made at least twice for every leaf sample.

This experiment was made on the first three leaves to appear on the plant axis.

Plants were grown as in A(a).

(c) T, LEAF AGE, LEAF POSITION AND ENZYME ACTIVITY (FIGURES 3, 4, 5, 6.)

Plants were grown in soil and supplemented daily with a Long Ashton Nutrient solution. On the fourth day the plants were thinned to leave only plants of equal height. Two growth chambers were used with a staggered light regime, so that sampling could be made twice a day, after the plants had received only four hours of light. Twenty-four hours prior to sampling the plants were supplied with 100ml. nitrate solution containing 500 p.p.m. nitrate.

Sampling commenced after the first leaf was longer than 10cms. This leaf was only fully expanded four days after the initial sample. The same procedure was followed for leaves 2, 3, and 4. Sampling continued for 31 days, samples being taken every second day.

Sixteen similar leaves in age and leaf position were used for each set of determinations, eight of which were used to determine the T, and the remainder for enzyme activity.

T determinations were conducted at 20,000 lux.

(d) ENZYME AND T FLUCTUATION DURING A SINGLE DAY. (FIGURES 7,8, 9.)

Plants were grown in a Long Ashton nutrient solution and sampled at the beginning of the light period and at varying intervals thereafter.

T determinations were made at 20,000 lux. Leaf 2 was used for these determinations.

(e) EFFECT OF VARIABLE NUTRIENT SUPPLY ON ENZYME ACTIVITY AND T. (FIGURES 10 AND 11).

Plants were grown in nutrient cultures varying in the quantity and form of nitrogen supply. The low nitrate experiments contained between 10 and 50 p.p.m. of nitrogen. The K and Ca levels were maintained by the supply of K_2SO_4 and $CaCl_2$. The high nitrate experiments contained 400 p.p.m. of nitrogen, with K and Ca ratio being maintained by a balanced supply of KNO_3 and $Ca(NO_3)_2$. Nitrogen supplied as ammonia was at the rate of 300p.p.m. with the ammonia supplied as $(NH_4)_2SO_4$ and K and Ca as in the low nitrate treatment.

Leaf 2 was used exclusively and first sampled on the 11th Day after germination. T was determined at 20,000 lux. Leaves were sampled as described previously in H(c).

(f) EFFECT OF HIGH CARBON DIOXIDE CONCENTRATIONS ON ENZYME ACTIVITY (FIGURE 12).

Plants were grown as in (a) and placed in a large perspex box through which a gas containing high CO_2 levels (1 to 3%) was passed. Leaf 1 was sampled 5 days after germination and 18 hours after being placed under high CO_2 conditions. A control plant outside the box was sampled concurently with the experimental plant. Sampling was as described in H(c). Sampling continued for five days.

(g) HIGH AND LOW OXYGEN EFFECTS ON ENZYME ACTIVITY (FIGURE 13).

Method was as in H(f) with O_2 levels maintained at $\pm$ 50% and <5%, keeping CO_2 concentrations below 500 p.p.m. PLANTS PLACED UNDER LOW O_2 CONDITIONS ONLY AFTER THE INITIAL SAMPLE TAKEN ON DAY 5.

(h) EFFECT OF HIGH AND LOW LIGHT INTENSITIES ON ENZYME ACTIVITY (FIGURES 14 AND 15)

Two slightly differing experiments were run:-

(i) Plants were grown as in (a) in the phytotron at varying light intensities. These being 8,000, 11,000 and 25,000 lux. Sampling was as described in H(c). T was determined at 20,000 lux for all light treatments.

(ii) Only two light intensities namely:- 10,000 and 25,000 lux were used in this experiment. T was again determined at 20,000 lux. The initial T and enzyme determinations on day 5 (fig. 15) were made on both the groups of plants growing under light conditions of 25,000 lux. Immediately after this determination one of the groups of plants was moved to light conditions of 10,000 lux.

(i) INCORPORATION OF $C^{14}O_2$ WITH RESPECT TO TIME (FIGURE 16)

Labelling with $C^{14}O_2$ of the material was carried out in the light as in (D). Leaf material was removed from the perspex box at intervals of 15 seconds, 60 seconds, 5 minutes, 10 minutes and 15 minutes and immediately extracted as in E(a). Light intensity was maintained at 20,000 lux and material was prepared as in (B (c)). and grown as described in (A (b)). A short period of 10 minutes in $C^{12}O_2$ was allowed for equilbration.

(j) EFFECT OF VARYING NUTRIENT SUPPLIES ON THE DISTRIBUTION OF C14. (FIGURE 17).

Labelling with $C^{14}O_2$ of the material was carried out for 10 minutes as described in (D) and immediately extracted as described in E(a). Light intensity was maintained at 20,000 lux. Plant material was prepared as described in B(c) and grown as described in H(e). A short period of 10 minutes in $C^{12}O_2$ was allowed for equilbration.

Leaf two was used exclusively and was cut soon after full expansion.

(k) EFFECT OF HIGH CO_2 CONCENTRATIONS ON THE DISTRIBUTION OF C14. (FIGURE 18).

Labelling procedure was conducted as in H(j) except for the presence of a high CO_2 concentration (> 1%) in the perspex box.

Material was grown as described in H(c) and prepared as described in B(c). A sample of the plants were further pre-treated, for 18 hours prior to labelling, in a high CO_2 concentration environment.

Leaf one was used exclusively for these experiments.

(l) EFFECT OF LOW OXYGEN CONCENTRATIONS ON THE DISTRIBUTION OF C14 (FIGURE 19).

Labelling procedure was conducted as described in H (j) with however low O_2 concentrations (< 5%) and normal CO_2 concentrations being maintained in the perspex box.

Material was grown as described in H(i) and prepared as desribed in B(c). Leaf one was used exclusively for these experiments.

(m) EFFECT OF HIGH AND LOW LIGHT ON THE DISTRIBUTION OF C14. (FIGURE 20).

Labelling procedure was again conducted as described in H(j). Two separate labelling experiments were carried out one at 10,000 lux and the other at 30,000 lux on leaves before (6 days) and after full expansion, (9 days) had occured.

Material was grown as described in H (i) and prepared as described in B(c). Leaf two was used exclusively for the experiment.

In all labelling experiments the material, after extraction, was separated as described in E (b) and radio-activity of samples was determined as described in (F).

All experiments were conducted at least twice and enzyme activities were tested for any effect the 'Box' (plate 4) may have had on their activity. The influence of the box was shown to be negligable.

RESULTS

Results are presented and are numbered and headed to correspond with the experiments described in the section under Methods.

H. (a) T AND LIGHT INTENSITY (FIGURE 1.)

T was observed to vary with light intensity (fig. 1.). As light intensity increased from 30,000 to 100,000 lux, T increased linearly, but the extent of T increase for different samples of leaves differed over a given range of light intensities above 30,000 lux. This is indicated by the slope. Below 30,000 lux the T response was varied. Change of T with light intensity from 10,000 to 30,000 lux was not always linear, in relation to increase of T above 30,000 lux. Furthermore for a given light intensity the T of a sample of leaves also varied.

The light intensity at which the lowest T observed was not consistant and varied between 10,000 and 20,000 lux for the different samples.

The variable response of T, to light intensity, observed with different groups of leaves suggest that the response of T to light intensity may be dependent on the physiological state of the leaves used.

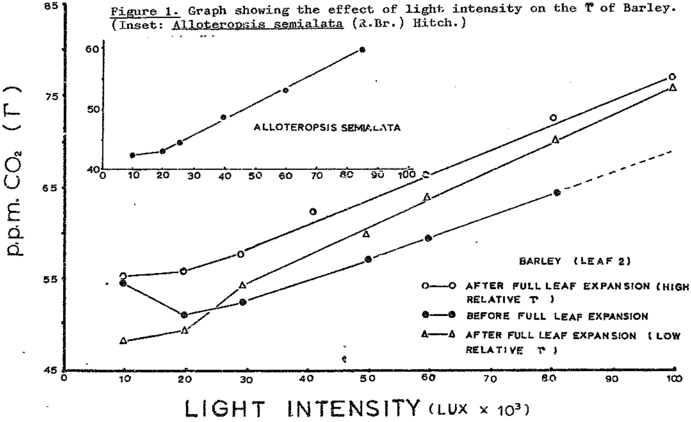
(b) T AND LEAF AGE (FIGURE 2.)

To further investigate the variable response of T to light intensity, the T response for given intensities was determined with leaves of increasing age.

The first three leaves to appear on the plant axis were measured at three light intensities, namely:- 30,000, 20,000, and 10,000 lux (Graphs a, b, and c respectively fig. 2.).

These three light intensities were chosen as they were the light intensities at which the anomalies in T response were observed in the previous experiment (fig. 1).

Figure 1. Graph showing the effect of light intensity on the Γ of Barley.
(Inset: *Alloteropsis semialata* (R.Br.) Hitch.)



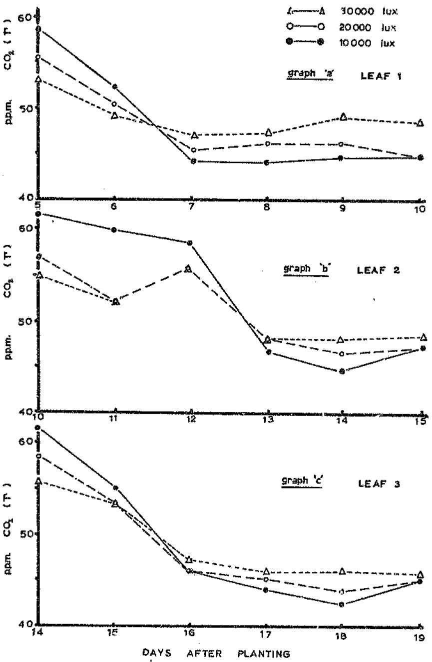


Figure 2. Graphs showing the variable response of Γ to light intensity with age in leaves one, two and three respectibely.

T in leaves 1, 2 and 3 was observed to be relatively high during the leaf expansion stage irrespective of the light intensity, decreasing to a relatively stable level as the leaf reached full expansion and remaining at this level during the remainder of the experimental period. The relationship between the T's at the three light intensities used was also observed to vary from day to day. During the early expansion phase of a leaf's growth the lowest T was obtained at the highest light intensity used (30,000 lux) and the highest T at the lowest light intensity (10,000 lux). With leaf development this relationship was reversed, i.e. 10,000 lux giving the lowest and 30,000 lux the highest T. The relative difference between the T's for the three light intensities used also varied from day to day, i.e. the T occasionally being the same for the 10,000 and 20,000 lux determinations, or for the 20,000 and 30,000 lux determinations (fig. 2). The average or mean level of T was the same for the three leaves studied after full expansion.

(c). T, LEAF, AGE, LEAF POSITION AND ENZYME ACTIVITY. (FIGURES 3, 4, 5, AND 6).

Having confirmed the highly variable natur of the T, even if only small in magnitude, the following experiment was designed to test the implied variability of the CO_2 uptake (photosynthesis) and major CO_2 output (photorespiration) mechanism of the plant in the light. In this experiment the nutrient level was maintained at optium levels by supplementing with a Long Ashton nutrient medium. The experiment was run for 30 days, a longer period than the previous experiments.

At this stage Mr. John Tew joined me and carried out the determination on ribulose 1-5, diphosphate

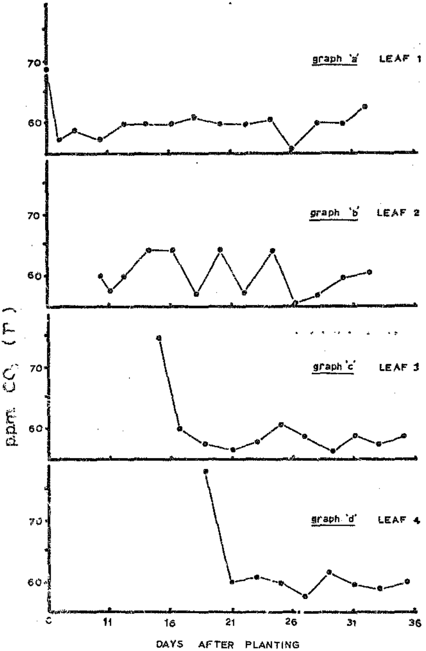


Figure 3. Graphsshowing the Γ with age in leaves one, two, three and four respectively.

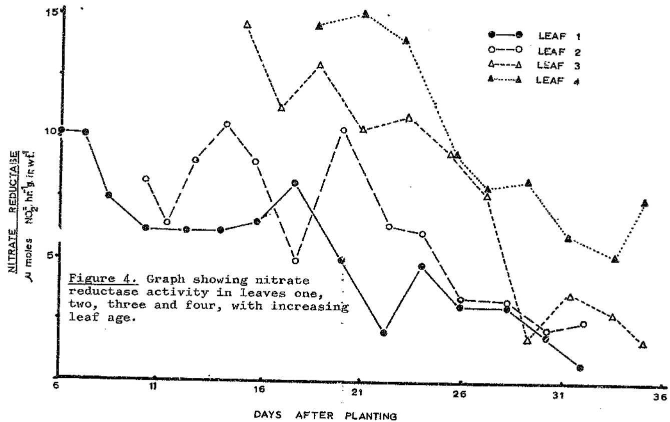
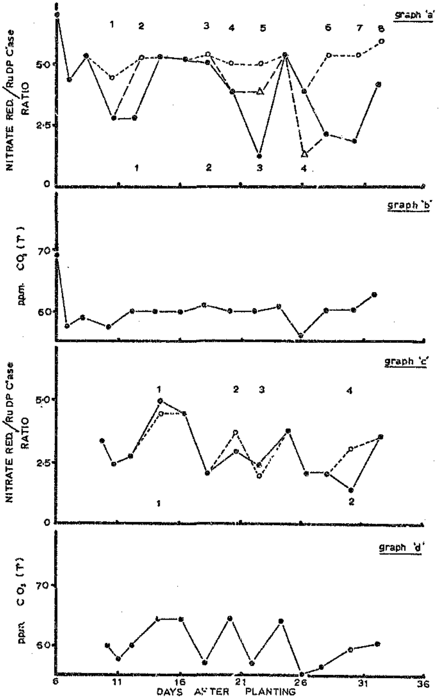


Figure 4. Graph showing nitrate reductase activity in leaves one, two, three and four, with increasing leaf age.

EXPLANATION OF FIGURES 5 AND 6 -

o----o show the position of the calculated nitrate reductase: RuDP carboxylase ratio that would give a 100% correlation with the measured T on a relative basis (i.e. the direction and the magnitude of change are considered). The upper set of numerals indicates the number of points of the ratio that do not correlate.

△----△ show the position of the calculated nitrate reductase: RuDP carboxylase ratio that would give a 100% correlation with the measured compensation point on a non-proportional basis (i.e. only the direction of change is considered and not the magnitude). The lower set of numerals indicates the number of points of the ratio that do not correlate.



<u>Figure 5.</u> Graphs showing Γ with time in days and nitrate reductase/ribulose 1-5,diphosphate carboxylase ratio in leaf 1 (graphs 'a' and 'b') and leaf 2 (graphs 'c' and 'd').

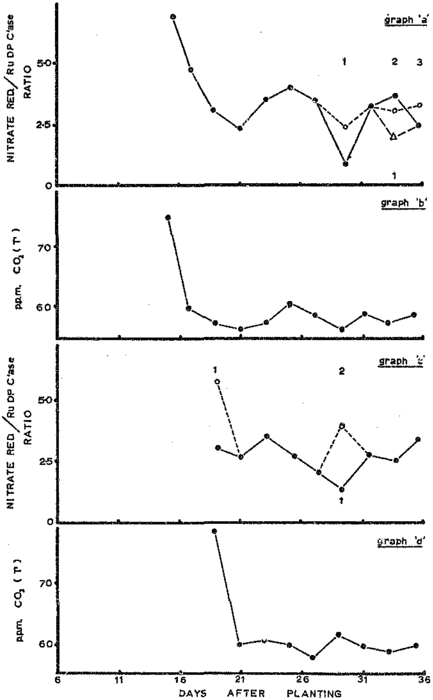


Figure 6. See figure 5. Leaves 3 and 4.respectively.

carboxylase activity and glycollate oxidase activity, the results of which are presented in the appendix.

(i) CO_2 COMPENSATION POINT (T)

As observed in the previous experiment the initial T's in leaves 1,3 and 4 are relatively high with the T decreasing to a lower level with full expansion of the leaf (fig. 3). The T then remaining relatively constant with individual T fluctuations about a mean. The T mean for all leaves studied was similar, being at or about 60 p.p.m. and is of particular interest when compared to the enzyme activities. The absence of a high initial T for leaf two, (fig. 3'b') may be explained by the fact that the leaf was relatively more developed than the other leaves, for the first determination.

The pattern of T fluctuation of the four leaves studied varied with the greatest fluctuation in T being observed in leaf two (fig. 3'b').

(ii) NITRATE REDUCTASE ACTIVITY.

Activity increased with each succeeding new leaf studied on the plant axis and decreased with leaf age (fig. 4). Day to day activity was variable with sharp rises and falls in activity. No regular pattern of activity was noted between the leaves studied other than the increase with leaf position and the overall decrease with age, mentioned above. No direct correlation was found between nitrate reductase activity and T. No. correlation with either ribulose 1-5, diphosphate carboxylase activity or glycollate oxidase activity and T was found (appendices 1 and 2). Furthermore the activities of all the enzymes decrease with age, but T remains at the same relative level (fig. 3).

(iii) RATIO OF NITRATE REDUCTASE ACTIVITY TO RIBULOSE 1-5, DIPHOSPHATE CARBOXYLASE ACTIVITY.

No correlation between T and the enzymes studied was observed, but it was felt that the ratios of the enzymes selected to represent the processes of photo-respiration and photosynthesis (see Introduction) should give a correlation. The nitrate reductase/ribulose 1-5, diphosphate carboxylase ratio (figs. 5 and 6) gave a high degree of correlation with T (Table 1), particularly in leaves 2, 3, and 4. A statistical analysis by linear regression analysis of these relations....(continued overleaf).

Leaf No.	% Correlation with T a(i)	b(i)	a(ii)	b(ii)
1	27	47	53	73
2	54	69	77	85
3	64	75	83	91
4	22	79	67	89

Table 1. The percentage positive correlation of the T with the calculated ratios of the four leaves sampled.

(a) The percentage correlation of the glycollate oxidase/ribulose 1-5, diphosphate carboxylase ratio.
   (i) on a relative basis
   (ii) on a non-proportional basis (i.e. when the direction of change and not the magnitude is considered).

(b). The percentage correlation of the nitrate reductase/ribulose 1-5, diphosphate carboxylase ratio (i) and (ii) as for a.

gave significant results at the 95% confidence level for leaves one (R = .535) and two (R = .639) and the 99% confidence level for leaf three (R = .819). Statistical correlation in leaf four was not significant. This however may be explained by the fact, that the first point (fig. 6, graph 'c') is so far 'out', and also the low level of the degrees of freedom.

Although the glycollate oxidase/ribulose 1-5, diphosphate carboxylase ratio (appendix 3 and 4) also gave satisfactory levels of correlation these were not as high as the nitrate reductase / ribulose 1-5, diphosphate carboxylase ratio. A regression analysis of this ratio showed no significant correlation with T for any of these leaves.

It is significant that the ratios of both nitrate reductase activity (fig. 5 and 6) and glycollate oxidase activity (appendix 3 and 4) to ribulose 1-5, diphosphate carboxylase do not show an overall drop as do the individual enzyme activities but remain about a mean as does the T of the leaves.

(d) ENZYME AND T FLUCTUATION DURING A SINGLE DAY (FIGURES 7, 8, and 9)

In an attempt to achieve a higher degree of correlation between the nitrate reductase/ribulose 1-5, diphosphate carboxylase ratio and T it was decided to monitor enzyme activity and T over a single light period.

The patterns of T and nitrate reductase activity varied from day to day (figs. 7, 8 and 9) as did the mean levels of T and nitrate reductase activity. Furthermore the T was observed to vary with the duration of the light treatment as seen in Figures 7 and 8. In the case of the younger leaf (fig.7) there was a general trend for the T to increase with length of light treatment, whereas the opposite was observed in the case of the older leaf (fig.8) whose T decreased with length of light treatments.

The initial T at the commencement of the light

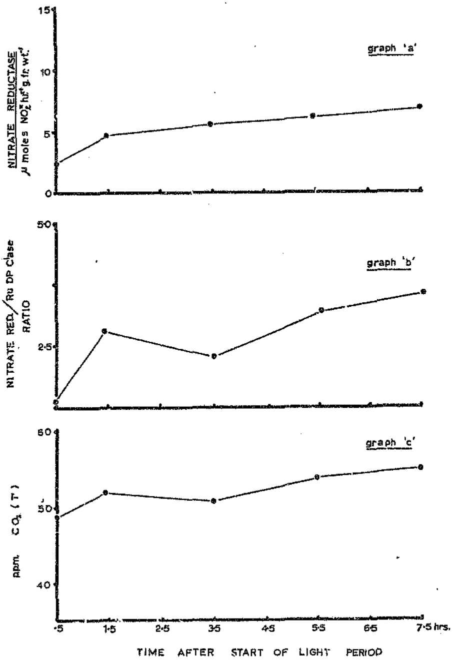


Figure 7. Graphs showing T fluctuation (graph 'c'), nitrate reductase/ribulose 1+5,diphosphate carboxylase ratio (graph 'b') and nitrate reductase activity (graph 'a'). Sampled 14 days after planting.

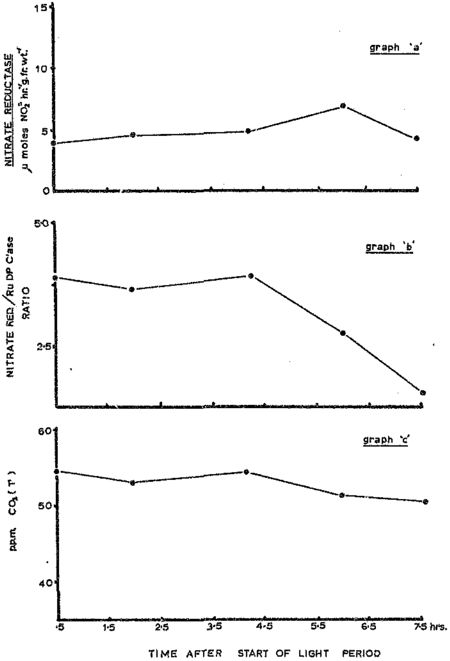


Figure 8. See figure 7. Sample 18 days after planting.

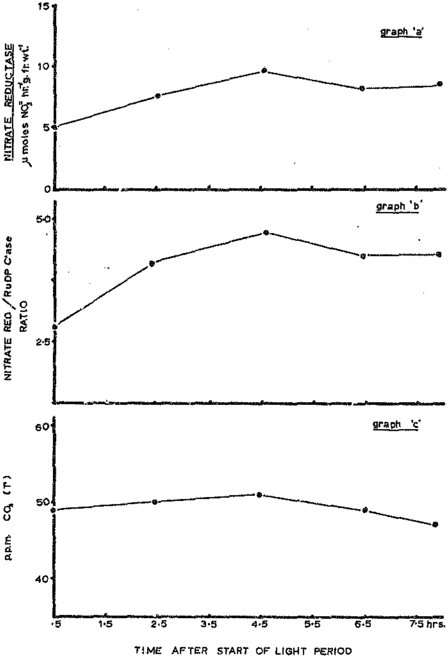


Figure 9. See figure 7. Graphs showing relatively poor correlation between the 'ratio' and T.

treatment was higher in the older leaf than observed in the younger leaf. These observations further emphasize the dynamic nature of the T.
A number of determinations with different leaf ages were made, with varying degrees of correlation. Perfect correlation between T and the nitrate reductase/ribulose 1-5, diphosphate carboxylase ratio was achieved for two determinations with leaf two, cut 14 and 18 days after planting (figs. 7 and 8). One determination had one point out and is presented in fig. 9.(cut 16 days after planting). A regression analysis of these ratios and T gave extremely high positive correlations at the 99.9% level for both the leaves cut 14 days (R = .994) and 18 days (R = .974) after planting.
Ribulose 1-5, diphosphate carboxylase and glycollate oxidase were again monitored by Mr. Tew (appendices 5, 6 and 7).

(e) <u>EFFECT OF VARIABLE NUTRIENT SUPPLY ON ENZYME ACTIVITY AND T (FIGURES 10 and 11)</u>

Having shown a possible relationship between nitrate reductase activity and T, and by implication photorespiration, it was decided to attempt to stimulate or suppress nitrate reductase activity, by growing the plants under varying levels of nitrogen supplied as nitrate. Nitrogen was also supplied as ammonia to study the effect of, bypassing the nitrate reduction step, on T.
Nitrate reductase activity was shown to be high under conditions of high nitrate supply and at minimal levels under the conditions of low nitrate supply used. As expected nitrate reductase activity was close to zero when nitrogen is supplied as ammonia (fig. 10).
For ease of comparison Mr. Tew's results of ribulose 1-5, diphosphate carboxylase and glycollate oxidase activity are also presented in fig. 10 and not in the appendix. Glycollate oxidase activity is

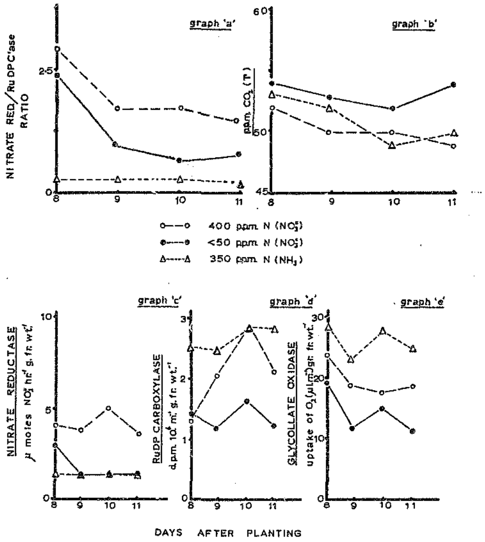


Figure 10. Graphs showing the effect of variable levels and modes of nitrogen supply on nitrate reductase activity, Γ, ribulose 1-5,diphosphate carboxylase, glycollate oxidase and the nitrate reductase/ribulose 1-5,diphosphate carboxylase ratios.

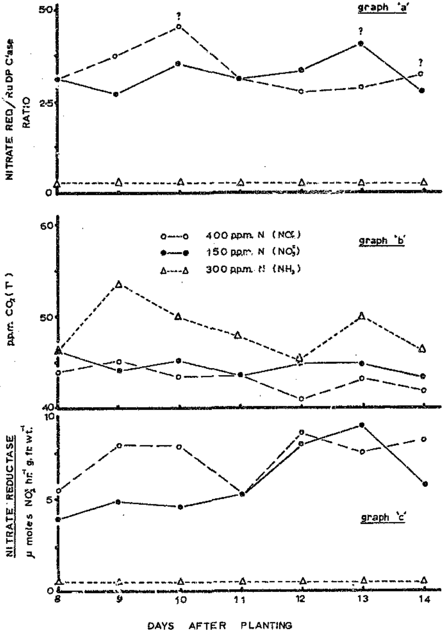


Figure 11. Graphs showing the effect of variable levels and modes of nitrogen supply on nitrate reductase activity, Γ, and nitrate reductase/ribulose 1-5,diphosphate carboxylase ratios

markedly stimulated by the high nitrogen levels, with the greatest stimulation observed when nitrogen is supplied as ammonia. Ribulose 1-5, diphosphate carboxylase activity is also stimulated by high nitrogen levels with the ammonia treatment again showing the greatest stimulation (fig. 10 graph 'd').

T's of the three treatments are not markedly different in this experiment. The T's of the low nitrate treatment were observed to be higher than those for the high nitrate and ammonia treatments. The ratios of the nitrate reductase ribulose 1-5, diphosphate carboxylase activities show a high degree of correlation to the observed T's with the obvious exception of the ammonia treatment. The correlation is one hundred percent in the high nitrate treatment and only the last point (day 11) out in the low nitrate treatment (fig. 10 graph 'a') The relative levels of the high and low ratios of nitrate reductase/ ribulose 1-5 diphosphate carboxylase are however reversed with respect to the observed T, with the mean of the high nitrate treatment being higher than the mean of the low nitrate treatment.

A second slightly modified experiment was conducted in which the ph of the nutrient solution was closely controlled. The low nitrate 'treatment' was also supplied with a level of nitrogen approximating to normal nutrient media values i.e. 150 p.p.m. nitrogen as opposed to 50 p.p.m. nitrogen in the previous experiment (fig. 11). Essentially similiar results to the previous experiment were obtained.

Once agin the 'high' nitrate treatment gave a higher mean nitrate reductase activity than the 'low' nitrate treatment, and no activity when nitrogen was supplied as ammonia (fig. 11). Glycollate oxidase and ribulose 1-5, diphosphate carboxylase activities were again stimulated by

increasing levels of nitrogen supply, with however a very marked stimulation in activity in the plants supplied with ammonia (appendix 8).
No significant difference in the T's of the high and low nitrate treatments was observed, but a very marked stimulation in the level of the T in the ammonia treated plants was noted (fig. 11).
This increase in the T's is in accordance with the very marked rise in glycollate oxidase activity observed (appendix 8).
This rise in glycollate oxidase activity is proportionately greater than the rise of activity in ribulose 1-5, diphosphate carboxylase and is supported by the plant's glycollate oxidase/ ribulose 1-5, diphosphate carboxylase activities ratio (appendix 8).
The nitrate reductase/ribulose 1-5, diphosphate carboxylase activities ratio for the high and low nitrate treatment again shows a high degree of correlation with the observed T's (fig. 11).
Two points, on days 10 and 14, in the high nitrate treatment are at variance with the T and one point on day 13, is 'out' in the low nitrate treatment.
When a simple linear regression analysis was applied to the data however, no correlation was obtained. This lack of statistical correlation may be explained by the small degrees of freedom and the resultant disproportionate effect of the ratio values obtained for day 10 in the 400 p.p.m. nitrogen treatment and day 8 in the 150 p.p.m. nitrogen treatment. When these points are adjusted to 3.4. units (400 p.p.m. treatment) and 4.4. units (150 p.p.m. treatment) correlations at the 95% (R = .725) and 99.9% (R = .956) confidence levels were obtained respectively.

(f) EFFECT OF HIGH CARBON DIOXIDE CONCENTRATIONS ON ENZYME ACTIVITY (FIGURE 12)

Having shown that factors known to affect nitrate reductase activity and assimilation had a similar

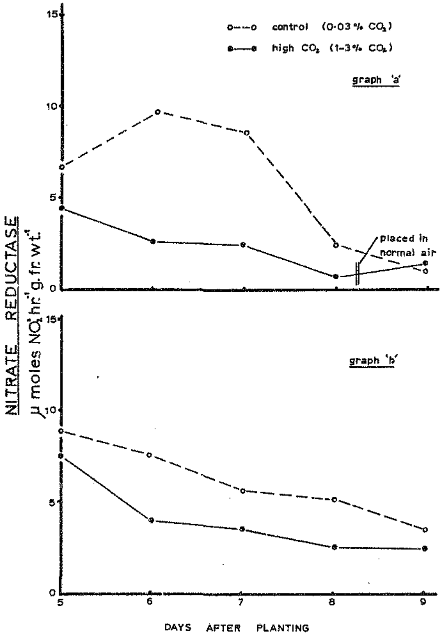


Figure 12. Graphs showing the effect of high CO_2 levels (1-3% CO_2) on nitrate reductase activity.

effect on enzymes of photorespiration and photosynthesis as well as T, it was decided to investigate the possibility of factors known to affect photosynthesis and photorespiration for their effects on nitrate reductase activity.
The first of these factors to be studied was the effect of high levels (>1%) of CO_2 on nitrate reductase. Nitrate reductase activity was sharply suppressed by high CO_2 (fig. 12 'a' and 'b') as was glycollate oxidase activity (appendix 9) while ribulose 1-5, diphosphate carboxylase activity was greatly stimulated (appendix 10). The suppression of nitrate reductase activity is apparent even on day 5 after only 18 hours of growth under high CO_2 conditions (fig. 12, graph 'a' and 'b'). The smaller degree of suppression in graph 'b' (fig. 12) of nitrate reductase activity may be due to a relatively lower concentration of carbon dioxide than that pertaining in graph 'a' (fig. 21). When the plants grown under high CO_2 levels were again placed in normal air the activities of the above enzymes returned to the level of the control plants (fig. 12, graph 'a' appendix 9 and 10 graph 'a').
The pattern of suppression or stimulation of the enzymes studied was maintained for the full five days of the experiment.
The plants grown under high CO_2 levels showed robust growth with however a distinct yellowing of the leaf tips.
Attempts to achieve a low relative T using plants with suppressed nitrate reductase and glycollate oxidase activities (fig. 12 and appendix 9 respectively) were unsuccessful. It was shown however that glycollate oxidase showed a 50% increase in activity in less than 60 minutes, when the CO_2 level was dropped to 300 p.p.m. CO_2 or below (Tew unpub. res.). It was not possible to achieve a T in less than 45 minutes after

removal of the plant material from the high CO_2 environment. As the nitrate reductase activity is influenced by the levels of amino acids and amides (Stewart 1972) and it is these compounds that are directly involved in the amination steps of the glycollate cycle, nitrate reductase activity itself would have no regulatory affect on this cycle. Nitrate reductase activity would still be a measure of glycollate cycle activity if sufficient time is allowed for the fluctuation of the amino acid pool size, caused by glycollate cycle activity to affect the nitrate reductase. Furthermore if nitrate reductase activity (nitrate assimilation) was actively suppressed (low nitrate supply), while a high demand for ammonia by the glycollate cycle existed, then nitrate assimilation could exert a controlling effect on the cycle.
Heath and Orchard (1967) have however shown that the light compensation point is reduced at high levels of CO_2.
Very high levels of CO_2 ($\pm$ 20%) were found to be toxic to the plants.

(g) HIGH AND LOW OXYGEN EFFECTS ON ENZYME ACTIVITY (FIG. 13).

The results for the experiment on the effects of high O_2 (42%) levels on nitrate reductase activity were unsatisfactory and are not presented. The high oxygen levels probably affected the assay procedure as very low levels of nitrate reductase activity [illegible] obtained were probably not a reflection of true activity as the normal assay colour was not obtained under these conditions. The plant however showed robust growth with no signs of yellowing, suggesting satisfactory nitrate assimilation.
Plants grown under low O_2 ($<5\%$) conditions showed a marked suppression of nitrate reductase activity (fig. 13). Glycollate oxidase activity and ribulose 1-5, diphosphate carboxylase activity were

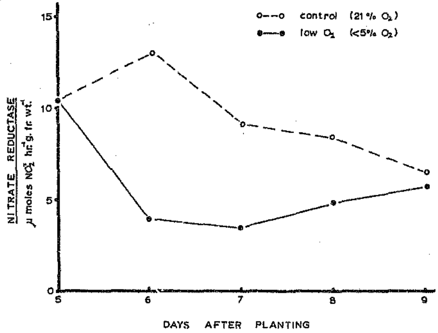


Figure 13. Graph showing the effect of low leve
(<5%) on nitrate reductase activity.

also suppressed by low O_2 levels (appendix 11). Ribulose 1-5, diphosphate carboxylase activity was however not so sharply suppressed as either nitrate reductase activity or glycollate oxidase activity. The initial activities on day five (fig. 13, appendix 11) were the same for both the control plants and the experimental plants as this determination was made on the plants before they were placed under low O_2 conditions.

(h) EFFECT OF 'HIGH' AND 'LOW' LIGHT INTENSITIES ON ENZYME ACTIVITIES (FIGURES 14 AND 15).

In previous experiments (figs. 1 and 2) as it was established that an increase in light intensity above 'limiting values' resulted in an increase in T and presumably photorespiration, it was decided to investigate the effect of increasing light intensities on nitrate reductase activity.
The initial experiment was conducted using three light intensities namely 8,000, 10,000, and 25,000 lux. Plants were grown under these intensities and sampled for a period of five days. Nitrate reductase activity rose with increasing light intensity (fig. 14) as did glycollate oxidase and ribulose 1-5, diphosphate carboxylase activity (appendix 12). The T's for the three light intensity treatments did not show any marked overall differences (fig. 14) T's were initially 'high' decreasing to lower levels with leaf age, which was consistent with previously observed patterns (fig. 2). Plots of the nitrate reductase/ribulose 1-5, diphosphate carboxylase activities ratios once again showed a high degree of correlation with the observed T's, particularly in overall patterns i.e. high levels dropping with leaf age (fig. 14). A linear regression analysis of the correlation gave confidence limits of 95% for the plants grown at 25,000 lux (R = .909) and 10,000 lux (R = .906) No correlation was obtained for the plants grown at 8,000 lux. This due to the ratio result obtained for day 5 (fig. 14).

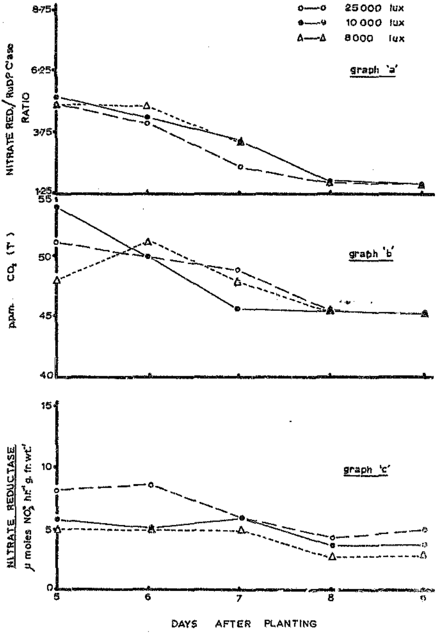


Figure 14. Graphs showing the effect of light intensity on the level of nitrate reductase activity, T, and nitrate reductase/ribulose 1-5,diphosphate carboxylase ratio.

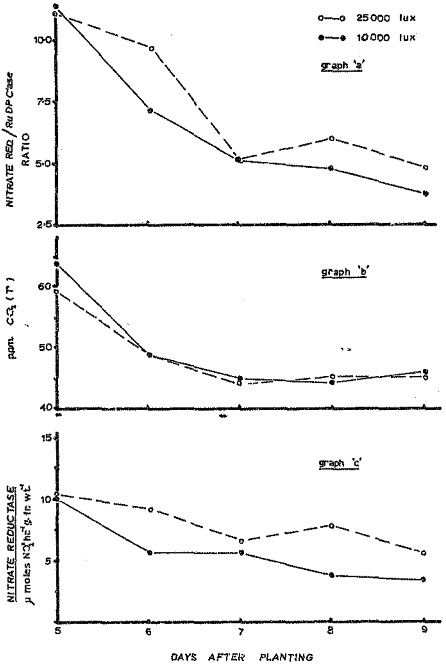


Figure 15. See figure 14.

In the second experiment presented, essentially similar results were obtained (fig. 15, appendix 13). Only two light intensities were used, namely 10,000 and 25,000 lux. In this experiment however both sets of plants were grown at 25,000 lux for the initial determination on day 5, before the one 'set' of plants was placed under the 'low' light conditions (10,000 lux). A good correlation between the nitrate reductase/ribulose 1-5, diphosphate carboxylase activities ratios and observed T was achieved particularly with the plants grown under low light intensity. A linear regression analysis gave a correlation at the 99.9% level for both 10,000 (R = .987) and 20,000 lux (R = .975).

The results presented above strongly support the possibility of a close connection between nitrate assimilation and photorespiration. This raises the possibility that one of the functions of the peroxisome or glycollate pathway is the supply of carbon skeletons for at least part of amino acid synthesis and subsequent protein metabolism in the light in the young rapidly expanding leaf. Furthermore in nitrate grown plants, nitrate assimilation could be expected to regulate the carbon flow through the glycollate pathway by means of the associated transaminase steps. To test this possibility a series of $C14O_2$ labelling experiments were conducted.

(i) INCORPORATION OF $C^{14}O_2$ WITH RESPECT TO TIME. ~~OR THE ASSOCIATED TRANSAMINASE STEPS~~ (FIGURE 16) AND TABLE 2).

In order to determine a satisfactory labelling period a time course incorporation of label, into young expanding leaves was conducted. After 15 seconds in $C^{14}O_2$ the major percentage of the total counts taken up are found in the 'lost' fraction (A_2 fraction contains up to 90% 3PGA - Canvin persr com. and Tew, unpub. data) and to a lesser extent in the organic acid fraction (fig. 16). Only a small

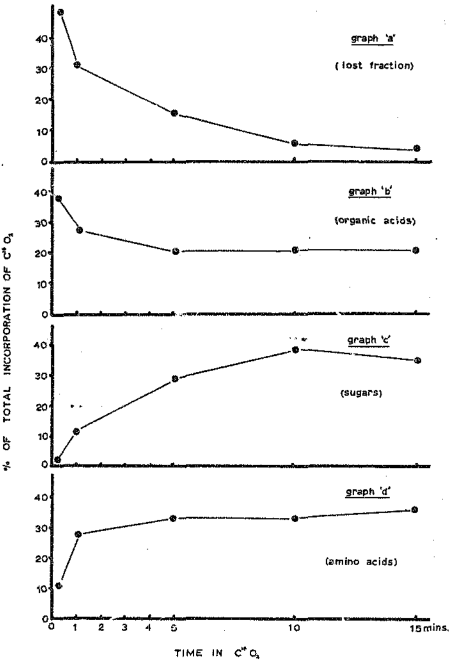


Figure 16. Graphs showing incorporation of $C^{14}O_2$ with respect to time -- leaf two (14 days after planting). Graph 'a'- lost fraction; Graph 'b'- organic acid fraction; Graph 'c'- sugar fraction; Graph 'd'- amino acid fraction.

RESULTS

TIME PERIOD IN $C^{14}O_2$	TOTAL COUNT D.P.M.	NEUTRAL FRACTION D.P.M.	ACID FRACTION D.P.M.	BASIC FRACTION D.P.M.	'LOST' FRACTION D.P.M.
15 secs	$.038 \times 10^6$	$.00075 \times 10^6$	$.0143 \times 10^6$	$.0044 \times 10^6$	$.0185 \times 10^6$
60 secs	$.036 \times 10^6$	$.012 \times 10^6$	$.036 \times 10^6$	$.036 \times 10^6$	$.052 \times 10^6$
5 mins	1.34×10^6	$.40 \times 10^6$	$.275 \times 10^6$	$.445 \times 10^6$	$.220 \times 10^6$
10 mins	2.31×10^6	$.90 \times 10^6$	$.500 \times 10^6$	$.750 \times 10^6$	$.160 \times 10^6$
15 mins	3.00×10^6	1.08×10^6	$.655 \times 10^6$	1.100×10^6	$.165 \times 10^6$

NOTE: The total count figures indicate that a relatively linear rate of $C^{14}O_2$ uptake occured with increasing time.

The figures for the 'Lost' fraction indicate that a relatively fixed ~~food~~ pool size for sugar phosphates exists, which is rapidly saturated (in $<$ 5 mins) with label.

TABLE 2: Disintergrations per minute obtained when leaf fractions were extracted and counted after being allowed to photosynthesize in $C^{14}O_2$ for varying time periods.

percentage of counts occuring in the amino acid and sugar fractions. With increased labelling time the percentage label in the 'lost count' decreases progressively as does the organic acid fraction, reaching steady state levels after ten and five minutes respectively. With increased labelling time the amino acid and sugar fractions show a steady rise in percentage label with a relatively steady state level being reached after 5 and 10 minutes respectively. The incorporation of label into the sugar fraction lags behind the amino acid fraction.

(j) EFFECT OF VARYING NUTRIENT SUPPLIES ON THE DISTRIBUTION OF C^{14} (FIGURE 17) AND TABLE 3)

Three levels of nitrogen were supplied as nitrate and one level of nitrogen was supplied, as ammonia, and the effect of these variable nutrient supplies on $C^{14}O_2$ distribution investigated. Low level of amino acid formation and high levels of sugar formation were found with low levels of nitrogen supply. Increasing levels of amino acid and decreasing levels of sugars were found, as nitrogen supply increased (fig. 17).

The plants treated with ammonia as a source of nitrogen do not give the high level of amino acid label (fig. 17) as would be expected from the enzyme activities (appendix 8). The label in sugars also drops slightly, relative to the high nitrate treatment (fig. 17).

The organic acid label increases with nitrogen supply when supplied as nitrate, but drops in the ammonia treatment. The 'lost' count is high in the low and high nitrate treatments and particularly so in the ammonia treatment. The normal nitrate treatment showing the lowest level of 'lost' count (fig. 17).

The pertinent enzyme activities are presented (fig. 17) and agree with the previously observed pattern (figs. 10 and 11 and appendix 5). Total counts, corrected for leaf weight, for each 'treatment' are also presented (fig. 17).

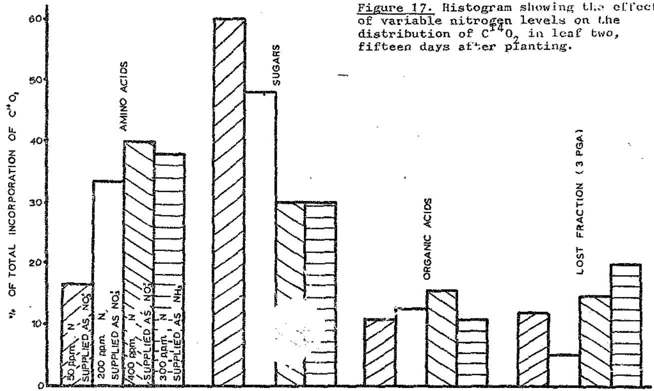


Figure 17. Histogram showing the effect of variable nitrogen levels on the distribution of $C^{14}O_2$ in leaf two, fifteen days after planting.

NUTRIENT CONDITIONS.	TOTAL COUNT D.P.M. X10^6	NEUTRAL FRACTION D.P.M. X10^6	ACID FRACTION D.P.M. X10^6	BASIC FRACTION D.P.M. X10^6	LOST FRACTION D.P.M. X10^6
[illegible] (NO_3)	3.00	1.8	.34	.5	.36
[illegible] (NO_3)	5.60	2.7	.72	1.9	.28
[illegible] (NO_3)	3.60	1.1	.57	1.45	.48
[illegible] (NH_4)	2.45	.74	.30	.93	.48

TABLE 3: Disintergrations per minute obtained when the leaf fractions were extracted and counted after leaves from plants growing under various nutrient conditions were allowed to photosynthesize in $C^{14}O_2$ for 10 minutes.

(k) EFFECT OF HIGH CO_2 CONCENTRATIONS ON THE DISTRIBUTION OF C^{14} (FIGURE 18) AND TABLE 6).

If the peroxisome was involved in the supply of carbon skeletons for amino acid metabolism, high CO_2 levels (>> 300 p.p.m.) known to suppress peroxisomal metabolism should also suppress the percentage label in amino acids and enhance the percentage label in sugars. This is clearly shown in fig. 18 where percentage amino acid label is suppressed by one third, when the plants are placed under high CO_2 levels (> 1%) 18 hours prior to labelling, of the control (0.03%). Percentage label in sugars is increased by a third over the control, while there is an equivalent drop in organic acids. The 'lost' count drops only marginally when compared to the control (fig. 18) High CO_2 levels during the labelling procedure also affected normal plants in a similar but less marked pattern when compared to the control (fig. 18).

(l) EFFECTS OF LOW OXYGEN CONCENTRATIONS ON THE DISTRIBUTION OF C14 (FIGURE 19) AND TABLE 6).

Low oxygen concentrations (<5%) are well known to suppress the T and should result in a reduced percentage label in amino acids, relative to a control. Percentage label in amino acids drops by one third of the control (similar to high CO_2) while percentage label in sugars rises only marginally (fig. 19). Percentage label in organic acids drops only slightly while there is a doubling of percentage label in the 'lost' count when compared to the control (fig. 19).

(m) EFFECTS OF 'HIGH' AND 'LOW' LIGHT INTENSITIES ON THE DISTRIBUTION OF C14 (FIGURE 20) AND TABLE 6).

Having established that a leaf of barley shows a variable response in T to light intensities with age (fig. 2) an experiment was conducted to determine whether this was reflected in the labelling patterns. Furthermore, as predicted from the T (fig. 1) and enzyme activities (fig. 14 and 15;

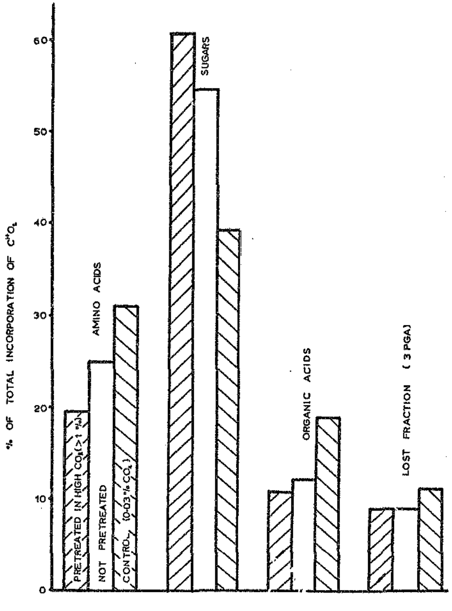


Figure 18. Histogram showing the distribution of $C^{14}O_2$ under conditions of high CO_2 concentrations (±1%) during the labelling period.

EXPERIMENTAL CONDITIONS.	TOTAL COUNT D.P.M. X10^6	NEUTRAL FRACTION D.P.M. X10^6	ACID FRACTION D.P.M. X10^6	BASIC FRACTION D.P.M. X10^6	LOST FRACTION D.P.M. X10^6
High CO_2 (Pre-treated)	.067	.041	.007	.013	.006
High CO_2 (Not pretreated)	.067	.036	.008	.017	.006
Control (.03% CO_2)	2.81	1.110	.540	.870	.290

NOTE: The relatively low level of D.P.M. recorded may be attributed to the dilution effect of the high CO_2 environment.

TABLE 4: Disintergrations per minute obtained when the leaf fractions were extracted and counted after leaves from plants were allowed to photosynthesize, in a high CO_2 concentration (> 1%) environment, in the presence of $C^{14}O_2$ for 10 minutes.

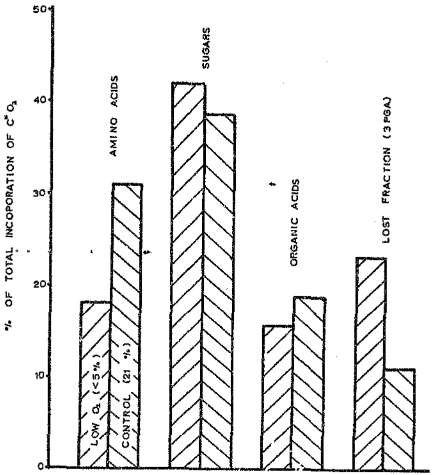


Figure 19. Histogram showing the distribution of $C^{14}O_2$ *under conditions of low* O_2 *concentrations (<5%)* during the labelling period.

ENVIRONMENTAL CONDITIONS	TOTAL COUNT D.P.M. $X10^6$	NEUTRAL FRACTION D.P.M. $X10^6$	ACID FRACTION D.P.M. $X10^6$	BASIC FRACTION D.P.M. $X10^6$	LOST FRACTION D.P.M. $X10^6$
Low O_2 (< 5%)	4.50	1.875	.724	.837	1.064
Control (21% O_2)	2.81	1.110	.540	.870	.290

TABLE 5: Disintergrations per minute obtained when the leaf fractions were extracted and counted after leaves from plants were allowed to photosynthesize, in a low O_2 concentration (< 5%) environment, in the presence of $C^{14}O_2$ for 10 minutes.

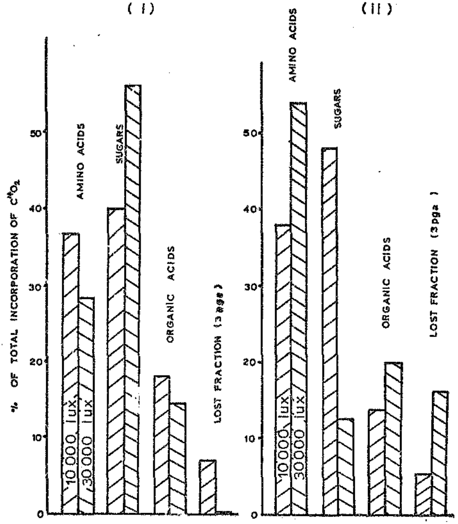


FIGURE 20. Histograms showing the distribution of $C^{14}O_2$ in (i) a young expanding leaf and (ii) a fully expanded leaf.

LIGHT REGIME	TOTAL COUNTS D.P.M. $X10^6$	NEUTRAL FRACTION D.P.M. $X10^6$	ACID FRACTION D.P.M. $X10^6$	BASIC FRACTION D.P.M. $X10^6$	LOST FRACTION D.P.M. $X10^6$
Hist. (i)					
10,000 lux	4.75	1.88	.76	1.77	.34
30,000 lux	5.30	3.10	.75	1.50	.00
Hist. (ii)					
10,000 lux	5.60	2.70	.72	1.90	.28
30,000 lux	2.55	.31	.49	1.40	.35

TABLE 6: Disintergrations per minute obtained when the leaf fractions were extracted and counted after leaves from plants were allowed to photosynthesize, under varying light regimes and at different stages of leaf growth, in the presence of $C^{14}O_2$ for 10 minutes.

appendices 12 and 13), in a fully developed leaf the supply of high light intensities would result in an increased percentage label in amino acids, when compared to the supply of lower light intensities. During the expansion stage of a leaf's development there is a relatively lower incorporation of label into the amino acid fraction at a light intensity of 30,000 lux when compared to the incorporation of label at 10,000 lux (fig. 20, list (i)). The same pattern is observed in percentage label incorporated into the organic acid and lost' count fractions. The sugars however show an enhanced incorporation of label at the higher light intensity when compared to the low light intensity 'treatment' (fig. 20, list (i)). When labelling was conducted on a fully grown leaf under high and low light conditions the reverse of the above pattern was obtained (fig. 20, list (i)).

No standard deviations are presented as the view is held that the parameters measured are dynamic and thus constantly fluctuating, according to the metabolic demands of the plant. Therefore a determination made later in the same day or on a different day would be expected to vary. It was therefore decided that eight leaves (an arbitary figure) would be used for any determination made thereby giving an average result for the particular group of leaves at a given time, and a given stage of growth. Determinations of enzyme activities on samples of eight leaves, taken from a group of plants at a given time and age, showed a standard deviation of less than one percent.

DISCUSSION

In the Review of Literature it was mentioned that previous experimental work suggested that the light intensities above limiting values had little influence on the T, (Heath 1969, Poskuta 1968, Jackson and Volk 1970). Samish and Koller (1968) suggested from calculations based on a mathematical model that T should increase with increasing light intensities; the present study presents experimental evidence suggesting that this is the case in _Hordeum vulgare_, and that this response varies with the age of the leaf, (Figure 1). In this experiment it was shown that there is an increase in T with increasing light intensity above 30,000 lux, although the extent of T increase was found to differ with the stage of leaf development. The least response was obtained from leaves which had not reached full expansion. All three sets of leaves at different stages of development (Figure 1) showed little or no increase in T between 10,000 and 20,000 lux. In the case of the leaves which had not completely expanded there was a slight decrease in T between 10,000 and 30,000 lux, whereas in the fully expanded leaves there was a slight increase in the same range of intensities, but not as great as for light intensities above 30,000 lux. These results suggested that the age of the leaf may be responsible for the differing responses of T to increased light intensity. Further, these results suggested that the influence of light intensity on T differs at light intensities below and above 30,000 lux. The influence of high light intensity on increasing T would appear not to be confined to _Hordeum vulgare_, as a similar response was obtained with another C-3 photosynthetic plant _Alloteropsis semialata_ (Figure 1).

The conflict of opinion as to the effect of light on T may be explained in two possible ways. One, that most work on light intensity determinations were not conducted at intensities much above 20,000 lux, at which level, changes in T are of the order of 1 to 4 p.p.m. CO_2 and may not have been considered significant (Figs. 1 and 2).

Secondly, the material used, may have been at a physiological stage of growth at which T response to light intensity was suppressed. This possibility is supported by the fact that the T does not always respond to an increase in light intensity. This is illustrated on day 10 in graph "a" (fig. 2), days 11 to 13 in graph "b" (fig. 2) and days 15 to 16 in graph "c" (fig. 2).

Although T increases with light intensity for each sample of leaves studied, and this increase is linear for each sample, the extent of the T increase for a given increase in light intensity, between each sample of leaves, varies (fig. 1). This suggests that the magnitude of the response to light varied with the leaf samples physiological state and is expressed by the varying slope of the graphs.

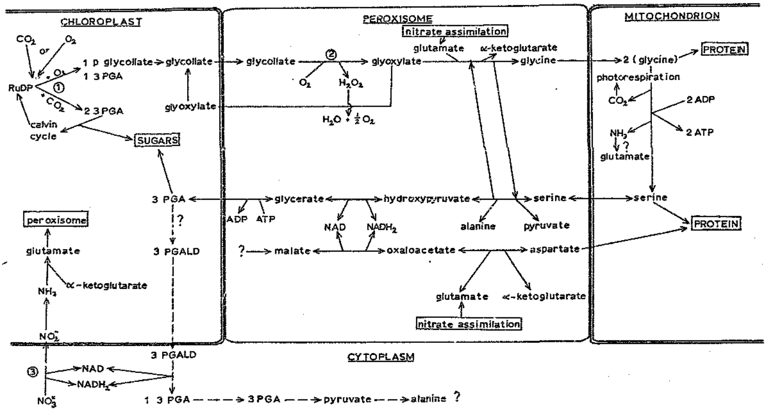
As mentioned above, it appeared from the initial experiment that the T response to increasing light intensity varied with leaf age, or the state of leaf development; for this reason, an experiment was carried out to investigate the influence of age and leaf position on the T response to light. The results of this experiment are presented in Figure 2. Irrespective of the leaf position the same result was obtained for all three leaf positions in that in the early stages of leaf development T was increased as the light intensity was decreased from 30,000 lux to 10,000 lux. This response of T to change in light intensity is reversed, with the development of the leaf; in the case of leaf 1 between day 6 and day 7 after planting; for leaf 2 between day 11 and day 13; and leaf 3 between day 15 and day 16. After this transition and for the duration of the experiment T increased when the light intensity was increased from 10,000 to 30,000 lux. These results suggest that the physiological response to light changes quite markedly between the stage of leaf development and full development.

In view of the influence of the results obtained from T, when plants were grown at a constant light intensity and measured at different light intensities, two experiments were carried out to investigate the influence of growing plants in different light intensities, and measuring the T at a constant light intensity.

These experiments established that T is influenced by the light intensity at which the plants were grown, (figures 14 and 15). During the development of the leaf the plants grown at the lower light intensities tended to give the higher T, and this tended to be reversed with the leaf attaining full expansion.

As the T is the carbon dioxide concentration at which the rate of carbon dioxide fixation and carbon dioxide output are equal, any change in T suggests that the process responsible for carbon dioxide fixation or carbon dioxide output or both may be changing. In an attempt to elucidate physiological factors associated with the measured T, an assessment of the level of carbon dioxide uptake by photosynthesis, and output by photorespiration was made by determining the enzyme activities associated with these two processes. The activity of the enzyme ribulose-1-5 diphosphate (RuDP) carboxylase was measured as it is generally accepted as the main photosynthetic carboxylating enzyme in C-3 photosynthetic plants. The selection of monitor enzymes for the decarboxylating process was more complex. Glycollate oxidase is considered to be one of the main terminal oxidases associated with photorespiration (Tolbert and Yamazaki, 1969). However, the measurement of its activity need not necessarily give a measure of the level of decarboxylation due to the glyoxylate-glycollate shuttle brought about by another peroxisomal enzyme, NADP glyoxylate reductase, or alternatively, the formation of glycine from glyoxylate and its removal from the peroxisome in this form. In a scheme proposed by Tolbert (1971) for peroxisome metabolism associated with photorespiration, glutamic acid plays a major role in the transamination processes implicated. Further, as glutamic acid is considered to be the prime product of reductive amination, it was decided to investigate the possibility of using the enzyme nitrate reductase as a measure of peroxisomal activity in plants grown with nitrate as the sole nitrogen source.

Figure 21. Proposed glycollate pathway adapted from the scheme proposed by Tolbert and Yamazaki (1969) including the suggested relationship between the glycollate pathway and nitrate assimilation. (1) Ribulose 1-5, diphosphate carboxylase; (2) Glycollate oxidase; (3) Nitrate reductase.



In the above experiments in which the plants were grown at different light intensities, and the T measured at a constant light intensity, in these experiments the activity of nitrate reductase and RuDP carboxylase activity was determined simultaneously with T determination. The activity of the enzyme nitrate reductase was found to be influenced by the level of the light intensity at which the plants were grown, (Figures 14 and 15) higher activities being obtained in the plants grown at the higher light intensities. The activity of nitrate reductase in both experiments decreased with the age of the leaf. The influence of light intensity on nitrate reductase activity is in agreement with the findings of Candela and Hewitt (1957) and Hageman and Flesher (1960). Nitrate reductase activity, however, showed a different response to RuDP carboxylase activity (Appendix 12 and 13) in that the activity decreased in plants with increase of age over the period measured irrespective of the light treatment received by the plants; in contrast, RuDP carboxylase activity increased over the same period. The calculated ratio of nitrate reductase activity to RuDP carboxylase activity was calculated for the first leaf of the plants grown at different light intensities, and the results are presented in Figures 14 and 15. The nitrate reductase RuDP carboxylase ratio appears to correspond very closely to the measured T in both experiments. By a linear regression analysis a correlation was obtained at the 99.9 per cent level for both 10,000 and 20,000 lux grown plants; in the other experiments a good correlation was also obtained. The correlation between T and nitrate reductase and RuDP carboxylase was good, and a similar correlation was not obtained with either glycollate oxidase RuDP carboxylase ratio, or catalase and RuDP carboxylase ratio. This observation suggested that nitrate reductase activity may be related to photorespiratory activity in plants receiving their nitrogen solely as nitrate. Further, this suggests that under the above experimental conditions nitrate reductase activity is more reliable than either glycollate oxidase activity or catalase activity as an indirect measure of photorespiration.

It is suggested that this may be due to the product of nitrate assimilation being probably associated with the amination steps thought to be associated with photorespiration. If this is the case then it is thought that a possible function of photorespiration could be the supply of carbon skeletons from glycollate for amino acid metabolism in the light. This suggestion is further supported by the findings that nitrate reductase was located on the membrane of the microbodies, and that nitrite reductase was located within the microbodies by Lips and Avissor (1972).

Having shown that the influence of light intensity on T varied with the age and state of the development of the leaf, an experiment was carried out to investigate the influence of leaf age and leaf position on T and enzyme activity of leaves of plants grown under controlled conditions. The enzyme nitrate reductase was found to be highest in the plants receiving the higher level of illumination (Figure 15). These observations are in agreement with the findings of Candela and Hewitt (1957) and Hagerman and Flesher (1960). A similar response had been obtained with RuDP carboxylase (Appendix 12 and 13); nitrate reductase activity however showed a different response to RuDP carboxylase activity in that the activity decreased in plants with increase of age over the period measured.

Nitrate reductase activity is well known to be dependant on light (Beevers and Hageman 1969, Hewitt 1970), however Hewitt (1970) mentions that the dependence of nitrate reductase activity on light is not wholly accounted for by its requirements for reducing potential and is related to the light intensity and its duration. Kannagera and Woolhouse (1967) demonstrated that the synthesis of nitrate reductase requires light and photosynthetic activity. Photosynthetic activity is known to be closely connected with peroxisomal activity.

When the ratio of nitrate reductase activity to RuDP carboxylase activity was calculated for the first leaf of the plants grown at different light intensities (Figures 14 and 15), the pattern of change of the ratio appears to correspond very closely to the measured T in both experiments.

When a linear regression analysis was carried out, a good correlation was obtained. In fact, the ratio of these two enzyme activities was found to give the best correlation with the pattern of measured T. These results suggested that nitrate reductase may be related to the photorespiratory activity in plants receiving their nitrogen solely as nitrate; this idea is supported by the findings of Lips and Avissor (1972) that the peroxisome is the intracellular site of nitrate reductase and nitrite reductase. It is suggested that the relationship between nitrate reductase activity and photorespiratory activity could be due to the product of nitrate assimilation being largely associated with the amination steps thought to be associated with photorespiration. If this is the case, then it is suggested that a possible function of photorespiration could be the supply of carbon skeleton from glycollate for amino acid metabolism in the light.

The initial experiments suggested that T is highly variable with respect to light intensity and leaf age. Further experiments were designed to study the variability of T; one of these was to investigate the influence of leaf age and leaf position on T, and the selected enzymes activities considered to be associated with CO_2 uptake, and output, the results are presented in Figure 3. The T was found to vary considerably under constant environmental conditions. This variation suggests that the equilibrium of carbon dioxide uptake and output is probably in a continual state of flux. This is in agreement with the observations of Steward, Craven, Weerasinghe and Bidwell (1971) who found that carbon dioxide uptake and output was affected by the age of the plant and previous environmental conditions although they did not relate their results to T.

In leaves 1, 3 and 4 (Figure 3) the initial T was at a higher level than at later stages of development; leaf 2 was slightly more mature when initially sampled compared to the other three leaves, and this probably accounts for the lower initial T. The average T for each of the leaves was found to be approximately the same for all the leaves, and it was found not to decrease with leaf age. This is of interest when compared to the measured enzyme activities.

When the level of nitrate reductase was determined for the different leaves at different stages of development (Figure 4) the level of nitrate reductase activity was found to increase with leaf position up the main axis, and the enzyme activity fluctuated as the leaf aged, with no regular pattern, but with a decrease in activity as ageing occurred. No correlation could be found between the measured T and the measured nitrate reductase activity.

The activity of RuDP carboxylase measured in this experiment (Appendix 1) was found to be similar to nitrate reductase activity in that it increased with ascending leaf position on the main axis, and decreased with leaf age. The activity of this enzyme was observed to fluctuate rhythmically on a frequency common to all four leaves. Like the nitrate reductase activity no correlation was found between the RuDP activity and the measured T.

It was of interest that the both carboxylating enzyme and nitrate reductase activities increased with leaf position, whereas the average T remained relatively constant. However, as in the previous experiment, a very high degree of correlation was obtained between measured T and the ratio of nitrate reductase and RuDP carboxylase activities (Table 1) (Figure 5, Figure 6). As previously mentioned above these observations suggested that nitrate reductase activity may be related to photorespiratory activity. In view of the good correlation obtained between measured T and the ratio of nitrate reductase activity and RuDP carboxylase activity, it would appear that enzyme activities may be used as a reliable indirect measure of a physiological phenomenon.

In view of the suggested relationship between nitrate reductase activity and photorespiratory activity, experiments were carried out to investigate the influence of nitrogen concentration and the form in which it was supplied on T.

The carbon dioxide compensation point appeared not to be influenced by the nitrate concentration in which the plants were grown (Figure 10, Figure 11). The plants grown in ammonia nitrogen exhibited a higher T than the plants grown in nitrate nitrogen (Figure 11).

It should be noted that if the pH of the culture solution of the ammonia grown plants was allowed to decrease to low pH levels (pH 3-4) the increase in Γ did not occur when the leaves were tested.

It was found in the previous experiments that the Γ may remain relatively constant even though the activities of the enzymes associated with carbon dioxide uptake and output increased or decreased, suggesting that the activities remain in balance with one another. This would appear to be the case with the plants grown in different concentrations of nitrate, in that the plants grown in the higher nitrate level showed a higher RuDP carboxylase activity (Appendix 8) as well as higher nitrate reductase activity.

Under ammonia nitrogen grown conditions the RuDP carboxylase activity was higher than under nitrate nitrogen conditions (Appendix 8). The increase in RuDP carboxylase activity with increasing nitrate supply is in agreement with the observations of Woycieska, Ursula, Ogren and Hagerman (1972), who showed a similar increase in RuDP carboxylase activity in soybean with increasing nitrate.

In these experiments no correlation was obtained between measured Γ and the ratio of nitrate reductase activity to RuDP carboxylase activity. This is largely due to the measured nitrate reductase activity in day 8 (Figure 11); when this measurement was ignored, a satisfactory correlation was obtained. This could possibly be explained by the nitrate reductase activity not being a satisfactory measure at that stage of the available ammonia for reductive amination, as the enzyme activity could be suppressed due to a build-up of ammonia, amino acids or amides in the plant tissue. If this was the case, one would obtain a relatively higher measured compensation point than indicated by the ratio of nitrate reductase to RuDP carboxylase. It is suggested that in the previous experiments where the rate of photorespiration has been influenced by other external factors such as age of leaf, leaf position and light intensity, the nitrate reductase activity is secondary, and possibly controlled by a demand for the products of nitrate assimilation in the photorespiratory pathway and differs from the present case where nitrate reductase activity is probably largely influenced by the level of nitrate available in the nutrient supply.

The increased measure T, and the considerably increased level of glycollate oxidase (Appendix 8) of the plants grown in ammonia nitrogen would further suggest that photorespiration may be linked to the nitrogen metabolism of the leaves of barley plants.

Oxygen concentrations above and below atmospheric levels have been shown to markedly affect the T. Forrester, Krotkov and Nelson (1966) using soya beans demonstrated that apparent photosynthesis (nett CO_2 uptake) was inhibited by increasing levels of oxygen, above atmospheric levels. A number of other workers have made similar observations, as well as showing that photorespiration was enhanced with increasing oxygen concentration (Tregunna, Krotkov and Nelson, 1966; Bjorkman, 1966; Zelitch, 1968; Hew and Krotkov, 1968; and Joliffe and Treguanna, 1968). Ellyard and Gibbs (1969) using isolated chloroplasts, have also demonstrated a decrease in net carbon dioxide fixation, in the presence of high oxygen concentrations, and its reversibility when the tissue is flushed with nitrogen. Furthermore, they found that high levels of bicarbonate would negate the high oxygen inhibition, and that low levels of bicarbonate enhanced this inhibition. Tregunna, Krotkov and Nelson (1966) have shown a linear relationship between ~~oxygen~~ oxygen concentration and T. Low oxygen levels (below 21 per cent) directly suppresses photorespiration with a corresponding drop in T. Fock, Becker and Egle (1970) and Goldsworthy (1968) suppressed carbon dioxide output by decreasing oxygen concentrations from 21 per cent to 1 per cent, obtaining T value close to zero for C-3 photosynthetic plants.

Interrelated with the effect of oxygen concentration on photorespiration is the influence of carbon dioxide concentration. Ellyard and Gibbs (1969) and Chollet and Ogren (1972) have demonstrated that the inhibition of apparent photosynthesis by high oxygen levels is reduced by increases in carbon dioxide concentrations above 300 p.p.m. Jolliffee and Tregunna (1968) found that at 21 per cent oxygen the rate of photosynthesis was increased by increasing the carbon dioxide concentration to 500 p.p.m. Egle and Fock (1967) failed to observe a post illumination carbon dioxide burst in the presence of 1200 p.p.m. carbon dioxide, and normal atmospheric oxygen concentrations, suggesting a suppression of photorespiration. Further, Zelitch and Walker (1964) and Zelitch (1965) have shown that the syntheses of glycollic acid is severely inhibited at carbon dioxide concentrations greater than 0.2 per cent, and there is considerable evidence for glycollate being the substrate for photorespiration.

In the present study the level of activity of nitrate reductase was found to be significantly suppressed when the plants were grown in a high carbon dioxide environment (Figure 12). It could be argued in view of similar results for glycollate oxidase (Appendix 9) that this was due to a general suppression in over-all metabolism due to extremely unfavourable growing conditions. However, this possibility is not supported by the change in RuDP carboxylase activity which increased markedly in the presence of the higher carbon dioxide environment.

These results are in agreement with the observations made on the effect of the external carbon dioxide concentration on photorespiration in short term experiments, namely, that high carbon dioxide concentrations suppress photorespiration as observed by Egle and Fock (1967) and Jolliffee and Tregunna (1968). It is therefore not surprising that if photorespiration is suppressed by high carbon dioxide levels that the levels of activity of these enzymes are suppressed when grown for longer periods under these conditions. It is further interesting to note that nitrate reductase behaved in a similar manner to glycollate oxidase which is well established as an enzyme component of the peroxisome.

Similarly, decreasing oxygen concentrations in short term experiments have been shown to decrease the level of photorespiration. Tregunna, Krotkov, and Nelson (1966), Bjorkman (1966), Zelitch (1968), and Hew and Krotkov (1968). *It is therefore again not surprising* in these longer term experiments to observe that the level of activity of nitrate reductase (Figure 13) and glycollate oxidase (Appendix 11) was also decreased in low oxygen (5%) concentrations. The level of RuDP carboxylase (Appendix 11) was also found to decrease in the plants grown under low oxygen concentration; however, this decrease was not as marked as that observed for the enzymes thought to be associated with photorespiration. Therefore, irrespective of whether high carbon dioxide concentrations or low oxygen concentrations were used for growing the plants, the enzymes associated with photorespiration were suppressed, and the responses of nitrate reductase activity to these conditions further suggests that this enzyme is associated with the photorespiratory activity of barley.

It can be argued that it is not surprising that a relationship exists between nitrate reductase activity and photorespiration when the plant is receiving its nitrogen in the form of nitrate. It is well established that amination occurs in the suggested *photorespiratory pathway* (*Tolbert and* Yamazaki 1969), and that glycine and serine [illegible] heavily labelled during $C^{14}O_2$ labelling experiments [illegible]olbert and Cohen 1953). Calvin and Bassham 1962; Ongur and Stocking 1965; Carr and Pate 1967). Furthermore, Ongun and Stocking 1965 have shown that serine is only metabolised in the light. The synthesis of these amino acids require the product of nitrate assimilation, which further suggests that nitrate reductase activity may well be linked to photorespiration. The formation of aspartate, another amino acid which readily incorporates $C^{14}O_2$ in photosynthetic tissue in the light, would also be formed in the peroxisomal pathway suggested by Tolbert and Yamazaki. Further indirect support for a possible connection between nitrate assimilation and peroxisomal metabolism is suggested by Ruis and Kindl (19[illegible]) [illegible] suggest from their results that "the leaf peroxiso[illegible] have more importance in amino acid photorespiration. Tregunna, Krotkov, and Nelson (1966), Bjorkman (1966), Zelitch (1968), and Hew and Krotkov (1968). It is therefore again not surprising in these longer term experiments to observe that the level of activity of nitrate reductase (Figure 13) and glycollate oxidase (Appendix 11) was also decreased in low oxygen (5%) concentrations. The level of RuDP carboxylase (Appendix 11) was also found to decrease in the plants grown under low oxygen concentration; however, this decrease was not as marked as that observed for the enzymes thought to be associated with photorespiration. Therefore, irrespective of whether high carbon dioxide concentrations or low oxygen concentrations were used for growing the plants, the enzymes associated with photorespiration were suppressed, and the responses of nitrate reductase activity to these conditions further suggests that this enzyme is associated with the photorespiratory activity of barley.

It can be argued that it is not surprising that a relationship exists between nitrate reductase activity and photorespiration when the plant is receiving its nitrogen in the form of nitrate. It is well established that amination occurs in the suggested photorespiratory pathway (Tolbert and Yamazaki 1969), and that glycine and serine are heavily labelled during $C^{14}O_2$ labelling experiments (Tolbert and Cohen 1953). Calvin and Bassham 1962; Ongun and Stocking 1965; Carr and Pate 1967). Furthermore, Ongun and Stocking 1965 have shown that serine is only metabolised in the light. The synthesis of these amino acids require the product of nitrate assimilation, which further suggests that nitrate reductase activity may well be linked to photorespiration. The formation of aspartate, another amino acid which readily incorporates $C^{14}O_2$ in photosynthetic tissue in the light, would also be formed in the peroxisomal pathway suggested by Tolbert and Yamazaki. Further direct support for a possible connection between nitrate assimilation and peroxisomal metabolism is suggested by Ruis and Kindl (19[illegible]) who suggest from their results that "the leaf peroxiso[illegible] have more importance in amino acid metabolism than was previously assumed."

It should be noted, however, that the significance of nitrate reductase activity as a possible indirect measure of photorespiration could be affected in one or all of the following ways:

(a) The influence of nitrogen supply for amino acid formation from sources other than nitrate assimilation in the leaf (e.g. uptake of NH_4^+).

(b) The formation of serine may occur through the reverse reaction sequence, namely 3 PGA - Glycerate - Hydroxypyruvate - Serine as shown in fig. 21 which would not result in the release of CO_2.

(c) Glycine may be utilized directly prior to the decarboxylating step as shown in fig. 21.

(d) Nitrate reductase activity may be suppressed by a pool build-up of amino acids and amides which are known to act as suppressors (Afridi and Hewitt 1965, Filmer 1966, Stewart 1972). Nitrogen however would still be available for the operation of the glycollate cycle due to build-up of substances such as glutamine (Tew Unpub. res.). From the above results there was a strong indication that nitrate assimilation is closely tied to photorespiration. A series of experimental work was thus conducted to further investigate this possible relationship.

The enzyme activity results strongly suggest a connection between photorespiration and nitrogen metabolism of leaves in the light. Preliminary $C^{14}O_2$ labelling experiments were carried out in an attempt to investigate whether the labelling pattern obtained under the known condition affecting photorespiration supported the above concept. The experimental approach for either stimulating or suppressing photorespiration as achieved with the enzyme experiments, was used. By this method it was hoped to obtain either an increase or decrease in percentage label in amino acids with a reciprocal decrease or increase in the percentage label in sugars.

The initial labelling experiment was merely a time course under standard conditions to ascertain the labelling patterns of various periods of exposure to $C^{14}O_2$. The results however proved to be pertinent to the hypothesis and are helpful in interpreting the later results. After 15 seconds of feeding $C^{14}O_2$ the highest percentage label (48.5%) occurs in the "lost" count (Figure 16). This count was found to be held on the basic column and led us to suspect that this probably represented a large proportion of the sugar-phosphate, more specifically, 3PGA. This observation is supported by the fact that it is well known that the first labelled product is 3PGA (in C^3 Photosynthesis) (Calvin and Bassham 1962). Hess and Tolbert (1966) have shown that using time course labelling experiments under similar conditions that at 4 seconds 3PGA was 100 times more labelled than glycollate, however at 4 to 11 seconds glycollate became uniformly labelled, presumably deriving its label from a product of 3PGA. Recent work has confirmed that the "lost count" is composed of sugar phosphates and mainly 3PGA (Tew - unpublished results, Canvin - pers. com.).

The second highest percentage label, after 15 seconds, is contained in the organic acid fraction (38%). In view of the findings of Hess and Tolbert (1966) and Tew (unpub. date), a high proportion of this fraction is probably glycollic acid (malic acid is also a significant proportion of this fraction). This fits well with the fact that a large proportion of newly fixed carbon passes through the peroxisomal pathway (Zelitch 1969, Tolbert and Yamazaki 1969, Tolbert 1971).

After 15 secs. a higher proportion of labelling was found to occur in the A.A. fraction (14.5%) than in the sugar fraction (2%). These results are in agreement with Carr and Pates's (1967) findings, in young leaves. The~~se~~ above results are not in conflict with the proposal that A.A. metabolism in the light and photorespiration are closely related. As a higher A.A. label would be expected if the high percentage organic acid label is composed of a high proportion of glycollate. With a progressively longer labelling period a steady drop in the percentage label of both the "lost" fraction and organic acid fraction was obtained, while a steady increase in the percentage label in the basic and neutral fractions also occured (Fig. 16). The results indicated that after ten minutes all the fractions were approximating to a steady state (Table 2). From this experiment it was decided to use a ten minute labelling time for all the following work.

It is well established that high CO_2 gas pressures result in a suppression of photorespiration. It was argued that $C^{14}O_2$ labelling under these conditions should result in a decrease of percentage label in amino acids with a reciprocal increase in sugars, if the suggestion of photorespiration associated with nitrogen metabolism is correct, as less carbon will flow through the glycollate cycle as postulated by Ogren and Bowes (1971).

The results obtained ~~form~~ from the experiment carried out under the above conditions agreed with the suggested labelling pattern (fig. 18). The effect of increased PERCENTAGE label in sugars and decrease in the percentage label in amino acids is enhanced when the plants were grown and labelled under high CO_2 conditions (fig. 18).

Furthermore the percentage label in the organic acid fraction (fig. 18) shows a drop under high CO_2 conditions, which may be explained by the organic acids glycollate and malate being involved in peroxisomal metabolism (fig. 21). A slight drop in the 'lost' count (17%) is also recorded.

Similarly it is well established that low O_2 pressures markedly decrease photorespiratory activity, for this reason $C^{14}O_2$ labelling experiments were carried out under low O_2 pressures ($< 5\%$). Under these conditions there was a marked decrease in the percentage label in the amino acids accompanied by only a slight increase in the percentage label in the sugars (fig. 19). These results like those obtained for labelling under high CO_2 conditions further support the proposed link ~~like~~ between peroxisomal metabolism and nitrage metabolism. Fock and co-workers (Fock, Egle, Schaub and Hilgenberg 1969, and Egle pers. com.) have also shown with their experiments on C_3 plants that under low O_2 (2%) concentrations, using $C^{14}O_2$, that sugar formation is favoured, and reciprocally that under high O_2 (42%) concentrations, amino acid formation is favoured.

In view of the suggestion that T may indicate a low or high demand for amino acid formation, as the T dropped or rose respectively, it was decided to label, with $C^{14}O_2$, leaves at various light intensities and stages of growth. Having established that the T responded in a reciprocal manner to varied light intensities, with age (fig. 2), a young expanding leaf and a fully expanded leaf were labelled at two light intensities, namely 10,000 lux and 25,000 lux. The results (fig. 20) show the changes in percentage label in amino acids and sugars for both young (fig. 20, hist i) and mature leaves (fig. 20, hist ii). The results show that under the high light intensity (25,000 lux) in the young leaf (high T, fig. 2) the label in the amino acid fraction is higher than in the amino acid fraction of the low light intensity (10,000 lux) leaf (low T, fig. 2). The reverse of this picture is obtained when a set of leaves is labelled under identical conditions, but at a stage at which full leaf expansion has occurred (fig. 20, hist ii).

The final labelling experiments were carried out on plants grown under varying levels of nitrogen supplied as nitrate and ammonia. This experiment was carried out in view of the previously established correlations between nitrate reductase activity as an indirect measure of the photorespiratory component of T and in addition the influence of ammonia nitrogen on T and glycollate oxidase activity.

The results (fig. 17) were entirely in accordance with the now familiar, reciprocal response of sugars and amino acids. Under low nitrogen conditions (± 50p.p.m.) the percentage label in sugars is high (60%) and low (16%) in the amino acids. The sugars dropping in percentage label and the amino acids rising as the available nitrate nitrogen increases. At the same time the organic acid level also rises.

The treatment in which nitrogen is supplied as the NH_4^+ ion did not give the results one would expect (i.e. very high A.A. levels) from the enzymatic experiments (fig. 10 and 11, appendix 8). The relatively low level of label in the A.A. may be explained if one vizualizes that the peroxisomal cycle (fig. 21) may turn a full cycle with the production of 3PGA and no amino acids being withdrawn directly from the cycle, resulting in a reduced label entering the amino acid fraction. This suggestion is supported by the fact that the label in sugars remains relatively low (fig. 17) while the percentage in the "lost count" rises, indicating possible 3PGA formation. Furthermore the T is considerably above the T of the nitrate treatments (fig. 11, graph "b") indicating a high level of peroxisomal activity as does the greatly enhanced glycollate oxidase activity (appendix 8, graph "b"). The drop in the organic acids may be due to a decrease in pool size of glycollate due to the very high demand for this substrate by the peroxisome. The level of unlabelled amino acid is also much higher in the

.....NH_3 grown page 75.

ammonium nitrogen ~~NH_3~~ grown plants than in all the nitrate grown plants (Tew unpub. res.). It is well established that ammonium nitrogen ~~NH_3~~ taken up by the roots is immediately metabolized and transported to the leaves as amino acids and amides (Pate 1966, Weissman 1972).

In a recent review, Bassham (1971) reports work using Chlorella in which nitrogen supplied as ammonium nitrogen ~~NH_3~~ results in the suppression of sucrose formation and a consequent build - up of sugar - phosphates. This finding is supported by our work with ammonium nitrogen ~~NH_3~~, as sugars are maintained at a relatively low level, while "lost count" (sugar-phosphates) increases.

All the results presented strongly support the suggestion that a primary function of the peroxisome is the supply of carbon skeletons in the light, for amino acid metabolism. Furthermore, that overall nitrate reductase activity is a possible measure of the peroxisomal activity within the limits mentioned in this discussion. It is also suggested that the overall demand for amino acids will affect the activity of the peroxisome. In the authors opinion it is thought that the overall level of T of a particular species is probably determined by the plants efficiency in concentrating CO_2 at the site of uptake, whereas fluctuations in T within a plant is related to the balance between photosynthesis and photorespiration, which in turn is a reflection of the fluctuation in demand for amino acids or sugars within the plant.

DISCUSSION ON T.

Having established the effect of varying light intensities and age, on T, in the barley plant, a number of points arising from this remain to be discussed.

(1) Why does the T rise with increasing light intensities (fig. 1)? The obvious explanation is that there is a change in the relationship of CO_2 uptake to CO_2 output. This explanation however does not answer the question of why this relationship should change. Two possible answers exist. One, that under increasing light regimes, a higher demand for protein is initiated, due possibly to faster breakdown of existing protein by way of photooxidation. A drawback in this explanation

is that it is hard to visualize how this effect could act so rapidly as to cause an immediate rise or drop in T (fig. 1). The second explanation and the one favoured is that under an increased light regime the production of O_2 by photosystem II would be enhanced, thereby increasing the effective O_2 concentration in the plants intracellular spaces and thus stimulating peroxisomal metabolism. The first possibility has a long term effect in stimulating photorespiration and probably accounts for the general rise in nitrate reductase activity observed when plants are grown under relatively high light regimes. (fig. 14 and 15).

(2) A second complex question which arises is the reason why under relatively high light intensities the percentage amino acid and T should drop in a young expanding leaf, but the reverse of this in an older fully expanded leaf (fig. 2). A possible explanation for this is that in the young leaf amino acid production is probably at full capacity - high T and nitrate reductase activity (fig. 2, 3, & 4). Carr and Pate (1968) state that photosynthesis in a young leaf is primarily involved in protein production, sucrose being transported to the developing leaf. If this is the case then any increase in reducing power (higher light intensity) would be utilized for carbohydrate production rather than amino acid production. This would have the effect of lowering the percentage of labelled $C^{14}O_2$ going to the peroxisome and amino acid metabolism and hence a lower T (fig. 2, fig. 20). The switch would occur when the point is reached where the level of peroxisomal metabolism has dropped sufficiently (i.e. low nitrate reductase activity and T), that any increase in light intensity, results in the increased O_2 level or demand for protein being accompanied by a stimulation in peroxisomal metabolism to accommodate the increased demand for amino acids. This of course would result in a percentage increase in newly fixed $C^{14}O_2$ passing through the peroxisome and hence an increase in percentage amino acid label (fig. 2, fig. 20).

(3) Finally, what affect could a fluctuating T have on a plant's physiology? This question raises a number of interesting ecological possibilities.

(a) T increase with light.

The ecological implications of the effect of high light intensities on photorespiration may offer a partial explanation for the remarkably distinct distribution of C_3 and C_4 plants.

Before discussing this distribution, a clear picture of the possible effect photorespiration may have on a plant under high light conditions should be presented. Photorespiration is the release of CO_2 in the light and as it has been shown, this release of CO_2, as expressed by T, increases with increasing light intensity. This naturally represents a rise in intracellular CO_2 concentration. Concurrent within an increase in light intensity, temperature and intracellular O_2 concentration would be expected to increase, with a consequent further increase in intracellular CO_2 due to enhanced photorespiration (Forrester et.al. 1966, Heath and Orchard 1957). Oxygen would increase due to increased photosystem II activity. The resulting increase in intracellular CO_2 concentration may then result in stomatal closure (Heath 1952, Heath and Orchard 1957, Raschke 1972, Pallaghy 1972). It is well known that many plants (C_3) exhibit mid-day closure of stomata, particularly in hot climates (Meidner and Mansfield 1968). Accepting the above line of reasoning it is apparent that photorespiration in C_3 plants may well play a significant ecological role in determining the distribution of C_3 plants.

It is apparent that as far as present knowledge allows, C_4 plants occur widely in dry tropical and sub-tropical climates (Hatch and Slack 1970, Bjorkman 1971, Black 1971). Particularly where rainfall occurs in great abundance over relatively short periods with virtually no rain during intervening months (Hatch and Slack 1970). This is particularly evident in deserts and on the Serengeti Plains. In this regard Grzimik (1957) states that the evaporation potential on the Serengeti Plains is such that more than twice the annual rainfall could be evaporated on these plains in one year. It is thus apparent that plant growth would have to occur during the relatively limited rainfall periods, when water is available in great quantities. Other tropical habitats of C_4 plants are hot swampy conditions such as the Okavango Swamps (Fair and Cresswell - unpub. data). With the exception of swamp conditions, all the prefered habitats of C_4 plants have short growing seasons and an abundant supply of water during this period. These facts tend to contradict Bjorkman (1971) and Black's (1971) suggestion that C_4 plants are primarily water conserving plants, but suggest rather that C_4 plants are designed for fast and sustained growth, possibly unaffected by serious stomatal closure during high light intensity periods, which could seriously curtail growth in C_3 plants, especially when one considers that in these regions high temperatures and light intensities are reached early in the day and will continue until late afternoon. Lack of apparent photorespiration in C_4 plants would thus be of great competitive value in these regions, due to low intracellular CO_2 levels being maintained in these plants during potential high growth periods.

Typical examples of C_4 dicotyledonous plants are the Atriplex and Euphorbiaceae found in dry desert conditions and the grasses, such as Themeda triandra , Hyparrhenia hirta, Panicum and Eragrostoid Spp. found on the dry African Savannah regions.

C_3 plants however are widespread in the more temperate regions where extremes of light intensity and temperature are less prevalant. Days are longer and rainfall is spread over a relatively longer period with less intense precipitation (Hatch and Slack 1970). It can thus be envisaged that in conditions of "constantly" available moisture but seldom abundant, that the closure of stomata during mid-day [illegible] a result of increased photosynthesis, would favour the C_3 plant, preventing excessive moisture loss during the heat of the day.
If this argument is accepted it would be correct to say tha[illegible] plants are adapted to high growth rates (Black 1971), in conditions of extreme high levels of light and temperature, and abundant moisture. C_4 plants however do not always exhibit higher growth rates than C_3 plants under all conditions.
C_3 plants however are adapted to efficient water utilization and growth under conditions of relatively low light and temperature levels, as well as limiting but constantly available moisture levels.

(b) <u>"Switch" with leaf age</u>.

This "switch" in response between the immature and mature leaf suggests an interesting ecological adaptation (fig. 2). As the young leaf in barley appears at the top of the plant it will be exposed to full sunlight with a minimal degree of shading. If at this stage the "normal" response existed and stomatal closure resulted because of the high T, the growth of the young leaf could be severly retarded with detrimental results to the development of the entire plant. This problem is however "neatly" by-passed by the "switch" which results in relatively high light intensities giving minimal T (see section 2). As the leaf grows the change to a more 'normal' response is again beneficial as the mature leaf is subject to shading as a result of new leaves growing above it, thus lowering the average light intensity that reaches the leaf surface. This phenomenon thus results in the greatest efficiency of CO_2 uptake in all leaves of the plant. At first sight this may seem contradictory in terms of the previous discussion on water conservation in C_3 plants, as the new leaf would be expected to respire heavily under high light intensities. In terms of the plant however the total area of a new leaf is small and consequently water loss to the entire plant would be minimal.

(c) <u>BALANCE BETWEEN CO_2 AND O_2 LEVELS IN THE ATMOSPHERE</u>.

It has been well established that the levels of either CO_2 or O_2 have marked effects on the balance between photosynthesis and photorespiration (Ogrun and Bowes 1971, Gibbs 1969, Jollife and Tregunna 1968, Egle and Fock 1967). Now if O_2 concentration was to rise, photorespiration is stimulated (Tregunna et.al. 1966, Forrester et. al. 1966, Ludwig 1968), resulting in a utilization of O_2 (fig. 21) and an output of CO_2. This would result in an increase in CO_2 accompanied by a decrease in O_2 levels which would result in a suppression of photorespiration (Egle and Fock 1967, Ogrun and Bowes 1971) and an increase in CO_2 uptake (fig. 21) thus decreasing the level of CO_2. This reciprocating action between O_2 and CO_2 levels would thus tend to maintain a relatively stable relationship between the level of CO_2 and O_2. This action of plants may well play a significant part in maintaining the constancy of CO_2 and O_2 in the atmosphere.
It should be pointed out that this relationship between CO_2 and O_2 levels and its effect on C_3 plants metabolism, must have serious implications in physiological work involving the 'Warburg' apparatus. Thus if the CO_2

level in the flasks rose above physiological values or the O_2 level varied from the 21% norm, results obtained would have to be cautiously interpreted.

Discussion on Glycollate Pathway.

Previous work on labelling patterns established that glycine, serine and alanine are important early labelled amino acids in green tissue in the light. (Calvin and Basham 1962, Carr and Pate 1967, Tolbert and Cohen 1953). The work presented in this thesis has established that the levels of amino acids and sugars are related to photo-respiratory activity. Using the proposed glycollate pathway (Tolbert and Yamazaki 1969) the production of glycine serine and aspartate is explained. However the early appearance of alanine is not explained - it would appear from Tolbert's work (1971) that alanine is not formed in the peroxisome. Its early appearance in labelling experiments may possibly be explained, if the 3PGA, formed by a complete turn of the glycollate cycle (fig. 21) is utilized by the plant to form alanine. Furthermore if the 3PGA produced in this way was first utilized to produce energy for the reduction of NO_3^- to NO_2^- as suggested by Klepper et.al. (1972) a balanced stoichiomentry, of one 3PGA, to one $NO_3^=$ molecule reduced, to one alanine formed, would exist. This possible pathway is included in the modified pathway presented in fig. 21. 3PGA for this purpose could of course come directly from the 3PGA formed during the ribulose 1-5, diphosphate carboxylase reaction.

To conclude this discussion it would seem appropriate to briefly review the function of the peroxisome in the light of the work presented in this thesis.

The peroxisome undoubtedly plays an important part in the metabolism of C_3 plants. They occur in all C_3 plants studied in large numbers and it is well established that at least 50% of newly fixed carbon passes through the glycollate cycle (Tolbert 1971, Zelitch 1966,). Electron micrographs further underline its importance and demonstrate the close spatial relationship between the chloroplast, peroxisome and mitochondrion (Trelease et.al. 1971, Frederick et.al. 1969).
Tolbert in a recent review (1971) suggests a number of possible functions for the peroxisome, none of which could account for the pre-eminence of the peroxisome on the evidence then available. Tolbert infact dismisses the possibility of the synthesis of glycine and serine being a major function of the peroxisome.
The work presented in this thesis however would seem to point strongly to a major function of the peroxisome being the supply of carbon skeletons for certain amino acid synthesis in the light, when the plant's nitrogen is supplied solely as nitrate. This conclusion is directly supported by the suggestion of Ruis and Kindl (1971) that the peroxisome may play an important part in amino acid metabolism. The glycollate cycle however has also been shown to produce energy in the form of 2ATP's during the conversion of 2 glycine molecules to 1 serine.(Bird et.al. 1972b) Bird et.al. (1972a) surmise that the production of energy may also be an important function of this pathway.

BIBLIOGRAPHY.

Afridi, M.M.R.K. and E.J. Hewitt. "The Inducible Formation and Stability of Nitrate Reductase in Higher Plants. I". J. Exp. Bot. 15: 251-271 (1964)

Afridi, M.M.R.K. and E.J. Hewitt. "The Inducible Formation and Stability of Nitrate Reductase in Higher Plants II". J. Exp. Bot. 16: 628-645 (1965)

Beevers, L. and R.H. Hageman. "Nitrate Reduction in Higher Plants".
An. Rev. of Plant Physiol. 20: 495-522 (1969)

Bidwell, R.G.S. , W.B. Levin, D.C. Shepard.
"Photosynthesis, Photorespiration and respiration of chloroplast, from Acetabularia mediterrania".
Plant Physiol. 44: 946-954 (1969)

Bird, I.F., M.J. Cornelius, A.J. Keys and C.P. Whittingham.
"Oxidation and phosphorylation associated with the conversion of glycine to serine ".
Phytochem. 2: 1587-1594 (1972a)

Bird, I.F., M.J. Cornelius, A.J. Keys and C.P. Whittingham.
"Adenosine Triphosphate Synthesis and the Natural Electron Acceptor for Synthesis of Serine from Glycine in Leaves".
Biochem. J. 128: 191-192 (1972b)

Bjorkman, O. "The effect of Oxygen concentration on photosynthesis in higher plants".
Physiol. Plantarum 19: 618-633 (1966)

Bjorkman, O. "Comparative Photosynthetic CO_2 exchange in higher plants".
In "Photosynthesis and Photorespiration". Eds. M.D. Hatch, C.B. Osmond, R.O. Slayter. Pub. Wiley-Interscience New York (1971).

* ADDITIONAL REFERENCES AT END OF BIBLIOGRAPHY, PAGE 95a.

Black, C.C. "Ecological Implications of Dividing Plants into Groups with Distinct Photosynthetic Production Capacities". "Advances in Ecological Research". Ed. J.B. Cragg. Academic Press. London and New York 7: 87-114 (1971).

Boyer, J.S. "Nonstomatal inhibition of photosynthesis in Sunflower at Low Leaf Water Potentials and High Light Intensities." Plant Physiol. 48: 532-536 (1971)

Bowes, G. and W.L. Ogren. " Oxygen Inhibition and Other Properties of Soybean Ribulose 1-5, - Diphosphate Carboxylase". J. Biol. Chem. 247: 2171-2176 (1972)

Bravdo, B.A. "Decrease in nett photosynthesis caused by respiration". Plant. Physiol. 43: 479-483 (1968)

Breidenbach, W.A. Kahn, and H. Beevers. " Characterization of glyoxysomes from castor bean endosperm". Plant. Physiol. 43: 705-713 (1968)

Calvin, M. and J.A. Bassham. "Photosynthesis of Carbon Compounds". Pub. W.A. Benjamin inc. New York (1962)

Canvin, D.T. Ph.d. Thesis, Purdue Univ. U.S.A. (1960)

Carr, D.J. and J.S. Pate. "Ageing in the whole plant", In Symposium of Soc. Exp. Bot. No. XXI, P. 559-599. "Aspects of the Biology of Ageing". Ed. H.W. Woolhouse. Cambridge Univ. Press (1967).

Chollet, R. and W.L. Ogren. "Oxygen Inhibits Maize Bundle Sheath Photosynthesis". Biochem. Biophys. Res. Com. 46: 2062-2066 (1972)

Cresswell, C.F. "An investigation into the nitrate, nitrite and Hydroxylamine metabolism in higher plants". Ph.d. Thesis Univ. Bristol (1961)

Decker, J.P. "A rapid postillumination deceleration of respiration in green leaves". Plant Physiol. 30: 82-84 (1955)

Decker, J.P. "Further evidence of increased CO_2 production accompanying photosynthesis". J. Solar Energy Sci. and Eng. 1: 30-33 (1957)

Decker, J.P. "Comparative responses of CO_2 outburst and uptake in tobacco". Plant. Physiol. 34: 100-102 (1959a)

Decker, J.P. "Some effects of temperature and CO_2 concentration on photosynthesis of MIMULUS" Plant. Physiol. 34: 103-106 (1959b)

Decker, J.P. and J.D. Wien. "Carbon dioxide surges in green leaves". J. Solar Energy Sci. and Eng. 2: 39-41 (1958)

Downtown, J. and R.O. Slayter. "Variation in levels of some leaf enzymes". Planta 96: 1-12 (1971)

Egle, K. and H. Fock. "Light Respiration - Correlation Between CO_2 Fixation, O_2 Pressure and Glycollate Concentration, In: "Biochemistry of Chloroplasts" Vol. II 78-87 Ed. Goodwin, T.W. Academic Press N.Y. (1967)

El-Sharkawy, and J. Hesketh. "Photosynthesis among species in relation to characteristics of leaf anatomy and CO_2 diffusive resistances." Crop. Science 5: 517-521 (1965)

Ellyard, P.W. and M. Gibbs. "Inhibition of Photosynthesis by O_2 in isolated spinach chloroplasts". Plant Physiol. 44: 1115-1121 (1969)

Filmer, P. "Regulation of nitrate reductase in cultured tobacco cells". *Biochem. Biophys. Acta Vol. 118.* 299-310 (1966).

Fock, H., J.D. Becker and K. Egle. "Use of labelled CO_2 for separation of CO_2 evolved from true CO_2 uptake by photosynthesizing Amaranthus and sunflower leaves" Can. J. Bot. 48: 1185-1190 (1970)

Fock, H. and K. Egle. "Über die Lichtatmung bei grunen Pflanzen I. Die von Sauerstoff und Kohldioxyd auf den CO_2 - G. während der Licht - und Dinkelphase". Beitr. Biol. Pfla. 2: (213-239) (1966)

Forrester, Krotkov and C.D. Nelson. "Effect of O_2 on photosynthesis, photorespiration, and respiration in detached leaves". Plant Physiol. 41: 422-427 (1966)

Frederick, S.E. and Newcomb E.H. "Cytochemical localization of catalase in leaf microbodies (peroxisomes)". J. Cell. Biol. 43: 343-53 (1969)

Frederick, S.E., E.H. Newcomb. "Ultrastructure and distribution of Microbodies in leaves of grasses with and without CO_2 - photorespiration". Planta 96: 152-174 (1971).

Goldney, D.C. and R.F.M. van Stevenirck "Changes in dark respiration, photorespiration, and dark fixation in detached leaves of Nymphoides indica". Aus. Soc. Plant Physiol. (12th Meeting) (Abst.) May 1971.

Goldsworthy, A. "Exp. in the origin of CO_2 released by tobacco leaf segments in the light".
Phytochem. 5: 1013 - 1019 (1966)

Goldsworthy, A. "Comparison of the kinetics of photosynthetic CO_2 fixation in maize, sugar cane, and tobacco, and its relation to photorespiration".
Nature 217: 62 (1968)

Grzimek, B. "Serengeti Shall not die".
Hamish Hamilton London (1960)

Hall, A.E. "A hypothesis concerning the relationship between photosynthesis and photorespiration".
Plant. Physiol. Suppl. 47:60 (1971)

Hanson, A.D., J. Edelman. "Photosynthesis by Carrot Tissue Cultures". Planta 102:11-25 (1972)

Harper, J.E. and R.H. Hageman. "Canopy and Seasonal Profiles of Nitrate Reductase in Soybeans (Glycine max L. Merr") Plant. Physiol. 49: 146-154 (1972)

Hatch, M.D. and C.R. Slack. "The C_4 - Dicarboxylic Acid Pathway of Photosynthesis".
In: "Progress in Phytochemistry" Vol. II.
Ed. L. Reinbold and Y. Luischitz. Pub. Wiley- Interscience London (1970)

Heath, O.V.S. "Studies in Stomatal Behaviour".
New Phytol. 51: 30-47 (1952)

Heath, O.V.S. "The physiological aspects of photosynthesis". Heinemann Educational Books Ltd.
London (1969)

Heath, O.V.S. and B. Orchard. "Temperature effects on the minimum intercellular space CO_2 concentration". Nature , London 180: 180-181 (1957)

Heath, O.V.S. and B. Orchard. "Compensation Points and Carbon dioxide enrichment for lettuce grown under glass in winter". J. Exp. Bot. 18: 746-751 (1967)

Herath, H.M.W. and D.P. Ormrod. "Temperature and Sulfur Nutrition. Effects on CO_2 compensation values in Barley, Peas and Rape. Plant. Physiol. 49: 443-444 (1972)

Hesketh, J. "Enhancement of photosynthetic CO_2 assimilation in the absence of O_2 as dependent upon species and temperature". Planta 76: 371-374 (1967)

Hess, J.L. and N.E. Tolbert. "Glycollate, Glycine, Serine, and Glycerate formation during photosynthesis by tobacco leaves". J. Biol. Chem. 241: 5705-5711 (1966)

Hew, C.S. and M. Gibbs. "A study of chloroplasts of Corn, Sorghum and Sugar Cane". Plant. Physiol. 44: 5-47 (1969)

Hew, C.S. and G. Krotkov. "Effect of O_2 on the rates of CO_2 evolution in light and in darkness by photosynthesizing and non-photosynthesizing leaves". Plant. Physiol. 43: 464-466 (1968)

Hewitt, E.J. "Sand and water culture methods used in the study of plant nutrition". Technical Communication No. 22 of the Commonwealth Bureau of Horticulture and Plantation Crops, East Malling, Maidstone, Kent. (1952)

Hewitt, E.J. "Nitrogen Nutrition of the Plant".
In: Agric. Chem. Sym; University of Leeds.
Ed. E.A. Kirkby (1970)

Hewitt, E.J. and D.J.D. Nicholas. In: "Modern methods of Plant Analysis". Vol. VII P. 67 Eds. H.F. Linskens, B.D. Samwal and M.V. Tracey. Springer-verlag, Heidelberg (1964).

Hoch, G., W.H. Owens and B. Kock. "Photosynthesis and Respiration". Arch. Biochem and Biophys. 101: 171-180 (1963)

Ingoersen, J. and S. Ivanko. "Investigation on the assimilation of N_2 by maize roots..."
Physiol. Plantarum 24: 199-204 (1971)

Ivanko, S. and Ingoersen, J. "Investigation on the assimilation of nitrogen by maize roots and the transport of some major nitrogen compounds by xylem sap". I. "Nitrate and ammonia uptake and assimilation in the major fractions of nitrogen in starved maize roots".
Physiol. Plantarum 24: 59-65 (1971)

Jackson, W.A. and R.J. Volk. "Photorespiration"
Ann. Rev. Plant Physiol. 21: 385-426 (1970)

Jolliffe, P.A. and E.B. Tregunna. "Effect of temperature CO_2 concentration and Light Intensity on O_2 inhibition of Photosynthesis in Wheat Leaves".
Plant. Physiol. 43: 902-906 (1968)

Jones, M.B. and T.A. Mansfield. "A circadian rhythm in the level of the CO_2 compensation point in Bryophyllum and Coffea". J. Exp. Bot. 21: 159-163 (1970)

Jordan, W.R. and R.C. Huffaker. "Influence of age and light on the distribution and development of nitrate reductase in greening barley leaves".
Physiol. Plantarum 26: 296-301 (1972)

Kannagera, C.G. and H.W. Woolhouse. "The role of CO_2, light and nitrate in the synthesis and degradation of nitrate reductase in leaves of Perilla frutescens".
New. Phytol. 66: 553-561 (1967)

Klepper, L. Donna Flesher and R.H. Hageman.
"Generation of reduced NAD for Nitrate Reduction in Green Leaves".
Plant. Physiol. 48: 580-590 (1971)

Krotkov, G., Runeckles, V.C. and Zlimar, K.V. "Effect of light on CO_2 absorption, and evolution by Kalanchoe, wheat and pea leaves". Plant. Physiol. 33: 289-292 (1958)

Laetch, M.W. "Relationship between chloroplasts structure and photosynthetic carbon fixation pathways".
Sci. Prog. 57: 323-351 (1969)

Levitt, J. "The Mechanism of Stomatal Action".
Planta 74: 101-118 (1967)

Lowry, O.H. , N.J. Rosebrough, A.L. Farr and R.J. Randall.
"Protein measurement with the folic phenol reagent".
J. Biol. Chem. 193: 265-275 (1951)

Lips, S.H. and H. Beevers. "Compartmentation of organic acids in corn roots (i) Differential labelling of two malate foods".
Plant. Physiol. 41: 709-712 (1966a)

Lips, S.H. and H. Beevers. "Compartmentation of organic acids in corn roots (ii) The cytoplasmic pool of malic acids".
Plant. Physiol. 41: 713-717 (1966b)

Lake, J.V. "Respiration of leaves by photorespiration I. Estimate for an electrical analogue". Aust. J. Biol. Sci. 20: 487-493 (1967)

Ludwig, L.J. and D.T. Canvin. "An open gas-exchange system for the simultaneous measurement of the CO_2 and 14 CO_2 fluxes from leaves". Can. J. Bot. 49: 1299-1313 (1971a)

Ludwig, L.J. and D.T. Canvin. "The Rate of Photorespiration during Photosynthesis and the Relationship of the Substrate of Light Respiration to the Products of Photosynthesis in Sunflower Leaves". Plant. Physiol. 48: 712-719 (1971b)

Meidner, H. "Effect of photoperiodic induction and debudding in Xanthium pennsylvanicum and of partial defoliation in Phaseolus vulgaris on rates of net photosynthesis and stomatal conductances". J. Exp. Bot. 21: 164-169 (1970)

Meidner, H. and T.A. Mansfield. "Physiology of Stomata" Pub. McGraw-Hill London (1968).

Moss, D.N. "The limiting CO_2 concentration for photosynthesis". Nature 193: 587 (1962)

Myers, J. and G.O. Burr. "Studies on photosynthesis. Some effects of light of high intensity on Chlorella". J. Cell. Physiol. 24: 45-67 (1940)

Nason, A. and H.J. Evens. "Triphosphopyridine nucleotide, nitrate reductase in Neurospora". J. Biol. Chem. 202: 655-673 (1953)

Ogren, W.L. and G. Bowes. "Ribulose Diphosphate Carboxylase regulates Soybean photorespiration". Nature New. Biology 230: 159-160 (1971)

Ongun, A. and C.R. Stocking. "Effect of light on the Incorporation of Serine into the Carbohydrates of Chloroplasts and Nonchloroplast Fractions of Tobacco Leaves". Plant. Physiol. 40: 819-824 (1965a)

Ongun, A. and C.R. Stocking. "Effect of light and dark on the intracellular fate of photosynthetic products ". Plant Physiol. 40: 825-831 (1965b)

Pallaghy, C.K. "Stomatal Movement and Potassium Transport in Epidermal Strips in Zea mays: The effect of CO_2". Planta 101: 287-295 (1972)

Pate, J.S. "Photosynthesizing Leaves and Nodulated Roots as Donors of Carbon to protein of the shoot of the field pea." An. Bot. 30: 93 (1966)

Poskuta, J. "Photosynthesis, photorespiration and respiration of detached spruce twi~~g~~s as influenced by oxygen concentration and light intensity". Physiol. Plantarum 22: 1129-1136 (1968)

Raschke, K. "Saturation Kinetics of the Velocity of Stomatal Closing in Response to CO_2. Plant Physiol. 49: 229-234 (1972)

Ruis, H. and H. Kindl "Formation of α,β - unsaturated carboxylic acids from amino acids in plant peroxisomes". Phytochem. 10: 2627-32 (1971)

Ryle, G.J.A. and J.D. Hesketh. "Carbon dioxide uptake in nitrogen deficient plants". Crop. Sci. 9: 451-454 (1969)

Samish, Y. and D. Koller. "Estimation of photorespiration of green plants and their mesophyll resistance to CO_2 uptake". An. Bot. 32: 687-694 (1968)

Steemann Nielsen, E. "Carbon dioxide concentration, respiration during photosynthesis, and maximum quantum yield of photosynthesis".
Physiol. Plantarum 6: 316-332 (1953)

Steemann Nielsen, E. "The interaction of photosynthesis and respiration and its importance for the determination of ^{14}C - discrimination in photosynthesis."
Physiol. Plantarum 8: 945-953 (1955)

Steward, F.C., G.H. Craven, S.P.R. Weerasinghe and R.G.S. Bidwell. "Effects of prior environmental conditions on the subsequent uptake and release of CO_2 in the light.
Can. J. Bot. 49: 1999-2008 (1971)

Stewart, G.R. "The regulation of Nitrate Reductase level in Lemma minor L."
J. Exp. Bot. 23: 171-183 (1972)

Sutcliffe, J. "Plants and Water".
Edward Arnold (Pub.) Ltd. London (1968)

Tolbert, N.E. "Glycollate pathway". In: "Photosynthetic Mechanisms of Green Plants".
Pub. 1145, NAS-NRC 648-662 (1963)

Tolbert, N.E. "Microbodies - Peroxisomes and Glyoxysomes".
An. Rev. Plant Physiol. 22: 45-72 (1971)

Tolbert, N.E. and M.S. Cohen. "Products formed from glycollic acid in plants".
J. Biol. Chem. 204: 649-654 (1953)

Tolbert, N.E. and R.K. Yamazaki. "Leaf peroxisomes and their relation to photorespiration and photosynthesis."
Annals of the New York Acad. of Sci. 168: 325-341 (1969)

Tregunna, E.B., G. Krotkov, and C.D. Nelson. "Evolution of CO_2 by tobacco leaves during the dark period following illumination with light of different intensities". Can. J. Bot. 39: 1045-1056 (1961)

Tregunna, E.B., G. Krotkov, and C.D. Nelson. "Further evidence on the effect of light on respiration during photosynthesis". Can. J. Bot. 42: 989-997 (1964).

Tregunna, E.B., Krotkov G. and Nelson, C.D. "Effect of O_2 on the Rate of Photorespiration in Detached Tobacco Leaves". Physiologia Plantarum 19: 723-733 (1966)

Trelease, R.N., W.M. Becker, P.J. Gruber and Eldon H. Newcomb. "Microbodies in Cucumber Cotyledons. Correlative Biochemical and Ultra-structural Study in Light and Dark-grown Seedlings". Plant. Physiol. 48: 461-475 (1971)

Weight, J.W., P.M. Warrington and M. Calvin. "The relation of photosynthesis to respiration". J. Amer. Chem. Soc. 73: 5058-5063 (1951)

Weissman, G.S. "Influence of Ammonium and Nitrate Nutrition on Enzymatic Activity in Soybean and Sunflower". Plant. Physiol. 49: 138-141 (1972)

Whiteman, P.C. and D. Koller. "Interactions of CO_2 concentrations, light intensity and temperature on plant resistances to water vapour and CO_2 diffusion." New Phytologists 66: 463-73 (1967)

Wolf, F.T. "Photorespiration, the C_4 pathway of photosynthesis and related phenomena". Advancing Frontiers of Plant Sciences. Ed. L. Chandra 26: 161-231 (1970)

Zelitch, I. "The relationship of glycollate to respiration and photosynthesis in tobacco leaves".
J. Biol. Chem. 234: (1959)

Zelitch, I. "The relation of glycollic acid synthesis to the primary photosynthetic carboxylation reaction in leaves".
J. Biol. Chem. 240: 1869-1876 (1965)

Zelitch, I. "Organic acids and respiration in photosynthetic tissue"
An. Rev. of Plant Physiol. 15: 121-142 (1966a)

Zelitch, I. "Increased rate of nett photosynthetic CO_2 uptake caused by the inhibition of glycollate oxidase".
Plant. Physiol. 41: 1623-1631 (1966b)

Zelitch, I. "Investigation in Photorespiration with a sensitive 14C - assay".
Plant Physiol. 43: 1829-1837 (1968)

Zelitch, I. and Walker, D.A. "The role of glycollic acid metabolism in opening of leaf stomata".
Plant. Physiol. 39: 856-862 (1964)

Zvi Plant and Gibbs, M. "Glycollate formation in intact spinach chloroplasts";
Plant. Physiol. 45: 470-474 (1970)

ADDITIONAL REFERENCES

Candela, M.C., Fisher, E.G. and Hewitt, E.J. "Molybdenum as a plant nutrient and some factors affecting the activity of nitrate reductase in cauliflower plants grown with different nitrogen sources and molybdenum levels in sand culture." Plant Physiol. Lancaster 32:280-288 (1957).

Fock, H., Egle, K., Schaub, H. and Hilgenburg, W. "Der einflub des saurstoff-partialdrucks auf die radioaktivitat der nach $C^{14}O_2$ -zufutterung entstehenden photosynthese-produkte" Z. Pflanzenphysiol. 61:261-263 (1969).

Lips, S.H. and Avissor, Y. "Plant-leaf microbodies as the intra-cellular site of nitrate reductase and nitrite reductase." Eur. Jour. Biochem. 29:20-24 (1972).

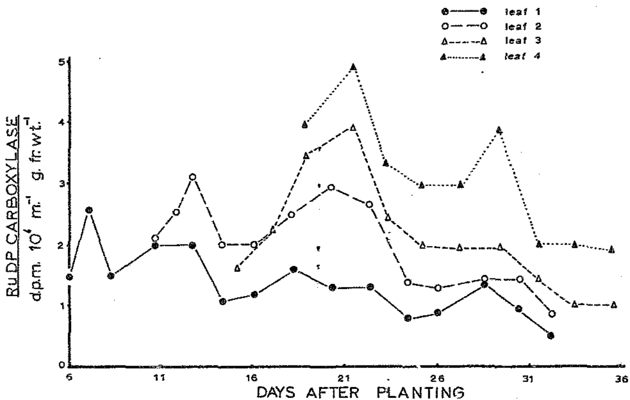
Sestak, Z., Catsky, J. and Jarvis, P.G., EDS. "Plant Photosynthetic Production." Manual of Methods. p.297. Pub. Springerverlage (1971).

Wojcieska, B., Ursula, Ogren, W.L. and Hagerman, R.H. "Photosynthetic properties of soybean grown at different nitrate concentrations." Plant Physiol. 49:suppl. 227 (1972).

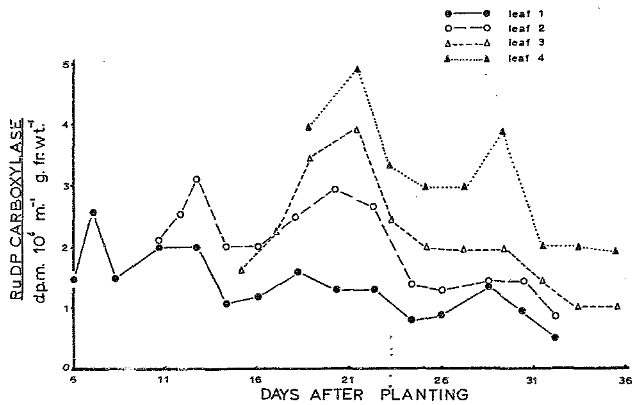
APPENDIX

TABLE OF APPENDIXES

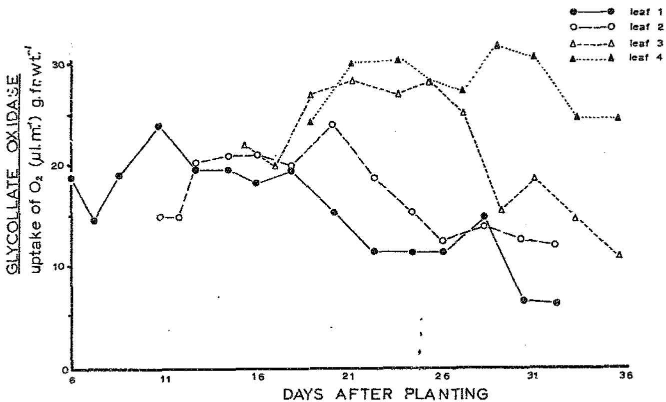
Appendix 1. Graph showing ribulose 1-5,diphosphate carboxylase activity with age in leaves 1, 2, 3 and 4.



Appendix 1. Graph showing ribulose 1-5, diphosphate carboxylase activity with age in leaves 1, 2, 3 and 4.



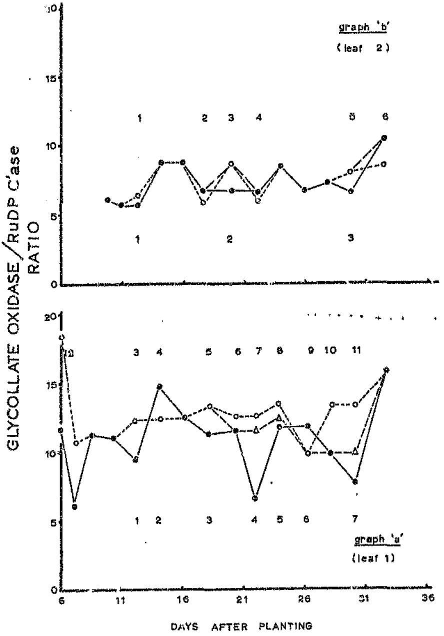
Appendix 2. Graph showing glycollate oxidase activity with age in leaves 1, 2, 3 and 4.



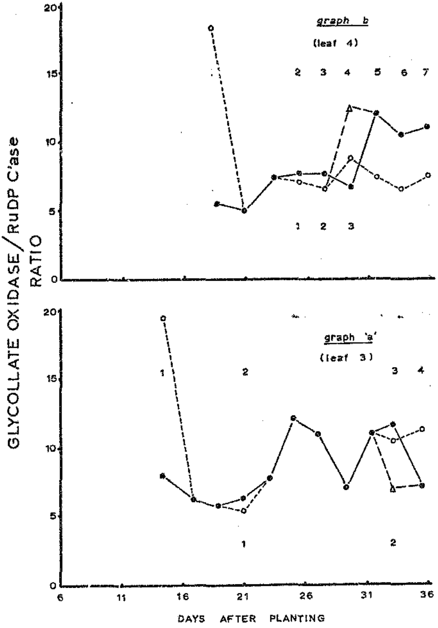
EXPLANATION OF APPENDICES 3 AND 4 -

o-----o show the position of the calculated glycollate oxidase: RuDP carboxylase ratio as described on page 30A.

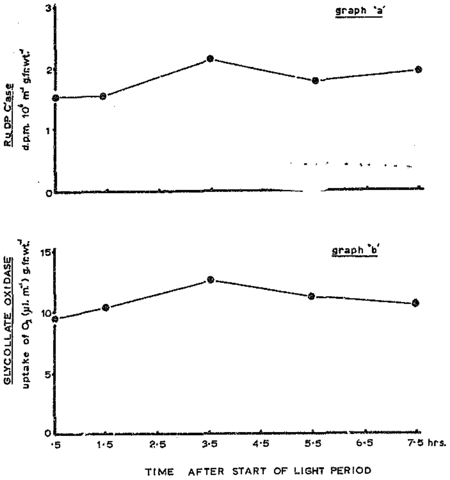
△——△ show the position of the calculated glycollate oxidase: RuDP ratio also as described on page 30A.



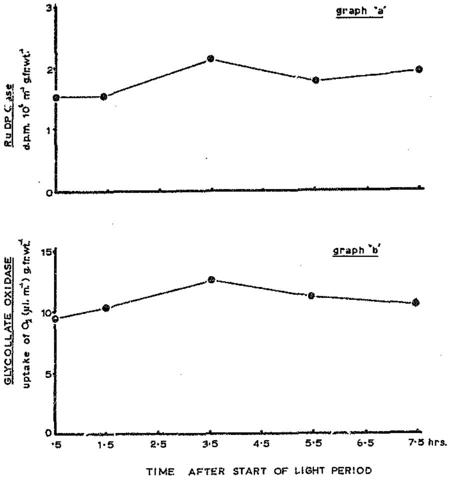
Appendix 3. Graphs showing glycollate oxidase/ribulose 1-5, diphosphate carboxylase ratios for leaves 1 and 2.



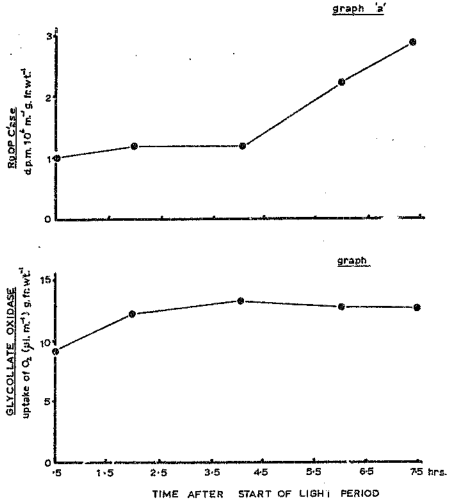
Appendix 4. See appendix 3. Leaves 3 and 4.



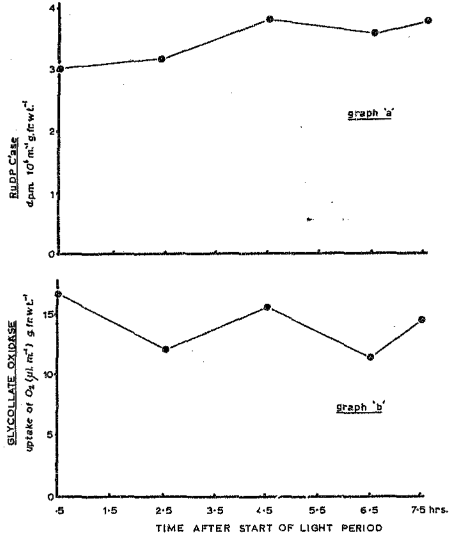
Appendix 5. Graphs showing activity of RuDP C'ase (graph 'a') and glycollate oxidase (graph 'b') during a single light period. 14 days after planting - leaf 2.



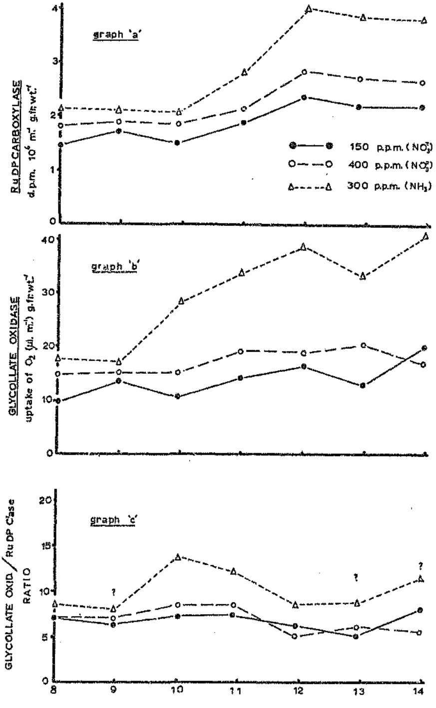
Appendix 5. Graphs showing activity of RuDP C'ase (graph 'a') and glycollate oxidase (graph 'b') during a single light period. 14 days after planting - leaf 2.



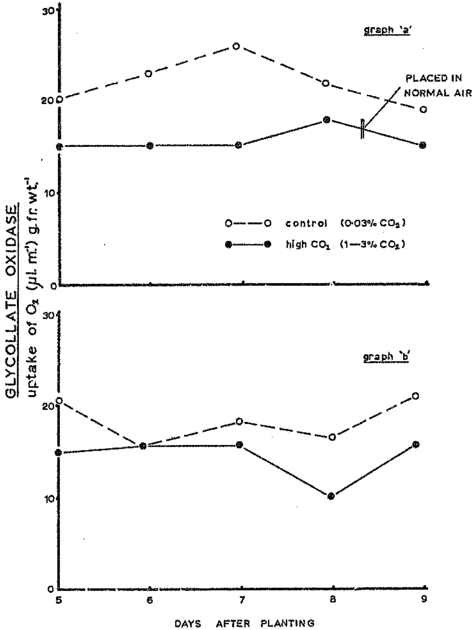
Appendix 6. See appendix 5. 18 days after planting - leaf 2.



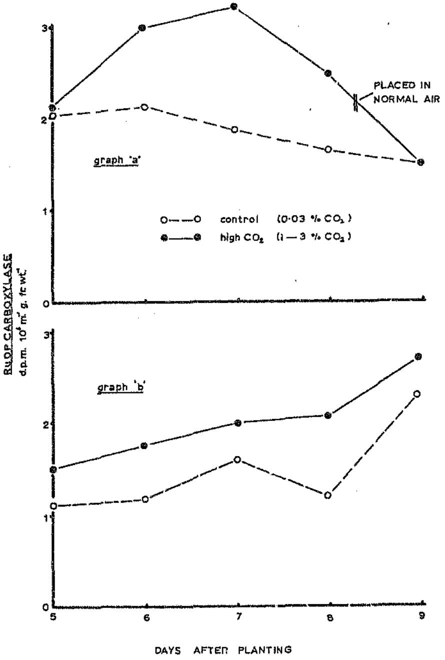
Appendix 7. Seeappendix 5. 16 days after planting - leaf 2.



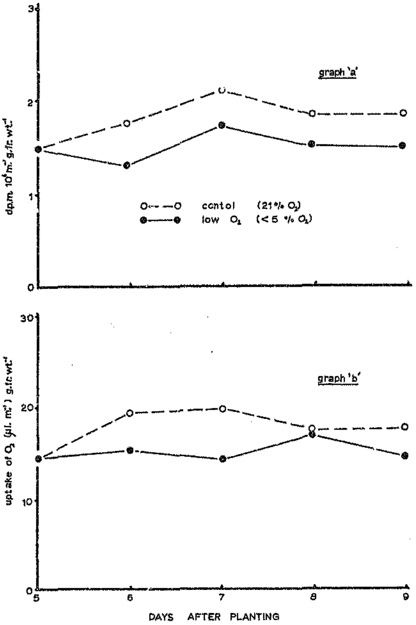
Appendix 8. Graphs showing the effect of varying nutrient supplies on RuDP C'ase (graph 'a') and glycollate oxidase (graph 'b'). Graph 'c' represents the ratio of the above enzymes.



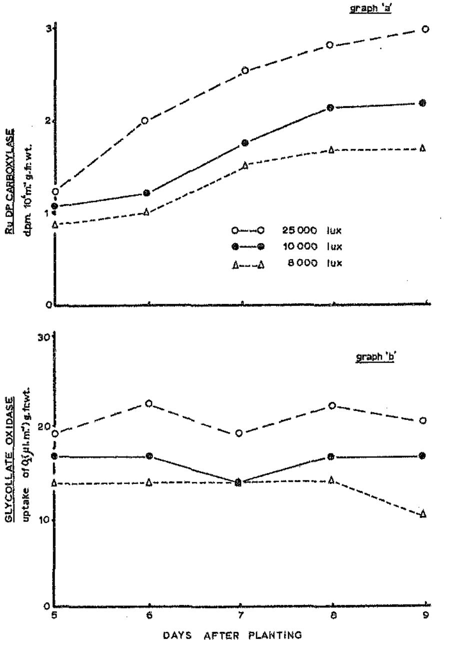
Appendix 9. Graphs showing the effect of high levels of CO_2 (1-3%) on glycollate oxidase activity.



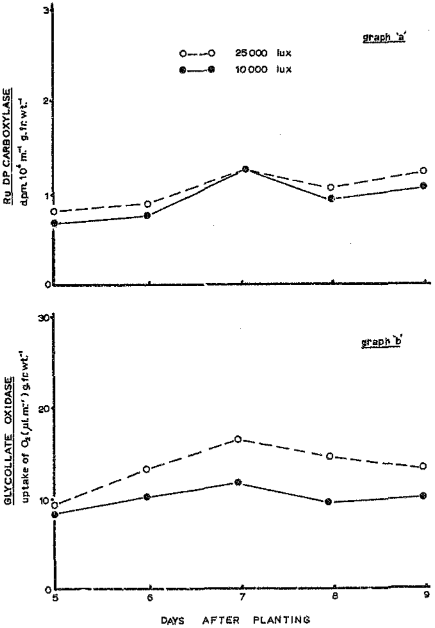
Appendix 10. Graphs showing the effect of high levels of CO_2 (1-3%) on ribulose 1-5,diphosphate carboxylase.



Appendix 11. Graphs showing the effect of low levels of O_2 (< 5%) on ribulose 1-5,diphosphate carboxylase (graph 'a') and glycollate oxidase (graph 'b').



Appendix 12. Graphs showing the effect of light intensity on the activity of ribulose 1-5,diphosphate carboxylase (graph 'a') and glycollate oxidase (graph 'b').



Appendix 13. See appendix 12.

LONG ASHTON NUTRIENT MEDIUM.

	gm/1
KNO_3	0.505
$Ca(NO_3)_2$	0.820
$Na\ H_2\ PO_4 2H_2O$	0.208
$Mg\ SO_4 \cdot 7H_2O$	0.369
$Mn\ SO_4$	0.00223
$Cu\ SO_4 \cdot 5H_2O$	0.00024
$ZN\ SO_4 \cdot 7H_2O$	0.000296
$H_3\ BO_3$	0.00186
$(NH_4)_6\ MO_7\ O_{24} \cdot 4H_2O$)	0.000035
$CO\ SO_4 \cdot 7H_2O$	0.000028
NaCl	0.00585

FERRIC EDTA	.5 gm/1
$Ca\ Cl_2$	.5 gm/1
$K_2\ SO_4$	.435 gm/1
$(NH_4)_2\ SO_4$	1.05 gm/1

Appendix 14

Detail of Long Ashton Nutrient Medium used.